

Neutral lipid storage disease: genetic disorders caused by mutations in adipose triglyceride lipase/PNPLA2 or CGI-58/ABHD5

Martina Schweiger, Achim Lass, Robert Zimmermann, Thomas O. Eichmann, and Rudolf Zechner

Institute of Molecular Biosciences, University of Graz, Graz, Austria

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Schweiger M, Lass A, Zimmermann R, Eichmann TO, Zechner R. Neutral lipid storage disease: genetic disorders caused by mutations in adipose triglyceride lipase/PNPLA2 or CGI-58/ABHD5. *Am J Physiol Endocrinol Metab* 297: E289–E296, 2009. First published April 28, 2009; doi:10.1152/ajpendo.00099.2009.— Neutral lipid storage disease (NLS) is a group of autosomal recessive disorders characterized by the excessive accumulation of neutral lipids in multiple tissues. Recently, two genes, *adipose triglyceride lipase (ATGL/PNPLA2)* and *comparative gene identification-58 (CGI-58/ABHD5)*, have been shown to cause NLS. ATGL specifically hydrolyzes the first fatty acid from triacylglycerols (TG) and CGI-58/ABHD5 stimulates ATGL activity by a currently unknown mechanism. Mutations in both the ATGL and the CGI-58 genes are associated with systemic TG accumulation, yet the resulting clinical manifestations are not identical. Patients with defective ATGL function suffer from more severe myopathy (NLSM) than patients with defective CGI-58 function. On the other hand, CGI-58 mutations are always associated with ichthyosis (NLSI), which was not observed in patients with defective ATGL function. These observations indicate an ATGL-independent function of CGI-58. This review summarizes recent findings with the goal of relating structural variants of ATGL and CGI-58 to functional consequences in lipid metabolism.

ichthyosis; myopathy; Jordans' anomaly; Chanarin-Dorfman syndrome; lipolysis

Neutral Lipid Storage Disease with Ichthyosis

NEUTRAL LIPID STORAGE DISEASE (NLS) is a rare nonlysosomal, autosomal recessive lipid storage disorder characterized by systemic triacylglycerol (TG) deposition in multiple tissues (40), including skin, muscle, liver, central nervous system, and blood leukocytes. One of the diagnostic characteristics of NLS, the lipid-containing vacuoles in leukocytes, was originally observed by Jordans et al. (29) in two brothers suffering from muscular dystrophy, and subsequently the condition was named Jordans' anomaly. In 1966, Rozenszajn et al. (45) described two sisters exhibiting the clinical manifestation of neutral lipid-containing vacuoles in leukocytes combined with severe ichthyosis. In the 1970s, Dorfman et al. (12) and Chanarin et al. (7) reported additional cases of NLS with ichthyosis and lipid accumulation in leukocytes, gastrointestinal epithelium, bone marrow, cultured fibroblasts, liver, and striated muscle. More variable clinical features included liver steatosis, hepatosplenomegaly, skeletal and cardiomyopathy, double-sided cataracts, growth retardation, ataxia, bilateral sensorineural hearing loss, horizontal nystagmus, and/or mental retardation (7, 12, 45). In recognition of these early works, NLS with ichthyosis was subsequently named Chanarin-Dorfman syndrome (CDS). The specific form of ichthyosis associated with CDS represents nonbullous congenital ichthyosiform erythroderma and is characterized by a pronounced

defect in the permeability barrier function of the skin (58). The condition is thought to result from the excessive incorporation of nonpolar lipids into the lamellar membrane (43). As a consequence, lipid microinclusions form a nonlamellar phase within the stratum corneum interstices, impairing the barrier function (10, 13).

In an excellent overview published in 1996, Igal et al. (26) comprehensively summarized data on 44 patients with NLS. The authors discriminated between two groups of patients, 26 individuals that were affected with ichthyosis and 18 without ichthyosis. They also showed that essentially all NLS patients without ichthyosis suffered from cardiomyopathy, whereas this condition was uncommon in CDS patients with ichthyosis. The distinct differences in clinical manifestations of NLS between the two groups of patients suggested that the underlying mutations might affect (at least) two different genes. However, the identity of these genes remained unknown until 2001.

