Table 1 Summary of lipid storage myopathies

Disease	Gene	Protein/ function	Clinical manifestations	Pathologic features	Laboratory findings[b]	Treatment
Primary carnitine deficiency (PCD)	*SLC22A5*	OCTN2/carnitine transport	Infantile-onset: hypotonia, hepatomegaly, encephalopathy, cardiomyopathy Later-onset: Myopathy, cardiomyopathy	Lipid accumulation (muscle, liver)	Free carnitine ↓↓ Acylcarnitines ↓↓ CK ↑, could be normal	Carnitine
Multiple acyl-CoA dehydrogenase deficiency (MADD)	*ETFA, ETFB, ETFDH*	α subunit of ETF, β subunit of ETF, ETF-QO/ electron transfer	Neonatal-onset: Congenital anomalies, hypotonia, hepatomegaly, encephalopathy, cardiomyopathy Later-onset: Myopathy, hepatomegaly, encephalopathy, episodic metabolic crisis	Lipid accumulation (muscle, liver)	Free carnitine→or↑ acylcarnitines ↑↑ C5-10 dicarboxylic aciduria with acylglycine derivatives CK ↑ to↑↑, could be normal	Riboflavin
Neutral lipid storage disease with ichthyosis (NLSDI)	*ABHD5*	CGI-58/activator of ATGL	Multisystem involvement, including ichthyosis, mild myopathy, hepatomegaly, intestinal and ophthalmologic symptoms, hearing loss, mental retardation, short stature, microcephaly	Lipid accumulation (muscle and various tissues); Jordans' anomaly (leukocyte)	CK: usually normal	None
Neutral lipid storage disease with myopathy (NLSDM)	*PNPLA2*	ATGL/triglyceride lipase	Myopathy and cardiomyopathy predominantly	Lipid accumulation (muscle and various tissues); Rimmed vacuoles[a] (muscle); Jordans' anomaly (leukocyte)	CK ↑or↑↑	None

[a] This finding was seen in some patients, not all.

b↑: mild elevation; ↑↑: moderate to marked elevation; →: no change;↓↓: moderate to marked decrease.

ATGL adipose triglyceride lipase; *CGI-58* comparative gene identification-58; *CK* creatine kinase; *ETF* electron-transfer flavoprotein; *ETF-QO* ETF-coenzyme Q oxidoreductase; *OCTN2* plasma membrane sodium-dependent carnitine transporter.

