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Clinical Research Article

Pelvis Magnetic Resonance Imaging to Diagnose Familial Partial Lipodystrophy

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Abstract

Context: The diagnosis of familial partial lipodystrophy (FPLD) is currently made based on clinical judgment.

Objective: There is a need for objective diagnostic tools that can diagnose FPLD accurately.

Methods: We have developed a new method that uses measurements from pelvic magnetic resonance imaging (MRI) at the pubis level. We evaluated measurements from a lipodystrophy cohort (n = 59; median age [25th-75th percentiles]: 32 [24-44]; 48 females and 11 males) and age- and sex-matched controls (n = 29). Another dataset included MRIs from 289 consecutive patients.

Results: Receiver operating characteristic curve analysis revealed a potential cut-point of ≤13 mm gluteal fat thickness for the diagnosis of FPLD. A combination of gluteal fat thickness ≤13 mm and pubic/gluteal fat ratio ≥2.5 (based on a receiver operating characteristic curve) provided 96.67% (95% CI, 82.78-99.92) sensitivity and 91.38% (95% CI, 81.02-97.14) specificity in the overall cohort and 100.00% (95% CI, 87.23-100.00) sensitivity and 90.00% (95% CI, 76.34-97.21) specificity in females for the diagnosis of FPLD. When this approach was tested in a larger dataset of random patients, FPLD was differentiated from subjects without lipodystrophy with 96.67% (95% CI, 82.78-99.92) sensitivity and 100.00% (95% CI, 98.73-100.00) specificity. When only women were analyzed, the sensitivity and the specificity was 100.00% (95% CI, 87.23-100.00 and 97.95-100.00, respectively). The performance of gluteal fat thickness and pubic/gluteal fat thickness ratio was comparable to readouts performed by radiologists with expertise in lipodystrophy.

Conclusion: The combined use of gluteal fat thickness and pubic/gluteal fat ratio from pelvic MRI is a promising method to diagnose FPLD that can reliably identify FPLD in women. Our findings need to be tested in larger populations and prospectively.

Key Words: familial partial lipodystrophy, gluteal fat, pubic fat, pelvic MRI

Abbreviations: ALT, alanine aminotransferase; APL, acquired partial lipodystrophy; AST, aspartate aminotransferase; AUC, area under curve; DXA, dual-energy X-ray absorptiometry; GL, generalized lipodystrophy; *LMNA*, lamin A/C; MRI, magnetic resonance imaging; *PPARG*, peroxisome proliferator-activated receptor gamma; ROC, receiver operating characteristic curve.

Lipodystrophy is a group of rare diseases characterized by near-total or partial lack of adipose tissue (1). Lipodystrophy is classified into 2 main categories as generalized lipodystrophy (GL) and partial lipodystrophy, both of which can be genetic or acquired (2).

Familial partial lipodystrophy (FPLD) covers a spectrum of heterogeneous diseases characterized by selective partial fat loss. The diagnosis of FPLD is established clinically; however, successful diagnosis requires knowledge and familiarity with the syndrome and a certain amount of clinical experience. The accurate diagnosis of FPLD can be challenging even for experts as the disease has various subtypes, can be modified because of environmental and other genetic modifiers, and not all causative genetic variants are known (3).

Although fat distribution may vary in different subtypes of FPLD, all patients selectively lose fat from the lower part of the body, which is a unique characteristic (4). The preservation of specific fat depots can be helpful to identify subtypes of lipodystrophy (5). Patients with some forms of FPLD classically preserve and/or accumulate fat in the face and neck and upper part of the body, especially posteriorly (6). The presence of pubic fat can be clinically observed in patients with FPLD (7). Many years ago, Kobberling and Dunnigan (8) reported the prominent appearance of genitalia with a considerable

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amount of fat on the mons pubis in a patient with FPLD. A recent study highlighted the presence of pubic fat in patients with FPLD. By using low-resolution dual-energy X-ray absorptiometry (DXA) images, Meral et al (9) identified an isolated high signal area corresponding to the mons pubis. Increased fat signal around the mons pubis region was observed in all patients with FPLD2 and 34% of the females with FPLDX (no causative genetic mutation found) but in none of the control subjects.

FPLD is a focus of increasing interest because the disease offers a model for conditions presenting with ectopic lipid deposition and insulin-resistant states. Further, development of novel therapeutic agents may offer disease-specific treatment options to those with the disease (10). Thus, it is critical to develop objective tools that can diagnose FPLD accurately. DXA-derived lower limb fat (%) was reported to be an objective anthropometric measure for diagnosing FPLD2 in females in a study comparing data from a cohort of patients with FPLD2 to sex- and age-matched controls from the National Health and Nutrition Examination Survey and unaffected females from the Dallas Heart Study (11). The ratio of percentage fat in the trunk to percentage of fat in the legs, namely fat mass ratio, from DXA scans may prove useful as a diagnostic tool, although there is some overlap between FPLD and subjects without lipodystrophy (12). Recently, DXA scan-derived "fat shadows" were proposed as a potential diagnostic tool. Our study conducted at the University of Michigan (9) found that "fat shadows" derived from DXA scans may improve the diagnosis of FPLD qualitatively, but not quantitatively because this method was developed recognizing the substantial overlap between healthy lean controls (especially lean athletic males) and subjects with lipodystrophy, and still required a degree of training and building clinical acumen on the part of the interpreting physicians.

