Prism 3130xl genetic analyzer. Sequence data were assembled into contigs using the SeqMan II program (DNASTAR®, Madison, WI).

3.7. Bioinformatics

To evaluate the effects of *GHRHR* IVS2 + 3a > g substitution on 5′-splice donor site strength, we used 5 web-based tools for splice-site analysis: the Splice Site Prediction by Neural Network (NN; http://www.fruitfly.org/seq_tools/splice.html), the Splice-Site Analyzer Tool (SS; http://ibis.tau.ac.il/ssat/SpliceSiteFrame.htm), the SD-Score Algorithm (SD, http://www.med.nagoya-u.ac.jp/neurogenetics/SD_Score/sd_score.html), the MaxEntScan score5ss program (http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html), and the HBond (Hydrogen Bond) Score Web-Interface (HBond, http://www.uni-duesseldorf.de/rna/html/hbond_score.php).

3.8. Ethical considerations

The patient's parents provided written informed consent for the genetic studies described above. The Ethical Committee of Kanagawa Children's Medical Center reviewed and approved the study procedures.

4. Results

4.1. Pharmacological GH stimulation tests

The patient showed impaired GH responses. GH peaked at 2.81 ng/mL in response to insulin-induced hypoglycemia, 3.78 ng/mL in response to arginine, 3.93 ng/mL in response to GHRH, and 1.35 ng/mL in response to GH-releasing peptide-2 (GHRP-2; [22,23]). The normal response to the above pharmacological stimulants is defined as above 6 ng/mL, except for GHRP-2, where normal response is defined as above 16 ng/mL [22].

4.2. Mutation detection

In the *GH-1* gene, no pathological mutations were identified. Sequencing of the *GHRHR* gene revealed compound heterozygous mutations consisting of a G to T transition in the 407th nucleotide in the 5th exon, substituting glycine (GGC) with valine (GTC) [p.G136V], and an A to G transition at position 3 in IVS 2 [IVS2 + 3a > g]. Her father was found to be heterozygous for p.G136V, while her mother was heterozygous for IVS2 + 3a > g (Fig. 1b). Neither mutation has been previously reported, nor were they found in 150 Japanese control individuals. The MLPA analysis did not detect any deletion or duplication at the exon level in each gene examined.

4.3. Functional characterization of the missense mutation in GHRHR (p.G136V)

The glycine to valine mutation at position 136 of the *GHRHR* gene is located in the first transmembrane-spanning domain, and is conserved in disparate species such as mouse, rat, chicken, and zebrafish (Fig. 2a,b). The effect of p.G136V was evaluated by GHRH-induced luciferase reporter assay. Fig. 2c shows that GHRH-stimulated luciferase activity was significantly impaired in p.G136V-GHRHR than in WT cells. HA-tagged WT and G136V receptors showed comparable surface distribution of fluorescence, indicating equivalent membrane receptor expression (Fig. 2d).

4.4. Bioinformatics

To predict the effects of the *GHRHR* IVS2 + 3a > g on mRNA splicing, we first used 5 in silico programs (Table 1). In general, these assessments predicted the intrinsic strength of the 5′-splice site of the WT sequence to be relatively low (NN = 0.31 and SS = 71.6,

Table 1
Comparison of 5′-splice donor sites.

	Position									Splice Site Prediction by Neural Network	Splice-site analyzer tool			SD-score algorithm		MaxEntScan::score5ss[a]				HBond score web-interface
	−3	−2	−1	+1	+2	+3	+4	+5	+6	Score	Score	No. of H-Bond between U1 and 5′ splice-site	Free energy of U1/5′ splice-site pairing	SD-score	Coefficient of variation	ME model	MDD Model	FM model	WM model	H-Bond score
Wild	*T*	*G*	*G*	*g*	*t*	*a*	*t*	*g*	*g*	0.31	71.63	7	−4.7	−3.46	0.72	6.23	8.68	5.39	4.88	11.7
IVS2 + 3a > *g*	*T*	*G*	*G*	*g*	*t*	*g*	*t*	*g*	*g*	0.01	66.67	7	−4.4	−4.80	0.68	1.27	7.28	2.07	3.50	8.3
Cryptic donor site1[b]	*C*	*A*	*G*	*g*	*t*	*g*	*g*	*t*	*g*	0.01	67.69	6	−5.8	−4.10	0.69	1.16	8.08	3.35	4.48	11.8
Cryptic donor site2[b]	*G*	*A*	*G*	*g*	*c*	*a*	*g*	*g*	*t*	0.01	69.73	7	−4.7	na	na	1.90	5.42	2.00	1.74	na
U1 snRNA 3′-	G	U	C	C	A	U	U	C	A	−5′										

na: not applicable. U1 snRNA: U(uridine-rich)1 small nuclear ribonucleic acid. Website of each tool used for splice-site analysis is given in the Materials and Method section.
[a] ME, maximum entropy; MDD, maximum dependence decomposition; FM, first-order Markov; WM, weight matrix.
[b] For the location of each cryptic donor site, see Fig. 3d.

calculated using the Neural Network approach and the Shapiro–Senapathy algorithm, respectively), and that IVS2 + 3a > g further reduces the strength (NN = 0.01 and SS = 66.7), potentially leading to aberrant splicing. The latter hypothesis was further corroborated by other methods. For example, based on the SD-score algorithm, the ΔSD-Score of this mutation was calculated as −1.336, and thus was predicted to cause aberrant splicing. A decrease in the HBond score (11.7 to 8.3) also suggested a reduced sequence complementarity to U1 snRNA (small nuclear RNA).

4.5. In vitro splicing assay

The effect of IVS2 + 3a > g on in vitro splicing efficiency was evaluated using minigene constructs, containing the *GHRHR* exons 2 and 3 with either the WT or mutant sequence (Fig. 3a). RT-PCR of the minigenes revealed that the WT construct generated 2 major splicing products of approximately 470- and 600-bp in size, with the former species being dominant (Fig. 3b). Subcloning and subsequent sequencing showed that the predominant lower band consisted of the expected normal splicing product of 471-bp containing exons 2 and 3 as well as the 474-bp product generated by alternative usage of a CAGCAG tandem acceptor of IVS1, and that the upper band consisted of spliced products but retained the 126-bp sequence of IVS2 (Fig. 3c).

The IVS2 + 3a > g construct also produced 2 bands (Fig. 3b, lane 4), with the 600-bp fragment being the predominant band (Fig. 3b,c). The smaller band (Fig 3b, lane 4) contained either an intronic 20- or 16-bp insertion, consistent with aberrant splicing events due to utilization of cryptic donor splice sites within intron 2 (Fig. 3d, Table 1). The 471/474-bp clones, reflective of normal splicing, were not detected.

Notably, comparison with reference human genomic sequences indicated that the 20-bp-inserted clones used the canonical (gt–ag) splice–site pair, whereas the 16-bp-inserted clones used a non-canonical (gc–ag) splice–site pair (Fig. 3d). In addition, several splicing prediction algorithms (e.g., SS and SD) showed higher 5′-splice site strength for these cryptic splice sites, compared to the mutated counterpart (Table 1). Taken together, our data suggested that IVS2 + 3a > g results in a significant decrease in normal splicing efficiency as well as the activation of cryptic donor splice sites.

5. Discussion

The phenotype of human *GHRHR* gene mutation is IGHD with autosomal recessive trait, classified as IGHD type 1B. First described in 1996 [17], more than 20 mutations have been reported, including a mutation in a POU1F1-binding site of the promoter region [24], missense mutations [8–11,24,25], nonsense mutations [7,8,12,26–28], microdeletions [13,25,29], and splice site mutations [6,14–16,19,26,30–33] (Table 2). Most cases were reported to be severely GH deficient, with extremely low IGF-1 levels and profoundly diminished GH responses to the pharmacological stimulants. Anterior pituitary hypoplasia was a common finding.

