



**HAL**  
open science

## **LIPE-related lipodystrophic syndrome: clinical features and disease modeling using adipose stem cells**

Camille Sollier, Emilie Capel, Caroline Aguilhon, Vasily Smirnov, Martine Auclair, Claire Douillard, Miriam Ladsous, Sabine Defoort-Dhellemmes, Jennifer Gorwood, Laura Braud, et al.

### ► To cite this version:

Camille Sollier, Emilie Capel, Caroline Aguilhon, Vasily Smirnov, Martine Auclair, et al.. LIPE-related lipodystrophic syndrome: clinical features and disease modeling using adipose stem cells. *European Journal of Endocrinology*, 2021, 184 (1), pp.155-168. 10.1530/EJE-20-1013 . inserm-03138972

**HAL Id: inserm-03138972**

**<https://inserm.hal.science/inserm-03138972v1>**

Submitted on 11 Feb 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 ***LIP*E-related lipodystrophic syndrome: clinical features and disease modelling using**  
2 **adipose stem cells**

3  
4 **Short title: Lipodystrophy due to HSL defects**

5  
6 Camille Sollier<sup>1,\*</sup>, Emilie Capel<sup>1,\*</sup>, Caroline Aguilhon<sup>2</sup>, Vasily Smirnov<sup>3</sup>, Martine Auclair<sup>1</sup>,  
7 Claire Douillard<sup>4</sup>, Miriam Ladsous<sup>5</sup>, Sabine Defoort-Dhellemmes<sup>3</sup>, Jennifer Gorwood<sup>1</sup>, Laura  
8 Braud<sup>6</sup>, Roberto Motterlini<sup>6</sup>, Camille Vatier<sup>1,7</sup>, Olivier Lascols<sup>1,8</sup>, Eric Renard<sup>2</sup>, Corinne  
9 Vigouroux<sup>1,7,8,#</sup>, Isabelle Jéru<sup>1,8,#</sup>

10

11 \* and # : equally contributing to this paper

12 Correspondance to Corinne Vigouroux and Isabelle Jéru, Sorbonne Université Faculté  
13 Médecine site Saint-Antoine, 27 rue Chaligny, 75571 Paris Cedex 12, France.  
14 corinne.vigouroux@inserm.fr, isabelle.jeru@aphp.fr

15

16

17 1. Sorbonne Université, Inserm UMRS\_938, Centre de Recherche Saint Antoine, Paris, France

18

19 2. CHU de Montpellier, Hôpital Lapeyronie, Service d'Endocrinologie-Diabétologie-Nutrition,  
20 and Institut de Génomique Fonctionnelle, CNRS, INSERM, Université de Montpellier,  
21 Montpellier, France

22

23 3. CHU de Lille, Exploration de la Vision et Neuro-Ophtalmologie, Lille, France ; Université  
24 de Lille, Faculté de Médecine, Lille, France

25

26 4. Hopital Huriez-CHU Lille, Service d'Endocrinologie-Diabétologie-Métabolisme, et Hôpital  
27 Jeanne de Flandres, Centre de Référence des Maladies Héritaires du Métabolisme, Clinique  
28 de Pédiatrie, Lille, France

29

30 5. Hôpital Jean Bernard, Service d'Endocrinologie-Diabétologie, Valenciennes, France.

31

32 6. Inserm U955, Faculté de Médecine, Université Paris-Est, Créteil, France

33

34 7. Assistance Publique-Hôpitaux de Paris, Hôpital Saint Antoine, Centre National de Référence  
35 des Pathologies Rares de l'Insulino-Sécrétion et de l'Insulino-Sensibilité (PRISIS), Service de  
36 Diabétologie et Endocrinologie de la Reproduction, Paris, France

37

38 8. Assistance Publique-Hôpitaux de Paris, Hôpital Saint Antoine, Laboratoire commun de  
39 Biologie et Génétique Moléculaires, Paris, France

