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COMPLEX LIPIDS

Inborn errors of cytoplasmic triglyceride metabolism

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Abstract Triglyceride (TG) synthesis, storage, and degradation together constitute cytoplasmic TG metabolism (CTGM). CTGM is mostly studied in adipocytes, where starting from glycerol-3-phosphate and fatty acyl (FA)-coenzyme A (CoA), TGs are synthesized then stored in cytoplasmic lipid droplets. TG hydrolysis proceeds sequentially, producing FAs and glycerol. Several reactions of CTGM can be catalyzed by more than one enzyme, creating great potential for complex tissue-specific physiology. In adipose tissue, CTGM provides FA as a systemic energy source during fasting and is related to obesity. Inborn errors and mouse models have demonstrated the importance of CTGM for non-adipose tissues, including skeletal muscle, myocardium and liver, because steatosis and dysfunction can occur. We discuss known inborn errors of CTGM, including deficiencies of: AGPAT2 (a form of generalized lipodystrophy), LPIN1 (childhood rhabdomyolysis), LPIN2 (an inflammatory condition, Majeed syndrome, described elsewhere in this issue), DGAT1 (protein loosing enteropathy), perilipin 1 (partial lipodystrophy), CGI-58 (gene *ABHD5*, neutral lipid storage disease (NLSD) with ichthyosis and "Jordan's anomaly" of vacuolated polymorphonuclear leukocytes), adipose triglyceride lipase (ATGL, gene *PNPLA2*, NLSD with myopathy, cardiomyopathy and Jordan's anomaly), hormone-sensitive lipase (HSL, gene *LIPE*, hypertriglyceridemia, and insulin resistance). Two inborn errors of glycerol metabolism are known: glycerol kinase (*GK*, causing pseudohypertriglyceridemia) and glycerol-3-phosphate dehydrogenase (*GPD1*, childhood hepatic steatosis). Mouse models often resemble human phenotypes but may diverge markedly. Inborn errors have been described for less than one-third of CTGM enzymes, and new phenotypes may yet be identified.

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Abbreviations

ATGL	Adipose triglyceride lipase
BAT	Brown adipose tissue
CTGM	Cytoplasmic TG metabolism
DG	Diglycerides
ER	Endoplasmic reticulum
FA	Fatty acid
G3P	Glycerol-3-phosphate
HSL	Hormone-sensitive lipase
LD	Lipid droplet
LPA	Lysophosphatidic acid
MG	Monoglycerides
MGL	Monoglyceride lipase
NLSD	Neutral lipid storage disease
NLSDI	NLSD with ichthyosis
NLSDM	NLSD with myopathy
PA	Phosphatidic acid
PLIN1	Perilipin 1

TG Triglycerides
WAT White adipose tissue.

Introduction

Certain inborn errors of metabolism are directly related to enzymes and structural proteins of intracellular triglyceride (TG) synthesis, storage or degradation. Cytoplasmic TG metabolism (CTGM) has been most studied in adipose tissue, which derives its identity from the capacity to accumulate TGs and to release FAs in response to physiological demands. Not surprisingly, the phenotype of most inborn errors of CTGM includes adipose tissue changes. However, non-adipose signs often dominate the clinical picture of inborn errors of CTGM.

In this review we briefly summarize the pathways of CTGM, using the adipocyte as an example, then describe the features of known inborn errors of CTGM. Inborn errors of extracellular and lysosomal TG metabolism (involving the production, transport, and uptake of TG-containing lipoproteins in liver and enterocytes) are not discussed. The clinical spectrum of CTGM is expanding. Some clinical descriptions are based on single case reports and description of phenotypes in experimental organisms, mainly mice, while the clinical spectrum of others conditions is much better defined.

The structure of acylglycerols

TGs are glycerol molecules in which a fatty acid (FA) esterified each of its three hydroxyl groups. Most TG molecules contain a saturated FA or oleic acid (C18:1) at position *sn*-1. Position *sn*-2 usually is occupied by an unsaturated FA, especially linoleic acid (C18:2). Position *sn*-3 is often occupied by longer-chain FAs. The American Oil Chemists' Society website provides interested readers with additional information (http://lipidlibrary.aocs.org/Lipids/tag1/index.htm).

Carbon 2 of the glycerol backbone of TGs is asymmetrical (Fig. 1a). This observation has important physiological consequences (Eichmann et al 2012): *sn*-1,3 and *sn*- 2,3 DGs are formed during TG degradation. sn-2,3 DGs are distinct from the *sn*-1,2 DGs that are generated from cell membrane phospholipids by phospholipase C, and that mediate cell signaling (Eichmann et al 2012).

Cytoplasmic TG metabolism

TGs are the largest energy reservoir of mammals. Each step of the canonical pathway of TG synthesis, storage and lipolysis can be mediated by more than one enzyme. The enzymes that catalyze a given reaction may differ in their tissue expression, physiological regulation, and affinity for specific FA esterified to glycerol. Figures 1b and 2 show the pathways of CTGM.