This report describes novel compound heterozygous mutations in the *GHRHR* gene in an IGHD patient without consanguinity. The first mutation was a G to T transition in the 407th nucleotide located in

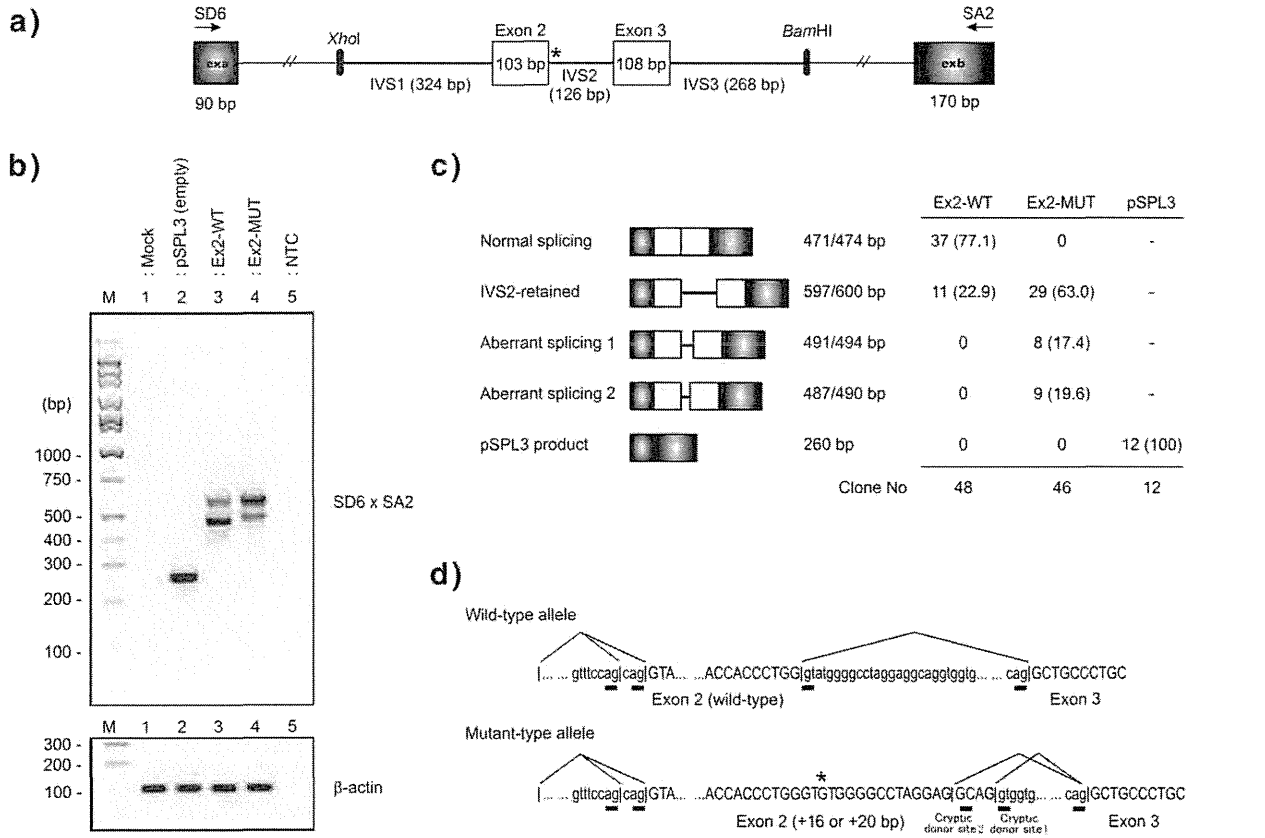

Fig. 3. In vitro splicing assay to verify the effect of the *IVS2 + 3a > g* mutation. a) Schematic diagrams of the *GHRHR* gene locus around the *IVS2 + 3a > g* mutation and the minigene constructs for in vitro splicing assay. SD6 and SA2 are exon-trapping vector pSPL3-specific primers. b) RT-PCR amplification of the GHRHR transcripts obtained from cells transfected with the plasmids pSPL-3-empty (lane 2), pSPL-3-GHRHR exons 2 and 3 wild type (WT; lane 3), pSPL-GHRHR exons 2 and 3 mutant (lane 4), and non-template control (NTC; lane 5). c) Schematic diagrams of WT, various aberrant splicing products, and the appearance frequency. Depending on the alternative usage of CAGCAG tandem in IVS1 acceptor site, the length of normal splice product was either 471 or 474 bp. d) Schematic representation of normal splicing and aberrant splicing with the cryptic 5′ splice sites usage. Underlined nucleotides indicate that both the authentic and cryptic splice sites are involved.

Table 2
Summary of the hitherto reported human mutations in the *GHRHR* gene.

Mutation (site)	Domain	Ethnicity	Consanguinity	Gender	Age at investigation	Height (SDS)	Peak GH response to pharmacological stimuli (ng/mL)					IGF-1 (ng/mL)	IGFBP3 (μg/mL)	Hypoplastic pituitary on MR imaging (age)	Ref.
							GHRH	insulin	arginine	L-DOPA	clonidine				
−124A > C (promotor) K329F (exon 11)	promoter 3rd IC loop	U	−	M	4y	−4.6	1.6	1.2						+ (4 y)	24
IVS1 + 1G > A/IVS1 + 1G > A		Brazil	+	M9, F13	5y − 20y (n = 22)	−2.7 – −8.4	0.63 ± 0.61	flat			flat	6.7 ± 2.8	0.43 ± 0.17	ND	6
IVS1 + 1G > A/IVS4-2A > G		Brazil	−	F	9.5 y	−6.2		1.5			1.9			+ (9 y)	16,30
IVS1 + 1G > A/ E382E (exon 12)		Brazil	−	F	7.1 y	−3.3		0.1						+ (16 y)	16
IVS1 + 2 T > G/IVS1 + 2 T > G		Morroco	+	M F	11y6m 9y4m	−6.6 −4.4		0.1 4.2		0.1 1.2		21 17.4		+ (14 y) + (9 y)	15
Q43X (exon 2) IVS3 + 1G > A	1st EC domain	U	−	M M	11y 8y9m	−5.5 −5.5	<0.05 <0.05		<0.05 <0.05		<0.05 <0.05	20 16		+ (20 y) + (18 y)	26
E72X/E72X (exon 3)	1st EC domain	Sri Lanka	−	M M	7.7 y 6.9 y	−4 −5	<1.5 <1.5					<38.5 <38.5		+ (10 y) + (9 y)	27
		Pakistan	+	M14, F3	3 y – 28 y (n = 17)	−7.2 ± 1.18	0.107 ± 0.053			0.255 ± 0.193	0.374 ± 0.406	5.2 ± 2.0 (n = 14)	0.42 ± 0.13 (n = 14)	ND	7
		U	U	F F	13.8 y 14.8 y	−8.6 −6.74		0.1 0.0		0.1 0.2		7.5 4.2	0.626 0.612	+ (13 y) + (14 y)	28
		India	+ in 2/5 families	M8, F5	4y – 17 y	−4.1 – −8.6	<0.96 (n = 7)			0.9, 1.0	<3.2 (n = 10)	10 – 15 (n = 4)	0.38, 0.42	+ in 4/4 + (4 y – 13 y)	12
		Asia/ Somalia	+	M4, F4	3 y – 15 y (n = 6)	−2.33 – −6.64		<0.1 – 2.2[a]				<−2.5SDS		+ (n = 6) − (n = 2; 4 y, 6 y)	8
E72X/R161W (exon6)	EC/1st IC loop	Asia Asia	− −	M F	5.2 y 4.9 y	−4.81 −4.48		0.7[a] 0.6[a]				<−3.5SD <−2.5SD		+ (5 y) − (4 y)	
R94L/R94L (exon4)	1st EC domain	Asia	U	F	5.8 y	−5.6		0.9[a]				−3.0SD		+ (5 y)	

