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Genetics of lipedema: new perspectives on genetic research and molecular diagnoses

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Abstract. – OBJECTIVE: The aim of this qualitative review is to provide an update on the current understanding of the genetic determinants of lipedema and to develop a genetic test to differentiate lipedema from other diagnoses.

MATERIALS AND METHODS: An electronic search was conducted in MEDLINE, PubMed, and Scopus for articles published in English up to March 2019. Lipedema and similar disorders included in the differential diagnosis of lipedema were searched in the clinical synopsis section of OMIM, in GeneCards, Orphanet, and MalaCards.

RESULTS: The search identified several genetic factors related to the onset of lipedema and highlighted the utility of developing genetic diagnostic testing to help differentiate lipedema from other diagnoses.

CONCLUSIONS: No genetic tests or guidelines for molecular diagnosis of lipedema are currently available, despite the fact that genetic testing is fundamental for the differential diagnosis of lipedema against Mendelian genetic obesity, primary lymphedema, and lipodystrophies.

Key Words:
Lipedema, Lipoedema, Differential diagnosis, Subcutaneous fat, Genetic diagnosis.

Introduction

Lipedema is an underdiagnosed chronic debilitating disease characterized by bruising and pain and excess of subcutaneous adipose tissue of the legs and/or arms in women during or after times of hormone change, especially in puberty[1]. A definition of lipedema has not yet been included in the 10th revision of the International Classification of Diseases (ICD) by the World Health Organization (WHO) although an ICD-11 code of EF02.2 has been proposed. The first guidelines on lipedema were proposed in 2015 in Germany and by others in 2017 using the international classification of functioning, disability and health[2-4]. Lipedema coexists frequently with obesity and can be easily confused with it. However, lipedema is distinguishable from obesity because it is located primarily in the lower limbs and upper extremities sparing the trunk; lipedema minimally responds to diets. Finally, women with lipedema can have a normal Body Mass Index (BMI)[5]. Lipedema can be confused with lymphedema. There are substantial differences between these two diseases:

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while lipedema is always bilateral, lymphedema can be unilateral or bilateral; distinctive features of lipedema are pain and bruising that are absent in lymphedema; and in lipedema, the Stemmer sign is negative[6]. Furthermore, lipedema may be associated with multiple lipomas on the arms and/or trunk which are not found in lymphedema[5,6]. The prevalence of lipedema has been reported to be 1-9/100000[7]. However, it is notable that epidemiological data can fail to recognize lipedema or include individuals who are misdiagnosed with other similar diseases, most commonly obesity, lymphedema, and lipodystrophies. Lipedema can be considered as one component of a spectrum of diseases that are characterized by the dysregulated proliferation of adipose tissue and the presence of pain. The *adiposis dolorosa* (painful fat) spectrum of diseases includes: 1) "Generalized diffuse form of Dercum disease" where painful pearl-sized nodular subcutaneous adipose tissue is widespread throughout the body without larger masses; 2) "generalized nodular form of Dercum disease" where larger painful nodules are found on the arms, trunk, and thighs; 3) "lipedema", a localized form of painful fat with characteristic distribution of pearl-sized nodular fat and larger masses on the limbs; 4) "localized nodular form of Dercum disease", usually found around joints (juxta-articular type); and 5) "Madelung disease" or multiple symmetric lipomatosis with nodular fat and lipomas on the upper aspect of the body although a "gynoid type" has been identified[8,9] (Figure 1). With a variety of diseases of painful fat, diagnosing painful lipedema may be difficult; it would be useful to develop a genetic test that analyses all the genes known for Mendelian non-syndromic genetic obesity, primary lymphedema, and lipodystrophies when trying to diagnose a patient with lipedema, due to the clinical findings these diseases hold in common[10]. This review aims to outline current knowledge about the genetics of lipedema and highlights the importance of genetic testing for a more precise diagnosis of lipedema to separate it from similar appearing conditions.

Materials and Methods

This is a "qualitative review" in which, information published about lipedema in referenced scientific journals until March 2019 were collected. An electronic search was conducted in MEDLINE, PubMed and Scopus, using different combinations of the search terms and concepts "lipedema", "lipedema genes"," lipedema etiology", "lipedema differential diagnosis", "lipedema and obesity", "lipedema and lymphedema", "lipedema and lipodystropy", "clinical manifestations of lipedema" and "lipedema genetic testing". Articles in English were first chosen by examining the title and abstract and, subsequently, analyzed by two independent readings of the whole text. Reference lists were scanned to retrieve other relevant articles. The reviewers assessed the full-text articles. A search for lipedema and similar disorders that overlap in the differential diagnoses was conducted in the clinical synopsis section of OMIM, GeneCards, Orphanet and MalaCards. Orphanet GeneReviews, and Genetic Home Reference were searched to find existing genetic diagnostic tests for lipedema. A general PubMed research was used to investigate clinical, diagnostic and genetic testing guidelines regarding lipedema. Any disagreements were solved through discussion until consensus.

