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The role of MTHFR polymorphisms in the risk of lipedema

P. GUALTIERI[1], M. AL-WADART[2], G.L. DE SANTIS[3], N. ALWADART[4], D. DELLA MORTE[5,6,7], C. CLARKE[8], T. BEST[8], C. SALIMEI[9], G. BIGIONI[10], R. CIANCI[11], A. DE LORENZO[1], L. DI RENZO[1,12]

[1]Department of Biomedicine and Prevention, Section of Clinical Nutrition and Nutrigenomics, University of Tor Vergata, Rome, Italy
[2]Department of Rehabilitation Sciences, Faculty of Applied Medical Sciences, Jordan University of Science and Technology, Irbid, Jordan
[3]School of Applied Medical-Surgical Sciences, University of Rome Tor Vergata, Rome, Italy
[4]Faculty of Applied Medical Sciences, Jerash University, Jerash, Jordan
[5]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy
[6]Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University, Rome, Italy
[7]Department of Neurology, Evelyn F. McKnight Brain Institute, University of Miami Miller School of Medicine, Miami, FL, USA
[8]Neuro Health Lab, Appleton Institute, School of Health, Medical and Applied Sciences, Central Queensland University, Brisbane, Australia
[9]Department of Clinical Sciences and Translational Medicine, University of Tor Vergata, Rome, Italy
[10]Department of Physics, University of Rome "La Sapienza", Rome, Italy
[11]Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "A. Gemelli", IRCCS, Rome, Italy
[12]School of Specialization in Food Science, University of Tor Vergata, Rome, Italy

P. Gualtieri and M. Al-Wadart contributed equally to this study

Abstract. – OBJECTIVE: This study examines the role of MTHFR gene polymorphism (rs1801133) in women with lipedema (LIPPY) body composition parameters compared to a control group (CTRL).

SUBJECTS AND METHODS: We carried out a study on a sample of 45 LIPPY and 50 women as a CTRL. Body composition parameters were examined by Dual-energy X-ray Absorptiometry (DXA). A genetic test was performed for the *MTHFR* polymorphism (*rs1801133, 677C>T*) using a saliva sample for LIPPY and CTRL groups. Mann-Whitney tests evaluated statistically significant differences between four groups (carriers and non-carriers of the *MTHFR* polymorphism for LIPPY and CTRL groups) on anthropometric/body composition parameters to identify patterns.

RESULTS: LIPPY showed significantly higher ($p<0.05$) anthropometric parameters (weight, BMI, waist, abdominal, hip circumferences) and lower waist/hip ratio ($p<0.05$) compared to the CTRL group. The association between the polymorphism alleles related to the *rs1801133 MTHFR* gene and the body composition values LIPPY carriers (+) showed an increase in fat tissue of legs and fat region of legs percentage, arm's fat mass (g), leg's fat mass (g), and leg's lean mass (g) ($p<0.05$) compared to CTRL (+). Lean/fat arms and lean/fat legs were lower ($p<0.05$) in LIPPY (+) than in CTRL (+). In the LIPPY (+), the risk of developing the lipedema disease was 2.85 times higher (OR=2.85; $p<0.05$; 95% confidence interval = 0.842-8.625) with respect to LIPPY (-) and CTRL.

CONCLUSIONS: The presence or absence of MTHFR polymorphism offers predictive parameters that could better characterize women with lipedema based on the association between body composition and MTHFR presence.

Key Words:
MTHFR, Polymorphisms, Lipedema, Obesity, Fat mass.

Introduction

Lipedema is a highly underdiagnosed adipose tissue pathology and is often confused with non-lipedema obesity and lymphedema[1]. Lipedema's histopathological or molecular characteristics have not been identified yet[2]. The lipedema diagnosis is clinically based on history, signs, and physical

Corresponding Author: Paola Gualtieri, Ph.D; e-mail: paola.gualtieri@uniroma2.it

exam[1]. Compared to obesity, which is often localized on the torso/trunk of the body, lipedema is predominately found in the buttock, and lower and upper limbs and minimally responds to dietary treatments[3]. Usually, individuals affected by lipedema present a disproportionate thickness of the legs compared to the upper part of the body, which affect the quality of life[4-6]. Evidence suggests that endocrine disruption may be associated with disease onset[5,7]. For many women, symptoms of the disorder arise or worsen during specific hormonal changes across the lifespan, such as puberty, pregnancy, or menopause. Estrogen plays a direct role in developing fat and other tissues through the presence of Estrogen Receptors (*ERs*)[8,9]. While the etiology and pathogenesis of lipedema are not entirely known, potential mechanisms – such as increased vascular permeability and damage (microangiopathy), excessive lipid peroxidation, disturbances in adipocyte metabolism, and production of inflammatory cytokines[9] – may underly the pathogenesis of lipedema. Consequently, a hypothesized link between genetic factors and estrogen in the onset of lipedema has emerged.