391delG/391delG (exon4)	1st EC domain	Egypt	+	F F F	5.9 y 5.9 y 5.9 y	−5.5 −5.74 −6.01			0.3		0.3	9 <6 <6		+ (5 y) − (4 y) + (5 y)	13
H137L (exon 5) del 1140-1144 (exon11)	1st TM 7th TM	U	−	F M	14 y 2m 11 y 6m	−7.0 −5.9		1.0 1.0						ND ND	25
L144H/L144H (exon 5)	1st TM	Spain	−	M M	5 y 3 y	−4.0 −3.3	0.41 2.0					22 40		+ (5 y) ND	9
		Brazil	+	F F	26 y 19 y	−7.4 −7.1	<0.3 <0.3					4.6 2.5		+ (26 y) + (19 y)	10
L144H/F242C (exon 7)	1st TM/ 4th TM	US	−	M M	17 y 6m 16 y 2m	−5.2 −4.5		3.8 2.3						ND ND	9
A176V/A176V (exon6)	2nd TM	Pakistan	+	M M	8.5 y 8.4 y	−4.5 −3.5	<1 mU/L		1.2 mU/L 2.0 mU/L					+ (8 y) + (8 y)	11
A222E/A222E (exon 7)	3rd TM	Pakistan	+	M M	11 y 2m 2 y10m	−6.8 −5.2								ND ND	9
		Asia	+	M F	3.2 y 2.0 y	−2.8 −2.98			0.4[a] 0.4[a]			<−3.5SD <−3.5SD		+ (3y) ND	8
IVS7 + 1G > C/IVS7 + 1G > C		Morroco	−	M F	16 y 14.9 y	−5.1 −7.3	absent absent		5.0 mU/L 1.3 mU/L			14.5 26.8		− (25 y) − (23 y)	31,32
IVS7-1G > A/IVS7-1G > A		Brazil	+	M F	10.1 y 3.5 y	−3.9 −2.5	2.6 1.76				1.3 3.49	18		ND ND	16
IVS8 + 1G > A/IVS8 + 1G		China	−	F F F	56 y 42 y 36 y	121.2 cm 119.5 cm 120.2 cm			0.15			31.5	<0.5	ND + (42 y) + (36 y)	33
W273S/W273S (exon9)	2nd EC loop	Asian Asian	U	F F	6.0 y 2.6 y	−4.92 −2.8			0.9[a]			−3.5SD −1.9SD		+ (6 y) − (2− y)	8
E382E/E382E (exon12)	intracellular tail	Japan	−	F F	7 y 2m 5 y9m	−5.2 −5.6	2.0		2.1					− (7 y) − (5 y)	19
del 1121-1124/ del 1121-1124 (exon12)	7th TM	Japan	−	M	3 y	−6.0	0.2		0.6	0.8	0.6	67.9		+ (3 y)	29
IVS12 + 2 T > A/IVS12 + 2 T > A		Pakistan	+	M F F	8 y 3 y 2 y	−3.6 −2.7 −2.5	0.6 0.7 1.0		0.4 1.7 0.5			49 41		− (8 y) − (4 y) + (3 y)	14

EC, extracellular; TM, transmembrane; U, unavailable.

[a] The stimulant was either insulin or glucagon.

exon 5, which led to p.G136V substitution. Functional analysis showed that the G136V mutant failed to elicit any discernible luciferase activity increment in response to GHRH stimulation, albeit normal membranous localization in the immunofluorescence study. Salvatori et al. reported a H137L mutation also located in the first GHRHR transmembrane domain and showed that this mutant receptor was normally expressed [25]. In addition, the first transmembrane domain has been shown to be important for ligand binding, both in GHRHR [34] and in the V2 vasopressin receptor [35]. These findings suggest that impairment of receptor function due to p.G136V must occur in the process from ligand binding to cAMP generation.

The second mutation, IVS2 + 3a > g, is an uncommon splice site mutation, considering that both adenosine (A) and guanidine (G) nucleotides are similarly conserved at the +3 position of the 5′-splice donor site in humans. However, several lines of evidence support our assumption that IVS2 + 3a > g is a disease-causing mutation. First, several examples of disease-associated a > g mutations at this position have been previously described [36,37]. In particular, Ohno et al. reported 10 cases of human disease associated with a > g substitutions at the +3 site [37]. Second, all the in-silico programs we utilized predicted that IVS2 + 3a > g would have resulted in aberrant splicing. Lastly, a splicing assay using a mini-gene construct and containing the mutant genomic DNA spanning exons 2 to 3 of the *GHRHR* gene demonstrated that aberrant splicing products, with a 20-bp or 16-bp insertion, were mainly generated from the allele containing the IVS2 + 3a > g mutation. The 20- and 16-bp insertions would cause a frameshift and result in truncated GHRHR with 98 and 107 amino acids, respectively. These truncated GHRHRs would lose biological activity, assuming the protein structures were profoundly altered.

The minigene constructs with both WT and the IVS2 + 3a > g mutant also generated the 600-bp product, which retained the 126-bp sequence of IVS2. According to the prediction tool for transmembrane helices in proteins (TMHMM v2.0: http://www.cbs.dtu.dk/services/TMHMM-2.0), the 600-bp products would produce GHRHR with an extended 42-amino acid sequence on its N-terminal (results not shown). Although the biological activity of the 600-bp (+42 amino acid) GHRHR is uncertain, and it is unknown whether the longer GHRHR would be generated in vivo, it may have some residual function, considering that it is normal structure except for the longer N-terminal.

In the majority of patients described with *GHRHR* gene mutations, GH responses to pharmacological stimulants had been completely or nearly absent (Table 2). However, our patient, despite severe growth failure and low IGF-1 levels, showed significant GH responses to the various stimulants. In particular, exogenous GHRH induced a GH response up to 3.93 ng/mL, which seemed paradoxical. For measuring circulating GH levels, we utilized the RIA kit with recombinant GH molecule as the standard. Therefore, the absolute values of serum GH level tended to be lower compared to the values determined by RIA/IRMA with older standards [21]. Thus, methodological differences in GH measurement cannot explain the significant GH response observed in our patient.

Another possible explanation for the GH response may be the younger age of our patient (2 years). As noted in Table 2, our patient was one of the youngest among the reported cases. In the setting of diminished GHRH stimulation, GH response to pharmacological stimulants may gradually decrease with advancing age. In addition, as suggested previously [38], age-dependent changes may also affect pituitary size in patients with *GHRHR* mutations. Whereas our patient was found to have normal pituitary size, previous morphological studies described hypoplastic pituitaries in patients older than 8 years of age [38]. Further research involving serial GH stimulation tests and MR imaging studies may help clarify the presence of age-dependent decline in GH response and pituitary size.

It is also possible that, unlike the in vitro results, splicing inefficiencies at the cryptic donor sites may result in normal splicing in vivo, albeit at very low frequency, and could account for low detection level of stimulated GH.

Yet another possible explanation for the GH response in our patient may be explained by the presence of the 600-bp GHRHR isoform discussed earlier. The increased expression of this form of GHRHR may result in preservation of some receptor function. In addition, different GHRHR isoforms originating from the 4th exon (359 and 337 amino acids) have been reported [39]. Given that these isoforms must also be generated in our patient, it may be the case that they exert some receptor functions.

The parents of our patient harboring heterozygous *GHRHR* mutation had normal stature. This finding is in accordance with other reports arguing against any effects of heterozygosity, although gene–dosage effect [7] or dominant negative effect [29,40] of the mutant *GHRHR* had been suggested previously.

In conclusion, we identified novel compound heterozygous *GHRHR* gene mutations in a Japanese IGHD patient without consanguinity. Although the clinical manifestation was compatible with severe GH deficiency, GH reactivity against pharmacological stimuli including GHRH was partially preserved. To elucidate this mechanism, re-testing after a significant time interval may be helpful.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Original Article

Abnormal Adipose Tissue Distribution with Unfavorable Metabolic Profile in Five Children Following Hematopoietic Stem Cell Transplantation: A New Etiology for Acquired Partial Lipodystrophy

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Abstract. We report five consecutive patients who underwent hematopoietic stem cell transplantation (HSCT) to treat leukemia or neuroblastoma early in their lives and later manifested abnormal patterns of adipose tissue distribution. Lipoatrophy was remarkable in the gluteal regions and extremities, whereas subcutaneous fat was preserved in the cheeks, neck, and abdomen. In addition, visceral fat deposition, fatty changes in the liver, and metabolic derangements such as insulin resistance and hypertriglyceridemia were evident. These features resemble Dunnigan-type familial partial lipodystrophy, which is a rare condition caused by *LMNA* gene mutation. These patients shared a common medical history involving HSCT, including conditioning with total body irradiation (TBI). They also received intensive chemotherapy because of multiple metastases (n = 3), relapse (n = 3), and repetitive HSCT (n = 3). We propose HSCT as a new etiology for acquired partial lipodystrophy and recommend that patients who undergo HSCT with TBI and intensive chemotherapy early in their lives must receive careful observation for the possible development of lipodystrophy and metabolic complications.

