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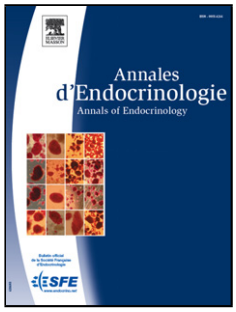

Proceedings of the annual meeting of the European Consortium of Lipodystrophies (ECLip) Cambridge, UK, 7-8 April 2022

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Proceedings of the annual meeting of the European Consortium of Lipodystrophies (ECLip) Cambridge, UK, 7-8 April 2022

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Abstract

Lipodystrophy syndromes are rare diseases with defects in the development or maintenance of adipose tissue, frequently leading to severe metabolic complications. They may be genetic or acquired, with variable clinical forms, and are largely underdiagnosed. The European Consortium of Lipodystrophies, ECLip, is a fully functional non-profit network of European centers of excellence working in the field of lipodystrophies. It provides a favorable environment to promote large Europe-wide and international collaborations to increase the basic scientific understanding and clinical management of these diseases. It works with patient advocacy groups to increase public awareness. The network also promotes a European Patient Registry of lipodystrophies, as a collaborative research platform for consortium members. The annual congress organized gives an update of the findings of network research groups, highlighting clinical and fundamental aspects. The talks presented during the meeting in Cambridge, UK, in 2022 are summarized in these minutes.

Keywords: lipodystrophy syndrome, fatty acid, total body irradiation, encephalopathy, miRNA, caveolin, therapeutic education, EPHX1, seipin, registry, metreleptin, antibody.

INTRODUCTION

The European Consortium of Lipodystrophies, ECLip, is a fully functional non-profit network of European centers of excellence in the field of lipodystrophies.
Lipodystrophy syndromes form a heterogeneous group of diseases characterized by defects in the development or maintenance of adipose tissue, frequently leading to metabolic complications associated with insulin resistance, in the absence of malnutritional or a catabolic state. They are classified in 4 major categories: congenital generalized lipodystrophy (CGL), familial partial lipodystrophy (FPL), acquired generalized lipodystrophy (AGL) and acquired

partial lipodystrophy (APL). Partial forms of lipoatrophy may be associated with regions of fat accumulation. Lipodystrophy can also occur as part of complex systemic diseases such as premature ageing syndromes (progeroid syndromes).
The ECLip network (https://www.eclip-web.org/lipodystrophies/) provides a favorable environment to promote and facilitate large Europe-wide and international collaborations to increase the basic scientific understanding and clinical management of lipodystrophy syndromes. It aims to improve diagnosis and prevention, to provide optimum medical care and, in collaboration with patient advocacy groups, to promote public awareness in the domain of lipodystrophies. The ECLip Patient Registry of lipodystrophies was set up to extract and share data and provide a collaborative research platform for consortium members [1].
The network organizes at least one annual meeting, updating the findings of network research groups and stimulating collaborations. The presentations given during the last ECLip meeting in Cambridge, UK, in 2022 are summarized in these minutes.

ABSTRACTS of the ECLIP meeting, Cambridge, 2022

Fatty acid trafficking in regional adipose tissues
Professor Fredrik Karpe, University of Oxford, UK
Pr. Fredrik Karpe coordinates several research programs in metabolic physiology and pathophysiology, based in particular on the Oxford Biobank general population cohort. He presented the results of his research assessing the storage capacities of excess energy in the form of lipids in adipose tissue. Measuring the entry of fatty acids into adipocytes, their esterification into triglycerides, and conversely, assessing the intra-adipocyte hydrolysis of triglycerides (lipolysis), the exit of fatty acids into the capillaries draining the adipose tissue, and the flow of fatty acids into the bloodstream (spillover) all require complex metabolic investigations. Fredrik Karpe discussed the interest and limitations of different methods: imaging (DEXA, PET-scan), and ingestion or intravenous administration of labeled fatty acids followed by measurement of labeled triglycerides in biopsy samples of adipose tissue. He presented the principles of measurement of arteriovenous difference in labeled fatty acid concentrations, enabling exploration of the flow of fatty acids in very precise anatomical territories, specific to abdominal and gluteal subcutaneous adipose tissue. This method makes it possible to dynamically explore the metabolic response of adipose tissue to several stimuli (for example, during a meal, or after an increase in insulin or other hormones). At the physiological level, it shows that femoro-gluteal adipose tissue is not very sensitive to lipolysis induced by catecholamines, but on the contrary very active in extracting fatty acids from circulating lipoproteins of hepatic origin (very low-density lipoprotein: VLDL) and storing them as triglycerides. This method also allowed his team to show, in 5 patients with lipodystrophy linked to *LMNA* variants versus 5 control subjects, that lipodystrophic patients have a lower capacity to extract fatty acids from circulating lipoproteins (chylomicrons and VLDL) to feed the adipocyte triglyceride storage pathway. Conversely, they have an increased flow of fatty acids and triglycerides into the bloodstream (spillover), which results from a decreased adipocyte triglyceride storage capacity.

