


From peroxisomal disorders to common neurodegenerative diseases – the role of ether phospholipids in the nervous system

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The emerging diverse roles of ether (phospho)lipids in nervous system development and function in health and disease are currently attracting growing interest. Plasmalogens, a subgroup of ether lipids, are important membrane components involved in vesicle fusion and membrane raft composition. They store polyunsaturated fatty acids and may serve as antioxidants. Ether lipid metabolites act as precursors for the formation of glycosyl-phosphatidyl-inositol anchors; others, like platelet-activating factor, are implicated in signaling functions. Consolidating the available information, we attempt to provide molecular explanations for the dramatic neurological phenotype in ether lipid-deficient human patients and mice by linking individual functional properties of ether lipids with pathological features. Furthermore, recent publications have identified altered ether lipid levels in the context of many acquired neurological disorders including Alzheimer's disease (AD) and autism. Finally, current efforts to restore ether lipids in peroxisomal disorders as well as AD are critically reviewed.

Keywords: autism; peroxisome; plasmalogen

Ether (phospho)lipids are a particularly common subgroup of glycerophospholipids in the mammalian nervous system and diseases involving ether lipid deficiency have most drastic consequences in nervous tissue. The distinctive chemical feature of ether lipids is the ether bond at the *sn*-1 position of the glycerol backbone, where a fatty alcohol is attached as opposed to the more common diacyl phospholipids, in which fatty acids are esterified at both the *sn*-1 and *sn*-2

position. The biosynthesis of ether lipids requires the concerted action of different organelles. The first steps of the pathway take place in peroxisomes, where the sequential activities of dihydroxyacetone phosphate acyltransferase (DHAPAT; gene name: glycerone phosphate acyltransferase, *GNPAT*) and alkyl-dihydroxyacetone phosphate synthase (ADHAPS; gene name: alkylglycerone phosphate synthase, *AGPS*) are needed to generate the ether bond [1]. A fatty alcohol

Abbreviations

AA, arachidonic acid; AADHAPR, acyl/alkyl-dihydroxyacetone phosphate reductase; AD, Alzheimer's disease; ADHAPS/AGPS, alkyl-dihydroxyacetone/alkylglycerone phosphate synthase; CHO, Chinese hamster ovary; CNS, central nervous system; DHA, docosahexaenoic acid; DHAP, dihydroxyacetone phosphate; DHAPAT/GNPAT, dihydroxyacetone/glycerone phosphate acyltransferase; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FAR, fatty acyl-CoA reductase; GPI, glycosyl-phosphatidyl-inositol; GSK, glycogen synthase kinase; KO, knockout; LPA, lysophosphatidic acid; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; PAF, platelet-activating factor; PEX, peroxin; PexRAP, peroxisomal reductase activating PPAR γ ; PlsCho, plasmenylcholine; PlsEtn, plasmenylethanolamine; PNS, peripheral nervous system; PTS, peroxisomal targeting signal; PUFA, polyunsaturated fatty acid; RCDP, rhizomelic chondrodysplasia punctata; RNAi, RNA interference; SNARE, soluble N-ethylmaleimide-sensitive receptor attachment protein receptor.

for the ADHAPS reaction is supplied by fatty acyl-CoA reductase (FAR), a peroxisomal tail-anchored protein [2], which catalyzes the step suggested to be rate limiting in ether lipid biosynthesis and whose activity is subject to feedback regulation by the cellular plasmalogen level [3,4]. The resulting precursor, alkyl-dihydroxyacetone phosphate (DHAP), is shuttled across the peroxisomal membrane by a currently unknown mechanism. Ether lipid biosynthesis proceeds at the outer (cytosolic) face of the peroxisomal membrane, where an acyl/alkyl-DHAP reductase (AAD-HAPR; alternative name: peroxisomal reductase activating PPAR γ , PexRAP) reduces the ketone group at the *sn*-2 position [5]. This step also occurs in the biosynthesis of diacyl phospholipids and can be performed at both peroxisomal and endoplasmic reticulum (ER) membranes [6,7]. All subsequent enzymatic steps take place at other organelles, depending on the type of ether lipid. The most abundant ether lipids are the plasmalogens (Fig. 1A), which are characterized by a vinyl ether bond (a double bond adjacent to the ether bond) at *sn*-1 and mostly carry either ethanolamine or choline as head group thereby forming compounds termed plasmenylethanolamine¹ (PlsEtn, ethanolamine plasmalogen) or plasmenylcholine (PlsCho, choline plasmalogen). Less abundant but also found in a variety of tissues, are the plasmanyl phospholipids (Fig. 1E), which differ from the plasmalogens by a simple ether bond at *sn*-1 instead of the vinyl ether. Other prominent types of ether lipids include the small inflammatory mediator platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; Fig. 1C) or the sulfoglycolipid seminolipid [8], which is found mainly in testis. For details on ether lipid structure and metabolism, we may refer the readers to previous reviews [1,9–11].

Plasmalogens are especially enriched in the nervous system, where, in mammals, ethanolamine is the dominant head group with PlsCho present only in trace amounts. In man and most animals, brain tissue levels of plasmalogens are reported to constitute between 15 and 30 mol% of total phospholipids [1,12], depending on the method and brain region used for analysis. Although also neurons contain considerable levels of these lipids, they are particularly enriched in the myelin sheath, with more than 30 mol% of all phospholipids and the majority of ethanolamine phospholipids in the plasmalogen form [12,13]. In the human central

nervous system (CNS), the levels of plasmalogens are relatively low at birth, but increase strongly during early development [14], concomitant with myelination [15]. Early studies implied that the amounts of plasmalogens in the human brain rise until the age of 30 years and then start to decline [16]. Analyses of plasmalogen levels in the peripheral nervous system (PNS) have mainly been restricted to the sciatic nerve, which, due to its extensive myelination, shows similarly high levels as brain white matter [12].

In the present review, we summarize the current knowledge on the biological roles of ether phospholipids in nervous tissue. Based on the phenotypic traits in the nervous system of human patients and animal models with ether lipid deficiency, we recapitulate the main cellular functions of these lipids in the search of explanations for the observed phenotypes. Lastly, we highlight potential roles of ether lipids in more common neurological diseases and discuss recent approaches to treat ether lipid deficiency.

Nervous system pathology in human ether lipid deficiency

In man, the inherited deficiency in ether lipid biosynthesis results in a rare (estimated incidence: 1 : 100 000) and fatal disease, rhizomelic chondrodysplasia punctata (RCDP). Currently, five types of RCDP, differing in the affected gene, are known. Type 1 (MIM #215100), the by far most common subtype, is caused by mutations in the peroxin (*PEX*) 7 gene [17–19], which encodes the cytosolic receptor for proteins containing a peroxisomal targeting signal (PTS) 2. Upon *PEX*7 deficiency, ADHAPS is not imported into the peroxisome leading to loss of its function. The other RCDP types are evoked by mutations in *GNPAT* (type 2, MIM #222765) [20], *AGPS* (type 3, MIM #600121) [21], *FARI* (type 4, MIM #616154) [22], and *PEX5* (type 5, MIM #616716) [23]. The latter subtype represents a peculiarity, as it only affects the isoform *PEX5L*, a long splice variant of the *PEX5* protein. *PEX5L* can also mediate peroxisomal import of PTS1-containing proteins [24,25] but is dispensable here providing the shorter *PEX5* isoform is intact. However, it is essential as a coreceptor for *PEX7* in the import of proteins relying on PTS2 [26] and, therefore, a defect in *PEX5L* has similar consequences as *PEX7* deficiency.

Inborn ether lipid deficiency is also a secondary feature of peroxisome biogenesis disorders of the Zellweger syndrome spectrum, in which all peroxisomal functions are impaired due to deficient biogenesis of the whole organelle and whose nervous system

¹In the present review, we use the term ‘plasmalogens’, when referring to all plasmalogens irrespective of head group, and the term ‘plasmenylethanolamine’ or ‘plasmenylcholine’ for species with a specific head group.

