Lipodystrophies: windows on adipose biology and metabolism

Robert A. Hegele, Tisha R. Joy, Salam Al-Attar and Brian K. Rutt

Robarts Research Institute and
Schulich School of Medicine and Dentistry,
University of Western Ontario
London, Ontario, Canada N6A 5K8

Correspondence:
Robert A. Hegele MD, FRCP(C), FACP
Blackburn Cardiovascular Genetics Laboratory
Robarts Research Institute
406-100 Perth Drive, London, ON, Canada N6A 5K8
Email: hegele@robarts.ca; Tel: 519-663-3461; Fax: 519-663-3037

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ABSTRACT

The lipodystrophies are characterized by loss of adipose tissue in some anatomical sites, frequently with fat accumulation in non-atrophic depots and ectopic sites such as liver and muscle. Molecularly characterized forms include Dunnigan-type familial partial lipodystrophy (FPLD), partial lipodystrophy with mandibuloacral dysplasia (MAD), Berardinelli-Seip congenital generalized lipodystrophy (CGL) and some cases with Barraquer-Simons acquired partial lipodystrophy (APL). The associated mutant gene products include: 1) nuclear lamin A in FPLD type 2 and MAD type A; 2) nuclear lamin B2 in APL; 3) nuclear hormone receptor peroxisome proliferator activated receptor γ (PPARγ) in FPLD type 3; 4) the lipid biosynthetic enzyme in 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2) in CGL type 1; 5) integral endoplasmic reticulum membrane protein seipin in CGL type 2; and 6) metalloproteinase ZMPSTE24 in MAD type B. An unresolved question is whether metabolic disturbances are secondary to adipose repartitioning or result from a direct effect of the mutant gene product. Careful analysis of clinical, biochemical and imaging phenotypes – using an approach called “phenomics” – reveals differences between genetically stratified subtypes that can be used to guide basic experiments and to improve understanding of common clinical entities, such as metabolic syndrome or the partial lipodystrophy syndrome associated with HIV infection (HIVPL).

Supplementary key words: fatty acid, metabolism, obesity, type 2 diabetes, insulin resistance, dyslipidemia, genetics, medical imaging, laminopathy, nuclear hormone receptors
Lipodystrophies are an interesting group of clinical disorders that are most often characterized by lipoatrophy or selective loss of adipose tissue from particular anatomical regions, ranging from localized to generalized (1). The extent of adipose loss usually determines the severity of the associated clinical and metabolic manifestations (2). Expansion of spared adipose stores in partial lipodystrophies is one likely mechanism that leads to clinical and metabolic manifestations (3). Patients with lipodystrophy often have some of the disturbances that define the common metabolic syndrome, such as increased visceral fat, dyslipidemia (elevated triglycerides and depressed high-density lipoprotein [HDL]), hypertension, dysglycemia and insulin resistance and sometimes increased predisposition to atherosclerosis (3).

Lipodystrophies can broadly be classified into “familial” or “genetic” and “acquired” types (1-4). The molecular basis of disease has been characterized in two subtypes of congenital generalized lipodystrophy (CGL), in two subtypes of familial partial lipodystrophy (FPLD) and in some patients with acquired partial lipodystrophy (APL). Lipodystrophy can also be a component of certain rare inherited multi-system syndromes (1). It can also appear to be “acquired” without an obvious germline basis, such as in some patients with acquired generalized lipodystrophy (AGL), and in the partial lipodystrophy syndrome that is associated with infection and treatment of HIV (HIVPL) (1). Localized lipodystrophies characterized by loss of subcutaneous fat from small regions of a limb can be drug-induced, pressure-induced, panniculitis-induced or idiopathic (1, 2). Although insulin resistance in lipodystrophies could be secondary to adipose
redistribution and/or central obesity, the altered products of the mutated causative genes might also act directly in pathogenesis, and might illuminate new causative mechanisms for common insulin resistance (3, 4).

Prior to the molecular genetic era, the classification of lipodystrophies was based on clinical features, mainly the pattern and extent of adipose tissue loss and the evidence for heritability. Defining the molecular genetic basis of certain lipodystrophies has since shown heterogeneity both with respect to causative genes and the range of mutations within causative genes. This review will begin with general – “pre-molecular” - clinical descriptions of various lipodystrophies, followed by summations of current molecular genetic understanding and then brief discussions of how post-genomic molecular stratification and careful evaluation using “phenomics” (4) including magnetic resonance imaging have revealed subtle differences (Table 1) that can suggest hypotheses for future cellular and molecular studies. Finally, other important aspects and forms of lipodystrophy, including HIVPL, will be discussed in the context of the molecular genetics of inherited lipodystrophies.

Congenital generalized lipodystrophy (CGL; Berardinelli-Seip syndrome)

Clinical features of CGL

Congenital generalized lipodystrophy (CGL) was first described more than a half-century ago by Berardinelli and later by Seip (5, 6). CGL is inherited in an autosomal recessive manner and is clinically characterized by generalized absence or near-absence of adipose tissue. Affected individuals are usually
recognized soon after birth because of almost complete lack of fat and prominent musculature. The childhood years are distinguished by a voracious appetite, accelerated linear growth, advanced bone age, and marked acanthosis nigricans (7, 8). Acromegaloid features, including enlargement of hands, feet, and jaw, are often present. Associated features include umbilical hernia, hepatomegaly secondary to hepatic steatosis that can progress to cirrhosis, splenomegaly, lymphadenopathy and focal lytic lesions of the appendicular bones (7-9). Cardiomyopathy and mental retardation may variably occur (10, 11). Metabolic complications include fasting hyperglycemia, diabetes (often with marked insulin resistance), hypertriglyceridemia (sometimes resulting in pancreatitis), depressed HDL cholesterol and markedly depressed plasma adiponectin and leptin (12). Affected women can have hirsutism, polycystic ovarian syndrome (PCOS) and menstrual irregularities while among men reproductive function appears to be unaffected (13).