Form vs function in adipose disorders: where does lipodystrophy stop?
Professor Robert Semple, University of Edinburgh, UK
Pr. Robert Semple, of the University of Edinburgh, discussed the challenge of developing sensitive and specific clinical diagnostic criteria for lipodystrophies: should the definition be based on adipose tissue anatomy? Or on its function? Or both? Organs are usually assessed both anatomically, on imaging and clinical examination, and functionally, by

direct measurement of organ function, or by biochemical biomarkers of function. It is usually functional assessment that drives clinical decision-making. A good example is the thyroid gland, where thyroid function determines treatment more commonly than thyroid anatomy.

Anatomically, lipodystrophies can readily be identified as long as patients are well nourished. In this case, general or regional adipose tissue deficiency is easily observed. However, this can be mimicked or masked by negative energy balance, which leads to “physiological” loss of adipose tissue. Where true lipodystrophy is present, important subtypes may be identified that help to disclose underlying pathological processes. Decreased adipose tissue is associated with increased muscle mass and strength in several developmental forms of lipodystrophy but, in contrast, with decreased muscle mass and bone density in lipodystrophies featuring accelerated mesodermal tissue aging, for example. In diagnosing lipodystrophy, anatomical abnormalities must be assessed in the context of markers of adipose tissue dysfunction. These include low blood concentrations of leptin, and adiponectin, high concentrations of insulin, metabolic dyslipidemia, increased liver lipid content, and systemic inflammation. None of these are specific for lipodystrophy, however.

The challenges in diagnosis were illustrated through discussion of two genetic disorders. *PIK3R1*-related SHORT syndrome features reduced amounts of adipose tissue and is commonly called a form of lipodystrophy. However, serum triglycerides and hepatic fat, serum leptin and adiponectin are usually normal. The mouse model similarly shows decreased adiposity but also decreased serum triglycerides, no systemic inflammation, and healthy adipose tissue histology and gene expression. It also has increased energy expenditure. Is reduced adiposity without evidence of functional adipose failure consistent with true lipodystrophy?

Conversely, Alström syndrome, a complex recessive syndrome, includes severe insulin resistance with low serum adiponectin, dyslipidemia and hepatic steatosis, and premature atherosclerosis, all closely mimicking the biochemical profile of adipose failure seen in severe lipodystrophy. Adiposity, however, is moderately increased. This suggests that Alström syndrome could be regarded as a *forme fruste* of lipodystrophy, but with relative rather than absolute adipose failure.

Thus, the definition of lipodystrophy cannot reliably be based on either form or function alone; lipodystrophies are a heterogeneous group of pathologies for which diagnosis would be accelerated by development of a better biochemical index of adipose failure.

Bone phenotype of familial partial lipodystrophy type 2 (FPLD2)
Professor MC Vantyghem, University of Lille, France

FPLD2 combines partial lipoatrophy, muscle hypertrophy, and skeletal abnormalities such as relatively short fingers and lower limbs. The bone phenotype of lipodystrophic syndromes is controversial, especially in terms of T-score measured in DEXA: no difference in T-score between FPLD2, FPLD1 and obese individuals [2], higher Z-score levels at least in one site, especially trabecular, in generalized lipoatrophy syndromes [3], or lower T-score in HIV-related lipodystrophies [4]. Radiological abnormalities have also been described in generalized lipodystrophies [5]. Given the links between adipocytes, myocytes and chondrocytes, all of which are derived from mesenchymal stem cells, the study of the bone phenotype of FPLD2 is of interest both for the individual management of these rare diseases and pathophysiologically.

Bone is an endocrine organ that secretes FGF23, a hormone that increases urine phosphates, and osteocalcin, a hormone that can increase insulin secretion, insulin sensitivity of adipose tissue, testosterone secretion in men and nutrient uptake by muscle. The main objective of this study was to evaluate the bone phenotype of FPLD2 women with *LMNA R482* mutation, by studying blood markers of bone remodeling (osteocalcin

and crosslaps), and phosphate and calcium parameters, and by assessing body composition by DEXA and MRI, in comparison with age- and sex-matched obese and thin women. The second objective was to specify the determinants of this phenotype.

Lipodystrophy induced by total body irradiation
Dr Anna Stears, University of Cambridge, UK.
Dr. Stears reported the case of 2 patients with a presentation of lipodystrophy following total body irradiation in the setting of hematologic malignancy. The first patient presented partial lipodystrophy, which started 2 years after irradiation. Eight years later, a picture of insulin resistance was revealed, with hypertriglyceridemia, diabetes and hepatic steatosis. Management was based on reducing carbohydrate and fat intake, stopping enteral nutrition and oral nutritional supplements, and increasing physical activity. These lifestyle changes resulted in improvements in diabetes and triglyceride and insulin levels. At last clinical evaluation, the hepatic steatosis had significantly improved, and the question of introducing metreleptin was raised. The second case was that of a patient who, in the aftermath of total body irradiation, showed a presentation of severe insulin resistance, with hypertriglyceridemia, acute pancreatitis, diabetes requiring high doses of ultraconcentrated insulin, and steatosis. In this context of uncontrolled metabolic situation despite a very low fat diet, metreleptin treatment was introduced at 5 mg/d. The metabolic situation was partially improved, with a decrease in triglycerides and in hunger. However, insulin levels remained very high. These two clinical situations illustrate the importance of early detection of metabolic complications following total body irradiation in pediatric cancer. Dietary measures such as a low-fat diet were effective. The role of metreleptin in these situations remains to be defined [6]–[9].