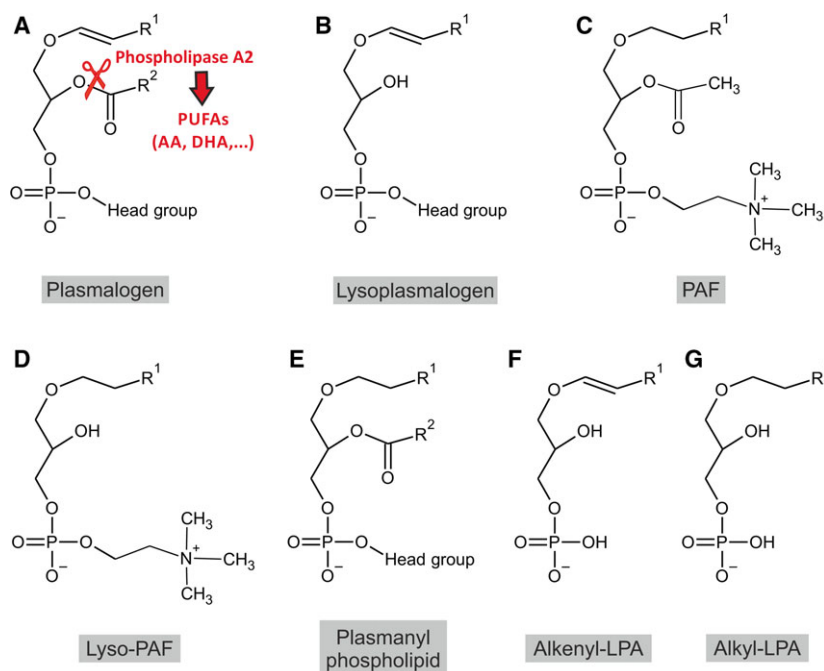


Fig. 1. Structure of ether lipids with reported involvement in signaling processes. A prototypic plasmalogen (A), lysoplasmalogen (B), PAF (C), lyso-PAF (D), plasmanyl phospholipid (E), alkenyl-LPA (F), and alkyl-LPA (G) are drawn disregarding stereochemistry. R¹ represents alkyl residues originating from the primary alcohols C16:0, C18:1, or C18:0; R² designates a wider range of saturated and unsaturated fatty acyl residues. The cleavage site for plasmalogen-specific phospholipase A2 is indicated in (A). Phospholipase A2 activity releases PUFAs, which can be converted into a variety of additional signaling mediators.

phenotype has been reviewed extensively elsewhere [27]. Formally, RCDP types 1 and 5 belong to the peroxisome biogenesis disorders, as not only ether lipid biosynthesis but also peroxisomal α -oxidation [28] and, in some tissues, peroxisomal β -oxidation [29] are compromised by the dysfunctional PTS2 protein import. However, due to the comparatively late onset of symptoms in α -oxidation deficiency (Refsum disease), the phenotype of RCDP type 1 and 5 patients can be with some rare exceptions ascribed exclusively to the deficiency in ether lipids. RCDP types 2–4 belong to the group of peroxisomal single enzyme or transporter deficiencies and result in similar phenotypes. Merely, the few patients described to suffer from the recently discovered RCDP types 4 and 5 display a somewhat milder phenotype compared with types 1–3. The clinical hallmarks of RCDP are rhizomelia, a shortening of the proximal long bones, and chondrodysplasia punctata, a type of skeletal dysplasia. Other typical symptoms involve congenital cataracts and joint contractures as well as profound growth and developmental retardation. In general, disease severity shows good inverse correlation with the residual activity of the affected enzyme and, therefore, the plasmalogen levels. Although the severe form of RCDP is usually

lethal within the first years of life, patients with a less severe (mild or intermediate) disease course may reach adolescence; these do not display the full spectrum of classical symptoms, and often have modestly reduced plasmalogen levels [30,31].

The nervous system is severely affected in RCDP patients with frequent intellectual disabilities and motor impairment. Developmental deficits manifest in microcephaly, and head growth ceases at 3 years of age in cases with particularly low plasmalogen levels [32]. A large proportion of patients develop epileptic seizures of varying type and frequency [33]. Main pathologic features commonly described in severe RCDP cases include myelination deficits, enlarged ventricles and subarachnoidal spaces, and cerebellar atrophy. White matter signal abnormalities, indicating myelin deficiency, have been detected by magnetic resonance imaging in different brain regions and often involve the parieto-occipital region [34–36]. The exact origin of impaired myelination is largely unclear. Cerebellar atrophy is usually progressive and presumably due to loss of Purkinje cells [37]. In individual cases, disturbances in neuronal migration have been reported resulting in polymicrogyria [38], pachygyria [39,40], agenesis of the corpus callosum [41], or dysplastic olivary bodies [42].

Spastic paresis is a frequent symptom in RCDP [43]. Hypotonia has been sporadically observed [22,44] but is not a common feature [43]. Peripheral neuropathy and retinitis pigmentosa were diagnosed in several atypical RCDP type 1 cases with a milder disease course that strikingly mimic Refsum disease, the disorder resulting from defects in peroxisomal α -oxidation [45–47]. Thus, the accumulation of phytanic acid, the main substrate for peroxisomal α -oxidation, is a likely contributor to the pathogenesis in these patients. In patients with the severe form of RCDP, stenosis of the cervical canal is prevalent [34,48]. Other commonly reported skeletal abnormalities of the spine include coronal clefts, likely caused by ossification defects, and scoliosis [22,49].

Lessons from animal models of ether lipid deficiency

Animal models are commonly used tools to unravel the pathomechanisms underlying human disorders. Here, we review the nervous system phenotype of animal models that are currently in use to study ether lipid deficiency.

Mouse models

Several widely used and well characterized mouse models of ether lipid deficiency have been generated by targeted inactivation of one of the genes encoding biosynthetic enzymes. Three different knockout (KO) mouse strains with complete ether lipid deficiency genetically mimic types 1, 2, and 3 of RCDP: *Pex7* [50], *Gnpat* [51], and *Agps* [52] KO mice, respectively, of which the first two have been more extensively described. Whereas, similarly to the human disease, *Pex7* mutations also affect α -oxidation (and potentially other pathways involving yet unknown PTS2-containing proteins), *Gnpat* and *Agps* KO mice represent models of isolated ether lipid deficiency.

The major pathological features in the CNS of RCDP patients are well reflected in these KO mouse models. Involvement of the cerebellum is typical of RCDP. Accordingly, investigations in *Gnpat* KO mice have revealed morphological alterations at early postnatal time points, particularly impaired cerebellar foliation and poorly developed fissures [53,54], which have been suggested to persist in some but not all animals over time [54]. The detailed results of these studies might depend on contributions from the genetic background of the mice used, as different strains vary considerably in their cerebellar foliation pattern [55]. The migration of granule cell precursors from the external granule layer is delayed in the *Gnpat* KO cerebellum

[53]; a finding that is complemented by the observation of an increased number of apoptotic cells in the external granular layer [54]. Furthermore, Purkinje cells, the major output neurons of the cerebellar cortex, show an increased density of spines and structurally altered innervation by parallel fibers (originating from granule cells) and climbing fibers (projecting from the inferior olivary nucleus) in *Gnpat* KO mice. The Purkinje cell axon often features signs of neurodegeneration in the form of axonal swellings that are filled with smooth ER-like membranes of enigmatic origin [53].

Also another pathological hallmark of RCDP brains, hypo- and dysmyelination, has been well documented in the *Gnpat* KO brain, particularly in the neocortex, cerebellum, and corpus callosum as well as in optic nerve [53,55]; but in contrast to a conditional mouse model of peroxisome biogenesis disorders (*Nestin-Pex5* KO), progressive demyelination was not observed [57]. In the cerebellum, hypomyelination ameliorates with age, but is still prominent, particularly in folium VI, by postnatal day 45 [53]. At the protein level, the amount of myelin basic protein was found to be reduced by 40–60% in affected areas [53,57].

Apparently, inflammatory processes do not play a major role in young ether lipid-deficient mice (up to 5 months), as indicated by the absence of microgliosis and only mild astrogliosis and axonal loss, when compared with the situation in the conditional *Pex5* KO mice [57]. However, our own observations indicate marked microglia activation in white matter tracts of *Gnpat* KO mice at 7 months of age (S. Forss-Petter & J. Bauer, unpublished observation). Neuroinflammation in the CNS is much more pronounced upon combined defects in ether lipid biosynthesis and peroxisomal β -oxidation (*Pex7/Abcd1* double KO) [58]. Recently, a thorough investigation of PNS (sciatic nerve) myelin in ether lipid-deficient (*Gnpat* and *Pex7* KO) mice revealed reduced thickness of the myelin sheath and a defect in the process of radial sorting, by which axons destined for myelination are separated from larger axon bundles, at postnatal day 5 [59]. Even though myelin thickness catches up in young adult mice, plasmalogen-deficient myelin appears less stable and Schwann cells, the myelinating cells of the PNS, show a reduced ability to remyelinate axons after sciatic nerve injury. In the PNS of aged (1.5 years old) mice, axonal loss and demyelination are pronounced and accompanied by partly abnormal Schwann cell morphology [59]. The myelination phenotype in the CNS and PNS of ether lipid-deficient mice manifests in reduced conduction velocities in the corpus callosum [53] and in motor nerves [59,60], respectively.

Mild impairment of neuronal migration was observed in the neocortex of *Pex7* KO embryos at embryonic day

18.5 (E18.5) [50], but could not be confirmed in *Gnpat* KO mice [54]. Next to background strain differences, additional defects in *Pex7* mice (as opposed to the isolated ether lipid deficiency in *Gnpat* KO mice), related to peroxisomal α -oxidation or still unknown proteins depending on PEX7 for peroxisomal import [61], might play a role in these discrepant findings.

