Biosynthesis of hFA and hFA-SL

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Sphingolipids containing fatty acid (acyl) residues that are hydroxylated at position C-2 (2-hydroxylated sphingolipids) are found in most, possibly all, eukaryotes and certain bacteria. 2-hydroxylated sphingolipids are present in many organs and cell types, though they are especially abundant in myelin and skin. The enzyme fatty acid 2-hydroxylase (FA2H in mammals or its orthologs in other classes) is involved in the synthesis of many but not all 2-hydroxylated sphingolipids in eukaryotes. Deficiency in FA2H causes a neurodegenerative disease known as hereditary spastic paraplegia 35 (HSP35/SPG35) or fatty acid hydroxylase-associated neurodegeneration (FAHN). FA2H likely also plays a role in other diseases, for example in different types of cancer.

cancer fatty acid hydroxylase-associated neurodegeneration fatty acid 2-hydroxylase leukodystrophy

1. Fatty Acid 2-Hydroxylase (FA2H)

The fatty acid 2-hydroxylase enzyme, which is encoded by the *FA2H* gene in humans, has orthologs in apparently all eukaryotes. The *FA2H* gene has been characterized in mammals [1][2], yeast (*SCS7*) [3][4], plants (*FAH1*, *FAH2*) [5] and protists [6]. The FA2H enzyme belongs to the fatty acid hydroxylase/desaturase gene family and is an NAD(P)H-dependent monooxygenase that localizes to the endoplasmic reticulum [2]. The catalytic center is composed of four conserved histidine motifs that form an essential di-metal ion center in the catalytic center of the enzyme [7] (**Figure 1**). The enzyme adds the hydroxyl group in a stereospecific manner and forms only the (R)-enantiomer [8]. While in most eukaryotic genomes only one *FA2H* gene is present, *Arabidopsis thaliana* and other plants express two related *FA2H* genes, *FAH1* and *FAH2* [9], which differ with respect to their substrate specificity. The FAH1 enzyme mainly uses mainly very long chain fatty acids (VLCFAs) as substrates, whereas FAH2 prefers long chain fatty acids (LCFAs) [10]. In animals, fungi and protists, the FA2H enzyme contains an N-terminal cytochrome b5-like domain that is responsible for electron transfer from NAD(P)H. In contrast, the plant enzymes lack this domain but interact with one of the separate cytochrome b5 proteins within the ER membrane [11]. Whether cytochrome b5 can functionally replace the cytochrome b5-like domain, e.g. in cases of FAHN with mutations in this domain is not known.

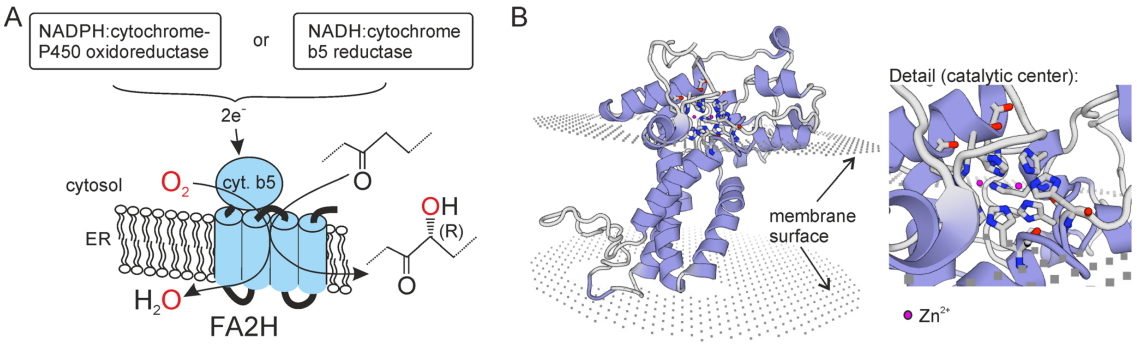

Figure 1. Enzymatic reaction catalyzed by FA2H and structure of the enzyme. (**A**) Reaction scheme of the 2-hydroxylation reaction of FA2H. (**B**) Model of the human FA2H enzyme (without cytochrome b5-like domain). Molecular modeling of human FA2H (Ac. Q7L5A8) was performed using Swiss Model (https://swissmodel.expasy.org/; accessed on 14 January 2023) with yeast FA2H (SCS7p; PDB file AZR0) as template. The histidine residues and the two zinc ions forming the catalytic center of the hydroxylase domain of the enzyme are highlighted and shown in the enlarged section on the right.