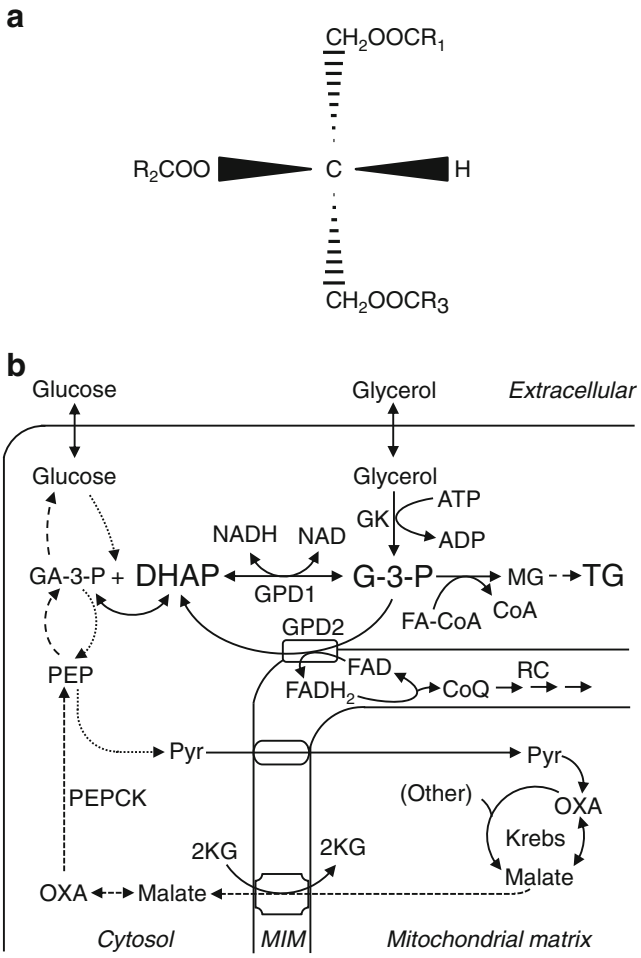

Fig. 1 Triglyceride (TG) structure and glycerol metabolism. **a** The chemical structure of TGs. The second carbon of the glycerol backbone is asymmetrical except if the R1 and R3 groups are identical. Different di- and mono-glyceride species play specific roles in metabolism (Eichmann et al 2012) (see text). **b** Glycerol metabolism in liver. Shown are the relationships to glycolysis and gluconeogenesis, to the redox state of mitochondria and cytosol and to glycerol supply for glycerolipid synthesis. Dihydroxyacetone phosphate (DHAP) and glycerol-3-phosphate (G3P) play central roles. G3P is an essential substrate for the first step of TG synthesis. G3P can be formed either: (*1*) by capture of circulating glycerol and its phosphorylation by glycerol kinase (GK) or (*2*) by reduction of dihydroxyacetone phosphate (DHAP) by glycerol phosphate dehydrogenase 1 (GPD1). DHAP is produced by glycolysis (dotted lines) or by *glyceroneogenesis*, which uses the first steps of gluconeogenesis (dashed lines). This simplified figure shows the Krebs cycle production of malate from gluconeogenic amino acids or from pyruvate, malate transport by the *SLC25A11* carrier and synthesis of phospho-*enol*-pyruvate (PEP) by PEP carboxykinase (PEPCK). Cycling between G3P and DHAP enables transport of cytoplasmic reducing equivalents to the mitochondrial matrix. Other abbreviations: MIM, mitochondrial inner membrane; OXA, oxaloacetate; RC, respiratory chain

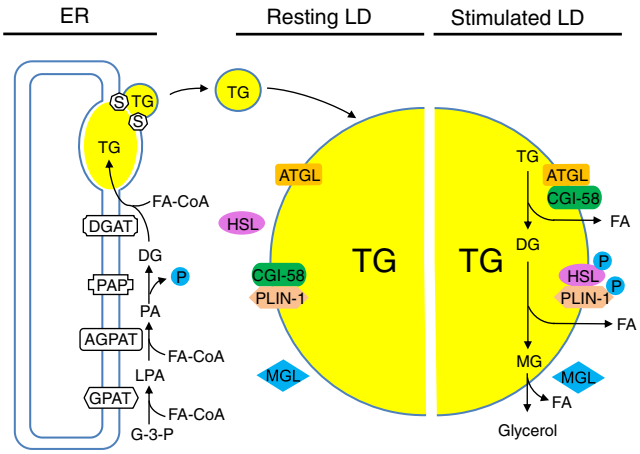

Fig. 2 Intracellular TG metabolism. (*left*) Pathways of acylglycerol synthesis and LD formation. (*center and right*) The LD. (*center*) In the resting state, lipolysis is low, PLIN1 is associated with CGI-58, neither HSL nor PLIN1 is phosphorylated and HSL is located in the cytoplasm, far from its substrate. (*right*) lipolytically-stimulated LD. After beta adrenergic stimulation, PKA phosphorylates HSL and PLIN1, HSL translocates to the LD surface and PLIN1 dissociates from CGI-58, which approaches and activates ATGL. Abbreviations: DG, diglycerides; ER, endoplasmic reticulum; FA, fatty acid; G3P, glycerol-3-phosphate; LD, lipid droplet; LPA, lysophosphatidic acid; MG, monoglycerides; P, inorganic phosphate; PA, phosphatidic acid; S, seipin; TG, triglycerides. The abbreviations for protein species are provided in the main text

Substrates: FA-CoAs and glycerol-3-phosphate

FAs enter the adipocyte by diffusion or by CD36, a plasma membrane transporter, then associate noncovalently with intracellular FA binding proteins. They are activated to fatty acyl-coenzyme A (CoA) esters by an ATP-requiring acyl-CoA synthetase. The 26 different acyl-CoA synthetase genes in the human genome produce enzymes with a range of substrate specificities (Watkins et al 2007).

Glycerol metabolism

Most of the energy content of TGs resides in their FAs, but CTGM formally involves both lipid and carbohydrate metabolism. Glycerol, which is derived from carbohydrates and from gluconeogenic amino acids, can be considered as part of CTGM and is briefly reviewed here. During fasting, glycerol released by adipocyte lipolysis provides a substrate for hepatic gluconeogenesis (Millward et al 2010). If glucose is scarce, glycerol may provide a substantial fraction of glucose production (Hetenyi Jr 1985; Wapnir and Stiel 1985). Conversely, TG synthesis requires glycerol-3-phosphate. Glycerol can be produced by a pathway leading from three-carbon intermediates of both glycolysis and gluconeogenesis (Fig. 1b). In adipocytes, G3P is derived almost exclusively from dihydroxyacetone phosphate (DHAP), which can be derived by glycolysis or by glyceroneogenesis (Fig. 1b). Glyceroneogenesis may be the main source of G3P in adipose tissue (Nye et al 2008). In other tissues, G3P can also be produced by phosphorylation of glycerol, mediated by glycerol kinase (GK, E.C. 2.7.1.30) (Millward et al 2010). Cycling between DHAP and G3P enables the transfer of reducing equivalents from the cytosol to mitochondria, similar to the malate-aspartate shuttle. This is mediated by two glycerol phosphate dehydrogenase enzymes encoded by evolutionarily-unrelated genes, *GPD1* (cytoplasmic GPD) and *GPD2* (mitochondrial, Fig. 1b). The GPD1 reaction is reversible and uses an $NADH/NAD^+$ cofactor; GPD2 is irreversible and uses a FADH cofactor.

TG synthesis

The four steps of TG synthesis are reviewed elsewhere in greater detail (Takeuchi and Reue 2009). The first is catalyzed by a glycerol-3-phosphate acyltransferase (GPAT, EC2.3.1.15) as follows:

$$\text{glycerol}-3-\text{phosphate} + \text{acyl}-\text{CoA} \rightarrow 1-\text{acylglycerol}-3-\text{phosphate (lysophosphatidate)} + \text{CoA}.$$

Four mammalian GPAT isoforms are known (GAPT1-4). GPAT1 and GPAT2 localize to the mitochondrial outer membrane; GPAT3 and GPAT4, to the endoplasmic reticulum (ER) membrane. The mitochondrial GPAT isoforms may compete with carnitine palmitoyltransferase I for long-chain acyl-CoA substrates, directing them toward TG synthesis as opposed to beta-oxidation in the case of carnitine palmitoyltransferase I (Takeuchi and Reue 2009). No GPAT-associated inborn errors are yet reported.

1-acylglycerol-3-phosphate O-acyltransferases (AGPATs, EC 2.3.1.51) (Takeuchi and Reue 2009) are endoplasmic reticulum proteins that catalyze the next reaction:

$$\text{lysophosphatidate} + \text{acyl}-\text{CoA} \rightarrow \text{sn}-1,2-\text{diacylglycerol phosphate (phosphatidic acid, PA)} + \text{CoA}.$$

The AGPAT gene family contains the least members (Takeuchi and Reue 2009). AGPAT enzyme activity has been demonstrated for AGPAT1 and AGPAT2. AGPAT3-9 show different tissue expression and substrate preferences and their functions have not yet been systemically defined (Takeuchi and Reue 2009).

