Familial Partial Lipodystrophy linked to a novel peroxisome proliferator activator receptor -γ (*PPARG*) mutation, H449L

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Abstract

Introduction

Familial partial lipodystrophy (FPL) is a rare genetic disorder characterized by a selective lack of subcutaneous fat that is associated with insulin resistance and diabetes. FPL has been reported to be caused by mutations in the *PPARG* gene, which encodes a key transcription factor that regulates adipocyte differentiation and insulin sensitivity.

Material and Methods

The objective of this study was i) to describe the phenotype associated with a novel heterozygous missense *PPARG* mutation, H449L, discovered in a Turkish family; and ii) to compare the fat distribution and metabolic characteristics of subjects with the *PPARG* H449L mutation (n=4) to that of a cluster of FPL patients with various *LMNA* mutations (n=5; R482W, R582H, L306V and T528M).

Results

Compared to patients with *LMNA* mutations, fat loss was generally less prominent in subjects with *PPARG* H449L mutation. Partial fat loss was limited to the extremities whilst truncal fat mass was preserved. The *PPARG* H449L mutation was associated with insulin resistance, hypertriglyceridemia and non-alcoholic fatty liver disease in all affected subjects but the severity was variable. Three of four mutation carriers were overtly diabetic or had impaired glucose tolerance. Pioglitazone therapy in these three individuals resulted in a modest improvement in their metabolic control, and regular menstrual cycles in both females.

Conclusion

We suggest that relatively modest fat loss in patients with *PPARG* mutations may render the recognition of the syndrome more difficult in routine clinical practice. The *PPARG* H449L mutation is associated with insulin resistance and metabolic complications; however the severity is variable among the affected subjects, suggesting that additional factors such as variations in other predisposing genes, gender, age and lifestyle factors might affect the clinical features in patients with *PPARG* mutations.

Keywords: Diabetes, insulin resistance, lipodystrophy, *PPARG*.

Introduction

Familial partial lipodystrophy (FPL) is a rare genetic lipodystrophy syndrome characterized by a selective lack of subcutaneous fat [1]. Fat loss is typically observed in the arms and legs. Subcutaneous fat may accumulate in other areas of the body such as face and neck in some forms of FPL. Visceral fat may also be increased. FPL is frequently associated with insulin resistant diabetes, elevated triglyceride levels and non-alcoholic fatty liver disease. Mutations in several genes have been identified in different subtypes of FPL, most of which are inherited as autosomal dominant traits [2-5]. The Dunnigan variety is the most common subtype of FPL, which is caused by heterozygous mutations in the lamin A/ C (*LMNA*) gene [2]. A mutation in the cell death-inducing DFFA-like effector C (*CIDEC*) gene causes autosomal recessive FPL that has only been reported in one individual in the literature [6]. Some FPL patients do not have mutations in any of these genes including those with Kobberling variety, suggesting that there should be additional genes to be identified.

Barroso et al. [3] were first to identify heterozygous missense *PPARG* mutations, P467L and V290M, in patients with severe insulin resistance, diabetes, dyslipidemia and hypertension. These patients were later noted to have FPL [7]. Since then, approximately 30 patients with FPL due to *PPARG* mutations have been reported [1]. *PPARG*, located on chromosome 3p25, is a member of the nuclear receptor family of ligand-activated transcription factors which encodes PPARG, a key transcription factor that regulates adipocyte differentiation and insulin sensitivity. It is most highly expressed in adipose tissue. Functional studies have shown that missense mutations in *PPARG* are linked to FPL as a result of defective differentiation of adipocytes [8-10]. It has been reported that FPL patients with *PPARG* mutations develop less severe lipodystrophy than those with classical *LMNA* mutations [1, 11].

Here, we report on three sisters and their father from the same Turkish family in whom FPL was caused by a novel heterozygous missense *PPARG* mutation, H449L. The patients were evaluated for fat distribution and metabolic features, and their clinical characteristics and fat distribution were compared to 5 FPL patients with various *LMNA* mutations (R482W, R582H, L306V and T528M).

Material and Methods

The subjects were enrolled from the Turkish Lipodystrophy Study Group (TuLip) registry, a national platform that is committed to furthering our knowledge on lipodystrophy syndromes. The study was approved by the Dokuz Eylul University Ethics Review Panel, and all the subjects gave informed consent.

FPL was clinically diagnosed based on fat loss in selected areas, and the diagnosis was supported by documenting fat distribution using whole body magnetic resonance imaging (WB MRI). The WB MRI was performed by using a 1.5-T MR device (Gyroscan Intera, release 8.1; Philips Medical Systems, Best, the Netherlands) with a 6 multichannel body coil. The WB MR images were performed from the top of the head to the end of the toes in several planes. The protocol included coronal and axial T1-weighted turbo spin echo (TSE) images (Time to repetition (TR)/time to echo (TE)/flip angle (FA)/echo train length (ETL)/field of view (FOV)/slice thickness (ST), 396/17/90°/6/50x180 cm/5 mm), coronal T2-weighted TSE images (TR/TE/FA/ETL/FOV/ST, 3495/90/90°/56/50x180 cm/5 mm) and coronal T2-weighted fat-saturated (SPIR) images (TR/TE/FA/ETL/FOV/ST, 3505/90/90°/56/50x180 cm/5 mm).