Key words: chemotherapy, dyslipidemia, hypertriglyceridemia, insulin resistance, total body irradiation

Introduction

Partial lipodystrophy refers to a pathological and unique fat distribution, characterized by lipoatrophy (loss of adipose tissue) and lipohypertrophy (abnormal fat accumulation) (1, 2). Metabolic complications such as insulin resistance, diabetes, hypertriglyceridemia, and fatty changes in the liver are additional hallmarks of partial lipodystrophy (1–4).

Familial partial lipodystrophy (FPLD) arises from genetic mutations, including mutations in the *LMNA* (5–7), *PPARγ* (8–10), *AKT2* (11) and *CIDEC* (12) genes. Specifically, FPLD caused by a *LMNA* mutation is referred to as Dunnigan-type FPLD, or FPLD2, and is characterized by lipoatrophy in the extremities and buttocks combined with fat accumulation in the face, neck

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and intra-abdominal areas. Among the acquired forms of partial lipodystrophy, the most prevalent one is highly active antiretroviral therapy (HAART)-associated lipodystrophy syndrome found in HIV-infected individuals (13, 14). Acquired partial lipodystrophy can also develop following viral infection, autoimmune disease or membranous proliferative glomerulonephritis (1).

We treated 5 consecutive patients who had previously undergone hematopoietic stem cell transplantation (HSCT) to treat malignancies at a younger age. These patients, later in their lives, manifested aberrant fat distribution patterns similar to those occurring in patients with FPLD2, as well as severe metabolic abnormalities.

Case Series

All the study procedures, including the control subjects in body composition analysis, were reviewed and approved by the ethics committee of Kanagawa Children's Medical Center. Patients 2, 3, 4, and 5 and the mother of patient 1 provided written informed consent for publication of their facial photographs.

Patient 1, female, acute myeloid leukemia (AML)

As a result of an evaluation of walking difficulties and repetitive, febrile episodes, a 1-yr-old girl was diagnosed with AML, classified as M4, with multiple extra-marrow involvements, including the central nervous system. Following successful induction chemotherapy, bone marrow transplantations (BMTs) from her mother were attempted twice, but were rejected. A third allogeneic BMT, with conditioning that included total body irradiation (TBI) of 10 Gy, was successful and resulted in long-term remission. However, the patient developed chemotherapy-related leukoencephalopathy, and suffered from intractable epilepsy. To suppress extensive, chronic, graft-versus-host disease (GVHD) (15), she had received steroid therapy for 3 yr (Table 1).

At 17 yr of age, the patient underwent her first endocrinological evaluation (Table 2) because she was short [130.3 cm, –5.3 SD for Japanese standards (16)] and prepubertal. Subcutaneous fat was rather abundant in her cheeks and neck, which resulted in a moon-face appearance (Fig. 1a). In addition, the patient exhibited remarkable abdominal distension, with an abdominal circumference of 69 cm at the navel level. However, both her extremities and buttocks showed marked reductions in subcutaneous fat tissue (Fig. 1b).

An oral glucose tolerance test (OGTT) showed a diabetic blood glucose pattern with tremendous hyperinsulinism (Fig. 2). Mildly elevated alanine aminotransferase (ALT) (56 IU/L) and γ glutamyl transpeptidase (γ GTP) (387 IU/L) levels were found, and fatty changes in the liver were suspected based on abdominal ultrasonography (US). Dyslipidemia was also evident, with fasting triglyceride (TG) levels of 675 mg/dL, high-density lipoprotein cholesterol (HDL-C) of 39 mg/dL and low-density lipoprotein cholesterol (LDL-C) of 168 mg/dL (Table 3).

A magnetic resonance imaging scan revealed a small pituitary gland, and the patient was found to have GH deficiency, subclinical hypothyroidism (TSH, 5.45 μIU/L; free T4, 0.93 ng/dL), and primary ovarian insufficiency (FSH, 60.9 IU/L) (Tables 1 and 2).

Patient 2, female, AML

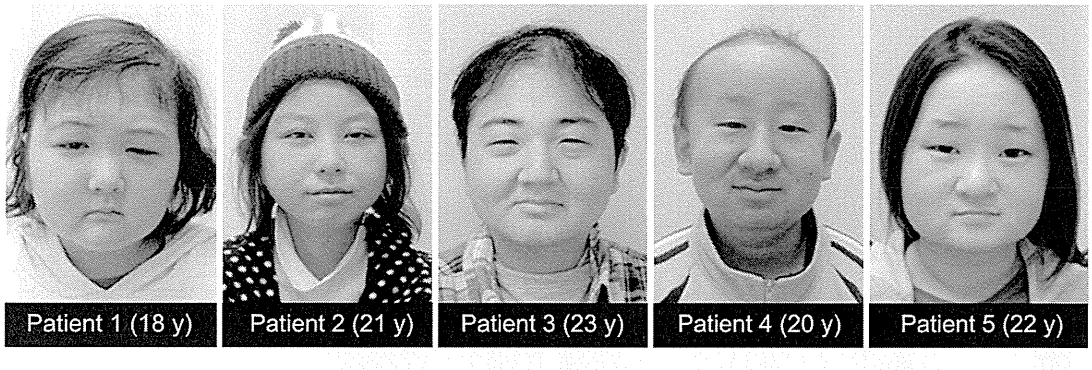
An 8-yr-old girl was diagnosed with AML (M2) through an evaluation for petechiae and epistaxis. Six mo after the first remission was achieved by chemotherapy, a marrow relapse was found. A BMT from a human leukocyte antigen (HLA)-identical donor was conducted, following conditioning that included TBI of 12 Gy. Chronic GVHD with pneumonitis, joint contractures and liver dysfunction required immunosuppressive treatment lasting more than a decade. She also suffered from right-sided femoral neck necrosis (with an onset at age 12), transient aplastic anemia following a parvovirus infection (at age 13) and multiple hepatic angiomas (at age 17).

Table 1 Treatment summaries for the original malignancies in the 5 patients

Pt (sex)	Primary disease (onset)	Treatment			Chronic graft-versus-host disease classification and treatment@	Treatment-related complications
		Chemo-therapy*	Radiation (site)	Transplantation (conditioning#)		
1 (F)	AML (1 yr)	VP-16, Ara-C, MIT, THP-ADR, ACR, VCR, MTX (it), HC (it)	ND	Failed 2 consecutive allogeneic BMTs (BSF, VP-16, L-PAM) Successful third allogeneic BMT (TBI 10 Gy, ATG, TT)	Extensive (skin, liver) CsA (for 1.5 yr), PSL (for 3 yr)	GH deficiency (untreated), hypothyroidism (treated from age 17 yr), leukoencephalopathy, epilepsy, primary hypogonadism (treated from age 17 yr)
2 (F)	AML (8 yr)	VP-16, Ara-C, MIT, IDR, MTX (it), HC (it)	ND	Unrelated BMT following marrow relapse (TBI 12 Gy, BSF, L-PAM)	Extensive (lung, joint, liver) PSL (for 12 yr), FK-506 (for 12 yr)	Femoral neck necrosis, aplastic anemia, hepatic angioma, hypothyroidism (treated from age 15 yr), primary hypogonadism (treated from age 19 yr)
3 (M)	ALL (0 yr)	VCR, THP-ADR, L-ASP, MTX, CPM, Ara-C, 6-MP, PSL, DEX	18 Gy (cranial)	2 consecutive allogeneic BMTs following marrow relapse [1) TBI 12 Gy, VP-16, Ara-C, CPM 2) BSF, VP-16, Ara-C,CPM]	Extensive (skin, joint) CsA (for 4 yr), AZA (for 9 yr), PSL (for 12 yr), MTX (for 1 yr)	GH treatment (from age 11 to 17 yr), chronic thyroiditis (no treatment)
4 (M)	NB (1 yr)	CPM, VP-16, THP-ADR, CDDP, CBDCA	23.4 Gy (cranial), 18 Gy (right orbit)	Scheduled PBSCT (TBI 12 Gy, CBDCA, VP-16, L-PAM)	None	Hypothyroidism (treated from age 4 yr), empty sella, GH deficiency (treated from age 14 to 20 yr), hypogonadism (treated from age 18 yr)
5 (F)	NB (1 yr)	CPM, VP-16, THP-ADR, CDDP, DTIC, IFM	19.8 Gy (epigastrium), 30 Gy (right iliac)	2 consecutive autologous BMTs [1) CBDCA, THP-ADR, L-PAM, 2) CBDCA, VP-16, THP-ADR, CPM], allogeneic BMT following regional and marrow relapse (TBI 12 Gy, TT, VP-16)	Extensive (liver, intestine) FK-506 (for 3 yr), PSL (for 3 yr)	GH deficiency (treated from age 11 to 17 yr), primary hypogonadism (treated from age 15 yr), high-frequency deafness, cataract