The preceding reactions are shared with those of glycerophospholipid synthesis. Phosphatidic acid

phosphatases (PAPs, *LPIN1-3*) catalyze the removal of the phosphate group, creating a sn-1,2-diacylglycerol. This is the first committed step of TG synthesis:

$$\text{Phosphatidic acid} \rightarrow \text{sn}-1,2-\text{diacylglycerol} + \text{inorganic phosphate.}$$

Diacylglycerol acyltransferases (DGATs) catalyze the final step of TG synthesis:

$$\text{sn}-1,2-\text{diacylglycerol} + \text{acyl}-\text{CoA} \rightarrow \text{TG} + \text{CoA.}$$

The two DGAT enzymes, DGAT1 and DGAT2 have marked structural differences and are encoded by two nonparalogous genes (Turchetto-Zolet et al 2011).

TG storage

Newly-synthesized TGs accumulate between the leaflets of the lipid bilayer of the ER membrane. Budding of small TG-filled lipid droplets (LDs), which are surrounded by a phospholipid monolayer, is a necessary step of LD formation. Successful budding appears to require seipin, an ER-localized protein (Magre et al 2001).

LDs have a tissue-specific proteome. Perilipins are found in all cells with LDs. The perilipin gene family has five members that are felt to contribute to the structure and organization of the surface of cytoplasmic LDs: perilipin 1 (the most abundant LD surface protein of adipocytes), ADRP (adipophilin, perilipin 2), TIP47 (PP17, perilipin 3), S3-12 (perilipin 4), OXPAT/MLDP (perilipin 5). Perilipins 1 and 2 are found only at the LD surface; the others can be located on the LD surface or in the cytoplasm.

Perilipins 1–4 are expressed in adipocytes (Dalen et al 2004) and some in other LD-containing tissues. For instance, perilipin 1 is also expressed in steroidogenic tissues (Servetnick et al 1995) and perilipin 2 in liver (Okumura 2011). Perilipin 5 is expressed in oxidative tissues including heart, liver, brown adipose tissue, and slow-twitch skeletal muscle fibers (Wolins et al 2006). Further discussion is beyond the scope of this article.

Lipolysis

Adipocyte lipolysis is a key pathway of fasting energy homeostasis. The principal TG lipase in adipocytes is adipocyte triglyceride lipase (ATGL; gene, *PNPLA2*). ATGL is situated at the LD surface. Interaction with CGI-58 (gene, *ABHD5*) activates ATGL, enhancing its lipolytic capacity. sn-1,3-DGs and to a lesser extent sn-2,3-DGs are produced by ATGL (Eichmann et al 2012). These DGs are hydrolyzed by hormone-sensitive lipase (HSL), producing mainly sn-1-MGs. A monoacylglyceride hydrolase(s) liberate(s) glycerol and a FA (Tornqvist and Belfrage 1976; Thomas et al 2013). FAs and glycerol produced by lipolysis are released from adipocytes to the circulation. A fraction of FAs produced by lipolysis is retained by the cell and recycled to acylglycerols (Forest et al 2003).

Perilipin 1 has multiple sites for protein kinase A (PKA)-mediated phosphorylation. Perilipin 1 coordinates the efficient suppression of lipolysis in the fed state and its activation of lipolysis by beta adrenergic stimulation and by low circulating insulin levels during fasting (Fig. 2).

Inborn errors of glycerol and cytoplasmic TG metabolism

The inborn errors of CTGM described to date in humans are summarized in Table 1. Adipose tissue is important for the study of inborn errors of TG synthesis but adipose-related phenotypes are often not carefully noted clinically. The major categories of lipodystrophies (Vigouroux et al 2011) are summarized in Table 2.

Diseases of glycerol metabolism

Glycerol kinase (GK) deficiency

GK deficiency (McCabe 2001; Zhang et al 2006) is an X-linked recessive trait. Several affected boys with episodes of fasting ketoacidosis, Reye-like syndrome, and in some cases mental retardation have been reported (McCabe 2001). Affected older males are usually asymptomatic and detected because of pseudohypertriglyceridemia. The commonest assay for plasma TGs involves hydrolysis, followed by measurement of free glycerol. GK-deficient patients are reported to have high levels of plasma TGs, typically measured at 2–8 mmol/L (Magre et al 2001). In comparison, normal fasting TG levels are <1.7 mmol/L and normal glycerol concentrations, 0.02 to 0.27 mmol/L (McCabe 2001). A clue to diagnosis is that plasma is transparent at measured TG levels expected to render it lactescent. The same assay as above, performed without the initial hydrolysis step, measuring the level of free glycerol. Marked glyceroluria is present on urine organic acid chromatography, up to 350 mmol/L, (McCabe 2001). These high levels of urinary glycerol may contribute to osmotic dehydration during symptomatic episodes. The GK gene on chromosome Xp21 may be involved in contiguous gene deletions, including the AHD gene, absence of which causes congenital adrenal hyperplasia, DMD, which causes Duchenne muscular dystrophy and OTC (ornithine transcarbamylase), causing hyperammonemia (Francke et al 1987).

Table 1 Human inborn errors of cytoplasmic triglyceride metabolism

	AGPAT2	LPIN-1	DGAT1	Perilipin 1 (*PLIN1*)[1]	CGI-58 (*ABHD5*)	ATGL (*PNPLA2*)	HSL (*LIPE*)
Fat	H & M: generalized lipodystrophy (M : ↓WAT & ↓BAT)	M: fld (fatty liver dystrophy) lipo-dystrophy strain	M: normal	H : partial lipodystrophy M : fat mass↓70 %, ↓ adipocyte size; ↑ basal & ↓ stimulated lipolysis		H: Mild hypertrophy M: ↑WAT mass; ↑ LD size; BAT, unilocular; ↑ mass	H: ↓ lipolysis. M: ↓↓ stimulated lipolysis, BAT: unilocular, ↑ mass
Liver	H: steatosis; M: steatosis	M: steatosis	M: ↓ fat content	H:steatosis; M: no apparent TG, ↓ fat	H: steatosis; cirrhosis; M: steatosis	H: ↑ mass, steatosis M: steatosis	H:steatosis; M: ↑ insulin sensitivity; ↓TG content
Muscle	M: no evident lipid accumulation	H: Rhab-domyolysis	M: ↓TG	M: ↑ lean mass	H : possible ↑ TG	H & M : lipid myopathy	H: NA; M: impaired insulin stimulated G uptake, ↑ DG
Heart	M: no evident lipid accumulation	M: cardiomyopathy	M: ↓TG	M: ↓fat		H & M: lipid cardiomyopathy; H: lipid coronary disease (1 pt).	H: NA
Other	M: insulin resistance, diabetes; large pancreatic islets	H:myoglobinuria M: neuropathy; insulin resistance; hyper-triglyceridemia; ↑ atherosclerosis	H: Infantile diarrhea & enteropathy M: ↓ diet-induced obesity; ↓↓ lactation; ↑ female lifespan		H, ichthyosis; M : ↓ growth, lethal skin barrier defect & ichthyosis; neonatal death	M: ↑ body mass, ↑ TGs in testis & kidney.	H: insulin resistance, ↑ triglyceridemia; M: male infertility