Mutation analysis of the genes *PPARG* and *LMNA* were carried out by direct automated DNA sequencing from the patients' genomic DNA. *LMNA* and *PPARG* mutation analyses were performed by sequencing of the coding exons and the exon-intron boundaries of the genes. Genomic DNA was isolated from peripheral blood cells using standard techniques. PCR primers used in order to amplify the regions of interests could be sent upon request. Sequencing was performed with Miseq V2 chemistry on MiSeq instrument (Illumina California, USA). Analysis was performed with IGV software.

According to the follow-up protocol of the TuLip, the patients had detailed physical examination, full biochemistry and urinalysis for protein content at the time of diagnosis for FPL, after which they had these tests on a regular basis approximately every 6 months. Height (m) and weight (kg) were measured under fasting conditions with subjects in light clothing and without shoes. BMI was calculated as body weight divided by height squared. Blood pressure was measured using a sphygmomanometer in the sitting position after 5 min rest. Blood was

taken from the cannulated antecubital vein between 8:00 a.m. and 9:00 a.m. after 8-h overnight fasting. 75-g OGTT was performed in order to evaluate carbohydrate intolerance, if needed. Diabetes was defined according to the recommendations of American Diabetes Association (ADA) [12].

Hepatic steatosis was evaluated by high resolution ultrasound (US), conventional MRI and MR spectroscopy (MRS). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and -γ-glutamyl transpeptidase (GGT) levels were measured. The US was obtained with convex transducers (frequency bandwidth 3-6 MHz). Conventional MRI and MRS were performed by using a 1.5-T MR device (Gyroscan Intera, release 8.1; Philips Medical Systems, Best, the Netherlands) with a phased-array coil. Initially, T2-weighted fat-saturated TSE (TR/TE/FA, 1600/70/90°), T1-weighted inphase gradient echo (GRE) (196/4.6/80°) and T1-weighted opposed-phase GRE (253/6.9/80°) images were obtained. Subsequently, a single voxel 1H MRS was performed for liver by PRESS sequence (TR/TE/, 1900/145). A 20x20x20 mm sized single voxel was placed on both right and left lobes of the liver with paying attention to avoid vascular and biliary structures. The spectroscopic analysis was performed on MR console by the spectroscopy software package of the MR system. On MRS data, water, cholin and lipid peaks were obtained at 4-5, 3.2, and 1-1.5 ppm, respectively. The hepatic triglyceride content was acquired with the measurement method that provides a ratio of signal from fat (f) to total signal from fat (f) and water (w) [f/(f + w)], as described previously [13].

Glucose levels were measured by a colorimetric method. Triglycerides, total cholesterol and high density lipoprotein (HDL) cholesterol were measured using an enzymatic colorimetric assay. Direct low density lipoprotein (LDL) cholesterol measurement was done. Insulin levels were measured by a chemiluminescent method. Homeostasis model assessment (HOMA) score was calculated as fasting serum insulin (μIU/ml) x fasting plasma glucose (mg/dl)/405. Fasting C-peptide levels were measured by a chemiluminescent immunoassay. Leptin and adiponectin levels were measured with enzyme-linked immunosorbant assay (ELISA) according to the manufacturer's instructions (Leptin: Boster Product Code: EK0439, Pleasanton, CA, USA;

sensitivity: < 8 pg/ml; Adiponectin: Boster Product Code: EK0595, Pleasanton, CA, USA; sensitivity: < <60 pg/ml).

Results

Subjects

PPARG family

Patient 1, a 26 year-old woman, first noticed that she lacked fat on her extremities when aged 16 years. Fat loss was limited to extremities. There was no increase in facial and abdominal fat. She developed overt diabetes when she was 22 years old. At that time, she was also discovered to have hypertriglyceridemia, low HDL-cholesterol levels and non-alcoholic fatty liver disease. She reported having had irregular menstrual cycles since the onset of puberty. Polycystic ovaries were detected on ultrasound. Her diabetes was treated with metformin and intensive insulin (insulin aspart 4 units three times a day and insulin detemir 10 units once a day). In view of her physical appearance, she was diagnosed with FPL when she was 23 years old. She was then commenced on pioglitazone. Although she reported no benefit of pioglitazone treatment on her fat distribution, the treatment resulted in improved metabolic control and more regular menstrual periods. Despite no change in her exercise and diet habits, HbA1c dropped from 8.4% to 5.7%. However, no significant change in her lipid levels was observed.

