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Glycerophospholipids: Roles in Cell Trafficking and Associated Inborn Errors

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ABSTRACT

Glycerophospholipids (GPLs) are the main lipid components of cellular membranes. They are implicated in membrane structure, vesicle trafficking, neurotransmission, and cell signalling. GPL molecules are amphiphilic, organized around the three carbons of glycerol. Positions *sn-1* and *sn-2* are each esterified to a fatty acid (FA). At position *sn-3*, a phosphate group is linked, which in turn can bind a polar head group, the most prevalent classes being phosphatidic acid (PA, phosphate alone as head group), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and cardiolipin (CL). Pathways of GPL biosynthesis span several cell compartments (endoplasmic reticulum (ER), Golgi mitochondria). Particularly important are mitochondria-associated membranes (MAMs), where the ER and mitochondrial outer membrane are in proximity. After synthesis, GPLs continuously undergo remodelling by FA hydrolysis and re-esterification. Esterification with different FAs alters membrane properties. Many steps in GPL synthesis and remodelling can be mediated by more than one enzyme, suggesting complexity that requires further exploration. The 38 known GPL-related inborn errors are clinically diverse. 23 (61%) have neurologic features, sometimes progressive and severe, particularly developmental delay/encephalopathy in 16 (42%) and spastic paraplegia in 12 (32%). Photoreceptor/neuroretinal disease occurs in 14 (37%). Three present skeletal dysplasias (8%). Most GPL inborn errors have been diagnosed by broad molecular testing. Lipidomics holds promise for diagnostic testing and for the discovery of functionally relevant metabolite profiles for monitoring natural history and treatment response.

1 | Introduction

Glycerophospholipids (GPLs) are the major lipid constituents of membranes, accounting for 50–60 mol% of membrane lipid content [1, 2]. They are responsible for the bilayer structure of membranes, and they influence membrane stability, fluidity, and permeability [1, 3], signal transduction, and vesicular trafficking. GPL composition differs among cell types, among the membranes of a single cell, and between the two leaflets of lipid bilayers. Because of the importance of GPLs to normal

Abbreviations: AA, arachidonic acid; CL, cardiolipin; DAG, diacylglycerol; DHA, docosahexaenoic acid; ER, endoplasmic reticulum; FA, fatty acid; GPL, glycerophospholipid; HSP, hereditary spastic paraplegia; IEM, inborn error of metabolism; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; MAM, mitochondria-associated membranes; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PItds, phosphoinositides; PLA_1, PLA_2, PLB, PLC and PLD, phospholipases A_1, A_2, B, C and D; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid.

membrane structure and function, it is predicted that inborn errors of GPL metabolism may affect membrane functions like myelination, trafficking, and signalling.

This article reviews GPL biochemistry, then describes GPL-related inborn errors. It emphasizes practical links to clinical or biochemical diagnosis and treatment, and to the normal functions of GPLs, including cell trafficking.

2 | Glycerophospholipid (GPL) Structure and Distribution

GPLs are the most abundant membrane lipids. For example, in human red blood cell membranes, the four major classes of GPLs comprise over 43% of lipid mass (phosphatidylcholine (PC, 17%), phosphatidylethanolamine (PE, 18%), phosphatidylserine (PS, 7%), phosphatidylinositol (PI, 1%)), followed by cholesterol (23%), sphingomyelin (18%), and other lipids [4]. PC generally accounts for 45–55 mol% of total cellular GPLs, PE 17%–25%, and PS ~5%. Total PI plus its phosphoinositide derivatives (PItds) vary from 2% to 20% in different systems [5–7].

Both the head group and the FA tails of membrane GPLs contribute to membrane properties.

2.1 | Head Group Structure

A diagram of the GPL structure is shown in Figure 1A. The simplest GPL head group is found on phosphatidic acid (PA) which has only phosphate as the head group, with a single negative charge. In most GPLs, the phosphate binds a small organic molecule: choline, ethanolamine, serine, and inositol, forming PC, PE, PS, and PI, respectively (Figure 1B). The molecular shape of GPLs depends on the size of the head group versus that of the acyl chains. For example, when the cross-sectional areas of the head group and the acyl chains are similar, the GPL is cylindrical, as in phosphatidylcholine (PC). If the area of the head group is smaller than that of the tail, this produces conical molecules such as PA, PE, and cardiolipin (CL, Figure S1) [8]. GPLs with head groups larger than their hydrophobic tails have an inverted conical shape. This is the case for lyso-GPLs, PI and PItds [9, 10]. The molecular shape of GPL can be altered by their head group cleavage by phospholipases C or D (Figure 1A), producing DAG or PA, respectively.

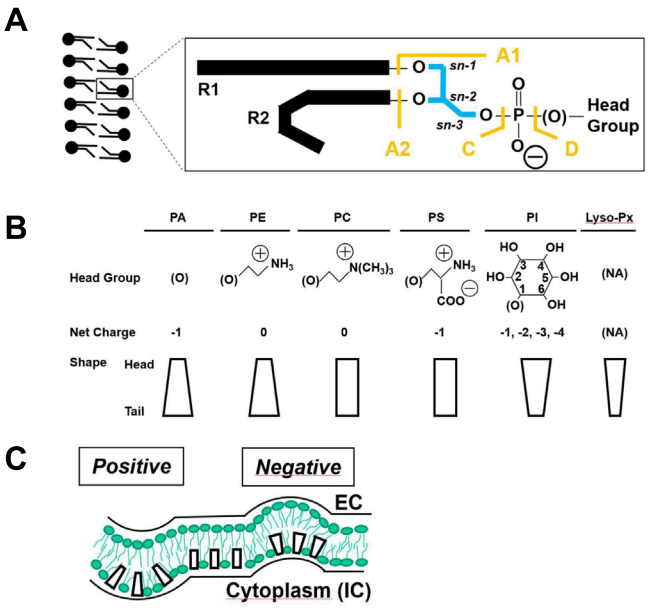

FIGURE 1 | Glycerophospholipid (GPL) structure and nomenclature. (A) GPL structure. A GPL bilayer is shown on the left and a single GPL molecule is detailed on the right. GPLs are structured around a glycerol backbone (blue). Two fatty acids (FA), R_1 and R_2 (thick black lines) are esterified, at the *sn*-1 and *sn*-2 positions. At the *sn-3* position a phosphate is linked, usually with an additional head group. Often, the R_1 FA is saturated and the R_2 FA is unsaturated. The FAs are hydrophobic and placed within the membrane. The phosphate and head groups are hydrophilic and align at the aqueous interface of the membrane, with the hydrophilic ends of neighboring GPLs. (B) Phospholipases are classified by their preferred site of cleavage, shown in *orange*. "Phospholipases A_1" (PLA_1) refers to enzymes that preferentially cleave the *sn*-1 ester bond; PLA_2, the *sn*-2 bond. Phospholipases B (PLB, not shown) cleave FAs from lyso-GPLs which have a single bound FA. Phospholipases C (PLC) cleave between glycerol and the phosphate, releasing a diacylglycerol (DAG). Phospholipases D (PLD), cleave between the phosphate and the head group, producing phosphatidic acid (PA). (B) Head groups, charges and shapes of GPLs. The main GPL head groups are shown: PA, phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI). PI can be phosphorylated on carbons 3, 4 and/or 5, in any combination, forming phosphoinositides (PItds), conferring different net charges as indicated. (C) Membrane curvature. The figure shows a plasma membrane. GPLs with conical shapes are mainly located on the inner leaflet. Their effect on curvature is shown. Curvature is defined with respect to the cytoplasm. A bulge in any membrane towards the cytoplasm is a positive curvature; a bulge away is negative. EC, extracellular; IC, intracellular (cytoplasmic).

2.2 | Fatty Acid Composition and Glycerophospholipids Structure

The FA composition of GPLs influences molecular shape, membrane thickness, stiffness, fluidity, packing, and curvature [1, 3].

The presence of double bond(s) in the *cis* conformation produces kink(s) in the FA chain, increasing molecular volume of the hydrophobic tail compared to that of a linear, saturated FA of the same length [9, 11, 12]. Membranes rich in saturated lipids are tightly packed, rigid, and ordered, whereas high contents of polyunsaturated fatty acids (PUFAs) yield more fluid, loosely packed membranes. Molecules in which glyceryl-phosphate head groups are esterified to only one FA are designated *lyso*-glycerophospholipids (e.g., *lyso*-phosphatidylcholine, LPC) and have an inverted cone shape [13].

A common lipidomics shorthand for GPL molecules indicates the head group, the total number of FA carbons, and the total number of unsaturated bonds in the FAs. For example, the most prevalent PI molecule has a combination of 18:0 (stearic acid, 18 carbons and no double bond) at the *sn*-1 position and 20:4 (arachidonic acid) at the *sn*-2 position, and is designated PI38:4 [11, 14].

3 | Glycerophospholipid Distribution and Membrane Properties

3.1 | Distribution of the Classes of GPLs

This article cannot review the great diversity seen in GPL contents in relationship to physiology, development, tissue [15] and intracellular compartment [7, 16–19], but some references and examples are provided. The mitochondrial inner membrane is a striking example, containing CL (~18% of total GPLs), which is absent elsewhere in cells, and a higher level of PE than other membranes (~34%).

An important source of membrane asymmetry is heterogeneity between the two membrane leaflets. In the cell membrane, PE, PS, and PI are nearly exclusively on the inner leaflet. PC is found on both leaflets but mostly in the outer leaflet [18] (Table S1).

Lipid rafts, although still somewhat controversial [15], illustrate lateral heterogeneity in the plane of the membrane. Diffusion within the plane of a leaflet is rapid, orders of magnitude faster than translocation between membrane leaflets [15]. In lipid rafts, sphingomyelin, cholesterol, and some GPLs concentrate into a small region, serving as platforms for protein localization, cell signalling, and vesicle formation [15, 20].

3.2 | Distribution and Diversity of the FAs of Glycerophospholipids

FA content of GPLs also differs by subcellular location and by organ. For example, the plasma membrane and *trans*-Golgi are enriched in GPLs with saturated FAs, allowing for tight lipid packing. In contrast, the nuclear, ER, and *cis*-Golgi membranes show higher fractions of unsaturated FAs [21]. In these membranes, a high unsaturated FA content of GPLs and a low level of cholesterol render the bilayer more flexible [22].

In rat, fatty acids at position *sn-2* vary according to the tissue. Brain PC is enriched in 18:0 and 18:1 FA, PE is enriched in 20:4 and 20:6, and PS contains mainly 20:6 FA. In heart, the predominant FA at the *sn-2* position is 20:4 for PC, and 20:4 and 22:6 for PE and PS. In the liver, 20:4 is the predominant FA esterified at the *sn*-2 position in PC, PE, and PS [23]. In mouse heart and skeletal muscle, CL is enriched in linoleic acid (C18:2), whereas in brain, oleic acid (C18:1) predominates. It has been shown that the presence of 18:1 in CL correlates with the esterification of a broad set of other FAs, leading to a more diverse molecular population of CL species in brain than in heart or skeletal muscle [8, 24–26].

In most tissues, FAs forming GPLs usually contain no or one PUFA. However, in the brain, most GPLs contain one of two PUFAs, docosahexaenoic acid (DHA) or arachidonic acid (AA) [21, 27, 28]. DHA is mostly found in PS and PE. Although most brain PC contains saturated FAs (see above), brain AA is mainly found in PC [29, 30]. The high PUFA levels in the brain reflect the enrichment of synaptic vesicle membranes in PUFA-containing GPLs. This enrichment facilitates membrane fusion, allows for the high degree of curvature of synaptic vesicles, and can serve as a source of signalling lipids [21, 31].

The fatty acids of GPLs are distributed asymmetrically between membrane leaflets. In the plasma membrane, the outer leaflet is enriched in saturated FAs [16], whereas the cytoplasmic leaflet is enriched in PUFAs. In human red blood cell membranes, external leaflet lipids on average contain 1.6 desaturations compared to 3.4 unsaturated bonds in inner leaflet lipids [18]. Surprisingly, an opposite trend is observed for PC acylation: PC in the external leaflet is more enriched in unsaturated FAs than is PC in the cytoplasmic leaflet [18].

The pathophysiological mechanisms of inborn errors of GPL metabolism are largely undefined, but their consequences for the structures of myelin and cellular membranes, which affect organelle fusion and fission, vesicular trafficking, and neurotransmission are prime candidates.

Lipidomics studies for comprehensive identification and quantification of GPLs in relevant cells and tissues have made rapid advances, providing insights into normal physiology and the underlying mechanisms of related inborn errors.

3.3 | Glycerophospholipids and Membrane Curvature

Membrane curvature is the result of complex interactions among structural lipids, membrane proteins, and forces acting on the membrane surfaces. By convention, membrane curvature is defined with respect to the cytoplasm. A bulge in any membrane towards the cytoplasm is a positive curvature; a bulge away from the cytoplasm is negative (Figure 1C) [32, 33].

In GPLs, the head group and the esterified FAs both contribute to molecular shape (Figure 1B). For example, in the cytoplasmic leaflet of the plasma membrane, high levels of PA and PE, which have conical shapes expanding from their head groups, would favor a negative curvature. Conversely, high inner leaflet concentrations of PI or lyso-GPLs, which have inverted conical shapes, could produce a positive curvature [32, 34, 35].

Asymmetry of the plasma membrane leaflets, with a concentration of anionic GPLs in the cytosolic leaflet and mostly neutral lipids in the extracellular leaflet, creates an electrostatic potential. The anionic head groups of cytoplasmic GPLs can attract basic amino acid residues on proteins. One example is the interaction of basic amino acid residues of the actin regulatory protein with $PI(4,5)P_2$ [36], described in detail elsewhere [2, 37].

