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Improvement of quality of life by intake of hydroxytyrosol in patients with lymphedema and association of lymphedema genes with obesity

K. DHULI[1], M.R. CECCARINI[2,3], V. PRECONE[1], P.E. MALTESE[4], G. BONETTI[1], S. PAOLACCI[4], A. DAUTAJ[4], G. GUERRI[4], G. MARCEDDU[1], T. BECCARI[2,3], S. MICHELINI[5], M. BERTELLI[1,4]

[1]MAGI EUREGIO, Bolzano, Italy
[2]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
[3]C.I.B., Consorzio Interuniversitario per le Biotecnologie, Trieste, Italy
[4]MAGI'S LAB, Rovereto (TN), Italy
[5]Vascular Diagnostics and Rehabilitation Service, Marino Hospital, ASL Roma 6, Marino, Italy

Abstract. – OBJECTIVE: Lymphedema is a debilitating disease and may be a comorbidity of obesity. New molecules have been investigated for the treatment of lymphedema; one of the most promising molecules is hydroxytyrosol. The aim of this study was to evaluate the association between mutations in genes mutated in lymphedema and the presence of obesity and making an estimate of the quality of life in lymphedema patients.

MATERIALS AND METHODS: We recruited 71 Caucasian individuals with the diagnosis of primary lymphedema, and they undertook a questionnaire to assess their quality life. For this purpose, we developed a NGS custom-made panel comprising genes associated with lymphedema.

RESULTS: An obesity rate of 20% was detected. The average Lymph-ICF-LL value for patients who consume olive oil daily was 20 with a better quality of life. Twenty-three patients resulted positive to the genetic test. Genetic variants with a likely association with obesity have been identified in *PROX1*, *FOXC2* and *FLT4*.

CONCLUSIONS: A obesity rate, higher than that reported by ISTAT, was detected. The use of olive oil enhances the quality of life of lymphedema patients. Moreover, a diagnostic approach by a NGS panel shows an association of lymphedema with obesity.

Key Words:
Lymphedema, Obesity, Hydroxytyrosol, Next generation sequencing panel.

Introduction

Lymphedema is a progressive disease resulting from an accumulation of fluid in the interstitial space as a result of failure of the lymphatic system. Primary lymphedema is congenital with typical Mendelian inheritance and is characterized by intrinsic defects in the lymphatic vasculature whereas secondary lymphedema is caused by an external factor, but genetic predisposition may be present[1]. Lymphedema affects especially the legs (80%), but it can also affect arms, face or external genitalia. Primary lymphedema affects 1 in 100,000 individuals while secondary lymphedema affects about 1 in 1000 individuals[2]. The lymphedema increases the risk of infections, causes debilitating pain, weakness, difficulty moving and decreases overall quality of life[3]. Patients with chronic lymphedema for ten years have a 10% higher risk of developing lymphangiosarcoma. This tumour is aggressive and has a very poor prognosis[4].

Obesity causes lymphatic dysfunction increasing risk of lymphedema[5,6] and lymphedema caused by obesity may not be reversible[7]. Scientific evidence[6] points out that obesity can increase the risk of secondary lymphedema. Lymphedema usually occurs as a side effect of cancer and cancer treatment. One in 5 women who survive breast cancer develop lymphedema[8], but the risk of developing lymphedema is about three times higher in presence of a body mass index (BMI) greater than 30[9,10]. Moreover, a connection between obesity and lymphedema in subjects without cancer was demonstrated. In fact, subjects with no other pre-existing condition or risk factors associated with lymphedema, with a BMI over 60 present an insufficient lymphatic flow in the lower extremities[11,12]. Therefore, the association between obesity and lymphedema provides a mechanism for the de-

