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LIPE-related lipodystrophic syndrome: clinical features and disease modelling using adipose stem cells

Short title: Lipodystrophy due to HSL defects

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Word count: 3611

Keywords: hormone sensitive lipase, Multiple Symmetric Lipomatosis, lipodystrophy, adipose stem cells, adipocyte differentiation

ABSTRACT

Objective

The term Multiple Symmetric Lipomatosis (MSL) describes a heterogeneous group of rare monogenic disorders and multifactorial conditions, characterized by upper-body adipose masses. Biallelic variants in *LIPE* encoding hormone sensitive lipase (HSL), a key lipolytic enzyme, were implicated in three families worldwide. We aimed to further delineate *LIPE*-related clinical features and pathophysiological determinants.

Methods

A gene panel was used to identify pathogenic variants. The disease features were reviewed at the French lipodystrophy reference center. The immunohistological, ultrastructural, and protein expression characteristics of lipomatous tissue were determined in surgical samples from one patient. The functional impact of variants was investigated by developing a model of adipose stem cells (ASCs) isolated from lipomatous tissue.

Results

We identified new biallelic *LIPE* null variants in three unrelated patients referred for MSL and/or partial lipodystrophy. The hallmarks of the disease, appearing in adulthood, included lower-limb lipoatrophy, upper-body and abdominal pseudolipomatous masses, diabetes and/or insulin resistance, hypertriglyceridemia, liver steatosis, high blood pressure, and neuromuscular manifestations. Ophthalmological investigations revealed numerous auto-fluorescent drusen-like retinal deposits in all patients. Lipomatous tissue and patient ASCs showed loss of HSL and decreased expression of adipogenic and mature adipocyte markers. *LIPE*-mutated ASCs displayed impaired adipocyte differentiation, decreased insulin response, defective lipolysis, and mitochondrial dysfunction.

Conclusions

Biallelic *LIPE* null variants result in a multisystemic disease requiring multidisciplinary care. Loss of HSL expression impairs adipocyte differentiation, consistent with the lipodystrophy/MSL phenotype and associated metabolic complications. Detailed ophthalmological examination could reveal retinal damage, further pointing to the nervous tissue as an important disease target.

SIGNIFICANCE STATEMENT

Multiple Symmetric Lipomatosis (MSL), characterized by upper-body pseudo-lipomatous masses, can be associated with alcoholism or due to rare monogenic disorders. Its pathophysiology remains elusive. We identified in three unrelated patients new biallelic variants in *LIPE,* encoding the key lipolytic enzyme hormone sensitive lipase (HSL), and defined the disease clinical features. MSL co-existed with peripheral lipoatrophy, diabetes and/or insulin resistance, hypertriglyceridemia, liver steatosis, hypertension, neuromuscular signs and previously undescribed drusen-like retinal deposits. We isolated adipose stem cells from pseudo-lipomas. HSL expression was abolished, leading to major defects in adipocyte differentiation, insulin response, lipolysis, and mitochondrial function.

Loss of HSL leads to a multisystemic disease with lipodystrophic syndrome and neurological alterations. Cellular modeling reveals the major role of HSL for adipocyte differentiation.

INTRODUCTION

Multiple Symmetric Lipomatosis (MSL), also known as Launois-Bensaude Lipomatosis or Madelung disease is a generic term used to describe several rare disorders. MSL is characterized by the progressive development of voluminous masses of adipose tissue, mostly localized in the neck and upper body, and frequently associated with metabolic complications and neurological manifestations. MSL was first described and most frequently encountered in alcoholic patients (1). A few genetic forms have also been characterized. MSL could affect ~20% of patients with the myoclonic epilepsy and ragged red fibers (MERRF) syndrome due to variants in the mitochondrial *MTTK* gene (2, 3, 4). Two autosomal recessive form of MSL have been described. Among them, MSL due to *MFN2* variants has been reported in a dozen unrelated families (5, 6, 7). *MFN2* encodes mitofusin 2, a protein involved in mitochondrial fusion and cellular energy metabolism (8). In addition, biallelic null variants in the *LIPE* gene have been reported in 2 unrelated families of Israeli-Arab and Italian origin, and in 3 patients from an Old Order Amish community with MSL (9, 10, 11, 12). *LIPE* encodes the hormone-sensitive lipase (HSL), a key enzyme for triglyceride hydrolysis expressed during adipocyte differentiation (13, 14, 15). At the clinical level, the different MSL subtypes cannot be easily distinguished. The pathophysiological mechanisms underlying the development of lipomatous masses remain also very poorly understood. A better understanding of these mechanisms is important for the management of patients and could also expand our knowledge on more common related polygenic conditions. As an example, the *LIPE* null variants responsible for the autosomal recessive form of MSL have been reported as susceptibility factors to hypertriglyceridemia and type 2 diabetes in the heterozygous state (9).