Results

Clinical Aspects of Lipedema

Lipedema is a chronic condition of deposits of painful fat, primarily in the gynoid region, occurring at times of hormone change in women, especially at the time of puberty, that causes gait and joint abnormalities, and which eventually can result in the development of lymphedema, called lipo-lymphedema. Clinically, lipedema has several features: abnormal and painful adipose tissue on the legs, thighs, buttocks, and on the arms in 80%, of cases with a BMI that can be normal or increased without abdominal obesity[11]; chronic pain that can significantly impact mobility; joint hypermobility associated with fatigue[1,12,13]; bruising due to increased capillary fragility[14]; edema in advanced stages or longstanding lipedema[15] and psychosocial stress and associated psychiatric disorders, especially anxiety and depression[16]. The main classifications of lipedema are Schingale classification (Types I, II, III, IV, and V) based on the different distribution of adipose tissue[2] and Schmeller and Meier-Vollrath classification (Stages I, II, III, and IV) depending on the severity of the disease[12]. Conservative treatments of lipedema include: manual lymphatic drainage as part of complex decongestive therapy, subcutaneous adipose tissue therapy to reduce fibrosis and increase

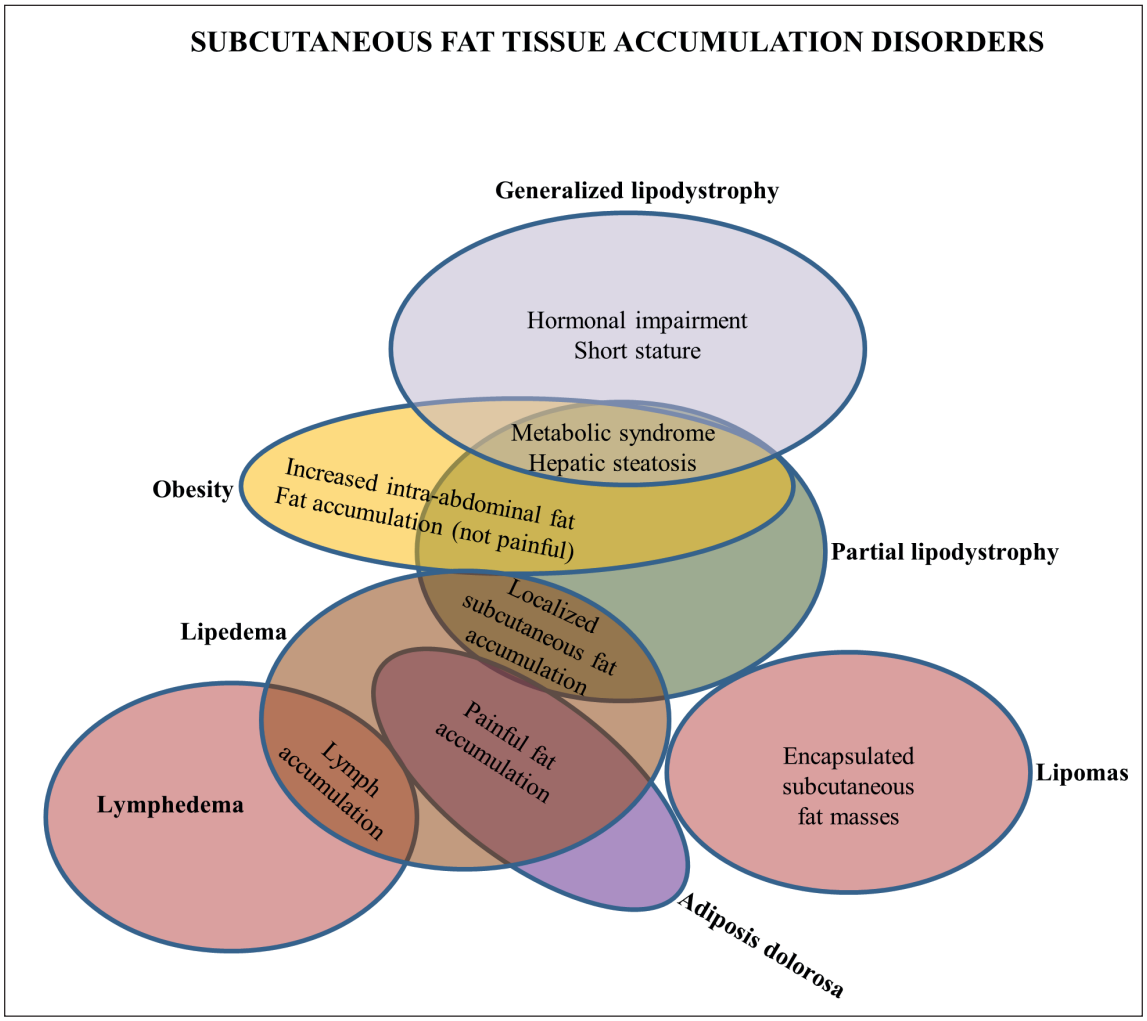

Figure 1. Depiction of phenotypic overlap of different conditions characterized by subcutaneous fat accumulation.