Corresponding Author: Stefano Paolacci, Ph.D; e-mail: stefano.paolacci@assomagi.org

velopment of primary lymphedema in superobese individuals. For obese individuals, in addition to a physical compression of lymphatic vessels by fatty tissue, local inflammation caused by the presence of excess fat is hypothesized to further damage the lymphatic vessels[13,14]. Moreover, the excessive fat increases the production of lymph and can also promote venous insufficiency, a condition that can cause swelling by itself[15]. There is a possible link, even genetic, between obesity and lymphedema. Experimental evidence linking lymphatic dysfunction and obesity were derived from human system and transgenic mice with a variety of mutations in their lymphatic system[6]. Several studies suggest that high fat diet induced obesity results in marked alterations in lymphatic endothelial cell gene expression. In fact, obese mice showed downregulated expression of genes that control the development and functionality of lymphatic vessels[16,17].

Lymphedema comprises more than ten Mendelian forms, of which the most frequent are Milroy disease (OMIM 153100) and Meige disease (OMIM 153200). Primary lymphedema is genetically heterogeneous, furthermore polymorphisms predisposing to secondary lymphedema have been identified[1].

Genetic testing can be useful for understanding the molecular mechanisms involved in the pathogenesis of lymphedema, especially for identifying new therapeutic targets[18]. Currently, benzopyrones are the only drug treatment for lymphedema. However, new targets are currently investigated, and we have supposed that a natural molecule, hydroxytyrosol, acts on pathways activated during lymphedema and could therefore be an ideal candidate compound[19,20]. Hydroxytyrosol is a phenolic phytochemical with antioxidant properties that can be found in olive oil, currently already studied for its beneficial effects in the treatment of obesity and metabolic disorders[21].

In this study, we made an estimate of physical activity and eating habits, including the intake of hydroxytyrosol, and performed a genetic analysis using a next-generation-sequencing (NGS) approach and a custom-made gene panel designed to include genes involved in lymphedema in 71 Italian patients with primary lymphedema.

Materials and Methods

Patients Selection and Clinical Evaluation

We recruited 71 Caucasian patients diagnosed with primary lymphedema in hospitals across Italy. The clinical diagnosis of lymphedema was confirmed by three-phase lymphoscintigraphy according to the protocol of Bourgeois, Munck, Becker, and Leduc[22]. Each patient undertook a questionnaire for the collection of the following data: weight and height from which the BMI was derived, level of physical activity, lifestyle by the administration of the International Physical Activity Questionnaire (IPAQ), MET minute/week questionnaire and Eating Habits questionnaire to calculate weekly and daily kcal intake[23], Lymphedema Functioning, Disability and Health Questionnaire for Lower Limb Lymphoedema (LYMPH ICF LL)[24] to know the level of influence of the disease on the subject's quality of life and the olive oil daily intake[25]. Pretest genetic counselling was carried out to evaluate each patient's personal and familial history. All patients gave their written informed consent to the study that was carried out according to the tenets of the Helsinki Declaration and approved by the Ethics Committee of Azienda Sanitaria dell'Alto Adige, Italy (Approval No. 132-2020).

Genetic Testing

Genetic testing was performed on genomic DNA extracted from peripheral blood of each proband using a commercial kit (SAMAG 120 BLOOD DNA Extraction Kit) according to the manufacturer's instructions. DNA quantity was evaluated using Quant-iT Picogreen dsDNA Assay Kit (Life Sciences, Marlborough, MA, USA) and a Varioskan LUX (Thermo Fisher Scientific, Waltham, MA, USA). A custom-made oligonucleotide probe library was designed to capture all coding exons and flanking exon/intron boundaries (~15bp) of genes known to be associated with lymphedema and obesity genes: namely *ADAMTS3* (OMIM *605011), *CELSR1* (OMIM *604523), *EPHB4* (OMIM *600011), *FAT4* (OMIM *612411), *FLT4* (OMIM *136352), *FOXC2* (OMIM *602402), *GATA2* (OMIM *137295), *GJA1* (OMIM *121014), *GJC2* (OMIM *608803), *HGF* (OMIM *142409), *KIF11* (OMIM *148760), *PIEZO1* (OMIM *611184), *PTPN14* (OMIM *603155), *SOX18* (OMIM *601618), *VEGFC* (OMIM *601528), *PROX1* (OMIM *601546) and *MET* (OMIM * 164860). The custom DNA probes were designed using Twist Bioscience technology (https://www.twistbioscience.com/). DNA samples were processed using MiSeq personal sequencer (Illumina, San Diego, CA) using a 150 bp paired-end long reads protocol. Each predicted pathogenic variant and target region coverage with less than 10 reads was confirmed by conventional Sanger sequencing according to the manufacturer's protocols (CEQ8800 Sequencer, Beckman Coulter).