Mutation analysis of the genes encoding PPARG and LMNA identified a novel *PPARG* H449L (c.1346A>T) mutation (Fig.1). She was wildtype for the *LMNA* gene. The *PPARG* mutation was predicted to be "probably damaging" by PolyPhen2 and "disease causing" by Mutation Taster prediction software. This mutation was also found in both her sister's (patients 2 and 3) and her father (patient 4). Fat distribution was similar in all affected subjects within the family. Patient 2, a 30 year-old woman, was not diabetic; however she was discovered to have hypertriglyceridemia and non-alcoholic fatty liver disease on ultrasound. Patient 3, a 28 year-old woman, had IFG + impaired glucose tolerance (IGT), hypertriglyceridemia, polycystic ovaries and non-alcoholic fatty liver disease. Pioglitazone was added to her current treatment with metformin, which resulted in more regular menstrual cycles. Patient 4, a 56 year-old man, had diabetes for 3 years. He also had hypertension, hypertriglyceridemia, low HDL cholesterol levels

and coronary artery disease (CAD). He had already been on pioglitazone for 2 years when he was diagnosed with FPL caused by the *PPARG* H449L mutation. Reportedly, pioglitazone was associated with a slight improvement in his glycemic control, but no change in his fat distribution. The mother was not lipodystrophic. However, she had impaired fasting glucose (IFG), hypertriglyceridemia (220 mg/dl) and hepatic steatosis. The clinical characteristics and recent laboratory results of the affected family members are summarized in tables 1 and 2.

Subjects with LMNA mutations

We have also recently identified five patients with FPL caused by *LMNA* mutations; patient 5 and patient 6 had classical codon 482 mutations (R482W). Patient 7 had a R582H mutation in exon 11, which is expected to affect lamin A only. Patient 8, a 47 year-old woman, first reported fat loss on the extremities and excess fat on the face and neck when she was 16 years old. She had polycystic ovaries. Because of her physical appearance, she was investigated for Cushing's syndrome but these tests were all negative. She also developed diabetes, hypertension, severe hypertriglyceridemia, low HDL cholesterol and non-alcoholic fatty liver disease. Her diabetes was poorly controlled by metformin, pioglitazone and insulin (insulin aspart 22 units three times a day and insulin glargine 44 units once a day). She eventually developed micro- (retinopathy and microalbuminuria) and macrovascular (CAD) complications of diabetes. There was no other feature associated with laminopathy. Mutation analysis of the gene *LMNA* identified a heterozygous missense *LMNA* T528M mutation in exon 9. The parents of patient 9, an 18 year-old woman, first noticed atypical fat distribution when she was 15. Fat loss was remarkable on the extremities, and there was excess fat on the face and neck. She was put on metformin after being diagnosed with IGT by a 75 g OGTT. She also had low HDL cholesterol levels. There was no other feature associated with laminopathy. Mutation analysis of *LMNA* revealed a novel heterozygous missense *LMNA* L306W mutation in exon 7. No clinical data and genotyping was available for the other members of the kindred. However, reportedly, father's sister was lipodystrohic.

All *LMNA* mutations were predicted to be "probably damaging" by PolyPhen2 and "disease causing" by Mutation Taster prediction softwares. The clinical characteristics and recent laboratory results of patients with *LMNA* mutations are shown in tables 1 and 2.

The comparison of fat distribution and metabolic characteristics

MRI investigations and biochemical analysis were performed in each subgroup (Fig.2). However, the study protocol could not be completed for *LMNA* L306W as patient 9 was discovered to be pregnant when she was scheduled for leptin and adiponectin measurements and MRI procedures.

Fat loss was limited to extremities in affected subjects with the *PPARG* H449L mutation (Figs. 2A, B: I, II). Fat excess was not apparent on the face and neck. Compared to people with *LMNA* mutations, fat loss was less prominent in subjects with the *PPARG* H449L mutation. Breast fat was preserved in *PPARG* mutation carriers whereas it was reduced in *LMNA* mutation carriers. Subcutaneous fat was reduced in the gluteal region compared to the control subject; however it was less severe than patients with LMNA mutations. (Figs. 2A-E: I-IV). The degree of hepatosteatosis was less severe compared to the patients with *LMNA* mutations (Figs. 2B-E: VI, VII). f/(f + w) was 0.75 (Fig. 2B: VIII). All patients with *LMNA* mutations manifested prominent fat excess on the face and neck. Patients with the most prevalent *LMNA* mutation (R482W) were characterized by prominent partial subcutaneous and visceral fat tissue loss that was most striking in the gluteal region, forearm and calves in the MR images (Figs. 2C: I, III, IV). Fat loss was also striking in the breast tissue (Fig. 2C: II). Dual phase T1 weighted image (WI) revealed high level fatty infiltration within the liver (Figs. 2C: VI, VII). f/(f + w) was 0.96 (Fig. 2C: VIII). Compared to FPL patients with *LMNA* R482W mutation, *LMNA* R582H and *LMNA* T528M mutations were associated with less severe fat loss (Figs. 2C-E: III, IV). Visceral fat tissue within the abdomen was increased in both patients compared to *LMNA* R482W, which was more evident in the carrier of the *LMNA* T528M mutation (Figs. 2D, E-III). Fatty degeneration was also remarkable in the muscles of the extremities; this was especially prominent in the carrier of the *LMNA* T528M mutation (Figs. 2D, E: V). Hepatosteatosis was less severe compared to *LMNA* R482W (Figs. 2D, E: VI, VII). f/(f + w) was 0.87 and 0.83, respectively (Figs. 2D, E: VIII).