Molecular genetics of CGL

CGL was first mapped genetically to chromosome 9q34 (14); this locus is now designated as CGL1 (MIM 608594) (14). BSCL1 is caused by mutations in the AGPAT2 gene, which encodes 1-acylglycerol-3-phosphate O-acyltransferase 2, also called LPA acyltransferase (LPAAT)-β (15), or 1-acyl-sn-glycerol-3-phosphate acetyltransferase (EC 2.3.1.51). AGPAT2 is important in the metabolism of lysophosphatidic acid (LPA), and was correlated with enhanced transcription and synthesis of interleukin-6 and tumor necrosis factor-α,
consistent with a link between adipocyte biology and cytokine expression (16). Of the genes so far discovered to be causative for the various lipodystrophy syndromes, AGPAT2 seems to be the most reasonable due to its biochemical activity, although the precise pathogenic mechanism causing a specific impact on adipose tissue remains undefined. AGPAT2 mutations in CGL1 are shown in Figure 1. Most AGPAT2 mutations in CGL1 are of the nonsense or aberrant splicing variety. Most would result in complete deficient enzyme function in the homozygous state. There is no obvious correlation of the mutation severity with phenotype severity. Murine studies have shown a lipodystrophy phenotype in mice in whom the closely related Agpat6 gene encoding the related enzyme AGPAT6 has been deleted (17), but screening of human lipodystrophy patients has shown no potentially disease-causing mutations in AGPAT6 (Hegele, unpublished observations).

Homozygosity mapping in CGL families from Lebanon and Norway identified a second locus on chromosome 11q13 (18), now designated CGL2 (MIM 269700). Using microsatellite markers, the minimal region for this locus was narrowed to a single causative gene that had 87% identity to the mouse 'gamma-3-linked gene' (Gng3lg) product and partial homology to Drosophila CG9904 protein (18). This open-reading frame of this gene, also called BSCL2 (MIM 606158), encodes a deduced 398-amino acid integral membrane protein localized to the endoplasmic reticulum of eukaryotic cells, dubbed “seipin” (18, 19). This protein is expressed mainly in brain and testes; it has at least two hydrophobic amino acid stretches, but as yet its function – and thus the
mechanism(s) by which altered function might lead to CGL2 - is largely unknown (19). To date, >12 mutations in the BSCL2 gene have been identified (Figure 2). Most BSCL2 mutations in CGL2 are of the nonsense or aberrant splicing variety. Most would result in complete deficient protein function in the homozygous state. There is no obvious correlation between mutation severity and phenotype severity. Interestingly, an unrelated neurological disorder, namely distal spinal muscular atrophy type 5 (Silver syndrome; MIM 600794) was found to be due to heterozygous mutations in BSCL2 (20). Screening studies indicate that ~50% of individuals with a clinical diagnosis of CGL have no sequence mutation in either AGPAT2 or BSCL2, suggesting the existence of other loci (Hegele, unpublished observations).

CGL1 and CGL2 phenotypes considered in light of molecular diagnosis

The literature contains fairly detailed clinical reports of >200 CGL patients, with approximately equal numbers of CGL1 and CGL2 subjects. These relatively large numbers have allowed for the comparisons of specific attributes between the two molecular types (Table 1). For instance, it would appear that hepatic dysfunction, hyperlipidemia, diabetes mellitus and hypertrophic cardiomyopathy were each significant contributors to morbidity to subjects with both CGL1 and CGL2, with no clear differences in their prevalence (21). However, CGL2 appeared to have a higher incidence of premature death than CGL1 and a pattern of lipodystrophy that was distinguished by earlier-onset and greater severity (21). Also, subjects with CGL2 had a significantly higher prevalence of
intellectual impairment than those with CGL1 or CGL with no detected molecular basis (21, 22). In addition, cystic angiomatosis with progressive incapacitating bone involvement was associated with mutations in AGPAT2 but not seipin (22), clarifying that a syndrome comprised of CGL with systemic cystic angiomatosis - sometimes called “Brunzell syndrome” (23) – was actually a subtype of CGL1. Cardiomyopathy appears to be more severe in CGL2 (11). A multinational study showed lower serum leptin and earlier diabetes onset in CGL2 compared to CGL1 (10), while a study in Brazilian patients showed lower serum leptin in CGL1 compared to CGL2, but earlier diabetes onset and higher serum insulin in CGL2 compared to CGL1 (24). Finally, both CGL1 and CGL2 subtypes demonstrate a lack of metabolically active adipose tissue within most subcutaneous, intermuscular, bone marrow, intra-abdominal, and intrathoracic sites (25). However, mechanical adipose tissue in palms, soles, orbits, scalp, and periarticular regions was absent in CGL2 but not in CGL1 (25). Together these findings indicate that CGL2 is a more severe phenotype than CGL1, with more extensive fat loss and biochemical changes, more severe cardiomyopathy and intellectual impairment, earlier diabetes onset and possibly earlier mortality.