GPLs can move between the leaflets of the plasma membrane. Specific proteins, flippases, floppases, and scramblases, mediate this [7, 26, 38]. Flippases are type P4-ATPases that catalyze the inward movement of PS and PE from the outer to the cytosolic leaflet. Floppases are ATP-binding cassette (ABC) transporters that move PC from the cytosolic to the outer leaflet [7, 26, 38]. Thus, both flippases and floppases maintain leaflet asymmetry in a concerted, energy-dependent fashion [21, 39, 40].

In contrast, scramblases are ATP-independent. They translocate phospholipids non-specifically and bidirectionally between membrane leaflets, collapsing membrane asymmetry [40, 41]. Loss of membrane asymmetry can trigger changes in curvature,

cell fusion and fission, apoptosis, membrane repair, and blood coagulation [41]. For instance, on red blood cells and platelets, exposure of PS on the surface substantially accelerates clotting and thrombosis [39].

GPLs can also move between membranes. Important examples of this occur at regions of proximity between the ER and other organelles. For instance, mitochondrial-associated membranes (MAMs) are hotspots for GPL exchange between the ER and mitochondria. At MAMs, the ER membrane and the mitochondrial outer membrane are positioned only 10 to 20 nm apart [42, 43]. Lipid trafficking at MAMs involves non-vesicular transport by lipid transfer proteins, which sequester lipids in hydrophobic pockets and rapidly move between membranes [32, 43].

3.4 | Glycerophospholipids and Vesicular Trafficking

Much of intracellular transport occurs by vesicular trafficking. GPL subclasses, having different geometries and electrostatic charges, can be concentrated in specific microdomains, affecting membrane curvature and charge. The interaction of charged GPLs with proteins that have membrane-deforming activity is an essential step in the development of membrane curvature, which is an essential early step in vesicle formation, stabilization, and trafficking. Although detailed proof of defective trafficking is often lacking for inborn errors of GPL metabolism, GPLs are clearly necessary for normal trafficking.

The nervous system is predicted to be particularly vulnerable if lipid trafficking is impaired. Two examples of this are neurotransmission and axonal transport in motoneurons. Neurotransmission is a special case of trafficking, with vesicle formation, movement, and membrane fusion leading to neurotransmitter release. The roles of $PI(4,5)P_2$, $PI(3,4,5)P_3$, DAG, and PA in neurotransmission are reviewed elsewhere [44, 45]. PItds mediate the recruitment of clathrin coats, an essential step of vesicle trafficking. At the presynaptic terminal, $PI(4,5)P_2$ is required for membrane budding, fusion, and neurotransmission. PItds thus control the rate of calcium-stimulated exocytosis of neurotransmitters [9, 46, 47]. The conversion of DAG to PA by diacylglycerol kinases (DGKs) alters membrane architecture and may help to lower the energy needed for fusion [48–50].

Neurons rely heavily on vesicular trafficking for transport over the long distances between the cell body and synapses. In Box 1 we discuss the frequent occurrence of hereditary spastic paraparesis (HSP) in inborn errors of GPL metabolism, and the high performance required for vesicular trafficking along axons in motoneurons, the cells responsible for HSPs.

3.5 | Glycerophospholipids and Cell Signalling

Lipid signals such as PUFAs can be produced by PLA2-mediated action on membrane GPLs [51]. Such GPL-dependent signalling can be activated by many stimuli, including neurotransmitters, neurotrophic factors, cytokines, membrane depolarization, and altered activity of ion channels [52] and regulates processes including inflammation, autophagy, and cell death. Examples include arachidonate and its hydroxylated derivatives 19- and 20-hydroxyeicosatetraenoic acids (19-HETE, 20-HETE), endocannabinoids, lysophospholipids, PItds, and diacylglycerol (DAG). DAG is the physiological activator of protein kinase C (PKC) [53] as discussed in the companion article about PI-related inborn errors [46].

BOX 1 | Hereditary spastic paraplegias and inborn errors of GPL metabolism.

Hereditary spastic paraplegias (HSPs) are genetically and clinically heterogeneous. They are due to axonal degeneration of upper motoneurons and are designated SPG 1 to 83 [169]. Some HSPs present isolated lower limb spasticity. In these patients, detectable involvement is limited to upper motoneurons ("pure" HSP). Other patients designated "complex" HSP have additional neurological signs, such as cognitive impairment, cerebellar ataxia, and peripheral neuropathy.

Why is HSP a recurrent finding in inborn errors of GPL metabolism and particularly in those of FA remodeling? Although direct mechanistic proof is lacking, the nervous system is predicted to be particularly vulnerable to diseases of cell trafficking, given the central roles of lipids in synaptic neurotransmission and vesicle formation and transport.

Vesicular transport in motoneuron axons, which measure up to 1 m in length in humans, requires highly efficient GPL metabolism in addition to motor and cytoskeleton proteins and adequate energy input. Essential macromolecules from the cell body must traffic along the axon to the synapse. Conversely, synaptic signalling complexes, other macromolecules, and organelles must migrate from the presynaptic terminal to the cell body for degradation or recycling [170]. By extension, motoneuron dysfunction may be a sensitive indicator of problems with vesicular trafficking.

Motoneurons are also sensitive to other lipid-related processes. Several causal genes of HSPs are related to GPL, sphingolipid, cholesterol and bile acid metabolism [170, 171].

4 | Glycerophospholipid Biosynthesis

GPL biosynthetic pathways often span different organelles, particularly the ER, Golgi, and mitochondria, as well as the cytoplasm.

4.1 | Phosphatidic Acid (PA) and Diacylglycerol (DAG)

PA is the precursor of GPL biosynthesis (Figure 2). *De novo* synthesis of PA starts from glycerol-3-phosphate (G-3-P) and a fatty acyl-CoA, with acylation at the *sn*-1 position, forming *sn*-1 acyl-lysophosphatidic acid (LPA), catalysed by glycerophosphate acyltransferase (GPAT, gene *GPAT3*). Next, using a second acyl-CoA, lysophosphatidic acid acyltransferase (LPAAT, gene *AGPAT3*) converts LPA to PA. PA serves directly in the synthesis

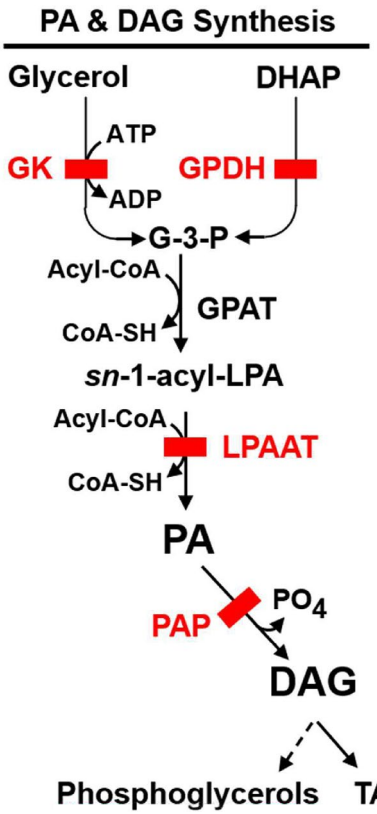

FIGURE 2 | Synthesis of phosphatidic acid (PA) and diacylglycerols (DAG). See text for details. Red bars indicate steps with known inborn errors. DHAP, dihydroxyacetone phosphate; GK, glycerol kinase; GPDH, glycerol-3-phosphate dehydrogenase; LPA, lysophosphatidic acid; LPAAT, lysophosphatidic acid acyltransferase; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; TAG, triacylglycerol.

of PI and CL. For other syntheses, PA is hydrolysed by a phosphatidic acid phosphatase (PAP, genes *LPIN1* or *LPIN2*) to form a 1,2-diacyl-*sn*-glycerol (DAG). The reverse reaction is catalysed by a diacylglycerol kinase (DAGK). Ten different DAGKs are encoded by separate genes [45].

DAG can also be derived from phosphatidic acid (PA) by the action of phosphatidic acid phosphatase (PAP) (Figure 2), or by cleavage of triacylglycerol (TAG) in lipid droplets by TAG lipases, or by cleavage of GPLs, particularly PI and PItds, by phospholipase C (PLC). As mentioned above, DAG is a potent lipid second messenger [46, 53].

4.2 | Phosphatidylcholine (PC)

Choline and ethanolamine are prerequisites for the Kennedy pathway of PC and PE synthesis of PC and PE. FLVCR1 and FLVCR2, encoded by the paralogous genes *FLVCR1* and *FLVCR2*, can transport choline and ethanolamine down concentration gradients into cells [52, 54] by a mechanism that requires the presence of a membrane potential. Plasma levels of ~10 μmol/L (choline) and ~2 μmol/L (ethanolamine) exceed intracytoplasmic levels because choline and ethanolamine are rapidly phosphorylated after cell entry [55]. Choline and ethanolamine [56] can also enter cells via the high-affinity choline transporters encoded by *SLC44A1* or *SLC44A2*, both of which are localized to the plasma membrane and to mitochondria [56]. Choline produced by PC degradation can be reused for GPL synthesis [57].

The Kennedy pathway of PC synthesis from choline (Figure 3) begins with phosphorylation of choline to phosphocholine (P-Choline), catalyzed by one of two choline kinases, CHKα (gene *CHKA*) or CHKβ (gene *CHKB*). Using cytidine triphosphate (CTP) and P-Choline as substrates, phosphocholine cytidylyltransferase (CCT) catalyzes the synthesis of CDP-phosphocholine (CDP-P-Choline). CCT is rate limiting for PC synthesis. CCT has two subunits, CCTα (gene *PCYT1A*) and CCTβ (gene *PCYT1B*). Finally, choline/ethanolamine phosphotransferase (CEPT1, gene *CEPT1*) transfers P-choline from CDP-choline to DAG, forming PC.

In hepatocytes, PC can also be synthesized by methylation of PE by phosphatidylethanolamine N-methyltransferase (PEMT, gene *PEMT*), a liver-specific enzyme that uses one S-adenosylmethionine molecule for each of the three methylation steps.

PC is the most abundant GPL, usually accounting for about 50% of all membrane GPLs [9]. In cell membranes, PC localizes preferentially to the outer plasma membrane leaflet, where 80% to 90% of GPLs consist of PCs [10].

The influence of hepatic PC synthesis on essential FA content of the brain is an interesting example of the interplay between head group and FA chemistry, well summarized by Harayama [58]. Briefly, the synthesis of PC from PE exhibits metabolic bias: the preferred substrate of PEMT is PE esterified to the essential polyunsaturated FA, DHA. The resulting PC is therefore enriched in DHA. The brain selectively imports DHA and other long-chain FAs as lyso-DHA-PCs; unbound DHA is not recognized. The transporter is major facilitator superfamily D2A (gene, *MFSD2A*) [59]. This pathway of DHA entry to brain uptake is likely physiologically significant because pathogenic variants in *MFSD2A* cause an encephalopathy in mice [59] and humans [60, 61].

PC plays important roles outside of cell membranes. It is the major lipid of pulmonary surfactant, reducing the surface tension in the lung and reducing the work of ventilation. Bile and high-density lipoproteins contain PC, which helps to maintain cholesterol in a soluble form.

In mammalian cells, PC is mainly esterified to saturated or monounsaturated C16 and C18 acyl chains (Table S2) but this varies in a tissue- and leaflet-specific fashion (Section 4.2).

4.3 | Phosphatidylethanolamine (PE)

PE is the second most abundant GPL in most mammalian tissues, after PC. Ethanolamine is an essential nutrient, found in the diet mainly as PE [62, 63]. It is transported into cells (see previous section). PE biosynthesis occurs in three enzymatic steps, analogous to those of PC synthesis (Figure 3). First, ethanolamine is phosphorylated to phosphoethanolamine (P-Eth), either by CHK (CHKα or CHKβ) or by one of the ethanolamine kinases EKI1

