Metabolism of Very Long-Chain Fatty Acids: Genes and Pathophysiology

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Abstract

Fatty acids (FAs) are highly diverse in terms of carbon (C) chain-length and number of double bonds. FAs with C>20 are called very long-chain fatty acids (VLCFAs). VLCFAs are found not only as constituents of cellular lipids such as sphingolipids and glycerophospholipids but also as precursors of lipid mediators. Our understanding on the function of VLCFAs is growing in parallel with the identification of enzymes involved in VLCFA synthesis or degradation. A variety of inherited diseases, such as ichthyosis, macular degeneration, myopathy, mental retardation, and demyelination, are caused by mutations in the genes encoding VLCFA metabolizing enzymes. In this review, we describe mammalian VLCFAs by highlighting their tissue distribution and metabolic pathways, and we discuss responsible genes and enzymes with reference to their roles in pathophysiology.

Key Words: Sphingolipids, Glycerophospholipids, ELOVL, Ceramide, Ichthyosis, Leukodystrophy

CELLULAR FATTY ACIDS (FAS): CHAIN-LENGTH AND NUMBER OF DOUBLE BONDS

FAs are classified according to their carbon (C) chain-length and the number of double bonds (Fig. 1). Long-chain FAs (LCFAs) have chain-lengths of C11-20, of which C16 and C18 LCFAs are the most abundant FA species in mammalian cells. FAs longer than C20 (C>20) are called very long-chain FAs (VLCFAs) and are less abundant than LCFAs. VLCFAs with C22 and C24 are found ubiquitously throughout the body. VLCFAs with C≥26 are often sub-classified into ultra long-chain FAs (ULCFAs) and are found in specific tissues, including the skin, retina, meibomian gland, testis, and brain.

Another classification of FAs is based on the number of double bonds. FAs are classified into saturated FAs (SFAs; no double bond), monounsaturated FAs (MUFA; one double bond) and polyunsaturated FAs (PUFAs; two or more double bonds) (Fig. 1). PUFAs are further sub-classified into n-3 (ω3) and n-6 (ω6) series depending on the position of the terminal double bond, i.e. the double bond most distant from the carboxyl group. In the n-x series, x indicates the ordinal number of carbon atom with a double bond from the end of the carbon chain.

By combining these two classifications, arachidonic acid for example, an n-6 FA with chain-length C20 and four double bonds is denoted by C20:4n-6. Linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) are essential FAs (EFAs) that must be consumed through food, since humans are unable to synthesize them.

ENZYMES RESPONSIBLE FOR FA ELONGATION

FAs are elongated in the form of acyl-CoA, in which FAs are covalently linked to coenzyme A via thioester bonds. Elongation of ≥C16 FAs that are either synthesized de novo by the FA synthase in the cytoplasm or absorbed from food, occurs in the endoplasmic reticulum (ER). FA elongation proceeds through repetition of the FA elongation cycle whereby two carbons are added to the carboxyl end in each cycle (Fig. 2) (Jakobsson et al., 2006; Guillou et al., 2010). The FA elongation cycle consists of four sequential reactions: condensation, reduction, dehydration, and reduction. The first condensation reaction is the rate-limiting step, in which 3-ketoacyl-CoA is produced by condensation of acyl-CoA with malonyl-CoA. This reaction is catalyzed by FA elongase. FA elongases constitute the ELOVL family of proteins, and there are seven isozymes (ELOVL1-7) in mammals (Jakobsson et al., 2006; Guillou et al., 2010; Ohno et al., 2010). In the second reduction step, 3-ketoacyl-CoA is converted to 3-hydroxyacyl-CoA using nicotinamide adenine
Fig. 1. Human FA elongation pathways. The FA elongation pathways of SFAs, MUFAs and PUFAs are illustrated. ELOVL isozymes (E1-E7) responsible for each elongation step are indicated. Parentheses denote ELOVLs that exhibit weak activity toward the indicated substrates. ∆5, ∆6 and ∆9 represent ∆5-, ∆6- and ∆9-desaturase, respectively. FA: fatty acid; SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA.

http://dx.doi.org/10.4062/biomolther.2014.017
acyl-CoA incorporates two carbon units from malonyl-CoA. In each step, the enzyme involved in each step is illustrated. In each cycle, acyl-CoA incorporates two carbon units from malonyl-CoA.