Mutations in CGI-58 Cause NLS with Ichthyosis

In homozygosity mapping of consanguineous families with autosomal recessive ichthyosis, nonbullous congenital ichthyosiform erythroderma was linked to a 7.7-cM (centimorgan) locus on chromosome 3p21 in six families (15). Finally, in 2001, Lefevre et al. (37) described eight different mutations in the human gene for *comparative gene identification-58 (CGI-58)*, also designated as α/β -hydrolase domain-containing protein 5 (*ABHD5*), in nine families as causative for NLS with ichthyosis (NLSI).

Address for reprint requests and other correspondence: R. Zechner, Institute of Molecular Biosciences, Univ. of Graz, Heinrichstrasse 31, A-8010 Graz, Austria (E-mail: rudolf.zechner@uni-graz.at).

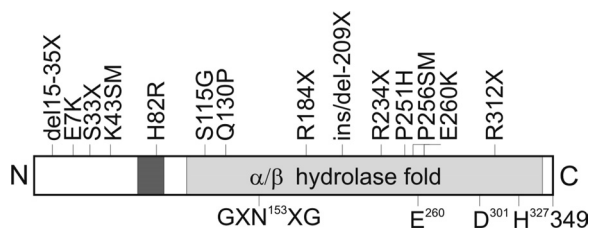


Fig. 1. Structural domains of human CGI-58 (comparative gene identification-58) protein. The α/β -hydrolase domain containing the consensus sequence GX¹⁵³SXG where serine is replaced by asparagine (N153). Histidine (H327), and the putative 3rd element of the catalytic triad, either glutamate (E260) or aspartate (D301), are shown. The dark-gray area marks the putative lipid-binding domain. Mutations associated with neutral lipid storage disease with ichthyosis (NLSDI) are indicated.

CGI-58 was originally identified by the comparative analysis of the human proteome and the proteome of *Caenorhabditis elegans* (33). The gene consists of 7 exons and has a length of 5370 bp. *CGI-58* mRNA comprises 1050 nucleotides and is translated into a 39-kDa polypeptide (349 amino acids). The protein belongs to an esterase/thioesterase/lipase gene family named α/β -hydrolase domain containing (ABHD) (39), which consists of 15 members. *CGI-58* is synonymous with ABHD5. Figure 1 displays the domain structure of *CGI-58*. The α/β -hydrolase fold is located between amino acids 104 and 345. The protein contains a “pseudo-catalytic triad” at positions 153(Asn)/327(His)/301(Asp). It is considered very unlikely that *CGI-58* exhibits hydrolase activity, because the nucleophilic serine usually found in the typical GX¹⁵³SXG esterase/lipase motif is replaced by an asparagine (Asn¹⁵³) (9, 36, 37, 50, 63). However, *CGI-58* contains a HX₄D motif between

amino acids 327 and 332 of the human protein that is present in proteins with acyltransferase activity (18, 21). A predicted lipid-binding motif corresponds to a highly hydrophobic region between amino acids 69 and 87 bordered by a conserved His⁸²/Gly⁸³ dipeptide (2, 50).

Figure 1 and Table 1 summarize the currently known, naturally occurring mutations in the gene for *CGI-58*. Sixteen mutations have been described (1, 37, 41, 49, 53, 54), including 10 single nucleotide mutations (7 transitions and 3 transversions) leading to missense mutations (7) and nonsense mutations (3). Additionally, two deletion mutations, two insertion mutations, and two splice site mutations leading to premature stop codons and truncated proteins have been shown to cause NLSD with ichthyosis. True null mutations have not been observed so far; however, several protein truncations lack the α/β -hydrolase domain, the lipid-binding region, the pseudo-catalytic triad, and the acyltransferase domain, suggesting that these patients completely lack functional *CGI-58* (37). The clinical phenotypes of patients suffering from NLSD with ichthyosis are summarized in Table 1. As mentioned above, all patients are affected with ichthyosis. Liver steatosis is seen only in eight of twelve patients and myopathy is quite rare.