In this study, we aimed to develop a reliable quantitative method to diagnose FPLD by using fat measurements from pelvic magnetic resonance imaging (MRI) at the pubis level. Although whole-body MRI is very informative in lipodystrophy (13, 14), it is only available in referral centers, requires special equipment, is time-consuming and expensive, and requires clinical and radiological expertise. On the other hand, pelvic MRI is easy to perform because it takes around 5 minutes to complete and can be performed elsewhere. We thought, if successful, this new approach would provide a simple quantitative assessment that could help diagnose FPLD accurately without the need to secure clinical expertise.

Materials and Methods

Dataset 1

We recruited 59 patients with various subtypes of lipodystrophy (median age [25th-75th percentiles]: 32 [24-44]; 48 females and 11 males). MRI scans were performed between 2013 and 2022 at Dokuz Eylul University. Lipodystrophy group consisted of 30 patients with FPLD (median age [25th-75th percentiles], 39 years [29-49]; 27 females and 3 males), 19 patients with GL (median age [25th-75th percentiles], 26 years [21-33]; 14 females and 5 males), and 10 patients with acquired partial lipodystrophy (APL) (median age [25th-75th percentiles]: 35 years [27-43]; 7 females and 3 males). Age- and sex-matched control subjects (n = 29; (median age [25th-75th percentiles], 31 years [22-39]; 19 females and 10 males) were selected from our radiology database in whom a pelvic MRI scan was ordered for clinical purposes, but no significant pathology was found. MRI was performed because of urinary tract symptoms in 7 (25.9%), genital pain in 13 (48.1%), gastrointestinal symptoms in 6 (22.2%), and for assessment of fat distribution (which turned out to be normal) in 1 (3.7%) patient.

Subjects who met any of the following criteria were not included in the study: any acute or chronic disease that can alter fat distribution (excluding altered fat distribution from lipodystrophy syndromes), lipodystrophy previously treated with metreleptin and/or subjects currently on treatment with metreleptin, treatment with any investigational medicinal product, HIV-positive subjects, radiation-induced lipodystrophy, Cushing syndrome, subjects who are immunocompromised or receiving immunomodulatory drugs (past 2 years), known history of drug or alcohol abuse, and pregnant women.

Dataset 2

MRI scans from 289 consecutive subjects without lipodystrophy (median age [25th-75th percentiles], 47 years [35-63]; 178 females and 111 males), who underwent an abdominopelvic or pelvic MRI for various reasons at Dokuz Eylul University Department of Radiology between 2021 and 2022, were included for confirmatory testing. MRI indications were gastrointestinal pathologies in 100 (34.6%), malignancies in 75 (26%), urinary tract pathologies in 53 (18.3%), genital tract pathologies in 45 (15.6%), benign tumors in 11 (3.8%), infectious diseases in 4 (1.4%) subjects, and assessment of fat distribution in 1 (0.3%) individual. Measurements from 30 patients with FPLD were added to this dataset.

Laboratory Measurements

Glucose, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides, and cholesterol levels were measured by standardized methods with appropriate quality control and quality assurance procedures. All laboratory assessments were performed after a minimum of 8 hours fasting. Direct low-density lipoprotein cholesterol measurement was performed.

MRI Acquisition

The pelvic MRI protocol included an axial T1-weighted turbo spin-echo image (time to repetition/time to echo, 396/17; flip angle, 90°; echo train length, 6; field of view, 50 × 180 cm; slice thickness, 5 mm). A single-slice axial MRI scan at the mons pubis level was selected from whole-body MRIs or abdominopelvic/pelvic MRIs obtained previously by using a 1.5-T MRI system with a 6-multichannel body coil (Gyroscan Achieva, release 8.1; Philips Medical Systems). Gluteal fat thickness was the average of the right gluteal and the left gluteal fat. On T1-weighted axial images, the mons pubis fat thickness was measured in the midline in sections passing through the level of the symphysis pubis joint. On both sides, the gluteal fat tissue thickness was measured at the midline for each hemigluteal region. Measurements were made in a single section. Each measurement was repeated 3 times and averaged. Spatial resolution was 0.1 mm. Pelvic MRI scans were also reviewed by radiologists with significant expertise

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in lipodystrophy. These experts were blinded to clinical diagnoses and fat measurements.

Ethics Statement

The study protocol was reviewed and approved by the Dokuz Eylul University Ethics Committee (Approval ID: 2022/25-05). All subjects gave written consent for genetic testing.

Role of the Funding Source

No sponsor was involved in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Statistical Analysis

Statistical analysis was performed using Statistical Package of Social Science (SPSS, IBM Corp), version 22.0 for Windows. Data were expressed as median (25th-75th percentiles). Categorical parameters were analyzed by using the χ^2 test. Mann-Whitney *U* test was used to compare continuous parameters. Receiver operating characteristic curve (ROC) curves were generated to assess the diagnostic ability of fat measurements at a variety of thresholds. Sensitivity (true positives/[true positives + false negatives]), specificity (true negatives/[false positives + true negatives]), positive predictive value (true positives/[true positives + false positives]), and negative predictive value (true negatives/[true negatives + false negatives]) were calculated. A *P* value of .05 or lower was considered statistically significant.