**Familial partial lipodystrophy (FPLD; Dunnigan or Kobberling syndrome)**

Clinical features of FPLD

FPLD, originally described in the 1970s independently by Kobberling (26) and Dunnigan (27), often shows autosomal dominant inheritance. FPLD is generally characterized by progressive and gradual subcutaneous adipose tissue
loss from the extremities, classically commencing in puberty (26-28). Thus, during infancy and childhood, affected individuals cannot be easily distinguished clinically from unaffected individuals. Across all FPLD types, the loss of adipose tissue from the extremities is accompanied by variable adipose tissue loss in the trunk and chest. As well, increased fat deposition within muscles and liver can occur (29-32). Metabolic manifestations of FPLD include hypertriglyceridemia, depressed HDL cholesterol, dysglycemia developing into diabetes, acanthosis nigricans, and among women, hirsutism, PCOS, and menstrual irregularities (33). The risk of developing diabetes is higher among women than men, particularly for multiparous women with excessive central adipose deposition (34).

Molecular genetics of FPLD

FPLD is subdivided into three forms – FPLD1 (Kobberling-type; MIM 608600), FPLD2 (Dunnigan-type; MIM 151660) and FPLD3 (MIM 603637) (31, 35, 36). FPLD1 has an unknown molecular basis. FPLD2 results from heterozygous mutations in the \textit{LMNA} gene encoding nuclear lamin A/C (MIM 150330) (35). The \textit{LMNA} mutations implicated in FPLD2 are shown in Figure 2. Most \textit{LMNA} mutations in FPLD2 are of the missense variety, with only one splicing mutation so far identified (Figure 2). Furthermore, screening for larger-scale genomic variants in \textit{LMNA} such as deletions and duplications in patients with lipodystrophy has so far revealed no mutations of this type (Hegele, unpublished observations). The family of diseases that result from >100 nuclear lamin mutations are called “laminopathies” and in addition to FPLD2 mutations in
LMNA can cause Hutchinson Gilford progeria syndrome (HGPS), mandibuloacral dysplasia (MAD), Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy, dilated cardiomyopathies, cardiac conduction defects, Charcot-Marie-Tooth disease, atypical Werner syndrome and various overlapping syndromes (37). Most FPLD2 mutations in LMNA are missense mutations within the 3’ end of the gene; exons 11 and 12 are specific for the lamin A isoform, so that missense mutations in these exons implicate the lamin A isoform in FPLD2 (37). Most LMNA mutations in FPLD2 are downstream of the nuclear localization sequence (NLS), which divides lamin A into the structural rod domain on the amino-terminal side and the DNA-binding domain on the carboxy-terminal side. This had led some to conjecture that the molecular disease mechanism in FPLD2 - and indeed the other laminopathies that are caused by mutations within or near the lamin DNA-binding domain – is related to altered interactions of transcription factors or other DNA-binding molecules, in contrast to a disease resulting from altered nuclear envelope structure and integrity from mutations that occur upstream of the NLS within the lamin A/C rod domain (37).

The mechanisms by which a mutation in LMNA leads specifically to dystrophy of adipose cells are incompletely defined. It is not clear which of the multiple normal roles of the nuclear lamina, such as maintenance of nuclear shape and structure, but also non-structural roles such as transcriptional regulation, nuclear pore positioning and function, and the organization of heterochromatin (38) become disrupted by FPLD2 mutations in LMNA. It is not even clear whether the same mechanism is responsible for the pathogenesis of
each laminopathy. One interesting development has been the demonstration that progeria mutations in \textit{LMNA} affect farnesylation of prelamin A, which behaves as an intracellular toxin (39). Indications are that treatment with oral farnesyl transferase inhibitors alters the natural progression of disease (40). If a similar pathogenic mechanism can be shown for the FPLD2 mutations in \textit{LMNA}, this would suggest a new approach to treatment for this disease.

Two recently described FPLD2 mutations, namely \textit{LMNA} D230N and R399C, occur upstream of the lamin A NLS (41), suggesting that the position of the mutation within the secondary and/or tertiary structure of lamin A might also be a key determinant of pathogenicity (37). Re-annotation of sequences of previously studied genes can identify new sequences to be screened, leading to discovery of new mutations (42). In this regard, a recent freeze of NCBI AceView, which annotates genes by aligning cDNA and expressed sequence tags, indicates that \textit{LMNA} is more variable at the transcriptional level than previously thought, with perhaps >40 exons and >10 distinct mRNA transcripts (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly). The evolving \textit{LMNA} sequence annotation might identify new regions that harbor mutations, helping to explain the range of laminopathy phenotypes, including FPLD2.

FPLD3 (MIM 604367) results from any one of more than a dozen heterozygous mutations in the \textit{PPARG} gene encoding peroxisome proliferator-activated receptor gamma (PPAR\textsubscript{γ}; MIM 601487) (43-51). The \textit{PPARG} mutations implicated in FPLD3 are shown in Figure 2. Both “dominant negative” and “haploinsufficiency” mechanisms have been proposed to explain the
pathogenicity of the *PPARG* mutations. According to the dominant negative hypothesis, the mutant allele disrupts wild type function by direct interference. In the case of PPARγ, the mutant receptor would compete with the wild type for DNA binding. In contrast, with haploinsufficiency 50% reduced gene expression results from one nonfunctional allele, compared to the greater reductions in gene expression and function resulting from dominant negative mutations. Careful cellular assays indicate that seven PPARγ mutations (C114R, C131Y, C162W, FS315X, R357X, P467L and V290M) act via a dominant negative mechanism (44, 51), while six (-14A>G, F388L, E138fsΔAATG, Y355X, R194W and R425C) act through haploinsufficiency (45-50) (Figure 2).