[1] Human *PLIN-1* deficiency is reported in five patients with heterozygous truncating mutations near the C-terminus; in mice, *Plin-1* deficiency refers to homozygous null alleles. Not mentioned in this table is deficiency of LIPN-2, which causes an inflammatory condition, Majeed syndrome, reviewed elsewhere in this issue. Abbreviations: H, human; M, mouse; LD, lipid droplet; NA, not available; WAT, white adipose tissue; BAT, brown adipose tissue

Table 2 Hereditary lipodystrophies in humans

	Generalized (Berardinelli-Seip congenital lipodystrophy)	Partial
Onset	Congenital	Postpubertal
Sex of reported patients	Female = male	Female > male
Genetic transmission	Autosomal recessive (usually)	Autosomal dominant (usually); sometimes recessive
Distribution of fat atrophy	Generalized	Atrophy of gynecoid depots (limbs & buttocks) with preservation of facial, neck and visceral fat.
Extra-adipocytic signs	Cardiac hypertrophy; muscular hypertrophy, insulin resistance, fatty liver.	Insulin resistance, fatty liver.
Genes	Seipin, AGPAT2, caveolin 1; cavin/PTRF.	PPAR-gamma; LMNA; AKT2, perilipin 1, Cell death-inducing DFF45-like effector C (CIDEC)

Note: Lipodystrophy also occurs as a feature of genetic syndromes like Hutchinson–Gilford progeria (gene, *LMNA*) and mandibuloacral dysplasia type B (genes ZMP-STE24), and in acquired conditions in which anti-adipose antibodies are produced (Lawrence syndrome). A centripetal shift of fat mass occurs during treatment with some antiretroviral drugs, in Cushing syndrome and in normal aging. See (Vigouroux et al 2011) for a clinically-based review

In WAT, GK expression is very low (Koschinsky et al 1971; Ryall and Goldrick 1977). As a result, the export of glycerol is near-complete, with little recycling to acylglycerol. In all tissues, G3P can also be derived from DHAP, an intermediate of glycolysis and gluconeogenesis (Fig. 1b).

Mice Glycerol kinase-deficient male mice appear normal at birth, but exhibit postnatal growth retardation, hyperglycerolemia (397±49 mg/dL vs normal, 5.9±1.0 mg/dL), elevated plasma FAs (1.5±0.16 mmol/L; normal, 0.5±0.06), and a threefold increase of plasma corticosterone. They die 3–4 days after birth. Heterozygous females are healthy and biochemically normal (Huq et al 1997).

Deficiency of glycerol-3-phosphate dehydrogenase 1 (GPD1, cytoplasmic GPD)

Humans Basel-Vanagaite et al (Basel-Vanagaite et al 2012) described ten individuals from an Israeli Arab geographic isolate and Joshi et al (Joshi et al 2014) reported one patient (designated here as patient 11); patients 1–10 are homozygous for a splicing mutation in *GPD1* predicted to cause premature termination and patient 11 is a genetic compound with no detectable GPD1 protein in liver.

Patients had hypertriglyceridemia (2.5–70 mmol/L at presentation), marked fatty liver, hepatomegaly from birth, and four- to eightfold elevations of serum aminotransferase and gamma glutamyltransferase levels. They tended to improve with age. Heterozygotes were unaffected (Basel-Vanagaite et al 2012). Although in patients 1–10, a tendency to fibrosis was suspected (Basel-Vanagaite et al 2012), patient 11 had no evidence of inflammation or fibrosis. In patient 11, extensive metabolic evaluations were otherwise normal except that the activities of carnitine palmityltransferase I and II were marginally low in fibroblasts. She had normal plasma bilirubin, brain magnetic resonance imaging, and normal neurological development until at least 2.5 years. The long term course of *GPD1* deficiency remains to be defined, but treatment with a low fat, high carbohydrate hypercaloric diet with medium chain triglyceride supplementation was provided, with improvement of the failure to thrive.

The observed phenotype of fatty liver and hypertriglyceridemia is not an obvious consequence of the pathway depicted in Fig. 1b. GPD1 deficiency might be expected to decrease TG synthesis, by restricting the supply of G3P from cellular glucose-derived metabolites. To explain the clinical phenotype, the authors (Joshi et al 2014) speculate that the peroxisomal pathway of DHAP acylation (Hajra 1997) may be activated. Perhaps this clinical observation will force reconsideration of the canonical pathway of TG synthesis. Alternatively, G3P production by liver GK may suffice to stimulate acylglycerol synthesis.

Deficiency of the mitochondrial *GPD2* enzyme has not to our knowledge been described in humans.

Mice Murine *Gpd1* deficiency occurs naturally in BALB/cHeA mice (Prochazka et al 1989), apparently without clinical consequence. Gene targeted, *Gpd2*-deficient mice show 50 % mortality, reduced WAT mass, but normal BAT function including cold tolerance. Mice deficient in both *Gpd1* and *Gpd2* develop severe hyperglycerolemia (30–50 mmol/L) and hyperglyceroluria, severe hypoglycemia, ketosis in the first day of life, have ~40 % reduction of hepatic ATP levels, ten- to 20-fold elevated liver G3P levels, and die within a few days (Brown et al 2002). Liver fat content was not mentioned for these acutely-ill animals. The authors speculated that because of the low lactose content of mouse milk in which it comprises only 2–5 % of calories, versus about 42 % in human milk, gluconeogenesis from TG glycerol may be critical for survival in newborn mice (Brown et al 2002). The acute phenotype of *Gpd1*/*Gpd2*–deficient mice would overshadow signs, if any, resembling the *GPD1*-deficiency phenotype seen in humans.

Inborn errors of Acyl-CoA synthesis

Two hereditary deficiencies of acyl-CoA synthetases have been reported. Mutations in ACSL4, a long chain FA-CoA synthetase expressed in brain, cause a nonspecific form of X-linked mental retardation (Meloni et al 2002; Longo et al 2003). In mice, deficiency of very long chain fatty acyl-CoA synthetase, a peroxisomal and endoplasmic reticulum enzyme, impairs the oxidation of very long chain fatty acids. This deficiency is well-tolerated clinically and does not worsen the phenotype of mice with combined deficiency of the X-linked adrenoleukodystrophy protein encoded by *ABCD1* (Heinzer et al 2003). These diseases show the importance of the CTGM pathway in tissues like brain that are not part of the canonical hierarchy of lipid energy flow and storage among WAT, liver, heart, and skeletal muscle. They are not considered further in this review.