In the third dehydration step, 3-hydroxyacyl-CoA is converted to trans-2-enoyl-CoA, and this reaction is catalyzed by 3-hydroxyacyl-CoA dehydratase (HACD). The last reduction step, trans-2-enoyl-CoA is converted to acyl-CoA, which is longer than the original acyl-CoA by two carbons. The trans-2-enoyl-CoA reductase responsible for this reaction is TER, and the reaction requires NADPH as a cofactor (Moon and Horton, 2003). In vitro FA elongation assays and knockdown or knockout (KO) of ELOVL1-7 genes uncovered that ELOVL1-7 elongates both saturated and unsaturated C16-C22 acyl-CoAs, with the highest activity toward C18-CoAs (Ohno et al., 2009). ELOVL3 and ELOVL7 elongate both saturated and unsaturated C16-C22 acyl-CoAs, with the highest activity toward C18-CoAs (Ohno et al., 2010). ELOVL4 also elongates both saturated and unsaturated acyl-CoAs, but it is specialized for the synthesis of ULCFAs (Ohno et al., 2010). In ELOVL4 KO mice and homozygous Stargardt disease 3 (STGD3) model mice that carry a pathogenic mutation in the ELOVL4 gene, ULCFAs in the epidermis were completely missing (Li et al., 2007; Vaisiredy et al., 2007). ELOVL6 elongates shorter acyl-CoAs as compared to other ELOVLs, with activity toward C12.0-C16.0 acyl-CoAs (Moon et al., 2001). Consistent with this, the levels of C16:0 and C16:1 FAs are increased whereas the levels of C18:0 and C18:1 FAs are reduced in the liver of ELOVL6 KO mice (Matsuzaka et al., 2007). Interestingly, ELOVL6 KO mice are protected from obesity-induced insulin resistance despite becoming obese and developing hepatosteatosis (Matsuzaka et al., 2007).

**Table 1. Substrate specificity and tissue distribution of mammalian CERS isozymes**

| Isozyme | Preferred substrates | mRNA expression |
|---------|----------------------|-----------------|
| CERS1   | C18                  | Brain, skeletal muscle |
| CERS2   | C22–C24              | Ubiquitous, high in liver, kidney, lung |
| CERS3   | ≥C26                 | Skin, testis |
| CERS4   | C18–C20              | Lung, heart |
| CERS5   | C16                  | Ubiquitous, high in brain, kidney, testis |
| CERS6   | C16                  | Ubiquitous, high in brain, liver, thymus |

**C24 VLCFAS IN SPHINGOLIPIDS**

Most saturated and monounsaturated VLCFAs are found as acyl moieties of sphingolipids. Sphingolipids contain ceramide (Cer) as a backbone, in which FAs are amide-linked to the sphingoid base. Cers are converted to sphingomyelin (SM) and glycosphingolipids by addition of a polar head group of phosphocholine and sugars, respectively.

Cer synthase (CERS) catalyzes an amide bond formation between acyl-CoA and the sphingoid base. Cers are converted to sphingomyelin (SM) and glycosphingolipids by addition of a polar head group of phosphocholine and sugars, respectively.

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microdomain; this association is necessary for neutrophil functions such as superoxide generation and migration (Iwabuchi et al., 2008). In HeLa cells, knockdown of ELOVL1 or CERS2 causes a shift in sphingolipid composition from C24 to C16 and increases susceptibility to apoptosis induced by diverse stimuli such as anticancer drugs (i.e. cisplatin), ultraviolet radiation, or C6 Cers (Sassa et al., 2012).