Because of the extremely well-studied role of PPARγ in adipocyte biology, the mechanistic link to FPLD3 is not as obtuse as for some of the other lipodystrophy genes. For the “dominant negative” *PPARG* mutations it has been reported that receptor mutants lacked DNA binding and transcriptional activity but could translocate to the nucleus to interact with PPARgamma coactivators and inhibit coexpressed wild-type receptor, with resultant attenuation of the expression of PPARγ target genes (44). This suggested that the mutants restricted wild-type PPARγ action via a non-DNA binding, transcriptional interference mechanism involving the sequestration of functionally limiting coactivators (44). Another proposed mechanism was reduced promoter turnover rate for certain “dominant negative” mutant PPARγ receptors, with the result that the mutant would eventually out-compete the wild-type receptor for promoter binding sites (49). In any event, the mechanism(s) through which mutant PPARγ...
receptors lead ultimately to the expression of a lipodystrophy phenotype are complex and likely varied.

About 50% of FPLD patients appear not to have mutations in either *LMNA* or *PPARG* genes by genomic DNA sequence analysis to screen known coding regions (Hegele, unpublished observations). The reasons for this may include: 1) presence of mutation types not detected by DNA sequence analysis, such as copy number variations; 2) genetic heterogeneity with new causative genes yet to be identified; and 3) the presence of mutations in unrecognized functionally important sequences of *LMNA* or *PPARG*.

FPLD2 and FPLD3 phenotypes considered in light of molecular diagnosis

There appears to be little difference in the severity of clinical presentation between the various *LMNA* missense mutations that lead to FPLD2. However, a single *LMNA* splicing mutation has been found in two sisters with a very severe FPLD2 phenotype (52). Other splicing mutations in *LMNA* also lead to severe phenotypes, such as the splicing mutation in exon 11 that underlies most cases of HGPS. It is perhaps noteworthy that HGPS patients have lipodystrophy as part of their cluster of systemic abnormalities (37). Also, the severity of the phenotype in FPLD2 can be modulated by the presence of other mutations: in one striking example, a patient with a severe form of FPLD2 with acromegaly and aggressive vascular disease was a compound heterozygote for the R482Q mutation and the V440M mutation, which on its own is not pathogenic (53).
Among individual PPARG mutations, there appears to be little correlation of mutation type with phenotype severity. The severity of adipose tissue loss and metabolic disturbances appear to be similar among individuals with dominant negative and haploinsufficiency mutations. All FPLD3 patients with PPARG haploinsufficiency mutations were ascertained based upon a clinical diagnosis of lipodystrophy (45-50). Thus, virtually every patient with a PPARG mutation has had partial lipodystrophy as a core phenotype.

Table 1 summarizes clinical features compiled from female subjects with FPLD2 and FPLD3. Subjects with FPLD2 were further subdivided according to the presence or absence of diabetes. At the clinical and biochemical levels, it would appear that FPLD3, compared to FPLD2, is associated with: 1) less extensive adipose loss clinically; 2) more severe and/or earlier clinical endpoints such as acanthosis nigricans, hepatic steatosis, PCOS and hirsutism; 3) more severe hypertension; 4) earlier onset of type 2 diabetes; 5) greater biochemical insulin resistance; 6) pronounced depression of adipocytokines; and 7) variable biochemical response to thiazolidinedione (TZD) treatment. One clear difference is the documentation of early heart disease among women with FPLD2 (54). The early atherosclerosis that was clearly seen in women with FPLD2 was less definitively shown in the smaller number of FPLD3 subjects accumulated so far. In aggregate, it would appear that the clinical and biochemical derangement in FPLD3 subjects is out of proportion to the extent of lipodystrophy when compared with FPLD2 subjects, suggesting that the PPARG mutations might have additional and independent effects on metabolism.
Acquired partial lipodystrophy (APL; Barraquer-Simons syndrome)

Acquired partial lipodystrophy (APL; MIM 608709) was initially reported >100 years ago (55-57). A family history is usually absent in APL, while a wide range of secondary factors and conditions is often associated (58). For instance, autoimmune disorders such as systemic lupus erythematosis, dermatomyositis, hypocomplementemia and membranoproliferative glomerulonephritis (MPGN) are sometimes seen in association with APL (58). The sporadic expression and frequent requirement for secondary factors indicate that APL is a complex trait, perhaps with a component of genetic susceptibility. Like some of the lipodystrophies described above, there is a female preponderance of ascertained cases at a ratio of ~4:1. Affected individuals develop adipose tissue loss affecting primarily the face, neck, arms, thorax, and upper abdomen in progressive cephalocaudal order, commencing in childhood or adolescence. The adipose stores of the gluteal regions and lower extremities (including soles) tend to be either preserved or increased, particularly among women. Variable fat loss of the palms, but no loss of intramarrow or retroorbital fat has been demonstrated. Patients with MPGN develop lipodystrophy at an earlier age compared to those without renal disease. Although the prevalence of diabetes has been shown to be only ~10%, diabetic APL patients were predominantly female (58). Clinical attributes of APL are shown in Table 1.