fluid flow through the tissue, compression garment therapy or wrapping to reduce tissue fluid, physical activity and healthy food plans to reduce any obesity component of lipedema, medical foods and supplements (such as selenium, Butcher's broom or diosmin), deep breathing and psychosocial counseling. For patients with minimal or no improvement with conservative treatment, surgical treatments including liposuction and reductive surgery are needed[2]. Lipedema is thought to be either primary (idiopathic, probably genetic) or secondary, associated with hormonal disease, connective tissue disorders and/or autoimmune disease[17,18]. The long-term effects of lipedema on lifespan are not known[12].

Etiopathogenesis of Lipedema

Lipedema is a chronic disease of lipid metabolism resulting in the symmetrical deposition of subcutaneous adipose tissue associated with hyperplasia and/or hypertrophy of fat cells[19]. Etiopathogenesis is unclear but may be associated with impairment of a hormonal axis, since lipedema mainly affects females and its onset is primarily around the time of puberty[20]. Estrogen has a direct effect on adipose tissue through estrogen receptors (ERs)[21]. Rarely, men are affected with lipedema. Men who develop lipedema tend to be hypogonadal or have liver disease resulting in relatively elevated estrogen levels[22]. Estrogen may,

therefore, play a role in the development of fat and other tissues that have sex hormone receptors[23]. Almost certainly an altered ER pattern or responsiveness exists centrally, not only in the adipose tissue, which may provide an additional explanation for difficulties in gynoid and arm fat loss in patients suffering from lipedema[10,20]. Additional mechanisms thought to play a role in the pathogenesis of lipedema include increased vascular permeability and damage (microangiopathy), excessive lipid peroxidation, and disturbances in adipocyte metabolism and cytokine production[20,21]. In a recent study, cells of the stromal vascular fraction (SVF), which includes adipose-derived stromal stem and other cells from 30 patients with lipedema and 22 controls, were characterized. Of note, the yield of stem cells was higher from lipedema fat, which might be explained by an enhanced expression of the mesenchymal (connective tissue) marker CD90 and the endothelial/pericytic marker CD146 suggestive of growth. These markers specifically suggest there may be a chronic capillary injury in lipedema leading to an increased need for repair and protection. Despite these findings, the SVF is thought to be a suitable option for autologous tissue regeneration strategies in lipedema patients[22]. In summary, lipedema is a heterogeneous likely genetic disease, which manifests in parallel with female hormonal changes and leads to vasculo- and lymphangiopathy. Inflammation of the peripheral nerves and sympathetic innervation abnormalities of the subcutaneous adipose tissue may be responsible for neuropathy with adipocyte hyperproliferation as a secondary phenomenon[20].

Genetics of Lipedema

Primary Lipedema

Lipedema is thought to be an inherited disease in most cases. Self-reported positive family history of lipedema is found for up to 64% of women, therefore, a genetic etiology for lipedema is strongly suggested[10]. A possible link between the genetic factors and the hormonal role of estrogens in the development of lipedema has been hypothesised[24]. Autosomal dominant inheritance with incomplete penetrance and sex limitation (most affected female family members) is the most likely mode of inheritance[12,25]. Lipedema may be differentiated into non-syndromic, syndromic and/or associated with comorbidities. To date, the gene associated with primary isolated lipedema has not been identified. For the diagnosis and research of the genetic basis of lipedema, it is necessary a multidisciplinary approach that evaluates the genome, the transcriptome, and the methylome.

Syndromic Lipedema

Lipedema may be present within a syndromic context. These diseases may be good models to determine the molecular pathways potentially involved in isolated non-syndromic lipedema.

Example 1: One report associated a germline missense variant, c.196C>T (p.Pro24Leu), in *POU1F1A* in a family with recurrence of short stature and swelling of legs which only affected females in four generations of the family. The only male found affected did not have a lipedema phenotype, only short stature. Affected family members also had growth hormone deficiency, secondary hypothyroidism and hypoprolactinemia[26]. *POU1F1A* encodes Pit-1, a transcription factor specifically expressed in the anterior pituitary gland, that regulates the expression of the growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone (TSH) beta-subunit genes[27,28].

A pathogenic mutual translocation 46; XX; t(10;15) (q25.2;q11.2) (ClinVar ID: SCV000320871.1) is associated with various conditions, including lipedema[29]. The 10q25.2 and 15q11.2 bands include several genes that are important for fat metabolism. For instance, changes in the chromosomal region 15q11.2, with loss of gene activity are associated with specific syndromes, such as Prader-Willi syndrome (PWS), characterized by pathologic hyperphagia and obesity[30]. The genes that map in this region may be good candidates for the onset of lipedema. This is also supported by the observation of one of the authors, that report that lipedema is present in 5% of PWS patients (personal observation).