The X-ray crystal structure of the baker's yeast FA2H enzyme (SCS7p) has been resolved, though without the N-terminal cytochrome b5-like domain [7]. This study confirmed the previously predicted four-transmembrane domain structure [1][2] of the enzyme, with N-terminal cytochrome b5 domain and C-terminus facing the cytosol. Although FA2H is regarded as a di-iron enzyme, the yeast ortholog contains two zinc ions in the di-metal ion binding site [7]. Based on structural differences between SCS7p and the functionally and structurally related stearoyl-CoA desaturase-1 (SCD1), it was concluded that acyl-CoAs are most likely not substrates for FA2H/Scs7p, whereas a ceramide fits well into the catalytic center of the enzyme. On the other hand, the only established in vitro enzymatic FA2H assay used free fatty acids as an efficient substrate [12]. It is an open question whether or to what extent free fatty acids may serve as substrates in vivo.

Heme is synthesized in mitochondria and thus must be transferred to the ER-localized FA2H protein when its cytochrome b5-like domain is folded into its native conformation. A screen for FA2H interaction partners identified progesterone receptor membrane component 1 (PGRMC1) as a binding partner of FA2H [13]. PGRMC1 binds heme and is a putative heme chaperon [14]. Its yeast homologue (Dap1) is known to be required for the activation of several CYP450 enzymes [15]. A PGRMC1 antagonist reduced FA2H activity [13], and PGRMC1 may be involved in the delivery of heme to the cytochrome b5 domain of FA2H.

2. Alternative Pathways of 2hFA Synthesis

FA2H is the most studied enzyme involved in the synthesis of 2hFA in eukaryotes. However, the FA2H enzyme is not the only enzyme capable of synthesizing 2hFA or 2hFA-ceramides. It is clear that mice lacking a functional *Fa2h* gene seem to be devoid of 2hFA-SL in the nervous system [16][17] yet still contain 2hFA-SL in various organs, such as the skin [18]. In addition, levels of 2hFA-sphingomyelin in lymphocytes and erythrocytes from FAHN/SPG35 patients with a mutation causing exon 5/6 skipping (which is expected to fully abolish FA2H activity) were not

reduced [19]. One alternative source for 2hFA is an α-oxidation pathway in the ER [20]. Through this pathway, 2-hydroxylated palmitic acid can be formed from phytosphingosine (**Figure 2**). However, this pathway mainly generates C16-2hFA (and C18-2hFA from C20-phytosphingosine base). Longer phytosphingosine bases that could potentially enable the synthesis of VLCFA 2hFA (>C_{20}) are rare. As *FA2H* knockout mice and FAHN patients still contain substantial amounts of VLCFA 2hFA-SL [18][19], it is very likely that additional enzymes, which have not yet been characterized, exist that are capable of synthesizing 2hFA/2hFA-SL, at least in mammals. The only other known mammalian fatty acid 2-hydroxylase, peroxisomal phytanoyl-CoA hydroxylase, appears to be unable to hydroxylate straight fatty acids. As CYP450 enzymes synthesize 2hFA in certain bacteria (see above), they are possible candidates for these currently unknown enzymes.

3. Degradation of 2hFA-SL

The degradation of 2hFA-SL occurs mainly in lysosomes (**Figure 2**). This is achieved by the same acid hydrolases that degrade their non-hydroxylated counterparts. The sphingolipid activator protein (saposin) D, one essential cofactor of acid ceramidase, seems to be mainly involved in extracting the 2hFA-ceramide from the membrane to enable its hydrolysis [21]. Alternatively, the amide bond of 2hFA-ceramide may be hydrolyzed by alkaline or neutral ceramidase outside the lysosome; however, to what extent this happens with the 2hFA-ceramide is unclear. The released 2hFA can likely be recycled through a salvage pathway (**Figure 2**). All six mammalian ceramide synthases (CerS1-6) accept 2hFA-CoA as substrate [22]. Alternatively, 2hFA can be degraded through peroxisomal α-oxidation. In addition to branched chain fatty acids, peroxisomal 2-hydroxyphytanoyl-CoA lyase is able to cleave 2-hydroxylated straight chain fatty acids [23]. Whether free 2hFA have a physiological function is currently not known. However, exogenously added 2hFA can dramatically affect cell physiology.