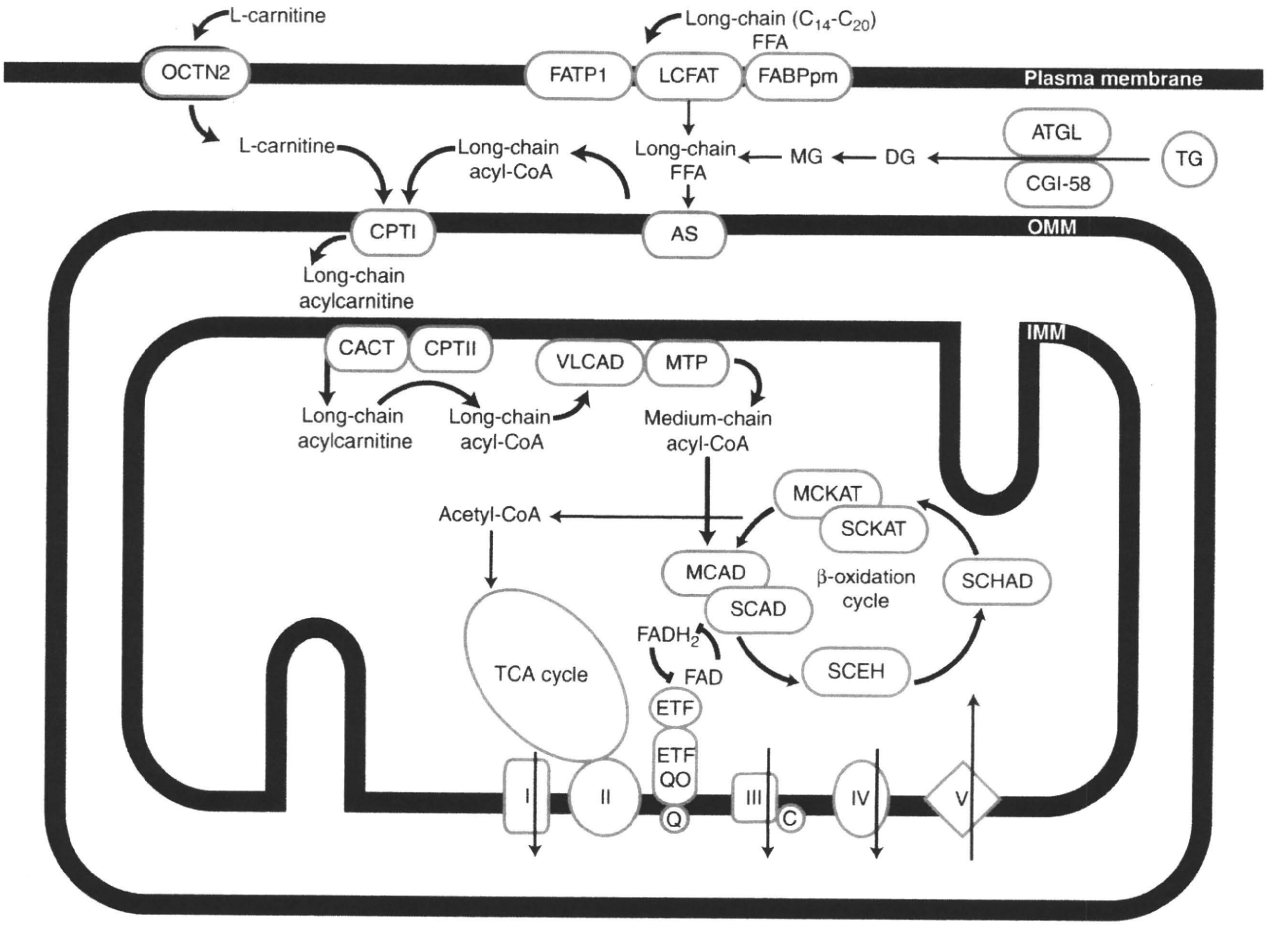

Fig. 1 Scheme of metabolic pathways of triglycerides and fatty acids. *AS* acyl-CoA synthetase; *ATGL* adipose triglyceride lipase; *C* cytochrome c; *CACT* carnitine-acylcarnitine translocase; *CGI-58* comparative gene identification-58; *CoA* coenzyme A; *CPTI* carnitine palmitoyltransferase I; *CPTII* carnitine palmitoyltransferase II; *DG* diglycerides; *ETF* electron-transfer flavoprotein; *ETF-QO* ETF-coenzyme Q oxidoreductase; *FABPpm* plasma membrane-associated fatty acid-binding protein; *FAD* flavin adenine dinucleotide; $FADH_2$ flavin adenine dinucleotide [reduced form]; *FATP1* fatty acid transporter protein 1; *FFA* free fatty acid; *IMM* inner mitochondrial membrane; *I~V* respiratory chain complex I~V; *LCFAT* long-chain fatty acid transporter; *MG* monoglyceride; *MTP* mitochondrial trifunctional protein; *OCTN2* plasma membrane sodium-dependent carnitine transporter; *OMM* outer mitochondrial membrane; *Q* coenzyme Q; *SCAD/MCAD* acyl-CoA dehydrogenases; *SCEH* enoyl-CoA hydratase; *SCHAD* 3-hydroxyacyl-CoA dehydrogenase; *SCKAT/MCKAT* 3-ketoacyl-CoA thiolase; *TCA* tricarboxylic acid; *TG* triglycerides; *VLCAD* very long chain acyl-CoA dehydrogenase

genases in fatty acid β-oxidation and, consequently, the disease is called MADD.

Homozygous or compound heterozygous mutations in *ETFA*, *ETFB*, or *ETFDH*, which encode α- and β-subunits of ETF and ETFDH, respectively [16], are responsible for MADD. Intriguingly, MADD seem to be common in the southern Chinese population due to a probable founder mutation, c.250G> A (p.A84T) in *ETFDH*, with an estimated carrier frequency of about 0.8% in Taiwanese [17, 18]. To date, all 20 reported patients from the southern Chinese population harbor this mutation, with 16 from Taiwan [19, 20]. In contrast, according to PubMed-listed reports, only 15 MADD patients with *ETFDH* mutations have been identified in Japan, which has a 5.5 times larger population than Taiwan. This finding indicates that the incidence of MADD is likely to be much higher than previously estimated and many MADD patients may actually be underdiagnosed at least among Southern Chinese, including Taiwanese people.

The clinical phenotype of MADD is quite heterogeneous and has been classified as neonatal-onset forms with or without congenital anomalies, and mild- and/or later-onset form. The congenital anomalies include facial dysmorphism, cystic renal dysplasia, and other features. Patients with neonatal-onset forms usually present with hypotonia, hepatomegaly, nonketotic hypoglycemia, and metabolic acidosis and usually die early in infancy. Later-onset patients manifest proximal myopathy often with hepatomegaly, encephalopathy, and episodic lethargy, vomiting and hypoglycemia; these episodes can be lethal [21]. Cardiomyopathy has also been

reported in both neonatal- and later-onset MADD patients [22]. Routine biochemical tests often show mildly to moderately elevated CK levels, especially during the episodes of metabolic decompensation.