Yeast sphingolipids consist exclusively of saturated C26 VLCFAs (Ejsing et al., 2009). VLCFA synthesis is essential for yeast viability, and viable mutant strains with limited VLCFA synthesis exhibit defects in vesicular transport mainly in the late endosome/multivesicular body (Obara et al., 2013). VLCFAs may have a conserved role in vesicular trafficking systems, where a highly curved membrane is constantly appearing and disappearing to support vesicle budding and fusion.

**ULCFAS IN EPIDERMAL CERS**

Saturated and monounsaturated ULCFAs are found in the skin. The stratum corneum (SC), the outermost layer of the skin epidermis, is essential for the epidermal permeability barrier, which protects terrestrial animals against desiccation by transepidermal water loss and invasion of pathogens and toxic chemicals (Proksch et al., 2008). In the SC, the intercellular spaces are filled with multimellar lipid layers called extracellular lipid lamella (ELL) (Breiden and Sandhoff, 2014; Rabionet et al., 2014). Cers account for ~50% of lipids in the ELL, although Cers are less abundant in most tissues since they are utilized as precursors of complex sphingolipids such as SMs and glycosphingolipids. C26-C36 ULCFAs are found in Cers in the ELL (Masukawa et al., 2008; t’Kindt et al., 2012). Most C≥30 ULCFAs in the epidermal Cers are ω-hydroxylated and esterified with linoleic acid (C18:2n-6) to form ω-O-acyl-Cers or covalently bound to proteins (mainly involucrin) of corneocytes, the latter form corneocyte lipid envelopes (CLEs) (Breiden and Sandhoff, 2014; Rabionet et al., 2014). EFA deficiency in human and other mammals causes ichthyosis-like cutaneous abnormalities including scaly skin and impaired permeability barriers (Prottey, 1977; Yamanaka et al., 1980). In EFA deficiency, linoleic acid (C18:2n-6) esterified with ω-OH ULCFAs in ω-O-acyl-Cers is replaced by oleic acid (C18:1n-9) with a concomitant decrease in the epidermal permeability barrier, suggesting an essential function of linoleate-esterified ω-O-acylCers in constructing and maintaining epidermal permeability barrier integrity (Prottey, 1977; Yamanaka et al., 1980).

Our understanding on the pathway(s) and the enzymes involved in the synthesis of epidermal Cers is still incomplete. However, recent studies using KO mice identified the enzymes responsible for the synthesis of ULC-Cers. ULC-Cers are essential for the epidermal permeability barrier, thus a deficiency in ULC-Cers synthesis can be easily identified in KO mice by examining transepidermal water loss and dye penetration, and epidermal histology. Among the six CERS isozymes, CERS3 is exclusively required for ULC-Cer synthesis (Jennemann et al., 2012). Epidermal ULC-Cers are completely lost in CerS3 KO mice, precluding the formation of the ELL and CLE, which leads to severe transepidermal water loss and early postnatal lethality (Jennemann et al., 2012). Consistent with the involvement of ELOVL4 in ULCFA synthesis (Fig. 1), Elov4 KO mice exhibit essentially identical skin phenotypes to that of CerS3 KO mice.

![Fig. 3. FA compositions of SM. FA compositions of SM in indicated mouse tissues determined by liquid chromatography-mass spectrometry analysis are illustrated. SM: sphingomyelin; BAT: brown adipose tissue; WAT: white adipose tissue.](http://dx.doi.org/10.4062/biomolther.2014.017)
KO mice (Li et al., 2007; Vasireddy et al., 2007). CERS3 and ELOVL4 mRNA increases during keratinocyte differentiation (Mizutani et al., 2013). Elovl1 KO mice also exhibit lethal epidermal permeability barrier deficiency (Sassa et al., 2013). ELOVL1 activity is regulated differently by CERS2 or CERS3 (Ohno et al., 2010; Mizutani et al., 2013; Sassa et al., 2013). In most tissues, ELOVL1 cooperates with CERS2 and elongates acyl-CoAs up to C24-CoAs, which are utilized for C24 sphingolipid synthesis by CERS2. However, in the upper epidermis, where ULC-Cers present in the ELL and CLE are synthesized, CERS3 is highly expressed and instructs ELOVL1 to elongate up to C26-CoAs, which are either utilized for the synthesis of C26-Cers by CERS3 or subject to further elongation up to C36-CoAs by ELOVL4 (Sassa et al., 2013).