Although the causal relationship between *CGI-58* and NLSD has been known since 2001 (37), the biochemical basis of NLSD caused by *CGI-58* mutations is only partially understood. Early studies with NLSD-derived fibroblasts demonstrated that the TG content is 2- to 20-fold higher in patient fibroblasts compared with cells from healthy individuals (22, 60). The accrual of neutral lipids was shown not to be caused by a defect in mitochondrial fatty acid (FA) uptake or defective β -oxidation (42, 60). Also, enzymatic activities of lipases,

Table 1. Clinical phenotypes caused by mutations in the gene for *CGI-58* from patients suffering NLSDI

Mutation in <i>CGI-58</i>	Origin	Ichthyosis	Cardiomyopathy	Myopathy	Liver Disease	Organs Affected by TG Accumulation	Other	Ref.
Exon 1 E7K (19G-A)	Morocco	Yes	NA	NA	NA	NA	NA	37
Exon 6 altered splice site P256SM (773-1G-A)	Tunisia	Yes	NA	NA	Hepatomegaly, liver steatosis	Skin, liver, bone marrow	Growth retardation, cataract	37
Exon 2 S33X (98C-G)	Algeria	Yes	NA	NA	None	Skin	Mental retardation, small ears, ectropion	37
Exon 3 altered splice site K43SM (135-2A-G), 2 patients	Algeria	Yes	NA	NA	None	None	Small ears	37
Exon 3 Q130P (389A-C)	Algeria	Yes	NA	NA	None	None	Mental retardation, sensory deafness	37
Exon 4 209X (594insC), 3 patients	Turkey	Yes	NA	NA	Hepatomegaly, liver steatosis	Skin	Growth retardation, small ears, strabismus	37
Exon 6 E260K (778G-A)	Turkey	Yes	NA	NA	Hepatomegaly	None	Ectropion, Alopecia	37
Exon 1 35X (46/47del AG)	France	Yes	NA	NA	Hepatomegaly	None	Ectropion, alopecia, strabismus	37
Exon 4 R184X (550C-T)	Japan	Yes	NA	NA	Liver cirrhosis	Skin	Growth and mental retardation,	2
Exon 5 P251H (752A-C)	East India	Yes	NA	NA	Liver steatosis	None	Growth and mental retardation, ectropion	54
Exon 5 R234X (700C-T); Exon 3 H82R (245A-G)	—	Yes	NA	Yes	NA	Muscle	Hearing loss, cataract	49
Exon 6 R312X (934C-T); Exon 4 209X (616insGGG, del 31bp)	—	Yes	NA	NA	Hepatomegaly	Skin	Erythrokeratoderma variabilis, splenomegaly	41
Exon 3 S115G (343A-G) and fibrosis	Turkey	Yes	NA	NA	Liver steatosis	Skin	Splenomegaly	53

NLSDI, neutral lipid storage disease with ichthyosis; NA., not analyzed.

carboxyesterases (25, 48, 59, 60), and enzymes for the glycerolipid synthesis (24, 59) were normal in most studies. Hilaire et al. (22), however, observed an impaired degradation of TG containing long-chain FA in NLSM cells, suggesting a defect in long-chain TG hydrolase activity. Importantly, Coleman and colleagues (24, 59) suggested that TG accumulation in NLSM is caused by a defect in the recycling of TG-derived acylglycerols into phospholipids.

More recent studies employed mutant CGI-58 to explore the functional role of this protein in TG accumulation. Yamaguchi et al. (62) demonstrated that the mutated CGI-58 variant Q130P is not recruited to lipid droplets (LD) due to an inability to interact with perilipin. Gosh et al. (18) tested whether the presence of the acyltransferase motif in CGI-58 has functional implications and showed that CGI-58 exhibits acyl-CoA-dependent acyltransferase activity for lysophosphatidic acid generating phosphatidic acid in vitro. This study also showed that mutant forms of CGI-58 (Q130P and E260K) associated with NLSM were not compromised in acyltransferase activity, indicating that this activity is not affected in NLSM. The physiological relevance of CGI-58 as acyltransferase and its putative role in lipid metabolism require elucidation. In 2006, our laboratory discovered that CGI-58 drastically enhanced the enzymatic activity of adipose triglyceride lipase (ATGL), suggesting that CGI-58 functions as an activator of lipolysis and intracellular mobilization of fat stores (36). Mutant CGI-58 variants (Q130P, E260K, and 190TER) failed to activate ATGL. These findings indicated for the first time that ATGL-mediated lipolysis might be crucially involved in cellular neutral lipid homeostasis and that a decreased ATGL activity in response to defective enzyme activation by CGI-58 results in systemic TG accumulation.