In order to resolve whether APL had a component of genetic susceptibility (58), we used candidate gene sequencing to identify genomic DNA sequence
mutations that were present in APL patients but absent in healthy individuals. In 2001 we used genomic information available at that time to screen candidate genes encoding nuclear envelope proteins, including LBR, LMNB1 and LMNB2, which encode lamin B receptor, lamin B1 and lamin B2, respectively (59). We then sequenced these candidate genes in patients with APL who had no mutations in LMNA (59). We identified several common polymorphisms, but found no disease-causing mutations. We concluded that sequence variants affecting the nuclear lamina proteome were not likely to be associated with APL (59). But recent observations suggest that early versions of mammalian genome maps underestimated the total numbers of exons. New computational algorithms have revealed thousands of previously unappreciated exons in mammalian genomes (60). Upon re-visiting the re-annotated genomic structures of nuclear proteome genes, we found that LMNB2 had only six exons identified in 2001 but 12 exons today. We thus developed reagents to interrogate the coding regions of the re-annotated LMNB2 gene (MIM 150341) in nine unrelated APL patients. In four of these patients we found three new rare LMNB2 mutations: intron 1 -6G>T, exon 5 p.R215Q (in two patients) and exon 8 p.A407T. Compared with a multi-ethnic control sample of 1100 subjects, the relative risk (RR) of APL for carriers of these mutations was 110 (95% confidence interval [CI] 36 to 271; P<0.00001). These novel heterozygous mutations were the first reported in LMNB2 and the first reported among patients with APL (42). There was no obvious genotype-phenotype correlation. However, the findings indicated how sequencing of a re-annotated candidate gene can reveal new disease-associated mutations (42).
**Acquired generalized lipodystrophy**

Like APL, acquired generalized lipodystrophy (AGL) does not follow classical Mendelian patterns of inheritance, but unlike APL, no genetic susceptibility component has been identified for AGL (61). Clinical features of AGL are summarized in Table 1. AGL is typically recognized in childhood and adolescence, with progressive loss of adipose tissue affecting the face and extremities with varying changes in intra-abdominal fat, sparing of retro-orbital and intramarrow adipose stores and variable loss of adipose tissue in the palms or soles (61). Females tend to be more often affected – or at least ascertained clinically - than males, with a ratio of 3:1. During childhood, affected individuals have a voracious appetite, acanthosis nigricans and hepatic steatosis. Metabolic changes include low plasma leptin and adiponectin, hyperinsulinemia, diabetes, hypertriglyceridemia and low plasma HDL. Females with AGL have compromised reproduction with menstrual irregularities and PCOS. AGL has been subclassified into three groups based largely on clinical attributes: AGL associated with autoimmune disorders, AGL associated with panniculitis, and idiopathic. Misra and Garg found the prevalence of diabetes and hypertriglyceridemia to be highest in both the autoimmune and idiopathic groups when compared to the panniculitis group: ~88% vs. 44% for diabetes and ~90% vs. 59% for hypertriglyceridemia (61). In order to resolve whether AGL had an underlying component of genetic susceptibility, we have extensively used candidate gene sequencing of known lipodystrophy genes *(AGPAT2, BSCL2,*
LMNA, PPARG and LMNB2) and also candidate genes encoding nuclear envelope proteins, but to date have found no putative causative or associated mutations (Hegele, unpublished observations).

HIV-related partial lipodystrophy (HIVPL)

HIV-related lipodystrophy is the most commonly ascertained form of lipodystrophy in the clinic (62). HIVPL affects males and females equally and has been related to the intensity and tonicity of antiretroviral therapy. Adipose redistribution among HIV-infected individuals is very common, affecting up to 50% of individuals, although there is no standard definition or clinical criteria for this diagnosis (62). Initially, patients with HIV-related lipodystrophy were mistakenly presumed to have Cushing syndrome, but careful evaluation revealed unaltered steroid hormone metabolism (63-65). Later reports linked the presence of peripheral lipoatrophy affecting the face and extremities with central lipohypertrophy affecting the dorsocervical and truncal regions. Prospective trials have shown that while peripheral lipoatrophy is common among HIV-infected individuals, truncal adipose distribution can range from lipoatrophy to lipohypertrophy, suggesting that peripheral lipoatrophy is not always linked with central lipohypertrophy (66-68). Other metabolic manifestations of HIV-related lipodystrophy include hypertriglyceridemia, low plasma HDL, insulin resistance, impaired glucose tolerance or diabetes, androgen deficiency and hepatic steatosis (69-71). PCOS is not found among HIV-infected women, and acanthosis nigricans is also rarely seen, despite the presence of significant
insulin resistance (72). In contrast to other forms of lipodystrophy, plasma leptin tends to be normal or even elevated, together with low adiponectin (73, 74).