Results

Characteristics of patients with lipodystrophy and healthy controls from dataset 1 are shown in Table 1. Among subjects with lipodystrophy, 40 had partial lipodystrophy. Among 30 subjects with FPLD, 2 with Lamin A/C (*LMNA)* c.1456A > G, p.(K486E) and homozygous *LMNA* c.1745G > A, p.(R582H) pathogenic variants presented with severe fat loss. Another patient with *LMNA* c.1045C > T, p.(R349W) variant, had progeroid features and an atypical fat loss pattern affecting the face and the limbs. Another 12 patients with heterozygous *LMNA* variants c.1583C > T, p.(T528M), c.1745G > A, p.(R582H), c.1444C > T, p.(R482W), c.1445G > A, p.(R482Q), c.916C > G, p.(L306V), and c.139G > A, p.(D47N) had classical Dunnigan syndrome fat loss pattern predominantly affecting the limbs with preservation/accumulation of fat in the upper part of the body and/or face and neck. Two patients had peroxisome proliferator-activated receptor gamma (*PPARG)* variants of c.452A > G, p.(Y151C) and c.1346A > T, p.(H477L). These 2 patients had relatively milder fat loss affecting the limbs. The remaining 13 subjects with FPLD phenotype had no disease-causing variants. Among them, 7 patients had a phenotype suggestive of Mendelian FPLD but no pathogenic variant in the genes currently associated with FPLD (FPLDX), and 6 had Kobberling variety (FPLD1) characterized by central obesity, metabolic disorders, fatty liver, and a deficiency of adipose tissue in the gluteal region and lower extremities without an identifiable causative gene. Whole exome/genome sequencing was not performed in any of these patients, but they were screened for known lipodystrophy genes by genetic panels and/or Sanger sequencing. Ten patients were diagnosed with APL. Among 19 patients with GL, 12 had homozygous 1-acylglycerol-3-phosphate-O-acyltransferase-2 variants c.202C > T, p.(R68X), n = 2; c.685G > T, (p.E229X), n = 3; c.514G > A, p.(E172K), n = 1; c.538_539delGA, p.(D180PfsX5), n = 1; c.144C > A, p.(C48X), n = 4; and c.662-2A > C, (IVS5-2 A > C), n = 1. Four had homozygous Berardinelli-Seip congenital lipodystrophy 2, otherwise known

Table 1. Clinical and laboratory features and MRI measurements of patients with lipodystrophy and controls, dataset 1

	All subjects			Females		
	Lipodystrophy (n = 59)	Control (n = 29)	*P* value	Lipodystrophy (n = 48)	Control (n = 19)	*P* value
Age, y	32 (24-44)	31 (22-39)	.239	34 (27-44)	29 (18-35)	.029
BMI, kg/m^2	21.7 (19.6-24.8)	22.6 (19.4-25.4)	.699	21.7 (19.6-25.0)	21.7 (18.7-25.4)	.636
Sex						
Female	48 (81.4%)	19 (65.5%)	.117			
Male	11 (18.6%)	10 (34.5%)				
Glucose, mg/dL	132 (94-174)	90 (82-98)	<.001	133 (94-171)	89 (82-96)	<.001
Creatinine, mg/dL	0.60 (0.49-0.77)	0.76 (0.61-0.87)	.008	0.57 (0.49-0.73)	0.67 (0.53-0.76)	.065
ALT, IU/L	25 (16-36)	17 (10-21)	.001	24 (15-33)	14 (9-19)	.001
AST, IU/L	23 (17-33)	19 (17-25)	.110	24 (16-34)	18 (15-21)	.024
Total cholesterol, mg/dL	187 (170-233)	187 (163-204)	.490	196 (173-235)	187 (171-208)	.543
Triglyceride, mg/dL	321 (151-619)	108 (76-157)	<.001	323 (172-691)	105 (74-144)	<.001
HDL cholesterol, mg/dL	32 (25-41)	49 (40-56)	<.001	33 (26-42)	54 (41-63)	.002
LDL cholesterol, mg/dL	107 (88-126)	114 (84-124)	.707	112 (86-142)	110 (89-130)	.922
Average gluteal fat thickness, mm	4.5 (1-9.5)	20.7 (17-29.4)	<.001	4.5 (2-7.8)	25.1 (20.7-30.0)	<.001
Pubic/gluteal fat ratio	3.8 (1-7.4)	0.8 (0.6-1.3)	<.001	4.1 (2-7.4)	0.7 (0.5-0.9)	<.001

All laboratory assessments were performed after a min. 8 hours of fasting. Data are expressed as median (Q1-Q3). Mann-Whitney *U* test was used to calculate *P* values for continuous parameters. Categorical parameters were assessed by χ^2 test. A *P* value of .05 or lower was considered statistically significant.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPLD, familial partial lipodystrophy; GL, generalized lipodystrophy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRI, magnetic resonance imaging.

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as Seipin, variants c.280C > T p.(Q94X), n = 2 and c.630 + 1G > A, (IVS4 + 1G > A), n = 2, and 2 had homozygous Caveolae Associated Protein 1, otherwise known as polymerase I and transcript release factor variants, c.259C > T, p.(Q87X), n = 1 and c.481_482insGTGA, p.(K161SfsX51), n = 1. One subject had acquired generalized lipodystrophy.