F, female; M, male; ND, not done; it, intrathecal injection; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; rt, right. *Abbreviations for agents: VP-16, etoposide; Ara-C, cytarabine; MIT, mitoxantrone hydrochloride; THP-ADR, tetrahydropyranyladriamycin; ACR, aclarubicin hydrochloride; VCR, vincristine sulfate; MTX, methotrexate; HC, hydrocortisone; IDR, idarubicin hydrochloride; L-ASP, L-asparaginase; CPM, cyclophosphamide; 6-MP, 6-mercaptopurine; PSL, prednisolone; DEX, dexamethasone; CDDP, cisplatin; CBDCA, carboplatin; DTIC, dacarbazine; IFM, ifosfamide. #BSF, busulfan; L-PAM, melphalan; ATG, antithymocyte globulin; TT, Thio-TEPA; @CsA, cyclosporin A; FK-506, tacrolimus; AZA, azathioprine.

a



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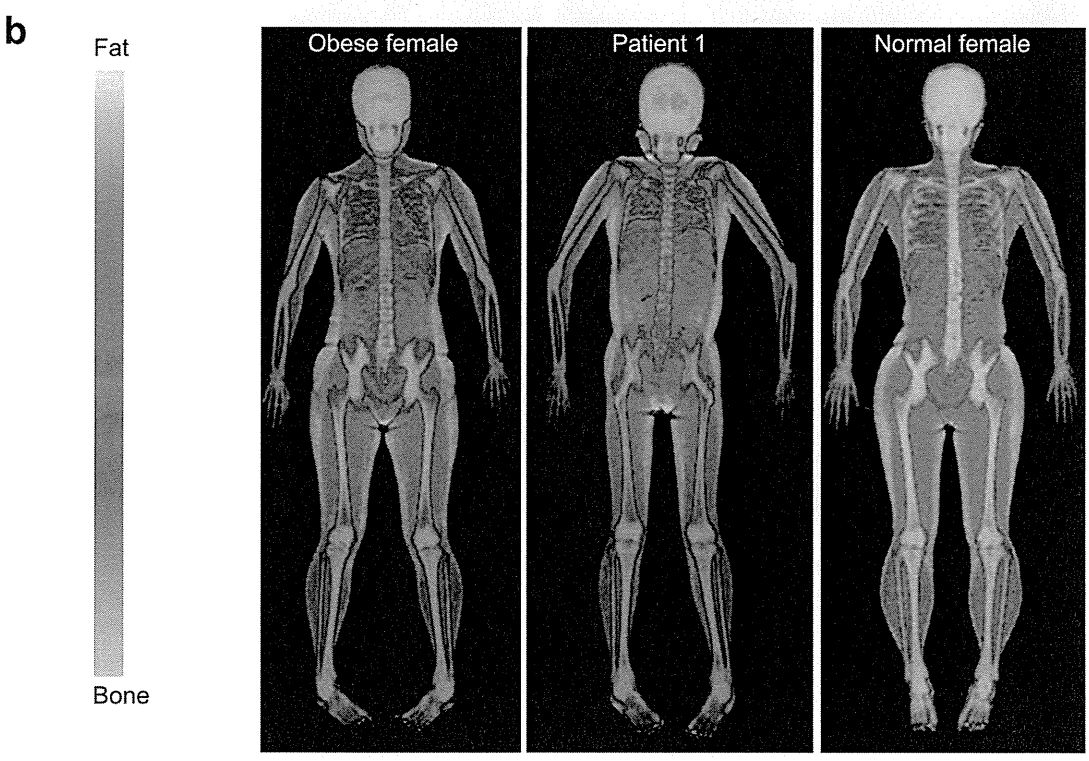

% fat	Obese female	Patient 1	Normal female
upper extremities	54.7	40.5	28.1
trunk	38.5	28.4	18.1
lower extremities	35.0	35.5	30.6
total body	37.2	31.2	23.7

When the patient was 15 yr old, abnormal fat distribution was ascertained by whole body computed tomography (CT) (Fig. 1c). An OGTT showed a normal blood glucose response but with hyperinsulinism (Fig. 2). Dyslipidemia and fatty changes in the liver were also noticed. An endocrinological evaluation revealed mild hypothyroidism (TSH, 9.28 μIU/mL; free T4, 0.89 ng/dL) with primary hypogonadism (FSH, 217.9 IU/L; E2, 5.6 pg/mL).

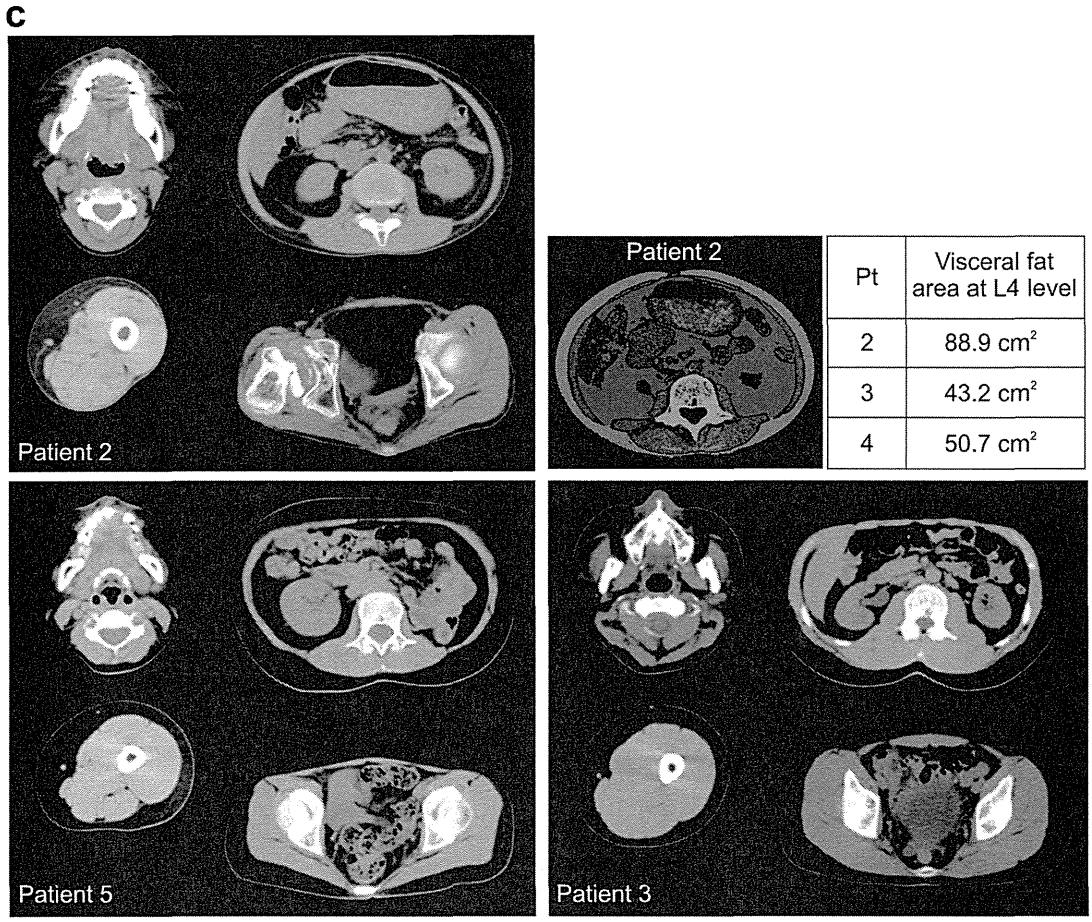

Fig. 1. Abnormal fat distribution observed in the patients. a) Facial photos of the patients. Note that the increased subcutaneous fat tissue in the cheeks gives the impression of a moon-face. b) Dual-energy X-ray absorptiometry image of patient 1 (middle) at age 18 yr. Compared with a 25-yr-old obese woman (left), lipoatrophy in the hips and legs and lipohypertrophy in the neck are evident. For comparison, an image of a nonobese, healthy, 29-yr-old woman is also shown (right). Fat content values of various portions of the body are provided under the images. c) Typical whole-body computed tomography scans of patient 2 at age 18 yr, patient 3 at age 19 yr, and patient 5 at age 18 yr. Increased subcutaneous cheek and visceral fat is evident, whereas loss of subcutaneous fat is remarkable in both the buttocks and thighs. In the inset table, the visceral fat area at the 4^{th} lumbar spine level in each patient is provided, along with the image obtained from patient 2.

