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The role of IL-6 gene polymorphisms in the risk of lipedema

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Abstract. – OBJECTIVE: Lipedema is a disorder of adipose tissue characterized by abnormal subcutaneous fat deposition, leading to swelling and enlargement of the lower limbs and trunk. The aim of this study was to evaluate the lipedema phenotype by investigating the role of polymorphisms related to IL-6 (rs1800795) gene in people with diagnosis of lipedema. The second aim was to identify indicators of body composition, useful for a differential analysis between subjects with lipedema and the control group.

PATIENTS AND METHODS: Two groups are involved in the study, 45 women with lipedema (LIPPY) and 50 women randomly chosen from the population as Control (CTRL). Clinical and demographical variables recorded include weight, height, body mass index (BMI) and circumference measurements. Body composition (Fat mass, FM; lean mass, LM) was assessed by Dual-energy X-ray Absorptiometry (DXA). The genetic tests for IL-6 (rs18oo795) gene were performed for both groups, using a saliva sample.

RESULTS: The study of the relationship between the IL-6 (rs1800795) gene polymorphism, the anthropometric values and the body composition indices has provided the following significant results: subjects with diagnosis of lipedema present statistically significant increased values with regard to weight, BMI, waist, abdomen and hip circumferences, arms, legs and whole FM (% and kg), gynoid FM (kg), legs LM (kg) and ASMMI. Moreover, the value of the waist hip ratio was found to be decreased.

CONCLUSIONS: For the first time, we suggested that IL-6 gene polymorphism could characterize subjects with lipedema respect to Normal Weight Obese and obese subjects. The intra-group comparisons (LIPPY carriers *vs.* LIPPY non-carriers and CTRL carriers *vs.* CTRL non-carriers) showed no statistically significant values. In contrast, the inter-group comparisons (LIPPY non-carriers *vs.* CTRL non-carriers and LIPPY carriers *vs.* CTRL carriers) resulted statistically significant. We have identified other indices, such as leg index, trunk index, abdominal index, total index, that could be promising clinical tools for diagnosis of the lipedema phenotype and for predicting the evolution of the disease.

Key Words:
Lipedema, BMI, Fat mass, Obesity, Polymorphism.

Abbreviations

BMI: Body Mass Index; CRP: C-reactive protein; CTRL: Control; DXA: Dual-Energy X-ray; FFM: Free Fat Mass; FM: Fat Mass; HWE: Hardy-Weinberg equilibrium; LIPPY: Lipedema; LM: Lean Mass; MetS: Metabolic Syndrome; MONW: Metabolically Obese Normal Weight; MHO: Metabolically Healthy obese; MUO: Metabolically unhealthy obese; NW: Normal Weight; NWO: Normal Weight Obese; WHR: Waist to Hip Ratio.

Introduction

Obesity is defined as a disease and several phenotypes have been proposed to classify it[1,2]. Different obesity phenotypes have been defined in

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the literature, taking into account both body mass index (BMI) values and total fat mass percentage (FM%)[3,4] direct measurements of percentage body fat (PBF: a) the normal weight obese (NWO)[5,6]; b) the metabolically obese normal weight (MONW)[7,8]; c) the metabolically healthy obese (MHO)[9,10]; d) the metabolically unhealthy obese (MUO), or "at risk" obese[11], with metabolic syndrome (MetS)[12].

Up to date, lipedema (or lipoedema) has never been considered among the obesity phenotypes instead of often mistakenly diagnosed as simple obesity or lymphedema. Genetic tests could be fundamental for a differential diagnosis between lipedema, genetic obesity, primary lymphedema and lipodystrophies[13-15].

Lipedema may coexist with other phenotypes of obesity, but should not be confused with metabolic obesity (metabolically unhealthy obesity, MUO)[16-18]. Prevalence of lipedema is estimated to be in the range of 7-11% of all adult women in Western countries[19,20]. Lipedema is a chronic and progressive adipose tissue disorder that affects mainly women[19], with a prevalence of 1-9/100000[15]. It is characterized by an abnormal deposition and distribution of adipose tissue in the subcutaneous region localized from the hips down to the ankles and sometimes also in the upper limbs[21].