The *PPARG* H449L mutation was clinically associated with insulin resistance and metabolic complications in all carriers. However, the severity was variable among the affected subjects. Two siblings with the *PPARG* H449L mutation were not overtly diabetic (patient 2 had normal

glucose tolerance, and patient 3 was diagnosed with IFG + IGT). Patient 9, in the *LMNA* group had also not developed overt diabetes at the time of our study. Although all patients either with *PPARG* or *LMNA* mutations had lipid abnormalities related to insulin resistance, patients with *LMNA* mutations, especially those with *LMNA* R482W mutation, had more severe hypertriglyceridemia and lower HDL cholesterol levels. Patients with the *PPARG* H449L mutation demonstrated varying levels of leptin and adiponectin from reduced to normal levels. *LMNA* R482W appeared to be associated with more severe hypoleptinemia, however no statistical analysis was performed because of the small number of patients in the study.

Discussion

We have described a novel heterogeneous missense *PPARG* mutation, H449L, in three sisters and their father from a Turkish family. We have also reported a novel heterozygous *LMNA* mutation (L306V) in a proband with FPL. Our study suggests that, compared to FPL patients with various *LMNA* mutations, fat loss is less prominent in subjects with the *PPARG* H449L mutation. Fat loss was limited to the extremities. The *PPARG* H449L mutation was associated with insulin resistance and metabolic complications. However, the severity was variable among the affected subjects. While the youngest sister and the father had overt diabetes, the older sisters were not diabetic but did exhibit signs of insulin resistance. Pioglitazone appeared to result in a modest improvement in metabolic control although this was not formally evaluated in a clinical trial setting. It was also associated with more regular menstrual periods in females either when used as add-on therapy to metformin or when used in conjunction with existing treatment with metformin and intensive insulin therapy.

Mutations in *LMNA* and *PPARG* cause FPL in the majority of reported cases [1, 2, 14]. A handful of patients with FPL due to some other mutations have also been described [4-6, 15]. On the other hand, there are some FPL patients, including those with Kobberling variety, without mutations in any of the genes known to cause lipodystrophies [1]. *LMNA* is the most common gene responsible for FPL. It leads to a somewhat heterogeneous partial lipodystrophy phenotype influenced by age, gender, diet and presumably other genetic variants. Although patients with FPL due to *LMNA* mutations share several core characteristics such as the loss of fat in the lower extremities and in the gluteal region, and fat accumulation in the face, they vary in the loss of subcutaneous

abdominal fat, and also in the severity of fat loss from the extremities. The degree of insulin resistance is also variable [16]. Many missense mutations have been reported in the *LMNA* gene in patients with FPL, most of which are located in exon 8 at the codon position 482, affecting both splice forms, lamins A and C [1]. As *LMNA* encodes proteins prelamin A and lamin C by alternative splicing in exon 10, mutations in exons 11 and 12 affect lamin A only. Garg et al. [16] noted that patients with *LMNA* exon 11 mutation had a less severe form of lipodystrophy. There are rare cases reported with *LMNA* mutations in exons 1, 7, and 9 [17-20]. Compared to classical codon 482 mutations, reported patients with *LMNA* mutations in exons 1, 7, and 9 had some differences. Fat loss was mild; even no clinical lipoatrophy was detectable in some cases [18]. Most of them were referred not for altered fat distribution, but for insulin-resistant diabetes [18-20]. Here, we have identified a novel heterozygous missense mutation in the *LMNA* gene which appeared to result in FPL (L306V in exon 7). Although no genotyping was available on the other members of the kindred including the parents, father's sister was reportedly lipodystrophic. In another patient, FPL was linked to a heterozygous *LMNA* T528M in exon 9. Savage et al. [21] previously described three FPL patients with subcutaneous fat loss on the trunk and limbs, who were compound heterozygotes for *LMNA* S583L and T528M mutation in exon 9. Interestingly, they reported that carriers of the T528M mutation in the same family were not clinically lipodystrophic. However, detailed imaging was not reported in this study. Furthermore, the authors mentioned that subjects with heterozygous missense *LMNA* T528M mutation had some features of insulin resistance such as increased fasting triglycerides, diabetes and hypertension.