Patient 3, male, acute lymphoid leukemia (ALL)

This patient was diagnosed with unclassified ALL at 11 mo of age as a result of an evaluation of petechiae. His first remission was achieved by chemotherapy and 18 Gy cranial radiation. A year later, marrow relapse was found. Following chemotherapy, two consecutive allogeneic BMTs were conducted, with 12 Gy TBI conditioning being provided before the first round of BMT (Table 1).

Chronic GVHD, with major symptoms of joint contractures and scleroderma, was treated with immunosuppressants. In accordance with

Table 2 Current status and information relevant to the etiology of lipodystrophy in the 5 patients

Patient	Current status			At diagnosis of lipodystrophy				Estimated onset of lipodystrophy* (yr)	*LMNA* mutation
	Age (yr)	BMI (kg/m^2)	Typical fat distribution$^{\$}$	Age (yr)	GH status	Thyroid status	Gonadal status		
1	18	17.7	+	17	Deficient	Hypothyroid	Hypogonadal	11	Absent
2	21	12.2	+	15	Sufficient	Hypothyroid	Hypogonadal	13	Absent
3	23	16.5	+	19	Sufficient$^{\#}$	Euthyroid	Eugonadal	12	Absent
4	21	18.3	+	19	Replacement therapy for 5 yr	Replacement therapy for 15 yr	Replacement therapy for 1 yr	15	ND
5	22	14.1	+	17	Replacement therapy for 5 yr	Euthyroid	Replacement therapy for 2 yr	14	Absent

+, present; BMI, body mass index; ND, not determined. $^{\$}$Typical fat distribution denotes lipoatrophy in the gluteal region and extremities coupled with preserved, or even prominent, subcutaneous fat in the cheeks, neck and abdomen. *Onset of lipodystrophy as deduced from the emergence of an elevated triglyceride level. (For details, refer to the Methods section.) $^{\#}$GH treatment was conducted from 11 to 17 yr of age despite normal GH secretion.

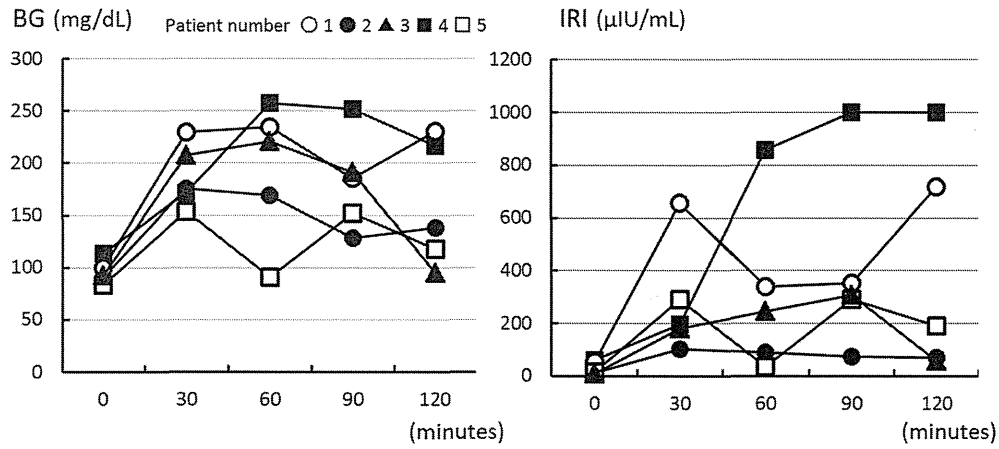

Fig. 2. Results of a 75-g oral glucose tolerance test in the 5 patients. Left panel, blood glucose (BG) response; right panel, insulin response. In the criteria developed by the Japanese Diabetes Society, the diabetic pattern is defined as the fasting blood glucose being higher than 126 mg/dL or the blood glucose level at 120 min being higher than 200 mg/dL. The normal pattern is defined as a fasting blood glucose less than 110 mg/dL and a blood glucose level of less than 140 mg/dL at 120 min.

his wishes, GH treatment was conducted for 6 yr beginning at age 11 despite normal GH secretion. Nevertheless, his final height was 142.4 cm (–4.9 SD).

Thinning of the extremities, due to subcutaneous fat loss, and a moon-face appearance were noticed when the patient was 13 yr old. A whole body CT scan taken at age 19 revealed fatty changes in the liver and an abnormal pattern of subcutaneous fat distribution (Fig. 1c). Dyslipidemia and hyperinsulinism were also evident.

Table 3 Summary of the metabolic profile of the 5 patients

Patient	Fatty liver		Lipid					Adipokines		Diabetes				
	US/CT	ALT (IU/L)	TC (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	Treatment	Leptin* (ng/mL)	Adiponectin# (μg/mL)	BG response in OGTT (age)	Insulinogenic index	HOMA-R	HbA1c	Treatment
1	+	56	322	168	675	39	none	18.7	1.6	DM pattern (17 y)	4.63	13.3	6.1%	none
2	+	102	375	203	965	50	none	9.5	6.8	normal (18 y)	1.06	2.92	5.4%	none
3	+	85	284	179	901	44	fenofibrate	10.7	8.5	normal (13 y)	1.04	2.11	5.3%	none
										normal (23 y)	1.47	1.98	5.1%	
4	+	137	314	176	1,073	44	none	17.9	1.7	DM pattern (19 y)	2.34	17.0	5.7%	none
5	+	250	203	124	402	35	none	11.9	3.8	normal (21 y)	3.89	3.15	5.2%	none

+, present; US, ultrasound; CT, computed tomography; ALT, alanine aminotransferase; TC, total cholesterol; LDL-C, LDL-cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; HOMA-R, homeostasis model assessment ratio; BG, blood glucose; DM, diabetes mellitus; ND, not determined. Each value is the highest one ever determined, except for HDL-C where the lowest value is presented. *Leptin levels in healthy Japanese adolescents were reported to be 1.65 ± 0.78 ng/mL in males and 6.03 ± 3.69 ng/mL in females (35). #Adiponectin levels in healthy young Japanese women were reported to be 6.2–10.0 μg/mL (36). In addition, adiponectin level lower than 6.65 μg/mL was suggested as a diagnostic marker of metabolic syndrome in Japanese children (37).

Patient 4, male, neuroblastoma

A 1-yr-old boy developed exophthalmos and neuroblastoma, originating from the left adrenal, with multiple bone and marrow metastases (stage IV_A). Following total resection of the primary lesion, complete remission was obtained by chemotherapy and radiation (whole skull, 23.4 Gy; right orbit, 18 Gy). Eight months after diagnosis, peripheral blood stemcell transplantation (PBSCT) was carried out, with conditioning that included 12 Gy TBI.

Since age 4, the patient has been receiving L-thyroxine because of primary hypothyroidism (TSH, 22.0 μIU/mL; free T4, 0.78 ng/dL). At age 14, an evaluation for growth failure revealed severe atrophy of the pituitary gland. At that time, abdominal US demonstrated fatty changes in his liver. After a diagnosis of complete GH deficiency, GH treatment was started. At age 18, testosterone administration was introduced due to primary hypogonadism (LH, 7.6 IU/L; FSH, 26.6 IU/L; testosterone, 51 ng/dL).

At 19 yr of age, abnormal liver function tests, dyslipidemia and a moon-face appearance prompted a metabolic reevaluation. Although the degree of aberrant fat distribution was modest compared with other patients, a CT scan demonstrated increased visceral fat with a markedly fatty liver. A diabetic pattern of blood glucose response was observed in an OGTT, as well as pronounced hyperinsulinism.