40

41 **Word count:** 3611

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

44 **ABSTRACT**

45

46 **Objective**

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

52

53 **Methods**

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

59

60 **Results**

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

70

71 **Conclusions**

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

77

78 **SIGNIFICANCE STATEMENT**

79

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

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

92

93 **INTRODUCTION**

94

95 Multiple Symmetric Lipomatosis (MSL), also known as Launois-Bensaude Lipomatosis or  
96 Madelung disease is a generic term used to describe several rare disorders. MSL is characterized  
97 by the progressive development of voluminous masses of adipose tissue, mostly localized in  
98 the neck and upper body, and frequently associated with metabolic complications and  
99 neurological manifestations. MSL was first described and most frequently encountered in  
100 alcoholic patients (1). A few genetic forms have also been characterized. MSL could affect  
101 ~20% of patients with the myoclonic epilepsy and ragged red fibers (MERRF) syndrome due  
102 to variants in the mitochondrial *MTTK* gene (2, 3, 4). Two autosomal recessive form of MSL  
103 have been described. Among them, MSL due to *MFN2* variants has been reported in a dozen  
104 unrelated families (5, 6, 7). *MFN2* encodes mitofusin 2, a protein involved in mitochondrial  
105 fusion and cellular energy metabolism (8). In addition, biallelic null variants in the *LIPE* gene  
106 have been reported in 2 unrelated families of Israeli-Arab and Italian origin, and in 3 patients  
107 from an Old Order Amish community with MSL (9, 10, 11, 12). *LIPE* encodes the hormone-  
108 sensitive lipase (HSL), a key enzyme for triglyceride hydrolysis expressed during adipocyte  
109 differentiation (13, 14, 15). At the clinical level, the different MSL subtypes cannot be easily  
110 distinguished. The pathophysiological mechanisms underlying the development of lipomatous  
111 masses remain also very poorly understood. A better understanding of these mechanisms is  
112 important for the management of patients and could also expand our knowledge on more  
113 common related polygenic conditions. As an example, the *LIPE* null variants responsible for  
114 the autosomal recessive form of MSL have been reported as susceptibility factors to  
115 hypertriglyceridemia and type 2 diabetes in the heterozygous state (9).  
116 We and others have previously shown that *MFN2*-related MSL is a multisystem disease  
117 characterized by pseudo-lipomatous masses but also by lipoatrophy, insulin resistance-

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

## 123 **METHODS**

124

### 125 **Patients**

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

131

### 132 **Genetic studies**

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

142

### 143 **Histological and immunohistological studies**

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

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

152

### 153 **Adipose stem cell isolation and culture**

154 Adipose stem cells were isolated from surgical samples of lipomatous masses in patient A and  
155 of subcutaneous abdominal adipose tissue in controls. Adipose tissue samples were  
156 enzymatically digested with collagenase B (0.2%). After centrifugation, stromal vascular  
157 fraction was filtered, rinsed, plated and cultured in  $\alpha$ -MEM with 10% Fetal Calf Serum (FCS),  
158 2 mmol/L glutamin, 1% Penicillin/streptomycin (10,000 UI/mL), 1% HEPES and Fibroblast  
159 Growth Factor-2 (145 nmol/L). After 24 h, only adipose stem cells adhered to plastic surfaces,  
160 while other cells were removed after culture medium replacement (17).

161

### 162 **Adipocyte differentiation of ASCs**

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

168

### 169 **Western blot analysis**

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



173 phosphor-Tyr204-ERK1/2 (#9101) from Cell Signalling Technology; SREBP1c (SC-366),  
174 PPAR $\gamma$  (SC-7273), phosphotyrosine residues ( $\alpha$ PY, SC-7020), Akt (SC-8312), phospho-  
175 Ser473-Akt (SC-7985-R), ATGL (SC-67355) from Santa Cruz Biotechnology; and C/EBP $\alpha$   
176 (#18311-1-AP, Proteintech), ADIPOQ (#MA1-054, Invitrogen), PLIN1 (ab3526, Abcam) and  
177 IRS1 (#07-247, Upsate). Tubulin (T5168, Sigma-Aldrich) was used as a loading control. The  
178 immune complexes were detected by chemiluminescence and quantified using the ImageJ  
179 (NIH) software.

180

### 181 **Oil Red-O staining, gene expression analyses, lipolysis, insulin response, and** 182 **mitochondrial function studies**

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

185

### 186 **Statistical analyses**

187 Data were expressed as means  $\pm$  standard error of the mean (SEM) using the GraphPad Prism  
188 6 software. Statistical significance was evaluated using non-parametric Mann-Whitney tests  
189 with a threshold at  $p < 0.05$ .