Furthermore, *Pex7* KO mice have been described to suffer from hypotonia, a condition that may or may not have a neurologic cause. Hypotonia has not been reported in single *Gnpat* KO mice, but was severe in mice with combined deficiency of *Gnpat* and *Hsd17b4* (encoding multifunctional protein 2, an enzyme involved in peroxisomal fatty acid β -oxidation) [54]. Recently, our own studies have unraveled a defect also at the level of the neuromuscular junction of *Gnpat* KO mice. In addition to abnormal nervous innervation of the diaphragm muscle, characterized by exaggerated phrenic nerve sprouting and widening of the endplate zone, we also identified alterations in neuromuscular transmission [62].

In terms of behavior, the combined CNS and PNS pathologies of *Gnpat* KO mice results in impaired motor performance and muscular strength [53,62] as well as hyperactivity in the open field paradigm (F. Dorninger, G. Zeitler, C. Pifl, S. Forss-Petter & J. Berger, unpublished observation). Further behavioral evaluation, for example, of cognitive function would be an interesting addition to these findings. However, reliable studies of behavior are hindered by the fact that the visual system of ether lipid-deficient mice is strongly impaired in all KO mouse models, including bilateral congenital cataracts. In addition, detailed studies in *Gnpat* KO mice have identified a variety of morphological lens abnormalities and developmental ocular defects, including microphthalmia, a persistent hyaloid artery (Bergmeister's papilla), dysgenesis of the anterior eye chamber, and optic nerve hypoplasia [51,56], as well as abnormal angiogenesis resulting in an aberrant vascular network [63].

In addition to the KO mice with complete deficiency, several models of partial ether lipid deficiency exist. Mice with an inducible knockdown of AAD-HAPR (PexRAP-iKO mice) have been used for immunological studies [64], but their nervous system has not yet been characterized. Two further mouse models with residual plasmalogen levels represent the milder forms of RCDP: *Pex7* hypomorphic mice [65] and *bs2* mice (carrying a spontaneous hypomorphic mutation in the *Agps* gene) [66]. As expected due to the residual ether lipid levels, neuronal migration is normal in *blind sterile 2* (*bs2*) mice. Both hypomorphic models, like the null mice, develop cataracts

[65,66], but currently no additional information is available on the nervous system status of these animals. However, a careful characterization would be of major significance in order to obtain additional knowledge on how reduced, but not completely abolished, levels of ether lipids influence the CNS and PNS. Valuable insights into the role of ether phospholipids in the nervous system may also be gained from cell type- or brain region-specific KO or knockdown models, which have yet to be generated.

Other animal models

Animal models of isolated ether lipid deficiency have been largely restricted to mice. Purely ether lipid-deficient variants of *Caenorhabditis elegans* have been reported [67–69] but, so far, no information has been provided on the potential role of ether lipids in their nervous system. Strikingly, the capability of ether lipid-deficient worms to develop into adults differs remarkably between studies. Although earlier reports using an RNA interference (RNAi) strategy against *Agps* described an arrest mainly at the larval stage [69,70], another study did not find any major impairment of development and viability was only reduced upon lower temperatures after chemical mutagenesis of different genes involved in ether lipid biosynthesis (including *Agps*) [67]. Although this discrepancy might be associated with differences in background strain and the mode and time point of genetic intervention, the differences are striking and the molecular basis for this outcome would be of general interest.

Organisms with an inability to form peroxisomes cannot synthesize ether lipids, either. In recent years, models of generalized peroxisome deficiency have been generated in *Mus musculus* (mouse, for example [71,72]), *Drosophila melanogaster* (fruitfly [73,74]), *C. elegans* (roundworm [69,75]), and *Danio rerio* (zebrafish [76]). However, due to the multiplicity of other metabolic disturbances in these animals, it is impossible to assign an individual phenotypic alteration or pathology in the nervous system to the defect in ether lipid biosynthesis. Therefore, we refrain from discussing these models here and may refer the reader to another excellent review handling this issue [77].

Molecular basis and potential mechanisms underlying the phenotypic traits of ether lipid deficiency

The molecular mechanisms causing the nervous system pathology in ether lipid-deficient individuals are largely

enigmatic. Therefore, we will next discuss the main biological functions that have been documented or proposed for ether lipids and try to envision how the loss of these functions and/or attempts of compensation might contribute to the nervous system abnormalities observed upon ether lipid deficiency.

The importance of plasmalogens as membrane constituents

Ether lipids, predominantly plasmalogens, are major components of the plasma membrane and various organellar membranes, in which they shape the physicochemical properties of the lipid bilayers. Plasmalogens exhibit biophysical properties, which distinguish them from other phospholipids. Model membranes composed of PlsEtn undergo the transition from gel to liquid-crystalline phase at lower temperatures than membranes made up of the corresponding diacyl phospholipids [78]. Fluorescence anisotropy studies indicate that the membranes of plasmalogen-deficient cells are more fluid and less ordered than those of control cells, implying that plasmalogens confer increased order and decrease lipid mobility [79]. Membranes consisting of plasmalogens had slightly reduced thickness in molecular dynamics simulations and the vinyl ether bond causes increased ordering and denser packing of the *sn*-1 and *sn*-2 chains as compared with diacyl phospholipids [80,81]. In addition to the vinyl ether bond, the fatty acyl chain at *sn*-2 by itself, with the increased occurrence of polyunsaturated fatty acids (PUFAs) in plasmalogens, has an impact on the biophysical role of these phospholipids and, thus, on membrane properties [82]. Another important aspect of plasmalogens in membrane biology lies in their 'fusogenic' activity. The hypothesis that these lipids favor membrane fusion and fission is derived from the fact that plasmalogens have an increased propensity to undergo the transition from a nonlamellar to a hexagonal phase [78,83]. Accordingly, plasmalogen-containing vesicles or membranes show an increased tendency toward fusion [84] and have reduced surface tension [85], implying an important role in various physiological processes, in particular, those relying on exo- and endocytosis [86]. Furthermore, it has been proposed that not only the plasmalogens themselves but also their lyso-derivatives are important players in membrane fusion and phase transition [87].

In line with the importance of plasmalogens for membrane properties, their deprivation strongly impairs cholesterol trafficking [88,89] resulting in accumulation of free cholesterol but reduced levels of esterified cholesterol in ether lipid-deficient mouse and

human fibroblasts as well as Chinese hamster ovary (CHO) cells [44,51,88]. Interestingly, plasmalogen levels also seem to have a direct effect on cholesterol homeostasis, as recently shown by an inverse correlation between the amount of plasmalogen and the extent of cholesterol biosynthesis [90].

Moreover, plasmalogens have been described to be enriched in so-called lipid rafts (also termed membrane rafts) [91], which are small and heterogeneous membrane domains that are highly dynamic and compartmentalize cellular processes like, for example, signal transduction [92]. These domains were originally defined *via* their resistance to detergents [93]. Interestingly, detergent-resistant membranes isolated from the brains of ether lipid-deficient mice showed abnormal levels of marker proteins like flotillin and disturbed migration in a sucrose gradient [51]. Similarly, caveolae, small membrane compartments facilitating endo- and exocytosis, as well as clathrin-coated pits, are morphologically altered and reduced numerically in fibroblasts derived from ether lipid-deficient human patients in comparison with those of healthy controls [44]. Consistent with these results, also the rate of endocytosis proved decreased.

When considering how these biochemical findings apply to the nervous system, numerous processes come to mind that could be affected by the altered membrane lipid composition evoked by ether lipid deficiency. Basically, every membrane-associated event could be modulated in some way, which has prompted speculation on the molecular basis of the neurologic phenotype of ether lipid-deficient mice and men. However, aside from the recently reported impairment of AKT signaling due to deficient recruitment of AKT kinase to the plasma membrane (see The versatile functions of ether lipids in signaling), detailed experimental data on specific molecular mechanisms are lacking.

The synaptic vesicle cycle, which orchestrates the release of neurotransmitters from synaptic vesicles and allows communication between neurons, comprises a series of membrane-associated steps that have long been subject to hypotheses in the context of ether lipid deficiency. The lipid environment plays an important role in the synaptic vesicle cycle. Lipid bilayers and their interplay with the involved proteins provide the framework for endo- and exocytotic processes and the detailed molecular composition of membranes regulates the dynamics of fusion events [94]. Plasmalogens were shown to be a major constituent of the membranes of synapses [95] as well as synaptic vesicles [96], which—together with their proposed role in membrane fusion and constriction processes—has led to

speculations about an essential role of plasmalogens in the synaptic vesicle cycle and, thus, in neurotransmission [10,56,97–99]. Another link between plasmalogens and the synaptic vesicle cycle involves lipid rafts. These plasmalogen-rich microdomains are presumed to be responsible for sorting and sequestration of the soluble N-ethylmaleimide-sensitive receptor attachment protein receptor (SNARE) proteins [100,101], which mediate synaptic vesicle exocytosis, and to provide the environment for many different postsynaptic receptors [102,103] and to play a role in synaptic plasticity [101,104]. Moreover, not only the biophysical properties of the plasmalogen backbone (i.e., the vinyl ether bond) but also their enrichment in PUFAs has to be considered. Arachidonic acid (AA), which is particularly enriched in nerve growth cones [105], and docosahexaenoic (DHA) are abundant in synaptic membranes, rendering these highly dynamic [94]. They modulate the endocannabinoid system, an important regulator of synaptic function, and, possibly, the assembly of SNARE proteins [106–108].