Compared to patients with *LMNA* mutations, less is known about the phenotype of FPL patients with *PPARG* mutations. Barroso et al. [3] were first to identify heterozygous missense *PPARG* mutations in patients with severe insulin resistance, diabetes, dyslipidemia and hypertension. Later, Savage et al. [7] reported that these three patients had FPL caused by two different *PPARG* mutations, P467L and V290M. They concluded that *PPARG* mutations differ from other partial lipodystrophies syndromes in the preservation of normal facial and abdominal fat depots. They also suggested that the lack of excess facial fat distinguishes FPL patients with *PPARG* mutations from those with LMNA mutations. Agarwal et al. [14] described limb and facial lipoatrophy in a Caucasian female with a heterozygous R425C mutation in *PPARG* gene. As far as *PPARG* mutation H449L is concerned, we similarly observed no abnormalities in

subcutaneous abdominal fat. There was no facial atrophy. Facial fat was not increased as observed in patients with *LMNA* mutations. Ludtke et al. [8] proposed that the presence of arterial hypertension appeared to be the main difference between subjects with FPL due to *LMNA* and *PPARG* mutations. They suggested that arterial hypertension was very common in subjects with *PPARG* mutations while it was only reported in some patients with *LMNA* mutations [3, 7, 8, 10, 22, 23]. On the contrary, none of the sisters with *PPARG* mutation H449L had hypertension in our study. It is very well known that *PPARG* has a key role in the control of insulin action [24]. It has been proposed that, despite a less distinct fat loss, subjects with *PPARG* mutations tend to develop more severe diabetes which occurs at a younger age. Savage et al. [7] suggested that FPL patients with *PPARG* mutations represent a human monogenic model of the metabolic syndrome. However, in this present study, *PPARG* H449L mutation was associated with a relatively mild metabolic syndrome phenotype. While the youngest sister had distinct diabetes which developed at a relatively young age, the severity of insulin resistance was variable in other affected siblings. Despite all showing the signs of insulin resistance, the other sisters were not overtly diabetic. Furthermore, none of the sisters was hypertensive. We conclude that the clinical phenotype appears to be heterogeneous in FPL patients with *PPARG* mutations, and it is yet not possible to describe a 'concrete' genotype-phenotype correlation because of the relatively small number of subjects described so far.

Thiazolidinediones, PPARG agonists, act by activating PPARs, with the greatest specificity for PPARG [25]. The treatment of patients with type 2 diabetes with PPARG agonists has been associated with a selective increase in subcutaneous adipose tissue [26]. Because the mutant receptors in patients with FPL caused by *PPARG* mutations have been shown to retain some residual ability to respond to PPARG agonists in vitro [3], the clinical use of these agents could improve fat distribution and metabolic outcomes. For this purpose, patient 1 was treated with pioglitazone for three years in addition to metformin and intensive insulin treatment with insulin aspart and detemir. Patient 3 also received pioglitazone for four months as add-on therapy to metformin. Both sisters reported improvements in the regularity of their menstrual cycles due to polycystic ovaries after pioglitazone. However, no study was done on their androgen levels. We also observed a positive effect of pioglitazone on the glycemic control of patient 1 (HbA1c dropped from 8.4% to 5.7%) without any attempt to change her diet, exercise and medications.

Savage et al. [7] treated two patients with FPL due to *PPARG* mutations with another PPARG agonist, rosiglitazone, which was later removed from the market because of its cardiovascular side effects. They observed a significant increase in the total body fat in both subjects, which was more remarkable in the limb and gluteal regions rather than in the trunk. Metabolic control was dramatically improved in one patient with a *PPARG* P467L mutation, who had more marked changes in the fat distribution. However, in the other patient with a different *PPARG* V290M mutation, rosiglitazone treatment resulted in no change in insulin resistance and a very slight reduction in HbA1c levels. Similarly, the father in our *PPARG* family reported a very slight reduction of HbA1c after pioglitazone. However, we should note that he was already on pioglitazone in the first appointment, so we could only evaluate the effect of the drug retrospectively.

In conclusion, we have described the phenotype of a novel heterogeneous missense *PPARG* mutation, H449L, in three Turkish sisters and their father. Fat loss was less prominent compared to FPL patients with *LMNA* mutations, which may render the recognition of the syndrome more difficult in routine daily practice. The *PPARG* H449L mutation was associated with insulin resistance and metabolic abnormalities; however the severity was variable among the affected subjects, suggesting that clinical features in patients with *PPARG* mutations may vary depending on additional factors such as age, gender, diet and other genetic variants. Pioglitazone is worth considering in the treatment of patients with FPL caused by mutations in the *PPARG* gene as it may lead to metabolic benefits.

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Tables

Table-1: The clinical characteristics of patients with FPL caused by PPAR-γ and LMNA mutations.