**Other syndromes with a lipodystrophy component**

**Mandibuloacral dysplasia (MAD; MIM 248370)**

MAD is an extremely rare autosomal recessive disorder characterized by multiple musculoskeletal abnormalities, progeroid features and lipodystrophy with insulin resistance, hypertriglyceridemia, depressed plasma HDL and impaired glucose tolerance (1, 75, 76). There are two molecular forms of MAD: type A (MADA; MIM 248370) due to homozygous missense mutations in LMNA (77) and type B (MADB; MIM 608612) due to compound heterozygous mutations in ZMPSTE24 (MIM 606480), which encodes a zinc metalloproteinase involved in proteolytic processing of prelamin A. Defective prelamin A maturation with mutant ZMPSTE24 leads to the generation of abnormalities in nuclear architecture that underlie the various phenotypes (78). MADA patients had loss of subcutaneous adipose tissue from the extremities with sparing of the neck and trunk, while the MADB patient had global subcutaneous adipose loss involving the face, trunk, and extremities (76).

**SHORT syndrome (MIM 269880)**

SHORT syndrome is an extremely rare disorder characterized by Short stature, Hyperextensible joints and/or inguinal hernia, Ocular depression, Rieger anomaly (defective development of cornea and iris) and Teething delay. There is
no sex predominance, obvious inheritance pattern or molecular genetic basis. Most affected individuals have depleted adipose stores in the face, upper extremities and trunk with less effect on lower extremities. Others have adipose tissue loss of the trunk, gluteal region, and elbows (79-81).

Progeria syndromes

Features of Hutchinson-Gilford progeria syndrome (HGPS; MIM 176670) due to mutant LMNA include growth delay, short stature, alopecia, osteolysis, elderly facial features and lipodystrophy beginning in the first year of life, involving the extremities, trunk, and face but sparing intra-abdominal adipose stores. Atherosclerosis is common and represents the major cause of death (82, 83).

Features of Werner syndrome (WS: MIM 277700) due to homozygous mutations in RECQL2 (MIM 604611), encoding a DNA helicase (84) include short stature, late-onset progeroid features, decreased subcutaneous adipose in the trunk, face and extremities with insulin resistance and diabetes, osteoporosis, cataracts, hypogonadism, numerous skin problems, calcified blood vessels and early death from cardiovascular disease or cancer (85, 86).

Wiedemann-Rautenstrauch neonatal progeroid syndrome (MIM 264090) or “neonatal progeroid syndrome” follows autosomal recessive inheritance. Affected individuals have progeroid features at birth, skull deformities and generalized lipodystrophy fat loss (87). The molecular basis is unknown, but
these patients had no mutations in known lipodystrophy genes or in candidate genes encoding nuclear envelope proteins (Hegele, unpublished observations).

**Phenomic studies of fat distribution using magnetic resonance imaging**

We have developed standardized methodology for semi-automated quantitation of subcutaneous adipose stores from magnetic resonance imaging (MRI) in order to study differences between subjects with lipodystrophy (62, 88, 89). Inspection of whole body MR images, and also regional and segmental scans showed remarkable differences between different types of lipodystrophy (Figure 3). After obtaining reference ranges for percent subcutaneous adipose tissue in normal control subjects for 6 anatomical sites (Figure 4), we quantified the percent adipose in patients with women with FPLD2 (six subjects), FPLD3 (two subjects), HIVPL (one subject), APL (one subject) and CGL (one subject).

FPLD2 has long been clinically characterized by decreased adipose deposition in the trunk and increased deposition in the neck and labia. Using MRI, we demonstrated significant differences in FPLD2 patients compared to controls, specifically increased supraclavicular and visceral adipose stores and decreased subcutaneous abdominal, gluteal, thigh and calf adipose stores (Figure 4). We also confirmed the subjective clinical impression of differences between FPLD2 and FPLD3 subjects, such as no increase in visceral adipose tissue, no decrease in abdominal subcutaneous adipose tissue and less dramatic depletion of subcutaneous gluteal, thigh and calf adipose in FPLD3 compared with FPLD2 patients (Figure 4). Finally, FPLD3 patients have relatively more
truncal adipose tissue and less attenuation of limb adipose tissue that FPLD2 patients; they may also have decreased to absent facial fat (Figure 4). This supports the clinical impression of less severe adipose tissue alteration but more severe clinical endocrine abnormalities in FPLD3 compared with FPLD2 patients.

We noted that the pattern of adipose repartitioning in the HIVPL subject closely resembled the pattern seen in the FPLD3 subjects (Figure 4). We documented reduced subcutaneous fat in the upper body, gluteal region and thigh, in both APL and CGL subjects, with increased and decreased calf fat in APL and CGL subjects, respectively (Figure 4). Finally, we noted consistently reduced fat in the mid-thigh across the lipodystrophies (Figure 4), suggesting that imaging of this bodily region could be a defining clinical biomarker that could help distinguish whether a patient is affected with some form of lipodystrophy when the clinical diagnosis is unclear (Figure 4). Studies are underway to acquire these quantified traits from a larger number of patients, including males with various lipodystrophy subtypes, both defined molecularly and not. Future application of this quantification method may include quantification of both thigh and calf depots for “garden variety” obesity, metabolic syndrome or diabetes. This approach might also be applicable to quantify metabolically important substrata of adipose tissue. Finally, the differences in adipose tissue distribution between lipodystrophy types might help to identify genetic programs of development or apoptosis related either to affected pathways linked to mutant gene products or to various environmental or pathogenic insults.
Conclusion