We and others have previously shown that *MFN2*-related MSL is a multisystem disease characterized by pseudo-lipomatous masses but also by lipoatrophy, insulin resistance-

associated metabolic abnormalities and neurological involvement (5, 6, 7). We report here the consequences of new biallelic pathogenic *LIPE* variants, identified in three unrelated patients, at the clinical, tissular and cellular levels. Cellular disease modelling was obtained by isolating adipose stem cells (ASCs) from lipomatous tissue and submitting them to adipocyte differentiation *in vitro*.

METHODS

Patients

This study included three unrelated women with biallelic variants in the *LIPE* gene. Clinical data collection was coordinated at the French reference center for rare diseases of insulin secretion and insulin sensitivity. We obtained written informed consent for all genetic studies as well as for the use of photographs shown in Figure 1. The study was approved by the institutional research ethics board (CPP Ile de France 5).

Genetic studies

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. A panel of genes used for routine diagnosis of lipodystrophic syndromes, including *LIPE* and other genes, was analyzed. Exons and flanking intronic sequences of 23 genes were captured from fragmented DNA with the SeqCapEZ enrichment protocol (Roche NimbleGen). Paired-end massively parallel sequencing was achieved on a MiSeq platform (Illumina). Bioinformatic analysis was performed using the Sophia Genetics DDM pipeline®. *LIPE* variants were confirmed by Sanger sequencing with the Big Dye Terminator v3.1 sequencing kit (Thermo Fisher) after PCR amplification and analyzed on a 3730XL device with the SeqScape v2.7 software (Applied Biosystems).

Histological and immunohistological studies

Samples of axillar lipomatous masses were obtained from patient A after surgical ablation for functional discomfort. Control adipose tissue was obtained from subcutaneous abdominal plastic surgery in 3 non-obese, non-diabetic women aged 40.5 ± 14.2 years (16). Light microscopy was performed on 10% zinc-formol–fixed paraffin-embedded tissue sections,

stained with hematoxylin-eosin, and with Sirius Red to detect collagen fibers. For immunohistochemical studies, tissue sections were probed with antibodies directed against CD68 (PA0273, Leica), and CD163 (NCL-CD163, Leica). Electron microscopy procedures are described in Supplemental Methods.

Adipose stem cell isolation and culture

Adipose stem cells were isolated from surgical samples of lipomatous masses in patient A and of subcutaneous abdominal adipose tissue in controls. Adipose tissue samples were enzymatically digested with collagenase B (0.2%). After centrifugation, stromal vascular fraction was filtered, rinsed, plated and cultured in α-MEM with 10% Fetal Calf Serum (FCS), 2 mmol/L glutamin, 1% Penicillin/streptomycin (10,000 UI/mL), 1% Hepes and Fibroblast Growth Factor-2 (145 nmol/L). After 24 h, only adipose stem cells adhered to plastic surfaces, while other cells were removed after culture medium replacement (17).

Adipocyte differentiation of ASCs

The differentiation of ASCs was induced in DMEM with 10% Fetal Bovine Serum, 1 µmol/L insulin, 500 µmol/L 3-isobutyl-1-methylxanthine (IBMX), 1 µmol/L dexamethasone and 1 µmol/L rosiglitazone. Cells were cultured in this medium for 7 days, then in adipogenic maintenance medium (DMEM with 1 µmol/L insulin) for an additional 7 days. Mature adipocytes were obtained within 14 days (17).

Western blot analysis

Frozen fat tissue or ASCs were solubilized in Laemmli buffer containing dithiothreitol. Lysates were subjected to SDS-PAGE, blotted onto nitrocellulose membranes, and probed with antibodies directed against HSL (#4107), FAS (#3180), IRβ (#3025), ERK1/2 (#9102),

phosphor-Tyr204-ERK1/2 (#9101) from Cell Signalling Technology; SREBP1c (SC-366), PPARγ (SC-7273), phosphotyrosine residues (αPY, SC-7020), Akt (SC-8312), phospho-Ser473-Akt (SC-7985-R), ATGL (SC-67355) from Santa Cruz Biotechnology; and C/EBPα (#18311-1-AP, Proteintech), ADIPOQ (#MA1-054, Invitrogen), PLIN1 (ab3526, Abcam) and IRS1 (#07-247, Upsate). Tubulin (T5168, Sigma-Aldrich) was used as a loading control. The immune complexes were detected by chemiluminescence and quantified using the ImageJ (NIH) software.