Of 30 patients with FPLD, 24 had diabetes and 22 had hypertriglyceridemia. Patients with FPLD had higher glucose (median, 129 mg/dL; 25th-75th percentiles, 93-164 mg/dL vs median, 90 mg/dL, 25th-75th percentiles, 82-98 mg/dL; $P = .002$), triglyceride (median, 314 mg/dL; 25th-75th percentiles, 146-458 mg/dL vs median, 108 mg/dL, 25th-75th percentiles, 76-157 mg/dL; $P < .001$), ALT (median, 21 IU/L; 25th-75th percentiles: 15-29 vs median, 17 IU/L; 25th-75th percentiles, 10-21 IU/L; $P = .037$), and lower high-density lipoprotein cholesterol (median, 37 mg/dL; 25th-75th percentiles, 30-46 mg/dL vs median, 49 mg/dL, 25th-75th percentiles, 40-56 mg/dL; $P = .011$) than controls. Patients with GL were younger and had lower body mass indexes than patients with FPLD and had more severe disease reflected by their higher levels of triglycerides, total cholesterol, ALT, and lower levels of high-density lipoprotein cholesterol (Table 2). Metabolic abnormalities were less common among patients with APL (data not shown).

Figure 1 shows representative T1-weighted pelvic MRI scans from subjects with different subtypes of lipodystrophy and healthy controls. Patients with FPLD had lower gluteal fat thickness compared with healthy controls and subjects with APL (FPLD: median, 5 mm, 25th-75th percentiles, 2-8 mm; controls: median, 21 mm, 25th-75th percentiles, 17-29 mm; APL: median, 16 mm, 25th-75thpercentiles, 14-24 mm; $P < .001$ for both comparisons). On the other hand, gluteal fat thickness was slightly higher in patients with FPLD compared with those with GL (Table 2). Figure 2A shows individual gluteal fat thickness measurements in patients with different types of lipodystrophy and healthy controls (dataset 1). Gluteal fat thickness measurements of females are presented in Fig. 2B. Eight females with FPLD (5 with *LMNA* variants, 1 with a *PPARG* variant, and 3 with no genetic mutation) were on pioglitazone, a PPARG agonist that could potentially lead to changes in fat distribution. Gluteal fat thickness and pubic/gluteal fat thickness were slightly higher in females treated with pioglitazone, but these differences were not statistically significant ($P > .05$).

ROC analysis revealed an area under curve (AUC) level of 0.718 (95% CI, 0.612-0.823; $P = .001$) for gluteal fat thickness to diagnose FPLD in our mixed cohort of subjects with lipodystrophy and healthy controls (dataset 1). The AUC level was 0.703 (95% CI, 0.576-0.830; $P = .005$) in females. ROC analysis identified 13 mm of gluteal fat thickness as a potential cut-point for the diagnosis of FPLD. When used alone, gluteal fat thickness of 13 mm had 100% sensitivity and 60% specificity in the overall cohort and 100% sensitivity and 60% specificity in females (Table 3).

To improve the diagnostic value of subcutaneous fat measurements from pelvic MRI scans, we calculated pubic/gluteal fat ratios and combined these calculations with gluteal fat thickness measurements. Pubic/gluteal fat ratios of patients with different types of lipodystrophy and healthy controls are shown in Fig. 2C (dataset 1). Pubic/gluteal fat ratios of females are presented in Fig. 2D. Pubic/gluteal fat ratio was significantly increased in subjects with FPLD compared with controls and patients with GL and APL (FPLD: median, 7.00, 25th-75th percentiles, 4.00-11.20; controls: median,

Table 2. Comparison of patients with FPLD and GL, dataset 1

	All subjects			Females		
	FPLD (n = 30)	GL (n = 19)	*P* value	FPLD (n = 27)	GL (n = 14)	*P* value
Age, y	39 (29-48)	26 (21-33)	.002	40 (30-48)	26 (18-30)	<.001
BMI, kg/m^2	22.9 (20.4-25.7)	19.6 (18.3-22.2)	.006	23.1 (20.4-26.2)	19.3 (17.2-20.3)	.001
Sex						
Female	27 (90%)	14 (73.7%)	.233	27 (100%)	14 (100%)	
Male	3 (10%)	5 (26.3%)		0 (0%)	0 (0%)	
Glucose, mg/dL	129 (93-164)	152 (119-235)	.043	133 (94-165)	156 (119-235)	.111
Creatinine, mg/dL	0.59 (0.51-0.72)	0.57 (0.46-0.77)	.861	0.59 (0.51-0.72)	0.51 (0.4-0.68)	.157
ALT, IU/L	21 (15-29)	31 (19-47)	.011	20 (14-29)	30 (18-44)	.052
AST, IU/L	24 (15-29)	25 (17-50)	.121	24 (14-30)	28 (16-50)	.160
Total cholesterol, mg/dL	196 (181-254)	166 (146-219)	.034	199 (182-259)	171 (139-233)	.115
Triglyceride, mg/dL	314 (146-458)	570 (259-763)	.121	324 (193-472)	467 (246-905)	.492
HDL cholesterol, mg/dL	37 (30-46)	27 (22-34)	.004	36 (30-46)	29 (22-35)	.056
LDL cholesterol, mg/dL	114 (90-133)	97 (64-107)	.029	113 (90-133)	99 (68-108)	.108
Average gluteal fat thickness, mm	5.0 (2.0-8.0)	1.0 (1.0-3.5)	.001	5.0 (2.0-8.0)	1.5 (1-3.5)	.003
Pubic/gluteal fat ratio	7.0 (4.0-11.2)	2.0 (1.0-2.0)	<.001	7 (4.2-11.2)	2 (1.0-2.0)	<.001