ATGL Is a CGI-58-Activated TG Hydrolase

ATGL was initially annotated as transport-secretion protein 2.2 (TTS 2.2). In 2004, three groups discovered independently that this protein was involved in adipose tissue TG breakdown, and the enzyme was designated adipose triglyceride lipase (ATGL) (66), desnutrin (56), and calcium-independent phospholipase (PL)A₂ζ (27). ATGL hydrolyzes TG and releases the first FA from the glycerol backbone. The enzyme exhibits high substrate specificity for TG and only low activity against diacylglycerols (DG) and phospholipids (27, 38, 66). Essentially no activity was observed against cholesteryl- and retinylester substrates (66). Jenkins et al. (27) and Lake et al. (34) showed that ATGL also acts as a DG transacylase. However, the physiological importance of this observation requires further investigation.

Figure 2 displays the domain structure of ATGL. Human ATGL comprises 504 amino acids and has a molecular mass of 56 kDa, sharing 86% homology with its murine ortholog (66). ATGL belongs to a class of α/β -proteins containing a patatin domain of three-layer sandwich architecture ($\alpha/\beta/\alpha$), which is located within the NH₂-terminal half of the protein (amino acid residues 1–251). The patatin domain is named after the potato tuber protein patatin, which was previously shown to exhibit weak acylhydrolase activity (46). The human genome harbors a family of nine proteins with a patatin domain subsumed under the name of patatin-like phospholipase domain containing (PNPLA) 1 to 9 (31, 61, 64, 65). The members of this

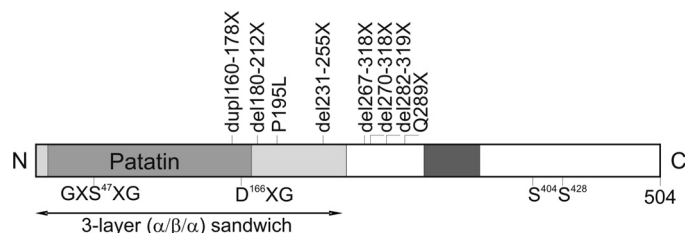


Fig. 2. Structural domains of human adipose triglyceride lipase (ATGL) protein. The 3-layer ($\alpha/\beta/\alpha$) sandwich contains the catalytic dyad consisting of the active site serine (S47) within the canonical GX SXG residues and the aspartic acid (D166). ATGL is phosphorylated on 2 serine residues (S404 and S428). The dark-gray area marks the putative lipid-binding domain. Mutations associated with NLSM with myopathy (NLSM) are indicated.

family most closely related to ATGL (PNPLA2) are PNPLA1, adiponutrin (PNPLA3), gene sequence-2 (GS2, PNPLA4), and GS2-like (PNPLA5). The physiological functions of these proteins remain mostly elusive.

The catalytic site of ATGL is located within the patatin domain and differs from the catalytic triad frequently found in serine hydrolases. It is characterized by an uncommon catalytic dyad similar to that of human cytosolic PLA₂ (cPLA₂) (11, 46). Mutational analysis revealed that in ATGL the catalytic dyad consists of Ser⁴⁷ (34, 36) located within a GX SXG motif and Asp¹⁶⁶ (our unpublished observation) outside the central β -sheet. Both patatin domain-containing proteins and cPLA₂ contain a Gly-Gly-X-Arg sequence motif that defines the oxyanion hole of the active site. The nitrogen atom in glycine stabilizes the oxyanion formed during the nucleophilic attack by the catalytic serine during substrate cleavage (46). For ATGL, the glycine-rich oxyanion hole is assembled by amino acid residues 14–18.

The COOH-terminal region of ATGL (amino acid residues 252–504) consists primarily of α -helical and loop regions. A putative lipid-binding region is located within a hydrophobic stretch at the COOH terminus of the protein (amino acid residues 315–360) (51). *Brummer* lipase, the ATGL orthologous enzyme in *Drosophila melanogaster* also contains a peptide region called *Brummer* box for localizing the protein to LD (19). The COOH-terminal region of ATGL includes two phosphorylation sites (Ser⁴⁰⁴ and Ser⁴²⁸, respectively). To date, however, it is unknown whether phosphorylation affects ATGL activity and which protein kinase phosphorylates the enzyme (4, 66). Recently, we reported that the COOH-terminal region of ATGL mediates LD binding and contains regions important for the regulation of enzyme activity (51). In the presence of the COOH-terminal protein region, the specific activity of ATGL is much lower than in its absence, suggesting an inhibitory effect of this region on hydrolase activity. This effect may be explained by the observed increased binding of CGI-58 to COOH-terminally truncated variants of ATGL compared with the full-length enzyme. However, the underlying mechanisms and the regulatory factors that are potentially involved are currently unknown.