Diseases of intracellular TG metabolism

AGPAT2 deficiency

Autosomal recessive deficiency of AGPAT2 underlies nearly half of cases of severe congenital generalized lipodystrophy (Vigouroux et al 2011) (Table 2). The most direct explanation for the lipodystrophy is a failure to synthesize TGs, although abnormalities of adipocyte dedifferentiation or accumulation of toxic lipids related to the AGPAT reaction cannot be eliminated (Takeuchi and Reue 2009).

The clinical presentation of congenital lipodystrophy has been clearly described (Van Maldergem 2012). Lipoatrophy is usually evident at birth, although the absence of facial fat may not be apparent for several months. In a person who presents with a lipodystrophy, the absence of clinical lipoatrophy in photographs taken after 1 year of age is strong evidence against the diagnosis of congenital lipodystrophy. The combination of muscle hypertrophy and lipoatrophy confers an athletic appearance to patients. Some patients have large hands and feet and prominent jaws, suggestive of acromegaly. The extremely low white adipose tissue (WAT) mass of patients leads to visceral steatosis, insulin resistance, hypertriglyceridemia, and low circulating levels of leptin and adiponectin. Insulin resistance is present, accompanied by hyperglycemia, hypertriglyceridemia which can be severe and acanthosis nigricans. Diabetes mellitus develops in ~30 % of patients during the second decade. Hepatomegaly is nearly universal, due to steatosis. Cirrhosis may develop. Hypertrophic cardiomyopathy is present in ~25 % of patients. It increases the risk of sudden death in young adults but it can be symptomatic even in infants. Clinically-severe patients may have failure to thrive or paradoxically, gigantism with hepatomegaly and macroglossia. In many clinical series, molecular diagnosis was not available; hence the precise risk for AGPAT2-deficient patients may differ from the above figures.

Mice Agpat2-deficient mice are lipodystrophic, showing a tenfold reduction in body TG content, hepatic steatosis, low leptin levels, and die at~3 weeks of age.

Diagnosis and differential diagnosis Clinical and biochemical parameters cannot reliably distinguish AGPAT2-deficient patients from others with severe congenital generalized lipodystrophy (Vigouroux et al 2011). The other major autosomal recessive form of severe generalized congenital lipodystrophy is associated with inactivating mutations in seipin (Magre et al 2001).

Although molecular testing of the AGPAT2 and seipin genes provides the most specific diagnostic approach, some clinical differences among the causes of congenital lipodystrophy are of note. Some studies report that AGPAT2-related congenital lipodystrophy may be more severe than that related to seipin deficiency, showing loss of fat tissue in mechanical areas such as palms, soles, orbits, scalp, and periarticular regions; both forms showed depletion in intermuscular, bone marrow, intraabdominal and intrathoracic adipose depots (Simha and Garg 2003). Developmental delay is reported less frequently in AGPAT2 deficiency (10 %) than in seipin deficiency (80 %) (Van Maldergem 2012). The occurrence of bone cysts, situated in the epiphyseal and metaphyseal regions of long bones, suggests AGPAT2 deficiency (10-20 % or patients) (Van Maldergem 2012). If creatine kinase levels are elevated, rarer forms of lipodystrophy should be suspected, due to deficiencies of the *CAV1* or *PTRF* genes (Van Maldergem 2012).

Treatment Adequate general nutrition and symptomatic treatment of insulin resistance/diabetes are important elements. A low fat diet (20–30 % of energy as lipids) can reduce circulating TG levels (Van Maldergem 2012). Leptin replacement therapy may improve hypertriglyceridemia and diabetes (Oral et al 2002; Ebihara et al 2007).

LPIN deficiencies

LPIN1 and *LPIN2* deficiencies each cause human phenotypes. *LPIN1* is expressed in adipose tissue and skeletal muscle. Autosomal recessive *LPIN1* deficiency is one of the commonest causes of severe recurrent rhabdomyolysis in childhood (Michot et al 2010, 2012). In a study of 141 patients with rhabdomyolysis, 18 (12.8 %) were found to have homozygous *LPIN1* mutations. Sixteen of 18 (89 %) were aged 2–6 years; the remaining two patients were aged 8 and 42 years at their first identified episode. Rhabdomyolysis was typically severe, with creatine kinase levels >10,000 IU/L, and often

occurred following exercise, febrile illness, anesthesia or fasting. Six of the *LPIN1*-deficient patients died (33 %) as well as five of their siblings who had not been investigated. The cause of death was cardiac arrest, sometimes with hyperkalemia. Three of six autopsies showed cardiomyopathy and one, hepatic steatosis. The frequency of rhabdomyolytic episodes decreased with age. Between episodes, patients are typically normal. Some were athletic, but four, including the two oldest (45 and 46 years old), showed mild, apparently chronic myolysis. Six patients had mild proximal weakness and one required a wheel chair. No patients with LPIN1 mutations were found among groups of patients who presented with milder rhabdomyolysis (CK<10,000) or with myalgia without rhabdomyolysis. Patients had normal WAT mass and distribution, normal plasma levels of cholesterol, TGs and adiponectin and no evidence of neuropathy. Intriguingly, single mutations of LPIN1 were found in 4/141 patients, including one who received statin therapy, and the authors speculated that rhabdomyolysis may occur under stress in some LPIN1 deficiency heterozygotes.

Mice Fld (fatty liver dystrophy) mice, which are deficient in *Lpin1*, develop lipodystrophy and neuropathy and lack PAP activity in WAT (Peterfy et al 2001). The excess of neutral fat in tissues may not be TGs, but rather non-TG fatty acyl esters such as cholesteryl esters (Zhang et al 2014). Heterozygous *Lpin1*-deficient mice are susceptible to statin-induced rhabdomyolysis (Zhang et al 2014). Mice with combined *Lpin1* and *Lpin2* deficiencies die prenatally at 12.5 days pc (Dwyer et al 2012).

Multiple mechanisms potentially underlie the clinical manifestations of *LPIN1* deficiency. *LPIN1* mediates the key bifurcation step of acylglycerol metabolism, at the branch point between phospholipid synthesis and TG storage. It also influences transcription: in adipocytes, it interacts with and directly modulates the actions of PPAR-gamma in the nucleus (Kim et al 2013). Interesting recent work (Zhang et al 2014) shows that *LPIN1* deficiency in muscle can simultaneously increase the early stages of autophagy and reduce the clearance of autophagic vacuoles.

Diagnosis and differential diagnosis of LPIN1 deficiency *LPIN1* deficiency should be considered in patients with recurrent episodes of severe rhabdomyolysis, especially if plasma acylcarnitine levels are normal. Occurrence in young children is an element in favor of *LPIN1* deficiency since many other causes occur mainly at older ages. Sequence analysis of *LPIN1* is the preferred diagnostic method and should be performed at an early stage of the evaluation of rhabdomyolysis. Muscle biopsy, if performed, should be obtained after recuperation from the rhabdomyolytic episode. Histology and biochemical testing of muscle are useful for ruling out glycogenoses and mitochondrial respiratory chain disorders, although ragged red fibers have been described in some biopsies of LPIN1-deficient patients.