Patient 5, female, neuroblastoma

A neuroblastoma, with multiple bone metastases (stage IV_A), was diagnosed in a 1-yr-old female during the evaluation of an abdominal mass. Following total removal of the primary tumor and chemotherapy, two consecutive autologous BMTs, without TBI, were performed 3-mo apart. A regional relapse in the right iliac bone, as well as marrow relapse, was found one year later. The patient was treated with an allogeneic BMT, the donor being her 2-locus mismatched sister, following 12 Gy TBI conditioning. Thereafter, complete remission was obtained, and immunosuppressants were withdrawn at age 8.

Partial GH deficiency was diagnosed at age 12, and GH treatment was conducted for 5 yr. The patient also exhibited primary hypogonadism, high-frequency deafness and the presence of cataracts. At around age 17, fatty

changes in the liver and hypertriglyceridemia developed, followed by a mild, abnormal pattern of subcutaneous fat distribution. An OGTT showed normal blood glucose responses, but with hyperinsulinism.

Methods and Results

Body composition analysis with dual-energy X-ray absorptiometry was performed in patient 1 using a Discovery® A Densitometer (Hologic, Inc., Bedford, MA, USA) in fan beam analysis mode and software version 13.3.0.1 (Fig. 1b). To illustrate fat distribution abnormality clearly, a 25-yr-old obese woman and a 29-yr-old healthy nonobese woman served as controls. In the former, this analysis was carried out as one of the routine medical evaluations for obesity.

Visceral fat area at the 4^{th} lumbar spine level was determined with FatVizCalc® (LISIT Co., Ltd., Tokyo, Japan) using the CT images of each patient (Fig. 1c).

Written informed consent for *LMNA* gene analysis was obtained from patients 2, 3 and 5 and from the mother of patient 1. Patient-specific gDNA was extracted from a nail specimen, and the common mutations in exons 8 and 11 of the *LMNA* gene, associated with FPLD2, were studied, as previously described (17). The mutation was absent in these patients (Table 2).

The onset of lipodystrophy is difficult to ascertain because the attending hematologists, as well as the patients themselves, are unaware of fat distribution abnormalities. Because serum triglyceride levels were routinely measured in the patients, we deduced the onset of lipodystrophy based on the timing of emergence of an elevated triglyceride level. As listed in Table 2, lipodystrophy seemed to develop about a decade after HSCT.

Discussion

All the described patients demonstrated a characteristic adipose tissue distribution pattern. Lipoatrophy was remarkable in the gluteal region and extremities, whereas subcutaneous fat was preserved, or even prominent, in the cheeks, neck and abdomen. Visceral fat deposition, as well as fatty changes in the livers of these patients, was also evident. This particular distribution pattern resembles that seen in FPLD2, which is caused by a *LMNA* gene mutation (1, 2, 5–7). Patients with this rare entity, which has an estimated prevalence of 1 in 15 million individuals (1), manifest a peculiar lipodystrophy after adolescence. In addition, metabolic complications, including insulin resistance, diabetes, hypertriglyceridemia, low HDL cholesterol levels and fatty liver, are prevalent in FPLD2 patients (3, 4). Female patients also have an increased risk for developing polycystic ovary syndrome and infertility (18). Compared with generalized lipodystrophy and other types of partial lipodystrophy, leptin and adiponectin levels in FPLD2 are only modestly decreased (19). However, FPLD2 is unlikely the cause of lipodystrophy in these patients, considering its low prevalence and the absence of *LMNA* gene mutations in the 4 patients tested.

Our patients and those with FPLD2 share similarities in fat distribution patterns and in metabolic derangements. Pronounced hypertriglyceridemia, coupled with decreased HDL cholesterol levels, was present in the 5 patients; elevated LDL cholesterol, defined as levels above 150 mg/dL, was present in 4 patients. The patients had high homeostasis model assessment ratios (HOMA-Rs), indicating insulin resistance, although acanthosis nigricans was not observed in any patients. Two patients had OGTTs that categorized them into the diabetic pattern according to the criteria developed by the Japanese Diabetes Society (see Fig. 2 legend). The patients with the diabetic pattern were found to have pronounced hyperinsulinemia with a peak insulin level exceeding 700 μIU/mL. In the present cases, the levels of leptin and adiponectin were modestly decreased.

The patients described in this report shared a common medical history that included HSCT

and conditioning with 10–12 Gy TBI. All of them also received intensive chemotherapy because of the severe nature of their diseases, including widespread metastases (patients 1, 4 and 5) and early relapses (patients 2, 3 and 4), and/or repetitive HSCT (patients 1, 3 and 5). Major surgery, however, was only conducted on those with neuroblastomas. Cranial radiation was performed on only 3 patients.

Based on the above observations, we propose HSCT as a new etiology for acquired partial lipodystrophy. Partial lipodystrophy seems to develop following HSCT, including TBI, especially in conjunction with intensive chemotherapy. This outcome appears to occur irrespective of other interventions such as surgery and cranial radiation. Younger age at the time of HSCT may be of significance, considering that 4 patients received transplants during infancy.

We speculate that TBI and/or intensive chemotherapy may damage the function of adipocytes in the subcutaneous fat, thereby limiting their lipid-storage capacity. This may lead to ectopic deposition of fat in visceral adipose tissue, muscle and liver. This hypothesis is reasonable because adipose tissue fibrosis, and the resultant ectopic lipid accumulation, has been demonstrated in obese individuals (20). Although the mechanism for the characteristic pattern of lipodystrophy is unclear, it may reflect the site-specific adipose tissue functions. In a subset of partial lipodystrophy accompanying glomerulonephritis, differential expression of complementary D by various adipose tissues is considered to cause the different degrees of lipodystrophy among the body (21).

It is essential to consider other potential factors that may be relevant to the development of lipodystrophy. Rooney and Ryan (22) reported a female patient who underwent allogeneic HSCT for relapsed ALL and developed partial lipodystrophy, with overt diabetes, 9 yr later. This patient had GVHD-associated scleroderma, and these authors speculated that there was a causative relationship between partial lipodystrophy and scleroderma. This hypothesis is very intriguing considering that decreased adiponectin levels have been described in systemic sclerodermas with autoimmune origins (23, 24). However, the severity of the GVHD varied among our patients, and scleroderma was present only in patient 3. GVHD was entirely absent in patient 4, who underwent autologous HSCT. Therefore, GVHD and GVHD-related scleroderma may not be a prerequisite but may be a predisposing factor for the development of partial lipodystrophy.

Another factor that should be considered is endocrinopathy. Four patients had endocrinological complications such as GHD, hypothyroidism and hypogonadism (Table 2). Although some of these endocrinopathies had been treated at the time of the investigation, hormone deficiency must be present for a significant period before the initiation of hormonal therapy. At present, endocrinopathy, *per se*, is not regarded as a definite cause of lipodystrophy (1, 2). However, endocrinological complications may be likely to modify the development and/or progress of lipodystrophy, considering that each hormone has its own receptor in the adipose tissue (25, 26), and the relationship between hormonal deficiency and metabolic complications is well-known (27, 28).

A causative relationship between HSCT and lipodystrophy may be disputed based on the absence of reports other than that of Rooney and Ryan (22). However, in accordance with our proposal, a high incidence of fatty liver was also reported in individuals who have undergone HSCT (29). In addition, radiation therapy, including TBI, is an established risk factor for developing metabolic syndrome (30, 31). Moreover, impaired glucose tolerance and dyslipidemia have been described as late complications following HSCT (32–34). We infer that a substantial number of partial lipodystrophy patients may have gone undiagnosed because careful observations are necessary to detect abnormal fat distribution

and because lipodystrophy is not a well-known condition, especially among pediatricians.

To clarify the incidence of HSCT-related lipodystrophy, as well as the contributions of GVHD, GVHD-scleroderma and endocrinopathies, further studies are clearly needed. Lipodystrophy appears to develop more than a decade after HSCT. In addition, the progress of lipodystrophy may be slow, considering that the OGTT results did not differ over a 10-yr interval in patient 3 (Table 3). Thus, prospective studies with long observation periods may be needed to clarify the reality of this potentially life-threatening complication in childhood cancer survivors.