Mutations in ATGL Cause NLSM

In 2006, Fischer et al. (16) described three individuals from the Netherlands, France, and Algeria who were affected with NLSM and exhibited mutations in the *ATGL* gene. To date, a total of eight ATGL gene mutations (summarized in Table 2

Table 2. Clinical phenotypes caused by mutations in the gene for ATGL from patients suffering NLSDM

Mutation in ATGL	Origin	Ichthyosis	Cardiomyopathy	Myopathy	Liver Disease	Organs Affected by TG Accumulation	Other	Ref.
Exon 5 P195L (584C-T); Exon 7 319X (808delC)	Netherlands	No	NA	Mild	Mild hepatomegaly	Muscle, skin	NA	37
Exon 7 319X (847delC)	France	No	Yes	Yes	Hepatomegaly, liver steatosis	Skin, muscle	Walking delay, diabetes,	16
Exon 7 Q289X (865C-T)	Algeria,	No	NA	Yes	None	Muscle	Muscle weakness	16
		No	Yes	Yes	NA	Muscle	Muscle atrophy	23
Exon 4 178X (475dupCTCC)	Japan	No	NA	Yes	None	Muscle	Walking delay, muscle weakness,	2
Exon 5 L255X (695delC)	Iran	No	NA	Yes	Hepatomegaly	Muscle	Muscle weakness diabetes	6
Exon 5 I212X (542delAC), 2 patients	Italy	No	Yes	Yes	None	Muscle	Muscle weakness	6
Exon 7 318X (799-802delGCCC)	—	No	Yes	Yes	Hepatomegaly	Liver, muscle	Muscle weakness, diabetes	32

NLSDM, NLS with myopathy.

and displayed in Fig. 2) were discovered by various laboratories (1, 6, 16, 23, 32). They include three single-nucleotide deletions, a dinucleotide deletion, and a deletion of four nucleotides. Additionally, a four-nucleotide duplication mutation and two single-nucleotide transversions have been reported. Besides one missense mutation (P195L), all known gene mutations result in premature stop codons due to translational frameshifts or nonsense mutations. True null mutations leading to the complete absence of ATGL have not been reported to date.

In addition to the shared features of systemic lipid accumulation and Jordans' anomaly, all patients were reported to suffer from muscle weakness and skeletal muscle myopathy; ichthyosis was not observed. Hepatomegaly was reported in some (6) but not all patients; instead, cardiomyopathy was common. In fact, cardiomyopathy was lethal in some patients or necessitated cardiac transplantation (23) (see below). Taken together, the clinical appearance of patients with ATGL gene mutations was consistent with the group of NLS patients without ichthyosis as classified by Igal et al. (26). To differentiate between these groups of patients and their clinical appearance, Fischer et al. (16) suggested that NLS that was caused by mutations in *CGI-58* and was always associated with ichthyosis be designated NLS with ichthyosis (NLSI) and that NLS that is caused by mutations in ATGL and associated with cardiac myopathy be designated NLS with myopathy (NLSM).

Interestingly, most ATGL mutations were detected in the COOH-terminal region of the patatin domain, leaving the proposed active site of the enzyme intact. To examine whether the mutated variants of the enzyme were still partially functional, our laboratory analyzed mutated ATGL from four patients originally reported by Fischer et al. (16). The single missense mutation at position 195 (P195L) led to the expression of an enzymatically inactive ATGL protein. LD binding or its interaction with CGI-58 was not affected. Unexpectedly, by use of artificial TG substrate in hydrolase assays in vitro, all COOH-terminal truncated ATGL proteins exhibited elevated

enzyme activity compared with the full-length enzyme and were stimulated by CGI-58. However, the truncated enzyme variants were drastically impaired in their ability to bind to cellular LD in intact cells (51). Consistent with these findings, Kobayashi et al. (32) reported impaired LD binding of a truncated version of ATGL identified in a Japanese patient (premature stop codon at amino acid 318). Together, this suggested that the COOH-terminal protein region of ATGL contains the LD binding region as well as a regulatory unit that affects the hydrolase activity of the enzyme.