Treatment The treatment of rhabdomyolysis in *LPIN1* deficiency is symptomatic: aggressive intravenous fluid administration and monitoring of electrolytes, kidney, and cardiac functions as for episodes of rhabdomyolysis of any cause (Bergounioux et al 2012). The relationship between *LPIN1* and inflammation is not well understood (Michot et al 2013). Suppression of inflammation may eventually prove to have therapeutic potential, but this is speculative.

Mutations in *LPIN2* cause Majeed syndrome (Ferguson et al 2005), an inflammatory condition reviewed elsewhere in this issue.

DGAT1 deficiency

Two siblings with severe watery diarrhea from birth and protein-losing enteropathy requiring long-term parenteral nutrition were found to be homozygous for a functionally-deleterious splicing mutation in *DGAT1* (Haas et al 2012). Birth weights were normal. The first patient, a girl, died of infection aged 17 months. In contrast, in the affected brother, diarrhea and intestinal protein loss subsided after 10 months of age and at 46 months he was thriving on a normal diet. Both siblings had a twofold increase in plasma TG and normal plasma cholesterol levels. Of note, DGAT1 is ubiquitously expressed in humans, with highest levels in intestine (Cases et al 1998).

Mice In mice, DGAT1 and DGAT2 together account for nearly all TG synthesis (Harris et al 2011). Both are expressed in murine intestine, but human intestine expresses mainly DGAT1 (Haas et al 2012). DGAT1-deficient mice have growth failure despite higher than normal energy intake, and have elevated energy expenditure. Diarrhea is not reported. DGAT1 knockout mice show low fat mass (Smith et al 2000). Human DGAT2 deficiency has not been described, but DGAT2-deficient mice have low tissue TG levels detectable prenatally, ichthyosis due to impaired cutaneous permeability barrier function and early postnatal death (Stone et al 2004).

Diagnosis and differential diagnosis In addition to the fascinating initial publication (Haas et al 2012), reports of additional unrelated patients will be necessary before formally assigning a phenotype to DGAT1 deficiency. Conversely, it is reasonable to consider DGAT1 deficiency in patients with unexplained congenital diarrhea and protein loosing enteropathy. Molecular analysis of leukocyte DNA provides the most convenient diagnostic method.

Perilipin 1 deficiency

Heterozygous frameshift mutations were found in *PLIN1* in five women with partial lipodystrophy (Table 2), who lacked a gynecoid pattern of fat distribution (Gandotra et al 2011). Patients were 25–54 years old and originated from three families. In the patients, total fat mass was about 30 % less than normal but a high lean mass was present, resulting in a normal body mass index. Other findings included fatty liver, elevated levels of plasma glucose and insulin, twofold elevation of plasma TGs and low levels of HDL cholesterol and adiponectin. One patient required insulin treatment. Adipose tissue biopsies showed reduced mean adipocyte diameter and increased macrophage infiltrate and fibrosis.

Mice Mice with homozygous *Plin1* deficiency have low total body fat mass, high lean mass, and a tendency to glucose intolerance and peripheral insulin resistance (Martinez-Botas et al 2000; Tansey et al 2001). Isolated *Plin1*-deficient adipocytes show elevated basal lipolysis but attenuated stimulated lipolysis (Martinez-Botas et al 2000; Tansey et al 2001). Heterozygous *Plin1*-deficient mice had a lower mean fat pad mass (~20 % less than wild type controls) but this was not statistically significant because of the variability of this parameter among mice of the same genotype (Tansey et al 2001).

Differential diagnosis Molecular diagnosis is the most specific diagnostic method. Clinically, the fat distribution reported in the small number of PLIN1-deficient patients is typical for partial lipodystrophy (Table 2). It differs from that of Dunnigan type familial partial lipodystrophy, in which fat accumulation in the face and neck is striking (Garg et al 1999).

Of note, the phenotypes of PLIN1-heterozygous humans and mice differ substantially. Patients with the autosomal dominant human form all had truncating mutations that removed the C-terminus of *PLIN1*, which is involved in CGI-58 binding, as opposed to null alleles in the mice. Although we do not have specific data in support of the following, perhaps the 3' truncating mutations described in humans may have a distinct dominant action. Alternatively, perhaps the differences between the mouse and human phenotypes relate to other differences in human and mouse physiology unrelated to PLIN1 itself.

ATGL and CGI-58 deficiencies: neutral lipid storage diseases (NLSDs)

Two forms of NLSDs are distinguished clinically as having either predominant muscle involvement (NLSDM) or ichthyosis (NLSDI) (Igal et al 1997). Generally, patients with NLSDM have ATGL deficiency and those with NLSDI, CGI-58 deficiency (Fischer et al 2007).

The Jordan anomaly of circulating polymorphonuclear leucocytes (Fig. 3a–c) is a sensitive, useful clinical finding in both NLSDM and NLSDI. Leucocytes show vacuoles that appear empty on routine Giemsa staining and red on oil red O staining for neutral lipid (Jordans 1953).

CGI-58 deficiency (NLSDI)

NLSDI patients have a nonbullous congenital ichthyosis of variable distribution and severity (Fig. 3d–f). Skin flexures, scalp and face are frequently affected. Hyperkeratosis of the palms and soles and pruritis often occur. Severe cases present as collodion babies, with a desquamating membrane and underlying erythroderma (Israeli et al 2012).

Because of the many causes of ichthyosis, noncutaneous findings are important for the clinical diagnosis of NLSDI. Jordan's anomaly is predicted to be a sensitive marker even in neonates. Hepatic steatosis is frequent, with two- to four-fold elevations of plasma aminotransferases. Steatohepatitis, fibrosis, and cirrhosis can occur (Mitra et al 2010). Myopathy may be present, with elevated serum creatine kinase, abnormal electromyographic studies and an excess of neutral lipid in types 1 and 2 fibers (Bruno et al 2008; Laforet and Vianey-Saban 2010). Liver and muscle findings can be detected clinically even in infancy and may be present even in clinically-asymptomatic patients (Cakmak et al 2012; Perrin et al 2013). The possibility of low-grade liver or muscle pathology should be recalled in patients with known NLSDI. Neurosensory deafness, cataract, nonprogressive psychomotor retardation, ataxia, spasticity, and cardiomyopathy are also well-documented in NLSDI, but do not occur in all patients.

Mice CGI-58-deficient mice have ichthyosis, hepatic steatosis, growth retardation, and neonatal death (Radner et al 2010).

Diagnosis and differential diagnosis Jordan's anomaly should be searched for in ichthyotic patients suspected of NLSDI. Skin biopsy may show the presence of neutral fat droplets, but these may be subtle. Frozen sections of the biopsy are necessary for lipid staining because lipids are removed by routine fixation. Therefore, non-identification of lipid droplets in skin biopsy does not exclude NLSDI. It is noteworthy that many diseases of complex lipid metabolism cause pediatric ichthyosis (reviewed in (Rizzo et al 2012)). The absence of clinical signs in liver or muscle does not exclude NLSDI. Molecular testing can provide specific diagnosis in many cases.