Progressive Encephalopathy with/without Lipodystrophy: an update
Professor David Araujo-Vilar, University of Santiago de Compostela, Spain
Progressive encephalopathy with or without lipodystrophy (PELD), or Celia encephalopathy (10), is a neurodegenerative disease with poor prognosis, associated with lipodystrophy or not. This genetic disease involves variants in the BSCL2 gene located on chromosome 11q12.3, and encoding the protein seipin, which has a highly conserved loop in the lumen of the endoplasmic reticulum, two transmembrane domains and a cytosolic C and N-terminal domain. Seipin forms oligomers to create an overall ring structure with the transmembrane domains in the periphery. There are 3 isoforms: BSCL2-203, BSCL2-205/207/210 and BSCL2-201. These isoforms are differentially expressed in tissues, with 88% of the BSCL2-203 isoform in the brain, and 78% of the BSCL2-205 form in adipose tissue. Seipin is involved in lipid droplet formation, adipogenesis, lipid homeostasis, mitochondrial function, neuronal function and spermatogenesis. The natural history of PELD is the appearance (or not) of congenital generalized lipodystrophy (CGL) in the first months of life, with hypertriglyceridemia, hepatic steatosis, followed by psychomotor retardation around 2 years of age, progressing to a neurodegenerative pathology around 4 years of age and convulsions around 4.5 years of age, with death around 8 years of age. Six BSCL2 variants have been identified in this syndrome: c.985C>T with 9 reported cases; c.974dupG with 11 cases, all CGL; c.1048C>T with 2 cases, all CGL and all neurodegenerative syndrome; c.1076dupC with 1 case; c.566T>A with 2 cases without CGL; and c.445C>G with 1 case. When the mutation affects exon 7 (the Celia seipin), seipin oligomerization is impaired, with formation of large aggregates localized in the nucleus and leading to activation of endoplasmic reticulum stress. This abnormal seipin may impair peroxisome function and biogenesis. PELD has a varied phenotypic spectrum; it is of recessive or dominant origin and always shows neurological involvement of variable degree (from language delay to fatal epileptic encephalopathy). In recessive forms, generalized lipodystrophy is frequent, and overexpression of the short transcript of BSCL2

could be responsible for the neurological damage. The pathological mechanisms of these neurological abnormalities are due to the formation of aggregates of this small seipin isoform, leading to reticulum stress and neuronal damage due to intra-nuclear inclusions. The mechanisms of the dominant forms remain unknown.

Familial partial lipodystrophy syndromes in Spain
Dr Antía Fernández-Pombo, University of Santiago de Compostela, Spain
Between 2001 and 2020, 92 patients were diagnosed with familial partial lipodystrophy syndrome in the Lipodystrophy Unit of Santiago de Compostela, Spain. Dr Antía Fernández-Pombo described this cohort.
Seventy-five of the 92 patients were followed for a median 4.7 years (range, 0.5-17.6); 82.7% were women; median age was 42.7 years. Sixty-six had a pathogenic variant in the *LMNA* gene (FPLD2), 5 in the *PPARG* gene (FPLD3) and 4 in the *PLIN1* gene (FPLD4). 70.7% had muscle hypertrophy, 64% had phlebomegaly, especially in FPLD2 patients (71.2%, versus 20% in FPLD3 patients and 0% in FPLD4), and 41.3% had insulin resistance. Among the FPLD2 patients, only those with the N466 variant had pancreatitis (20%); triglycerides were higher in FPLD2 patients with the N466 variant; and skinfolds were thinner in the FPLD2 R482 group than in the N466 group. Thus, some phenotypic and metabolic characteristics in FPLD2 patients suggest heterogeneity even in forms due to *LMNA* exon 8 variants.
Adipose tissue loss was less severe in FPLD4 and FPLD3 patients, which may explain their later diagnosis. FPLD3 patients had a higher prevalence of metabolic complications, suggesting that their metabolic severity is not due solely to the defect in adipose tissue expandability. The prevalence of metabolic complications in this cohort is consistent with that previously described in the literature. However, fewer cardiovascular complications were found in these subjects (although prevalence was higher than in the general population).