Example 2: Sotos syndrome 1 is caused by germline mutations in *NSD1*. A novel missense mutation, (p.Cys2175Ser) has been reported in a patient with a familial form of Sotos syndrome characterized by normal intelligence, insulin-dependent diabetes, bronchial asthma, and lipedema. *NSD1* encodes a protein that, together with associated co-regulators, enhances androgen receptor transactivation, therefore it may be partly responsible for the estrogen-mediated lipedema tissue growth[31].

Example 3: Williams-Beuren syndrome is a multi-systemic neurodevelopmental and metabolic disorder. In some cases, lipedema can be present[17,32]. This syndrome is caused by deletion of a stretch of 27 genes in the chromosomal region 7q11.23[33] and the best candidates to explain the

involvement of subcutaneous fat tissue are *ELN*[34], *FZD9*[35], *MLXIPL*[36].

Example 4: Pseudoxanthoma elasticum (PXE) is another syndrome in which lipedema can be present. PXE mainly affects skin, eyes, and the cardiovascular and gastrointestinal systems and patients show a progressive mineralization of elastic fibers. Abnormal elastic fibers similar to those found in PXE have been observed in skin lesions of patients with lipedema; furthermore, PXE fibroblasts show an increased cholesterol biosynthesis which could be important in the development of lipedema fat tissue[37]. PXE can be either autosomal dominant or recessive and is due to germline mutations in *ABCC6* and a subclinical form of PXE has been reported as predisposing to lipedema[18,38]. Although, to date, no mutations have been reported in *ABCC6*, in association with lipedema.

Example 5: Cutis laxa type III is a multisystem connective tissue disorder characterized by wrinkled, inelastic skin, associated with a neurologic component, retinopathy and the presence of abnormal fat pads on the buttocks and upper thighs. Cutis laxa type III can be either autosomal recessive or dominant and is caused by germline mutations in *ALDH18A1*[39], a gene that encodes an enzyme that catalyzes the reduction of glutamate to delta1-pyrroline-5-carboxylate, a critical step in the *de novo* biosynthesis of proline, ornithine, and arginine.

One study reports that hypermobility may be associated with lipedema; in this report, about 50% of women with lipedema had joint hypermobility suggesting that they may also have the common disorder known as hypermobile Ehlers Danlos syndrome (hEDS) formerly hypermobility type or type III[13,21]. Germline truncating mutations in *TNXB* and a single germline variant in *COL3A1*, (p.Gly637Ser) have been found to cause the Ehlers-Danlos syndrome type III, to date, no mutations have been found in those two genes in association with lipedema[40,41].

Clinically Overlapping Conditions with Lipedema

In thinking about a possible genetic test for lipedema, it is important to consider the genes mutated in conditions that may be phenotypically overlapping or confused with lipedema for differential diagnosis, such as lipomas or familial multiple lipomatosis.

For instance, disruption of *HMGA2*, located in the chromosomal band 12q14.3, has frequently been detected in human benign tumors of mesenchymal origin, including lipomas[42,43].

A rare case of autosomal dominant familial multiple subcutaneous lipomatosis (FML) and increased predilection to cancers co-segregating with a novel germline truncating mutation in *PALB2* (c.2716delT) has been reported[44].

Two patients with encephalocraniocutaneous lipomatosis, a congenital hamartomatous disorder characterized by unilateral skin lesions, lipomas, and ipsilateral ophthamological and cerebral malformations, have been found to carry a *KRAS* somatic mutation c.436G>A (p.Ala146Thr) and a *NF1 de novo* germline truncating mutation, respectively[44-47].

PIK3CA somatic activating mutations, previously reported in cancer and overgrowth syndromes, have been discovered in the affected tissue of individuals with facial infiltrating lipomatosis[48]. The syndrome of multiple symmetric lipomatosis, partial lipodystrophy and insulin resistance is caused by germline mutations in *LIPE*. These mutations result in marked inhibition of lipolysis from adipose tissue depots[49] which could explain fat expansion in lipedema.

The Pierpont syndrome, a plantar lipomatosis with limb abnormalities, unusual facies and developmental delay is caused by a *de novo* heterozygous missense mutation (p.Tyr446Cys) in *TBL1XR1*[50]. In two cases, two different mutations have been reported p.Cys325Tyr and p.Tyr446His[51].

Hereditary retinoblastoma is caused by variants in *RB1* (40% of mutations are germline, inherited or *de novo*; 60% of mutations are somatic). Retinoblastoma, an embryonic malignant neoplasm of retinal origin, is found in association with lipomatosis in less than 4% of the affected subjects[52]. Lipomatosis predisposition in hereditary retinoblastoma is not associated with specific *RB1* variants but is probably determined by modifying factors[53]. Interestingly, the retinoblastoma interacting zinc finger gene *RIZ* codes for a protein that is a downstream effector of estrogen action and is involved in an alternative intracellular pathway, mediating the mitogenic effect of estradiol[54,55], a finding that may be pertinent to lipedema.