A familial genetic association in up to 64% of women has been reported following the self-reported family history of lipedema[10,11]. Similarly, genetic hallmarks have been studied in lipedema[10], as caveolin 1 (*CAV-1*), matrix metalloproteinase 14 (*MMP-14*), *ER*, and *IL-6* have been proposed to have a role in the development of lipedema[12,13]. However, literature poorly explored specific genetic polymorphisms related to obesity and other disease states in lipedema, which may assist in differentiating lipedema from genetic obesity, lymphedema, and lipodystrophy.

The methylenetetrahydrofolate reductase (*MTHFR*) gene and its polymorphisms are among the most studied genetic polymorphisms in obesity, with the *C677T* (*rs1801133*) polymorphism associated with an increased risk of several diseases[14]. This polymorphic variant decreases enzymatic functionality, resulting in altered levels of methylation and DNA synthesis[14]. The expression or presence of the *C677T* (*rs1801133*) *MTHFR* gene polymorphism is also highly related to ethnicity and geographic location, with a higher prevalence in Italian and Hispanic populations and a lower prevalence in African American populations[15].

A meta-analysis by Fu et al[16] showed that in patients carrying the polymorphism, there is a strong relationship between homocysteine levels (Hcy) and increased risk of obesity. The association between the polymorphisms of the *MTHFR* gene (*rs1801133*) and obesity, defined by body mass index (BMI), and lean mass, has been demonstrated by observing a link between BMI and lean mass on chromosome 1p36, where the gene is located[17]. Moreover, in subjects affected by normal weight obese syndrome, this polymorphism could represent a genetic risk marker in these patients[17]. Understanding underlying genetic factors and identifying diagnostic markers of lipedema is currently an important area of research. However, it still needs to be understood how the *MTHFR* gene polymorphism (*rs1801133*) is related to the anthropometric presentation of lipedema.

This study aimed to assess the potential relationship between *MTHFR* gene polymorphism (*rs1801133*) in women with lipedema compared to a control group to differentiate obesity and the risk of developing lipedema. The second aim was to evaluate the correlation among the *MTHFR* gene polymorphism (*rs1801133*), anthropometric measurements (such as weight, height, BMI, circumferences), and body composition parameters (such as total body and segmental fat and lean masses).

Subjects and Methods

Subjects

The study was conducted between 2018 and 2021 at the Section of Clinical Nutrition and Nutrigenomics, Department of Biomedicine and Prevention of the University of Rome Tor Vergata, Italy. The initial sample was 100 Italian women, randomly enrolled among the participants in other active studies in the same period. To be eligible, each individual had to belong to the Caucasian ethnicity, be Italian, and be aged >18 and <65 years. Exclusion criteria included pregnancy, breastfeeding, diabetes, Hepatitis C and B, HIV, and other infectious diseases. Informed consent was obtained from all subjects according to the principles of the Declaration of Helsinki of 2013.

The Ethical Committee approved the study protocol of the Calabria Region Center Area Section (Register Protocol No. 146 17/05/2018), and the trial registration protocol is ClinicalTrials.gov Id: NCT01890070.

After a 12-hour overnight fast, all subjects underwent anthropometric evaluation (body weight, height, waist, and hip circumferences) accord-

ing to the standard methods[18]. Body Mass Index (BMI) was calculated using the following formula: BMI= body weight (Kg)/height (m)^2. Body composition parameters in terms of fat mass (FM) and lean body mass (LBM) expressed in g and % were measured by Dual-energy X-ray Absorptiometry (DXA) (Osteosys Primus Nemomedical s.r.l., Seoul, Korea). Subjects were categorized according to BMI, and percentage (%) of total body fat mass (FM) into normal weight Lean (NW Lean) women, with a BMI <25kg/m^2 and % FM <30); normal weight obese (NWO) women with a BMI <25 kg/m^2, and FM% ≥30%; pre-obese and obese (OB) women, with a BMI ≥25 kg/m^2 and FM>30%[19].