Main symptoms are the symmetrical fat accumulation in the extremities associated with swelling, pain and tenderness in the affected areas. Despite caloric restriction or intense physical activity fat tissue enlargement is continuing[19,22,23]. Differently in diabetic and obese patients, personalized diets with caloric restrictions have led to a noticeable weight decrease[24-25]. Recently, our review highlighted that lipedema has a significant negative effect on health-related quality of life and general physiological status[26]. The aetiology and pathogenesis of lipedema are not yet fully understood. To date, it is considered a multifactorial condition. Several theories in the literature try to explain the role of different elements involved in the onset and development of this disease.

Since adipose tissue plays an endocrine role in the release of proinflammatory cytokines, including interleukin 6 (IL-6), it is of great interest to study the role of genetic variants of IL-6 in lipedema[27].

The IL-6 gene located on chromosome 1 in position q23.1, encodes interleukin 6, a cytokine with well-defined pro-inflammatory properties also involved in the maturation of B lymphocytes[27]. IL-6 is produced following acute and/or chronic inflammation. Accumulative evidence indicates that obesity may represent a low-grade chronic inflammatory state. This is reflected by the elevation of inflammatory markers in the serum such as IL-6 and C-reactive protein (CRP)[26-28]. Several polymorphisms have been located in the 5 'region of the IL-6 gene. However, the majority of studies have focused on the G/C substitution at position -174. since it has the greatest impact on cytokine effects[29]. In the European population the frequency of the two alleles (G and C) is 54% and 46% respectively[30]. IL-6 plays a role in the regulation of body fat. In fact, Wallenius et al[31] conducted on mice have shown that IL-6 deficiencies lead to the onset of obesity, probably related to high levels of leptin.

Therefore, the first aim was to investigate the role of IL-6 (rs1800795) gene polymorphism in people suffering of lipedema useful for a classification of a new phenotype of obesity, and differential diagnosis between women with lipedema and a control group. The second aim was to identify indicators of body composition, obtainable from anthropometric measurements (body weight, height, circumferences) and body composition (total body fat mass, FM, total body lean mass, LM, fat free mass, FFM) useful for a differential analysis between subjects with lipedema and a control group.

Patients and Methods

Subjects

The sample study was composed of 95 Caucasian women from the Italian population between 20 and 60 years. They were divided into two groups: the first group with lipedema (LIPPY) was represented by 45 women enrolled on the basis of the diagnosis made at the San Giovanni Battista Hospital, in Rome (Italy); the second was the control group (CTRL) represented by 50 women without lipedema randomly enrolled among participants in clinical trial going on in the same period at the Section of Clinical Nutrition and Nutrigenomics of Biomedicine Department and Prevention of the Faculty of Medicine of Tor Vergata. Informed consent was obtained from all subjects in accordance with principles of the Declaration of Helsinki. All the enrolled subjects signed an informed consent, and the study protocol was approved by the Ethical Committee of the Calabria Region Center Area Section (Register Protocol No. 146 17/05/2018). Exclusion

criteria included pregnancy, breast-feeding, type 1 diabetes, Hepatitis C and B virus and HIV. After a 12-hour overnight fast, all subjects underwent anthropometric evaluation (body weight, height, waist and hip circumferences), according to standard method[32]. All the individuals were instructed to take off their clothes and shoes before undergoing the measurements, BMI was calculated using the following formula: BMI = body weight (kg)/height (m)^2. FM and LM were measured by Dual-energy X-ray Absorptiometry (DXA) (Primus, X-ray densitometer we; Software Version 1.2.2, Osteosys Co., Ltd, Guro-gu, Seoul, Korea)[33]; data were expressed in percentage (%) and in kilogram (kg). We categorized the subjects according to BMI, and % of total body fat mass (FM) into normal weight (NW) lean women with a BMI <25 kg/m^2 and FM% <30%; NWO women with a BMI <25 kg/m^2, and FM% ≥30%; pre-obese and obese women with a BMI ≥25 kg/m^2 and FM% ≥30%[1].