190

191

## 192 **RESULTS**

193

### 194 **Identification of rare pathogenic variants in *LIPE***

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

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

204

### 205 **Clinical picture of patients with *LIPE* pathogenic variants**

206 **Patient A** was a 54-year old woman born from asymptomatic first-cousin parents. She had  
207 progressively developed lipomatous masses in the nape, shoulders, back, axillae, arms,  
208 abdomen and pubis since the age of 40, leading to iterative surgical ablation for functional  
209 discomfort. On clinical examination, she also presented with lipoatrophy of lower limbs and  
210 forearms (Table 1, Fig. 1B). She was diagnosed with insulin resistance (fasting insulinemia  
211 13.8 mU/L, increased to 133 mU/L after 2h-oral glucose tolerance test), hypertriglyceridemia  
212 and low HDL-cholesterol (1.05 mmol/L) at age 35, hypertension at age 49, and fasting  
213 hyperglycemia at age 53. Hypercortisolism was ruled out. Liver steatosis with increased liver  
214 enzymes was present since the age of 45, in the absence of alcohol consumption. She had *pes*  
215 *cavus* and complained of paresthesia. Cardiac examination and resting electrocardiogram were  
216 normal. While visual acuity was normal, ophthalmological fundus monitoring revealed  
217 numerous disseminated drusen-like retinal flecks at the age of 45. They appeared as bilateral  
218 sub-retinal auto-fluorescent deposits of the posterior pole, without neovascularization, and were  
219 confirmed by optical coherence tomography.

220

221

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

238

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

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

259

### 260 **Characteristics of patient lipomatous tissue**

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

267 Western blot studies revealed a complete loss of HSL expression in lipomatous tissue (Fig. 3B).

268 The protein expression of the adipocyte differentiation marker peroxisome proliferator-  
269 activated receptor gamma (PPAR $\gamma$ ) was decreased in adipose tissue from patient A as compared  
270 to controls. The protein expression of two markers of mature adipocyte, adiponectin (ADIPOQ)  
271 and fatty acid synthase (FAS), was severely reduced (Fig. 3C). Electron microscopy of

272 lipomatous masses revealed no abnormalities in size and morphology of mitochondria (Fig.  
273 3D).

274

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

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

280 Protein expression of HSL was detectable from day 7 onwards in control cells and further  
281 increased during differentiation. In contrast, as expected from lipomatous tissue data, HSL  
282 expression was completely absent in patient's ASCs submitted to adipocyte differentiation (Fig.  
283 4A). Adipocyte differentiation was confirmed by accumulation of cytoplasmic lipid droplets  
284 stained by Oil Red-O after 14 days of differentiation in control ASCs. In contrast, lipid  
285 accumulation was markedly impaired in ASCs from patient A (Fig. 4B). Consistently, and in  
286 accordance with the data obtained in the patient's lipomatous tissue, the protein expression of  
287 mature adipocyte markers (ADIPOQ, FAS, PLIN1 and ATGL) was strongly decreased in ASCs  
288 from patient A as compared to control ASCs after 14 days of *in vitro* adipocyte differentiation.  
289 Mutated ASCs showed a low to normal expression of adipogenic proteins (PPAR $\gamma$ , C/EBP $\alpha$ ,  
290 SREBP1c) (Fig. 4C). In addition, the mRNA expression level of *LIPE* and of adipogenic and  
291 mature adipocyte genes was decreased in patient ASCs as compared to controls (Fig. 4D).  
292 Taken together, our results strongly suggest that *LIPE* null variants impair the gene activation  
293 cascade required for proper adipocyte differentiation.

294

### 295 **Altered function of adipocytes harboring *LIPE* pathogenic variants**

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

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

306

### 307 **Mitochondrial dysfunction in ASCs harboring pathogenic *LIPE* variants**

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

320

321

322 **DISCUSSION**

323

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

331

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

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

353

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

365

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



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

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

410

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

418

419 In conclusion, biallelic null variants in *LIPE* lead to a multisystem disease associating features  
420 of MSL and lipodystrophy syndrome with neuromuscular involvement which requires  
421 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.