DNA Isolation and RTq-PCR Analysis

The phenol-chloroform technique extracted the genomic DNA from the saliva swab. The real-time PCR technique was used to determine *MTHFR* promoter polymorphism (*rs1801133*), also known as *C677T*. This method provides the fluorescent emission by fluorophores linked to specific probes. The amplifications were carried out following the Applied Biosystem protocol, using the TaqMan™ Genotyping Master Mix that contains DNA polymerase (AmpliTac-Gold®), buffer, and dNTPs. Also, according to the manufacturer, SNP genotyping was executed using the Real-Time PCR method [Applied Biosystem StepOnePlusTM (Real-Time PCR System) thermal cycler, Life Technologies, Carlsbad, CA, USA] instructions. The samples were subjected to 40 amplification cycles. Each cycle is divided into two phases: a) denaturation phase at 95°C for 15', to permit the separation of the two DNA strands due to the breaking of the hydrogen bonds; b) single annealing-extension phase at 60°C for 1'. The first cycle of the total 40 is preceded by a different phase, in which a temperature of 95°C is reached and maintained for 45'. The lattest step is necessary to activate the polymerase[13].

Statistical Analysis

The statistical analysis was performed using SPSS v.12.0 (Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) for the *MTHFR* polymorphism was calculated using the SNP-HWE program. This equation expresses this equilibrium: p2+2pq+q2=1 where p and q describe frequencies of allele 1 and 2, p2 indicates the frequency in the genotype 1/1, q2 defines the frequency of the genotype 2/2, and 2pq express the frequency of genotype ½. The χ^2 test was used as a statistical analysis to determine the risk of developing. The Kolmogorov-Smirnov test showed non-normally distributed data on continuous data. Therefore, the Mann-Whitney test was used to determine group differences in anthropometric characteristics. A *p*-value less than 0.05 was considered significant.

Results

Of the 100 subjects enrolled, 5 were excluded from the study because they did not meet the eligibility criteria. Finally, 95 subjects were considered for this study: 45 women with lipedema, the lipedema group (LIPPY), and 50 women without lipedema, the control group (CTRL).

We categorized our population according to phenotype classification, based in BMI and % FM, as follows: underweight (UW) (BMI<18.50); normal-weight (NW) (18.50≤BMI<25 and % FM lower than 30%); normal weight obese (NWO) (18.50≤BMI<25 and % FM higher than 30%); Preobese (PreOb) (25≤BMI<30 and % FM higher than 30%); Obesity (30≤BMI and % FM higher than 30%). According to the phenotype classification, based on BMI and % FM, we obtained: 6.7% of NW Lean, 42.2% of NWO, and 51.1% of OB women in the LIPPY; 10.0% of NW Lean, 58.0% of NWO, and 32.0% of OB women were in the CTRL.

Table I summarizes the main anthropometric characteristics of the study population. The statistical analysis showed that compared to the CTRL, the LIPPY had statistically significant higher weight, BMI, waist, abdominal, and hip circumferences, except for waist/hip ratio (WHR) (p<0.01), which was lower in comparison to the CTRL.

LIPPY and CTRL groups were further categorized into whether the carrier allele of the *MTHFR* polymorphism was present (+), (CT, TT), or absent (-), (CC), creating four groups in total for comparison: LIPPY (-) (n = 13), LIPPY (+) (n = 32), CTRL (-) (n = 12), and CTRL (+) (n = 38).

Table II shows anthropometric parameters associated with the presence or absence of the carrier allele of the *MTHFR* polymorphism in the LIPPY and CTRL. Results showed that the LIPPY (+) had significantly greater median values of weight (p<0.05), BMI (p<0.05), and Hip Circumference (p<0.01), and a lower median value of WHR (p<0.05), compared to the CTRL (+). Comparisons between other groups were non-significant.

Table I. Anthropometric characteristics of the study population.