Importantly, Akiyama et al. (1) found a Japanese case of NLSM that was caused by a duplication mutation (475dupCTCC), leading to a frameshift at amino acid 160 within the patatin domain. This genetic defect leads to a truncated ATGL that lacks 19 amino acids of the patatin domain, including the aspartic acid at position 166 (D-166), which is essential for catalytic activity. This patient developed severe myopathy at the age of 33 yr, together with massive LD accumulation in the sarcoplasm and leukocytes, as well as splenomegaly. Hepatomegaly or fatty liver was not observed. Since this mutation is very likely to result in a completely inactive enzyme, it seems likely that patients with complete ATGL deficiency may exist.

Comparing Phenotypes of NLSI, NLSM, and ATGL-Deficient Mice

As described above, mutations in the genes of *CGI-58* and *ATGL* are associated with the development of NLSI and NLSM, respectively. Figure 3 displays an overview of the lipolytic pathway and the clinical manifestations associated with these diseases. Lipolysis is currently seen as a three-step, three-enzyme process. ATGL and hormone-sensitive lipase (HSL) are responsible for the first step in TG degradation generating FA and DG. ATGL hydrolytic activity largely depends on the presence of its activator protein CGI-58 (for review see Ref. 64). The formed DG is the preferred substrate for HSL, leading to the formation of FA and monoglycerides

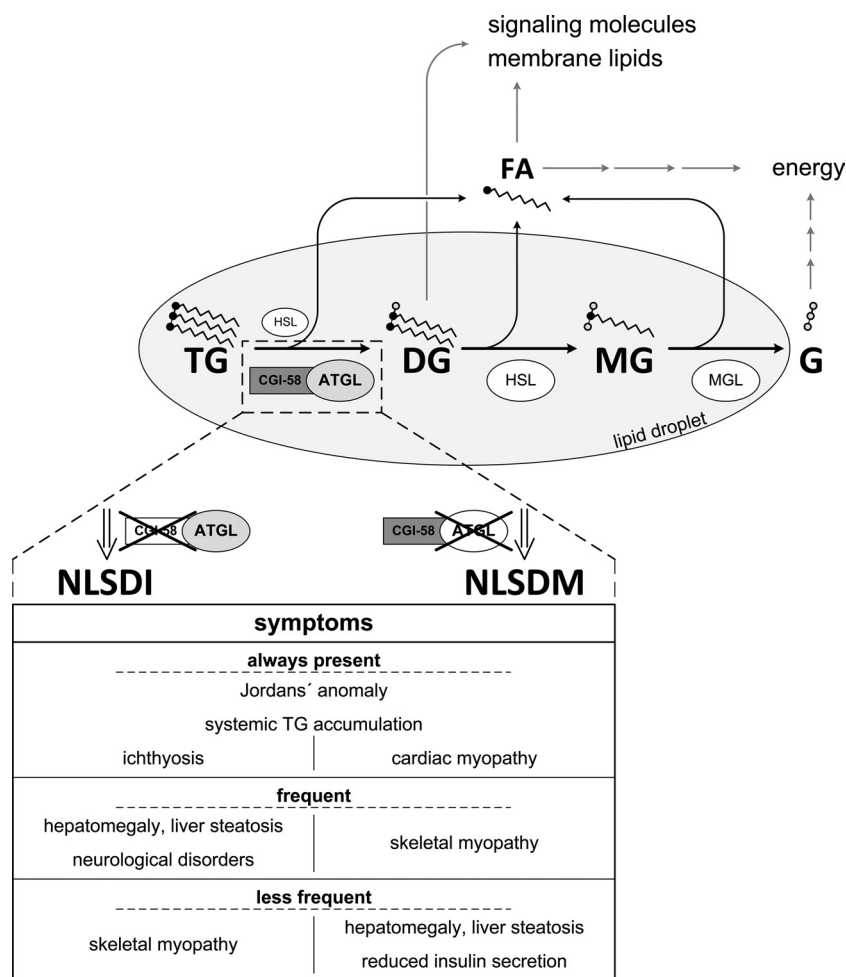


Fig. 3. Scheme of the lipolytic pathway and the clinical features observed in NLSDM and NLSDI. ATGL and hormone-sensitive lipase (HSL) are responsible for the hydrolysis of triglycerides (TG) generating free fatty acids (FA) and diglycerides (DG). DG is the major substrate for HSL activity, which leads to formation of FA and monoglycerides (MG). In the last step of lipolysis, monoglyceride lipase (MGL) catabolizes MG to FA and glycerol. Generated lipid intermediates are major energy substrates, precursors of membrane lipids, and signaling molecules. Defective ATGL and CGI-58 are associated with development of NLSDM and NLSDI, respectively. Symptoms, observed in patients suffering from NLSDM and NLSDI, which vary in frequency, are indicated.