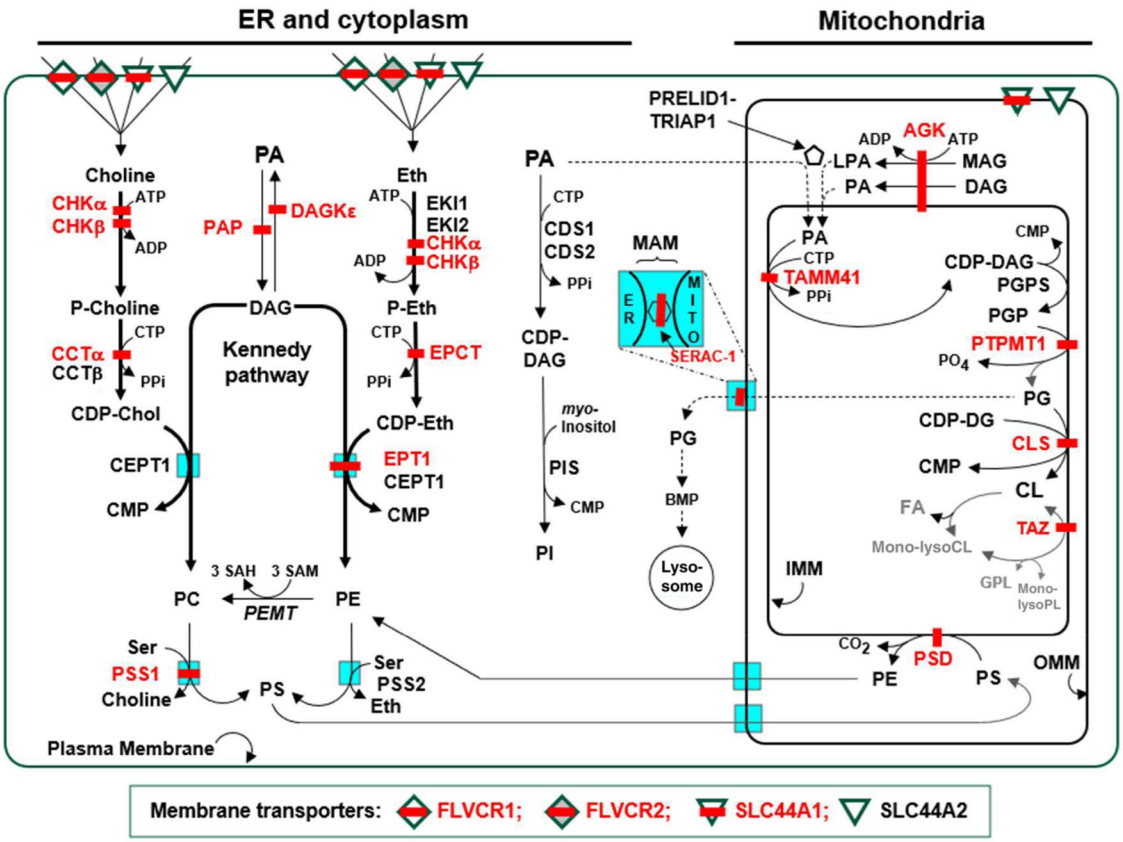

FIGURE 3 | Glycerophospholipid (GPL) synthesis pathways span the cytoplasm, ER and mitochondria. Sites of known inborn errors are shown by red bars and proteins with associated inborn errors are in red bars. Cytidine diphosphate (CDP) is the key activating molecule of GPL synthesis. In the Kennedy pathway of PC and PE synthesis (shown in bold lines), choline and ethanolamine are imported to the cytoplasm or recuperated from GPL degradation (not shown). They are phosphorylated, then activated as CDP derivatives, followed by DAG addition. PS synthesis and degradation occur at mitochondria-associated membranes (MAMs, indicated as blue boxes), where lipid exchanges between the ER and mitochondria are facilitated. PS synthesis proceeds by head group replacement of either PC or PE. PS conversion to PE occurs at the inner mitochondrial membrane via PS decarboxylase (PSD, gene *PISD*). PC synthesis from PE occurs via three liver-specific methylation reactions catalysed by the liver-specific PE methyltransferase (gene, *PEMT*). In contrast to the Kennedy pathway, other PGL syntheses involve DAG activation by CDP: For PI this occurs in cytoplasm and for cardiolipin (CL), in the mitochondrial matrix. Cardiolipin (CL) synthesis starts from PA, which is produced in the intermembranous space by acylglycerol kinase (AGK) or imported from the cytoplasm. The PRELID1-TRIAP1 heterodimer mediates PA transfer to the inner membrane, where it is activated to CDP-DAG by TAMM41. Phosphatidylglycerol phosphate (PGP) synthesis is catalysed by PGP synthase (gene *PGS1*) then dephosphorylation by protein tyrosine phosphatase PTPMT1 (gene *PTPMT1*) to form phosphatidylglycerol (PG). PG is a precursor of bis(monoacylglycero) phosphate (BMP) in lysosomes. SERAC1, a MAM protein, facilitates FA remodelling. In mitochondria, PG is a substrate of cardiolipin synthase (CLS). After synthesis, PGLs undergo continuous FA remodelling (see Figure 4A) For CL, see Figure S1.

and EKI2 (genes *ETNK1* and *ETNK2*). The next step is catalyzed by ethanolamine-phosphate cytidylyltransferase (EPCT, gene *PCYT2*). Finally, PE synthesis from CDP-ethanolamine plus DAG is catalyzed either by choline/ethanolamine phosphotransferase 1 (CEPT1) or by ethanolamine phosphotransferase 1 (EPT1, also called selenoprotein I, gene *SELENOI*). EPT1 is located in the Golgi apparatus and can produce long-chain PE and plasmanyl-PE from DAG and 1-alkyl-2-acylglycerol, respectively [64]. PE can also be synthesized from PS by the mitochondrial enzyme phosphatidylserine decarboxylase (PSD, gene *PISD*, Figure 3).

PE constitutes about 15%–20% of total GPLs in most mammalian cells. In neural cells, it can account for 45% [62, 65]. PE is particularly enriched in the inner leaflet of the mitochondrial membrane [10, 66, 67]. In mammalian cells, PE is more enriched than PC in unsaturated FA (Table S2).

The CDP-ethanolamine pathway is also important for the synthesis of PE etherphospholipids (plasmalogens), both PE-plasmanyl and PE-plasmenyl [68].

4.4 | Phosphatidylserine (PS)

As shown in Figure 3, much of the synthesis of PS, an anionic GPL, is mitochondrial. The pathway does not involve CTP intermediates. Instead, PS synthesis uses intracellular pools of PC and PE, substituting their head groups with serine. For PC, this is catalysed by phosphatidylserine synthase 1 (PSS1, gene *PTDSS1*). For PE, it is catalysed by PSS2 (gene *PTDSS2*).

PS typically comprises about 5% of total cellular GPLs in mammalian cells but reaches higher levels in the brain cortex [69].

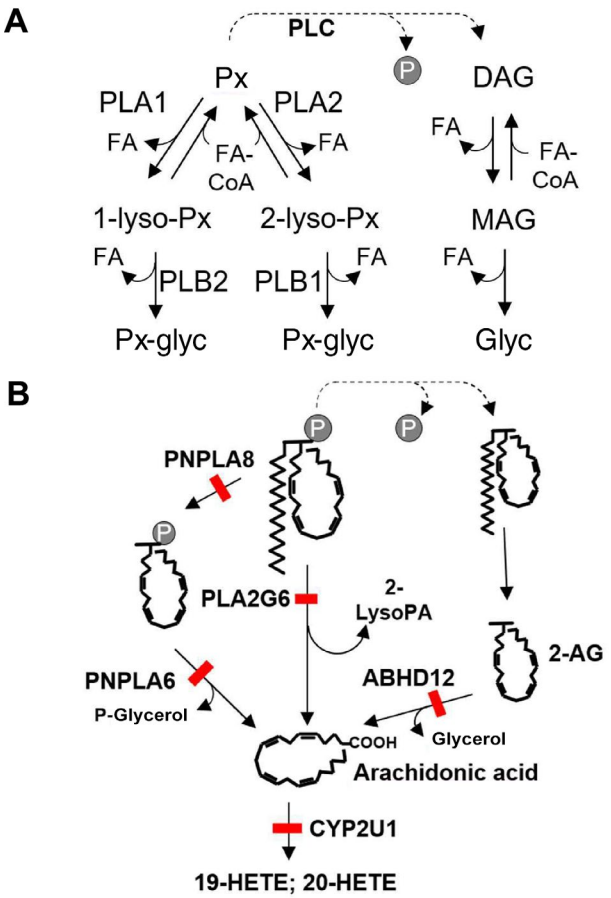

FIGURE 4 | The Lands cycle, FA remodelling of GPLs and some related inborn errors. (A) Schematic view of GPL remodelling. The *sn*-1 and *sn*-2 positions are considered individually, with PLB and PLC cleavages shown. Nonstandard abbreviation: Px, a generic GPL with headgroup not specified. (B) IEMs relating to arachidonate cleavage and liberation. Different pathways can lead to the liberation of the same molecules. For details, see the text. Nonstandard abbreviations: 2-AG, 2-arachidonoylglycerol; P-glyc, phosphatidylglycerol;19-HETE, 20-HETE, 19- and 20-hydroxyeicosatetraenoic acids.

PS has a higher fraction of polyunsaturated acyl chains than PC (Table S2).

PS has unique biological roles [70]. In the plasma membrane, PS is maintained nearly exclusively in the cytoplasmic leaflet. The presence of PS on the external leaflet of the plasma membrane strongly promotes key biological processes, including interaction with fusogenic proteins during neurotransmitter release [3], efferocytosis [71], apoptosis [72], coagulation [73] and activation of protein kinase C family members [74]. PS plays roles in bone development and in vision, which are discussed later with PS-related inborn errors.

4.5 | Pathways Involving CDP-DAG: Cytoplasmic Phosphatidylinositol (PI) Synthesis and Mitochondrial GPL Synthesis

The synthesis of GPLs other than PC, PE, and PS proceeds by the formation of CDP-DAG (Figure 3). This activates DAG for transfer to other molecules. CDP-DAG synthesis from cytidine triphosphate (CTP) and phosphatidic acid (PA) is catalysed by CDP-DAG synthases CDS1 and CDS2 (genes *CDS1* and *CDS2*), which are integral ER membrane proteins.

In the ER, CDP-DAG is an essential intermediate in the synthesis of PI from PA via PI synthase (PIS, gene *CDIPT*) and thence of phosphoinositides (PItds) [46]. PI is ubiquitously present in the cytosolic leaflet of all cellular membranes and many organelles [11, 14, 46, 75].

In mitochondria, PA can come from the cytoplasm or be synthesized from PA generated by phosphorylation of DAG by acylglycerol kinase, AGK (gene *AGK*, Figure 3) [76]. AGK is an inner mitochondrial membrane-bound protein with a catalytic domain projecting into the intermembrane space. AGK also moonlights functionally as an assembly factor for TIM22 translocase [77].

PRELID1 and TRIAP1 form a heterodimer in the mitochondrial intermembrane space. Direct in vitro assays showed that, in the presence of PRELID1-TRIAP1, PA but not other GPLs was selectively transferred from donor to recipient liposomes [78].

CDP-DAG synthesis in mitochondria is catalysed by the inner membrane-bound protein TAMM41 (gene *TAMM41*, Figure 3) [79]. CDP-DAG is converted in turn to phosphatidylglycerophosphate (PGP, Figure S1B) by PGP-synthase (gene *PGS1*) and then to phosphatidylglycerol (PG) by protein tyrosine phosphatase mitochondrial 1 (PTPMT1, gene *PTPMT1*). CDP-DAG provides substrate for cardiolipin synthesis (Figure S1B).

Serine active site-containing protein 1 (SERAC1, gene *SERAC1*), a MAM protein, aids FA remodeling of PG to form phosphatidylglycerol 36:1 (PG36:1). PG36:1 is a precursor of bis(monoacylglycerol) phosphate (BMP), an important GPL of late endosomes and lysosomes [80]. Inside mitochondria, PG is a substrate for cardiolipin synthase (CLS, gene *CRLS1*).

Interestingly, mice deficient in Lgpat1, which encodes an ER acyltransferase that catalyzes PG remodeling and that facilitates the transport of PG from ER to mitochondria, develop a MEGDHEL-like syndrome [81]. Also, *Serac1* knockout mice are deficient in mitochondrial serine transport and single carbon metabolism [82]. Taken together, these data suggest that SERAC1, perhaps in concert with other proteins like LGPAT1, may have a broad role in lipid and amino acid transport at MAMs.

5 | Glycerophospholipid Remodelling

Membrane GPLs are continuously remodeled by FA cleavage and re-esterification. This pathway, called the Lands cycle, includes the excision of a FA by a PLA_1 or PLA_2 and replacement by another FA in a reaction catalyzed by lysophospholipid acyltransferases (LPLATs, Figure 4) [83, 84]. LPLATs belong to the membrane-bound O-acyltransferase (MBOAT) or acylglycerol-3-phosphate *O*-acyltransferase (AGPAT) families [83, 85].

The Lands cycle serves several purposes. One is the replacement of oxidized FAs, permitting the salvage of the undamaged

remainder of the molecule. Another is the release of bioactive lipids for signaling. Remodeling also changes the FA content of GPLs and hence membrane properties such as fluidity and curvature [12, 29, 44, 86].

Diagrams of the Lands cycle are deceptively simple. They hide underlying complexities at several levels. Single enzymes participating in the Lands cycle can often act upon different substrates. For example, enzymes with PLA activity act preferentially at either the *sn*-1 or the *sn*-2 position, but some have activity at the other position. Some enzymes may act optimally upon certain FAs, such as arachidonate at the *sn-2* position (Figure 4) but also act upon other FAs at that position. Some phospholipases can cleave FA from different GPL classes or even non-GPL molecules with similar esterified FAs. Importantly, as well, identical reactions can sometimes be performed by more than one enzyme.

Transesterification is a different process but has a similar effect. In transesterification, a single enzyme removes an esterified FA from a GPL, then re-esterifies the FA to a lyso-GPL. Taffazin is a transesterase that is discussed elsewhere in this issue [8]. Lipidomics studies under normal physiological conditions have elegantly shown that the FA content of GPLs can be influenced primarily by the local availability of lipid substrates rather than by enzyme specificity [24]. Once synthesized, CL continually undergoes transesterification by Tafazzin (gene *TAFAZZIN*) [25, 26].

Together, enzyme multiplicity, substrate promiscuity, and substrate availability contribute to the complexity of lipid content observed in biological membranes. Because of these considerations, in Lands cycle-related inborn errors, caution is necessary before attributing a clinical finding to a particular substrate or reaction.

6 | Inborn Errors of GPL Metabolism

6.1 | Disorders of Choline and Ethanolamine Transport

Both FLVCR1 and FLVCR2 can transport choline and ethanolamine into cells [55]. Both are expressed in all tissues tested [54]. It is unknown how different syndromes arise from variants in *FLVCR1* and *FLVCR2*. It is tempting to attribute the mechanism to choline and/or ethanolamine availability, but the transporters may have other substrates as well that could influence clinical features. Supplementation with choline and ethanolamine has been suggested as a potential treatment [55] although no results of such treatment are available to our knowledge.

6.1.1 | FLVCR1 Deficiency (Gene *FLVCR1*)

In patients with biallelic variants, progressive gait difficulties due to lack of proprioception and night blindness due to retinitis pigmentosa can usually be detected in the first decade. This combination of clinical signs has been named PCARP (posterior column ataxia and retinitis pigmentosa). Homozygous null variants of *Flvcr1* in mice are prenatally lethal [54]. Perhaps the variants of human patients have some activity, allowing survival.