	LIPPY	CONTROL	*p*
Height (cm.)	163 [148-169.5]	162 [146.5-196.5]	0.810
Weight (kg.)	79.4 [54-153]	69.1 [42.4-136.78]	**0.002**
BMI	30.04 [20.34-62.87]	25.48 [17.6-51.47]	**0.001**
Age (years)	42.93 [18.00-71.63]	40 [20-80]	0.243
Waist Circumference	83.5 [63-116]	79.5 [56.5-117]	**0.044**
Abdominal Circumference	97 [74-155]	93.5 [71-140]	**0.035**
Hip Circumference	115 [66-175]	101 [82-144]	**0.000**
Waist/Hip Ratio	0.73 [0.36-1.2]	0.79 [0.49-0.97]	**0.001**
Number of patients	45	50	

Values are expressed as median and minimum and maximum in square brackets (M [min-max]). BMI: body mass index. The Mann-Whitney test was performed to evaluate the variable distribution. Variables are considered non-normally distributed for $p<0.05$ (in bold).

In Table III, according to the presence or absence of *MTHFR* gene polymorphism (*rs1801133*), the comparison between the body composition parameters of fat mass in LIPPY and CTRL groups was reported. Leg fat tissue % ($p<0.05$), whole fat tissue % ($p<0.05$), leg fat region % ($p<0.05$) values were significantly higher in CTRL (-) *vs.* CTRL (+). Leg fat tissue % ($p<0.05$), leg fat region % ($p<0.05$), arm fat (g) ($p<0.05$) and leg fat (g) ($p<0.005$) values were significantly higher in LIPPY (+) *vs.* CTRL (+). The values of android fat tissue % ($p<0.05$), android fat region % ($p<0.05$), android fat (g) ($p<0.05$) were significantly lower in LIPPY (-) *vs.* CTRL (-). No statistical significance for any parameter was found in LIPPY (+) *vs.* LIPPY (-).

Table IV compares the body composition values relative to lean body mass and lean/fat ratios in LIPPY and CTRL according to the presence or absence of *MTHFR* gene polymorphism (*rs1801133*) was reported. Comparisons between LIPPY (+) and CTRL (+) showed statistically significant higher values of leg lean mass (g) ($p<0.05$) in LIPPY (+) group, while the values of lean/fat arms ($p<0.05$) and lean/fat legs ($p<0.05$) are lower in LIPPY (+) patients. When comparing LIPPY (-) to CTRL (-), values of lean/fat android ($p<0.05$) were significantly higher in LIPPY (-). No statistical significance in the comparison of LIPPY (+) *vs.* LIPPY (-) and CTRL (+) *vs.* CTRL (-) was found.

According to the Odds Ratio analysis, the risk of developing Lipedema disease was 2.85 times higher in the LIPPY (+), with respect to LIPPY (-) and CTRL (+) or (-), (OR=2.85; 95% confidence interval CI = 8.42-8.625, $p<0.05$).

Table II. Association of polymorphism of MTHFR rs1801133 between lipedema (LIPPY) and control (CTRL) groups and anthropometric measurements. Subjects were divided in carriers (CT and TT) and non-carriers (CC) for the risk allele. p1: LIPPY (-) *vs*. LIPPY (+), p2: CTRL (-) *vs.* CTRL (+), p3: LIP (+) *vs*. CTRL (+), p4: LIPPY (-) CTRL (-).

LIPPY MTHFR – (CC)		LIPPY MTHFR + (CT, TT)	CTRL MTHFR – (CC)	CTRL MTHFR + (CT, TT)	*p1*	*p2*	*p3*	*p4*
69.1 [64.7-133.3]	**Weight (kg.)**	83.5 [54-153]	72.65 [49.3-128.5]	68.85 [42.4-124.4]	0.427	0.565	**0.024**	0.465
29.9 [23.56-55.48]	**BMI**	32.3 [20.34-62.8]	27.48 [19.78-51.47]	25.27 [17.6-45.28]	0.630	0.188	**0.010**	0.572
79.5 [73-102]	**Waist Circumference**	87.25 [63-116]	80.1 [64.5-117]	78 [56.5-114]	0.206	0.220	0.056	0.588
96 [80-118]	**Abdominal Circumference**	102.85 [74-155]	97 [77-116]	93 [72.5-140]	0.371	0.300	0.117	0.947
104 [66-144]	**Hip Circumference**	118.5 [91-175]	105 [87-144]	100 [85-142]	0.109	0.136	**0.000**	1.000
0.76 [0.36-1.2]	**Waist/Hip Ratio**	0.73 [0.57-0.92]	0.8 [0.68-0.91]	0.78 [0.6-0.96]	0.590	0.536	**0.050**	0.161

Values are expressed as median and minimum and maximum in square brackets (M [min-max]). BMI: body mass index. The Mann-Whitney test was performed to evaluate the variable distribution. Variables are considered statistically significant for $p<0.05$ (in bold).