Oil Red-O staining, gene expression analyses, lipolysis, insulin response, and mitochondrial function studies

These experiments, performed on ASCs submitted to adipocyte differentiation for 14 days, are described in the Supplemental Methods section.

Statistical analyses

Data were expressed as means ± standard error of the mean (SEM) using the GraphPad Prism 6 software. Statistical significance was evaluated using non-parametric Mann-Whitney tests with a threshold at $p < 0.05$.

RESULTS

Identification of rare pathogenic variants in *LIPE*

Three women referred for MSL (patient A) and/or partial lipodystrophy (patients B and C) were found to carry new germline biallelic null variants in *LIPE* (Fig. 1A). Patient A was homozygous for the frameshift variant c.2828del; p.(Glu943Glyfs*22). Patient B was

compound heterozygous for two frameshift variants: c.1890_1891del; p.(Leu631Glyfs*57), and c.2077del; p.(Arg693Valfs*76). Patient C carried a nonsense variant in the homozygous state: c.1261C>T; p.(Gln421*). The variants identified in patient A and B were absent from databases reporting variants from the general population (gnomAD, ExAC, Exome Variant server). The variant identified in patient C was present in gnomAD at very low frequency (6.10^{-5}), but was never observed in the homozygous state.

Clinical picture of patients with *LIPE* pathogenic variants

Patient A was a 54-year old woman born from asymptomatic first-cousin parents. She had progressively developed lipomatous masses in the nape, shoulders, back, axillae, arms, abdomen and pubis since the age of 40, leading to iterative surgical ablation for functional discomfort. On clinical examination, she also presented with lipoatrophy of lower limbs and forearms (Table 1, Fig. 1B). She was diagnosed with insulin resistance (fasting insulinemia 13.8 mU/L, increased to 133 mU/L after 2h-oral glucose tolerance test), hypertriglyceridemia and low HDL-cholesterol (1.05 mmol/L) at age 35, hypertension at age 49, and fasting hyperglycemia at age 53. Hypercortisolism was ruled out. Liver steatosis with increased liver enzymes was present since the age of 45, in the absence of alcohol consumption. She had *pes cavus* and complained of paresthesia. Cardiac examination and resting electrocardiogram were normal. While visual acuity was normal, ophthalmological fundus monitoring revealed numerous disseminated drusen-like retinal flecks at the age of 45. They appeared as bilateral sub-retinal auto-fluorescent deposits of the posterior pole, without neovascularization, and were confirmed by optical coherence tomography.

Patient B, born from asymptomatic non-consanguineous parents, was referred at age 76 for fat accumulation in face and neck, lipomatous masses in axillar, abdominal and pubic areas, and lower limb lipoatrophy (Table 1). She was investigated for cushingoid morphotype, infertility, spaniomenorrhea and hirsutism at the age of 30, and hypercortisolism was ruled out. Lipomatous masses had progressively appeared since the age of 40. Diabetes with insulin resistance (fasting insulinemia 16.4 mU/L) was diagnosed at the age of 55. She also had hypertriglyceridemia, low HDL-cholesterol, and high blood pressure. She displayed paroxysmal atrial fibrillation at the age of 76 without sign of cardiomyopathy. Liver enzymes were increased and echography showed hepatosplenomegaly. She complained of walking difficulties due to asymmetric painful hypertrophy of the calves and *pes cavus*. Creatine phosphokinase was increased and electromyogram revealed axonal sensorimotor polyneuropathy. Magnetic resonance imaging (MRI) showed increased fat depots and dystrophic features of the right gastrocnemius muscle (Table 1, Fig. 2B). She underwent cataract surgery at the age of 67. Fundus examination at the age of 68 showed bilateral small sub-retinal deposits disseminated throughout the posterior pole, in the absence of decreased visual acuity.