Recently, recessive mutations in the CERS3 gene on chromosome 15 and the ELOVL4 gene on chromosome 6 have been identified in ichthyosis patients (Table 2) (Aldahmesh et al., 2011; Eckl et al., 2013; Radner et al., 2013). Lipid analysis of the CERS3 mutant keratinocytes derived from the patients revealed severe reductions in the levels of ULC-Cers including ω-O-acylCers and protein bound ω-OH Cers (Eckl et al., 2013; Radner et al., 2013).

Inherited ichthyoses form part of a large clinically and etiologically heterogeneous group of disorders of cornification, and most ichthyoses are associated with the impaired epidermal permeability barrier (Oji et al., 2010; Elias et al., 2012). Notably, along with CERS3 and ELOVL4, some non-syndromic and syndromic forms of inherited ichthyosis include genes involved in lipid metabolism. Given the crucial role of ULC-Cers in epidermal permeability barrier, it is plausible that at least some of these genes may be involved in the synthesis or transport of ULC-Cers necessary for the formation of the ELL and CLE. Indeed, the ABCA12 gene, which is mutated in harlequin ichthyosis, encodes a member of the ATP-binding cassette (ABC) transporters likely involved in the transport of glucosylCers (GlcCers) into lamellar bodies (LB) (Table 2) (Akiyama et al., 2005; Akiyama, 2014). GlcCers in LBs are precursors of Cers including ω-O-acylCers and are converted to Cers upon secretion in the SC (Akiyama, 2014; Breiden and Sandhoff, 2014; Rabionet et al., 2014).

VLCFAS IN THE MEIBUM

The ocular surface is covered by a structure called the tear film (TF), which consists of aqueous tears and a mixture of diverse lipids (meibum) that are produced by lachrymal glands and meibomian glands, respectively (Butovich, 2013). The physiological functions of the TF are the maintenance of a smooth surface for light refraction, and, in analogy with the epidermal permeability barrier, the protection of underlying ocular structures including the cornea and conjunctiva from desiccation and infection. In humans, there are 30-40 and 20-30 meibomian glands in the upper and lower eyelids, respectively. In dry eye patients, the number of functional (yielding liquid secretion) meibomian glands is significantly decreased compared with asymptomatic controls (Korb and Blackie, 2008).

The major components of meibum are cholesteryl esters and wax esters (WEs), each accounting for ~30% of the total meibum lipids (Ohashi et al., 2006). The FA residues of human meibum cholesteryl esters are ~80% saturated, and their chain-lengths range from C18 up to C32, with saturated C24-C27 VLCFAs being the major species (Butovich, 2010). WEs are esters of FAs with fatty alcohols. The major WE species in human meibum have saturated C24-C26 VLC fatty alcohols that are esterified to oleic acid (C18:1) (Butovich et al., 2009). Meibum also contains ω-O-acylVLCFAs, which consist of ω-OH monounsaturated VLCFAs (C30-C34) esterified with monounsaturated C16 or C18 FA (Butovich et al., 2009). However, neither ω-O-acylCers nor regular Cers were found in the human meibum, and FAs (palmitoleic acid, C16:1n-7 and oleic acid, C18:1n-9) esterified to ω-OH VLCFAs are different from those (linoleic acid, C18:2n-6) esterified to epidermal ω-OH Cers (Butovich et al., 2009).