Bioinformatics

Fastq (forward-reverse) files were obtained after sequencing. The sequencing reads were mapped to the genome by the Burrow-Wheeler Aligner (BWA version 0.7.17-r1188) software. Duplicates were removed using the SAMBAMBA (version 0.6.7) program and MarkDuplicates GATK tool (version 4.0.0.0). The BAM alignment files generated were refined by local realignment and base quality score recalibration using the RealignerTargetCreator and IndelRealigner GATK tools. When each variant was found, we searched for it in the dbSNP database (www.ncbi.nlm.nih.gov/SNP/) and in the Human Gene Mutation Database professional (HGMD; http://www.biobase-international.com/product/hgmd). In silico evaluation of the pathogenicity of all exonic variants was performed using the Variant Effect Predictor tool (http://www.ensembl.org/Tools/VEP) and VarSome (https://varsome.com/). Finally, minor allele frequencies (MAFs) were checked in the Genome Aggregation Database (GnomAD) (http://GnomAD.broadinstitute.org/) and all variants were evaluated according to American College of Medical Genetics and Genomics guidelines (https://www.acmg.net). Variants were also verified on ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and OMIM (https://www.omim.org/).

Statistical Analysis

The results are expressed as means ± SDs and interquartile range or as percentage. Statistical analysis was performed using the chi-square for the categorical variables and the Students' *t*-test for analysis of differences in average values. A p-value < 0.05 was considered significant. Statistical calculations were performed with SPSS software package version 25.0 (Chicago, IL, USA).

Results

Seventy-one Italian patients with lymphedema were analysed with a NGS custom panel, including genes associated with lymphedema. The recruited population included 16 males and 55 females. Their median age was 40 ± 17 years for males and 47 ± 12 years for females. The median BMI was 27 ± 6 (25 ± 4 for males and 27 ± 7 for females). Twenty percent of them were obese. The clinical data of the patients analyzed in this study are shown in Table I. There is a statistically significant correlation between the daily intake of olive oil and the quality of life in patients with primary lymphedema as shown in Figure 1. In particular, the average Lymph-ICF-LL value for patients who consume olive oil daily (7.50 mg of hydroxytyrosol/day which corresponds to about 20 grams of Italian extra virgin olive oil) was 20 (range: 8-30) with 14/18 patients with a score related to less severe issues and 4/18 patients with a score related to moderate problems; while the average score for patients who did not consume olive oil daily was 42 (range: 31-85) with 28/53 patients with moderate problem and 25/53 patients with severe problems.

For each subject all genes of the NGS panel were analyzed. Twenty-three primary lymphedema patients (32%) resulted positive for the molecular test. Four positive patients had a BMI ≥ 30 related to obesity (patient 3, patient 4, patient 15 and patient 21) (Table II).

Table I. Clinical data of the primary lymphedema patients recruited in the study.