Patient C, born from asymptomatic first-cousin parents, had progressively developed upper-body adipose overgrowth since the age of 35. She underwent a surgical resection of cervical and axillar pseudo-lipomatous masses at the age of 40 and 51. Clinical examination at the age of 57 showed accumulation of fat in anterior and posterior neck, shoulders, arms and axillar folds, contrasting with lipoatrophy of lower limbs (Fig. 1C, Fig. 2C). Patient C was diagnosed with dyslipidemia (hypertriglyceridemia and low HDL-cholesterol) at the age of 40, then with diabetes at the age of 47. She had severe hypertension since the age of 28, with macronodular adrenocortical hyperplasia, in the absence of specific hormonal alterations. Abdominal MRI

showed liver steatosis. Liver function tests were normal except for increased gamma glutamyl transferase levels, in the absence of alcohol consumption. Neuromuscular examination revealed *pes cavus*. Cardiac investigations showed left ventricular hypertrophy linked to high blood pressure. Routine ophthalmological examination at the age of 50, in the absence of visual complaints, revealed a very high number of symmetrical sub-retinal yellowish deposits disseminated in the macula, mid-peripheral and far-peripheral retina. These deposits were intensely auto-fluorescent, and induced abnormalities in the structure of retinal pigmentary epithelium, as assessed by optical coherence tomography (Table 1, Fig. 2D). Corrected visual acuity and anterior segments were normal, as well as electro-retinogram and electro-oculogram. Sequencing of a panel of genes involved in macular or retinal dystrophies with drusens/flecks, including *ABCA4*, *BEST1*, *EFEMP1*, *IMPG1*, *IMPG2*, *PLA2G5*, *PRPH2*, *RDH5*, *RLBP1*, and *TIMP3*, did not reveal any pathogenic variant.

Characteristics of patient lipomatous tissue

We studied surgical axillar lipomatous samples from patient A. Although it was not enclosed in fibrous capsules, pseudo-lipomatous tissue displayed a particular architectural organization with numerous lobules visible after Sirius red staining (marker of collagen), evocative of areas of cell regeneration (Fig. 3A, a). Pseudo-lipomatous tissue was composed of unilocular white adipocytes (Fig. 3A, b). It displayed inflammatory features with infiltrates of monocytes and macrophages, as assessed by the positivity of CD68 and CD163 staining (Fig. 3A, c,d).

Western blot studies revealed a complete loss of HSL expression in lipomatous tissue (Fig. 3B). The protein expression of the adipocyte differentiation marker peroxisome proliferator-activated receptor gamma (PPARγ) was decreased in adipose tissue from patient A as compared to controls. The protein expression of two markers of mature adipocyte, adiponectin (ADIPOQ) and fatty acid synthase (FAS), was severely reduced (Fig. 3C). Electron microscopy of

lipomatous masses revealed no abnormalities in size and morphology of mitochondria (Fig. 3D).

Defect in adipocyte differentiation of patient adipose stem cells (ASCs)

The decrease in the expression of adipogenic and mature adipocyte markers in pseudo-lipomatous tissue suggested a defect in adipocyte differentiation. To address this issue, we isolated ASCs from lipomatous tissue of patient A and compared their *in vitro* adipocyte differentiation with that of control ASCs.

Protein expression of HSL was detectable from day 7 onwards in control cells and further increased during differentiation. In contrast, as expected from lipomatous tissue data, HSL expression was completely absent in patient's ASCs submitted to adipocyte differentiation (Fig. 4A). Adipocyte differentiation was confirmed by accumulation of cytoplasmic lipid droplets stained by Oil Red-O after 14 days of differentiation in control ASCs. In contrast, lipid accumulation was markedly impaired in ASCs from patient A (Fig. 4B). Consistently, and in accordance with the data obtained in the patient's lipomatous tissue, the protein expression of mature adipocyte markers (ADIPOQ, FAS,PLIN1 and ATGL) was strongly decreased in ASCs from patient A as compared to control ASCs after 14 days of *in vitro* adipocyte differentiation. Mutated ASCs showed a low to normal expression of adipogenic proteins (PPARγ, C/EBPα, SREBP1c) (Fig. 4C). In addition, the mRNA expression level of *LIPE* and of adipogenic and mature adipocyte genes was decreased in patient ASCs as compared to controls (Fig. 4D). Taken together, our results strongly suggest that *LIPE* null variants impair the gene activation cascade required for proper adipocyte differentiation.

Altered function of adipocytes harboring *LIPE* pathogenic variants

We then studied the functional characteristics of patient ASCs after 14 days of adipocyte differentiation. We first evaluated the efficiency of lipolysis, known to be strongly regulated by

HSL. Glycerol, a product of triglyceride lipolysis, was measured in cell supernatants in the basal state and after stimulation by forskolin, an adenylate cyclase activator. Basal lipolysis was not different in patient and controls (Fig. 4E). However, whereas we observed a 3-fold increase in the release of glycerol after forskolin treatment in control cells, the level of glycerol remained unchanged in the supernatants of ASCs from patient A, indicating an altered lipolytic response to cAMP (Fig. 4E). We then investigated the cellular insulin response. Upon insulin stimulation, the activation of proximal and distal signaling intermediates (IRβ, IRS1, Akt/PKB, and ERK1/2) was decreased in ASCs from patient A as compared to control ASCs (Fig. 4F).