	PPAR-γ				LMNA				
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Current age	26	30	28	56	30	32	41	47	18
Gender	Female	Female	Female	Male	Female	Female	Female	Female	Female
Mutation	PPAR-γ, H449L	PPAR-γ, H449L	PPAR-γ, H449L	PPAR-γ, H449L	LMNA, R482W	LMNA, R482W	LMNA, R582H	LMNA, T528M	LMNA, L306W
The age when fat loss was first noticed (years)	16	13	13	?	13	17	20	16	15
The age when FPL was first diagnosed (years)	23	28	26	55	19	27	33	43	17
Follow-up (months)	36	24	24	12	132		96	48	12
Diabetes duration (years)	4	Not diabetic	IFG + IGT	3	10	9	6	18	IGT
BMI (kg/m^2)	23.6	19.9	23.7	22.1	20.8	31	27.3	30.6	20.4
Hypertrigliseridemia	+	+	+	+	+	+	+	+	-
Low HDL	+	-	-	+	-	+	+	+	+
HT	-	-	-	+	+	+	-	+	-
PCO	+	-	+	NA	+	-	+	+	-
Acanthosis nigricans	+	-	+	+	+	+	+	+	-
Hepatosteatosis	+	+	+	+	+	+	+	+	+
Macrovascular complications	-	-	-	CAD	-	-	-	CAD	-
Microvascular complications	-	-	-	+	-	Proteinuria	-	Retinopathy, microalbuminuria	-
Current treatment	Metformin, pioglitazone, insulin	NA	Metformin, pioglitazone	Metformin, pioglitazone, sitagliptin, ramipril	Metformin, pioglitazone, gliclazide, gemfibrozil, irbesartan	Metformin, insulin, fenofibrat, fish oil, ramipril	Metformin, pioglitazone, fenofibrat	Metformin, pioglitazone, insulin, fenofibrat, irbesartan	Metformin
Insulin dose (per day)	22 units	NA	NA	NA	NA	102 units	NA	110 units	NA

FPL: Familial partial lipodystrophy, PPAR-γ: Peroxisome proliferator-activated receptor -γ, LMNA: Lamin A/C, IFG: Impaired fasting glucose, IGT: Impaired glucose tolerance, BMI: Body mass index, HDL: High density lipoprotein, HT: Hypertension, PCO: Polycystic ovaries, CAD: Coronary artery disease, NA: Not available.

Table-2: Laboratory levels of patients with FPL caused by PPAR-γ and LMNA mutations.

	PPAR-γ					LMNA			
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Fasting glucose (mg/dL)	74	91	113	155	116	236	117	173	80
HbA1c (%)	5.7	NA	6	8,3	7.6	10.2	6.2	8.7	5.7
Total cholesterol (mg/dL)	195	218	164	170	195	175	183	293	148
Triglyceride (mg/dL)	179	208	211	179	575*	799*	169*	313*	93
HDL cholesterol (mg/dL)	45	51	50	37	33	21	38	42	29
LDL cholesterol (mg/dL)	114	125	72	97	126	61	111	188	100
Creatinin (mg/dL)	0.63	0.64	0.56	0,84	0.59	0.6	0.71	0.68	0.5
ALT (U/L)	29	21	102	118	35	21	32	71	12
GGT (U/L)	18	13	47	129	39	22	14	76	27
Protein excretion (mg/L)	6.7	9.1	9.6	171,96	4.5	313	5	30.25	4
C3 (mg/dL)	129	121	153	158,2	114	154	129	144	NA
C4 (mg/dL)	25	16,4	27	24,6	28.8	20	20.5	46.2	NA
Fasting insulin (µIU/mL)	NA**	6.69	96.88	27,76	20.77	NA**	26.5	NA**	33
HOMA	NA**	1,35	26,79	10,33	5,73	NA**	7,51	NA**	6,52
Fasting C-peptid (ng/mL)	5.2	2.38	10.2	5,88	5.24	1.9	4.93	1.88	4.34
Fasting leptin (ng/ml)	7.83	16.52	6.7	14.88	3.72	3.66	11.86	9.77	NA***
Fasting adiponectin (µg/ml)	3.93	33.62	8.57	8.03	8.57	4.04	9.9	10.27	NA***