The impact of ether lipids, or the lack thereof, on neurotransmission *in vivo* has not yet been fully elucidated. A study by the group of Wilhelm Just using synaptosomes isolated from *Gnpat*-deficient mice has provided first clues: While calcium-dependent neurotransmitter release was decreased in ether lipid-deficient nerve terminals, calcium-independent transmitter outflow was increased [109], which may derive from a reversal in ion transport [110]. In addition, ether lipid-deficient synaptosomes exhibited decreases in the rate of respiration and ATP levels [109] probably impacting energy-dependent processes like the vesicular uptake of neurotransmitters.

Also, our electrophysiological studies on the neuromuscular junction in ether lipid-deficient mice support a role of ether lipids in vesicular exocytosis at the synapse [62]. Here, we identified a reduced spontaneous fusion rate of vesicles in *Gnpat* KO mice. Also in line with the hypothesis of reduced fusion capacity under conditions of ether lipid deficiency, the quantal content after electrical stimulation was decreased, indicating that a lower number of vesicles fuse in each release event. Furthermore, we discovered abnormal clustering of acetylcholine receptors in ether lipid-deficient myotubes differentiated *in vitro*, possibly associated with altered lipid raft properties evoked by ether lipid deficiency.

Plasmalogens have also been proposed to contribute to the structure of myelin, the highly specialized membrane of oligodendrocytes. This is supported by the relative enrichment of PlsEtn in white matter compared with gray matter [12]. Specifically, plasmalogens

may ensure membrane compaction. This hypothesis is based on the rigidity and dense packing conferred by the vinyl ether bond [80,111] and the preferred esterification of saturated or monounsaturated fatty acids to the *sn*-2 position in white matter tissue [112,113].

Interestingly, also seminolipid-like sulfated glyco-glycerolipids of the alkylacyl type (i.e., containing an ether bond) were detected in the brain of adult rats, but upon aging the levels decreased [114]. Based on their temporal occurrence during development, parallel to plasmalogens, a role of these lipids (along with sulfatides) in myelination was suggested but apparently never investigated in further detail.

The versatile functions of ether lipids in signaling

It has repeatedly been shown that PUFAs are enriched at the *sn*-2 position of plasmalogens [12,115,116]. In the nervous system, particularly gray matter plasmalogens are rich in PUFAs [97]. These fatty acids can be easily released from phospholipids by the action of phospholipase A2 (Fig. 1A), for which a plasmalogen-selective, calcium-independent subtype has been identified and purified [117]. Among the PUFAs, AA (ω -6) and DHA (ω -3) are of particular biological and physiological importance. In mammals, they can be biosynthesized only through the metabolization of essential precursors. Consequently, mammals rely on the dietary intake of AA and DHA or their precursors linoleic acid and α -linolenic acid, respectively. Both PUFAs are vital for brain development and, especially DHA, for normal neural functioning [118]. AA serves as a precursor for a variety of inflammatory mediators like leukotrienes, lipoxins, epoxyeicosatrienoic acids, thromboxanes, and prostaglandins. DHA in turn can be metabolized to resolvins, protectins, and maresins, all of which have immunomodulatory effects as well [119]. Generally, ω -6 and ω -3 PUFAs are often portrayed as antagonistic players with ω -6 and ω -3 PUFAs (and their metabolites) adopting proinflammatory and anti-inflammatory functions, respectively [120], although this view may be oversimplified.

Gnpat KO mice show a defect in retinal vascularization, presumably due to erroneous astrocyte template formation [63]. Based on the similarity of this phenotype and that of a mouse model with inhibited calcium-independent phospholipase A2, the authors hypothesized that PUFAs released from plasmalogens are essential for proper development of the retinal vasculature [63,121]. In neocortex and hippocampus of *Gnpat* KO mice, however, ether lipid deficiency does not lead to a general loss of PUFAs. Instead, there is a shift from DHA- to AA-containing phospholipids

resulting in reduced amounts of DHA in total ethanolamine phospholipids (Fig. 2) [51,122], which may have physiological consequences on its own. DHA is a highly versatile compound that has attracted considerable attention, particularly in the field of neuronal development and memory formation. A role of this PUFA has been suggested in various molecular events regulating neurite outgrowth, neuro-, and synaptogenesis, as well as synaptic plasticity and learning- and memory-related processes [123]. Based on the available data, the DHA depletion in ether lipid-deficient brains is relatively modest and has arguably less drastic effects than complete deprivation of DHA (or all ω -3 PUFAs; reviewed in ref. [124,125]), as described in many studies investigating the biological relevance of DHA. However, a moderate global DHA deficit and the loss of DHA-containing plasmalogens as a source of substrates for plasmalogen-specific phospholipase A2 may be additional puzzle pieces that modulate the pathogenic events under conditions of complete or partial ether lipid deficiency in the nervous system.

Increasing evidence indicates that several key signal transduction pathways are impacted by the lack of ether lipids. Recent reports have shown that phosphorylation of both AKT (protein kinase B) [59] and extracellular signal-regulated kinase (ERK; a mitogen-activated protein kinase, MAPK) [126] is reduced in nervous tissue of ether lipid-deficient mice. In Schwann cells, the defect in ether lipid biosynthesis was

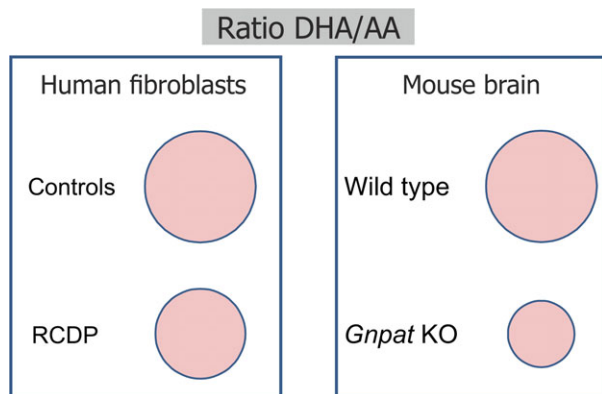


Fig. 2. Shift from DHA- to AA-containing ethanolamine phospholipids under conditions of ether lipid deficiency. The ratio between DHA- and AA-containing ethanolamine phospholipids in human fibroblasts of healthy controls and RCDP patients (left) and in gray matter-enriched brain tissue of adult wild-type and ether lipid-deficient (*Gnpat* KO) mice (right) is indicated by circles drawn to scale. Upon ether lipid deficiency, the diminished levels of DHA-containing ethanolamine phospholipids are generally compensated by an increase in AA-containing species. The diagrams are based on the data published in ref. [122].

demonstrated to impair the recruitment of AKT to the plasma membrane thereby inhibiting its proper phosphorylation, which in turn causes aberrant phosphorylation and overt activation of the downstream kinase glycogen synthase kinase (GSK) 3 β and finally impaired myelin formation and maintenance [59]. However, a recent study of mice with Schwann cell-selective KO of *Pex5* did not reveal such abnormalities in myelination [127] suggesting that the inherent defect in Schwann cells is only one aspect of the myelination problem and that also interaction with other compartments (e.g., axons) with additional disturbances is involved in mice with global ether lipid deficiency. Another study found decreased phosphorylation of both AKT and ERK phosphorylation after injection of lentiviral shRNAs against the *Gnpat* mRNA into the cerebral cortex of mice [126]; while exogenous supplementation with plasmalogens in cultured neuronal cells was suggested to enhance AKT and ERK phosphorylation [128] *via* the induction of G protein-coupled receptors [129]. AKT as well as MAPK signaling play indispensable roles in nervous system development and function [130–133]. However, future studies will be required to establish to what extent different cell types are affected by the reported defects and the impact on specific downstream targets. Nevertheless, these findings open interesting new perspectives in plasmalogen research and may enable the dissection of crucial molecular networks underlying the phenotypes in ether lipid-deficient individuals.

Most of the phenotypic traits of ether lipid-deficient patients and animal models have been attributed to the lack of plasmalogens in recent literature. Still, particularly in the context of signal transduction, a variety of other ether lipid compounds may contribute to pathology. The release of the fatty acyl group from the *sn*-2 position of plasmalogens does not only produce free fatty acids, which can mediate signaling functions but also lysoplasmalogens (Fig. 1B), which can also serve as second messengers. Lysoplasmalogens with an ethanolamine head group have the potential to act as self antigens in the stimulation and maturation of semi-invariant natural killer cells [134]; those with a choline head group are able to induce increased adherence of neutrophils to human endothelial cells [135], possibly by activating a cAMP-dependent protein kinase [136]. The current knowledge on the functions of lysoplasmalogen in the nervous system is limited, but presumably some roles in signaling apply as well here.