425

426

427 **ACKNOWLEDGEMENTS**

428 We thank the patients who took part in this study. We also thank Dr Soraya Fellahi and Pr Jean-  
429 Philippe Bastard (AP-HP, Hôpitaux universitaires Henri Mondor, Département de biochimie-  
430 pharmacologie-biologie moléculaire-génétique médicale, Créteil, France) for adiponectin and  
431 leptin measurements, Dr Guillaume Lefebvre (Service de Radiologie et Imagerie musculo-  
432 squelettique, Hôpital R. Salengro, CHRU de Lille, France) for Magnetic Resonance Imagery  
433 data, and Drs Isabelle Aubry (Centre d’Ophtalmologie des Arceaux, Montpellier, France) and  
434 Emna Saihi-Chaouch (Service d’Ophtalmologie, Valenciennes, France) for ophthalmological  
435 examination. We also thank Dr Pascale Cervera, Fatiha Merabtene and Romain Morichon  
436 (UMS30 Lumic, Sorbonne Université, Paris, France) for histological and immunohistological  
437 data, Marie-Christine Verpont (Inserm UMR\_S1155, Hôpital Tenon, Paris, France) for electron  
438 microscopy study, and Matthieu Mantecon, Dr Véronique Béréziat and Dr Claire Lagathu  
439 (Inserm UMRS\_938, Paris, France) for providing control ASCs.

440

441 **DISCLOSURE**

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

444

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

449

450 **REFERENCES**

451

- 452 1. Enzi G, Busetto L, Ceschin E, Coin A, Digito M & Pigozzo S. Multiple symmetric  
453 lipomatosis: clinical aspects and outcome in a long-term longitudinal study. *Int J Obes Relat*  
454 *Metab Disord* 2002 **26** 253-261.
- 455 2. Altmann J, Buchner B, Nadaj-Pakleza A, Schafer J, Jackson S, Lehmann D, Deschauer  
456 M, Kopajtich R, Lautenschlager R, Kuhn KA *et al.* Expanded phenotypic spectrum of the  
457 m.8344A>G "MERRF" mutation: data from the German mitoNET registry. *J Neurol* 2016 **263**  
458 961-972.
- 459 3. Mancuso M, Orsucci D, Angelini C, Bertini E, Carelli V, Comi GP, Minetti C, Moggio  
460 M, Mongini T, Servidei S *et al.* Phenotypic heterogeneity of the 8344A>G mtDNA "MERRF"  
461 mutation. *Neurology* 2013 **80** 2049-2054.
- 462 4. Musumeci O, Barca E, Lamperti C, Servidei S, Comi GP, Moggio M, Mongini T,  
463 Siciliano G, Filosto M, Pegoraro E *et al.* Lipomatosis Incidence and Characteristics in an Italian  
464 Cohort of Mitochondrial Patients. *Front Neurol* 2019 **10** 160.
- 465 5. Capel E, Vatier C, Cervera P, Stojkovic T, Disse E, Cottureau AS, Auclair M, Verpont  
466 MC, Mosbah H, Gourdy P *et al.* *MFN2*-associated lipomatosis: Clinical spectrum and impact  
467 on adipose tissue. *J Clin Lipidol* 2018 **12** 1420-1435.
- 468 6. Rocha N, Bulger DA, Frontini A, Titheradge H, Gribsholt SB, Knox R, Page M, Harris  
469 J, Payne F, Adams C *et al.* Human biallelic *MFN2* mutations induce mitochondrial dysfunction,  
470 upper body adipose hyperplasia, and suppression of leptin expression. *Elife* 2017 **6**.
- 471 7. Sawyer SL, Cheuk-Him Ng A, Innes AM, Wagner JD, Dyment DA, Tetreault M,  
472 Care4Rare Canada Consortium, Majewski J, Boycott KM, Sreaton RA *et al.* Homozygous  
473 mutations in *MFN2* cause multiple symmetric lipomatosis associated with neuropathy. *Hum*  
474 *Mol Genet* 2015 **24** 5109-5114.
- 475 8. Hoppins S, Lackner L & Nunnari J. The machines that divide and fuse mitochondria.  
476 *Annu Rev Biochem* 2007 **76** 751-780.