Mitochondrial dysfunction in ASCs harboring pathogenic *LIPE* variants

Since mitochondrial function contributes to adipocyte differentiation (18, 19), and since genes encoding mitochondrial proteins have been previously involved in monogenic forms of MSL, we hypothesized that *LIPE* pathogenic variants could alter mitochondrial functions. The analysis of basal oxygen consumption rates (OCR) of ASCs from patient A and controls did not reveal any significant difference. However, the patient's ASCs displayed a major decrease in their maximal respiratory capacity, as indicated by a significantly lower increase in OCR after addition of the uncoupling agent FCCP (Fig. 5A). Of note, the mitochondrial mass evaluated by MitoTracker staining was not decreased (data not shown). The mitochondrial membrane potential, evaluated by the capacity of the JC1 cationic dye to form aggregates, was significantly decreased (Fig. 5B). In addition, oxidative stress, assessed by the production of reactive oxygen species (ROS), was increased in cells from patient as compared to control cells (Fig. 5C).

DISCUSSION

The *LIPE*-associated MSL syndrome is a very rare disease since only 6 families have been reported worldwide, if we include patients from the current study and previous datasets (Table 1). The variants identified in each index case are different and patients are from various origins (French, Israeli-Arab, Italian, American Amish), consistent with independent mutational events. All the molecular defects in *LIPE* reported to date are null variants including four frameshift variants and one nonsense mutation. Consistent with another report (9), our data show that biallelic frameshift variants result in a complete loss of HSL expression.

Considering the very low number of patients reported with *LIPE* pathogenic variants, we integrated our clinical data with those described previously to draw the picture of the disease (Table 1). *LIPE*-related MSL is a late-onset disease, since the age at referral for diagnosis ranges from 23 to 76 years. Among the clinical signs, the following fours were the most common: lipoatrophy, lipomatosis, metabolic complications and neuromuscular manifestations. Unexpectedly, lipoatrophy is the predominant hallmark of the disease, since it was reported in most patients. It usually affects lower limbs and sometimes forearms. In contrast, pseudo-lipomatosis or areas of fat accumulation, present in all three patients from our study, was reported in only half of previously published cases. Adipose masses are most frequently observed in the neck, armpits, abdomen, and pubis. The metabolic complications observed in nearly all patients are similar to those observed in lipoatrophic syndromes and include insulin resistance and/or diabetes, hypertriglyceridemia, and liver steatosis. High blood pressure is also frequent. These characteristics suggest that the term “lipodystrophic syndrome” would be more appropriate than “multiple symmetric lipomatosis (MSL)” to describe the disease in patients with *LIPE* variants. Notably, the term “lipomatous” has also been used to describe areas of fat

accumulation in genetically-characterized lipodystrophy (20), further stressing the clinical continuum between so-called "MSL" and lipodystrophic syndromes. Apart from lipodystrophy, neuromuscular manifestations are present in nearly half of patients. Importantly, *pes cavus* was described in the absence of diabetes, in two patients with biallelic *LIPE* variants reported by Zolotov *et al*. (12) and in patient A from our study, arguing for a direct neuromuscular impact of *LIPE* variants.

The three patients from our study present with countless sub-retinal yellowish deposits disseminated in the macula and the peripheral retina that did not match with any description of drusen or flecked-retina disorders (21). This is the first description of ophthalmological signs in patients with *LIPE* pathogenic variants. Since they did not lead to visual complaints, their diagnosis requires a systematic fundus examination. Their bilateral and symmetric disposition, and their diagnosis at a young age in two patients (45-50 years old), strongly suggest that they could be of genetic origin. Several clues argue for a role of *LIPE* pathogenic variants in the development of these peculiar ophthalmological alterations: (i) HSL is expressed not only in adipocytes, but also in other organs including neurological tissues such as retina (22) (ii) HSL hydrolyzes triglycerides, but also retinyl and cholesterol esters, and alteration of neutral lipid and cholesterol homeostasis is one of the mechanisms involved in drusen biogenesis (23, 24).

The study of the consequences of *LIPE* variants is of importance not only to improve diagnosis and management of lipodystrophic syndromes, but also to better understand the role of HSL in humans. HSL is one of the three enzymes of triglyceride lipolysis. Adipose triglyceride lipase (ATGL) is able to catalyze the three successive enzymatic steps to release all non-esterified fatty acids molecules from triacylglycerol, then HSL and monoglyceride lipase (MGL) are thought to predominantly hydrolyze diglycerides and monoglycerides, respectively (13).