All laboratory assessments were performed after a minimum of 8 hours of fasting. Data are expressed as median (Q1-Q3). Mann-Whitney *U* test was used to calculate *P* values for continuous parameters. Categorical parameters were assessed by χ^2 test. A *P* value of .05 or lower was considered statistically significant. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPLD, familial partial lipodystrophy; GL, generalized lipodystrophy; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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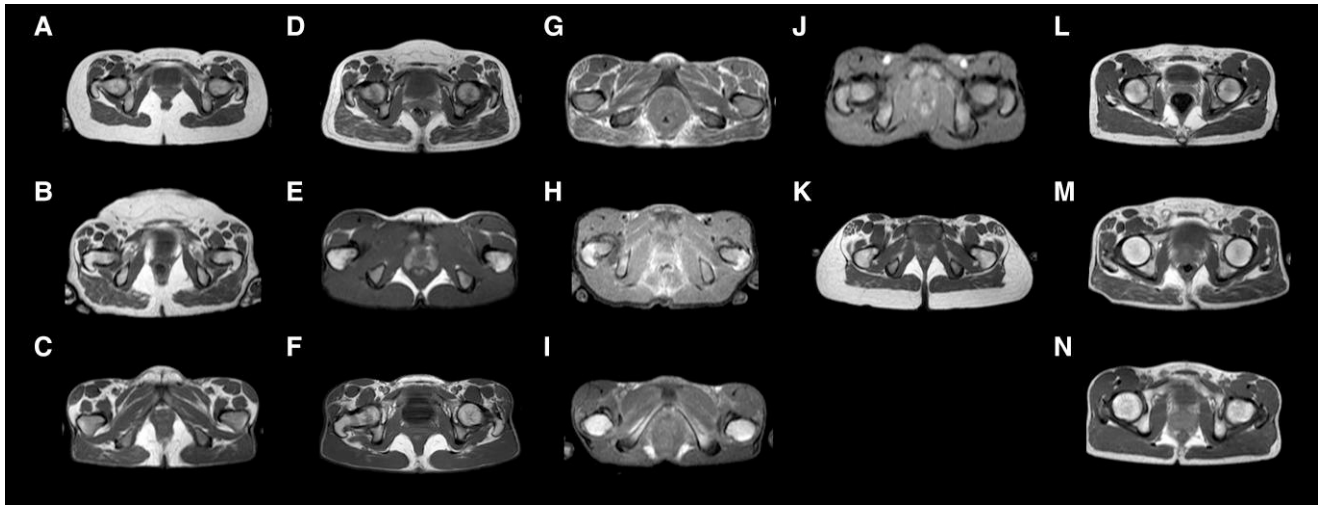

Figure 1. Pelvic MRI scans showing pubic and gluteal fat in healthy controls and patients with various subtypes of lipodystrophy. (A) Healthy control (28-year-old female). (B) FPLD1 (55-year-old female). (C) FPLD2 resulting from *LMNA* c.1444C > T, p.(R482W) variant (37-year-old female). (D) FPLD3 resulting from PPARG c.452A > G, p.(Y151C) variant (27-year-old female). (E) FPLD with no disease-causing variant (28-year-old female). (F) Fat distribution is similar to FPLD2 in this subject, but sequencing of the *LMNA* gene did not reveal any variant. (G) Severe fat loss because of homozygous *LMNA* c.1745G > A, p.(R582H) variant (30-year-old female). (H) CGL1 resulting from 1-acylglycerol-3-phosphate-O-acyltransferase-2 c.144C > A, p.(C48X) variant (27-year-old female). (I) CGL2 resulting from Berardinelli-Seip congenital lipodystrophy 2 c.630 + 1G > A, (IVS4 + 1G > A) variant (30-year-old female). (J) CGL4 resulting from Caveolae Associated Protein 1/polymerase I and transcript release factor c.481_482insGTGA, p.(K161SfsX51) variant (21-year-old female). (K) Acquired generalized lipodystrophy (17-year-old female). APL (19-year-old female). (L) Healthy control (26-year-old male). (M) FPLD2 resulting from *LMNA* c.1444C > T, p.(R482W) variant (62-year-old male). (N) Atypical FPLD with progeroid features due to *LMNA* R349W variant (24-year-old male).