DNA Isolation and RTq-PCR Analysis

The phenol-chloroform extraction was used to extract the Genomic DNA[34], which was collected, in turn, *via* saliva swab. Determination of the IL-6 promoter polymorphism (rs1800795), formerly known as 174G/C, was carried out from genomic DNA using Real-Time PCR with sequence-specific primers, using the TaqMan® probe method, a detection technique that allows to determine the genotype based on the fluorescence emission by fluorophores linked to allele-specific probes. To prepare the DNA for the genotyping, a two allele-specific fluorescent probe including a PCR primer pair (TaqMan SNP Genotyping Assays, Life Technologies, Carlsbad, CA, USA) and a master mix including dNTPs and Taq DNA Polymerase (TaqPath ProAmp Master mix Life Technologies, CA, USA) were used. The amplifications were carried out following the protocol provided by the Applied Biosystems, which provides for the use of: 1) TaqMan™ Genotyping Master Mix (Life Technologies, Carlsbad, CA, USA) which in turn contains: a) DNA polymerase (AmpliTac-Gold®, Life Technologies, Carlsbad, CA, USA); b) Buffer used to keep the pH constant; d) dNTPs. TaqMan SNP Genotyping Assays. which contains a) two specific primers for the sequence and the polymorphism of interest; b) allele specific TaqMan probes (MGB) (Life Technologies, Carlsbad, CA, USA) for the detection of alleles and for the specific polymorphism of interest. Each probe in turn is made up of a reporter (R) dye at its 5′ end, the VIC® (Life Technologies, Carlsbad, CA, USA) dye, tied to the 5′ end of the allele 1 probe and the FAM™ dye, tied to the 5′ end of the allele 2 probe. Based on this ATCGATT [G/T] ATCC sequence, the probe labelled with VIC® dye of allele 1 corresponds to the first nucleotide inside the sequence in the square bracket (G), while the probe labelled with FAM™ dye of allele 2 corresponds to the second nucleotide (T).

SNP genotyping assessment was executed using a Real-Time PCR analysis (Applied Biosystems Step One Plus TM Real-Time PCR thermal cycler, Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. The samples were subjected to 40 amplification cycles; each cycle is divided into two phases: 1) denaturation phase at 95°C for 15 seconds, allows the separation of the two DNA strands thanks to the breaking of the hydrogen bonds; 2) single annealing-extension phase at 60°C for 1 minute. The first cycle of the total 40 is preceded by a further phase, in which a temperature of 95°C is reached and maintained for 45 minutes; this step is necessary to activate the polymerase.

Statistical Analysis

The distribution of collected variables was preliminary evaluated with the Kolmogorov-Smirnov test. Therefore, the data was not normally distributed; the non-parametric tests were used to compare the differences between the two groups using the Mann-Whitney test. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using the SPSS-22 software package (IBM, Armonk, NY, USA).

The SNP-HWE program was used to calculate the Hardy-Weinberg equilibrium (HWE) for IL-6 promoter polymorphism (rs1800795). This equilibrium for a bi-allelic locus (alleles 1 and 2) is expressed by the following equation: p2 + 2pq + q2 = 1 where p and q indicate the frequencies of alleles 1 and 2, while p2 indicates the frequency in the population of the genotype 1/1, while q2 the frequency of the genotype 2/2 and 2pq is the frequency of the genotype 1/2. The result was tested using the χ^2-analysis.

Results

According to BMI and FM % evaluations, in LIPPY there were 6.7% of NW Lean, 42.2% of NWO, 51.1% of Obese. In CTRL group there were

Table I. The anthropometric values of lipedema (LIPPY) and Control (CTRL) group.

	LIPPY (N = 45) Median (Min-Max)	CTRL (N = 50) Median (Min-Max)	p
Height (cm)	163.00 (148.50-169.50)	162.00 (146.50-196.55)	0.814
Weight (kg)	79.40 (54.00-153.50)	69.1 (42.40-136.78)	0.002
BMI (kg/m^2)	30.04 (20.34-62.87)	25.48 (17.66-51.47)	0.001
Age (years)	42.93 (18.00-71.69)	40.50 (20.00-80.00)	0.243
C. Waist	85.50 (66.5-114.55)	79.50 (56.50-117.50)	0.044
C. Abdomen	97.60 (74.50-155.50)	93.50 (71.00-140.65)	0.035
C. Hips	115.50 (66.50-175.60)	101.60 (82.50-144.50)	0.000
WHR	0.73 (0.36-1.20)	0.79 (0.49-0.97)	0.001

LIPPY: lipedema group; CTRL: control group; Body Mass Index (BMI); Waist circumference (c. waist); Abdomen circumference (C. Abdomen); Hip circumference (C. Hip); Waist to Hip ratio (WHR). The data are presented as median (minimum-maximum). Statistical significance was assigned for a *p* less than 0.05.