Conclusion

Five pediatric patients manifesting aberrant fat distribution patterns similar to those observed in FPLD2 patients and severe metabolic abnormalities were described. Patients undergoing HSCT, especially when performed early in their lives and in conjunction with TBI and intensive chemotherapy, warrant careful observation for the potential development of partial lipodystrophy.

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CASE REPORT Open Access

Classic Bartter syndrome complicated with profound growth hormone deficiency: a case report

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Abstract

Introduction: Classic Bartter syndrome is a salt-wasting tubulopathy caused by mutations in the *CLCNKB* (chloride channel Kb) gene. Although growth hormone deficiency has been suggested as a cause for persistent growth failure in patients with classic Bartter syndrome, in our opinion the diagnoses of growth hormone deficiency has been unconvincing in some reports. Moreover, Gitelman syndrome seems to have been confused with Bartter syndrome in some cases in the literature. In the present work, we describe a new case with *CLCNKB* gene mutations and review the reported cases of classic Bartter syndrome associated with growth hormone deficiency.

Case presentation: Our patient was a Japanese boy diagnosed as having classic Bartter syndrome at eight months of age. The diagnosis of Bartter syndrome was confirmed by *CLCNKB* gene analysis, which revealed compound heterozygous mutations with deletion of exons 1 to 3 (derived from his mother) and ΔL130 (derived from his father). His medical therapy consisted of potassium (K), sodium chloride, spironolactone, and anti-inflammatory agents; this regime was started at eight months of age. Our patient was very short (131.1cm, -4.9 standard deviation) at 14.3 years and showed profoundly impaired growth hormone responses to pharmacological stimulants: 0.15μg/L to insulin-induced hypoglycemia and 0.39μg/L to arginine. His growth response to growth hormone therapy was excellent.

Conclusions: The present case strengthens the association between classic Bartter syndrome and growth hormone deficiency. We propose that growth hormone status should be considered while treating children with classic Bartter syndrome.

Keywords: Bartter syndrome, Salt-losing tubulopathy, Hypokalemia, Gitelman syndrome, Growth failure

Introduction

Classic Bartter syndrome (BS), also referred to as type III Bartter syndrome, is a rare genetic disorder characterized by salt wasting from the renal tubules, mainly the thick ascending loop of Henle [1]. It is caused by mutations in the *CLCNKB* gene that encodes the type b kidney chloride channel (ClC-Kb). Patients with classic BS fail to thrive from infancy and exhibit hypokalemia, metabolic alkalosis, hyperactive renin-aldosterone system, and overproduction of prostaglandins. Although potassium supplements, anti-aldosterone agents, and/or indomethacin are the mainstay of therapy, management of growth failure and hypokalemia is still challenging [1,2].

The association of growth hormone deficiency (GHD) with classic BS has been anecdotally reported, and GHD may be one of the causes of persistent growth failure frequently observed in patients with classic BS [2-8]. However, the degrees of GHD in the reported cases have been diverse, and hence, GHD has not yet been regarded as a definite complication of BS. In addition, most of the reported cases of BS accompanying GHD were not investigated on a molecular basis [3,7,8]. Moreover, Gitelman syndrome (GS) seems to have been confused with BS in older reports in the literature [4-6]. Here, we report a case of classic BS with documented *CLCNKB* gene mutations in a boy who was found to have profound

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GHD. We also present a literature review on the association between classic BS and GHD.

Case presentation

Our patient was a Japanese boy born at 41 weeks of gestation via spontaneous cephalic delivery, with a birth weight of 3,680g. His family history was remarkable in that his elder sister, who was five years older than him, had been diagnosed as having classic BS when she was five months old: her final height was 147.0cm (−2.1 standard deviation [SD]) and at a recent assessment her insulin-like growth factor 1 (IGF-1) level was 286ng/mL (normal range for her age, 168 to 459ng/mL).

At eight months of age, our patient was diagnosed as having classic BS based on the following findings: failure to thrive, metabolic alkalosis (pH 7.423; HCO_3^-, 33.6mmol/L; base excess, +8.2), hypokalemia (2.9mEq/L), and hyperactive renin-aldosterone system (plasma renin activity (PRA), 270ng/mL/h; normal value for his age, 2.58 ± 1.41ng/mL/h); aldosterone level, 850pg/mL (2,358pmol/L; normal value for his age, 173.7 ± 96.3pg/mL). The diagnosis of BS was confirmed by *CLCNKB* gene analysis, which revealed compound heterozygous mutations with deletion of exons 1 to 3 (derived from his mother) and ΔL130 (derived from his father), the latter of which has been reported previously by the authors TT and MA. Medical therapy consisting of potassium (K), sodium chloride, spironolactone, and anti-inflammatory agents was initiated at eight months of age and is still ongoing. However, as depicted in Figure 1, his serum K level remained considerably low because he was unable to consume large amounts of drugs, especially potassium preparations. Our patient also displayed mild intellectual impairment: he could only speak meaningful words by the age of three, and required specialized primary education.

Table 1 Results of pharmacological growth hormone stimulation tests in our patient at 14 years of age

	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes
Insulin-induced hypoglycemia:					
Blood glucose (mg/dL)	94	54	93	99	92
Growth hormone (μg/L)	0.11	0.07	0.15	0.13	0.08
Arginine:					
Growth hormone (μg/L)	0.11	0.26	0.39	0.28	0.17

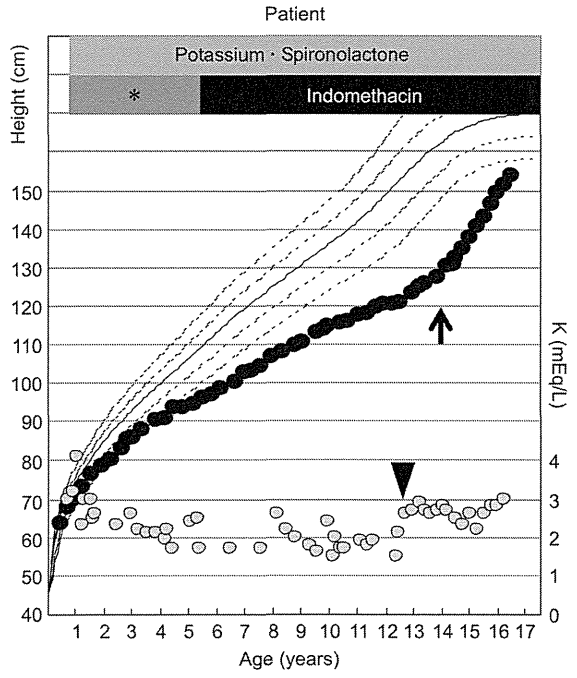

Figure 1 Growth charts of our patient superimposed with variations in his serum potassium levels. Black circles indicate heights; gray circles indicate potassium levels. The arrowhead indicates the age at which our patient could ingest potassium tablets, allowing higher potassium levels than before. Arrow indicates the initiation of growth hormone therapy. *Non-indomethacin anti-inflammatory agents such as tolmetin sodium and mefenamic acid.

When he was 11 years old, an investigation for macrohematuria led to the detection of renal stones with nephrocalcinosis. This complication resolved following the amelioration of hypokalemia, which was achieved by our patient's increased efforts to ingest potassium tablets.

At 14.3 years of age, his severe short stature (131.1cm, -4.9SD) prompted us to evaluate his growth

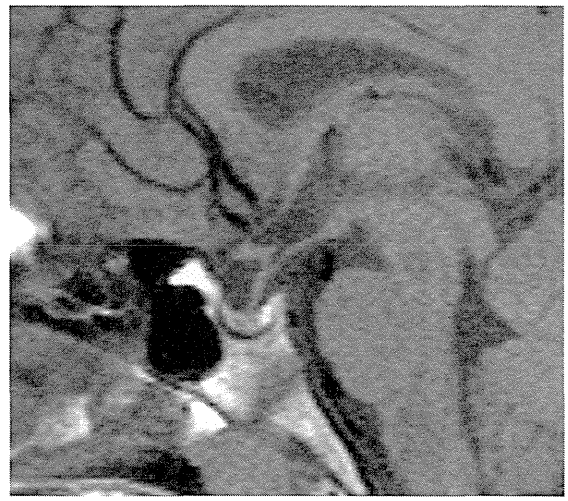

Figure 2 Magnetic resonance imaging scan of the pituitary gland of our patient.