Table III. Association of polymorphism of MTHFR rs1801133 between lipedema (LIPPY) and control (CTRL) groups and body composition on fat mass evaluated by DEXA. Subjects were divided in carriers (CT and TT) and non-carriers (CC) for the risk allele.

	LIPPY MTHFR – (CC)	LIPPY MTHFR + (CT, TT)	CTRL MTHFR – (CC)	CTRL MTHFR + (CT, TT)	*p1*	*p2*	*p3*	*p4*
Arm fat tissue %	44.1 [38.5-56.1]	48.9 [26.1-61.1]	47.15 [42-57.5]	43.2 [29.1-59]	0.831	0.159	0.144	0.413
Leg fat tissue %	47 [38.2-61.6]	49.3 [33-62.4]	54.15 [42-63.1]	44.6 [31-55.4]	0.887	**0.038**	**0.05**	0.501
Android fat tissue %	40.9 [23.9-56.9]	46.8 [18-65.8]	54.7 [41-55.9]	50.5 [33-62.1]	0.419	0.411	0.523	**0.039**
Gynoid fat tissue %	45.8 [36-59.6]	50.85 [30.7-63.5]	48 [44.9-57.4]	49.5 [37.3-59]	0.865	0.061	0.457	0.841
Whole fat tissue %	41.4 [31.5-55.3]	45.55 [26.7-58.7]	50.05 [42.6-59.2]	43.5 [31.7-57]	0.712	**0.031**	0.441	0,075
Arm fat region %	42.9 [37.2-54.7]	47.2 [24.8-60]	45.5 [40.6-56.5]	41.7 [27.8-57.9]	0.820	0.137	0.157	0.441
Leg fat region %	45.7 [37-60.6]	48.05 [31.6-61.6]	52.45 [40.6-61.6]	43.1 [29.7-53.9]	0.820	**0.041**	**0.040**	0.564
Android fat region %	40.6 [23.7-56.6]	46.35 [17.7-65.5]	54.4 [48.8-55.7]	50.2 [32.7-61.8]	0.461	0.411	0.463	**0.039**
Gynoid fat region %	44.8 [35.2-59]	50.1 [30-62.9]	47.2 [43.9-56.4]	48.6 [36.3-57.9]	0.865	0.666	0.413	0.841
Whole fat region %	40.2 30.4-54.3]	44.2 [25.7-57.5]	42.5 [20.3-58]	40.9 [21.9-56]	0.702	0.260	0.106	0.863
Arm Fat (g)	2950 [2535-6176]	3986.5 [1224-8027]	3600 [950-6126]	2984 [850-9675]	0.67	0.315	**0.019**	0.888
Leg Fat (g)	13162 [10035-31487]	15181.5 [6981-34276]	11638.5 [1869-30314]	10133 [4434-23139]	0.843	0.834	**0.005**	0.157
Android Fat (g)	1964 [960-5528]	2753.5 [592-7839]	3755 [3518-5637]	3569 [824-6718]	0.478	0.256	0.594	**0.014**
Gynoid Fat (g)	5332 [3932-13794]	6473.5 [2734-17302]	7658 [5613-8274]	5928 [3191-9764]	0.650	0.170	0.457	0.125
Whole fat (g)	28586 [21373-73045]	36075.5 [12018-86041]	40572.5 [9904-73611]	32530 [9763-69338]	0.691	0.103	0.176	0.322

Values are expressed as median and minimum and maximum values in square brackets (M [min-max]). The Mann-Whitney test was performed to evaluate the variable distribution. Variables are considered statistically significant for $p<0.05$ (in bold). *p*1: LIPPY (-) *vs.* LIPPY (+), *p*2: CTRL(-) *vs.* CTRL(+), *p*3: LIPPY (+) *vs.* CTRL(+), *p*4: LIPPY (-) *vs.* CTRL(-).