### Table 2. VLCFA-related genes mutated in inherited diseases

| Gene     | Chromosome | Function                        | Disease                                           |
|----------|------------|---------------------------------|---------------------------------------------------|
| ELOVL4   | 6          | FA elongase (condensation)      | Stargardt-like macular dystrophy (STGD3) (dominant) |
| HACD1 (PTPLA) | 10       | 3-Hydroxyacyl-CoA dehydratase   | Ichthyosis, nervous system abnormalities (recessive) |
| TER (TECR) | 19        | Trans-2-enoyl-CoA reductase     | Non-syndromic mental retardation                   |
| CERS3    | 15         | Cer synthase                    | Ichthyosis                                        |
| ABCA12   | 2          | Glucosylceramide transport into LB | Ichthyosis                                        |
| FA2H     | 16         | 2-Hydroxylation of FA           | Leukodystrophy with spastic paraparesis and dystonia |
| ABCD1    | X          | VLCFA-CoA transport into peroxisome | X-linked adrenoleukodystrophy (X-ALD)             |
| ACOX1    | 17         | VLCFA ω-oxidation in peroxisome | Leukodystrophy, other nervous system abnormalities |
| HSD17β4  | 5          | VLCFA ω-oxidation in peroxisome | Leukodystrophy, other nervous system abnormalities |

VLC-PUFAs include DHA (C22:6n-3) (Fig. 1). DHA is abundant in glycerophospholipids in the testis, brain, and retina. In the spermatozoa, DHA is almost exclusively found in phosphatidyethanolamine (PE), whereas n-6 PUFAs, including docosapentaenoic acid (DPA; C22:5n-6), are uniformly distributed in various glycerophospholipid species (Lin et al., 1993). In the brain gray matter, DHA is predominantly found in phosphatidylethanolamine (PS) and PE (Sastry, 1985). About 50-60% of the PE in the brain is present in the form of plasmalogen, which contains a vinyl ether bond at the sn-1 position instead of an ester bond. DHA is enriched in both PE and PE plasmalogen (Sastry, 1985). In
the retina, DHA is the major FA (~30%) in the disc membrane of retinal photoreceptor outer segment, where photopigment rhodopsin processes phototransduction (Sangiovanni and Chew, 2005). DHA accounts for 20-30% of the FAs in PC, PE, and PS of outer segment disc membranes. Newborns with n-3 FA deficiency exhibit reduced light sensitivity of retinal rod photoreceptors, and DHA supplementation enhances visual resolution acuity (Uauy et al., 2001).

One of the functions of DHA is to generate lipid mediators, which actively turn off the inflammatory responses in tissues. The lipid mediators formed from DHA include D-series resolvin (D-series resolvin D1), protectin D1 (neuroprotectin D1), and maresin 1 (Bannenberg and Serhan, 2010). These molecules constitute novel families of lipid mediators that are structurally unrelated to authentic eicosanoids, such as prostaglandin or leukotriene, and that display potent anti-inflammatory and tissue-protective actions such as reduced neutrophil migration and activation of phagocytosis by macrophages (Bannenberg and Serhan, 2010).

In the retina, continuous light absorption by photoreceptors induces oxidative stress in the photoreceptor outer segments. To replace the damaged outer segments, photoreceptors shed the distal tips of the outer segments, which are phagocytosed by retinal pigment epithelial (RPE) cells. RPE cells respond to oxidative stress by synthesizing protectin D1 from DHA in the phagocytosed outer segment membranes (Bazan et al., 2010). Protectin D1 promotes the survival of RPE cells, and, as a consequence, photoreceptor cell integrity (Mukherjee et al., 2007).

ULC-PUFAS IN THE TESTIS, BRAIN, AND RETINA

ULC-PUFAs are found in the testis, brain, and retina (Agbagha et al., 2010). In the mammalian testis and spermatooza, n-6 and n-3 ULC-PUFAs with chain-lengths of C26-C32 and 3-6 double bonds are present uniquely in sphingolipids including SMs, Cers, and fucosylated glycosphingolipids (FGSLs) (Sandhoff et al., 2005; Furland et al., 2007b; Rabionet et al., 2008). The level of ULC-PUFAs in these sphingolipids increase with the onset of spermatogenesis, and ULC-PUFAs account for up to 15% and 40% of rat testicular SMs and Cers, respectively (Furland et al., 2007b). Gaigt1 gene KO mice (encoding GM2 synthase) lack a subset of ULC-PUFAs-containing FGSLs and are infertile with an arrest of spermatogenesis at the stage of round spermatids (Sandhoff et al., 2005). Interestingly, ULC-PUFAs-containing SMs and Cers are enriched in the head of the spermatooza, and ULC-PUFAs-containing SMs are converted to the corresponding Cers during sperm capacitation in vitro, suggesting a role for ULC-PUFAs-containing SMs and Cers in acquiring competency for fertilization (Furland et al., 2007a).