Lipodystrophy UK (LDUK), Rebecca Sanders
The English Lipodystrophy Association, LDUK, represented by Rebecca Sanders, presented the objectives of its organization: 1) to increase awareness of the condition so that appropriate diagnosis can be made as early as possible, to improve longer-term prognosis; 2) to help improve the quality of life of patients and their families; and 3) to build a sustainable active organization. The association has set up a website: www.lipodystrophyuk.org. It participates in various research projects. For example, an evaluation of chronic fatigue is proposed to lipodystrophy patients in collaboration with the Pain Institute, using mixed quantitative and qualitative methods. The objective is to include 50 patients, who will be evaluated 3 times over a period of 6 to 9 months. This study will start in the summer of 2022. In addition, a survey was conducted to evaluate the care pathway of patients with lipodystrophy. Twenty-five patients and 17 clinicians were interviewed (UK, USA, Canada). Patients reported a difficult journey to final diagnosis. Outside of the specialized service, the patients described the lack of local care. They also reported a lack of coordination between care services. Patients most often have to explain to the health professional what lipodystrophy is. The main conclusions of this work are the need for better knowledge of lipodystrophy within the health care community, and the need for better coordination between the different actors in the care of these patients [11]

Reading WAT dysfunction and lipodystrophy through the lens of dysregulated miRNA
Dr Margherita Maffei, National Research Council, Institute of Clinical Physiology, Pisa, Italy

The general objective of this study is to uncover novel signatures of lipodystrophic (LD) syndromes by identifying differentially expressed circulating microRNAs (DE cmiRNAs) and then to correlate the abundance of these novel biomarkers with typical metabolic derangements of the disease and with WAT/adipocyte dysfunction.
An unbiased approach based on miRNOME profiling performed in a small cohort of LD subjects and matched controls (n=8) identified 8 DE cmiRNAs. The subsequent steps of validation and characterization were performed in larger cohorts (n=30) by quantitative PCR, and the results for 6 miRNAs are presented in more detail, including 5 members of the miRNA family 320 (a-e) and miRNA 196a5p.
Members a-3p, b, c, e of the 320 family were upregulated, while 320d and 196a-5p were downregulated in LD subjects. The difference remained statistically significant on analysis of subsets defined by specific LD subtypes (e.g., CGL, APL, FPLD2, etc.), except for FPLD1, which showed a distribution of circulating miRNAs 320 and 196a-5p totally overlapping with that of the controls. Expression of the 6 miRNAs was detected in the adipocyte.
CmiRs-320a-3p showed significant inverse relationships with plasma leptin, typically low in LD, while downregulation of cmiR-320d predicted an altered hepatic profile and greater inflammation. Gene ontology analysis revealed cell-cell adhesion as a process regulated by 320 miRNAs targets.
Interestingly, expression of miR196a-5p was reduced not only in the blood of LD subjects but in LD adipose tissue biopsies. Further, its expression was regulated during the adipogenesis of the human preadipocyte cell line SGBS, and transfection of a miR 196a5p mimic in normal condition or upon exposure to protease inhibitors, a treatment reported to impair adipogenesis, resulted in upregulation of typical markers of terminal adipocyte differentiation.
In conclusion, we established a correlation between LD syndromes and an altered abundance of specific circulating miRs 320a-e and 196a-5p. Their expression by WAT and the adipocyte is now demonstrated, and studies are ongoing to better elucidate the specific source or sources of dysregulation and to understand whether they constitute molecular switches implicated in LD pathogenesis.

Genome architecture during adipogenesis
Professor Philippe Collas, University of Oslo, Norway
Pr. Philippe Collas works on the **dynamic remodeling of chromatin during adipogenesis** (differentiation of precursor cells into adipocytes). His work shows that chromatin dynamically interacts with the nuclear submembrane protein network of A- and B-type lamins, defining specific functional regions called LADs (lamina-associated domains). LADs, located at the periphery of the cell nucleus, are poor in genes and enriched in heterochromatin: i.e., DNA regions with little transcriptional activity (generating little gene expression). During adipocyte differentiation, LADs are repositioned along chromosomes and can be modified, leading to the expression of genes that were repressed in undifferentiated stem cells, and conversely to the extinction of pluripotency genes. These "waves" of changes in specific gene expression commit the undifferentiated cell to a specific differentiated lineage (e.g., adipocyte or endothelial). Mutations in the *LMNA* gene, by modifying LAD structure, also modify these waves of epigenetic regulation of gene expression, which disrupts cell differentiation not only into adipocytes but also into endothelial cells.

Dissociating obesity and metabolic complications
Professor Toni Vidal-Puig, University of Cambridge, UK
Pr. Vidal-Puig's work focuses on the expansibility of adipose tissue. The expansion capacity of adipose tissue is not infinite. If the point of maximum expansion is reached,

fatty acids are stored ectopically, leading to metabolic complications; this is the concept of lipotoxicity. Adipose tissue fibrosis is linked to local inflammation, leading to an imbalance between collagen synthesis and degradation. GWAS (Genome-Wide Association Studies) techniques have identified regions of the genome involved in insulin resistance, such as the locus near the *PEPD (Peptidase D)* gene. The protein produced by this gene is a peptidase that plays a role in the degradation of collagen, and is also a ligand of *EGFR (Epithelial Growth Factor Receptor).* Decreased expression of this enzyme in human adipose, and especially visceral, tissue is associated with release of the enzyme, fibrosis and type-2 diabetes. In mouse models, pharmacological inhibition of PEPD was associated with an increase in fibrosis, blood glucose and insulin resistance. PEPD is also expressed in macrophages, immune cells involved in adipose tissue inflammation. The decrease in the enzymatic activity of PEPD in macrophages contributes to adipose tissue dysfunction, hepatic steatosis and insulin resistance. In adipose tissue, release of the protein by macrophages leads, via its role as an EGFR ligand, to an alteration of adipogenesis, fibro-inflammation and insulin resistance of adipose tissue (autocrine and paracrine pathways) [12], [13].