6.1.2 | FLVCR2 Deficiency (Gene *FLVCR2*)

Biallelic variants in *FLVCR2* cause Fowler syndrome, an autosomal recessive condition with hydrancephaly, hypokinesia, and arthrogryposis, and a characteristic glomeruloid proliferative vasculopathy in the brain and spinal cord, often with brain calcifications. The vascular lesions can occur in normal-appearing tissue and have been hypothesized to be primary and to lead to neurodegeneration. Most cases can be detected by prenatal ultrasound of the brain. Most patients have had severe neurological impairment with death in infancy [87], although some survive longer [88].

6.1.3 | SLC44A1 Deficiency (Gene *SLC44A1*)

Five patients were described from three different families. They presented with ataxia, tremor, dysarthria, dysphagia, cognitive decline, optic atrophy, and urinary/bowel incontinence. In three patients for whom it was available, birth head circumference was normal or high (+1.87 to +3 SD) but progressive microcephaly ensued. Brain MRI showed T_2-hyperintense leukoencephalopathy and progressive atrophy of the cerebral cortex and cerebellum. Susceptibility-weighted imaging (SWI) showed low signal intensity in the globus pallidus and substantia nigra with hyperintense streaking in the globus pallidus [57]. Cultured patient fibroblasts incubated with choline showed subtle improvement in some measurements, but treatment of patients with choline and with glycerophosphocholine did not noticeably affect disease progression. It was continued in a presymptomatic patient to see whether it might have a preventive effect.

Why does SLC44A1 cause yet another syndrome in addition to FLVCR1 and 2 deficiencies? We speculate that it may relate to the presence of SLC44A1 in mitochondria, which is not reported for the other transporters, or to the transport of substrates other than choline. These different diseases reveal previously unsuspected complexity in choline transport.

6.2 | IEMs of Phosphatidylcholine Synthesis

6.2.1 | Choline Kinase Deficiencies

Deficiencies of each of the two choline kinase isoforms, CHKα and CHKβ, are reported [89, 90].

6.2.1.1 | Choline Kinase, Isoform α Deficiency (CHKα; Gene *CHKA*). Six children from five families with biallelic pathogenic variants of *CHKA* presented severe to profound global developmental delay and progressive microcephaly (−4 to −7 standard deviations) detectable within the first months of life. Walking was delayed or absent. Speech was absent. Epilepsy developed in all before 3 years of age. Axial hypotonia and hyperreflexia were present. In one patient who had detailed ophthalmological testing, retinal dysfunction was documented. Brain MRIs of three patients showed only subtle changes, delayed myelination, or parietooccipital white matter hyperintensity on T_2-weighted images, apparently non-progressive. Muscle biopsy, performed in one patient, revealed enlarged and dense

mitochondria in the periphery of fibres, but functional mitochondrial testing was described as normal [89].

6.2.1.2 | Choline Kinase, Isoform β Deficiency (CHKβ; Gene *CHKB*). To date, 47 patients with pathogenic biallelic variants of *CHKB* have been reported and associated with megaconial muscular dystrophy [90, 91]. Symptoms appear before age 4 years, typically proximal limb weakness with myopathy and delays in walking, intellectual disability, and autistic or behavioral problems. Nonmuscular signs have included skin abnormalities (ichthyosis, vitiligo), dilated cardiomyopathy, and seizures. MRI was usually reported as normal, but a thin corpus callosum was reported in one patient. In all reported patients, serum creatine kinase activity shows continuous moderate elevation. Muscle biopsy is characteristic, with dystrophic fibers and enlarged mitochondria in the periphery and in the center of muscle fibres [91, 92].

6.2.2 | Choline-Phosphate Cytidylyltransferase α Deficiency (CCTα, Gene *PCYT1A*)

Biallelic loss-of-function variants in *PCYT1A*, encoding CCTα, have been associated with two distinct autosomal recessive phenotypes: spondylometaphyseal dysplasia with cone-rod dystrophy [93, 94], and partial lipodystrophy [95].

Spondylometaphyseal dysplasia with cone-rod dystrophy, reported in 12 patients [93, 94], causes disproportionate postnatal short stature with platyspondyly, short tubular bones producing rhizomelia, and bowing of the long bones of the legs. Most patients developed progressive early-onset visual impairment related to pigmentary maculopathy and cone-rod dystrophy. An apparently isolated infantile-onset retinal dystrophy clinically resembling Leber congenital amaurosis has been described in three patients from two Italian families with pathogenic *PCYT1A* variants [96].

Two unrelated females with *PCYT1A* variants presented with partial lipodystrophy and insulin resistance, without skeletal or visual abnormalities. Both were heterozygous for the same in-frame deletion of Glu280, plus a second variant on the other allele, different in each patient [97]. Further observations will be necessary to understand whether this form of lipodystrophy is a variant-specific phenotype.

Of note, inborn errors of PS metabolism are also associated with skeletal and retinal problems (Section 5.4). CCTα is positioned to determine the synthesis of PC, and PC is an important source of PS. In this context, we speculate that a PS-related mechanism may play a role in the skeletal pathology of CCTα deficiency.

6.3 | IEMs of Phosphatidylethanolamine Synthesis

6.3.1 | CTP-Phosphoethanolamine Cytidylyltransferase Deficiency (EPCT, Gene *PCYT2*)

Seven individuals from four families have been reported with biallelic loss-of-function variants in *PCYT2*. All presented complex spastic paraplegia, including mild to severe global developmental delay detectable in the first year of life and loss of previously acquired skills. All developed paraparesis and epilepsy between 2 and 16 years of age, with progressive cerebellar atrophy on MRI. Two brothers developed axonal neuropathy [97]. Nystagmus was reported in two patients and optic atrophy and microcephaly in one each [98, 99]. Lipidomic analysis of fibroblasts and plasma from patients showed both an accumulation of PC-etherphospholipids (mainly plasmanyl-PC) and also a deficiency of PUFA-containing species PE, PS, and PI, suggesting a possibly diagnostic pattern of lipid biomarkers [98, 100].

6.3.2 | Ethanolamine Phosphotransferase Deficiency (EPT1, Gene *SELENOI*)

This condition is caused by biallelic variants in *SELENOI* [101]. All eight patients reported to date [64, 102] presented with a complex progressive spastic paraplegia beginning in infancy or early childhood, with slow motor development and then regression. Mild to severe non-progressive intellectual impairment was present in all cases. Language delay and dysarthria occurred. Variably present features included microcephaly, seizures, retinitis pigmentosa, and bifid uvula with or without cleft palate [101]. Cerebral MRI showed increased T_2 signal intensity in the periventricular region in all patients tested.

6.4 | IEMs of Phosphatidylserine Synthesis

General considerations. *PS and bone*. Bone mineralization starts at nucleation sites in which hydroxyapatite crystals deposit and that are rich in PS, calcium, phosphate, and the protein annexin [103, 104]. PS is enriched in the matrix vesicles which are secreted by osteoblasts, and its anionic phosphate can bind calcium [105]. Accumulation of PS in osteoclasts has been reported to impair their function [106]. These observations may be relevant for understanding the skeletal abnormalities resulting from inborn errors of PS metabolism. *PS and the eye*. Under normal conditions, PS becomes exposed on the outer leaflet of the oldest (outermost) layers of the outer segment of photoreceptor cells, which are sloughed then phagocytosed by surrounding retinal pigment epithelium cells [107]. Intriguingly, retinal degeneration, sometimes specifically involving photoreceptor cells, recurs in two diseases of PS metabolism (Table 1) and also in deficiency of *PCYT1A* (CCT α), a condition that can be considered "upstream" to PS synthesis (Figure 2).

6.4.1 | Phosphatidylserine Synthase 1 Gain of Function (PSS1, Gene *PTDSS1*)

Heterozygous gain-of-function variants in *PTDSS1*, reported in 14 patients, cause Lenz-Majewski syndrome [108]. Patients have sclerosing bone dysplasia with brachydactyly, syndactyly, and symphalangism. At birth, patients have a progeroid appearance, prominent forehead, large ears, and cutis laxa. Fontanelle closure is delayed. Severe growth retardation and intellectual disability are present in all reported patients [109]. Hyperostosis is progressive, affecting the cranium, vertebrae, and the diaphyses of long bones. Hydrocephalus is a frequent and treatable feature. Hypoplasia or absence of the middle and proximal phalanges, but sparing of the thumbs, has been reported in some patients [110]. One three-year-old girl with

TABLE 1 | Inborn errors of GPL metabolism. Gene, protein, genetic transmission, and clinical features.

Gene	Protein name and function	Trans-mission	Inborn error: Name(s) and clinical features	References
IEMs of PA and DAG synthesis and degradation.				
GPD1	Glycerol-3-phosphate dehydrogenase 1 (GPDH)	AR	Hepatic steatosis with possible fibrosis, elevated aminotransferases, moderate hypertriglyceridemia.	[172]
GK	Glycerol Kinase (GK)	XL	Hyperglycerolemia with pseudo-hypertriglyceridemia (TG measurements spuriously elevated by hyperglycerolemia). Possible contiguous gene synd including Duchenne muscular dystrophy (*DMD*) and congenital adrenal hyperplasia (*NR0B1*). Vomiting, metabolic acidosis. suspect in older patients with high blood TGs without obesity or metabolic synd.	[173, 174]
AGPAT2	1-Acylglycerol-3-Phosphate O-Acyltransferase 2 (AGPAT)	AR	AR generalized congenital lipodystrophy.	[175]
LPIN1	Lipin-1, a PA phosphatase (PAP)	AR, (AD)	Recurrent childhood rhabdomyolysis with myoglobinuria. Occasional adult rhabdomyolysis. Heterozygote effects reported.	[177]
LPIN2	Lipin-2, a PA phosphatase (PAP)	AR, AD	Majeed Synd. AR recurrent multifocal non-infectious autoimmune osteomyelitis, anaemia and skin pustules.	[178]
DGKE	Diacylglycerol Kinase ε	AR	Atypical haemolytic uremic synd with proteinuria, frequently in infancy and post-viral, with slow progression. Some patients develop membranoproliferative glomerulonephritis without thrombotic microangiopathy.	[179]
LIPH	Lipase H, a PA phospholipase A1 (PLA_1)	AR	Associated with woolly hair, with or without hypotrichosis.	[190]
IEMs related to structural proteins facilitating lipid trafficking				
BSCL2	Seipin, a transmembrane endoplasmic reticulum protein important in lipid droplet formation	AR, AD	Seipinopathies. Variant-dependent phenotypes, with incomplete penetrance. AR: Berardinelli-Seip congenital generalized lipodystrophy (BSCL). AD: progressive neurological diseases ± lipodystrophy. Upper and/or lower motor neuron degeneration (onset childhood to geriatric); enceph ("Celia's enceph").	[176, 180]
IEMs of GPL head group metabolism, Phosphatidylcholine.				
FLVCR1	Feline leukemia virus subgroup C cellular receptor 1, a choline and ethanolamine transporter	AR	Posterior column ataxia with retinitis pigmentosa (PCARP). Sensory neuropathy. Hyperintense lesions in the posterior columns on inversion recovery MRI imaging. Gait difficulty with loss of position sense in first or second decade. Possible decreased pain sensation in the extremities.	[54, 55]
FLVCR2	Feline leukemia virus subgroup C cellular receptor 2, a choline and ethanolamine transporter	AR	Fowler synd: hydrancephaly and severe enceph with arthrogryposis. Characteristic proliferative vasculopathy ("glomeruloid"). Some patients survive infancy.	[87, 88]
SLC44A1	Choline transporter-like protein 1 (CTL1).	AR	Four patients described. Neurodegeneration in infancy. Ataxia, tremor, dysarthria, dysphagia, later cognitive decline, optic atrophy, urinary/bowel incontinence. Brain MRI: T2-hyperintense cerebral and cerebellar leukoenceph and progressive atrophy. On susceptibility-weighted imaging, low signal intensity in the globus pallidus and substantia nigra with hyperintense streaking in globus pallidus.	[56, 57]

(Continues)

TABLE 1 | (Continued)