The results of several studies have implicated an involvement of PAF (Fig. 1C) and its receptor in the processes of synaptic transmission and synaptic

plasticity associated with long-term potentiation (LTP). In details, however, the reports are conflicting. PAF has been suggested to induce or enhance LTP by acting as a retrograde messenger that is released from postsynaptic neurons and enhances presynaptic release [137–140], likely through the activation of several protein kinases [141]. PAF is also able to stimulate ATP release from PC12 cells and causes influx of calcium in PC12 cells and hippocampal neurons *in vitro* [142,143]. These observations fit the finding that LTP is weakened in hippocampal slices from mice with a deficiency in the G protein-coupled PAF receptor [144]. In line with these data, the infusion of a PAF analog into several brain regions improved the performance of mice in different memory-related tasks; whereas conversely, application of a PAF antagonist impaired memory function [145]. In stark contrast, other authors found no consequence of PAF receptor deficiency for hippocampal LTP [146] or even a negative effect of PAF on LTP mediated by tyrosine kinases [147]. However, different models and different PAF concentrations were used in these studies, and might have caused the contradictory findings, with low concentrations having a stimulating effect on LTP and high concentrations being rather inhibitory [147].

Similar to its role in peripheral tissues and in the circulation, under pathological conditions, PAF promotes inflammatory responses in the nervous system [148] and may induce neuronal apoptosis in both PAF receptor-dependent [149] and -independent ways [150]. Accordingly, PAF receptor inhibition or deficiency protects from neuroinflammation in different disease models [151,152].

In addition, PAF has been ascribed a role in brain development. PAF receptor activation has the ability to inhibit neuronal migration *in vitro* [153], and mice with a heterozygous deletion in the β -subunit of PAF acetylhydrolase, the enzyme that releases the acetyl group of PAF to produce lyso-PAF (Fig. 1D), display aberrant neuronal layering in several brain regions [154]. In contrast, PAF receptor KO mice do not exhibit major brain malformations [155] suggesting that either PAF receptor-independent actions of PAF or additional, PAF-unrelated functions of the acetylhydrolase affect neuronal migration. However, subsequent, more detailed studies of PAF receptor KO mice revealed a thickened external granular layer in the cerebellum and decreased migration capability of cerebellar granule cells *in vitro* and *in vivo* [156]—a phenotype that is also reproduced in *Gnpat* KO mice [53]. The regulation of the levels of PAF and its downstream actions, thus, seem to be important modulators of neuronal development.

Also some less abundant ether-linked lipids have been described to mediate signaling. Plasmalogen phospholipids (containing an ether, but not a vinyl ether bond; Fig. 1E) serve as ligands for the nuclear peroxisome proliferator-activated receptor (PPAR) γ [157]. Alkenyl-lysophosphatidic acid (alkenyl-LPA; 1-O-cis-alk-1'-enyl-2-lyso-sn-glycero-3-phosphate; Fig. 1F) elicits activation of the MAPKs ERK1 and ERK2 in 3T3 mouse fibroblasts [158]; and alkyl-LPA (Fig. 1G) participates in the activation of semi-invariant natural killer cells (similarly as lysoplasmalogens) [134]. Alkyl-LPA has been detected in considerable amounts in rat brain [159] and appears to be able to activate microglia *via* the LPA₅ receptor [160,161].

From a signaling perspective, ether lipid deficiency may, thus, have opposing effects on inflammatory processes in the nervous system (and elsewhere). On one hand, plasmalogen deficiency leads to an increased ratio between AA and DHA (Fig. 2), likely shifting the immunological balance in the proinflammatory direction [122]. Moreover, exogenously applied plasmalogens were reported to prevent serum starvation-induced neuronal apoptosis in cell culture experiments [128,162] and to ameliorate systemically induced neuroinflammation in mice, when coadministered intraperitoneally with lipopolysaccharide [163]. Conversely, plasmalogen deficiency may render neurons more vulnerable to stress conditions and inflammation. One study reported signs of neuroinflammation to be largely absent in the brain of younger (up to 5 months) *Gnpat* KO mice [57] but microglia activation may become apparent only with advancing age (S. Forss-Petter & J. Bauer, unpublished observation). However, we do not exclude that in constitutive KO models adaptive compensatory processes may occur, as we have previously argued in the case of neutropenia, which seems to be present in an inducible KO model of ether lipid deficiency but not in (constitutive) *Gnpat* KO mice [64,164]. Acute reduction in plasmalogen levels, as evaluated after *in vivo* knockdown of *Gnpat*, was described to promote microglia activation and upregulation of inflammatory cytokines in adult mice [126]. On the other hand, the lack of proinflammatory mediators like PAF or alkyl-LPA may dampen the immune response and protect neurons from death signaling in the ether lipid-deficient nervous system.

Taken together, signaling alterations are highly likely to play a crucial role in the nervous system phenotype elicited by ether lipid deficiency, but many of the underlying molecular mechanisms still need to be unraveled in detail. We want to emphasize that the perception of ether lipid deficiency should not be restricted only to plasmalogens but rather expanded to

comprise also less common signaling mediators that have often been neglected in the discussion.

The enigmatic role of ether lipid components in GPI-anchored proteins

A relatively new addition to the proposed roles of ether lipids is emerging from the discovery of an ether-bonded lipid derivative in the glycosyl-phosphatidylinositol (GPI) anchor, a post-translational modification of membrane proteins that tethers these proteins to the outer leaflet of the plasma membrane [165]. In eukaryotes, about 0.5% of all proteins are GPI-anchored [166], including prominent examples in the nervous system like the prion protein or a particular isoform of acetylcholinesterase. The detailed structure of the GPI anchor differs across species, but a conserved core is formed by a lipid part derived from phosphatidylinositol, a sequence of sugar residues and ethanolamine phosphate linking the anchor to the C terminus of the protein [167]. Remarkably, although phosphatidylinositol occurs mostly in the diacyl form, the lipid part of several GPI-anchored proteins was described already decades ago to involve 1-alkyl-2-acylglycerol (alkylacylglycerol), an ether lipid [168–170]. Later, a systematic study confirmed these findings and identified alkylacylglycerol as the dominant lipid variant in mammals, but not in other species [171,172]. The reason for the differential lipid composition of mature GPI-anchored proteins and phosphatidylinositol appears to be a remodeling step, in which diacylglycerol is exchanged for an alkylacyl moiety. The latter is derived from a peroxisomal precursor, as it has been shown that alkyl group-bearing GPI anchors cannot be formed in the absence of peroxisomal ether lipid biosynthesis [173]. The biological significance of the ether bond in the GPI anchor has not yet been identified. In ether lipid-deficient CHO cells and human fibroblasts, the alkylacylglycerol moiety is replaced by a diacyl lipid. Remarkably, in these cells, the surface levels of the GPI-anchored protein urokinase-type plasminogen activator receptor were even drastically increased in comparison with control cells [174]. However, a functional evaluation of the shift from alkylacyl to diacyl moiety is still missing. Intriguingly, these observations have only scarcely been picked up by the scientific community, likely because there is no obvious impairment due to the absence of the alkylacyl moiety in the GPI anchor. However, already subtle modifications of GPI-anchored protein function could have drastic consequences for the nervous system. Several GPI-anchored proteins are highly expressed in the nervous system, for example, cell

adhesion molecules, mostly members of the immunoglobulin superfamily (like contactin 1 and neural cell adhesion molecule) [175], which are critical for neuronal growth and nervous system development. Also, ephrinA proteins, GPI-anchored ligands for receptor tyrosine kinases of the EphA family, are crucial cell–cell signaling mediators and key players in neural development, regulating such essential processes as neuronal migration, axon guidance, and synaptogenesis [176]. So far, any contribution of GPI anchor dysfunction to the pathology in ether lipid-deficient organisms remains speculative, but this issue demands further attention and may bring some valuable insights.

The controversial function of plasmalogens as antioxidants

The defense against oxidative stress was initially proposed to be a major function of plasmalogens. Experiments carried out *in vitro*—by different research groups—provided evidence that the vinyl ether bond protects against various stress conditions, among others exposure to UV light [177] or hypoxia [178], *in vitro*. Plasmalogens act as scavengers of singlet oxygen and free radicals [179] and, thus, have the capability to protect PUFAs from oxidative modification [180,181]. It was repeatedly shown that this occurs at the cost of the plasmalogens themselves, which were preferentially destroyed by the different oxidative agents; and plasmalogen-deficient cells proved less resistant to stress conditions than control cells [182,183]. Also, the findings of decreased plasmalogen levels, mostly in the circulation, in several pathological conditions that are associated with enhanced oxidative stress (e.g., hyperlipidemia or sepsis) has been used as an argument in favor of the proposed antioxidative capacity of these compounds [184,185]. In line with these considerations, a recent study using myelin isolated from *Pex7* KO mice showed that plasmalogen deficiency confers increased vulnerability of myelin to oxidative attack by hydroxyl radicals *in vitro*, possibly due to enhanced oxidative damage of myelin membrane proteins and/or lipids; the authors concluded that plasmalogens are able to protect myelin from oxidative stress [186].