In support of the hypothesis that multiple symmetric lipomatosis is caused by mitochondrial mutations[10,56], there are several studies. A study reported that 32.4% (11/34) of cases affected by myoclonic epilepsy with ragged-red fibers (MERRF) show the presence of multiple lipomatosis.

Of them, only 6 had isolated lipomatosis with no myopathic complaints and/or CNS involvement. All of these patients carried the m.8344A>G mutation in the MTTK, transcripted in the tRNA$^{Lys57-59}$. In a multigenerational Canadian family with 8 affected members, the complete co-segregation of the rare mitochondrial variant in *MTTK* (8344A>G) and the phenotype was reported[60].

Multiple symmetric lipomatosis (Madelung disease), a rare disorder of adipocyte differentiation characterized by benign, diffuse lipomatosis in the cephalic, cervical, and upper thoracic subcutaneous depots, can carry biallelic mutations in *MFN2,* a gene encoding a mitochondrial protein already associated with Charcot-Marie-Tooth syndrome[45,61].

Familial Partial Lipodystrophies and Lipedema

It is important to point out that in some cases of partial lipodystrophy there may be an accumulation of subcutaneous fat tissue in specific depots coupled with the reduction of fat in other regions. The majority of familial partial lipodystrophy (FPLD) syndromes are autosomal dominant. Phenotypically, patients with FPLD, type 2 and some of the other partial lipodystrophies lack limb and gluteal subcutaneous fat, while in FPLD, type 1, the abdomen shows weight gain and the limbs and buttocks lose fat[62]. In most cases, body fat redistribution occurs during puberty. Adipose tissue in the face, neck and intra-abdominal areas may be increased in FPLD, type 2. Diabetes and metabolic complications, such as coronary artery disease, occur in adulthood in these disorders[63].

FPLD, type 2 is caused by autosomal dominant mutations in *LMNA*, the most common being p.Arg482Gln; this gene encodes the nuclear lamin A/C protein. Fat loss typically occurs in the buttocks and limbs, and gain of fat occurs to the face, neck, abdominal viscera, and labia, although there is phenotypic variability. Patients may also have lipomas[63]. While lipedema may be misdiagnosed as lipodystrophy, the metabolic differences between lipedema, which confers low cardiovascular risk, and FPLD, type 2, which confers high cardiovascular risk, are clear.

FPLD, type 3 is caused by heterozygous missense mutations in *PPARG*. Fat loss usually occurs with loss of fat from the trunk, buttocks, and limbs and there is no increase in head and neck fat. However, there may be a gain of fat on the abdomen[63,64]. The phenotype of women with FPLD3 is different from that of women with lipedema, with upper body obesity compared to gynoid fat deposits, respectively.

FPLD4 is caused by heterozygous truncating mutations in *PLIN1*, encoding perilipin-1. This protein covers lipid storage droplets in adipocytes, protecting them until they can be broken down by hormone-sensitive lipases. Perilipin-1 is the major cAMP-dependent protein kinase substrate in adipocytes and may play a role in the inhibition of lipolysis[63]. The phenotype of women with FPLD4 is different from that of women with lipedema, with upper body obesity compared to gynoid fat deposits, respectively.

FPLD5 has been reported in a single case caused by a homozygous truncating mutation in *CIDEC* (p.Glu186*). Perilipin-1 and CIDEC are involved in the structure and function of adipocyte lipid droplets[63].

FPLD6 is caused by homozygous truncating mutations in *LIPE*, encoding hormone-sensitive lipase, which is involved in the regulation of lipolysis[63].

Akt2-linked lipodystrophy was described in a single family and is caused by heterozygous missense mutations in *AKT2*, which is a key mediator of insulin signaling downstream of the insulin receptor[65]. The phenotype of women with FPLD6 is different from that of women with lipedema, with upper body obesity compared to gynoid fat deposits, respectively.

Familial partial lipodystrophies share common phenotypes. The typical loss of fat occurs to the buttocks and limbs and a gain of fat to the face, neck, and abdomen. Metabolic complications of the familial partial lipodystrophies include insulin resistance, diabetes mellitus, hypertriglyceridemia, hepatic steatosis, hypertension, and coronary artery disease, none of which correlates with the phenotype of lipedema. However, genes important in partial lipodystrophy may be important in modifying the phenotype of lipedema.

In an African-American pedigree with a novel autosomal dominant atypical FPLD, Garg et al[66] performed linkage analysis for candidate regions and whole-exome sequencing to identify the disease-causing mutation. The affected individuals had marked loss of the fat from the extremities, with excess fat in the face and neck, and metabolic complications. The authors identified a mutation (c.202C>T, p.Leu68Phe) in the adrenoceptor α 2A (ADRA2A) in all affected subjects but not in the unaffected. ADRA2A regulates norepinephrine release and its activation reduces lipolysis in adipocytes. The mutation p.Leu68Phe presumably

causes a rare atypical FPLD by inducing excessive lipolysis in certain adipose tissue depots[66].