Gene	Protein name and function	Trans-mission	Inborn error: Name(s) and clinical features	References
CHKA	Choline Kinase α (CHKα)	AR	Epileptic enceph, microcephaly (−3 to −7 SD), marked ID, possible movement disorder, hyperreflexia, nystagmus. Brain MRI unremarkable except possible hypomyelination.	[89]
CHKB	Choline Kinase β (CHKβ)	AR	Megaconial congenital muscular dystrophy. Developmental delay, loss of walking, variable intellectual disability, autism spectrum disorder, attention-deficit/hyperactivity. Possible dilated cardiomyopathy, epilepsy, ichthyosis. Older onset patients: limb girdle muscular dystrophy.	[90–92]
PCYT1A	Phosphate Cytidylyltransferase 1A, Choline, choline-phosphate cytidylyltransferase α (CCTα)	AR	Two phenotypes described, (1) Spondylometaphyseal Dysplasia with Cone-Rod Dystrophy (SMDCRD) and (2) partial lipodystrophy	[93–96]
IEMs of GPL head group metabolism, Phosphatidylethanolamine.				
PCYT2	Phosphate Cytidylyltransferase 2, Ethanolamine; Ethanolamine Phosphate Cytidylyltransferase 1 (EPCT1)	AR	Five patients described. SP in all, onset 2–16 years. Mild to severe developmental delay by 1 year. Progressive loss of skills. Progressive cerebellar atrophy on serial cerebral RMI. Nystagmus, optic atrophy, microcephaly in some.	[97–100]
PTDSS1	Phosphatidylserine synthase 1 (PSS1)	AD	Gain of function variants cause Lenz-Majewski hyperostotic short stature, with translucent lax skin, intellectual disability, sensorineural deafness	[108–112]
SELENOI	Selenoprotein I/Ethanolamine phosphotransferase (EPT1)	AR	AR complex progressive SP 81 (SPG81). Variable microcephaly, convulsions, retinitis pigmentosa, bifid uvula, optic atrophy, neurosensory deafness.	[64, 101]
IEMs of GPL head group metabolism, Phosphatidylserine.				
PISD	Phosphatidylserine decarboxylase. (PSD)	AR	Liberfarb synd, AR spondyloepimetaphyseal dysplasia with joint laxity and dislocations, retinal degeneration, hearing loss and progressive microcephaly.	[113–116]
IEMs of mitochondrial GPL metabolism				
AGK	Acylglycerol kinase (AGK)	AR	Sengers synd. Congenital or early-onset myopathy, hypertrophic cardiomyopathy, congenital cataract (isolated, in some cases), possible hyperlactatemia.	[118–122]
TAMM41	TAM41 (mitochondrial translocator assembly and maintenance homolog 41), a CDP-DAG synthase	AR	Three reported patients. Early severe hypotonia, myopathy, motor delay, areflexia. Possible ptosis, chronic progressive external ophthalmoplegia, dysphagia/GE reflux; attention deficit disorder; normal intellectual development possible at least in childhood.	[181]
SERAC1	Serine active site containing protein (SERAC1), an outer mitochondrial membrane component of serine transport necessary for mitochondrial nucleotide synthesis	AR	MEGDHEL Synd: AR Methylglutaconic aciduria, Deafness, Hepatic involvement, Enceph, and Leigh synd. Reversible neonatal liver failure; infantile muscular hypotonia, spasticity and dystonia. Possible optic atrophy. Neurosensory deafness. 3-methylglutaconic aciduria. Characteristic cerebral MRI with "eye"-like image of putamen.	[126–130]
CRLS1	Cardiolipin synthase 1 (CLS)	AR	Enceph, sometimes with epilepsy, progressive cerebral atrophy, sensorineural deafness, maculopathy, nystagmus, left ventricular noncompaction, hypertrophic cardiomyopathy.	[131]

(Continues)

TABLE 1 | (Continued)

Gene	Protein name and function	Trans-mission	Inborn error: Name(s) and clinical features	References
TAFAZZIN	Taffazin.	XL	Barth synd. X-linked cardiomyopathy and neutropenia.	[8, 26]
IEMs of GPL fatty acid hydrolysis, esterification and maintenance.				
ABHD12	α/β hydrolase domain-containing protein 12 (ABHD12)	AR	PHARC synd: Polyneuropathy, Hearing loss, Ataxia, Retinitis pigmentosa and Cataract. Onset: preschool to 20s. Spasticity, cerebellar ataxia and atrophy, polyneuropathy with absent reflexes, retinitis pigmentosa, sensorineural deafness, cataract by 3rd decade.	[182]
PLA2G6	PLA2G6, a calcium-dependent phospholipase A2, also named Patatin-like Phospholipase domain containing protein 9, PNPLA9	AR	Continuum of phenotypes: (1, infantile) Infantile Neuroaxonal Dystrophy type 1 (Seitelberger disease) with spasticity, ID, optic atrophy, neuropathy; (2, preschool) Neurodegeneration with Brain Iron Accumulation type 2B (Karak synd) with language difficulties, autism, spasticity, dystonia and cerebellar signs, (3, 20–40 y) AR Parkinson disease type 14, possible dystonia, spasticity, myoclonus and cerebellar signs, cerebral/cerebellar atrophy. Brain iron usually normal. (4, infantile/childhood) pontocerebellar hypoplasia.	[132–144]
PNPLA8	Mitochondrial calcium independent phospholipase A2γ (iPLA2γ), also named Patatin-like Phospholipase domain containing protein 8	AR	AR mitochondrial myopathy, dystonia, convulsions with microcephaly, hypertonia, spasticity, progressive atrophy of cerebral cortex, basal ganglion and brainstem atrophy, epilepsy, hyperlactatemia. Ataxia and polyneuropathy in later-onset forms.	[183, 184]
PNPLA6	Neuropathy Target Esterase (NTE), mitochondrial calcium-independent phospholipase A2γ	AR	Four major features, not always present: spinocerebellar signs with motor neuron disease (upper ± lower motor neuron) & SP (SPG39), ataxia, chorioretinal degeneration, hypogonadotrophic hypogonadism. Possible short stature, trichomegaly (long thick eyelashes), synophrys. Multiple eponymous clinically overlapping subforms: Boucher-Neuhauser; Gordon-Holms; Oliver-McFarlane; Laurence-Moon synd.	[145–149]
CYP2U1	CYP2U1, a cytochrome P450 expressed in thymus and brain that performs 19- and 20-hydroxylation of arachidonic acid	AR	SP with basal ganglion calcification, spastic paraplegia 56 (SPG56). Pseudoxanthoma elasticum in some patients.	[150, 161–165]
DDHD1	DDHD1, or Death domain homologous domain 1, which has PLA1 activity toward PIs	AR	AR Hereditary Spastic Paraplegia 28, SPG28. Spasticity, incontinence, possible lower motor neuron involvement. Possible retinal degeneration, neurodegeneration with brain iron accumulation (NBIA).	[152, 153]
MBOAT7	Lysophosphatidyl-inositol acyltransferase (LPIAT1) or Membrane-bound O-acyltransferase 7 (MBOAT7)	AR	AR ID, autism and epilepsy. Possible hepatic steatosis.	[185]
DDHD2	DDHD2, or Death domain homologous domain 2, involved in cerebral triglyceride hydrolysis	AR	AR Hereditary Spastic Paraplegia 54, SPG54. Typically, congenital or infantile onset motor and intellectual delay. Characteristic MRS lipid peak (90%), periventricular white matter (90%), thin corpus callosum (70%), ataxia. Adult onset: SP ± ataxia.	[150, 156–160]
IEMs of GPL membrane leaflet position				
ATP11C	ATP-dependent active transport of aminophospholipids (such as PS) from the outer to the inner leaflet	XL	Mild hemolytic anemia with reduced flippase activity for PS.	[186]

(Continues)

TABLE 1 | (Continued)

Gene	Protein name and function	Trans-mission	Inborn error: Name(s) and clinical features	References
ANO6	Anoctamin 6, or transmembrane protein 16F (TMEM16F), is essential for Ca(2+)-dependent exposure of PS on the platelet surface	AR	Scott synd, defective exposition of PS on platelet outer membrane leaflet with bleeding diathesis, particularly menstrual and postpartum bleeding and bleeding after dental extraction.	[73, 187]
ATP11A	P4 type ATPase, class VI, type 11A, a PL flippase that flips PE & PS from outer to inner plasma membrane leaflet	AD	AD deafness (isolated in some patients); hypomyelinating leucodystrophy	[188, 189]
ATP8A2	Catalytic component of a P4-ATPase PS flippase complex	AR	Cerebellar ataxia, impaired intellectual development and disequilibrium synd 4 (CAMRQ4). Ataxia/chorea, optic atrophy, tremors and seizures are frequent.	[191]
IEMs of GPL receptors or transport				
LPAR6	Lysophosphatidic acid receptor 6	AR	Woolly hair, with or without hypotrichosis.	[190]
MFSD2A	Major facilitator superfamily domain-containing protein 2A, a LPC transmembrane transporter	AR	Moderate to severe ID. Microcephaly. Brain MRI: simplified gyri, white matter thinning, ventricular dilatation, corpus callosum hypoplasia, pontine/vermal hypoplasia. Seizures and spasticity. Talipes equinovarus.	[59–61]

Note: This table lists known disorders of GPL synthesis, degradation, signaling, and transport.
Abbreviations: AD, autosomal dominant; AR, autosomal recessive; Enceph, encephalopathy; ID, intellectual deficiency; SP, spastic paraplegia; synd, syndrome; XL, X-linked.

normal growth, mild-to-moderate developmental delay, pulmonary stenosis, a supernumerary rib, and mildly elevated 3-methylglutaconic acid in urine was found on trio exome sequencing to have a heterozygous *de novo* loss-of-function variant in *PTDSS1*; the relationship of this variant with the phenotype remains to be established [111]. To date, all known variants causing Lenz-Majewski syndrome have occurred *de novo*. PS normally inhibits phosphatidylserine synthase 1 (PSS1), avoiding PS accumulation [112]. However, in Lenz-Majewski syndrome, there is a loss of this feedback control [112]. PS is important for bone mineralization, binding calcium within matrix vesicles, and enhancing hydroxyapatite crystal formation [105]. Of note, expression of the causal *PTDSS1* variants reduces levels of phosphatidylinositol-4-phosphate (PI4P), suggesting possible PI-related mechanisms [112].

6.4.2 | Phosphatidylserine Decarboxylase Deficiency (PSD, Gene *PISD*)

The seven reported patients [113, 114] had low birth length and progressive severe short stature (−4 to −9 SD), with a spondyloepimetaphyseal dysplasia producing marked kyphoscoliosis and associated with joint hyperlaxity and multiple dislocations (hip, knee, elbow). Some patients had progressive acquired microcephaly with mild to moderate intellectual disability, chorioretinal degeneration, and sensorineural hearing loss. MRI showed bilateral optic nerve atrophy and cerebellar atrophy. Cultured fibroblasts of one patient showed fragmented mitochondria [115]. Cerebral MRI of another patient showed T_2 white matter hyperintensity, progressive hypomyelination, and thinning of the corpus callosum [116].

PSD is a mitochondrial PS decarboxylase that synthesizes PE, and high levels of PE in the inner mitochondrial membrane are essential for mitochondrial biogenesis and fusion [117], suggesting a possible mitochondrial origin for at least some of the clinical features.

6.5 | IEMs of Mitochondrial GPL Metabolism

General considerations. Mitochondria play vital roles in GPL metabolism. Mitochondria are necessary for normal cellular PS and PE metabolism and for the synthesis of the lysosomal GPL derivative BMP. Mitochondria synthesize CL endogenously, plus PE, PA, and PG. Other GPLs (PS, PI and PC) are supplied to mitochondria by non-vesicular transport at MAMs. In the mitochondrial inner membrane, GPLs interact with the respiratory chain components to aid the assembly of respiratory chain supercomplexes [66].

6.5.1 | Acylglycerol Kinase Deficiency (AGK, Gene *AGK*)

Sengers syndrome [120] is an autosomal recessive disorder due to biallelic pathogenic variants in *AGK*. Four clinical forms are described: (1) Late onset with hypertrophic cardiomyopathy, congenital cataract, skeletal myopathy, exercise intolerance, hyperlactacidemia, and normal mental development, with death

in the fourth or fifth decade principally from cardiomyopathy [119, 120]; (2) Fatal neonatal-onset encephalomyopathy, including abnormal basal ganglia, brainstem, and cerebellar hypoplasia and possible cortical infarction; (3) Fatal neonatal liver dysfunction; (4) Isolated cataracts [121].

AGK encodes mitochondrial acylglycerol kinase (AGK), which produces PA, a precursor for mitochondrial GPL biosynthesis [119]. AGK also stabilizes the TIM22 complex, which imports transmembrane mitochondrial proteins [77]. Secondary TIM22 deficiency has been suggested to explain the deficiency of the transmembrane adenine nucleotide transporter 1 (ANT1) observed in Sengers syndrome patients [122].

6.5.2 | Protein-Tyrosine Phosphatase, Mitochondrial 1 Deficiency (PTPMT1, Gene *PTPMT1*)

Six patients from three families are described, each homozygous for variants in *PTPMT1* [123]. In five, the variant is c.255G>C, which shows markedly abnormal splicing. Disease was suspected before 15 months of age due to developmental delay and nystagmus, and sometimes neonatally with hypotonia and no eye contact. The disease evolves to include developmental delay in all, followed by regression, cerebellar ataxia (5 patients), progressive microcephaly (5), nystagmus (4), head bobbing when sitting (4), optic atrophy (3), sensorineural deafness (2), spasticity (2) and seizures (2). Plasma creatine kinase has been normal, and blood lactate was normal or marginally elevated. MRI images show cerebral and cerebellar atrophy and white matter signs (thin corpus callosum, abnormal signal intensities). The one patient with another variant, c.65A>C (p.Tyr22Ser), was the mildest clinically, but unlike the others, had elevated plasma aminotransferases, suggesting possible variant-specific effects on phenotype. In muscle, she had respiratory complex I deficiency.

Patient fibroblasts showed mitochondrial fragmentation, and there was a tendency for low CL levels in muscle and blood spots, although the diagnostic utility of this is unproven. Previous work shows that PTPMT1 is a protein tyrosine phosphatase that can also cleave phosphate groups from PItds [124] and PGP. *Ptpmt1* knockout in mice is embryonically lethal. Muscle- and heart-specific *Ptpmt1* knockout mice develop myopathy and heart failure, respectively, but *Ptpmt1* knockout is tolerated in liver [125].

6.5.3 | SERAC1 Deficiency (Gene *SERAC1*)

MEGDHEL is caused by biallelic pathogenic variants in *SERAC1* [126]. "MEGDHEL" is an acronym for 3-methylglutaconic aciduria, sensorineural deafness, hepatic involvement, encephalopathy, and Leigh syndrome. Hepatic involvement ranges from neonatal hypoglycaemia or transient cholestasis to fulminant hepatic failure [127]. On cerebral MRI, 98% of patients have bilateral basal ganglia involvement, particularly of the putamen [128]. Optic atrophy is reported in 25% [128]. Epilepsy can begin as early as the neonatal period [129]. Milder forms manifest as slowly progressive juvenile-onset complex spastic paraplegia and non-progressive mild cognitive deficit [130].