	Total (n=71)
Body mass index (kg/m^2)	27 ±7
Familial history	37 (52%)
Physical activity	High: 19 (27%) Moderate: 28 (40%) Low: 24 (33%)
KCal/ die intake	2228 +/- 2311
Level of activity (MET)/ die	2112 +/- 2346
Level of activity (MET)/ week	302 +/- 335
KCal consumed daily	372 +/- 418
KCal consumed weekly	2603 +/- 2929
Influence of the disease on the subject's quality of life (LYMPH ICF LL)	42 +/- 19

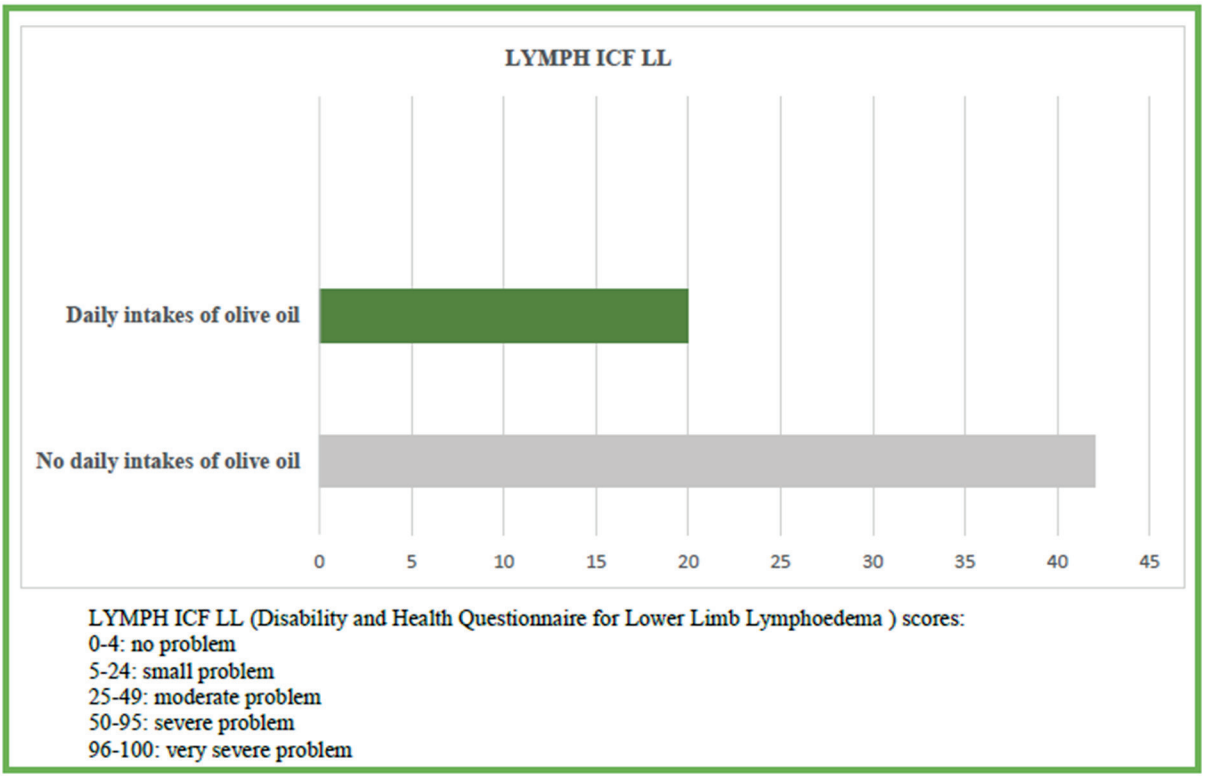


Figure 1. Quality of life of the primary lymphedema patients analyzed in relation to daily intake of hydroxytyrosol (p = 0,0002). Twenty grams of Italian extra virgin olive oil contain 7.50 mg of hydroxytyrosol.