Candidate Genes from Genome-Wide Association and Animal Models Studies

Several types of studies have been conducted to identify candidate genes associated with lipedema: genome-wide association studies (GWAS) and studies on animal models.

LYPLAL1, TBX15, HOXC13, RSPO3, CPEB4, VEGFA, STAB1, GRB14, ZNRF3, and *PIGC* have been associated with subcutaneous adipose depots by genome-wide association studies and confirmed by functional analyses. The association of these genes with body fat distribution is independent of any effects of BMI and/or obesity, demonstrating genetic control of body fat distribution, distinct from that of overall adiposity[67,68].

ATXN1 and *UBE2E2* play a physiological role in adipogenesis. In support of these genes as candidate genes for lipedema, seven *loci* associated with ectopic fat traits have been identified in a multi-ethnic, sample-size-weighted, fixed-effects genome-wide association meta-analysis study at the genomic level: *ATXN1, UBE2E2, EBF1, RREB1, GSDMB, GRAMD3*, *ENSA*. Functional analyses of *ATXN1* and *UBE2E* showed that loss of *Atxn1* or *Ube2e2* in primary mouse adipose progenitor cells hindered differentiation of adipocytes[69].

VEGFR3 signaling is fundamental for the development and maintenance of the lymphatic vascular system[70]. *VEGFR3* is associated with the development of lymphedema in humans[71]. Mice with *VEGFR-3* gene heterozygous inactivating missense mutations have hypoplastic dermal lymphatics and thickening of the subcutaneous adipose tissue. Vascular endothelial growth factors *VEGFC* and *VEGFD* stimulate lymphangiogenesis, whereas *VEGFR3* is a potent inhibitor of *VEGFC/VEGFD* signaling. In the skin of transgenic mice, VEGFR3 inhibits fetal lymphangiogenesis and induces regression of already formed lymphatic vessels, though the blood vasculature remains normal. The transgenic K14-VEGFR3–Ig mice show, besides other features, increased deposition of subcutaneous fat[70].

The ablation of *PRDM16* in mice adipocytes has significant reductions in thermogenic gene expression and O_2 consumption of white adipose tissue, both in the basal state and following stimulation with cold and β3-adrenergic agonist treatment resulting in marked enlargement of the subcutaneous adipose tissue. These animals developed obesity on a high-fat diet, with severe insulin resistance and hepatic steatosis, which is, however, not seen in lipedema in the absence of obesity. However, they also showed altered fat distribution[72].

Fat-specific *Sirt6* knockout mice are sensitized to high-fat diet–induced obesity resulting in adipocyte hypertrophy caused by compromised lipolytic activity due to the decreased expression of the lipolytic enzyme, adipose tissue triglyceride lipase (ATGL). Fat-specific *Sirt6* KO also showed increased inflammation in adipose tissue, which contributes to insulin resistance in high-fat diet–fed mice. These findings are consistent with the reduced expression of *SIRT6* and *ATGL* observed in obese patients[73]. Recently, in humans, it has been observed that, in the subcutaneous adipose tissue, increased body mass index modifies the expression of the genes encoding sirtuins and their target genes, which are metabolic regulators of adipose tissue[74]. Interestingly, SIRT6 is widely expressed in retinoblastomas, a tumor that in 4% of cases shows the presence of lipomatosis.

FGF21 improves systemic insulin sensitivity by promoting the healthy expansion of subcutaneous adipose tissue. *FGF21* knockout mice show less subcutaneous adipose tissue and are more insulin-resistant when fed with a high-fat diet. Moreover, serum *FGF21* levels positively correlate with the subcutaneous adipose tissue area in insulin-sensitive obese individuals[75].

IL18 receptor 1- knockout mice have an increased susceptibility to dietary-induced obesity. It was observed by Pazos et al[76] that *Il18* knockout mice were extremely dietary obesity-prone and did not develop diet-induced thermogenesis as assessed by brown adipose tissue and white adipose tissue Ucp1 mRNA levels. Interestingly, women with lipedema have reported cold areas in fat tissue.

PANX1 encodes a glycoprotein, pannexin 1, which plays a key role in regulating the formation of adipocytes and fat accumulation. Increased adipocyte size and decreased adipocyte numbers were observed in subcutaneous fat of the *Panx1* knockout mice compared to wild-type mice. Knockout mice lacking *Panx1* gene have significantly greater total fat mass and reduced lean mass under a normal diet. By metabolic cage data, it was observed that *Panx1* knockout mice on a high-fat diet display significantly increased activity levels, higher ambulatory activity, and reduced sleep duration with respect to wild type mice. Absence of the Panx1 protein also leads to increased insulin and blood glucose levels, which increase the risk of type 2 diabetes[77].