In the brain, n-6 and n-3 series of ULC-PUFAs with chain-lengths of C26-C38 and 4-6 double bonds are found in PCs at the sn-1 position, with n-6 series ULC-PUFAs being predominant (Poulos et al., 1988). In rats, the levels of PCs containing ULC-PUFAs in the brain are higher in the neonatal and early postnatal periods than in adults, suggesting a role in postnatal brain development (Robinson et al., 1990).

ELOVL4 is involved in the synthesis of ULC-PUFAs as well as saturated and monounsaturated ULCFAs (Fig. 1). The recessive mutations in the ELOVL4 gene mentioned above are associated with not only ichthyosis but also seizures, intellectual abnormality, and spastic quadriplegia, suggesting the importance of ULC-PUFAs in brain development and physiology (Aldahmesh et al., 2011).

In the retina, similar to the brain, ULC-PUFAs are found at the sn-1 position of PCs. These ULC-PUFAs are predominant-ly n-3 series with chain-lengths of C26-C36 and 3-6 double bonds (Aveldano and Sprecher, 1987). These ULC-PUFAs-containing PCs also have PUFAs (predominantly DHA) at the sn-2 position, thus as many as 12 double bonds are present in a single PC molecule (Aveldano and Sprecher, 1987). In retinal-specific Elov4 KO mice, ULC-PUFA-containing PCs in the adult retina were severely decreased (Harkewicz et al., 2012).

STGD3 is an early onset, autosomal dominant form of macular degeneration that is characterized by decreased visual acuity, flecks in fundus flavimaculatus, and macular dystrophy (Donoso et al., 2001). STGD3 is caused by mutations in the ELOVL4 gene on chromosome 6 (Table 2) (Zhang et al., 2001; Vasireddy et al., 2010). All three STGD3 causative mutations are located in the last exon (exon 6) of ELOVL4 and result in the production of C-terminally truncated proteins lacking the ER retention signal. These dominant mutations are located closer to the C-terminus of the ELOVL4 protein than the recessive mutations that result in ichthyosis and other nervous system abnormalities, and it may explain the differences in the symptoms and the mode of inheritance. A knock-in mouse model carrying one of the STGD3 mutations was generated and displayed characteristic features associated with the STGD3, such as the accumulation of lipofuscin in RPE and photoreceptor degeneration (Vasireddy et al., 2006). STGD3 mutant ELOVL4 proteins exhibited no activity in vitro and were misrouted to perinuclear agrgresomes (Karao et al., 2005; Okuda et al., 2010). Moreover, STGD3 mutant ELOVL4 proteins are able to form hetero-oligomeric complexes with other components of the elongation machinery as well as homo-oligomeric complex with wild type ELOVL4 (Okuda et al., 2010).

2-HYDROXYLATED (2-OH) VLCFAS IN THE MYELIN

A subset of sphingolipids contains 2-OH VLCFAs. 2-OH VLCFAs are found almost exclusively in sphingolipids and are abundant in Cers in the epidermis and GalCers in the brain and kidney (Hama, 2010). In the brain, myelin contains abundant amounts of C24 GalCers and C24 sulfatides. The levels of 2-OH GalCers and 2-OH sulfatides increase during the course of myelination (Alderson et al., 2006).