However, the role of plasmalogens as antioxidants has been repeatedly challenged: Some opponents argue that a potential antioxidative effect might be derived from PUFAs esterified to the *sn*-2 position of plasmalogens, rather than from the vinyl ether bond [187,188]. No protective effect of plasmalogens could be determined in cultured astrocytes exposed to lactic

acid stress [189]. Furthermore, a study using primary skin fibroblasts derived from human patients with plasmalogen deficiency did not detect any difference in sensitivity to reactive oxygen species [190]. Other adversaries contend that the oxidative degradation of plasmalogens produces compounds that could themselves be harmful for the organism [187,191,192]. For example, plasmalogens were identified as a preferred target of reactive chlorinating species produced by neutrophils thereby generating α -chloro fatty aldehydes, which might promote inflammation [193,194]. One study even reported a pro-oxidative effect of plasmalogens in a bulk lipid system [195].

Analyses of the nervous system in ether lipid-deficient mice have rather supported the hypothesis that plasmalogen deficiency does not necessarily cause enhanced oxidative stress. In the cerebellum of *Gnpat* KO mice, the oxidative stress markers 3-nitrotyrosine and 4-hydroxynonenal were consistently detected in earlier developmental stages (3 weeks), but only occasionally in advanced ones (12 weeks and 5 months) [57]. Another study found the overall antioxidative potential to be unaltered and even decreased levels of thiobarbiturate-reactive substances (markers of lipid peroxidation) in the cortex and cerebellum of *Gnpat* KO mice, thus contradicting the notion of increased vulnerability resulting from chronic lack of plasmalogens [109]. However, in the same report, primary fibroblasts derived from these mice were shown to be more susceptible to oxidative damage *in vitro*. These findings reinforce that caution is warranted when attempting to translate the data derived from *in vitro* experiments to the complex situation *in vivo*. The role of plasmalogens in oxidative defense remains controversial and the antioxidant capacity of these compounds appears to be strongly dependent on *sn*-2 composition and the cellular context [188]. Therefore, based on the current state of knowledge, there is no indication that oxidative stress would be a major inherent factor in the nervous system pathology of ether lipid-deficient mice or human patients. However, it is plausible that under certain circumstances, for example, upon exogenous oxidative attack triggered by additional toxic or pathogenic events, a lack of plasmalogens could be detrimental.

Potential involvement of ether lipids in more common neurologic diseases

In addition to the inborn deficiencies in ether lipid biosynthesis, many other, often more common, diseases have been associated with ether phospholipids, for example, due to abnormal levels or composition in

certain tissues of patients or animal models. Table 1 provides an overview of neurological disorders with a reported link to ether lipids and specifies the nature of the proposed associations. Many of the effects were not anticipated and open novel perspectives that may reveal potential therapeutic targets. Although some of the reported alterations and connections must still be verified and/or their clinical relevance has to be established, the plethora of reports dealing with the role of ether lipids in various neurological diseases underscores their importance for the nervous system. However, we are also aware that changes in lipid levels, like those listed in Table 1, also have their drawbacks: The reported involvement of ether lipids in many cases may not be of causative nature, but rather secondary to other pathologic processes. This applies particularly to alterations of the levels of ether lipids in peripheral blood, which often allow only limited conclusions to be drawn on the pathological mechanisms underlying neurological diseases. In addition, lipid profiles are often dependent on the diet or other lifestyle parameters and, therefore, might be population-specific.

Alzheimer's disease

More than in any other sporadic neurological disease, a role of plasmalogen deficiency in pathogenesis is discussed in the context of Alzheimer's disease (AD), the most abundant neurodegenerative disease and cause of dementia worldwide. AD is estimated currently to affect more than 35 million people [196] with a strong rise in the number of patients predicted [197]. Several reports have stated considerably reduced levels of PlsEtn [112,198–200] and PlsCho [201,202] in *postmortem* brain samples of AD patients (see also Table 1). Similarly, moderate deficits in PlsEtn were also detected in the brain of mouse models of the disease [203,204]. Interestingly, also increased levels of PlsEtn in brains of AD patients were reported in one study, but this finding has never been confirmed [205]. Decreased plasmalogen levels are not a general feature of neurodegenerative diseases, as this was not detected in brain samples of patients with Parkinson's disease (PD) or Huntington's disease [206]. However, to a lesser extent, it is associated with the normal aging process [16]. In AD, the deficit is present in white matter, where it appears very early in the disease course (i.e., in subjects with a clinical dementia rating of 0.5) [112], as well as in gray matter, where it correlates with cognitive decline [97] and neuropathological staging [199].

Several reports also state a reduction in some plasmalogen species in the circulation of AD patients [207,208] and, consequently, their utility as a

Table 1. List of neurologic disorders with suggested involvement of ether lipids.

Disease	Reported involvement of ether lipids	Reference
Alzheimer's disease	Reduced PlsEtn levels in <i>postmortem</i> brain tissue of AD patients (including both gray and white matter)	[112,198–200]
	Increased PlsEtn levels in <i>postmortem</i> brain tissue of AD patients	[205]
	Decreased PlsCho levels in prefrontal cortex autopsy tissue of patients	[201]
	Decreased PlsEtn levels in plasma of AD patients and in serum of patients with cognitive impairment	[207,208,210]
	Decreased PlsEtn levels in brain tissue derived from mouse models of the disease (APPswe/PS1-dE9 transgenic; APPswe/tauP301L transgenic)	[203,204]
	Reduced ADHAPS protein levels (but increased AGPS mRNA levels) in brain tissue of patients; dysregulation of expression of ether lipid biosynthetic enzymes in brain and cultured cells of mouse models deprived of A β peptides (PS 1/2 KO, APP KO, APP/APLP 2 KO)	[215]
	Increased activity of plasmalogen-selective phospholipase A2 in several brain regions of AD patients	[214]
	Reduced activity of γ -secretase upon treatment with different PlsEtn or PlsCho species <i>in vitro</i>	[218]
	Accumulation of C16:0-PAF and C16:0-lyso-PAF in posterior/entorhinal cortex of AD patients and of an AD mouse model (TgCRND8; transgenic APPswe/Ind double mutation)	[223]
	Impaired binding of PAF to platelets of patients with AD or multiinfarct dementia	[238]
Parkinson's disease	Reduced levels of plasmalogens with C16:0 at <i>sr-1</i> in plasma of PD patients	[239]
	Reduced levels of plasmalogens in lipid rafts isolated from cortical gray matter of PD patients	[240]
	Reduced PlsEtn levels in serum of MPTP-treated mice (a model of PD)	[241]
	Relief of dyskinetic side effects of levodopa in Parkinsonian monkeys by treatment with a plasmalogen precursor	[242]
Down syndrome	Reduced PlsEtn levels in frontal cortex and cerebellum of patients (but associated with a general decrease in ethanalamine phospholipids)	[243]
	Reduced plasmalogen levels in erythrocytes of patients	[244]
Schizophrenia	Elevated levels of some PlsCho and PlsEtn species in frontal cortex of patients and of a mouse model of the disease (G72/G30 transgenic)	[245,246]
	Reduced plasmalogen levels in plasma of first episode and recurrent patients	[247]
	Reduced PlsEtn levels in plasma and platelets of patients; decreased PlsCho levels in plasma, but increased levels in platelets of patients	[248]
	Reduced levels of several plasmalogen species in fibroblasts from patients (particularly under stress conditions)	[249]
	Lower PlsEtn levels in erythrocyte membranes of patients, particularly in individuals with low sphingomyelin levels	[250]
Autism	Genetic association between mutations or polymorphisms in <i>PEX7</i> and autism spectrum disorder	[225,226]
	Reduced plasmalogen levels in plasma and in erythrocytes of patients	[228,229]
Depression/anxiety	Reduced PlsEtn levels in brain of propionic acid-infused rats (a model of autism)	[230]
	Reduction of several ether-bonded phospholipid species in plasma of patients with major depressive disorder	[251]
	Inverse correlation between symptoms of depression and anxiety and plasmalogen C36:4 levels	[252]
Epilepsy	Genetic overlap between levels of ether-linked AA-containing choline phospholipids and major depressive disorder	[253]
	Increased levels of PAF in rat brain upon convulsive treatment	[254]
	Increased levels of PAF and PAF receptor overactivation as cause for neuronal circuit dysfunction in rodent models of limbic epileptogenesis	[255]
Multiple sclerosis (MS)	Reduction of PlsEtn levels in demyelinated areas of the cerebral cortex in MS patients	[256]
	Reduction of the levels of DHA-containing PlsEtn in serum of MS patients	[257]
	Reduction of plasmalogen levels in brain of rats with experimental autoimmune encephalomyelitis (EAE), along with reduced DHAPAT activity in brain peroxisomes and reduced <i>Gprat</i> mRNA levels in the spinal cord	[258]

Table 1. (Continued).