Discussion

Lymphedema is the progressive enlargement of tissues due to inadequate lymphatic function and may be associated with obesity. In addition, in obese patients, large fatty deposits can compress the lymphatic channels, creating either a mechanical disruption or complete obstruction. When the disruption becomes profound, the lymphatic fluid exceeds transport capacity, and irreversible lymphedema may occur[5-7]. This clinical association between obesity and lymphedema has led us to assume that there may be a common genetic ground. Therefore, we performed a genetic test in 71 Italian patients with primary lymphedema using a NGS custom-made gene panel designed to include genes involved in lymphedema in order to see whether there was an involvement in obesity. Interestingly, in our cohort, 20% of patients were obese with a BMI >30, a higher percentage than the 10% reported in the general population by the Italian National Institute of Statistics (ISTAT - https://www.istat.it/en/archQivio/obesity).

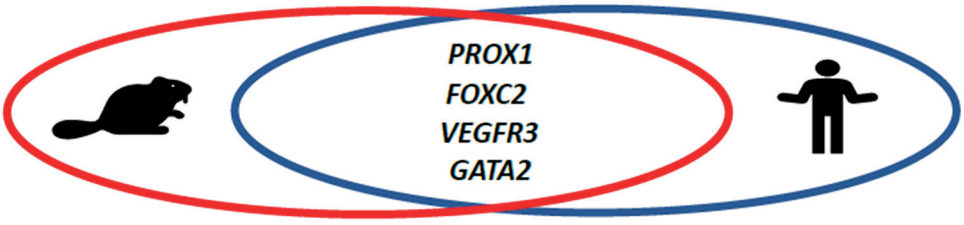
Our cohort underwent several tests: IPAQ and MET minute/week questionnaire to calculate weekly and daily kcal intake, Eating Habits questionnaire to determine daily calorie intake and LYMPH ICF LL questionnaire to know the level of influence of the disease on the subject's quality of life (Table I). Obesity causes perilymphatic inflammation and lymphatic dysfunction. Aerobic exercise, regardless of weight loss, reduces the accumulation of perilymphatic inflammatory cells, improves lymphatic function, and reverses pathological changes in gene expression in lymphatic endothelial cells[26]. The mean value of MET/week was 302 ± 335 and only 43/71 (61%) of the tested patients did not carry out an adequate volume of physical activity (over 600 MET-min/week), of which, only 26/71 patients undertake high volume of physical activity (over 1500 MET-min/week). Therefore, in our population it has emerged that it would be necessary to increase physical exercise, especially taking into account the results obtained from the Lymph-ICF-LL test, which is a descriptive questionnaire for assessing impairments in functioning, activity limitations, and daily life participation restrictions. In fact, for 21/71 (30%) patients of the present cohort, lymphedema is an invalidating disease. For none of the lymphede-

ma patients examined there was problem, only for 12/71 (17%) lymphedema patients the problem was small and for 38/71 (56%) lymphedema patients, instead, the problem was medium.

The intake of hydroxytyrosol in olive oil has also been evaluated because it has been suggested that hydroxytyrosol could be useful at treating the secondary effects of lymphedema, in particular inflammation. Lymphedema deregulates at least six molecular pathways and hydroxytyrosol, a compound with antioxidant activity, is able to improve endothelial dysfunction, hemostatic and lipid profiles and decreases oxidative stress and inflammation by inhibiting the activity of leukotriene B4[19,20]. In particular, only 18/71 (25%) lymphedema patients of the studied population regularly took olive oil every day. Interestingly, the average Lymph-ICF-LL value for patients

Table II. Genetic variants identified in the positive primary lymphedema patients.