Mutations in *ETFA* and *ETFB* tend to cause neonatal-onset forms, whereas *ETFDH* mutations often present with the later-onset form [23, 24], but disease severity may not solely depend upon the primary gene defect but also upon other factors [25•]. It has been long known that a group of MADD patients are riboflavin-responsive but others are not. Recent report suggests that all riboflavin-responsive MADD (RR-MADD) are associated with *ETFDH* mutations [26•]. We also confirmed this in our own series [11, 17]. *ETFDH* mutations have also been associated with the myopathic form of coenzyme Q_{10} (CoQ_{10}) deficiency [27]. However, this association is not consistent because CoQ_{10} levels are not decreased in some MADD patients due to *ETFDH* mutations [17].

Muscle pathology is characterized by increased lipid droplets in muscle fibers as in PCD. Regenerating fibers may also be seen if muscle biopsy is performed after metabolic crisis episode, suggesting mild rhabdomyolytic events can occur during metabolic decompensation in MADD. On electron microscopy, lipid droplets are often present next to mitochondria.

Measurement of plasma carnitine, acylcarnitines, and urinary organic acid profiles is useful to make a diagnosis. Blood acylcarnitine analysis usually displays elevated concentrations of all-chain-length, but mainly medium- and long-chain acylcarnitines. Plasma free carnitine level is usually decreased but can sometimes be normal. Urine organic acid analysis typically shows C5 to C10 dicarboxylic aciduria and acylglycine derivatives. In addition, reduced biochemical activities of other mitochondrial enzymes, including flavin-dependent and respiratory chain enzymes, have also been reported in MADD [26•, 28, 29], although it is still unknown if the mitochondrial dysfunction is directly associated with *ETFDH* mutations or caused by other factors. Of note, biochemical assays occasionally show normal results between episodes of metabolic decompensation; thus, mutation analyses of *ETFA*, *ETFB*, and *ETFDH* may be the most reliable diagnostic method for MADD at this moment.

Although the molecular mechanism of MADD is still unclear, riboflavin supplementation (100–400 mg/d) has been known to strikingly improve the clinical symptoms and metabolic profiles in a group of MADD patients, particularly those with the late-onset form. As mentioned earlier, the RR-MADD patients seem to have *ETFDH* mutations. Accordingly, riboflavin should be tried for all MADD patients. There is still a controversy about the combination therapy with carnitine. We think that L-carnitine supplementation helps when secondary carnitine deficiency is present. CoQ_{10} supplementation has also been reported to improve muscle weakness in patients with secondary CoQ_{10} deficiency, together with riboflavin use. However, this is still in dispute. Probably, CoQ_{10} supplementation should be considered only when secondary CoQ_{10} deficiency is confirmed.

Neutral Lipid Storage Diseases with Ichthyosis and Myopathy

Neutral lipid storage disease (NLSD) is a rare lipid storage disorder caused by a defect in either an adipose triglyceride lipase (ATGL; also called patatin-like phospholipase domain-containing 2 [PNPLA2]) or alpha/beta-hydrolase domain-containing protein 5 (ABHD5; also called comparative gene identification-58 [CGI-58]). ATGL catabolizes TG and releases the first fatty acid from the glycerol backbone and produces diglyceride (DG). The enzyme exhibits high substrate specificity for TG, but not DG or other lipids [30•]. ABHD5 activates ATGL and acylates lysophosphatidic acid. Activation of ATGL initiates the hydrolytic catabolism of cellular TG stores to glycerol and nonesterified fatty acids (Fig. 1). Naturally, dysfunction of these two proteins prevents the degradation of TG, resulting in the accumulation of TG in the cytoplasm of various organs including skeletal muscle in which TG accumulation is recognized as increased lipid droplets in muscle fibers.

NLSD is characterized by systemic TG deposition in multiple tissues, including skin, muscle, liver, central nervous system, and blood leukocytes. Not surprisingly, NLSD patients present with a wide variety of clinical manifestations, including myopathy, hepatomegaly, variable ophthalmologic symptoms (cataract, nystagmus, strabismus), neurosensory hearing loss, mental retardation, short stature, microcephaly, and intestinal involvement [31–33].