Several HSL-deficient mice models, which do not fully recapitulate the human disease, have been generated (25, 26, 27, 28, 29). Although HSL-deficient mice show reduced abdominal fat mass with normal body weight and decreased cAMP-stimulated lipolysis, they display decreased levels of circulating triglycerides, and their glucose homeostasis, insulin sensitivity, and liver lipid metabolism are very heterogeneous. This might be due, at least partly, to their different genetic backgrounds. However, adipose-specific HSL knockout mice have been described with age-dependent lipodystrophy, adipose tissue inflammation, systemic insulin resistance and hepatic steatosis, suggesting that the functions of HSL in adipose tissue strongly influence the whole-body metabolic homeostasis (29). How could decreased lipolysis due to HSL deficiency be associated with insulin resistance and fatty liver in humans? Since HSL plays a crucial role for proper adipocyte function and since its expression increases during adipocyte differentiation (15), we made the hypothesis that *LIPE* null variants could alter adipocyte differentiation. Human ASCs represent an appropriate model to address this issue. During adipose differentiation of ASC *in vitro, LIPE* pathogenic variants lead to a severe decrease in intracellular lipid droplets formation and in expression of adipocyte-specific markers. Consistently, expression of adipocyte proteins were also downregulated in lipomatous tissue obtained from a patient, consistent with data published by Albert *et al* (9). In accordance, the insulin-mediated signaling pathways were strongly impaired in cells harboring pathogenic *LIPE* variants. All these data were in agreement with the pathophysiology of other genetically-determined lipodystrophic syndromes, which is mainly driven by primary defects in differentiation, maintenance and/or functions of adipocytes (30). The mechanistic link between HSL deficiency and adipocyte differentiation defects remains to be clarified. It was proposed that HSL could act as a retinyl ester hydrolase, which provides ligands to activate the central adipogenic factor PPARγ and/or the Retinoid X Receptor (RXR) (31, 32). Defective HSL-mediated PPARγ activation could therefore be an important mechanism leading to defects in

adipocyte differentiation and maturation. Increased adipose tissue inflammation, described in our study as well as by Albert *et al* (9), could also contribute to adipocyte dysfunction and impaired differentiation. Finally, using the ASC model, we demonstrated the deleterious effect of *LIPE* pathogenic variants on mitochondrial function, including decreased mitochondrial respiration, decreased mitochondrial membrane potential and increased ROS production. These data are consistent with those obtained previously in a model of hMADS adipocytes treated with an HSL inhibitor, which severely decreased mitochondrial respiration (33). Mitochondrial dysfunction is known to impair adipocyte differentiation and to contribute to metabolic diseases such as obesity, diabetes and lipodystrophic syndromes (19, 34, 35). Other known genetic forms of MSL are due to variants in *MTTK*, a mtDNA gene necessary for proper oxidative phosphorylation and in *MFN2*, playing a major role in mitochondrial dynamics. Mitochondrial dysfunction could therefore represent a pathophysiological mechanism shared by *MTTK*-, *MFN2*-, and *LIPE*-related MSL.

The current study has some limitations. We only had the opportunity to study lipomatous tissue and ASCs from one of the three patients with *LIPE* pathogenic variants. Control and lipomatous tissue were both of subcutaneous origin, but originated from different depots, and we did not obtain similar lipomatous masses from patients without *LIPE* variants for comparison. However, these original clinical and cell modeling data, which increase the knowledge on the consequences of *LIPE* pathogenic variants in humans, pave the way for future additional studies.

In conclusion, biallelic null variants in *LIPE* lead to a multisystem disease associating features of MSL and lipodystrophy syndrome with neuromuscular involvement which requires multidisciplinary care. Attention should be drawn to possible specific retinal lesions that justify

422 further studies on the pathophysiological role of HSL in the retina. This work reveals the
423 multiple functional impacts of *LIPE* pathogenic variants on adipocyte differentiation and
424 functions and points to unexpected roles of hormone sensitive lipase in other organs.
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ACKNOWLEDGEMENTS