Patient	Gene	Nucleotide variant; amino acid variant	VarSome prediction	Obesity (BMI>30)
1	*FLT4* (NM_182925)	c.1906C>G; p.(Leu636Val)	Uncertain significance	-
	MET (NM_001127500)	c.2962C>T; p.(Arg988Cys)	Likely benign	
	GJC2 (NM_020435)	c.1027G>T; p.(Ala343Ser)	Uncertain significance	
2	*GATA2* (NM_032638.4)	c.414_417del; p.(Ser139Cysfs*78)	Pathogenic	-
3	*FLT4* (NM_182925)	c.2560G>A; p.(Gly854Ser)	Uncertain significance	+
4	*FOXC2* (NM_005251)	c.638delT; p.(Ile213Thfrs*19)	Likely pathogenic	+
5	*FOXC2* (NM_005251)	c.374C>T; p.(Ser125Leu)	Likely pathogenic	-
6	*FOXC2* (NM_005251)	c.595dup; p.(His199Profs*264)	Likely pathogenic	-
7	*MET* (NM_001127500)	c.2962C>T p.(Arg988Cys)	Likely benign	-
8	*MET* (NM_000245)	c.3650_3651del; p.(Thr1217Serfs*5)	Pathogenic	-
9	*FLT4* (NM_182925)	c.2740G>C; p.(Gly914Arg)	Uncertain significance	-
10	*FOXC2* (NM_005251)	c.1258C>T; p.(Gln420*)	Pathogenic	-
11	*FLT4* (NM_182925)	c.2670 C>G; p.(His890Gln)	Benign	-
		c.3437G>A; p.(Arg1146His)	Benign	
	FOXC2 (NM_005251)	c.1475C>T; p.(Ala492Val)	Uncertain significance	
12	*FLT4* (NM_182925)	c.2670 C>G; p.(His890Gln)	Benign	-
		c.3122G>A; p.(Arg1041Gln)	Likely pathogenic	
13	*FLT4* (NM_182925)	c.2670C>G; p.(His890Gln)	Benign	-
		c.2740G>T; p.(Gly914Trp)	Uncertain significance	
14	*FLT4* (NM_182925)	c.3437 G>A; p.(Arg1146His)	Benign	-
		c.2575 G>A; p.(Val859Met)	Uncertain significance	
15	*FOXC2* (NM_005251)	c.1109G>C; p.(Ser370Thr)	Uncertain significance	+
16	*FLT4* (NM_182925)	c.2777T>C; p.(Ile926Thr)	Uncertain significance	-
17	*FOXC2* (NM_005251)	c.1258C>T; p.(Gln420*)	Pathogenic	-
18	*FLT4* (NM_182925)	c.3460G>A; p.(Gly1154Arg)	Benign	-
19	*GJC2* (NM_020435)	c.1150C>T; p.(Pro384Ser)	Uncertain significance	-
20	*FOXC2* (NM_005251)	c.637A>G; p.(Ile213Val)	Uncertain significance	-
	SOX18 (NM_018419)	c.961G>A; p.(Asp321Asn)	Uncertain significance	
21	*FOXC2* (NM_005251)	c.238C>T; p.(Leu80Phe)	Likely pathogenic	+
22	*FLT4* (NM_182925)	c.2777T>C; p.(Ile926Thr)	Uncertain significance	-
23	*PROX1* (NM_001270616.1)	c.1769T>A; p.(Leu590His)	Uncertain significance	-



Gene (OMIM ID)	Description	Phenotype (OMIM ID)	Inheritance	Reference
PROX1 (*601546)	Prospero Omeobox 1	In mice, heterozygous inactivation of PROX1 leads to adult-onset obesitydue to lymph leakage. In humans, SNPs in genes associated with lymphatic development could predispose to adult-onset obesity		27
FOXC2 (*602402)	Forkhead Box C2	Lymphedema-distichiasis syndrome with renal disease and diabetes mellitus (153400)	AD	28-33
VEGFR3/FLT4 (*136352)	Vascular Endothelial Growth Factor Receptor 3	Lymphatic malformation 1 (153100)	AD	35-38
GATA2 (*137295)	GATA Binding Protein 2	Emberger syndrome (614038)	AD	39,40

Figure 2. Genes associated with lymphedema that are most likely also associated with obesity.