Treatment is symptomatic. Case reports describe some improvement of ichthyosis and mild reduction in aminotransferase levels following treatment with retinoids (Israeli et al 2012; Srinivasaraghavan et al 2014). Dietary fat restriction has not been tested extensively (Srinivasaraghavan et al 2014).

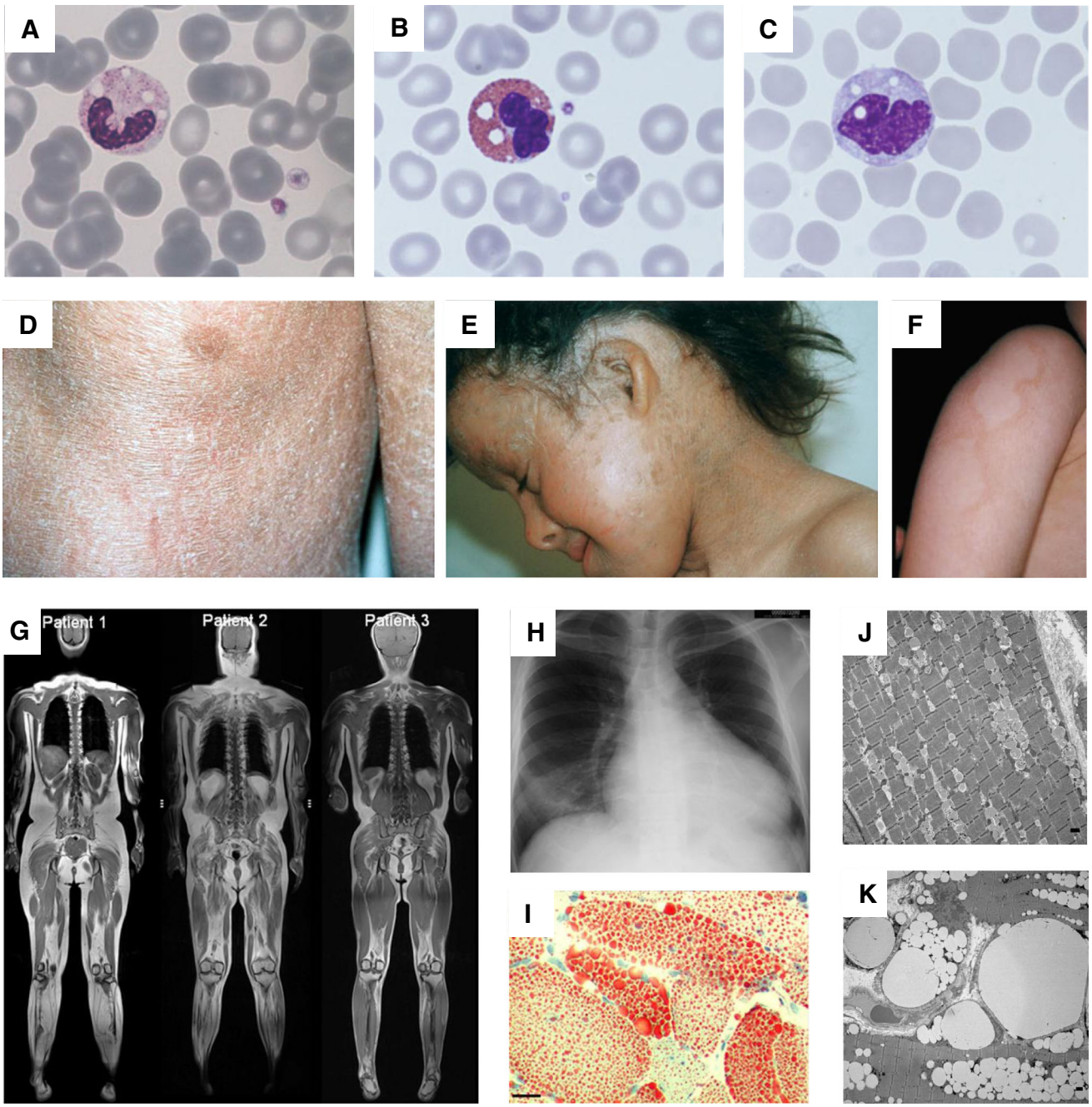

Fig. 3 Clinical images of neutral lipid storage diseases. (a-c) Giemsa-stained peripheral blood smears showing Jordan's anomaly in (**a**) neutrophils, (**b**) eosinophils, and (**c**) basophils (from (Piva et al 2009)). (**d**–**f**) NLSDI, showing diffuse ichthyosis (**d**,**e**) (Lefevre et al 2001), and a variant resembling erythrokeratoderma (**f**) (Pujol et al 2005). (**g**–**i**) NLSDM. (**g**) magnetic resonance images of NLSDM patients (from (Laforet et al 2013)), T1-weighted whole body views of three NLSDM patients; the two patients on the left have more severe involvement, showing marked fatty replacement in the scapular girdle, especially the supra- and infra-spinatus muscles, and in the legs; (**h**) cardiomyopathy with increased cardiothoracic ratio on chest radiography, from (Ohkuma et al 2008) and (**i**) skeletal muscle biopsy, from (Reilich et al 2011) showing the accumulation of neutral lipid (oil red O stain; bar, 10 μm. (**j**–**k**) Electron microscopic images from (Janssen et al 2013) of two muscle biopsies from a symptomatic NLSDM heterozygote taken in childhood (**j**) and at age 21 (**k**); bar, 1 μm

ATGL deficiency (NLSDM)

Patients with ATGL deficiency (Hirano et al 2008; Reilich et al 2011; Janssen et al 2013) typically present as young adults with weakness and fatty infiltration of muscle (Fig. 3g) or cardiomyopathy (Fig. 3h). Weakness can be proximal, distal or generalized. It is progressive. Some patients report having been athletic in childhood. Among 18 patients (Janssen et al 2013), cardiomyopathy, 9/18 (50 %); hyperlipidemia, 8/18 (44 %); hepatomegaly, 5/14 (36 %), and

diabetes, 4/16 (25 %) were observed. Serum creatine kinase levels were elevated in all 17 patients tested (100 %, mostly ~200-2000 units/L; maximum 4700). Lipid accumulates mainly in type I (oxidative slow twitch) fibers (Fig. 3i). Dilated cardiomyopathy occurs to a variable extent in some ATGL-deficient patients, and may require cardiac transplantation (Hirano et al 2008). One patient showed lipid accumulation throughout the coronary arteries (Hirano et al 2008). A detailed study of three patients (Natali et al 2013) revealed high visceral and pancreatic fat and impaired glucose-stimulated insulin secretion. These patients showed diffuse steatosis of variable severity in skeletal muscle, heart, and liver. Insulin sensitivity was preserved. Heart metabolism shifted from lipid to carbohydrate-related substrates. Fasting nonesterified FA level, basal lipolytic rate, and insulin-mediated suppression of lipolysis were normal, but norepinephrine-stimulated lipolysis was impaired. Episodes of rhabdomyolysis are not reported in NSLDM.