One important role of SERAC1 is the production of phosphatidylglycerol 36:1 (PG36:1). Lipidomics of MEGDHEL fibroblasts show a high level of PG34:1 with low levels of PG36:1 and BMP, as well as accumulations of cardiolipin species derived from PG34:1. Also, the accumulation of free intracellular cholesterol has been demonstrated by a positive filipin test [129].

6.5.4 | Cardiolipin Synthase 1 Deficiency (CLS1, Gene *CRLS1*)

Four affected individuals were reported [131]. They experienced early infant death or progressive neurological impairment including encephalopathy with microcephaly and hearing impairment or auditory neuropathy. Visual impairment with bull's eye maculopathy was a recurrent and distinctive feature. Brain stem dysfunction with hypoventilation and temperature instability can occur. Some had diabetes insipidus and cardiomyopathy. Prenatal hydrops or neonatal cardiomyopathy with noncompaction occurred, from which some patients improved. Lipidomics analysis showed low levels of cardiolipin, elevated levels of PG and of the PG/CL ratio, and atypical patterns of FA composition in the CL fraction [131].

6.5.5 | Cardiolipin Remodeling Enzyme Deficiency (Gene *TAFFAZIN*)

Barth syndrome (Table 1) is discussed in detail elsewhere in this issue [14].

6.6 | IEMs of GPL Remodeling

IEMs of lipid remodeling are an emerging group of conditions, about which much remains to be learned. Several genes encode phospholipases with PLA_1, PLA_2, or PLB activities that participate in the Lands cycle. Multiple enzymes can catalyse the same reactions. Some of these reactions cause inborn errors if deficient. Among these conditions, hereditary spastic paraplegia (HSP) is a recurrent observation (Box 1).

6.6.1 | Phospholipase A_2 Group VI Deficiency (PNPL9, Gene *PLA2G6*)

PLA2G6-related diseases present in two overlapping fashions: *PLA2G6*-associated neurodegeneration (PLAN) and hereditary spastic paraplegia (HSP). HSP is often a major component of PLAN [132, 133]. PLAN has a different clinical course depending on age of onset. (1) Infantile neuroaxonal dystrophy (INAD) presents between 6 and 36 months with psychomotor delay or regression and hypotonia. Progressive spasticity, cognitive decline, and visual impairment develop later, with death before 10 years [134–136]. (2) Atypical neuroaxonal dystrophy (ANAD) shows later onset and greater clinical variability than INAD. It may at first resemble stable mild cerebral palsy, with unsteady gait, ataxia, dystonia, spasticity, speech delay, attention deficit disorder, or autistic features, but progressive deterioration occurs between 7 and 12 years of age [133]. (3) *PLA2G6*-related dystonia-parkinsonism (PARK14, autosomal recessive

early-onset parkinsonism, AREP) [137] typically presents in adolescents or young adults, beginning with psychological instability or difficulty walking and progressing to dystonia (particularly of the extremities), parkinsonism, and sometimes mental decline. Motor delay or regression is consistently reported at onset [132, 137–139]. In all forms, ocular findings such as strabismus, nystagmus, and/or optic atrophy can occur [134]. (4) Infantile- or childhood-onset pontocerebellar hypoplasia (PCH1) with anterior horn cell degeneration was described in two siblings with cerebellar signs and cerebellar vermal atrophy [140]. On neuropathology, all forms of PLA2G6 deficiency have iron accumulation in the globus pallidus and substantia nigra. Usually, characteristic spheroids are present in axons throughout the brain [136, 141]. In some dystonia/parkinsonism patients, spheroids were absent, but α-synuclein accumulation with Lewy bodies and tau hyperphosphorylation were demonstrated, an intriguing similarity to Parkinson disease [139].

PLA2G6 is also known as patatin-like phospholipase domain-containing protein-9 (PNPLA9) and as iPLA$_2$β [138, 142]. It is highly expressed in substantia nigra, cerebral cortex, and hippocampus [136, 138, 141]. PLA2G6 can cleave the *sn*-2 position of PC, PE, and PA, especially if it is occupied by a PUFA, but can also cleave *sn*-1 lyso-GPLs and even FA-CoAs. PLA2G6 can also mediate a salvage pathway, hydrolyzing GPLs that contain oxidized FAs and allowing for replacement with a non-oxidized FA [142]. Pathological variants causing INAD or ANAD have been shown to produce loss of function, decreasing the release of arachidonic and docosahexaenoic acids [143]. In contrast, by *in silico* modeling, later-onset variants are predicted to maintain catalytic function. It is speculated that they may modify the substrate preferences or regulatory mechanisms of PLA2G6 [143, 144].

6.6.2 | Neuropathy-Targeted Esterase Deficiency (Gene, *PNPLA6*)

Neuropathy target esterase (NTE) was initially identified as the target of the toxins that cause organophosphate-induced delayed neuropathy. Variants in *PNPLA6* cause several autosomal recessive conditions initially felt to be unrelated, including a childhood-onset progressive spastic paraplegia (HSP) with peripheral neuropathy and distal muscle wasting, SPG type 39 [145]. Four overlapping neuroendocrine conditions with hypogonadotropic hypogonadism have been associated with *PNPLA6* variants: Boucher-Neuhauser, Gordon Holmes [146], Oliver-McFarlane, and Laurence-Moon syndromes [147]. The first two show spinocerebellar ataxia beginning in the second to fourth decades, with chorioretinal dystrophy developing between the first and the sixth decades. The syndromes differ in that Boucher-Neuhauser patients show cerebellar atrophy and pituitary hypoplasia, whereas Gordon-Holmes syndrome patients have progressive cognitive decline, dementia, and variable adult-onset movement disorders. Both Oliver-McFarlane and Laurence-Moon syndromes feature chorioretinal atrophy, typically noted before 5 years of age, and multiple pituitary hormone deficiencies (growth hormone, thyroid-stimulating hormone, with hypogonadotropic hypogonadism in nearly all patients) and spinocerebellar involvement in half. Oliver-McFarlane but not Laurence-Moon syndrome patients have long eyelashes (trichomegaly), sometimes with bushy eyebrows [147].

NTE shows phospholipase B activity, particularly towards membrane lyso-PCs, from which it generates glycerophosphocholine and a FA [148]. This reaction is thought to be important for normal vesicular trafficking and release. Compatible with this is the increased intracytosolic vesiculation observed in mammalian cells containing inactivated NTE homologues [149].

6.6.3 | *DDHD1* and *DDHD2* Variants

DDHD1 and *DDHD2* each encode DDHD domain-containing A$_1$ type phospholipases, although their substrate specificities differ. Pathogenic variants in *DDHD1* or in *DDHD2* produce autosomal recessive hereditary spastic paraplegias (HSP), often associated with other neurological signs. Phenotypic spectra are not yet well established.

6.6.3.1 | DDHD1 Deficiency (Gene *DDHD1*). *DDHD1* pathogenic variants are associated with autosomal recessive hereditary spastic paraplegia 28 (SPG28), first identified in three families with adolescent-onset isolated HSP [150]. In subsequent reports, some patients also showed axonal neuropathy with distal sensory loss, cerebellar and oculomotor disturbances with saccadic eye pursuit [151], retinal dystrophy, thin corpus callosum, and brain iron accumulation (NBIA) [152]. DDHD1 has been designated as a PA-preferring phospholipase A$_1$ and is also suggested to hydrolyse PI and PS at the *sn*-1 position, producing lysoPI and lysoPS [153]; in support of this, the brains of aged *Ddhd1* deficient mice have low *sn*-2 lysophosphatidylinositol with arachidonic acid (20:4) but correspondingly high levels of PI species [153]. *DDHD1* variants impair mitochondrial fragmentation, suggesting a role for *DDHD1* in mitochondrial fusion and fission [154].

6.6.3.2 | DDHD2 Deficiency (Gene *DDHD2*). *DDHD2* variants are associated with an early-onset autosomal recessive hereditary spastic paraplegia (SPG54), and with progressive complex HSP [155]. About 40 patients have been reported to date. Among eight patients with *DDHD2* variants, six presented with congenital or infantile HSP, and two showed onset of HSP in the fourth decade [156]. In early onset SPG54, HSP is accompanied or preceded by intellectual and motor delay, cerebellar ataxia with progressive loss of ambulation, and abnormal eye movements in 50% [157]. Neuroimaging reveals optic nerve hypoplasia, thinning of the corpus callosum (90%), subtle periventricular white-matter hyperintensities on T$_2$-weighted MRI images (70%) and on cerebral proton magnetic resonance spectroscopy, 90% of patients show a prominent lipid peak in the basal ganglia and the thalamus. Of note, in patients with adult onset, cognitive function has been normal [157].

DDHD2 can hydrolyse a range of lipid esters in vitro [158], including PC but also triglycerides, diglycerides, and monoglycerides. Mice with genetic DDHD2 deficiency have lipid droplet accumulation in brain, and data suggest that this finding may relate in whole or in part to the accumulation of triglycerides rather than of GPLs [158, 159]. Recent cellular data suggest that stimulation of lipophagy may reduce TG accumulation in DDHD2 deficiency [160].

6.6.4 | CYP2U1 Deficiency (Gene *CYP2U1*)

Patients with biallelic pathogenic *CYP2U1* variants have presented with early-onset HSP (birth to 8 years), frequently involving the upper limbs and sometimes associated with dystonic postures or cognitive alterations [150]. Degenerative pigmentary maculopathy was reported in three patients [150, 161] On brain MRI, thinning of the corpus callosum and delayed myelination, and other white matter lesions are reported [162]. Globus pallidus hypointensities on T_1-weighted cerebral MRI correspond to areas of calcification. Spinal cord MRI revealed hydromyelia of the thoracic spinal cord in one patient [163]. Clinical severity varies widely even within families, with no obvious genotype–phenotype relationship. For example, in one family, two patients never walked, while a third was fully autonomous in his fourth decade but had difficulty with running. Initially named SPG49, this condition is now designated SPG56 [150].

Pseudoxanthoma elasticum (PXE) has also been associated with pathogenic variants in *CYP2U1* [165]. In a retrospective study of 46 patients with *ABCC6*-negative biopsy-proven PXE, three patients (6.4%) harbored biallelic pathogenic variants in *CYP2U1*. These patients also suffered from complicated spastic paraplegia, with maculopathy and loss of visual acuity.

CYP2U1 encodes cytochrome P450 2U1 (Figure 4). CYP2U1 acts in a tissue-specific fashion to perform ω and $\omega-1$ hydroxylation of AA, producing active derivatives, such as 19- and 20-hydroxyeicosatetraenoic acids (19-HETE, 20-HETE) [165]. Their biological effects include control of vascular tone and blood pressure [150]. *CYP2U1* variants should be suspected in HSP patients with basal ganglia calcifications. Therefore, spastic paraplegia and liberation or activation of AA derivatives may be common threads linking several IEMs of GPL metabolism (Figure 4B). The correlation between AA and paraplegia is striking, but currently there is no formal proof of a causal association.

7 | Discussion

Knowledge about inborn errors of GPL metabolism is rapidly expanding. To 38 such conditions at the time of writing (Table 1) should be added the 34 other genes of PI or PItds metabolism that have been associated with clinical phenotypes [46]. Several steps of GPL metabolism and several enzymes with GPL substrates are not yet associated with an inborn error.

A vast literature clearly shows the physiological importance of GPLs for vesicular trafficking, cell signalling, blood clotting, apoptosis, and other vital pathways, but it does not suggest general methods for the diagnosis and treatment of genetic deficiencies of these pathways. No single biochemical test currently identifies a large fraction of these conditions. Broad molecular testing has been the main method of discovery and diagnosis.

The known inborn errors of GPL metabolism permit a bird's-eye clinical overview of the field. Neurological, ophthalmological, and skeletal features are recurrent findings. Neurological signs occur in 23 of the 38 conditions listed in Table 1 (61%), particularly intellectual deficiency (16/38, 42%) and spastic paraplegia (12/38, 32%). Perhaps the high prevalence of these features reflects the importance of GPLs for vesicular trafficking at synapses and in motoneuron axons. Epilepsy (8 conditions, 21%), basal ganglia pathology (8, 21%) and peripheral neuropathy (5,13%) are recurrent findings.

Eye involvement affecting photoreceptor cells and/or the neuroretina occurs in 14/38 conditions (37%), with retinopathy in 5, optic atrophy in 6, maculopathy in 2, and rod-cone dystrophy in one. Skeletal dysplasia occurs in three inborn errors of GPL metabolism, two involving PS metabolism. Although the numbers are small, the association of skeletal dysplasia plus retinal or optic nerve disease should evoke an inborn error of GPL metabolism.

In patients presenting with more than one of the above findings, a disorder of GPL metabolism is a consideration. However, the clinical presentations of most patients would not strongly suggest a specific diagnosis. For the foreseeable future, molecular testing, such as exome or genome sequencing, will likely remain the principal diagnostic method.

Metabolic diagrams involving GPLs are oversimplified. At many steps, the same reaction can be mediated by more than one transporter or enzyme, suggesting redundant functions. In many instances, deficiency of one or more of these proteins causes a distinct inborn error(s). Perhaps this is due to differences in tissue or subcellular expression. Alternatively, perhaps it depends on a similar reaction towards another substrate or upon completely different functional roles such as that of AGK in stabilizing TIM22. These observations underscore the importance of documenting new inborn errors of GPL metabolism, each of which provides empirical proof of the biological importance of the gene.