who drank olive oil daily was 20 (range: 8-30) with 14/18 patients with a score related to a small problem (relative range: 5-24) and 4/18 patients with a score related to a moderate problem (relative range: 25-49), while the average score for patients who did not consume olive oil daily was 42 (range: 31-85) with 28/53 patients with a score related to moderate problem (relative range: 25-49) and 25/53 patients with a score related to severe problem (relative range: 50-95), with a statistically significant difference (p = 0.0002). Therefore, these data confirm that regular intake of of hydroxytyrosol contained in olive oil may be able to improve the state and quality of life of patients with lymphedema (Figure 1). In particular, although physical activity is important, the accumulation of adipose tissue is independent from physical activity. Furthermore, the diet must exclude saturated fatty acids and must be supplemented with hydroxytyrosol. There is evidence in the literature on the interaction between the genes that control the development of lymphatic vessels and those that contribute to the onset of adiposity and obesity. The first and most striking evidence come from *Prox1* +/− mice that have a defective lymphatic vascular system and develop late-onset obesity. It is possible to hypothesize that the lymph leaking from the ruptured lymphatic vessels in *Prox1* +/- mice could potentially contain an adipogenic stimulus. Effects of loss of lymphatic function on lipid metabolism were also observed in humans with chronic lymphedema associated with PROSPERO homeobox 1 gene (*PROX1*) variants. Other variants of *PROX1* have been associated with hyperglycemia, type 2 diabetes and increased triglyceride levels. Moreover, PROX1 upregulates the expression of CPT1A which encodes an enzyme that controls the rate of β-oxidation of fatty acids in transgenic mouse models, decreases the production of acetyl-CoA and alters the lymphatic system development. Furthermore, in human cells PROX1 interacts with histone acetyltransferase p300 to increase the transcription of lymphangiogenic genes[27]. We identified c.1769T>A (p.Leu590His) variant in *PROX1*[27] (Table II). *FOXC2* (Forkhead Box C2) variants have been described in association with BMI and body fat percentage[28]. Furthermore, mice in which the expression of FOXC2 and its target genes in white adipose tissue is significantly higher, there is also a higher energy expenditure. It has also been observed that the expression of Egr-1 in

white adipose tissue represses the expression of FOXC2, promoting the accumulation of energy in WAT and favoring the development of obesity in conditions of high energy intake[29]. FOXC2 belongs to the forkhead family of transcription factors which is characterized by a distinct DNA-binding forkhead domain. The transcription factor encoded by *FOXC2* has been identified as a key regulator of adipocyte metabolism in mice. The exact function of FOXC2 in humans remains unknown. FOXC2 mRNA levels in visceral fat and skeletal muscle correlated with measures of insulin sensitivity[30]. FOXC2 is associated with lymphedema-distichiasis syndrome[31]. In our population, we identified various *FOXC2* variants (Table II): c.638delT; p.(Ile213Thfrs*19), c.1109G>C; p.(Ser370Thr), c.238C>T; p.(Leu80Phe), c.374C>T; p.(Ser125Leu)[32], c.595dup; p.(His199Profs*264)[33], c.1258C>T; p.(Gln420*), c.1258C>T; p.(Gln420*) and c.637A>G; p.(Ile213Val). The first three variants listed were detected in patients with BMI >30.