(MG). In the last step of lipolysis, MGL hydrolyzes MG to FA and glycerol. The metabolic intermediates provided by the consecutive actions of all three lipases provide energy substrate, precursors for membrane lipids, and bioactive lipid molecules that regulate cell signaling processes and gene transcription.

As CGI-58 and ATGL function in the same biochemical pathway, it is reasonable to assume that mutations in *CGI-58* and in *ATGL* are associated with similar clinical phenotypes. Notably, however, the clinical phenotypes vary markedly between patients with CGI-58 deficiency (NLSDI) and ATGL deficiency (NLSDM) (summarized in Fig. 3). This suggests that our current understanding of the biochemical function of CGI-58 and/or ATGL is incomplete. The following section of the review discusses the similarities and differences in the clinical manifestations observed in patients suffering from NLSDI and NLSDM.

Ichthyosis. The fact that all patients with NLSDI carrying mutations in the gene for *CGI-58* develop ichthyosis, whereas those suffering from NLSDM do not, supports the concept of an ATGL-independent function of CGI-58 in the skin and possibly in other cells and organs. As in humans, no skin defect is observed in mice lacking ATGL, arguing against the involvement of this enzyme in the development of ichthyosis. Unfortunately, the phenotype of CGI-58-deficient mice has not been reported so far. The nature of the ATGL-independent

function of CGI-58 in the epidermis is currently unknown. Several scenarios are conceivable: 1) CGI-58 may participate in the synthesis pathway of glycerophospholipids (24), and 2) CGI-58 may activate other lipases including patatin domain-containing lipases that are structurally related to ATGL. For example, adiponutrin and GS2 were already shown to exhibit TG hydrolase activity (34), and GS2, which is also expressed in human skin, was shown to exhibit retinyl ester hydrolase activity (17).

Neurological disorders. Neurosensory diseases and mental retardation are commonly seen in NLSDI but have not been observed in NLSDM. Similar to an independent role of CGI-58 in the development of ichthyosis, the clinical manifestation of neurosensory disturbances in NLSDI argues for an ATGL-independent function of CGI-58, presumably in brain-lipid homeostasis.

Liver disease. More than 70% of NLSDI patients and about one-half of the NLSDM patients suffer from liver disease, hepatomegaly, or liver steatosis, suggesting a prominent role of both factors in hepatic lipid metabolism. Brown et al. (5) showed that siRNA-mediated knockdown of CGI-58 in liver cells leads to a reduced secretion of TG, suggesting that CGI-58 is important in hepatic lipoprotein biogenesis. ATGL deficiency in mice led to hepatosteatosis in fasted animals due to a reduction in liver TG hydrolase activity (20). Reid et al. (44) evaluated the effect of ATGL overexpression on TG and

apoB secretion in murine liver cells. They found that ATGL-mediated liver TG hydrolysis does not correlate with increased lipoprotein secretion, suggesting that FA mobilized by ATGL are directed to β -oxidation rather than VLDL secretion.

Skeletal myopathy. All patients with mutations in the *ATGL* gene reported so far show substantial TG accumulation in muscle associated with myopathy and muscle weakness. ATGL is specifically expressed in type I oxidative (slow-twitch) fibers of skeletal muscle (28), and it is believed that the lack of enzyme activity is responsible for the pronounced TG accumulation. Similarly, ATGL-deficient mice also exhibited severe accrual of fat as a result of decreased TG hydrolase activity in skeletal muscle (20). In rats, siRNA-mediated knockdown of ATGL in skeletal muscle reduced TG hydrolase activity and led to TG accumulation (57). Conversely, ATGL overexpression led to the accumulation of DG, the product of the ATGL reaction. Apparently, the endogenous HSL activity is not sufficient to hydrolyze these elevated levels of DG produced by overexpressed ATGL (57). A recent study reported by Alsted et al. (3) elucidated the role of ATGL in human skeletal muscle TG degradation during physical exercise. They found that inhibition of HSL in skeletal muscle led to a 42% reduction in total TG hydrolase activity and that the remaining activity is ATGL. Moreover, after endurance exercise training, intramuscular TG levels were decreased and ATGL protein content was increased. These findings, together with the observation that NLSM patients suffer from myopathy, assign a pivotal role to ATGL in skeletal muscle lipolysis and in the provision of FA for energy production.