Discussion

Lipedema is commonly misdiagnosed and mistreated as obesity. However, the pathophysiology of lipedema is distinguished from obesity due to the localized deposit of adipose tissue in the buttock and lower limbs and arms. In contrast, obesity affects the whole body and increases visceral fat deposition and consequent metabolic comorbidities. Previous research[11] has found that lipedema was associated with a low risk of diabetes (2%), dyslipidemia (11.7%), and hypertension (13%) despite an average BMI of 35.3 kg/m^2. Moreover, lipedema does not typically respond to low-calorie regimens, which is difficult for those diagnosed as dietary advice is often recommended to reduce further weight gain and increased symptoms of the disease[20].

To date, the association between the risk of obesity and *MTHFR rs1801133* polymorphism expression has been contradictory across different groups of people. For example, Leal-Ugarte et al[21] showed no association between *MTHFR* and obesity in individuals from Mexico. In contrast, the results highlighted that those individuals carrying the *TT* genotype had a lower risk of developing high triglycerides and cholesterol levels. However, in the Chinese Han population, the *MTHFR* polymorphism is highly correlated to overweight and obese conditions, especially in females, as

Table IV. Association of polymorphism of MTHFR rs1801133 between lipedema (LIPPY) and control (CTRL) groups and body composition on lean body mass evaluated by DEXA. Subjects were divided in carriers (CT and TT) and non-carriers (CC) of the risk of the allele. ASMMI: Appendicular Skeletal muscle mass.

	LIPPY MTHFR – (CC)	LIPPY MTHFR + (CT, TT)	CTRL MTHFR – (CC)	CTRL MTHFR + (CT, TT)	*p1*	*p2*	*p3*	*p4*
Lean Arm (g)	4131 [3244-5352]	4347.5 [2999-7008]	4069.5 [2895-7829]	4196.5 [2841-7524]	0.478	0.601	0.892	0.509
Lean Leg (g)	14835 [12723-19627]	16022 [10519-28566]	14926 [10778-21875]	13478.5 [9714-21435]	0.478	0.369	0.032	0.671
Lean Android (g)	2693 [2323-4638]	3021.5 [2326-4412]	3893 [2909-4441]	3571 [1673-4357]	0.103	0.356	0.661	0.053
Lean Gynoid (g)	6035 [5148-56303]	6678.5 [5066-10034]	6669 [6095-8378]	5899 [3748-9284]	0.570	0.327	0.237	0.386
Lean Whole (g)	41286 [35108-59074]	44967.5 [31492-63352]	44905 [33189-70351]	43221.5 [31448-63689]	0.281	0.587	0.417	0.637
Lean/Fat Whole	1.41 [0.81-2.15]	1.2 [0.7-3.31]	1.21 [0.67-3.68]	1.38 [0.75-3.35]	0.755	0.143	0.144	0.540
Lean/Fat Arms	1.27 [0.78-1.6]	1.05 [0.64-2.83]	1.35 [0.6-6.59]	1.48 [0.7-9.66]	0.820	0.601	**0.010**	0.300
Lean/Fat Legs	1.13 [0.62-1.62]	1.05 [0.6-2.03]	1.36 [0.55-6.75]	1.33 [0.8-2.95]	0.755	0.676	**0.023**	0.110
Lean/Fat Android	1.44 [0.76-3.18]	1.14 [0.52-4.56]	0.83 [0.79-1.04]	0.98 [0.61-2.03]	0.427	0.411	0.505	**0.040**
Lean/Fat Gynoid	1.22 [0.68-7.04]	1 [0.57-2.26]	1.08 [0.74-1.23]	1.02 [0.7-1.68]	0.395	0.611	0.620	0.386
ASMMI	7.3 [6.44-10.4]	7.62 [5.54-13.21]	7.49 [5.36-11.9]	6.81 [4.94-12.29]	0.813	0.117	0.360	0.962
T-Score	1.6 [-1.2-2.3]	1.1 [-1.1-3.1]	0.95 [-0.1-2.1]	0 [-2.5-4.3]	0.977	0.279	0.054	0.768

The Mann-Whitney test was performed to evaluate the variable distribution. Variables are considered statistically significant for $p<0.05$ (in bold). p1: LIPPY (-) *vs.* LIPPY (+), *p*2: CTRL (-) *vs.* CTRL (+), *p*3: LIPPY (+) *vs.* CTRL (+), *p*4: LIPPY (-) *vs.* CTRL (-).

well as triglycerides and cholesterol serum levels[22]. Similarly, in individuals from Iran an equal distribution of *MTHFR* gene polymorphism in patients with diabetes or obesity has been shown and confirms *MTHFR* gene polymorphism association with serum homocysteine[23]obese, diabetic and obese and diabetics.