2-Hydroxylation of FAs is catalyzed by the fatty acid 2-hydroxylase (FA2H) (Mizutani et al., 2001). STDG3 is caused by mutations in the ELOVL4 gene on chromosome 6 (Table 2) (Zhang et al., 2001; Vasireddy et al., 2010). All three STGD3 causative mutations are located in the last exon (exon 6) of ELOVL4 and result in the production of C-terminally truncated proteins lacking the ER retention signal. These dominant mutations are located closer to the C-terminus of the ELOVL4 protein than the recessive mutations that result in ichthyosis and other nervous system abnormalities, and it may explain the differences in the symptoms and the mode of inheritance. A knock-in mouse model carrying one of the STGD3 mutations was generated and displayed characteristic features associated with the STGD3, such as the accumulation of lipofuscin in RPE and photoreceptor degeneration (Vasireddy et al., 2006). STGD3 mutant ELOVL4 proteins exhibited no activity in vitro and were misrouted to perinuclear agrgresomes (Karao et al., 2005; Okuda et al., 2010). Moreover, STGD3 mutant ELOVL4 proteins are able to form hetero-oligomeric complexes with other components of the elongation machinery as well as homo-oligomeric complex with wild type ELOVL4 (Okuda et al., 2010).

OTHER DISEASE-ASSOCIATED MUTATIONS IN THE VLCFA SYNTHESIS PATHWAY

A mutation in the HACD1 gene (PTPLA) on chromosome 10 was identified in congenital myopathy characterized by
the severe hypotonicity and the absence of deep tendon reflexes (Table 2) (Muhammad et al., 2013). HACD1 is one of the four mammalian HACD isozymes (HACD1-4) and catalyzes the third step of the FA elongation cycle: dehydrogenation of 3-hydroxyacyl-CoA to trans-2-enoyl-CoA (Fig. 2) (Ikeda et al., 2008). The expression of HACD1 gene is highly specific to the heart and skeletal muscle (Li et al., 2000). The mutant HACD1 protein, which is C-terminally truncated by the introduction of nonsense codon at the Tyr residue 248 (Tyr248Stop), exhibits no activity toward 3-hydroxy C16:0-CoA in vitro (Muhammad et al., 2013). A mutation of the dog HACD1 gene caused by the insertion of a short interspersed element (SINE) in exon 2 also causes myopathy (Pelé et al., 2005). SINE insertion leads to multiple splicing defects and severely reduces the amount of wild type transcripts.

A mutation in the TER (TECR) gene on chromosome 19 encoding trans-2-enoyl-CoA reductase has been identified in an autosomal recessive form of non-syndromic mental retardation (Table 2) (Çalişkan et al., 2011). TER catalyzes the fourth step and last of the FA elongation cycle: NADPH-dependent reduction of trans-2-enoyl-CoA to acyl-CoA (Fig. 2) (Moon and Horton, 2003). The mutation substitutes the Pro residue at 182 to Leu (P182L) in the TER protein (Çalişkan et al., 2011). TER is an ER resident membrane protein with six predicted membrane-spanning domains, and the Pro-182 residue is likely to be located in the second luminal loop. The P182L mutation reduces both the activity and stability of the TER protein, thereby impairing the FA elongation cycle (Abe et al., 2013). Lipid analysis of B-lymphoblastoid cell lines derived from patients revealed a change in the sphingolipid profile and decreased levels of C24 Cers and C24 SMs (Abe et al., 2013).