Disease	Reported involvement of ether lipids	Reference
Pelizaeus–Merzbacher disease	Reduction of PlsEtn levels in oligodendrocytes of a mouse model of the disease and, of some species, in patient lymphocytes	[259]
X-linked adrenoleukodystrophy (X-ALD)	Reduction of PlsEtn levels in white matter of patients with cerebral ALD (not restricted to lesions), in brains of <i>Abcd1</i> KO mice and in primary mouse astrocytes upon silencing of <i>Abcd1</i> and <i>Abcd2</i>	[260]
Prion diseases	PAF as suggested mediator of prion-induced toxicity <i>in vitro</i>	[221]
Head trauma/spinal cord trauma	Increased levels of ether-bonded choline and (by trend) ethanolamine phospholipids in the hippocampus; decreased levels of ether-bonded ethanolamine phospholipids in cortex and plasma and of ether-bonded choline phospholipids in cerebellum of a mouse model of traumatic brain injury	[261]
	Increased levels of some ether-bonded species, particularly such with choline head groups in murine optic nerve after repetitive mild traumatic brain injury	[262]
	Reduced PlsEtn levels in spinal cord after laminectomy and spinal cord compression in a cat model	[263]
Spinal cord ischemia	Reduction of PlsEtn levels (and other ethanolamine phospholipids) in spinal cord of rabbits after ischemia	[264]
Ischemic stroke	Reduction of PlsEtn levels in synaptosomes from rat striatum after injection of endothelin-1 (producing an ischemia-like lesion)	[265]
	Increased levels of PAF in blood of patients	[266]
	Correlation between PAF binding to platelets and infarct volume as well as neurological outcome	[267]

APLP, amyloid precursor protein-like protein; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin; MS, multiple sclerosis; PS, presenilin.

biomarker for cognitive decline has been suggested [209,210]. Taking into account that lipid levels in the blood are considerably influenced by external factors like diet, lifestyle, medications, or age and that the observed differences in plasmalogen levels are subtle, the putative use of plasmalogens as a single prognostic or diagnostic marker should be treated with caution. However, individual plasmalogen species may be valuable as components of a biomarker panel, as proposed recently [211].

The origin of plasmalogen depletion in AD is so far unresolved: A generalized dysfunction of peroxisomes evoked by aging in general or by AD pathology could lead to impaired plasmalogen biosynthesis [199,212,213]. In particular, the preferential degradation of these lipids under oxidative stress, a condition also found in AD [97], might account for the observed changes. Others suggest that amyloid-beta ($A\beta$) is responsible for the plasmalogen reduction, either by stimulation of plasmalogen-selective phospholipase A2 [214] or by destabilization of the ADHAPS protein, thus limiting plasmalogen synthesis [215]. Alternatively, based on the neuroinflammation present in AD [216] and results obtained in an AD mouse model, a recent report claims that the inflammatory response downregulates *Gnpat* expression via NF- κ B signaling and c-Myc binding to the *Gnpat* promoter [126].

It is not clear whether the plasmalogen deficits represent a secondary phenomenon or play a causative role in the disease process and further aggravate the disease symptoms. In line with the proposed role of plasmalogens as radical scavengers, their depletion might amplify the oxidative stress burden in the AD brain. However, we may again emphasize the controversy around the role of plasmalogens in oxidative stress prevention and defense (see The controversial function of plasmalogens as antioxidants). Other authors hypothesize that the elevated activity of plasmalogen-selective phospholipase A2 stimulates the production of lysoplasmalogens, resulting in excessive vesicular fusion and eventually causing synaptic failure [104]. Furthermore, signaling defects resulting from plasmalogen reduction (like those discovered in the ERK and AKT pathways) may cause additional damage in AD pathology, as suggested based on experiments involving RNAi to downregulate *Gnpat* mRNA levels in the cortex of mice [126]. Intriguing, in this context, is the impaired phosphorylation of AKT and, therefore, loss of inhibitory phosphorylation of GSK 3 β observed in the PNS of plasmalogen-deficient mice [59]. GSK 3 β is one of the kinases implicated in tau phosphorylation [217]; and increased GSK 3 β activity upon plasmalogen deficiency may well contribute to

the characteristic hyperphosphorylation of tau protein in AD.

In addition, plasmalogens may have a direct effect on the production of A β peptides. A recent study showed that in purified membranes from neuronal cells, supplementation with plasmalogens stimulates the activity of α -secretase, which releases nontoxic fragments of the amyloid precursor protein (APP), and restricts the activity of γ -secretase, which—in concert with β -secretase—is responsible for the formation of the A β peptides that aggregate in amyloid plaques, a hallmark of AD brains [218]. Reduced levels of plasmalogens could therefore promote the formation of A β leading to the assembly of plaques in AD. However, our own results from the APP^{swe}-PS1^{dE9} double transgenic AD mouse model on a *Gnpat* KO background show no increase in amyloidogenic A β plaque or peptide levels and, thus, do not support the concept that ether lipid deficiency amplifies A β production or deposition (S. Forss-Petter & J. Bauer, unpublished observation). Furthermore, cleavage of APP has been suggested to take place in lipid rafts [219]. Therefore, the potential disturbance of these membrane domains caused by the lack of plasmalogens may also influence the generation of A β peptides.

Docosahexaenoic acid deficits may play a role in AD, as elaborated in recent reviews [106,123]; and given that ether lipid deficiency also causes a decline in brain DHA levels in mice [51,122], it is tempting to speculate that the decrease in ether lipids in AD contributes to the pathogenic processes in part also *via* DHA depletion. However, considering the relatively modest plasmalogen reduction in AD (as compared with complete ether lipid deficiency), the modulatory effect on DHA levels due to plasmalogen deficits may not be of major relevance for the pathogenesis in AD.

Altogether, it appears plausible that plasmalogens are a modulating factor in AD pathology and play an important role in disease progression. However, in our opinion, it is safe to say that plasmalogen deficiency is not the main cause of disease in AD. Consequently, supplementation of plasmalogens alone as a treatment strategy for AD, as proposed recently [220], may not be of major benefit for affected patients (see also Overcoming ether lipid deficiency in the nervous system).

In addition to plasmalogens, PAF might also be involved in the etiology of AD. It has been suggested previously that PAF is a mediator of A β -induced neurotoxicity. PAF receptor antagonists are beneficial for neuronal survival after A β treatment *in vitro* [221,222]. This is in line with more recent findings showing increased levels of specific PAF and lyso-PAF species, the latter possibly generated in attempts to get rid of

excessive PAF, in the brain of AD patients and transgenic mouse models of the disease [223]. The same study found PAF but not lyso-PAF to be upregulated by A β exposure in a human cell culture model of terminally differentiated neurons leading to increased neuronal death, which could be rescued by inhibition of PAF signaling. In addition, PAF induced the AD-characteristic hyperphosphorylation of tau, mediated by cyclin-dependent kinase 5 [223]. Exactly how the action of PAF fits into the sequence of pathologic events in AD still remains to be elucidated.

Neurodevelopmental disorders

Already in 1999, a case report first mentioned signs of autism, a neurodevelopmental disorder involving lack of social interaction, impaired verbal and nonverbal communication, and repetitive behavioral patterns, in a patient suffering from RCDP [224]. However, most likely due to the broad range of life-threatening symptoms particularly in severe RCDP cases, autistic features did not gain much attention as potential consequence of ether lipid deficiency until, recently, a whole exome sequencing study indicated a genetic link between *PEX7* and autism spectrum disorders [225]. The consortium behind that study identified a mutation affecting the WD-40 domain of *PEX7* in three affected children, but not in the nonaffected siblings, of consanguineous parents. This discovery supports the findings in an earlier paper, which proposed an association between single nucleotide polymorphisms in *PEX7* and autism [226]. Furthermore, retrospective screening of the clinical records of previously reported RCDP patients [227] revealed that two patients with less severe disease course had later been diagnosed with a neurodevelopmental condition (autism and attention deficit hyperactivity disorder) [225]. An additional observation linking ether lipid deficiency and neurodevelopmental disorders is the hyperactive behavior displayed by *Gnpat* KO mice in their home cage as well as in paradigms specifically testing for hyperactivity like, for example, the open field test (F. Dorninger, G. Zeitler, C. Pifl, S. Forss-Petter & J. Berger, unpublished observation). Moreover, the total levels of plasmalogens were found reduced by about 15–20% in the plasma of autistic patients in two independent studies [228,229] and, similarly, ethanolamine plasmalogens were decreased by about 15% in the brain of a rat model of autism [230], further corroborating the hypothesis of an association between ether lipid deficiency and autism. How ether lipids and autism spectrum disorders are connected mechanistically, is still unclear. However, in the future, raised awareness of the topic may increase the attention of clinicians for

potential autistic features in RCDP, particularly in patients affected by the milder forms.