Lipidomics holds much promise for diagnosis and therapeutic monitoring. It has already revealed specific profiles for some inborn errors of GPL metabolism. Examples include PCYT2-associated spastic paraplegia [100], MEGDHEL, and Barth syndrome [166, 167]. Lipidomics studies in the tissues of animal models may suggest new disease mechanisms, as in *Ddhd1*-knockout mice [168]. Together with careful clinical description and focused studies of trafficking and other cell functions, this will define the clinical and biochemical spectra of known inborn errors of GPL metabolism, identify new diseases, and facilitate the much-needed development of effective treatments.

Author Contributions

Foudil Lamari: Initial concept and design, first draft of the manuscript, critical revision of the manuscript. Francis Rossignol: Concept and design, critical revision of the manuscript. Grant A. Mitchell: Concept and design, critical revision of the manuscript.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. G. Van Meer, D. R. Voelker, and G. W. Feigenson, "Membrane Lipids: Where They Are and How They Behave," *Nature Reviews. Molecular Cell Biology* 9 (2008): 112–124.

2. F. M. Hughson and K. M. Reinisch, "Structure and Mechanism in Membrane Trafficking," *Current Opinion in Cell Biology* 22, no. 4 (2010): 454–460, https://doi.org/10.1016/j.ceb.2010.03.011.

3. M. Hermansson, K. Hokynar, and P. Somerharju, "Mechanisms of Glycerophospholipid Homeostasis in Mammalian Cells," *Progress in Lipid Research* 50, no. 3 (2011): 240–257, https://doi.org/10.1016/j.plipres.2011.02.004.

4. W. Dowhan and M. Bogdabov, "Functional Roles of Lipids in Membranes," in *Biochemistry of Lipids, Lipoproteins and Membranes*, 4th ed., ed. D. E. Vance and J. E. Vance (Elsevier Science B.V, 2002), 1–35.

5. J. E. Vance, "Phospholipid Synthesis and Transport in Mammalian Cells," *Traffic* 16, no. 1 (2015): 1–18, https://doi.org/10.1111/tra.12230.

6. T. Balla, "Phosphoinositides: Tiny Lipids With Giant Impact on Cell Regulation," *Physiological Reviews* 93, no. 3 (2013): 1019–1137, https://doi.org/10.1152/physrev.00028.2012.

7. Y. Yang, M. Lee, and G. D. Fairn, "Phospholipid Subcellular Localization and Dynamics," *Journal of Biological Chemistry* 293, no. 17 (2018): 6230–6240, https://doi.org/10.1074/jbc.R117.000582.

8. M. Messina, F. M. Vaz, and S. Rahman, "Mitochondrial Membrane Synthesis, Remodelling and Cellular Trafficking," *Journal of Inherited Metabolic Disease* 48, no. 1 (2025): e12766, https://doi.org/10.1002/jimd.12766.

9. S. Suetsugu, S. Kurisu, and T. Takenawa, "Dynamic Shaping of Cellular Membranes by Phospholipids and Membrane-Deforming Proteins," *Physiological Reviews* 94, no. 4 (2014): 1219–1248, https://doi.org/10.1152/physrev.00040.2013.

10. W. Basu Ball, J. K. Neff, and V. M. Gohil, "The Role of Nonbilayer Phospholipids in Mitochondrial Structure and Function," *FEBS Letters* 592, no. 8 (2018): 1273–1290, https://doi.org/10.1002/1873-3468.12887.

11. Y. J. Kim, N. Sengupta, M. Sohn, et al., "Metabolic Routing Maintains the Unique Fatty Acid Composition of Phosphoinositides," *EMBO Reports* 23, no. 7 (2022): e54532, https://doi.org/10.15252/embr.202154532.

12. O. Ali and A. Szabó, "Review of Eukaryote Cellular Membrane Lipid Composition, With Special Attention to the Fatty Acids," *International Journal of Molecular Sciences* 24, no. 21 (2023): 15693, https://doi.org/10.3390/ijms242115693.

13. S. Y. Morita and Y. Ikeda, "Regulation of Membrane Phospholipid Biosynthesis in Mammalian Cells," *Biochemical Pharmacology* 206 (2022): 115296, https://doi.org/10.1016/j.bcp.2022.115296.

14. A. Traynor-Kaplan, M. Kruse, E. J. Dickson, et al., "Fatty-Acyl Chain Profiles of Cellular Phosphoinositides," *Biochimica et Biophysica Acta* 1862 (2017): 513–522, https://doi.org/10.1016/j.bbalip.2017.02.002.

15. I. Levental, K. R. Levental, and F. A. Heberle, "Lipid Rafts: Controversies Resolved, Mysteries Remain," *Trends in Cell Biology* 30, no. 5 (2020): 341–353, https://doi.org/10.1016/j.tcb.2020.01.009.

16. S. C. D. van IJzendoorn, J. Agnetti, and A. Gassama-Diagne, "Mechanisms Behind the Polarized Distribution of Lipids in Epithelial Cells," *Biochimica et Biophysica Acta - Biomembranes* 1862, no. 2 (2020): 183145, https://doi.org/10.1016/j.bbamem.2019.183145.

17. A. Zachowski, "Phospholipids in Animal Eukaryotic Membranes: Transverse Asymmetry and Movement," *Biochemical Journal* 294 (1993): 1–14, https://doi.org/10.1042/bj2940001.

18. J. H. Lorent, K. R. Levental, L. Ganesan, et al., "Plasma Membranes Are Asymmetric in Lipid Unsaturation, Packing and Protein Shape," *Nature Chemical Biology* 16, no. 6 (2020): 644–652, https://doi.org/10.1038/s41589-020-0529-6.

19. J. L. Symons, K. J. Cho, J. T. Chang, et al., "Lipidomic Atlas of Mammalian Cell Membranes Reveals Hierarchical Variation Induced by Culture Conditions, Subcellular Membranes, and Cell Lineages," *Soft Matter* 17, no. 2 (2021): 288–297, https://doi.org/10.1039/d0sm00404a.

20. A. Zhukov and M. Vereshchagin, "Polar Glycerolipids and Membrane Lipid Rafts," *International Journal of Molecular Sciences* 25, no. 15 (2024): 8325, https://doi.org/10.3390/ijms25158325.

21. M. Di Miceli, C. Bosch-Bouju, and S. Layé, "PUFA and Their Derivatives in Neurotransmission and Synapses: A New Hallmark of Synaptopathies," *Proceedings of the Nutrition Society* 79, no. 4 (2020): 388–403, https://doi.org/10.1017/S0029665120000129.

22. G. Pabst and S. Keller, "Exploring Membrane Asymmetry and Its Effects on Membrane Proteins," *Trends in Biochemical Sciences* 49, no. 4 (2024): 333–345, https://doi.org/10.1016/j.tibs.2024.01.007.

23. J. Choi, T. Yin, K. Shinozaki, et al., "Comprehensive Analysis of Phospholipids in the Brain, Heart, Kidney, and Liver: Brain Phospholipids Are Least Enriched With Polyunsaturated Fatty Acids," *Molecular and Cellular Biochemistry* 442, no. 1–2 (2018): 187–201, https://doi.org/10.1007/s11010-017-3203-x.

24. G. Oemer, J. Koch, Y. Wohlfarter, et al., "Phospholipid Acyl Chain Diversity Controls the Tissue-Specific Assembly of Mitochondrial Cardiolipins," *Cell Reports* 30, no. 12 (2020): 4281–4291, https://doi.org/10.1016/j.celrep.2020.02.115.

25. J. Cao, Y. Liu, J. Lockwood, P. Burn, and Y. Shi, "A Novel Cardiolipin-Remodeling Pathway Revealed by a Gene Encoding an Endoplasmic Reticulum-Associated Acyl-CoA:Lysocardiolipin Acyltransferase (ALCAT1) in Mouse," *Journal of Biological Chemistry* 279 (2004): 31727–31734, https://doi.org/10.1074/jbc.M402930200.

26. Y. Xu, A. Malhotra, M. Ren, and M. Schlame, "The Enzymatic Function of Tafazzin," *Journal of Biological Chemistry* 281 (2006): 39217–39224, https://doi.org/10.1074/jbc.M606100200.

27. R. J. Clarke, K. R. Hossain, and K. Cao, "Physiological Roles of Transverse Lipid Asymmetry of Animal Membranes," *Biochimica et Biophysica Acta - Biomembranes* 1862, no. 10 (2020): 183382, https://doi.org/10.1016/j.bbamem.2020.183382.

28. R. P. Bazinet and S. Layé, "Polyunsaturated Fatty Acids and Their Metabolites in Brain Function and Disease," *Nature Reviews. Neuroscience* 15 (2014): 771–785, https://doi.org/10.1038/nrn3820.

29. S. R. Wassall and W. Stillwell, "Polyunsaturated Fatty Acid-Cholesterol Interactions: Domain Formation in Membranes," *Biochimica et Biophysica Acta* 1788 (2009): 24–32.

30. H. Takeda, Y. Matsuzawa, M. Takeuchi, et al., "MS-DIAL 5 Multimodal Mass Spectrometry Data Mining Unveils Lipidome Complexities," *Nature Communications* 15, no. 1 (2024): 9903, https://doi.org/10.1038/s41467-024-54137-w.

31. E. Lauwers, R. Goodchild, and P. Verstreken, "Membrane Lipids in Presynaptic Function and Disease," *Neuron* 90, no. 1 (2016): 11–25, https://doi.org/10.1016/j.neuron.2016.02.033.

32. C. L. Jackson, L. Walch, and J. M. Verbavatz, "Lipids and Their Trafficking: An Integral Part of Cellular Organization," *Developmental Cell* 39, no. 2 (2016): 139–153, https://doi.org/10.1016/j.devcel.2016.09.030.

33. T. R. Graham and M. M. Kozlov, "Interplay of Proteins and Lipids in Generating Membrane Curvature," *Current Opinion in Cell Biology* 22, no. 4 (2010): 430–436, https://doi.org/10.1016/j.ceb.2010.05.002.

34. H. T. McMahon and E. J. Boucrot, "Membrane Curvature at a Glance," *Cell Science* 128, no. 6 (2015): 1065–1070, https://doi.org/10.1242/jcs.114454.

35. B. Binotti, R. Jahn, and Á. Pérez-Lara, "An Overview of the Synaptic Vesicle Lipid Composition," *Archives of Biochemistry and Biophysics* 709 (2021): 108966, https://doi.org/10.1016/j.abb.2021.108966.

36. A. S. Sechi and J. Wehland, "The Actin Cytoskeleton and Plasma Membrane Connection: PtdIns(4,5)P2 Influences Cytoskeletal Protein Activity at the Plasma Membrane," *Journal of Cell Science* 113, no. 21 (2000): 3685–3695, https://doi.org/10.1242/jcs.113.21.3685.

37. A. Kumar, D. Baycin-Hizal, Y. Zhang, M. A. Bowen, and M. J. Betenbaugh, "Cellular Traffic Cops: The Interplay Between Lipids and Proteins Regulates Vesicular Formation, Trafficking, and Signaling in Mammalian Cells," *Current Opinion in Biotechnology* 36 (2015): 215–221, https://doi.org/10.1016/j.copbio.2015.09.006.

38. P. F. Devaux, "Static and Dynamic Lipid Asymmetry in Cell Membranes," *Biochemistry* 30, no. 5 (1991): 1163–1173, https://doi.org/10.1021/bi00219a001.

39. H. M. Hankins, R. D. Baldridge, P. Xu, and T. R. Graham, "Role of Flippases, Scramblases and Transfer Proteins in Phosphatidylserine Subcellular Distribution," *Traffic* 16, no. 1 (2015): 35–47, https://doi.org/10.1111/tra.12233.

40. M. Caputo, D. Ivanova, S. Chasserot-Golaz, et al., "Phospholipid Scramblase 1 Controls Efficient Neurotransmission and Synaptic Vesicle Retrieval at Cerebellar Synapses," *Journal of Neuroscience* 44, no. 27 (2024): e0042242024, https://doi.org/10.1523/JNEUROSCI.0042-24.2024.

41. G. Csordás, C. Renken, P. Várnai, et al., "Structural and Functional Features and Significance of the Physical Linkage Between ER and Mitochondria," *Journal of Cell Biology* 174, no. 7 (2006): 915–921, https://doi.org/10.1083/jcb.200604016.

42. T. Sakuragi and S. Nagata, "Regulation of Phospholipid Distribution in the Lipid Bilayer by Flippases and Scramblases," *Nature Reviews. Molecular Cell Biology* 24, no. 8 (2023): 576–596, https://doi.org/10.1038/s41580-023-00604-z.

43. S. Lev, "Non-Vesicular Lipid Transport by Lipid-Transfer Proteins and Beyond," *Nature Reviews. Molecular Cell Biology* 11, no. 10 (2010): 739–750, https://doi.org/10.1038/nrm2971.

44. D. M. Raben and C. N. Barber, "Phosphatidic Acid and Neurotransmission," *Advances in Biological Regulation* 63 (2017): 15–21, https://doi.org/10.1016/j.jbior.2016.09.004.

45. C. N. Barber and D. M. Raben, "Roles of DGKs in Neurons: Postsynaptic Functions?," *Advances in Biological Regulation* 75 (2020): 100688, https://doi.org/10.1016/j.jbior.2019.100688.

46. F. Rossignol, F. Lamari, and G. A. Mitchell, "Phosphoinositide Metabolism: Biochemistry, Physiology and Genetic Disorders," *Journal of Inherited Metabolic Disease* 48, no. 2 (2025): e70008, https://doi.org/10.1002/jimd.70008.

47. R. P. Huijbregts, L. Topalof, and V. A. Bankaitis, "Lipid Metabolism and Regulation of Membrane Trafficking," *Traffic* 1, no. 3 (2000): 195–202, https://doi.org/10.1034/j.1600-0854.2000.010301.x.