We thank the patients who took part in this study. We also thank Dr Soraya Fellahi and Pr Jean-Philippe Bastard (AP-HP, Hôpitaux universitaires Henri Mondor, Département de biochimie-pharmacologie-biologie moléculaire-génétique médicale, Créteil, France) for adiponectin and leptin measurements, Dr Guillaume Lefebvre (Service de Radiologie et Imagerie musculo-squelettique, Hôpital R. Salengro, CHRU de Lille, France) for Magnetic Resonance Imagery data, and Drs Isabelle Aubry (Centre d'Ophtalmologie des Arceaux, Montpellier, France) and Emna Saihi-Chaouch (Service d'Ophtalmologie, Valenciennes, France) for ophthalmological examination. We also thank Dr Pascale Cervera, Fatiha Merabtene and Romain Morichon (UMS30 Lumic, Sorbonne Université, Paris, France) for histological and immunohistological data, Marie-Christine Verpont (Inserm UMR_S1155, Hôpital Tenon, Paris, France) for electron microscopy study, and Matthieu Mantecon, Dr Véronique Béréziat and Dr Claire Lagathu (Inserm UMRS_938, Paris, France) for providing control ASCs.

DISCLOSURE

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

This work was supported by Institute of Cardiometabolism and Nutrition, Institut National de la Santé et de la Recherche Médicale (Inserm), and Sorbonne Université. Camille Sollier was the recipient of a master grant from Assistance Publique-Hôpitaux de Paris (Fonds d'Etude et de Recherche du Corps Médical).

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554

555 **FIGURE LEGENDS**

556

557 **Figure 1. Genetic and clinical features in patients with biallelic *LIPE* pathogenic variants**

558 **A.** Human *LIPE* cDNA structure with positions of the *LIPE* variants identified in patients from
559 the current study (patients A, B and C) and from previous reports. The organization of hormone-
560 sensitive lipase (HSL) domains of are shown below.

561 **B.** Pictures of patient A showing lipomatous masses in the nape, shoulders, back, axillae, arms,
562 and abdomen (arrows) with lipoatrophy of lower limbs and forearms (arrowhead).

563 **C.** Pictures of patient C showing lipomatous masses in the anterior and posterior cervical areas,
564 as well as shoulders and armpits (black arrows).

565

566 **Figure 2. Imaging features in patients with biallelic *LIPE* pathogenic variants**

567 **A.** Axial computed tomography showing posterior cervical lipomatous regions in patient A
568 (arrow).

569 **B.** Coronal (a) and axial (b, c) T1-weighted magnetic resonance imaging of calves in patient B
570 showing an increased volume of the right medial gastrocnemius muscle, with increased fat
571 depots and dystrophic features, and muscular mild gadolinium contrast enhancement (c).

572 **C.** T1-weighted magnetic resonance imaging in patient C, showing accumulation of fat in the
573 sub-mandibular area (a, b: sagittal section; c: coronal section) with slight adipose infiltration of
574 the tongue muscle (a, arrow). Fat accumulation is also observed in the posterior cervical areas,
575 anterior and posterior thoracic regions (b), and in neck, shoulders, and axillary regions (d:
576 coronal section).

577 **D.** Ophthalmological multimodal fundus imaging of patient C. Fundus picture (a) showing
578 multiple yellow flecks with irregular edges disseminated in the macula and mid-peripheral
579 retina. Optic disc and retinal vasculature have a normal aspect. Short-wavelength fundus auto-

fluorescence (b). Most flecks are intensely auto-fluorescent. Larger flecks are surrounded by a hypofluorescent halo. Spectral domain optical coherence tomography (c, d, e). Flecks correspond to mound-shaped elevations of retinal pigmentary epithelium filled with iso- to hyper-reflective content. Interruption of ellipsoid zone and internal limiting membrane are observed on the apex of the fleck (d, arrow). Focal interruptions of outer retinal layers are present (e, arrows).

Figure 3. Characteristics of lipomatous tissue in patient A

A. Histological and immunohistological features of lipomatous tissue from patient A (*LIPE*-MSL), as compared to subcutaneous adipose tissue from one representative control. The different panels (a-d) correspond to specific stainings, as indicated on the left. HE: hematoxylin-eosin; SR: Sirius red. CD68 and CD163 are monocyte-macrophage markers.

B. Protein expression obtained by Western blotting of hormone-sensitive lipase in two samples of lipomatous tissue from patient A, as compared to subcutaneous adipose tissue from two representative controls. HSL: hormone-sensitive lipase.

C. Protein expression obtained by Western blotting of adipocyte markers in lipomatous tissue from patient A, in comparison to subcutaneous adipose tissue from two representative controls. PPARγ: peroxisome proliferator-activated receptor gamma; ADIPOQ: adiponectin ; FAS: fatty acid synthase.