Natural history and disease progression in generalized lipodystrophy
Dr. Baris Akinci, Dokuz Eylul University, Turkey

Dr. Baris Akinci presented findings from the Turkish Lipodystrophy Registry regarding the natural history of congenital (CGL) or acquired (AGL) generalized lipodystrophy. Unfortunately, Turkey has little access to treatments to help control metabolic disorders in patients with lipodystrophy. In particular, in Turkey, the prescription of GLP1 agonists is limited to cases of obesity, which prevents patients with lipodystrophy from benefiting from them. Metreleptin is also difficult to access. Thus, the longitudinal description of Turkish patients with lipodystrophy is somewhat similar to that of the spontaneous course of the disease. The study of the Turkish cohort, comprising more than 70 patients with different forms of generalized lipodystrophy (GL) (CGL or AGL; over 60% women), indicates that the disease is severe, leading to early metabolic complications (diabetes in more than half the patients, hypertriglyceridemia in nearly 90%, liver steatosis in more than 80%; all commonly diagnosed in the first decades of life). Also, end-organ complications are prevalent in GL and can develop early in life. These organ complications may lead to serious morbidity and mortality at relatively young ages. Patients with CGL2 generally have earlier and more severe liver disease than patients with CGL1. In the CGL1 group, women were younger than men at diagnosis of diabetes and had higher HbA1c and triglyceride concentrations. In contrast to previous reports, patients with CGL4 may have overt diabetes. Bone cysts associated with CGL can be complicated by fractures. The most common causes of mortality are related to metabolic and/or infectious complications.

The Spanish Association of Families and Patients with Lipodystrophy, AELIP
Ms. Naca Perez de Tudela and Mr. Juan Carrion Tudela

AELIP is an international association of relatives and people affected by lipodystrophies that was created in 2012 to continue the legacy of Celia Carrión Pérez de Tudela; the main objective is to improve the quality of life of people and families living with lipodystrophy worldwide.

There are 2 priority areas.

Firstly, AELIP supports and promotes research into lipodystrophies, assigning 75% of its annual budget to the 2 lines of research that currently exist in Spain. Since its foundation, AELIP has donated a total of €240,000 to lipodystrophy research projects.

Secondly, AELIP implements and develops a portfolio of services to respond to all the social and health needs of people and families affected by infrequent lipodystrophy syndrome: information and orientation in lipodystrophies, psychological support, dietetic

counseling, and legal advice. AELIP works hand in hand with an international panel of experts that responds to the medical consultation reports it receives, positioning it as a reference entity at a global level.
AELIP considers networking to be essential, which is why it has been coordinating and implementing World Lipodystrophy Day since its foundation, commemorated since March 31, 2013.
AELIP is also a member of the Spanish Federation of Rare Diseases (FEDER), Spanish Society of Lipodystrophies (SEL), Spanish Society of Endocrinology and Nutrition (SEEN), EURORDIS and the European Consortium of Lipodystrophies (ECLIP) at European level and, at international level, the Ibero-American Alliance of Rare Diseases (ALIBER) and the World Network of Rare Diseases (RDI). More information at www.aelip.org [14].

Caveolin-1, caveolae and congenital generalized lipodystrophy
Corinne Vigouroux, CRMR PRISIS, Paris, France
The *CAV1* gene encodes caveolin-1, a major protein of caveolae, which are plasma membrane microdomains involved in cell signaling. Until recently, only one patient had been described with a homozygous null variant of *CAV1*, associated with generalized congenital lipodystrophy (CGL3, Kim et al., JCEM 2008). We explored a consanguineous family referred for generalized congenital lipodystrophy by next-generation sequencing, and clinical, radiological and metabolic investigations were performed. We studied skin fibroblasts from the index case and the previously reported patient. We also identified a novel homozygous *CAV1* p.(His79Glnfs*3) variant, which predicts the synthesis of a protein truncated in its N-terminal portion, in 4 patients aged 8 months to 18 years with generalized lipodystrophy, insulin resistance, low HDL cholesterol and/or high triglycerides. Dysphagia due to esophageal achalasia was diagnosed in the 2 affected adolescents, aged 15 and 18 years; heterozygous relatives (n=9) were asymptomatic. Cultured fibroblasts from 2 patients showed complete absence of caveolae at the plasma membrane and loss of protein expression of caveolin-1 and its partners caveolin-2 and cavin-1. These abnormalities were accompanied by insulin resistance, with increased oxidative stress and premature cell senescence. This study showed that pathogenic variants of *CAV1* lead to a syndrome of congenital lipodystrophy with metabolic complications, with autosomal recessive transmission. The defect in caveolin-1 expression and/or the absence of caveolae induce specific clinical manifestations, including esophageal achalasia requiring specific management. In addition, other heterozygous variants of the *CAV1* gene which predict alterations of the C-terminal protein have been associated with other phenotypes: partial lipodystrophy with neurologic disorders (1 family), pulmonary arterial hypertension (a few families), and neonatal progeroid syndrome in 2 patients (heterozygous *de novo* variants) [15].