**MUTATIONS IN THE VLCFA DEGRADATION PATHWAY**

Impairment in VLCFA degradation pathway leads to several diseases (Table 2). VLCFAs are transported, as VLCFA-CoAs, into peroxisomes, where they are subjected to β-oxidation (Wanders, 2014). Peroxisomal membrane protein ABCD1, a member of ABC transporters encoded by the ABCD1 gene on X chromosome, transports VLCFA-CoAs into peroxisomes (van Rennemund et al., 2008; Morita and Imanaka, 2012). Mutations in the ABCD1 gene cause X-linked adrenoleukodystrophy (X-ALD), the most common peroxisomal disorder with more than 600 different mutations characterized by progressive demyelination and adrenal insufficiency (Table 2) (Mossner et al., 1993; Berger et al., 2010; Engelen et al., 2012). A defect in ABCD1 impairs the VLCFA degradation process, resulting in elevated levels of saturated C24-C26 VLCFAs in the plasma, brain, adrenal gland, and other tissues (Berger et al., 2010; Kemp et al., 2012). The accumulation of saturated VLCFAs is believed to play a crucial role in the pathogenesis of X-ALD such as demyelination with inflammation (Paintilla et al., 2003). As mentioned earlier, ELOVL1 is responsible for the synthesis of saturated and monounsaturated C24-C26 VLCFAs such as those accumulated in X-ALD. In fibroblasts derived from X-ALD patients, suppression of VLCFA synthesis by knock-down of ELOVL1 partially restores C26:0 levels, suggesting ELOVL1 is a potential target for the treatment of X-ALD (Ofman et al., 2010). Lorenzo’s oil, a 4:1 mixture of glycerol trioleate and glyceryl trierucate, has been used to reduce the saturated VLCFA level in the plasma of X-ALD patients (Rizzo et al., 1989; Berger et al., 2010). Biochemical analysis demonstrated that oleic and erucic acids inhibit ELOVL1, suggesting that inhibition of ELOVL1 may be an underlying mechanism by which Lorenzo’s oil exerts its action (Sassa et al., 2014).

Peroxisomal β-oxidation of VLCFAs consists of four sequential reactions: dehydrogenation, hydration, dehydrogenation, and thiolytic cleavage (Wanders, 2014). The first dehydrogenation step, which converts acyl-CoA to trans-2-enoyl-CoA, is catalyzed by acyl-CoA oxidase 1 (ACOX1). Mutations in the ACOX1 gene on chromosome 17 result in autosomal recessive peroxisomal acyl-CoA deficiency (or pseudoneonatal adrenoleukodystrophy) with similar clinical features to those of X-ALD including the accumulation of VLCFAs and leukodystrophy (Table 2) (Poll-The et al., 1988). Both the second and third steps of VLCFA β-oxidation, hydration of trans-2-enoyl-CoA, and dehydrogenation of 3-hydroxyacyl-CoA, respectively, are catalyzed by single enzyme 17β-hydroxysteroid dehydrogenase 4 (HSD17β4; D-bifunctional protein). Homozygous mutations in the HSD17β4 gene on chromosome 5 have been identified in the disorder called D-bifunctional protein deficiency with clinical features similar to those of peroxisomal acyl-CoA deficiency and X-ALD (Table 2) (Watkins et al., 1995). The fourth step, which converts 3-ketoacyl-CoA to acyl-CoA, is catalyzed by peroxisomal 3-oxoacyl-CoA thiolase encoded by the acetyl-CoA acyltransferase 1 (ACAA1) gene on chromosome 3 (Wanders, 2014). No mutations in the ACAA1 gene associated with disorders have been identified.

**CONCLUSIONS**

VLCFAs are FAs with a chain-length of C>20. VLCFAs with C≥26 are sub-classified as ULCFAs and found in limited tissues including the skin, retina, brain, testis, and meibomian gland. VLCFAs are variously present in sphingolipids, glycerophospholipids, and other forms of lipids including ω-O-acyl-ULCFAs. VLCFAs are synthesized by the FA elongation cycle in the ER. Identification of the enzymes that participate in FA elongation, modification, transport, and degradation enabled the study of specific VLCFA species. VLCFAs play multiple roles not substituted by LCFAs. KO mice for VLCFA-related genes exhibit various phenotypes, and mutations in VLCFA-related genes cause inherited disorders including ichthyosis, macular dystrophy, myopathy, mental retardation, and demyelination. VLCFAs may regulate cellular functions by affecting membrane properties including membrane fluidity, permeability, curvature, and lipid microdomain formation. Nevertheless, our present knowledge of VLCFAs is still the tip of the iceberg. Further identification of novel VLCFA species, VLCFA-related metabolic pathways, genes, and disorders will reveal the diverse and unique functions of VLCFAs, and may lead to intervention for lipid-associated diseases.

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