10.0% of NW Lean, 58.0% of NWO, 32.0% of Obese. The anthropometric measurements of LIPPY were statistically significant respect to CTRL regarding weight (p=0.002), BMI (p=0.001), waist circumference (p=0.044) abdomen circumference (p=0.035) and hip circumference (p<0.001). The value of Waist to Hip ratio (WHR) was decreased (p=0.001). The characteristics of the participants are presented in Table I. The statistically significant values of the body composition measured by DEXA between LIPPY and CTRL group were % arms FM (p=0.002), % legs FM (p<0.001), % whole FM (p=0.012), whole FM (kg) (p=0.007), legs FM (kg) (p<0.001), gynoid FM (kg) (p=0.008) and legs LM (kg) (p<0.001). In LIPPY subjects, all values were increased (Table II). Furthermore, the two groups were divided into carriers and non-carriers of allele risk, and data were compared within the group (LIPPY carriers *vs.* LIPPY non-carriers; CTRL carriers *vs.* CTRL non-carriers) and between the groups (LIPPY carriers *vs.* CTRL carriers; LIPPY non-carriers *vs.* CTRL non-carriers). In Table III, we have presented the association of the polymorphism related to the IL-6 gene (rs1800795) with the anthropometric measurements in LIPPY and CTRL groups. The LIPPY, respect to CTRL, presented statistically significant increased values of weight (p<0.001), BMI (p<0.001), waist circumference (p<0.05), abdomen circumference (p<0.05), hip circumference (p<0.001). In CTRL carriers respect to CTRL non-carriers the value of the circumference of the abdomen (p<0.05) was significantly decreased. In LIPPY carriers, the weight values (p<0.05), BMI (p<0.05), abdomen circumference (p<0.05) and hip circumference (p<0.01) were significantly higher respect to CTRL carriers. In Table IV, the comparison between the body composition values in LIPPY and CTRL groups according to IL-6 gene polymorphism (rs1800795) was reported. The HWE (p>0.05) in both LIPPY and CTRL groups was respected.

Table II. Body composition values measured by DXA in Lipedema (LIPPY) and Control (CTRL) groups.

	LIPPY (N = 45) Median (Min-Max)	CTRL (N = 50) Median (Min-Max)	p
Arms FM (%)	47.60 (26.10-61.10)	41.85 (23.70-59.00)	0.002
Legs FM (%)	48.90 (33.00-62.40)	42.55 (25.90-63.9)	0.000
Android FM (%)	44.20 (18.00-65.80)	48.50 (19.60-64.13)	0.961
Gynoid FM (%)	50.40 (30.70-63.50)	49.20 (28.90-64.35)	0.548
Whole FM (%)	43.40 (25.70-57.50)	39.19 (30.30-58.00)	0.012
Fat arms (kg)	3.92 (1.22-8.02)	2.73 (0.85-10.17)	0.000
Fat legs (kg)	15.04 (6.98-34.27)	11.99 (1.86-30.31)	0.000
Android FM (kg)	2.39 (0.59-7.83)	2.15 (0.55-7.29)	0.335
Gynoid FM (kg)	6.21 (2.73-17.30)	4.90 (2.78-13.17)	0.008
Whole FM (kg)	32.50 (12.01-86.04)	25.53 (9.76-73.76)	0.007
Arms LM (kg)	4.27 (2.99-7.00)	3.96 (2.70-8.37)	0.133
Legs LM (kg)	15.85 (10.51-28.56)	13.17 (8.56-29.23)	0.000

LIPPY: lipedema group; CTRL: control group; FM: Fat Mass; LM: Lean Mass. The data are presented as median (minimum-maximum). Statistical significance was assigned for a p less than 0.05.

Table III. Comparison between the anthropometric values in Lipedema (LIP) and control (CTRL) group according to polymorphism related to the IL-6 gene polymorphism (rs1800795).