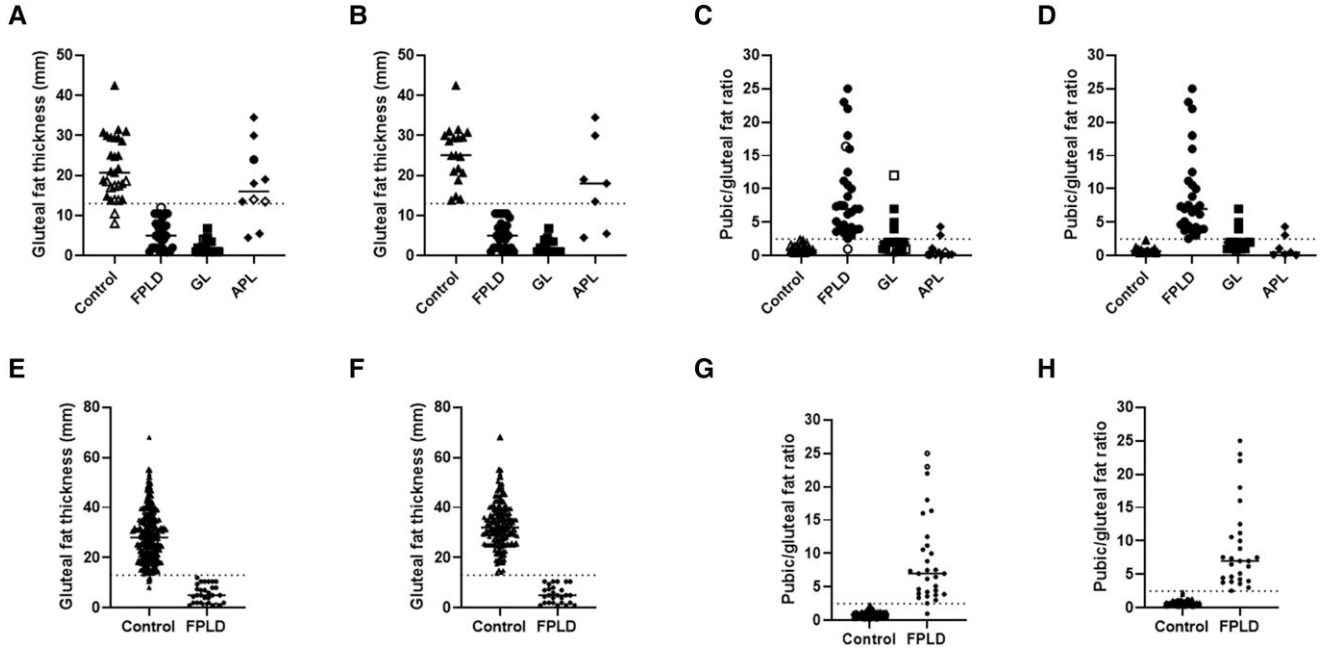

Figure 2. Gluteal fat thickness and pubic/gluteal fat ratios. (A) Gluteal fat thickness in patients with different types of lipodystrophy and healthy controls (dataset 1), all subjects (males shown with open symbols). (B) Gluteal fat thickness in patients with different types of lipodystrophy and healthy controls (dataset 1), females. (C) Pubic/gluteal fat ratios in patients with different types of lipodystrophy and healthy controls (dataset 1), all subjects (males shown with open symbols). (D) Pubic/gluteal fat ratios in patients with different types of lipodystrophy and healthy controls (dataset 1), females. (E) Gluteal fat thickness in patients with FPLD and subjects with no lipodystrophy (dataset 2), all subjects. (F) Gluteal fat thickness in patients with FPLD and subjects with no lipodystrophy (dataset 2), all females. (G) Pubic/gluteal fat thickness ratio in patients with FPLD and subjects with no lipodystrophy (dataset 2), all subjects. (H) Pubic/gluteal fat thickness ratio in patients with FPLD and subjects with no lipodystrophy (dataset 2), females.

0.82, 25th-75th percentiles, 0.61-1.32; GL: median, 2.00; 25th-75th percentiles, 1.00-2.00; APL: median, 0.40, 25th-75th percentiles, 0.19-1.11; $P < .001$ for all comparisons).

ROC analysis revealed an AUC level of 0.948 (95% CI, 0.901-0.995; $P < .001$) for pubic/gluteal fat thickness ratio for the presence of FPLD in dataset 1. A combination of gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ratio

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Table 3. Sensitivity, specificity, positive and negative predictive values of gluteal fat thickness ≤13 mm, combination of gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ≥2.5, and expert assessments for the diagnosis of FPLD in dataset 1

	Sensitivity	Specificity	PPV	NPV
Both sexes				
Gluteal fat thickness ≤13 mm	100.00% (95% CI, 88.43-100.00)	60.34% (95% CI, 46.64-72.95)	56.60% (95% CI, 48.71-64.18)	100.00%
Gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ≥2.5	96.67% (95% CI, 82.78-99.92)	91.38% (95% CI, 81.02-97.14)	85.29% (95% CI, 71.45-93.08)	98.15% (95% CI, 88.51-99.73)
Expert 1	80.00% (95% CI, 61.43-92.29)	100.00% (95% CI, 93.84-100.00)	100.00%	90.63% (95% CI, 82.53-95.19)
Expert 2	83.33% (95% CI, 65.28-94.36)	100.00% (95% CI, 93.84-100.00)	100.00%	92.06% (95% CI, 83.90-96.27)
Expert 3	83.33% (95% CI, 65.28-94.36)	100.00% (95% CI, 93.40-100.00)	100.00%	91.53% (95% CI, 82.91-96.01)
Females				
Gluteal fat thickness ≤13 mm	100.00% (95% CI, 87.23-100.00)	60.00% (95% CI, 43.33-75.14)	62.79% (95% CI, 53.59-71.15)	100.00%
Gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ≥2.5	100.00% (95% CI, 87.23-100.00)	90.00% (95% CI, 76.34-97.21)	87.10% (95% CI, 72.71-94.48)	100.00%
Expert 1	85.19% (95% CI, 66.27-95.81)	100.00% (95% CI, 91.19-100.00)	100.00%	90.91% (95% CI, 80.19-96.11)
Expert 2	88.89% (95% CI, 70.84-97.65)	100.00% (95% CI, 91.19-100.00)	100.00%	93.02% (95% CI, 82.10-97.48)
Expert 3	88.89% (95% CI, 70.84-97.65)	100.00% (95% CI, 90.26-100.00)	100.00%	92.31% (95% CI, 80.50-97.21)

Abbreviations: FPLD, familial partial lipodystrophy; NPV, negative predictive value; PPV, positive predictive value.