Set-up of a therapeutic education program on lipodystrophy syndromes
Dr Camille Vatier, Sorbonne University Paris
Therapeutic patient education (TPE) is defined by the World Health Organization as helping patients acquire or maintain the skills they need to manage their lives with a chronic disease. The WHO estimates that 80% of patients followed in the community have a chronic disease, so the challenge of TPE is major. Numerous publications have shown the beneficial effect of TPE on objective parameters and on patients' quality of life in many chronic diseases, including diabetes, with a decrease in HbA1c from 0.1% to 0.9%, better compliance with treatment and a better quality of life. For rare diseases, few data exist to date. Lipodystrophic syndromes combine metabolic and cardiovascular complications, low self-esteem, and a significant impact on quality of life. In France, the rare disease plan finances of TPE projects, including the LIPEA program for children and adults with lipodystrophic syndromes. This program was developed jointly with the medical, nursing,

dietetic and psychology teams of the endocrinology departments of the Saint Antoine and Robert Debré hospitals in Paris, the regional hospital of Lille and the university hospital of La Réunion. Based on the needs of the patients, identified using a questionnaire distributed by the AFLIP patient association, the program was built around 4 axes, with 1 whole day in small groups of 4 to 6 patients suffering from congenital generalized lipodystrophy (CGL) or familial partial lipodystrophy (FPL), of the same gender and age group. The first workshop is medical, to understand the disease, with adipocytes to be placed on body diagrams of non-lipodystrophic and lipodystrophic patients. The second is dietetic, to define together how to eat healthily and to draw up recipes. The third is psychological, to talk about the disease with emoticons as tools. And the last is socio-esthetic, to work on body image. Since 2021, 17 patients have taken part in Paris. Impact will soon be evaluated on metabolic parameters and on quality of life, anxiety, self-image and self-esteem.

Metabolic effects of metreleptin in patients with lipodystrophy: real-life experience in a French cohort
Dr Héléna Mosbah, PRISIS, Hôpital Saint Antoine, Paris
This is a retrospective French study, conducted within the PRISIS network, in patients with lipodystrophy who were treated with metreleptin between 2009 and 2020 [16]. Clinical and biological data were collected before and 1 year after treatment initiation and at long-term. Forty-seven patients were included: 28 with generalized lipodystrophy and 19 with partial lipodystrophy. Median overall follow-up was 37 months. In patients with generalized lipodystrophy, HbA1c and triglycerides decreased significantly at 1 year and beyond. Body mass index (BMI), liver enzymes and albuminuria were significantly reduced at 1 year. In patients with partial lipodystrophy, the course was different: HbA1c did not decrease, while triglycerides and BMI decreased significantly at 1 year. Patients with uncontrolled diabetes and/or high triglyceride level were mostly responsive to treatment. In patients with partial lipodystrophy, patients who responded to treatment were less often on insulin and had lower serum leptin levels than non-responders.

EPHX1 mutations cause lipoatrophic diabetes syndrome due to impaired epoxide hydrolysis and increased cellular senescence
Dr Isabelle Jéru, National Reference Centre for Rare Diseases of Insulin Secretion and Insulin Sensitivity, Assistance Publique-Hôpitaux de Paris, France
The discovery of the first epoxide hydrolase (EH), EPHX1, dates back more than 40 years and today 5 genes encoding EHs are known. EHs are enzymes that regulate cellular homeostasis by hydrolyzing various epoxide substrates into less reactive diols. The functions of EHs not fully known, and no pathogenic variants in this class of genes have been reported in humans to date. This study describes two independent families that were subjected to trio exome analyses. The effect of the identified EPHX1 variants was modeled in different cell systems: HEK293 cells expressing normal and mutated forms of the protein, murine 3T3-L1 pre-adipocyte cells and human adipocyte stem cells (ASCs) in which EPHX1 was inactivated by a CRISPR-Cas9 system, as well as patient fibroblasts. The authors identified 2 de-novo pathogenic variants located in the catalytic site of EPHX1 in patients with complex lipoatrophic diabetes characterized by adipose tissue loss, insulin resistance, and other multiple organ involvement. Analysis of HEK293 cells revealed that these variants led to aggregation of the protein in the endoplasmic reticulum and a loss of its epoxide hydrolysis activity. Knockout of Ephx1 in 3T3-L1 and ASCs abolishes adipocyte differentiation and inhibits the insulin response. The study of these two cell types also revealed major oxidative stress and cellular senescence, confirmed on patient fibroblasts. Finally, a beneficial effect of metreleptin treatment was observed. This translational study

underlines the importance of epoxide regulation in adipocyte function and insulin resistance and provides new insights into the physiological roles of EHs in humans.