This study shows an association between *MTHFR* polymorphism and body composition in Italian individuals with lipedema. Our data shows that LIPPY (+) has a higher weight, BMI, and hip circumference and lower WHR than CTRL (+). This result confirms the gynoid phenotype of obesity in lipedema and the mutation's presence. Hence, in LIPPY (+), there was a positive correlation between weight and weight distribution and the *MTHFR* polymorphism.

In our study, likewise in Mlodzik-Czyzewska et al[22], no differences in anthropometric parameters between LIPPY (+) and LIPPY (-) were highlighted. However, unlike Mlodzik-Czyzewska et al[24], in which *MTHFR* polymorphism has been associated with central and abdominal adiposity, we found more fat mass (g) on the legs and arms in LIPPY (+) with respect to CTRL (+) group. Comparing the two (+) groups, LIPPY (+) women had more fat than the CTRL (+) group and more lean mass on arms, legs, android, and gynoid regions. Analyzing LBM, LIPPY (+) had more lean mass on legs concerning CTRL (+). This is inconsistent with the results obtained in a study by Khanal et al[25], they observed that the association between three specific gene variants (*ACTN3 rs1815739, MTHFR rs1801131, and MTHFR rs1537516*) and the onset of sarcopenia in obese elderly women. Khanal et al[25] highlighted that woman with obesity carrying the *MTHFR rs1537516 A* allele had 2.8 times higher risk of being sarcopenic than homozygous *GG*. Similarly, significantly increased association between the *MTHFR rs1801133* polymorphism carriers and sarcopenic risk[25].

Liu et al[26] in a cohort of 405 Caucasian families, found that *MTHFR* polymorphism is probably more associated with LBM than FM or obesity. According to Liu et al[26], in our CTRL group, CTRL (+) *vs*. CTRL (-) had less total body and leg fat mass (%), while LBM, even if not significant, is lower in CTRL (+). *MTHFR* gene polymorphism increases the amount of fat mass in LIPPY since LIPPY (+) *vs*. CTRL (+) showed that FM % and g of legs and FM g of arms were significantly higher.

Di Renzo et al[17] highlighted the *T* (+) allele as a "risk allele" for pathologies like osteoporosis and sarcopenia. Moreover, we observed a significantly increased of 2.85-fold association between the *MTHFR rs1801133* polymorphism and the risk of the onset of lipedema.

Limitations

This study has some limitations. The number of subjects is small, since lipedema is a rare disease but also due to the lack of a correct diagnosis, since it is often confused with lymphedema or obesity. Furthermore, only one *MTHFR* polymorphism was considered, but we cannot exclude the involvement of other polymorphisms and genes in the pathogenesis a priori.

Conclusions

To our knowledge, this is the first study exploring the *MTHFR* gene expression in an Italian sample of patients affected by lipedema. Lipedema is a rare disease, and sometimes women are unaware of it. This aspect hinders the possibility of diagnosis and studying the disease in an adequate sample of patients. Our study shows that the *MTHFR rs1801133* null genotype may increase lipedema risk. We highlighted the *MTHFR* polymorphism as a genetic risk factor for lipedema development and a helpful tool for diagnosing this disease as it leads to higher amounts of appendicular fat mass. This study provides important preliminary insights into the potential link between *MTHFR* polymorphism and the presentation of lipedema. Further research into different populations and ethnicities which differ in the presence of the *MTHFR* polymorphism is suggested to further study this link.

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Authors' Contribution

P.G., and M.A-W. drafted the manuscript; G.L.D.S., N.A, performed the experiments and collected data;
G.B. analyzed the data; R.C., C.C., and T.B. reviewed the text; L.D.R. conceived and designed the experiments; M.A-W, L.D.R., G.P, had primary responsibility for the final content.
All the authors read and approved the final manuscript. All the authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

The authors declare that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The Ethical Committee approved the study protocol of the Calabria Region Center Area Section (Register Protocol No. 146 17/05/2018) and the trial registration protocol is ClinicalTrials.gov Id: NCT01890070.

Informed Consent

All participants provided fully informed written consent at time of recruitment.

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