Vascular endothelial growth factors (VEGFs) are instead key regulators of angiogenesis and lymphangiogenesis, with different roles in pathological conditions, such as inflammation. Serum concentrations of different VEGFs appear to be altered during the development of obesity. It has recently been identified that the alteration of vascular endothelial growth factor C (VEGF-C) and vascular endothelial growth factor D (VEGF-D) in the subcutaneous adipose tissue during the development of obesity improves metabolic parameters and insulin sensitivity in mice. Elevated VEGF-C levels contribute to metabolic deterioration and the development of insulin resistance and blocking VEGF-C in obesity is a suitable approach to alleviate the development of insulin resistance[34]. Inhibition of angiogenesis by vascular endothelial growth factor receptor-2 (VEGFR2) blocking antibodies not only reduces angiogenesis and tissue growth, but also inhibits preadipocyte differentiation. Thus, there is a mutual regulation of adipogenesis and angiogenesis. Vascular endothelial growth factor receptor-3 (VEGFR-3) is the major receptor for VEGF-C and VEGF-D. A reduced transcription of VEGFR-3 can be considered a key mechanism by which obesity decreases lymphatic function. This hypothesis is supported by the increased expression of VEGF-C in the tissues and serum of obese mice and patients[35]. K14-VEGFR-3-Ig mice, constitutively expressing-VEGFR-3-Ig, are protected from obesity-induced insulin resistance and hepatic lipid accumulation. VEGF-C and -D play a fundamental role in mediating the inflammation of the adipose tissue associated with the metabolic syndrome. Blocking these lymphangiogenic factors could constitute a new therapeutic strategy for the prevention of insulin resistance associated with obesity[36]. Interestingly, several variants in *FLT4* were identified in the positive patients of our study population (Table II): c.2560G>A; p.(Gly854Ser)[37] in a patient with BMI >30, c.2740G>C; p.(Gly914Arg)[38] in two patients, c.1906C>G; p.(Leu636Val), c.2670 C>G; p.(His890Gln) in three patients, c.3437G>A; p.(Arg1146His) in two patients; c.3122G>A; p.(Arg1041Gln) [38], c.2575G>A; p.(Val859Met), c.2777T>C; p.(Ile926Thr) in two patients, and c.3460G>A; p.(Gly1154Arg).

GATA factors have a fundamental role in adipogenesis. GATA2 is expressed in white and brown adipose tissue and GATA3 is expressed only in white adipose tissue. Both GATA-2 and GATA-3 expression are under-regulated in the white adipose tissues of obese mice. GATA-2 and GATA-3 are specifically expressed in murine preadipocytes. The inhibitory effect of GATA on adipogenesis is mediated primarily by the suppression of adipogenic factor promoters[39].

Various *GATA2* variants associated with lipid metabolism, obesity and lymphedema were identified[40]. In our study, we detected the *GATA2* variant c.414_417del; p.Ser139Cysfs*78 in one patient.

The genes associated with lymphedema that could be likely associated with obesity from studies on mouse and human models are shown in Figure 2. Furthermore, there are evidence in literature that associates lymphedema-related genes with obesity, such as *PIEZO1* that is highly expressed in adipose tissue and regulates diet-induced adipose inflammation and systemic insulin resistance[41], *CELSR1* associated with fatty acid metabolism[42], and *HGF*, which has been shown to play a key role in in insulin resistance[43]. Therefore, the genes responsible for primary lymphedema can also predispose to obesity.

Conclusions

Obesity is an important risk factor for the development of lymphedema[5-7]. An obesity rate of 20%, higher than the percentage of 10% in the general population reported by ISTAT, was detected in our cohort[44]. A diagnostic approach by a NGS custom-made panel can allow for the molecular diagnosis of lymphedema and elucidate their pos-

sible association with obesity. In fact, our analysis strengthens the thesis that there is a link between lymphedema and obesity. This is the first clinical and genetic study in humans that demonstrates that the genes responsible for primary lymphedema may also predispose to obesity due to the accumulation of adipose tissue caused by disturbances in lymphatic circulation. Physical activity has anti-inflammatory effects and genetic markers that can predict the exercise efficacy in obese and lymphedema patients would be essential to find which individuals would have benefits from the exercise[45]. In the population analysed in this study, there is a trend toward the worsening of the quality of life in patients who do not perform physical activity. However, in our analysis the accumulation of adipose tissue responds poorly to physical activity and diet must exclude saturated fatty acids and must be supplement with hydroxytyrosol. In fact, the use of hydroxytyrosol in olive oil may help in the treatment of the negative effects of lymph accumulation as we previously reviewed[19-22] and, in this study, we observed a significative enhancing of quality life in association with hydroxytyrosol daily intake.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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