An update on lipodystrophy research from Michigan: two evolving stories
Professor Elif Oral, University of Michigan, USA
Pr. Elif Oral reported on the 3 missions of his research group: to discover new forms of lipodystrophy, discover new mechanisms and discover new treatments.
In total through 2018, 269 patients were referred to his center for suspected lipodystrophy. 191 were patients with lipodystrophy or relatives, 43 had no real lipodystrophy and 35 had other atypical pathologies. In these patients, his team was able to reveal new clinical phenotypes, with variants of known genes (*LMNA, PPARG, MFN2,* (lipodystrophy, lipomatosis), *MC4R (*monogenic obesity)), new variants of known genes (*PI3KR1, FBN1* (lipodystrophies), *SH2B, SRC1* (heterozygous monogenic obesity)), groupings of phenotypes without gene association (acquired generalized lipodystrophy with immune dysregulation, abrupt onset obesity with autoimmune terrain, patients with suprasellar tumor and fat distribution abnormality), or new target genes in families or index cases *(EBF2*, the expression of which is thought to be important for adipogenesis).

Investigating potential therapeutic approaches and molecular pathophysiology in lipodystrophic seipin-deficient mice
Professor Justin Rochford, University of Aberdeen, UK
Pr. Rochford presented the recent results obtained by his research group using the seipin gene-validated mouse model. This model mimics the phenotype of congenital generalized lipodystrophy, with the exception of hypertriglyceridemia, which is not observed in the mouse model of the disease. The increase in beta-pancreatic cell mass in this model is indicative of the pancreatic response to extreme insulin resistance. Increased susceptibility to bacterial infection, reported to be an important cause of mortality in patients with congenital generalized lipodystrophy (CGL), was not found in the mouse model, in which seipin invalidation was restricted to myeloid cells. This suggests that the infectious pathologies in these patients are not due to seipin deficiency, but more likely result from underlying metabolic disorder. The mouse model has also allowed exploration of several therapeutic avenues in CGL.

Anti-metreleptin antibody: what do we know?
Professor Ferruccio Santini and Professor Giovanni Ceccarini, Obesity and Lipodystrophy Center at the University Hospital of Pisa, Italy
The Italian team recalled that any protein treatment could potentially induce the production of antibodies, even if the treatment was initiated when the protein is not totally absent from the organism (examples of hormonal treatments by insulin, ACTH, PTH, EPO or by cytokines: TNF, interferons). The antibodies produced are polyclonal, of different isotypes (IgM, Ig G, IgE) and of different subclasses. They are observed in up to 70% of patients and can bind to the protein and sometimes neutralize its activity. Metreleptin is a 16kDa protein synthesized by E. coli. In the American obese or lipodystrophic FHA101 cohort, treated with metreleptin, antibodies measured by ELISA were found in a large majority of patients: 96-100% of obese patients and 86-92% of patients with lipodystrophy. Antibody concentration usually peaks between 3 and 6 months after treatment initiation but sometimes even after 3 years' treatment, and decreases thereafter. The presence of antibodies affects the measurement of leptin concentration. Three patients with obesity developed neutralizing antibodies concomitantly with weight regain. Four patients with generalized lipodystrophy also developed neutralizing antibodies concomitantly with worsening of metabolic control; in 1 of these, neutralizing antibodies disappeared during metreleptin treatment.

The team discussed clinical cases characterized by the appearance of neutralizing antibodies, and reported 1 case of disappearance of antibodies after continuing metreleptin treatment at increased dose. Because of the difficulty of standardizing the measurement of leptin antibodies, an alternative would be to measure the concentration of leptin in serum after precipitation with polyethylene glycol (PEG), an easy and rapid method. Many questions remain concerning these antibodies: do they interfere with the half-life of metreleptin? Do they induce leptin-resistance and, if so, by what mechanism or mechanisms: blood-brain barrier transport? binding to its receptors? When and how is it necessary to measure them and what is the best strategy to encompass leptin resistance induced by neutralizing antibodies? [17], [18]