	LIPPY non-carriers (CC) Median (Min-Max)	LIPPY carriers (CG. GG) Median (Min-Max)	CTRL non-carriers (CC) Median (Min-Max)	CTRL carriers (CG. GG) Median (Min-Max)	(lip– *vs.* lip+) *p*	(ctrl– *vs.* ctrl+) *p*	(lippy+ *vs.* ctrl+) *p*	(lippy– *vs.* ctrl–) *p*
Weight ()	82.50 (64.55-133.54)	79.15 (54.55-153.60)	72.65 (50.68-110.40)	68.70 (42.40-128.50)	0.613	0.372	0.027	0.154
BMI (kg/m²)	29.94 (24.46-51.95)	31.10 (20.34-62.87)	26.93 (19.78-41.03)	23.86 (17.65-51.47)	0.745	0.152	0.013	0.342
C. Waist	79.50 (69.55-114.55)	84.51 (63.50-116.65)	81.56 (64.50-113.50)	74.00 (59.50-117.60)	0.731	0.094	0.056	0.887
C. Abdomen	96.60 (86.65-150.65)	97.75 (74.54-155.50)	96.56 (81.50-127.00)	88.80 (71.60-127.50)	0.626	0.036	0.016	0.759
C. Hips	109.10 (66.52-155.56)	115.75 (91.58-175.60)	104.51 (87.10-124.50)	98.89 (84.50-44.00)	0.957	0.162	0.001	0.286
WHR	0.76 (0.64-1.2)	0.73 (0.36-0.92)	0.81 (0.68-0.91)	0.77 (0.69-.0.96)	0.329	0.105	0.064	0.644

LIPPY: lipedema group; CTRL: control group; Body Mass Index (BMI); C. waist. waist circumference; C. Abdomen Abdomen circumference; C. Hip. Hip circumference; WHR. Waist to Hip ratio. The subjects were divided into carriers (CG and GG) and non-carriers (CC) of the risk allele: IL-6 polymorphism (rs1800795) non-carriers (CC); (+) IL-6 polymorphism (rs1800795) carriers (CG. GG). The data are presented as median (minimum-maximum). Statistical significance was assigned for a *p* less than 0.05.

Table IV. Comparison between the values of body composition in Lipedema (LIPPY) and control (CTRL) group according to polymorphism related to the IL-6 gene polymorphism (rs1800795).

	LIP IL-6 non-carriers Median (Min-Max)	LIP IL-6 carriers Median (Min-Max)	CTRL IL-6 non-carriers Median (Min-Max)	CTRL IL-6 carriers Median (Min-Max)	(lip– *vs.* lip+) *p*	(ctrl– *vs.* ctrl+) *p*	(lip+ *vs.* ctrl+) *p*	(lip– *vs.* ctrl–) *p*
Arms FM (%)	48.80 (39.60-57.65)	46.85 (26.15-61.18)	47.15 (42.96-57.5)	43.25 (29.10-59.55)	0.691	0.159	0.173	0.77
Legs FM (%)	48.60 (43.30-59.95)	49.30 (33.35-62.40)	54.15 (42.65-63.15)	44.66 (31.65-55.45)	0.705	0.038	0.053	0.661
Android FM (%)	39.10 (28.70-65.34)	44.4 0 (18.00-65.85)	54.75 (49.15-55.95)	50.55 (33.45-62.15)	0.986	0.411	0.299	0.602
Gynoid FM (%)	48.30 (43.70-61.55)	51.10 (30.71-63.56)	48.00 (44.95-57.46)	49.56 (37.35-59.96)	1.000	0.61	0.507	0.917
Whole FM (%)	40.90 (35.75-57.50)	43.40 (25.70-56.75)	42.50 (30.35-58.65)	40.52 (33.26-56.25)	0.745	0.902	0.286	0.594
Fat arms (kg)	3.65 (2.53-8.02)	3.94 (1.22-6.31)	3.25 (0.85-6.12)	3.10 (1.21-9.67)	0.348	0.771	0.164	0.197
Fat legs (kg)	15.08 (11.10-30.27)	13.28 (6.98-34.27)	10.37 (1.86-30.31)	11.10 (5.82-23.14)	0.493	0.366	0.017	0.057
Android FM (kg)	2.05 (1.02-6.48)	2.41 (0.59-7.83)	3.75 (3.51-5.63)	3.57 (0.82-6.71)	0.718	0.256	0.32	0.602
Gynoid FM (kg)	6.25 (4.62-12.58)	6.18 (2.73-17.30)	7.65 (5.61-8.27)	5.93 (3.20-9.76)	0.613	0.17	0.613	0.754
Whole FM (kg)	33.56 (24.37-75.98)	32.07 (12.01-86.04)	39.82 (9.76-73.61)	35.05 (14.60-69.33)	0.516	0.88	0.683	0.587
Arms LM (kg)	4.86 (3.55-7.00)	4.26 (2.99-5.86)	3.75 (2.84-7.82)	4.71 (3.10-7.52)	0.348	0.143	0.116	0.377
Legs LM (kg)	16.92 (13.35-22.83)	15.77 (10.51-28.56)	13.32 (10.77-21.87)	14.81 (9.71-21.43)	0.427	0.401	0.451	0.077