- 477 9. Albert JS, Yerges-Armstrong LM, Horenstein RB, Pollin TI, Sreenivasan UT, Chai S,  
478 Blaner WS, Snitker S, O'Connell JR, Gong DW *et al.* Null mutation in hormone-sensitive lipase  
479 gene and risk of type 2 diabetes. *N Engl J Med* 2014 **370** 2307-2315.
- 480 10. Farhan SM, Robinson JF, McIntyre AD, Marrosu MG, Ticca AF, Loddo S, Carboni N,  
481 Brancati F & Hegele RA. A novel *LIPE* nonsense mutation found using exome sequencing in  
482 siblings with late-onset familial partial lipodystrophy. *Can J Cardiol* 2014 **30** 1649-1654, 11.
- 483 11. Carboni N, Brancati F, Cocco E, Solla E, D'Apice MR, Mateddu A, McIntyre A, Fadda  
484 E, Mura M, Lattanzi G *et al.* Partial lipodystrophy associated with muscular dystrophy of  
485 unknown genetic origin. *Muscle Nerve* 2014 **49** 928-930.
- 486 12. Zolotov S, Xing C, Mahamid R, Shalata A, Sheikh-Ahmad M & Garg A. Homozygous  
487 *LIPE* mutation in siblings with multiple symmetric lipomatosis, partial lipodystrophy, and  
488 myopathy. *Am J Med Genet A* 2017 **173** 190-194.
- 489 13. Arner P & Langin D. The role of neutral lipases in human adipose tissue lipolysis. *Curr*  
490 *Opin Lipidol* 2007 **18** 246-250.
- 491 14. Kolditz CI & Langin D. Adipose tissue lipolysis. *Curr Opin Clin Nutr Metab Care* 2010  
492 **13** 377-381.
- 493 15. Ambele MA, Dessels C, Durandt C & Pepper MS. Genome-wide analysis of gene  
494 expression during adipogenesis in human adipose-derived stromal cells reveals novel patterns  
495 of gene expression during adipocyte differentiation. *Stem Cell Res* 2016 **16** 725-734.
- 496 16. Gandotra S, Le Dour C, Bottomley W, Cervera P, Giral P, Reznik Y, Charpentier G,  
497 Auclair M, Delepine M, Barroso I *et al.* Perilipin deficiency and autosomal dominant partial  
498 lipodystrophy. *N Engl J Med* 2011 **364** 740-748.
- 499 17. Gorwood J, Bourgeois C, Mantecon M, Atlan M, Pourcher V, Pourcher G, Le Grand R,  
500 Desjardins D, Fève B, Lambotte O *et al.* Impact of HIV/simian immunodeficiency virus  
501 infection and viral proteins on adipose tissue fibrosis and adipogenesis. *AIDS* 2019 **33** 953-964.

- 502 18. Wilson-Fritch L, Burkart A, Bell G, Mendelson K, Leszyk J, Nicoloso S, Czech M &  
503 Corvera S. Mitochondrial biogenesis and remodeling during adipogenesis and in response to  
504 the insulin sensitizer rosiglitazone. *Mol Cell Biol* 2003 **23** 1085-1094.
- 505 19. De Pauw A, Tejerina S, Raes M, Keijzer J & Arnould T. Mitochondrial (dys)function in  
506 adipocyte (de)differentiation and systemic metabolic alterations. *Am J Pathol* 2009 **175** 927-  
507 939.
- 508 20. Selting K, Hansen NJ & Harrison WL. Massive labial lipomatous hypertrophy in  
509 familial partial lipodystrophy seen on computed tomographic angiography. *Radiol Case Rep*  
510 2019 **14** 1311-1313.
- 511 21. Khan KN, Mahroo OA, Khan RS, Mohamed MD, McKibbin M, Bird A, Michaelides  
512 M, Tufail A & Moore AT. Differentiating drusen: Drusen and drusen-like appearances  
513 associated with ageing, age-related macular degeneration, inherited eye disease and other  
514 pathological processes. *Prog Retin Eye Res* 2016 **53** 70-106.
- 515 22. Farkas MH, Au ED, Sousa ME & Pierce EA. RNA-Seq: Improving Our Understanding  
516 of Retinal Biology and Disease. *Cold Spring Harb Perspect Med* 2015 **5** a017152.
- 517 23. Jun S, Datta S, Wang L, Pegany R, Cano M & Handa JT. The impact of lipids, lipid  
518 oxidation, and inflammation on AMD, and the potential role of miRNAs on lipid metabolism  
519 in the RPE. *Exp Eye Res* 2019 **181** 346-355.
- 520 24. Pikuleva IA & Curcio CA. Cholesterol in the retina: the best is yet to come. *Prog Retin*  
521 *Eye Res* 2014 **41** 64-89.
- 522 25. Wang SP, Laurin N, Himms-Hagen J, Rudnicki MA, Levy E, Robert MF, Pan L, Oligny  
523 L & Mitchell GA. The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice.  
524 *Obes Res* 2001 **9** 119-128.
- 525 26. Voshol PJ, Haemmerle G, Ouwens DM, Zimmermann R, Zechner R, Teusink B,  
526 Maassen JA, Havekes LM & Romijn JA. Increased hepatic insulin sensitivity together with