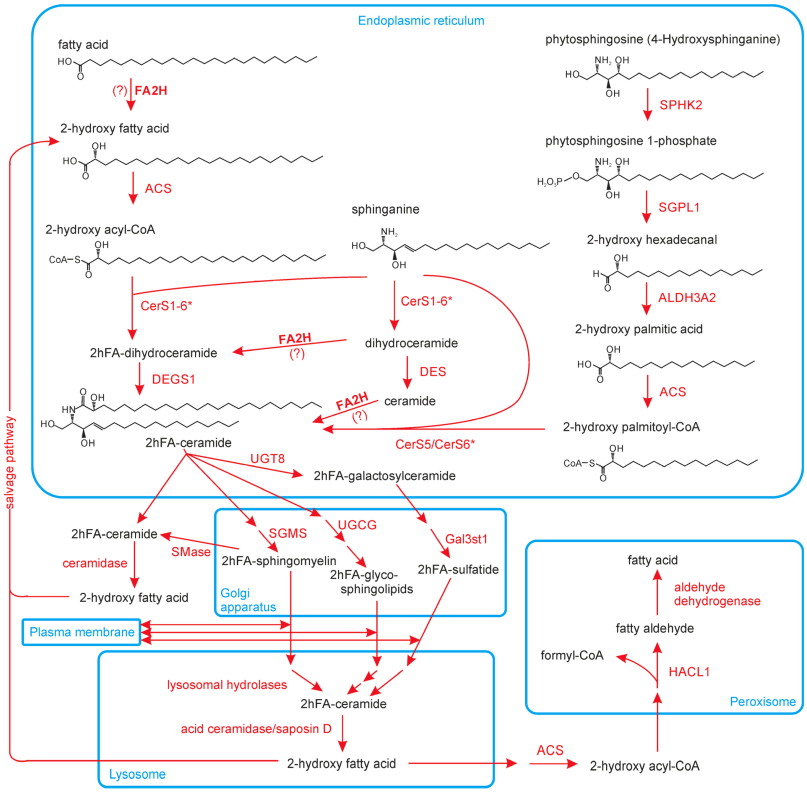

Figure 2. Overview of the metabolism of 2hFA-SL. As the physiological substrate(s) of FA2H is/are not yet clear, FA2H-dependent reactions are found here at different steps of the pathway, and the reactions are labeled with question marks (?) to indicate this fact. CerS1-6*/CerS5/CerS6*: In mammals, all six known ceramide synthases (CerS1 to CerS6) are capable of using 2-hFA-CoA as substrate. However, only CerS5 and CerS6 accept C16 acyl-CoA [22]. The FA2H-dependent reactions are shown with a C24 fatty acid/acyl residue; however, the enzyme apparently accepts acyl residues or fatty acids of various chain lengths. The α-oxidation of phytosphingosine can also metabolize longer sphingoid bases and thus generate longer 2hFA [20]. Note that many reactions, enzymes and metabolites, especially in the Golgi apparatus and lysosomes, are not specified in this Figure. Abbreviations/gene names and Enzyme Commission numbers: ACS—acyl-CoA synthetase (EC 6.2.1.2./6.2.1.3.); ALDH3A2—aldehyde dehydrogenase 3A2 (EC 1.2.1.3); (acid) ceramidase (EC 3.5.1.23); CerS(1-6)—ceramide synthase 1 to 6 (EC 2.3.1.24); DEGS1—sphingolipid-4-desaturase (EC 1.14.19.17); FA2H—fatty acid 2-hydroxylase (EC 1.14.18.6); Gal3st1—galactose-3-O-sulfotransferase 1 (EC 2.8.2.11); HACL1—2-hydroxylacyl-CoA lyase 1 (EC 4.1.2.63); SGMS—sphingomyelin synthase (EC 2.7.8.27); SGPL1—sphingosine-1-phosphate lyase 1 (EC 4.1.2.27); SMase—sphingomyelin phosphodiesterase (EC 3.1.4.12); SPHK2—sphingosine kinase 2 (EC 2.7.1.91); UGCG—UDP-glucose:ceramide glucosyltransferase (EC 2.4.1.80); UGT8—UDP-galactose:ceramide galactosyltransferase (EC 2.4.1.47).

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