LIPPY: lipedema group; CTRL: control group; FM: Fat Mass; LM: Lean Mass. The subjects are divided into carriers (CG and GG) and non-carriers (CC) of the risk allele: (–) non-carriers (+) carriers. The data are presented as median (minimum-maximum). Statistical significance was assigned for a p less than 0.05.

In CTRL carriers respect to CTRL non-carriers the value of Legs Fat Tissue % and Whole Fat Tissue % ($p<0.05$) were significantly decreased. In the comparison between LIPPY carriers and CTRL carriers the value of Fat Legs (kg) ($p<0.01$) was significantly increased. According to Odds Ratio analysis the risk for LIPPY *vs.* controls is 5.92 (OR=5.92; $p<0.001$; 95% confidence interval =1.983-17.711).

Discussion

Lipedema is a complex and multifaceted condition that has important physical, psychological and social implications[26].The etiology and pathogenesis of lipedema are not yet fully understood, as demonstrated by the fact that subjects with lipedema are mostly females and the onset of the disease is generally at puberty[35]. The lack of knowledge of the etiopathogenetic mechanisms could be due to the inability to carry out research due to the absence of animal models or substrates suitable for *in vitro* examinations. Also, the lack of identified biomarkers for this disease hinders the ability to separate correctly the cohorts affected family members for genetic study. Currently, no racial or ethnic group correlations are known, although according to some experts the disorder seems to be practically absent among Asian women. Prevalence estimates, although vague, suggest that a considerable fraction of Western adult female population, often misdiagnosed as obese or as having lymphedema, has instead lipedema.

Difficulties in recognition and lack of consensus on classification systems have led some to consider lipedema a rare disease, when instead some research, mainly on the German population, estimates a prevalence and a global distribution in the female population that is not rare[15,36,37]. Likely, discordant data on the prevalence of the disease could depend on the classification of particularly mild forms of lipedema that would overlap with problems typical of cellulite (aesthetics) and the gynoid phenotype of obesity[38]. In 89% of cases, maternal or paternal familiarity had been present for at least three generations[39].

However, the exact nature of the type of transmission is difficult to determine and has been supposed to be either a dominant inheritance linked to the X chromosome, or an autosomal dominant with sexual limitation. Also, it has been hypothesized that this genetic mutation could lead to the failure of male births, given the female predominance of the condition[40].

Higher BMI is associated with lipedema survivals, but in case of lipedema BMI may be inaccurate to reflect the adiposity index. In addition, BMI is limited to measure body fat and poorly distinguishes fat from lean or bone mass[4,28,29].

For the first time, in the present study, we analysed the body composition of Caucasian Italian women affected by lipedema compared to the control group represented by women without lipedema randomly enrolled among participants in ongoing clinical trials, in the same period for the evaluation of nutritional status.

According to De Lorenzo et al[3], we categorized our population based on phenotype classification through BMI and % of FM, in NW, NWO, and OB women, regardless of the diagnosis of lipedema. As regards a first differential analysis, based on anthropometric and body composition indices (Tables I and II) between the LIPPY and the CTRL groups, results have emerged in line with the characteristics already known in the literature. In other words, as far as lipedema is concerned, fat accumulation in the lower limbs, upper limbs, arms, legs and gynoid fat has been confirmed[21,41]. Among LIPPY, 42.2% of women was of NWO, and 51.1% was obese. Similar results were obtained in CTRL group, in which 58.0% was NWO, and 32.0% was obese.