Symptomatic heterozygotes Janssen et al reviewed 21 symptomatic heterozygotes and their associated mutations in *PNPLA2* (Janssen et al 2013). Some heterozygotes have lipid accumulation in muscle, leukocytes (Jordan's anomaly), and basal keratinocytes. The fraction of heterozygotes that develop symptoms is unknown. Signs in heterozygotes tended to be milder than in homozygotes, but clinically-significant findings were frequent: lipid myopathy, 16/21 (76 %) (Fig. 3j–k); cardiomyopathy, 9/21 (42 %) including ventricular tachycardia in one patient requiring placement of a pacemaker; hepatomegaly, 4/21 (19 %); insulin resistance or diabetes, 3/21 (14 %).

Mice Mice with complete ATGL deficiency die at 4–5 months of hypertrophic lipid cardiomyopathy and lipid myopathy (Haemmerle et al 2006). TG accumulation occurs in numerous cell types including vascular smooth muscle cells (Lin et al 2013). Liver-specific ATGL knockout mice show steatosis, suggesting an important role for ATGL in liver (Wu et al 2011). Adipocyte-specific ATGL knockout mice show low lipolytic capacity and rapid onset of hypothermia, lethargy and a torpor-like state during fasting (Wu et al 2012).

Diagnosis and differential diagnosis ATGL deficiency should be suspected in patients with myopathy and/or cardiomyopathy, particularly if biopsy is available and shows neutral lipid accumulation. Jordan's anomaly is a sensitive and specific finding for NLSDs. Other causes of lipid myopathy such as disorders of mitochondrial acylcarnitine transport or of FA oxidation can be distinguished by their characteristic profiles of acylcarnitines and other metabolites. In patients with lipid myopathy and who have normal levels of plasma acylcarnitines, molecular testing for *PLPNA2* mutations should be considered.

Treatment The cardiomyopathy of ATGL-deficient mice resolved following treatment with the PPAR-alpha agonist Wy14643 (Haemmerle et al 2011). PPAR transcription factors regulate many genes of lipid and energy metabolism and FAs are their natural ligands. The authors suggested that PPARs interacted preferentially with the pool of FAs derived from lipolysis. PPAR alpha agonist treatment was less successful in humans (van de Weijer et al 2013). A 37 year-old woman with known *PNPLA2* mutations, progressive weakness since childhood, biventricular cardiomyopathy, reduced ejection fraction, and episodic ventricular tachycardia requiring indwelling defibrillator placement, received a 28-week course of bezafibrate (400 mg/day). Leg strength improved somewhat but grip strength and cardiac ultrasound were unchanged. Although anecdotal, this report shows that PPAR agonist treatment is not universally effective in human NLSDM. Rodents tend to be more sensitive than humans to PPAR agonist treatment (Bility et al 2004), but given the success with murine ATGL deficiency, further study of PPAR agonist effects seems warranted.

During fasting, mice with adipocyte-specific ATGL deficiency show marked reduction of muscle mass, presumably reflecting proteolysis to provide a gluconeogenic substrate in the lack of sufficient FAs from lipolysis (Wu et al 2012). If a similar phenomenon occurs in human NLSDM, it could worsen the myopathy. Pending specific studies in humans, it seems prudent to recommend a generous protein intake and avoidance of prolonged fasting in NLSDM patients.

The possibility of symptomatic heterozygosity should be recalled during family assessment. Siblings and parents should be tested clinically for Jordan's anomaly, heart dysfunction and muscle weakness, and molecular testing should be considered for first-degree relatives of patients.

HSL deficiency

Two HSL-deficient humans have been reported (Albert et al 2014). In a population study of plasma TG levels, a 19 base pair frameshift deletion in *LIPE*, was found to be prevalent in the Old Order Amish population. Studies in WAT of a homozygote revealed absence of HSL protein, small adipocytes, impaired lipolysis, increased DG content, inflammation, and down-regulation of peroxisome-proliferator–activated receptor γ (PPAR-γ) target genes. Heterozygotes revealed mildly increased plasma TG levels compared to controls (109.9± 71.0 in heterozygotes versus 84.6±59.6 mg/dL in normal (p=0.003) and 145 in the one reported homozygote) and insulin resistance, but no differences in fat mass or blood pressure.

Mice In WAT, HSL-deficient mice have been produced. In WAT, they show low mass, inflammation (Cinti et al 2005), and reduced TG synthesis (Zimmermann et al 2003), with

heterogeneity of adipocyte size, normal fasting lipolysis (Fortier et al 2004) BAT mass is increased. HSL-deficient mice have numerous extra-adipose manifestations including adrenal degeneration with generally-preserved adrenocorticoid synthesis (Li et al 2002) and male infertility (Chung et al 2001).

Diagnosis Heterozygous HSL deficiency is on the borderline between classic inborn errors and common metabolic traits like hypertriglyceridemia and insulin resistance. It is too early to suggest a general diagnostic approach for severe HSL deficiency, because of lack of clinical data about extra-adipose manifestations, if any, in homozygous humans.

Conclusion

Inborn errors of CTGM occupy a frontier zone, producing certain rare phenotypes and also altering common metabolic parameters such as insulin resistance, diabetes, and obesity. They provide insight into common genetic metabolic diseases related to adipocytes, like obesity and diabetes. However, among the relatively small number of inborn errors of CTGM identified to date, the clinical signs related to adipose tissue are usually mild compared to those in organs like skeletal muscle, liver, and heart. These are major organs of FA flux and metabolism and CTGM is shown to be critical for extra-adipose energy homeostasis. Furthermore, clinical findings in other organs, like ichthyosis, diarrhea, psychomotor retardation, and Jordan's anomaly of leucocytes, reveal a widespread importance for CTGM or perhaps suggest unconventional roles for CTGM proteins in the affected tissues. Hereditary deficiencies have been described for less than one-third of CTGM-related genes. Further CTGM-related phenotypes may emerge from genome-wide diagnostic procedures in patients with unexplained clinical symptoms.

Molecular testing is the most precise diagnostic method for inborn errors of CTGM. In many diseases of CTGM, diagnostic pattern of circulating or excreted metabolites are useful diagnostically. Diagnostic enzyme assays are not well-established for these conditions. The development of reliable biochemical testing would be valuable and would fill the predictable diagnostic void for some patients with atypical clinical presentations or molecular findings of unknown significance.

Heterozygotes for some inborn errors of CTGM may develop similar symptoms to those of homozygotes (e.g., LPIN1, ATGL, HSL, PLIN1). Interestingly, the notion of synergistic heterozygosity was developed by consideration of inborn errors of fatty acid oxidation, a related group of diseases, to explain the reports of symptomatic patients or animals that have only one identifiable abnormal allele (Schuler et al 2005). The occurrence of clinical signs in patients with partial enzyme deficiencies suggests that diseases of CTGM and of lipid energy metabolism in general may provide interesting models of the importance of environment and of other metabolic pathways on clinical manifestations of disease.

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Compliance with ethics guidelines

Conflict of interest None.

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