SCD1 encodes an enzyme that catalyzes the synthesis of monounsaturated fatty acids and is an important regulator of whole-body energy homeostasis. Knockout mice lacking *Scd1* gene display marked sebaceous gland hypoplasia, depletion of sebaceous lipids, increased energy expenditure and protection from high-fat diet-induced obesity, and severe cold intolerance. mRNA levels of *SCD1* in subcutaneous adipose tissue correlate with BMI in morbidly obese subjects[78]. *SCD1* mRNA levels have not been tested in lipedema but loss of SCD1 activity was inferred due to the lower levels of unsaturated fatty acids from subcutaneous adipose tissue of people with fat disorders[79].

PROX1 is a gene correlated to leaky lymphatic vessels as observed by studying mice with functional inactivation of a single allele of this homeobox gene. *PROX1* is fundamental for lymphatic vascular development and *Prox1* knockout mice are obese. *Prox1* haploinsufficiency results in subcutaneous and intra-abdominal fat accumulation in mice[80], a potential subclinical cause for the accumulation of lipedema fat tissue.

ZPF423 encodes a multi-zinc-finger transcription factor expressed in preadipocytes and mature adipocytes *in vivo* and is responsible for the terminal differentiation of subcutaneous white adipose tissue. Knocking out *Zfp423* in mice results in white-to-beige phenotypic adipocytes switch. When *Zfp423* is removed from precursors and/or actively differentiating cells, there is a defect in the terminal differentiation of white adipocytes. The loss of lipid in the adipocytes in the absence of *Zfp423* is due to lower expression of *PPARγ* and other adipocyte-selective genes. Therefore, *ZFP423* has a fundamental role in the establishment and maintenance of the white adipose lineage[81] and could be speculated to play a role in maintenance of lipedema fat.

Lipedema Diagnosis and Genetic Test

The diagnosis of lipedema is mainly based on the medical history and physical examination of the patient[2]. Multiple imaging techniques such as ultrasound, magnetic resonance imaging (MRI) that allows identification of subclinical nodules, computed tomography, lymphoangioscintigraphy, indirect lymphangiography, and dual energy X-ray absorptiometry have all been applied in the diagnosis and study of lipedema. Fluorescence micrography makes it possible to detect multiple microaneurysms in lymphatic capillaries in the skin regions affected by lipedema not detectable in other pathologies, such as scleroderma and venous insufficiency[82]. In some cases, the MRI analysis of excised lipomatosis nodules revealed that they were angiolipomas identifiable with MRI[83]. In another work, using MRI in women with lipedema, higher levels of sodium were found in the fat tissue with even early stage lipedema compared to controls[84] suggesting a strong role for lymphatic dysfunction in lipedema[85].

The Streeten test may be considered as a functional test to differentiate lipedema from lymphedema[2]. General blood tests are important because patients with lipedema may have diseases that are responsible for the worsening of their symptoms[2].

Characterization of family history and clinical phenotype will be fundamental for genetic testing in lipedema, as a causal mutation is thought to be important in this disease[25]. Currently, genetic tests and guidelines for the molecular diagnosis of lipedema are not available. However, genetic testing to exclude overlapping conditions or syndromic lipedema would be of fundamental importance. Lipedema is often misdiagnosed because of its similarity with primary lymphedema, Mendelian non-syndromic obesity, and generalized and partial lipodystrophies. We propose the use of a genetic test based on the next generation sequencing of a panel of genes that could be involved in syndromic lipedema and are involved in phenotypically overlapping disorders (obesity, lymphedema, and lipodystrophies) with similar adiposity to exclude these pathologies. After the exclusion of the presence of known pathogenic mutations in both blood (germline mutations) and affected tissue (somatic mutations), whole exome/genome sequencing would be required to identify the still-unknown gene (2). In Figure 2, a possible and ideal diagnostic genetic test for lipedema is schematized.

Conclusions

Lipedema is a very disabling disease that affects the quality of life, but unfortunately, it may be easily misdiagnosed[1]. It belongs to a spectrum of the localized painful depots of subcutaneous adipose tissue (*adiposis dolorosa*) that includes a generalized/localized/diffuse/nodular form of Dercum disease, Madelung disease (multiple symmetric lipomatosis), partial lipodystrophy, familial multiple lipomatosis, and lymphedema. A timely and accurate diagnosis of lipedema is essential for early intervention and treatment of the disease to prevent the development

of co-morbidities. For this purpose, it is important to develop a genetic test for lipedema based on increasing knowledge of causative genes for other disorders and rapid advances in genetic screening technologies. Lipedema is an inherited disease expressed mainly in females, with males serving as carriers[27,86]. Lipedema is a relatively poorly studied disease, in fact, the mechanisms concerning the etiopathogenesis and the genetics of lipedema are not yet fully known and there are no current reliable biomarkers or imaging modalities. Currently, the gene/s associated with primary lipedema are not known, whereas some genes associated with syndromic lipedema are known. Genome-wide association and animal models studies have made it possible to identify possible candidate genes and genetic pathways involved in the onset of lipedema. The advent of new molecular technologies will improve research and diagnostic