There are two well-characterized NLSDs: NLSDI and NLSDM. In NLSDI, which is also known as Chanarin-Dorfman syndrome, patients typically have rather extensive nonbullous congenital ichthyosiform erythroderma and thus it is called NLSDI, whereas no ichthyosis is seen in NLSDM. Of note, in NLSDI, myopathy can be seen but the weakness is usually mild. In NLSDM, patients develop slowly progressive myopathy, which can be either proximal- or distal-dominant. Importantly, cardiomyopathy is exclusively found in almost half of the patients with NLSDM [33], but not NLSDI, whereas neurosensory defects and mental retardation are commonly seen in NLSDI but not NLSDM. The CK level is usually mildly to moderately elevated. In both NLSDs, lipid accumulation is observed in leukocytes, which is called Jordans' anomaly. This intracytoplasmic lipid storage is visible on peripheral blood smear. In skeletal muscles, increased lipid droplets can be easily recognized even during the presymptomatic period.

On muscle pathology, lipid droplets are increased in both size and number. Interestingly, rimmed vacuoles are shown in the muscle fibers in a significant number of patients with NLSDM, unlike PCD and MADD [11, 34]. In addition, fiber size variation seems to be more significant than PCD and MADD. These features suggest a different pathomechanism of the disease. Because DG is a source for phospholipid, these changes might be associated with membrane phospholipid abnormalities caused by decreased DG availability, although no solid evidence is available at this moment.

NLSDI is caused by mutations in *ABHD5* [35] and NLSDM by mutations in *PNPLA2* [36•]. Null mutations in *ABHD5* have never been reported, but several mutations removing the functional domains have been identified, suggesting that the mutant ABHD5 is not completely deficient but functionally impaired [35]. Interestingly, almost all mutations in *PNPLA2* are located on the C-terminal region, leaving the probable active site of the enzyme intact but impairing the binding ability to cellular lipid droplets in vitro [37].

Up to now, there is still no effective treatment for NLSD. Although *ATGL* and ABHD5 have been known to play a crucial role in lipid metabolism, many aspects of their functions are incompletely understood. However, the appearance of ichthyosis indicates an ATGL-independent function of ABHD5 in skin and/or other organs. Mice lacking ATGL have shown defective lipolysis and altered energy metabolism, which mimics human phenotype [38], providing an opportunity to enhance understanding of NLSDM. The phenotype of ABHD5-deficient mice has not been reported yet. More detailed genetic and clinical characterization of NLSDI patients may be helpful to elucidate the biological role of CGI-58 in lipid dysmetabolism and to develop promising therapeutic strategy.

Conclusions

LSM is pathologically defined by excessive lipid deposition in muscles. However, to identify the underlying disease, detailed characterization of clinical features combined with distinctive results of biochemical assays is required. In addition, mutation analyses are usually helpful for making the final diagnosis, especially when clinical phenotype and laboratory tests show indistinguishable and nonspecific findings. Prompt diagnosis is important for treatment of patients because carnitine for PCD and riboflavin for RR-MADD have demonstrated excellent efficacy in eliminating the clinical symptoms. Although no specific therapeutic management is available for NLSD so far, accurate diagnosis is necessary to predict disease course, to provide genetic counseling, and to advance further research.

To date, except for PCD, MADD, NLSDI, and NLSDM discussed in this review, the causative genes remain unknown in the majority of patients with LSM [11]. Although secondary change due to variable metabolic alterations can also generate significant lipid accumulation in the muscles, it may be mainly caused by other undetermined primary defects in lipid metabolism. Thus, further studies for the proteins involved in lipid metabolism are crucial for discovering the novel causative genes, probing molecular mechanisms, and developing useful therapeutic strategies.

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Disclosure Conflicts of interest: W.-C. Liang: none; I. Nishino: is an employee of the National Institute of Neuroscience, NCNP; is a Guest Professor at Waseda University Faculty of Science and Engineering, Tokyo, Japan; is an Executive Board member for the World Muscle Society; is a Secretary, Executive Board member, and Founding Board member for the Asian Oceanian Myology Center; is an Executive Board member for the Japanese Society of Neurology; is an Editorial Board member for the Neuromuscular Conference in Japan; is a Founding member and Executive Board member for the Myositis Workshop in Japan; is a Scientific Advisory Board member for the Patients Association for Distal Myopathies in Japan; he reads muscle biopsies sent from all over Japan and abroad as part of his job at the National Institute of Neuroscience, NCNP; and his travel expenses were paid by the Neuromuscular Foundation when he was a speaker for the HIBM Workshop held in Los Angeles, CA, in 2009. He also has patents pending for the following: 1) The method to develop a model mouse for distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy. 2) The development of the therapy for distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy. 3) The method to diagnose congenital muscular dystrophy with mitochondrial structural abnormalities.

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