D. Ultrastructural characteristics of lipomatous tissue from patient A, as compared to subcutaneous adipose tissue from one representative control. Double arrows indicate the thickness of the cytoplasmic rim. m: mitochondria, L: lipid droplet.

Figure 4. Defects in adipocyte differentiation and functions in ASCs harboring pathogenic *LIPE* variants

A. Protein expression of hormone-sensitive lipase (HSL) obtained by Western blotting during adipocyte differentiation (day 0 to day 14) in patient A (*LIPE*-MSL) and control ASCs.

B. Adipocyte differentiation assessed by Oil Red-O lipid staining. ASCs from patient A (*LIPE*-MSL) and controls were studied after adipocyte differentiation for 14 days. Top: Representative images of fluorescence microscopy after staining of intracellular lipids (Oil Red-O, red) and nuclei (DAPI, blue). Bottom: Pictures of cell dishes stained by Oil Red-O and quantification of Oil Red-O fluorescence normalized to DNA content (DAPI). Results are expressed as means ± SEM of four independent experiments.

C. Protein expression of adipocyte markers obtained by Western blotting in patient A (*LIPE*-MSL) and control ASCs after *in vitro* adipocyte differentiation for 14 days. Results are representative of three independent experiments.

PPARγ: peroxisome proliferator-activated receptor gamma; C/EBPα: CCAAT/enhancer binding protein alpha; SREBP1c: sterol regulatory element-binding protein 1c; ADIPOQ: adiponectin; FAS: fatty acid synthase; PLIN1: perilipin-1 ; ATGL: adipose triglyceride lipase.

D. Gene expression of adipocyte markers obtained by RT-qPCR in patient A (*LIPE*-MSL) and control ASCs submitted to adipocyte differentiation for 14 days. Results are expressed as means ± SEM of three independent experiments. **: $p < 0.01$. *PNPLA2* encodes adipose triglyceride lipase (ATGL).

E. Lipolytic function in patient (*LIPE*-MSL) and control ASCs submitted to adipocyte differentiation for 14 days. Cells were incubated or not for 4 h with the adenylate cyclase activator forskolin (FSK) as indicated. Lipolysis was evaluated by the production of glycerol in supernatants relative to the protein content in each well. Results are expressed as means ± SEM of three independent experiments. ns: non significant; *: $p < 0.05$; **: $p < 0.01$.

F. Activation of insulin signaling in patient (*LIPE*-MSL) and control ASCs submitted to adipocyte differentiation for 14 days. Insulin-induced phosphorylation of insulin receptor β-

630 subunit (IRβ), insulin receptor substrate-1 (IRS1), protein kinase B (Akt/PKB), and
631 extracellular-regulated kinase (ERK)1/2 was evaluated by Western blotting. Results are
632 representative of three independent experiments.
633 PY: phosphotyrosine residues; Phospho-Akt/PKB: phospho-Ser473-Akt; Phospho-ERK1/2:
634 phospho-Tyr204-ERK1/2.
635
636 **Figure 5. Mitochondrial dysfunction in ASCs harboring pathogenic *LIPE* variants**
637 **A.** Cellular respiration assessed by the measurement of oxygen consumption rate (OCR) in
638 patient A (*LIPE*-MSL*, grey diamonds*) and control ASCs (*open squares*) previously submitted
639 to adipocyte differentiation for 14 days, using Seahorse at baseline and after sequential addition
640 of oligomycin (inhibitor of ATP synthase), carbonyl cyanide 4-
641 trifluoromethoxyphenylhydrazone (FCCP, uncoupling agent), and rotenone/antimycin A
642 (AA/R, inhibitors of the respiratory chain complexes I and III), as described in Supplemental
643 Methods. Results are means ± SEM of three independent experiments. *: $p < 0.05$.
644 **B.** Mitochondrial membrane potential assessed by JC-1 (tetrachloro-tetra-ethyl-
645 benzimidazolyl-carbocyanine iodide) cationic dye staining (aggregates to monomers ratio) in
646 patient (*LIPE*-MSL) and control ASCs previously submitted to adipocyte differentiation for 14
647 days. Results are expressed as means ± SEM of five independent experiments. **: $p < 0.01$.
648 **C.** Reactive oxygen species (ROS) production assessed by oxidation of 5-6-chloromethyl-2,7-
649 dichlorodihydro-fluorescein diacetate (CM-H2DCFDA) in patient and control ASCs previously
650 submitted to adipocyte differentiation for 14 days. Results normalized to DNA content
651 measured by DAPI, are expressed as means ± SEM of four independent experiments *: $p <$
652 0.05.
653
654