Table 4. Sensitivity, specificity, positive and negative predictive values of combination of gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ≥2.5 for the diagnosis of FPLD in dataset 2

	Sensitivity	Specificity	PPV	NPV
Both sexes				
Gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ≥2.5	96.67% (95% CI, 82.7-99.92)	100.00% (95% CI, 98.73-100.00)	100.00%	99.66% (95% CI, 97.68-99.95)
Females				
Gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ≥2.5	100.00% (95% CI, 87.23-100.00)	100.00% (95% CI, 97.95-100.00)	100.00%	100.00%

Abbreviations: FPLD, familial partial lipodystrophy; NPV, negative predictive value; PPV, positive predictive value.

≥2.5 provided 97% sensitivity and 91% specificity in the overall cohort and 100% sensitivity and 90% specificity in females for the diagnosis of FPLD (Table 3).

Then, we tested our approach in a larger cohort of random subjects. Dataset 2 included MRIs from 289 consecutive patients who underwent an abdominopelvic or pelvic MRI for various reasons at Dokuz Eylul University Radiology Department. Single-slice MRIs at the mons pubis level were retrieved from these diagnostic procedures and used as a confirmatory analysis of our findings from dataset 1. When images from patients with FPLD were combined with 289 random MRI scans, FPLD was differentiated from lipodystrophic subjects without lipodystrophy with 97% sensitivity and 100% specificity by using a combination of gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ratio – ≥2.5. Sensitivity was 100% and specificity was 100% in females (Table 4). Figure 2 shows distribution of gluteal fat thickness (E) and pubic/gluteal fat thickness ratio (F) in patients with FPLD and subjects with no lipodystrophy in dataset 2. Gluteal fat thickness measurements of females are presented in Fig. 2G and pubic/gluteal fat ratios in Fig. 2H (dataset 2).

Finally, 3 independent radiologists with expertise in lipodystrophy blinded to clinical diagnoses and fat thickness measurements evaluated MRI scans from dataset 1 for the diagnosis of FPLD. Overall, these results were comparable to the performance of gluteal fat thickness and pubic/gluteal fat thickness ratio measured by a radiologist with no previous experience in lipodystrophy. The combination of gluteal fat thickness ≤13 mm and pubic/gluteal fat thickness ratio ≥2.5 yielded better sensitivity but slightly lower specificity (Table 3).

Discussion

Our findings suggest that the combination of gluteal thickness and pubic/gluteal fat ratio from a pelvic MRI is a promising method to diagnose FPLD. This new approach may prove

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useful as an easy and efficient method to distinguish patients with FPLD from subjects without lipodystrophy and from other subtypes of lipodystrophy. This would allow physicians with limited experience in lipodystrophy to identify subjects with FPLD with a simple test that is within the reach of most health care centers.

Selective loss of fat from the lower part of the body is a shared finding in all subtypes of FPLD (15, 16). The presence of pubic fat had been noted clinically and this finding was recently highlighted by Meral et al (9) in DXA images. The fat signal was markedly increased in subjects with Dunnigan subtype of FPLD indicating potential pubic fat excess. Based on these findings, Meral et al (9) opted to call this phenomenon "Dunnigan sign." A similar appearance was observed in a group of females with FPLD but no *LMNA* variants, although this finding was not present in any of the control subjects (9).

DXA is a useful tool to evaluate general fat distribution. The fat shadow method was developed as an overall image capturing fat distribution as the reporting researchers recognized the substantial overlap between lean healthy controls (especially in males) with any quantitative tool and wanted to enrich the diagnostic capability with a global image which can then be used by machine learning algorithms. MRI, on the other hand, provides high-resolution images that would perform much better than DXA to assess anatomical compartments of subcutaneous fat (5). When we reviewed patient images from our registry with our clinical acumen, we realized that MRI scans could be used to demonstrate and document the region-specific fat deposition in different subtypes of FPLD. This observation led us to explore the potential of combined measurements from gluteal and pubic fat to diagnose FPLD. First, we measured gluteal fat thickness and tried to identify a cut-point that has high sensitivity that can later be combined with a pubic/gluteal fat ratio to improve its specificity. When pubic/gluteal fat thickness was added to the method, the measurements showed an improved diagnostic value, which was confirmed in further datasets.

FPLD is a subtype of a rare heterogeneous group of diseases encompassed in lipodystrophy, with limited number of expert centers treating patients worldwide (17). As with any other rare disease setting, diagnostic evaluations require a certain amount of expertise in lipodystrophy, and this is also true for radiological assessments. For this reason, we wanted to test our method against clinical readings from radiologists with expertise in lipodystrophy that revealed comparable results. Because measurements proposed in this study are standardized and do not require any previous expertise in lipodystrophy, we believe the use of gluteal thickness and pubic/gluteal fat ratio may help everyone with limited expertise to identify subjects with FPLD. The expert assessments had perfect specificity, whereas the use of gluteal fat thickness pubic/gluteal fat thickness ratio yielded better sensitivity. However, we should note that FPLD is a heterogeneous disease and atypical clinical presentations can still be challenging (18, 19). Although our tool may improve the diagnosis of FPLD, we still consider specialist center consultations essential for the diagnosis and management of FPLD.