Patients with lipodystrophy represent the ultimate in vivo “experiment-of-nature” with regard to human adipose tissue development, dysfunction and programmed cell death. For the first century since after their initial description, these disorders were classified based on clinical and biochemical features. But ever since the first causative mutations in FPLD2 were reported less than eight years ago, the power of molecular genetics and biology has rapidly created of a new classification framework that enables examination these disorders from genotypic perspective. The associated mutations occur in genes encoding two nuclear envelope structural components (LMNA and LMNB2), a nuclear hormone receptor (PPARG), a metalloproteinase (ZMPSTE24), an integral endoplasmic reticulum membrane protein (BSCL2) and a lipid biosynthetic enzyme (AGPAT2). There are no obvious unifying mechanistic links or pathways that account for this range of gene products. Further molecular heterogeneity is likely to be discovered among patients who presently lack a molecular diagnosis. An unresolved question is whether the metabolic disturbances develop secondarily to adipose tissue repartitioning or result from a direct effect of the individual mutant gene product. Phenomic studies have revealed clinical differences between CGL1 and CGL2 and between FPLD2 and FPLD3, which may soon be translatable into differences in diagnosis, prognosis and treatment. The example of the lipodystrophies indicate how combining genomic and phenomic perspectives can guide future experiments and perhaps improve understanding of common clinical entities, such as metabolic syndrome or HIVPL.
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References


mutations in AGPAT2 or Seipin genes among Berardinelli-Seip congenital lipodystrophy patients. *J. Inherit. Metab. Dis.* **28**: 1123-1131.


Figure 1. Genomic maps of A) *AGPAT2* and B) *BSCL2* showing reported mutations in patients with CGL1 and CGL2, respectively. Exons are shown as numbered boxes. IVS, intron.

Figure 2. Genomic maps of A) *LMNA* and B) *PPARG* showing reported mutations in patients with FPLD2 and FPLD3, respectively. For *LMNA*, the single FPLD2 splicing mutation is shown below the genomic map. For *PPARG*, FPLD3 mutations that appear to act through a dominant negative mechanism are shown above the genomic map, while FPLD3 mutations that act through a dominant negative mechanism are shown below the genomic map. The promoters for the tissue-specific *PPARG* mRNA isoforms are indicated $\gamma_1$, $\gamma_3$, $\gamma_2$ and $\gamma_4$ and their associated untranslated exons are A1, A2, B and E1, respectively.

Figure 3. Phenomic evaluation of adipose tissue deposition in females with various lipodystrophies using magnetic resonance image (MRI) analysis. A) Control 28 year-old female, body mass index (BMI) 22.4 kg/m$^2$. B) Control 50 year-old female, BMI 34.8 kg/m$^2$. C) familial partial lipodystrophy type 2 (FPLD2 due to *LMNA* R482Q heterozygosity) 63 year-old female, BMI 24.8 kg/m$^2$; D) familial partial lipodystrophy type 3 (FPLD3 due to *PPARG* F388L heterozygosity) 49 year-old female, BMI 33.4 kg/m$^2$; E) acquired partial lipodystrophy (APL due to *LMNB2* R215Q heterozygosity) 65 year-old female, BMI 20.3 kg/m$^2$; F) HIV-associated partial lipodystrophy (HIVPL, no mutation) 40 year-old female, BMI 28.3 kg/m$^2$; G) congenital generalized lipodystrophy (CGL due to *BSCL2* frameshift mutation fs108insA homozygosity) 41 year-old female, BMI 22.9 kg/m$^2$. The top row of images is comprised of composite whole body MRI scans of patients in coronal section. The middle row of images is comprised of sagittal sections of the head and neck to visualize the dorsocervical fat pad. The bottom row of images shows cross sections through the mid-thigh (left side). Quantification of the regional scans of adipose tissues was used to produce the values in Figure 4.
Figure 4. Percent subcutaneous and visceral adipose tissue values in different body segments of lipodystrophy patients and healthy female controls from imaging studies shown qualitatively in Figure 3. Shaded region represents the range of adipose tissue values of the normal control group (10 females). Horizontal line and whiskers represent the mean and standard deviation of adipose tissue percentages seen in normal controls. Horizontal line in FPLD2 plots represents the mean value of adipose tissue in these subjects. (a) Percent subcutaneous adipose tissue in the upper back and shoulders. (b) Percent subcutaneous adipose tissue in the abdomen at the level of the fourth lumbar vertebra (L4). (c) Percent visceral adipose tissue in the abdomen-L4 region. (d) Percent subcutaneous adipose tissue in the gluteal region. (e) Percent subcutaneous adipose tissue in the thighs. Values for left and right thigh are plotted as two separate points for each subject. (f) Percent subcutaneous adipose tissue in the calves. Values for left and right calf are plotted as two separate points for each subject.
Table 1: Features of selected congenital and acquired forms of lipodystrophy