Therefore, lipedema, NWO and obese phenotype can coexist in the same patient, making the diagnosis of lipedema even more difficult. Furthermore, in the LIPPY an increase in total fat percentage was observed, probably due to the fact that many of them were also in a condition of obesity. Hence, we have to consider lipedema as a separate entity with peculiar phenotypic characteristics, exclusively for the type of subcutaneous deposition of subcutaneous fat, mainly in the lower limbs and sometimes in the upper limbs. It is therefore not surprising that in obese LIPPY waist and abdomen circumferences were increased. For the amount and percentage of total body fat mass, the LIPPY are similar to the obese. Obese LIPPY have an additional accumulation of fat with a gynoid-type distribution, beyond the increased hip circumference, characteristic of this disease. This causes a reduction in the WHR compared to the control ($p<0.001$). Subsequently, with increasing fat mass and in conjunction with menopause, it is possible to

find a combination of both phenotypes, android and gynoid, with both central and peripheral fat distribution. To our knowledge, this study is the first to approach complex genetic interactions in relation to body fat accumulation in patients with lipedema. Therefore, genetic studies on this disease related to inflammatory processes could be useful for defining the lipedema phenotype[13]. The analysis aimed at identifying genetic indicators for the differential analysis between LIPPY and CTRL groups, and it has led to a confirmation of fat distribution in the characteristic locations of lipedema. Significant values were highlighted in the comparison between carriers of both groups, but not in the intra-group comparison (Tables III and IV). IL-6 plays a role in the regulation of body fat. In fact, Wallenius et al[31] conducted on mice have shown that IL 6 deficiencies lead to the onset of obesity since related to high levels of leptin. Furthermore, several studies[29,42,43] show that IL-6 levels are elevated in obesity indicating the presence of an inflammatory state, IL-6 is released by adipocytes, and in obese subjects high levels of IL-6 are evident. Several polymorphisms located in the 5' region of the IL-6 gene have been identified, but the majority of studies have focused on the G/C substitution at position-174. The C allele of rs1800795 involves a lower production of interleukin 6 compared to G allele[37]. IL-6 gene polymorphism (rs1800795), influencing IL-6 transcription, is the cause of the onset of overweight and increased sensitivity to insulin[29]. Moreover, Di Renzo et al[29] analysed the role of this polymorphism in Italian women with the NWO syndrome and reported that the concentration of IL-6 was positively correlated with the % of fat mass in the GG genotype and negatively correlated in the CC genotype[29,42,43].

A typical feature of lipedema is the presence of chronic inflammation. For this reason, the IL-6 gene polymorphism (rs1800795) was chosen. In fact, IL-6 is one of the main factors released during chronic inflammation. Based on the data obtained, significant differences between carriers or not of the IL-6 gene polymorphism (rs1800795) and fat distribution in women with lipedema were found. Moreover, being carrier of the mutation increases the risk of developing lipedema by 5.92 times ($p<0.0001$). However, these results should be confirmed on a larger population of different ethnicity. As reported by Sun et al[44] pathologic expansion of subcutaneous adipose tissue (SAT) is associated with rapid growth of fat tissue and inadequate vascularization leading to fibrosis and secretion of high levels of inflammatory cytokines[43,44].

Since patients with lipedema suffer of an inflammatory state of subcutaneous fat, and due to the pro-inflammatory effect of IL-6, a role of this cytokine in the disease under consideration could be hypothesized. A subsequent analysis should be performed to correlate the circulating IL-6 levels with the anthropometric and body composition parameters in LIPPY. It is considered a duty of all physicians to recognize this condition and adequately meet the needs of their patients. These women do not deserve to be rejected by medical personnel and simply stigmatized as "obese"[16], but they deserve precise diagnostic evaluations and appropriate therapeutic interventions.

Conclusions

For the first time, we identified new significant indices and predictive parameters based on body composition and IL-6 gene polymorphism (rs1800795) that could characterize subjects with lipedema respect to NWO and obese subjects. We believe that leg index, abdominal index, trunk index, total index to gather with genetic analysis of IL-6 gene polymorphism (rs1800795) are promising clinical tools for the diagnosis of the lipedema phenotype and for predicting the evolution of the disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

LDR drafted the manuscript, conceived and designed the experiments; PG, SZ, GLDS, MM, MGT, performed the experiments and collected data; SM, enrolled the LIPPY group; MC, collected saliva samples, for genetic analysis; LR analysed the data; PG, AC, AP, FF reviewed the text; LDR, GM, 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.

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