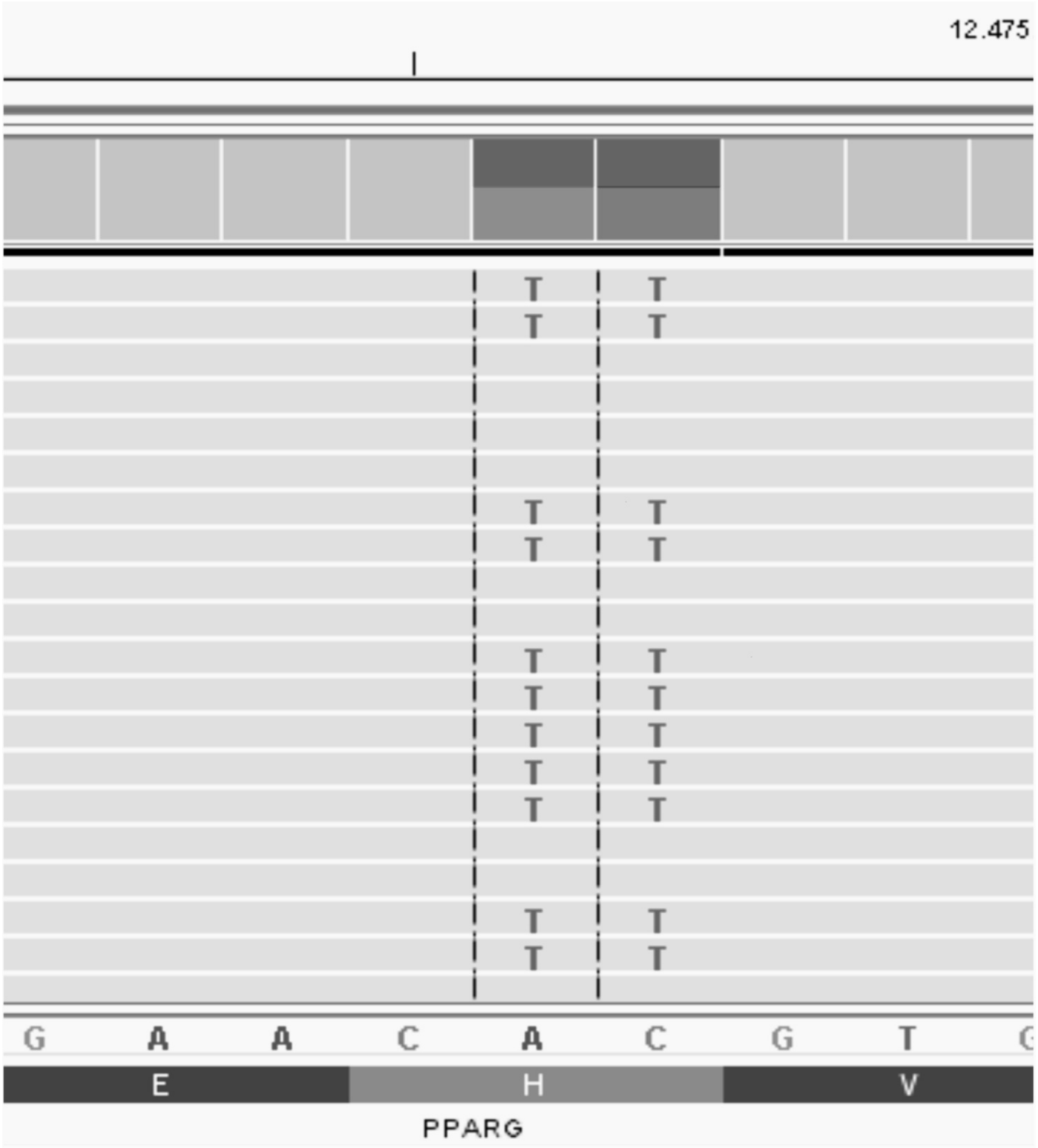
FPL: Familial partial lipodystrophy, PPAR-γ: Peroxisome proliferator-activated receptor -γ, LMNA: Lamin A/C, HDL: High density lipoprotein, LDL: Low density lipoprotein, ALT: Alanine transaminase, GGT: Gamma-glutamyl transferase, HOMA: Homeostasis model assessment, C3: Complement component 3, C4: Complement component 4. * Lipid levels were taken under lipid lowering treatment. **Insulin and HOMA scores were not available for selected patients as they were being treated with insulin injections. ***Leptin and adiponectin levels were not available for patient 9 as she was discovered to be pregnant when she was scheduled for the test.

Figure legend

Figure 1: PPAR-γ, H449L (c.1346 A>T) mutation detected in the affected Turkish family.

Figure 2: MR images and MR spectroscopy findings of the control and patients with FPL in each subgroup.