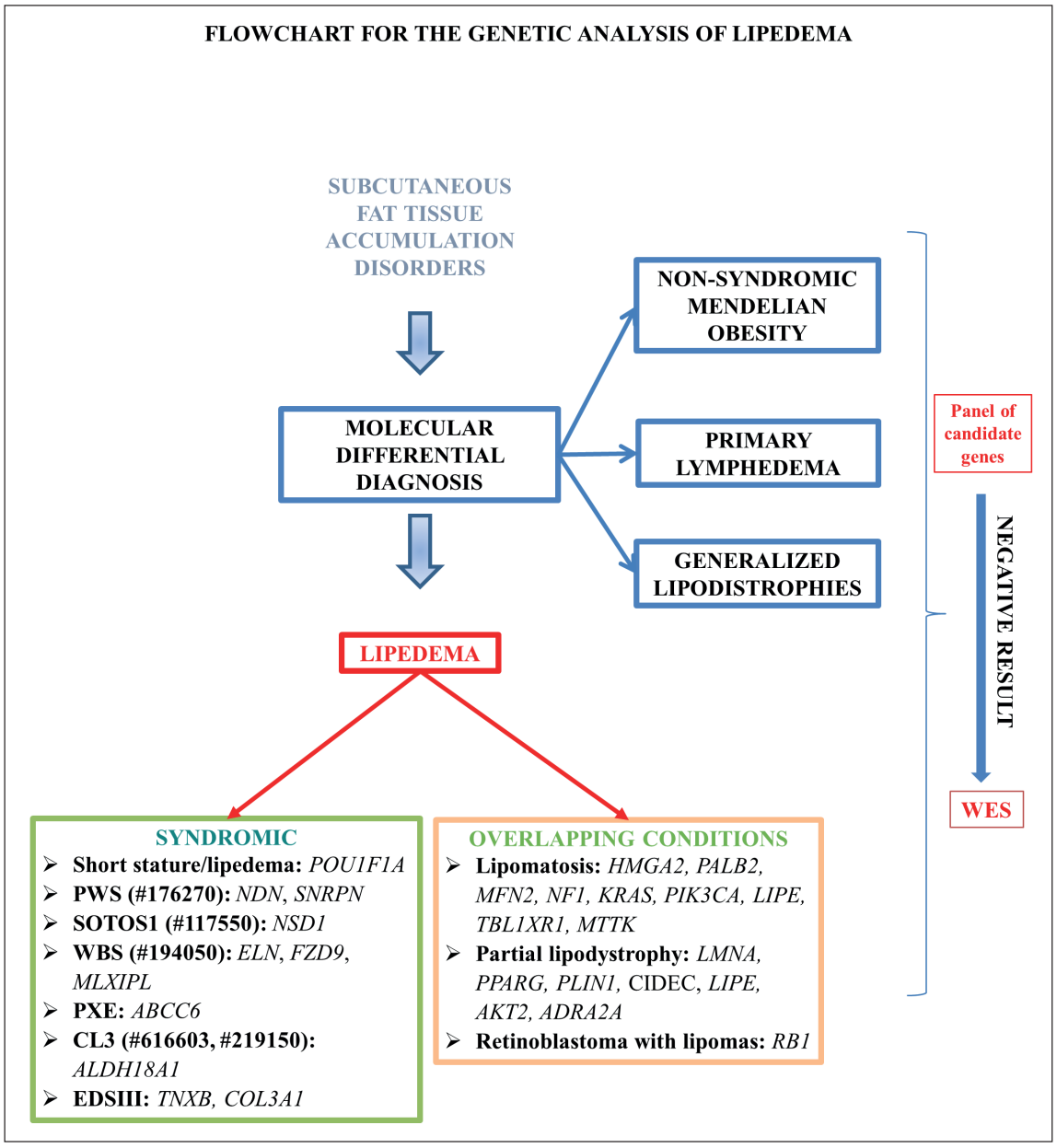

Figure 2. Scheme of a possible and ideal diagnostic genetic test for genetic subcutaneous fat accumulation.

process, ensuring the possibility of more effective prevention and treatment with clear improvements in the management of lipedema patients. The development of a genetic test containing candidate genes for lipedema and causative genes of diseases that can be confused with lipedema will be fundamental to find the etiology of lipedema and to provide patients with more targeted care and follow-up. By analysis of the whole exome with NGS technology in the patient and their parents could allow identification of new candidate genes for lipedema[87] but the phenotype must be clearly defined. A multidisciplinary approach and with different technologies would be needed. Understanding the molecular mechanisms underlying the onset of lipedema requires a multi-pronged approach based on, for instance, genome, transcriptome and methylome studies. Hopefully, the use of NGS technologies will help clinicians classify people that meet the criteria for the *adiposis dolorosa* spectrum.

Conflict of Interests

The Authors declare that there are no conflicts of interest.

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