<table>
<thead>
<tr>
<th></th>
<th>Congenital generalized lipodystrophy (CGL)</th>
<th>Familial partial lipodystrophy (FPLD)</th>
<th>Acquired generalized lipodystrophy</th>
<th>Acquired partial lipodystrophy</th>
<th>HIV-related lipodystrophy</th>
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<tbody>
<tr>
<td><strong>DEMOGRAPHICS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at Onset</td>
<td>Shortly after Birth</td>
<td>Puberty</td>
<td>Puberty to Adulthood</td>
<td>Typically &lt;20 y</td>
<td>Any age</td>
</tr>
<tr>
<td><strong>ADIPOSE DISTRIBUTION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (based on WHO criteria)</td>
<td>Normal to underweight</td>
<td>Normal</td>
<td>Normal to overweight</td>
<td>Normal to underweight</td>
<td>Normal typically</td>
</tr>
<tr>
<td>Facial Fat Loss</td>
<td>++++</td>
<td>0</td>
<td>0 to ++</td>
<td>+++</td>
<td>0 to +++</td>
</tr>
<tr>
<td>Mechanical Fat Loss (retroorbital/palm/sole)</td>
<td>++++</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>++ to +++</td>
</tr>
<tr>
<td>Limb Fat Loss</td>
<td>++++</td>
<td>+++</td>
<td>++++ (distal predominantly)</td>
<td>+++</td>
<td>++ to +++</td>
</tr>
<tr>
<td>Trunk Fat</td>
<td>↓↓↓↓</td>
<td>↑↑↑↑</td>
<td>0 to ↑↑↑</td>
<td>↓↓↓↓</td>
<td>↑↑↑↑ to ↑↑↑</td>
</tr>
<tr>
<td>Gluteal Fat</td>
<td>↓↓↓↓</td>
<td>↓↓↓↓</td>
<td>0 to ↓↓↓</td>
<td>↓↓↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Bone Marrow Fat</td>
<td>+++</td>
<td>0 to ++</td>
<td>++ to +++</td>
<td>+++</td>
<td>Rare</td>
</tr>
<tr>
<td>Hepatic Steatosis</td>
<td>+++</td>
<td>0 to ++</td>
<td>++ to +++</td>
<td>+++</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>CLINICAL FEATURES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Diabetes</td>
<td>Very common</td>
<td>Present</td>
<td>Typically present</td>
<td>Common; if autoimmune disorders present</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Age at Onset of Diabetes</td>
<td>Adolescence</td>
<td>&lt;10 years</td>
<td>~35-45 years</td>
<td>Adolescence to Adulthood</td>
<td>Adulthood (&lt;45)</td>
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<tr>
<td>Hypertension</td>
<td>Rare</td>
<td>Present</td>
<td>Present</td>
<td>Adolescence to Adulthood</td>
<td>Adulthood (&lt;45)</td>
</tr>
<tr>
<td>Acanthosis Nigricans</td>
<td>Present</td>
<td>Present</td>
<td>Typically Present</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>Can be present</td>
<td>Can be present</td>
<td>Rare</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Sexual Development/Function</td>
<td>No changes</td>
<td>- External genitalia pseudohypertrophy can be present</td>
<td>PCOS</td>
<td>Menstrual anomalies or PCOS often present</td>
<td>PCOS</td>
</tr>
<tr>
<td>Early CAD</td>
<td>Unknown</td>
<td>~50%</td>
<td>Rare</td>
<td>Typically rare</td>
<td>Possible</td>
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## METABOLIC PARAMETERS

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<th>++</th>
<th>+</th>
<th>++++</th>
<th>+++</th>
<th>0 to ++</th>
<th>++ to +++</th>
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</thead>
<tbody>
<tr>
<td>Increased Fasting Insulin</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0 to ++</td>
<td>++ to +++</td>
</tr>
<tr>
<td>Increased Triglyceride</td>
<td>0 to ++*</td>
<td>0 to +++</td>
<td>0 to +</td>
<td>0 to +</td>
<td>0 to ++</td>
<td>0 to +</td>
<td>0 to ++</td>
<td>0 to ++</td>
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<tr>
<td>Decreased HDL</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0 to +</td>
<td>0 to +</td>
<td>0 to +</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0 to ++</td>
</tr>
<tr>
<td>Increased Free Fatty Acids</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0 to +</td>
<td>0 to +</td>
<td>0 to +</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0 to ++</td>
</tr>
<tr>
<td>Leptin</td>
<td>↓↓↓</td>
<td>↓↓↓</td>
<td>↓↓</td>
<td>↓</td>
<td>0 to ↓↓↓</td>
<td>↓</td>
<td>↓</td>
<td>0 to ↑</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>↓↓↓↓*</td>
<td>↓↓↓</td>
<td>↓↓</td>
<td>↓</td>
<td>↓↓↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
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<tr>
<td>Elevated C-Reactive Protein</td>
<td>Unknown</td>
<td>Unknown</td>
<td>+</td>
<td>++</td>
<td>0 to +</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0 to ++</td>
</tr>
</tbody>
</table>

## OTHER FEATURES

- Not associated with mental retardation; cardiomyopathy can be present; bone cysts can be present
- Mental retardation possible; higher risk of cardiomyopathy than CGL1; bone cysts may be present
- Subgroups: Associated with autoimmune conditions
- Associated with idiopathic
- Low C3 complement
- Autoimmune disorders
- MPGN

* Summary characteristics are not further subdivided according to mutation type; abbreviations: 0, absent; + to ++++, present or increased to varying degrees as indicated; ↓ to ↓↓↓↓ depressed to varying degrees as indicated; PCOS, polycystic ovarian syndrome; CAD, coronary artery disease; HDL, high-density lipoprotein; MPGN, membranoproliferative glomerulonephritis; C3, complement factor C3
Figure 2

**LMNA**

- R28W
- R133L
- R62G
- D230N
- R399C
- G465D
- V440M
- K486D
- S573L
- R582H
- S583L
- R584H
- IVS8 +5G>C

**PPARG**

- C114R
- C131Y
- C162W
- V290M
- fs315X
- R357X
- R467L
- -14A>G
- E138fsAATG
- R194W
- Y355X
- F388L
- R425C