A: Female control, age: 28 years, height: 167 cm, weight: 58 kg, BMI: 20.8 kg/m2, waist: 70 cm, hip: 94 cm, waist to hip ratio: 0.75. B: Patient 3, PPAR-γ, H449L; C: Patient 5, LMNA R482W; D: Patient 8, LMNA T528M; and E: Patient 7, LMNA R582H. Whole body T1 WI (I), axial T1 WIs at the level of breast (II), abdomen (III), gluteal region (IV) and thigh (V) reveal fat distribution of the body and extremities. Dual phase T1 WIs (VI and VII) show signal loss on out of phase images (VII) in affected patients which is in consistent with variable degree of hepatosteatosis. MRS spectra of affected patients indicate the variable degree of hepatosteatosis (VIII, arrows).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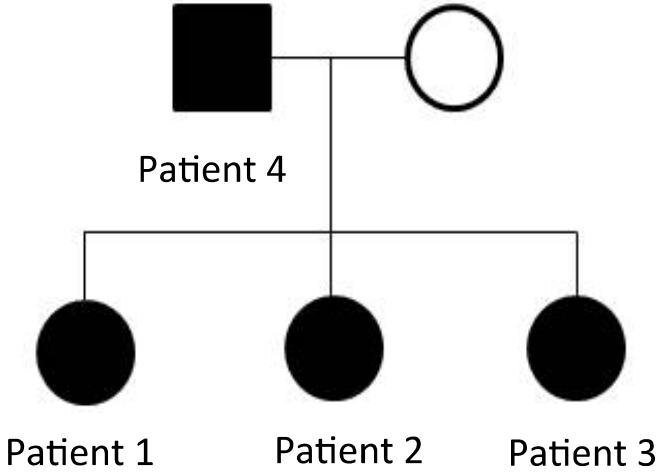
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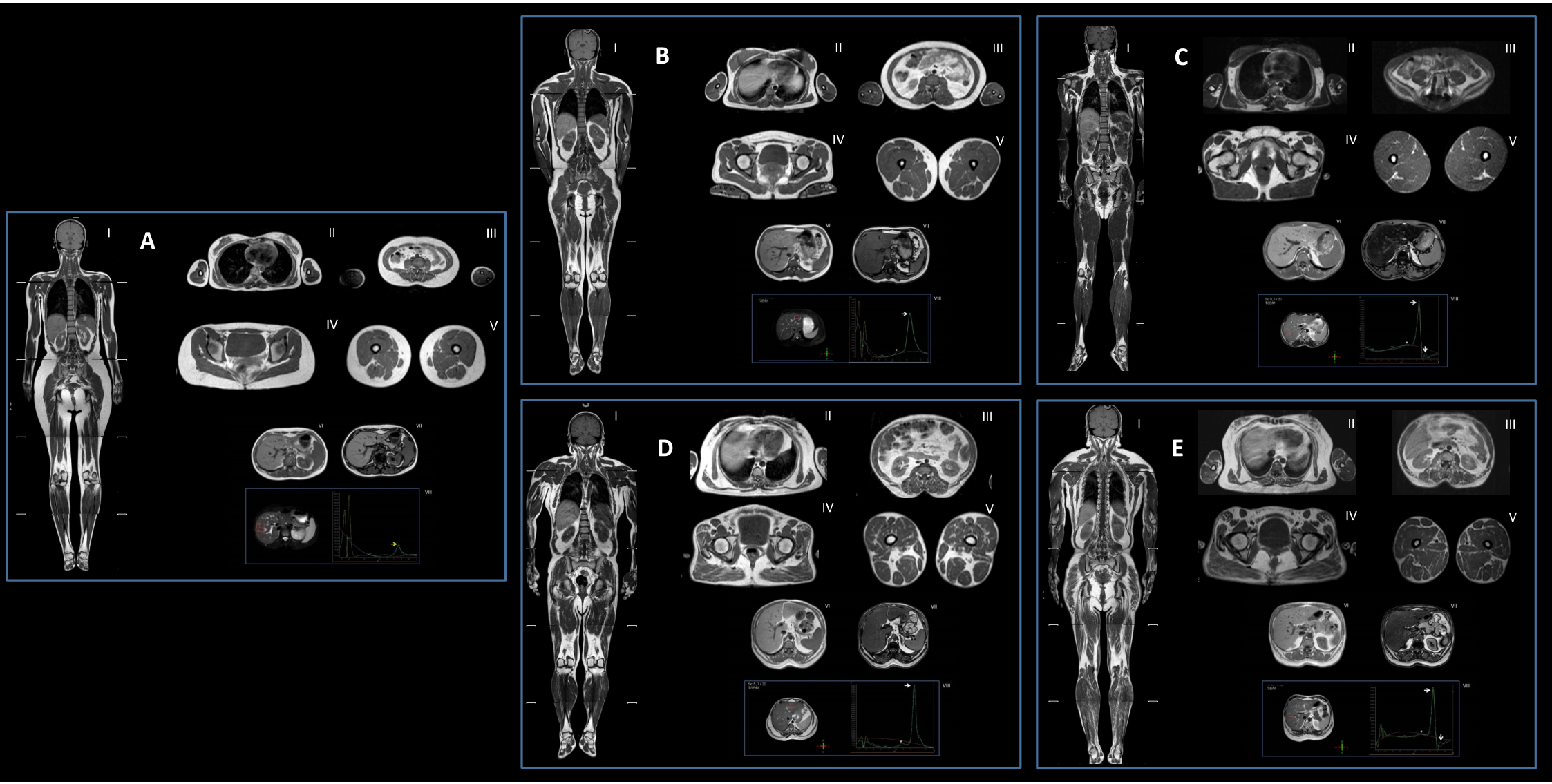
Patient 1
Patient 2
Patient 3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