Cardiomyopathy. In addition to skeletal myopathy, ~50% of all NLSM patients suffer from severe cardiomyopathy, a phenotype that is not observed in NLSDI. Hirano et al. (23) reported a case with NLSM exhibiting vacuoles in the cytoplasm of cardiomyocytes that stained positive for Oil red O and massively increased TG levels of the ventricles and the coronary arteries. Due to heart failure, the patient underwent cardiac transplantation. This observation is in accord with the phenotype seen in ATGL-deficient mice (20). The cardiac muscle of these animals accumulates large amounts of TG in cardiomyocytes, which leads to cardiac insufficiency and premature death starting at the age of 12 wk. Apparently, ATGL is indispensable for normal cardiac TG catabolism.

Obesity. Despite the fact that both ATGL and CGI-58 are involved in lipolysis and highly expressed in adipose tissue, patients with NLSDI or NLSM are not obese (26). In contrast, ATGL-deficient mice develop obesity as is evident from increased body weight and fat mass even when fed a normal chow diet (20). These species-specific differences may suggest that ATGL is less important as TG hydrolase in human adipose tissue than in murine adipose tissue. In murine white adipose tissue, ATGL and HSL are the major lipases responsible for more than 90% of the lipolytic capacity (52), but the relative contribution of these enzymes for TG catabolism in human adipose tissue remains unclear. Langin et al. (35) found a complete ablation of catecholamine-stimulated lipolysis and a 50% reduction in basal lipolysis in the presence of a specific HSL inhibitor. Consistent with these observations, a study by Ryden et al. (47), employing siRNA-mediated knockdown of ATGL in human fat, showed that ATGL deficiency decreased basal lipolysis but had no effect on catecholamine-stimulated lipolysis. Those authors concluded that ATGL contributes far

less than HSL to human catecholamine-induced lipolysis (35, 47). In contrast, immunoprecipitation experiments by Steinberg et al. (55) revealed that the absence of ATGL results in a 70–80% decrease in total TG hydrolase activity, indicating that ATGL substantially contributes to TG hydrolysis. In conclusion, current data on the role of ATGL in human adipose lipolysis are controversial and need further study. The analysis of specific enzyme activities in biopsy samples obtained from patients with NLSDI and NLSM may help clarify the tissue-specific roles of ATGL and HSL in human adipose tissue.

Diabetes. One third of the patients suffering from NLSM also develop diabetes, suggesting an interesting connection between lipid and carbohydrate metabolism in these patients. In an attempt to investigate the nature and origin of diabetes in NLSM, Kobayashi et al. (32) performed glucose loading tests and euglycemic-hyperinsulinemic clamp studies in a patient with ATGL deficiency. Glucose tolerance and insulin sensitivity in the patient were normal, supporting the hypothesis that ectopic lipid accumulation does not induce insulin resistance. Interestingly, plasma insulin levels were significantly decreased, arguing for defective insulin secretion. Reduced insulin levels were also observed in ATGL-knockout mice, implying that ATGL is involved in the control of pancreatic insulin production (20). Fex et al. (14) reported that ATGL is expressed in murine pancreatic β -cells. It is conceivable that ATGL generates lipid metabolites such as long-chain FA and DG, which are potent signaling molecules for insulin secretion (8, 30). Thus, the diabetic state in NLSM patients may represent type 1 rather than type 2 diabetes, which is characterized by insulin resistance and high insulin levels.

Taken together, the clinical phenotypes associated with ATGL and CGI-58 deficiency provide conclusive evidence for a crucial role of both proteins in human lipid metabolism. However, many aspects of ATGL and CGI-58 function are incompletely understood. These include the specific role of ATGL in human adipose lipolysis, its role in insulin secretion, and the specific ATGL-independent function of CGI-58 in the skin and other organs. Detailed characterization of NLSM and NLSDI patients will provide a unique opportunity to improve our understanding of the role of these proteins in human lipid metabolism and in the pathogenesis of metabolic diseases. This may eventually lead to improved treatment of patients affected with NLSDI or NLSM.

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