527 decreased hepatic triglyceride stores in hormone-sensitive lipase-deficient mice. *Endocrinology*  
528 2003 **144** 3456-3462.

529 27. Grousse A & Langin D. Adipocyte lipases and lipid droplet-associated proteins: insight  
530 from transgenic mouse models. *Int J Obes (Lond)* 2012 **36** 581-594.

531 28. Grousse A, Tavernier G, Valle C, Moro C, Mejhert N, Dinel AL, Houssier M, Roussel  
532 B, Besse-Patin A, Combes M *et al.* Partial inhibition of adipose tissue lipolysis improves  
533 glucose metabolism and insulin sensitivity without alteration of fat mass. *PLoS Biol* 2013 **11**  
534 e1001485.

535 29. Xia B, Cai GH, Yang H, Wang SP, Mitchell GA & Wu JW. Adipose tissue deficiency  
536 of hormone-sensitive lipase causes fatty liver in mice. *PLoS Genet* 2017 **13** e1007110.

537 30. Sollier C, Vatier C, Capel E, Lascols O, Auclair M, Janmaat S, Fève B, Jéru I &  
538 Vigouroux C. Lipodystrophic syndromes: From diagnosis to treatment. *Ann Endocrinol (Paris)*  
539 2020 **81** 51-60.

540 31. Strom K, Gundersen TE, Hansson O, Lucas S, Fernandez C, Blomhoff R & Holm C.  
541 Hormone-sensitive lipase (HSL) is also a retinyl ester hydrolase: evidence from mice lacking  
542 HSL. *FASEB J* 2009 **23** 2307-2316.

543 32. Shen WJ, Yu Z, Patel S, Jue D, Liu LF & Kraemer FB. Hormone-sensitive lipase  
544 modulates adipose metabolism through PPARgamma. *Biochim Biophys Acta* 2011 **1811** 9-16.

545 33. Jocken JW, Goossens GH, Popeijus H, Essers Y, Hoebbers N & Blaak EE. Contribution  
546 of lipase deficiency to mitochondrial dysfunction and insulin resistance in hMADS adipocytes.  
547 *Int J Obes (Lond)* 2016 **40** 507-513.

548 34. Ding L, Yang X, Tian H, Liang J, Zhang F, Wang G, Wang Y, Ding M, Shui G & Huang  
549 X. Seipin regulates lipid homeostasis by ensuring calcium-dependent mitochondrial  
550 metabolism. *EMBO J* 2018 **37**.



551 35. Vigouroux C, Caron-Debarle M, Le Dour C, Magré J & Capeau J. Molecular  
552 mechanisms of human lipodystrophies: from adipocyte lipid droplet to oxidative stress and  
553 lipotoxicity. *Int J Biochem Cell Biol* 2011 **43** 862-876.  
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-

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

586

### 587 **Figure 3. Characteristics of lipomatous tissue in patient A**

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

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

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

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

602

### 603 **Figure 4. Defects in adipocyte differentiation and functions in ASCs harboring pathogenic**

604 ***LIPE* variants**

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

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

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

616 PPAR $\gamma$ : peroxisome proliferator-activated receptor gamma; C/EBP $\alpha$ : CCAAT/enhancer  
617 binding protein alpha; SREBP1c: sterol regulatory element-binding protein 1c; ADIPOQ:  
618 adiponectin; FAS: fatty acid synthase; PLIN1: perilipin-1 ; ATGL: adipose triglyceride lipase.

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

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

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

630 subunit (IR $\beta$ ), 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 trifluoromethoxyphenylhydrazine (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  $\pm$  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  $\pm$  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-H<sub>2</sub>DCFDA) 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  $\pm$  SEM of four independent experiments \*:  $p <$   
652 0.05.

653

654