Seipin localizes at endoplasmic-reticulum/mitochondria contact sites to control mitochondrial calcium import and metabolism in adipocytes
Professor Xavier Prieur, University of Nantes, France
Loss-of-function variants of the *BSCL2* gene encoding the seipin protein localized in the endoplasmic reticulum (ER) lead to generalized lipodystrophy. The objective of this study was to better understand the associated pathophysiological mechanisms, which remain poorly understood. The study describes mitochondrial abnormalities and altered oxygen consumption in cells of seipin-deficient patients. Seipin enrichment is observed at ER-mitochondrial contact sites (MAMs) in human and mouse cells. Immunoprecipitation followed by mass spectrometry analysis showed an interaction between seipin and calcium regulators, in particular SERCA2, IP3R and VDAC. In a 3T3-L1 cell model with *BSCL2* gene siRNA, loss of seipin resulted in a defect in mitochondrial calcium import accompanied by generalized reduction in Krebs cycle metabolites and ATP levels. The authors then sought to determine the effect of nutritional status on seipin function using a proximity ligation assay. Association of seipin with MAM calcium regulators was stimulated by fasting-type stimuli, whereas association of seipin with lipid droplets was promoted by lipid loading. In a KO mouse model, inducible deletion of seipin resulted in mitochondrial dysfunction preceding the development of metabolic complications. Taken together, these data suggest that seipin controls mitochondrial energy metabolism by regulating mitochondrial calcium influx at MAMs. In seipin-deficient adipose tissue, the reduced production of ATP alters the properties of adipocytes, and could thus contribute to the pathogenesis of lipodystrophy.

Natural course of lipodystrophy syndromes: a 10 year-observational study
Ekaterina Sorkina, MD, PhD, Endocrinology Research Centre, Moscow, Russia
In 2011 the first family with familial partial lipodystrophy type 2 (FPL2) was diagnosed by Pr. Sorkina's team in Russia. Since 2012, an active search for patients with different lipodystrophy syndromes was started and, by April 8, 2022, 100 patients had been identified, 70 of whom are still being actively followed up. Seven died and 23 were lost to follow-up. To date, only 1 of the patients with congenital generalized lipodystrophy type 4 (CGL4) received metreleptin therapy; 5 FPL patients are in the process of getting access to this therapy.
The aim of this project was to identify the main types of lipodystrophy syndrome in Russia and the main causes of death in this population.
The structure of lipodystrophy syndromes in the 70 surviving patients in this cohort was the following:
-Generalized lipodystrophy (GL): 10 patients; 5 with CGL (3 genetically proven: 2 *AGPAT2*, 1 *BSCL2*) and 5 with acquired GL (AGL);
-Partial lipodystrophy (PL): 50 patients; 4 with APL, 46 with FPL, with 23 genetically proven: 11 FPL2 (*LMNA*), 7 FPL3 (*PPARG*), and 5 FPL4 (*PLIN1*).
-Multiple symmetric lipomatosis: 2 patients;

- Lipodystrophy associated with progeroid syndromes: 8 patients; 5 *LMNA*, 1 *WRN*, 1 *POLD1*, and 1 other type.

The causes of death in the 7 deceased patients were:
- gastrointestinal bleeding in a 40 y.o. CGL4 female with gigantic trophic ulcers;
- acute thrombosis after COVID-19 in a 45 y.o. *WRN* patient;
- acute respiratory failure due to H1N1-associated pneumonia in a 5 y.o. male with AGL and APS1;
- pancreatic cancer in a 52 y.o. male with FPL of unknown etiology;
- multiple organ system failure in a 36 y.o. male with AGL + neurofibromatosis type 1;
- acute myocardial infarction, heart failure in a 60 y.o. male (father of the patients) with FPL2 (LMNA p.R482W);
- extensive myocardial infarction in a 65 y.o. male (uncle of the patients) with FPL3, (PPARG p.R212Q).

Four-year results of the ECLip Registry
Professor Martin Wabitsch and Dr Julia von Schnurbein, University of Ulm, Germany

Dr. Julia Von Schnurbein presented some results from the European ECLip registry, which she coordinates with Pr. Martin Wabitsch at the University of Ulm (Germany) and Pr. David Araujo-Vilar (Santiago de Compostela, Spain). The ECLip lipodystrophy registry was set up in late 2017, and the principles of its operation were published in Orphanet, Journal of Rare Diseases [1]. Currently 17 European clinical centers contribute to the registry, and 517 patients have been included. Six new centers will join the project soon. Eighty percent of the patients included are women, 54% have familial partial lipodystrophy and 18% have congenital generalized lipodystrophy. At the molecular level, the main etiological form of lipodystrophy is partial lipodystrophy related to *LMNA* variants (FPLD2), which represents 39% of the patients. Metabolic complications are frequent, with nearly 80% of patients presenting dyslipidemia, more than 50% diabetes, and more than 50% liver steatosis. Cross-sectional data from the registry will be published in the future under the auspices of ECLip.

CONCLUSION and PERSPECTIVES

This publication allows the ECLip network to accomplish one of its missions: to increase knowledge in the field in cooperation with patient advocacy groups. Clinical experience and fundamental research presented during the Cambridge meeting showed the advances in the field, with cooperation between East and West. Noticeably, the data from the national registries and European registry are now sufficient to show that lipodystrophy syndromes are present everywhere, with wide clinical and genetic heterogeneity, but are often underdiagnosed. New specific causes of lipodystrophy and mechanisms of insulin resistance have been identified, and further research is ongoing. The ECLip network is currently working on a new classification of lipodystrophy syndromes, to help diagnosis. Future collaborations aim to improve therapeutic strategies, and define new approaches based on better understanding of the mechanisms involved.

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