Overcoming ether lipid deficiency in the nervous system

Treatment approaches targeting ether lipid deficiency, either in the inborn peroxisomal disorders or in one of the more common neurologic diseases, are limited. Currently, treatment of RCDP is mainly symptomatic and strategies to overcome the core of the problem, that is, ether lipid deficiency, are urgently needed. Based on the severe pathology in nervous tissue of patients with peroxisomal disorders (see Nervous system pathology in human ether lipid deficiency) and the involvement of reduced ether lipid levels in neurological disorders (see Potential involvement of ether lipids in more common neurologic diseases), the success of any treatment strategy is critically dependent on the ability to ameliorate ether lipid deficiency in the nervous system. This might be achieved either by restoring ether lipids themselves or by circumventing the consequences of their shortage.

For the first strategy, the replenishment of ether lipids, treatment with alkylglycerols is an obvious candidate, as these compounds are commonly believed to have the capability to restore the full spectrum of ether lipid species. Alkylglycerols already carry an ether bond at the *sn*-1 position and, therefore, the peroxisomal steps of ether lipid biosynthesis are not required. Their ability to restore plasmalogen levels is well documented in ether lipid-deficient cultured cells [122,231,232]. As a logical consequence, dietary supplementation of alkylglycerols, either in the form of chymyl alcohol (hexadecylglycerol; C16:0 chain at *sn*-1) or batyl alcohol (octadecylglycerol; C18:0 at *sn*-1), was proposed as potential treatment strategy for ether lipid deficiency already in the last century. In 2006, a registered trial was started in the Netherlands to evaluate the effects of batyl alcohol in humans (ISRCTN4 4820021), but the results have not yet been published. However, earlier studies in wild-type rats indicated that alkylglycerol replaces plasmalogens in various peripheral tissues but not in the brain [233,234]. This finding was later confirmed in a systematic preclinical trial in *Pex7* KO mice, which demonstrated that, upon oral batyl alcohol treatment, the levels of C18:0 plasmalogens are restored in a variety of organs, but not in the nervous system [60]. Other groups have tried oral administration of different alkylglycerol-based ether lipid precursors with DHA attached to the *sn*-2 position and lipoic acid at *sn*-3, in the hypomorphic *Pex7* mouse model but, again, incorporation into the

brain was modest; the ability of these substances to alleviate ether lipid deficiency in fully deficient KO animals has not been tested [235].

Hence, apparently, plasmalogens and their precursors either cannot efficiently cross the blood–brain barrier (at least not in the form, in which they are present in the blood) or brain tissue relies exclusively on its own ether lipid biosynthesis and does not incorporate exogenous ether lipids. However, the observation that upon intracerebral injections of radioactively labeled chymyl alcohol, radioactive plasmalogens appeared in the brain in a time-dependent manner [236], does not support the latter possibility.

In light of the findings suggesting that ether lipids do not overcome the blood–brain barrier, a recent report that treatment with plasmalogens isolated from scallops improves cognitive performance in a subgroup of mild AD patients is particularly puzzling [220]. Currently, it is unclear how orally administered plasmalogens would affect brain function. Therefore, until this discrepancy is resolved, claims of cognitive improvement owing to plasmalogen treatment should be considered with caution.

Besides the therapeutics aimed at restoring ether lipid levels, drugs targeting the downstream pathways affected by ether lipid deficiency may offer viable alternatives. A seminal study for such an approach recently applied inhibitors of GSK 3 β , mainly lithium chloride, to treat ether lipid-deficient mice [59]. By using this strategy, which may also be relevant for the CNS, the investigators were able to reverse the downstream effects of impaired AKT signaling (see The versatile functions of ether lipids in signaling) and ameliorate the dysmyelination of peripheral nerves in *Gnpat* KO mice. An obvious caveat of similar approaches is that they may not restore the whole range of functions covered by ether lipids including, for example, the shaping of membrane properties. Also, some concerns have been expressed about potential adverse effects of the long-term use of GSK 3 β inhibitors [237]. Nevertheless, as lithium chloride (and other specific GSK 3 β inhibitors) can cross the blood–brain barrier and is already in clinical use as a mood stabilizer, the results are exciting and might bring at least some relief for ether lipid-deficient patients. In particular, if therapeutic attempts to restore ether lipids in the CNS keep failing, inhibition of GSK 3 β may be the most promising pharmacological target at the moment.

Concluding remarks and perspectives

Ether lipids are a versatile group of lipids that fulfill a wide range of tasks and, thus, have the ability to shape

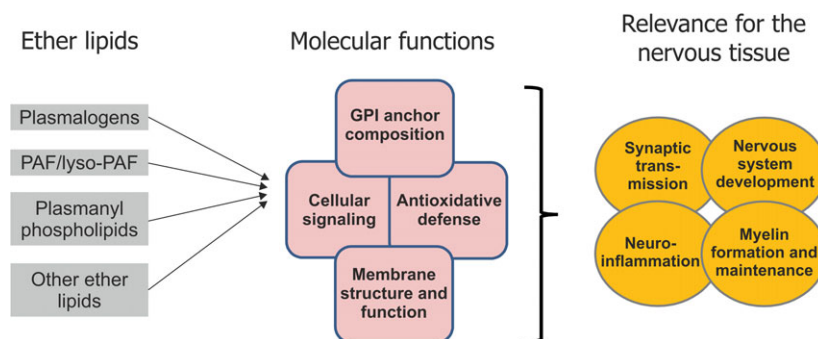


Fig. 3. The relevance of ether lipids for the nervous system. To the left, the main molecular functions proposed to involve ether lipids in the nervous system include: the fine-tuning of membrane properties and functions (see The importance of plasmalogens as membrane constituents), the mediation of signaling processes (see The versatile functions of ether lipids in signaling), the composition of GPI anchors (see The enigmatic role of ether lipid components in GPI-anchored proteins) and, presumably, the defense against oxidative damage (see The controversial function of plasmalogens as antioxidants). Thus, in a physiological context, ether lipids modulate crucial processes like nervous system development, myelin formation and maintenance, synaptic transmission, and neuroinflammation. As indicated by the overlap of circles or squares, these functions and activities are interdependent.

development and function of a complex tissue like the nervous system in a number of ways (Fig. 3). As we have also pointed out in the present review, the diversity of ether lipid functions is granted by the vast number of subspecies of these lipids. Although previous research has mainly focused on plasmalogens, which certainly are of major importance for the mammalian nervous system, recent studies have demonstrated that also a number of other ether lipid species like PAF or alkenyl- and alkyl-LPAs fulfill biologically relevant tasks in the nervous system. Accumulating research has also overturned the view that ether lipids are merely antioxidants and shape membrane properties but has uncovered exciting new functions like those in crucial signaling pathways, for example, the AKT, ERK, or PPAR γ pathways, or the assembly of GPI anchors.

A remarkable development is the increased number of papers reporting alterations, most often reductions, of ether lipid levels in different neurological disorders, particularly in AD. Considering these findings, it will be of major importance to determine the impact of such modest changes in the levels of ether lipids. Although current lipidome analyses, which have focused mainly on plasmalogens, point to a generalized reduction of ether lipids, some ether lipid subspecies might be affected more severely. On the other hand, a slight reduction in certain species may have more drastic consequences than that of others.

Concerning the proposed association between ether lipid deficiency and neurodevelopmental disorders, subtle impairments in ether lipid synthesis that do not manifest in the severe symptoms seen in RCDP, might still influence brain circuitry and function. Complete ether

lipid deficiency can cause neuronal migration defects or, at least in the mouse model, aberrant axonal branching at the neuromuscular junction. In the CNS, plasmalogen levels are relatively low at birth, but increase strongly during infancy. Thus, particularly during this early postnatal period of high demand for plasmalogen synthesis, a small reduction in these lipids might already increase the susceptibility for disturbances in postnatal fine-tuning and strengthening of neuronal interactions, thereby conferring a later risk for neurodevelopmental disorders. We expect that the progress in diagnostic techniques, like next-generation sequencing, will reveal new phenotypes evoked by moderate reductions in ether lipid levels, as this may enable the identification of patients with mutations affecting ether lipid biosynthesis that might be disregarded when using classical diagnostic strategies. Inevitably upcoming results of large-scale sequencing studies will show whether associations as described for *PEX7* polymorphisms and autism will indeed be substantiated. Also, further characterization and generation of hypomorphic and cell type-specific animal models of ether lipid deficiency should provide valuable clues.

Drugs to restore ether lipid function in the brain are of utmost importance in the treatment of peroxisomal disorders and the observation that ether lipid levels are also decreased in more common disease like AD creates an additional interest in such treatment strategies. Currently, therapeutic success is still limited, but increased knowledge on lipid transport across the blood–brain barrier and lipid homeostasis in the nervous system as well as the development of novel delivery systems will hopefully pave the way to overcome ether lipid deficiency in the nervous system.

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