Because the diagnosis of FPLD is currently clinical and requires a certain amount of expertise, it has been a challenge to correctly identify patients with FPLD in the absence of robust objective criteria. Analysis of patient photographs is very helpful in supporting the diagnosis of FPLD. As previously shown by Meral et al (9), trained physicians can accurately identify patients with FPLD by reviewing visual DXA scans. However, none of these approaches is quantitative but qualitative and subject to disagreement. Clinical trials in the field of FPLD, on the other hand, require robust inclusion criteria which are currently missing. Thus, the development of an objective tool is critical for clinical trials to enroll the correct patient populations. Leptin levels are, unfortunately, not diagnostic for FPLD (20), and skinfolds used in recent trials (21) and fat mass ratio has some overlap between FPLD and subjects without lipodystrophy (12). If confirmed in other populations, our method may serve as an alternative objective tool to identify eligible subjects for future clinical trials in the field of FPLD. Also, this approach can serve as remote diagnostics that can enable remote diagnosis, and improve access to care among individuals and regions that do not have easy access. Finally, the tool would allow screening of MRIs obtained for other purposes and help identify undiagnosed patients with FPLD.

Our study has several limitations. First of all, this is a retrospectively evaluated dataset with all subjects from the same geographic area. Second, the study included a limited number of males, and it is the lack of sufficient images from the lean athletic males (as well as females) that poses the hardest obstacle to the development of objective diagnostic criteria because the overlap is seen with these individuals in all previous attempts. In fact, it was the recognition of this overlap that pushed Meral et al (9) to overall imaging analysis rather than region-specific imaging or a search for a single quantitative cutoff. Although our tool revealed good specificity and sensitivity for the diagnosis of FPLD in females, our sample size is too small to determine the diagnostic value in males with FPLD as in other previous studies. Thus, our findings need to be confirmed in populations with various ethnic backgrounds from both sexes. We are currently collaborating with other centers and working on retrieving further MRI scans from men with FPLD to understand whether our method would reliably identify FPLD in men as well. Third, there was a limited number of subjects with typical Kobberling (FPLD1) phenotype in our cohort, again another patient group that brings differential diagnosis challenge to physicians (22) (because differentiating this group from more common truncal obesity and insulin resistance is difficult). Our group in Turkey has been hesitant to diagnose FPLD1 unless there is a very typical phenotype, which may explain the low number of subjects with FPLD1 analyzed in the study. However, we are collaborating with other centers to identify additional typical cases to test our approach in a greater number of women with FPLD1. Also, monogenic disease was limited to *LMNA* and *PPARG* variants. It is possible that our method would lose efficacy in atypical forms of FPLD. There were 2 patients with severe fat loss because of *LMNA* variants in our cohort, and gluteal thickness and pubic/gluteal fat ratio successfully identified these 2 patients. However, the method did not identify another male subject with atypical FPLD resulting from the *LMNA* p.R349W variant who exhibited progeroid features. Fat loss pattern was quite different in this case affecting the face and the limbs. Contrary to other *LMNA* variants, we did not observe any fat accumulation in the pubic area of this patient in part because of the specificity of fat loss in this mutation as reported by others. Therefore, our findings are generalizable for those with typical FPLD patterns and not for those with atypical fat loss patterns. Finally, MRI acquisition was not designed as a prospective single-slice

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T1-weighted pelvis MRI in this study; instead, we used images from existing libraries. The feasibility of T1-weighted image acquisition for lipodystrophy in a prospective study needs to be further assessed.

In conclusion, our study suggests that measurements from pelvis MRI scans can be used as an objective tool to identify patients with FPLD. A combination of gluteal thickness and pubic/gluteal fat ratio seems to be a promising method to diagnose FPLD, although our findings need to be confirmed in larger datasets that include subjects from both sexes with different phenotypes and genotypes from various ethnic backgrounds. We are excited by the possibility of remote diagnosis to facilitate uniformity in clinical trials and to enable accurate diagnosis of currently undiagnosed cases in the new era of telemedicine where the patients are not being evaluated physically by their treating providers. Improving remote diagnosis may also improve health equity across the globe.

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Author Contributions

B.A. designed the study. S.C.A. and B.A. performed data analyses and created tables and figures. C.A., E.R.A., I.B., and M.S. performed radiological studies. E.A.O. provided oversight in the execution of the study and manuscript writing. All other authors (B.Y., I.Y.S., and T.A.) contributed to data collection and analysis. All authors read and approved the final version of the letter. B.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Disclosures

B.A. has attended Scientific Advisory Board Meetings organized by Aegerion Pharmaceuticals (now Amryt Pharmaceuticals) and Regeneron Pharmaceuticals and has served as a consultant and/or received honoraria as a speaker from Third Rock Ventures, Amryt Pharma, Regeneron, AstraZeneca, Lilly, Merck, Novartis, Novo Nordisk, Boehringer Ingelheim, Servier, and Sanofi-Aventis. E.A.O. reports the following conflicts: grant support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Ionis Pharmaceuticals, Ionis Pharmaceuticals, Gemphire Therapeutics, GI Dynamics (current), AstraZeneca (past 2 years); consultant or advisor: AstraZeneca, TheraTec, and Bristol-Myers Squibb (past), Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Regeneron Pharmaceuticals (current); drug support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Akcea Therapeutics, Rhythm Pharmaceuticals (all current); and other support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Regeneron Pharmaceuticals (current). The other authors report no conflicts of interest.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Affirmation of Originality and Authorship

The authors affirm that the work submitted for publication is original and has not been published other than as an abstract or preprint in any language or format and has not been submitted elsewhere for print or electronic publication consideration.

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