48. T. Shemesh, A. Luini, V. Malhotra, K. N. Burger, M. M. Kozlov, and K. N. J. Burger, "Prefission Constriction of Golgi Tubular Carriers Driven by Local Lipid Metabolism: A Theoretical Model," *Biophysical Journal* 85, no. 6 (2003): 3813–3827, https://doi.org/10.1016/S0006-3495(03)74796-1.

49. S. Xie, N. Naslavsky, and S. Caplan, "Diacylglycerol Kinases in Membrane Trafficking," *Cellular Logistics* 5, no. 2 (2015): e1078431, https://doi.org/10.1080/21592799.2015.1078431.

50. S. Yao, S. C. Kim, J. Li, S. Tang, and X. Wang, "Phosphatidic Acid Signaling and Function in Nuclei," *Progress in Lipid Research* 93 (2024): 101267, https://doi.org/10.1016/j.plipres.2023.101267.

51. P. A. Postila and T. Róg, "A Perspective: Active Role of Lipids in Neurotransmitter Dynamics," *Molecular Neurobiology* 57, no. 2 (2020): 910–925, https://doi.org/10.1007/s12035-019-01775-7.

52. M. Tsuchiya, N. Tachibana, K. Nagao, T. Tamura, and I. Hamachi, "Organelle-Selective Click Labeling Coupled With Flow Cytometry Allows Pooled CRISPR Screening of Genes Involved in Phosphatidylcholine Metabolism," *Cell Metabolism* 35, no. 6 (2023): 1072–1083, https://doi.org/10.1016/j.cmet.2023.02.014.

53. M. J. Wakelam, "Diacylglycerol–When Is It an Intracellular Messenger?," *Biochimica et Biophysica Acta* 1436, no. 1–2 (1998): 117–126, https://doi.org/10.1016/s0005-2760(98)00123-4.

54. T. C. Kenny, A. Khan, Y. Son, et al., "Integrative Genetic Analysis Identifies FLVCR1 as a Plasma-Membrane Choline Transporter in Mammals," *Cell Metabolism* 35, no. 6 (2023): 1057–1071, https://doi.org/10.1016/j.cmet.2023.04.003.

55. Y. Son, T. C. Kenny, A. Khan, K. Birsoy, and R. K. Hite, "Structural Basis of Lipid Head Group Entry to the Kennedy Pathway by FLVCR1," *Nature* 629, no. 8012 (2024): 710–716, https://doi.org/10.1038/s41586-024-07374-4.

56. A. Taylor, S. Grapentine, J. Ichhpuniani, and M. Bakovic, "Choline Transporter-Like Proteins 1 and 2 Are Newly Identified Plasma Membrane and Mitochondrial Ethanolamine Transporters," *Journal of Biological Chemistry* 296 (2021): 100604, https://doi.org/10.1016/j.jbc.2021.100604.

57. C. R. Fagerberg, A. Taylor, F. Distelmaier, et al., "Choline Transporter-Like 1 Deficiency Causes a New Type of Childhood-Onset Neurodegeneration," *Brain* 143, no. 1 (2020): 94–111, https://doi.org/10.1093/brain/awz376.

58. T. Harayama and H. Riezman, "Understanding the Diversity of Membrane Lipid Composition," *Nature Reviews. Molecular Cell Biology* 19, no. 5 (2018): 281–296, https://doi.org/10.1038/nrm.2017.138.

59. L. N. Nguyen, D. Ma, G. Shui, et al., "Mfsd2a Is a Transporter for the Essential Omega-3 Fatty Acid Docosahexaenoic Acid," *Nature* 509, no. 7501 (2014): 503–506, https://doi.org/10.1038/nature13241.

60. V. Alakbarzade, A. Hameed, D. Q. Quek, et al., "A Partially Inactivating Mutation in the Sodium-Dependent Lysophosphatidylcholine Transporter MFSD2A Causes a Non-Lethal Microcephaly Syndrome," *Nature Genetics* 47, no. 7 (2015): 814–817, https://doi.org/10.1038/ng.3313.

61. A. Guemez-Gamboa, L. N. Nguyen, H. Yang, et al., "Inactivating Mutations in MFSD2A, Required for Omega-3 Fatty Acid Transport in Brain, Cause a Lethal Microcephaly Syndrome," *Nature Genetics* 47, no. 7 (2015): 809–813, https://doi.org/10.1038/ng.3311.

62. D. Patel and S. N. Witt, "Ethanolamine and Phosphatidylethanolamine: Partners in Health and Disease," *Oxidative Medicine and Cellular Longevity* 2017 (2017): 4829180, https://doi.org/10.1155/2017/4829180.

63. E. Calzada, O. Onguka, and S. M. Claypool, "Phosphatidylethanolamine Metabolism in Health and Disease," *International Review of Cell and Molecular Biology* 321 (2016): 29–88, https://doi.org/10.1016/bs.ircmb.2015.10.001.

64. A. S. Sarma, B. Siddardha, P. L. T, P. Ranganath, and A. Dalal, "A Novel Homozygous Synonymous Splicing Variant in SELENOI Gene Causes Spastic Paraplegia 81," *Journal of Gene Medicine* 25, no. 7 (2023): e3501, https://doi.org/10.1002/jgm.3501.

65. J. E. Vance and G. Tasseva, "Formation and Function of Phosphatidylserine and Phosphatidylethanolamine in Mammalian Cells," *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1831 (2013): 543–554, https://doi.org/10.1016/j.bbalip.2012.08.016.

66. M. G. Acoba, N. Senoo, and S. M. Claypool, "Phospholipid Ebb and Flow Makes Mitochondria Go," *Journal of Cell Biology* 219, no. 8 (2020): e202003131, https://doi.org/10.1083/jcb.202003131.

67. M. St Germain, R. Iraji, and M. Bakovic, "Phosphatidylethanolamine Homeostasis Under Conditions of Impaired CDP-Ethanolamine Pathway or Phosphatidylserine Decarboxylation," *Frontiers in Nutrition* 9 (2023): 1094273, https://doi.org/10.3389/fnut.2022.1094273.

68. Y. Horibata, O. Elpeleg, A. Eran, et al., "EPT1 (Selenoprotein I) is Critical for the Neural Development and Maintenance of Plasmalogen in Humans," *Journal of Lipid Research* 59, no. 6 (2018): 1015–1026, https://doi.org/10.1194/jlr.P081620.

69. L. Svennerholm, "Distribution and Fatty Acid Composition of Phosphoglycerides in Normal Human Brain," *Journal of Lipid Research* 9 (1968): 570–579.

70. J. E. Vance and R. Steenbergen, "Metabolism and Functions of Phosphatidylserine," *Progress in Lipid Research* 44, no. 4 (2005): 207–234, https://doi.org/10.1016/j.plipres.2005.05.001.

71. Y. Ge, M. Huang, and Y. M. Yao, "Efferocytosis and Its Role in Inflammatory Disorders," *Frontiers in Cell and Development Biology* 10 (2022): 839248, https://doi.org/10.3389/fcell.2022.839248.

72. K. Segawa, S. Kurata, Y. Yanagihashi, T. R. Brummelkamp, F. Matsuda, and S. Nagata, "Caspase-Mediated Cleavage of Phospholipid Flippase for Apoptotic Phosphatidylserine Exposure," *Science* 344, no. 6188 (2014): 1164–1168, https://doi.org/10.1126/science.1252809.

73. R. F. Zwaal, P. Comfurius, and E. M. Bevers, "Scott Syndrome, a Bleeding Disorder Caused by Defective Scrambling of Membrane Phospholipids," *Biochimica et Biophysica Acta* 1636, no. 2–3 (2004): 119–128, https://doi.org/10.1016/j.bbalip.2003.07.003.

74. C. J. Swanson, R. F. Sommese, K. J. Petersen, et al., "Calcium Stimulates Self-Assembly of Protein Kinase C α In Vitro," *PLoS One* 11, no. 10 (2016): e0162331, https://doi.org/10.1371/journal.pone.0162331.

75. K. D'Souza and R. M. Epand, "Enrichment of Phosphatidylinositols With Specific Acyl Chains," *Biochimica et Biophysica Acta - Biomembranes* 1838 (2014): 1501–1508, https://doi.org/10.1016/j.bbamem.2013.10.003.

76. M. Bektas, S. G. Payne, H. Liu, S. Goparaju, S. Milstien, and S. Spiegel, "A Novel Acylglycerol Kinase That Produces Lysophosphatidic Acid Modulates Cross Talk With EGFR in Prostate Cancer Cells," *Journal of Cell Biology* 169, no. 5 (2005): 801–811, https://doi.org/10.1083/jcb.200407123.

77. C. U. Mårtensson and T. Becker, "Acylglycerol Kinase: Mitochondrial Protein Transport Meets Lipid Biosynthesis," *Trends in Cell Biology* 27, no. 10 (2017): 700–702, https://doi.org/10.1016/j.tcb.2017.08.006.

78. C. Potting, T. Tatsuta, T. König, et al., "TRIAP1/PRELI Complexes Prevent Apoptosis by Mediating Intramitochondrial Transport of Phosphatidic Acid," *Cell Metabolism* 18, no. 2 (2013): 287–295, https://doi.org/10.1016/j.cmet.2013.07.008.

79. Y. Tamura, Y. Harada, S. Nishikawa, et al., "Tam41 Is a CDP-Diacylglycerol Synthase Required for Cardiolipin Biosynthesis in Mitochondria," *Cell Metabolism* 17, no. 5 (2013): 709–718, https://doi.org/10.1016/j.cmet.2013.03.018.

80. J. Chen, A. Cazenave-Gassiot, Y. Xu, et al., "Lysosomal Phospholipase A2 Contributes to the Biosynthesis of the Atypical Late Endosome Lipid Bis(Monoacylglycero)phosphate," *Communications Biology* 6, no. 1 (2023): 210, https://doi.org/10.1038/s42003-023-04573-z.

81. H. Sun, J. Zhang, Q. Ye, et al., "LPGAT1 Controls MEGDEL Syndrome by Coupling Phosphatidylglycerol Remodeling With Mitochondrial Transport," *Cell Reports* 42, no. 11 (2023): 113214, https://doi.org/10.1016/j.celrep.2023.113214.

82. H. Fang, A. Xie, M. Du, et al., "SERAC1 Is a Component of the Mitochondrial Serine Transporter Complex Required for the Maintenance of Mitochondrial DNA," *Science Translational Medicine* 14, no. 634 (2022): eabl6992, https://doi.org/10.1126/scitranslmed.abl6992.

83. B. Wang and P. Tontonoz, "Phospholipid Remodeling in Physiology and Disease," *Annual Review of Physiology* 81 (2019): 165–188, https://doi.org/10.1146/annurev-physiol-020518-114444.

84. H. Kawana, M. Ozawa, T. Shibata, et al., "Identification and Characterization of LPLAT7 as an Sn-1-Specific Lysophospholipid Acyltransferase," *Journal of Lipid Research* 63, no. 10 (2022): 100271, https://doi.org/10.1016/j.jlr.2022.100271.

85. D. Hishikawa, T. Hashidate, T. Shimizu, and H. Shindou, "Diversity and Function of Membrane Glycerophospholipids Generated by the Remodeling Pathway in Mammalian Cells," *Journal of Lipid Research* 55, no. 5 (2014): 799–807, https://doi.org/10.1194/jlr.R046094.

86. V. B. O'Donnell, "New Appreciation for an Old Pathway: The Lands Cycle Moves Into New Arenas in Health and Disease," *Biochemical Society Transactions* 50, no. 1 (2022): 1–11, https://doi.org/10.1042/BST20210579.

87. E. Meyer, C. Ricketts, N. V. Morgan, et al., "Mutations in FLVCR2 Are Associated With Proliferative Vasculopathy and Hydranencephaly-Hydrocephaly Syndrome (Fowler Syndrome)," *American Journal of Human Genetics* 86, no. 3 (2010): 471–478, https://doi.org/10.1016/j.ajhg.2010.02.004.

88. M. Kvarnung, F. Taylan, D. Nilsson, et al., "Mutations in FLVCR2 Associated With Fowler Syndrome and Survival Beyond Infancy," *Clinical Genetics* 89, no. 1 (2016): 99–103, https://doi.org/10.1111/cge.12565.

89. C. Klöckner, J. P. Fernández-Murray, M. Tavasoli, et al., "Bi-Allelic Variants in CHKA Cause a Neurodevelopmental Disorder With Epilepsy and Microcephaly," *Brain* 145, no. 6 (2022): 1916–1923, https://doi.org/10.1093/brain/awac074.

90. S. Mitsuhashi, A. Ohkuma, B. Talim, et al., "A Congenital Muscular Dystrophy With Mitochondrial Structural Abnormalities Caused by Defective de Novo Phosphatidylcholine Biosynthesis," *American Journal of Human Genetics* 88, no. 6 (2011): 845–851, https://doi.org/10.1016/j.ajhg.2011.05.010.

91. F. Magri, S. Antognozzi, M. Ripolone, et al., "Megaconial Congenital Muscular Dystrophy due to Novel CHKB Variants: A Case Report and Literature Review," *Skeletal Muscle* 12, no. 1 (2022): 23, https://doi.org/10.1186/s13395-022-00306-8.

92. S. H. S. Chan and I. Nishino, "CHKB-Related Muscular Dystrophy," in *Gene Reviews*, ed. M. P. Adam, J. Feldman, G. M. Mirzaa, et al. (University of Washington, 2023).

93. J. Hoover-Fong, N. Sobreira, J. Jurgens, et al., "Mutations in PCYT1A, Encoding a Key Regulator of Phosphatidylcholine Metabolism, Cause Spondylometaphyseal Dysplasia With Cone-Rod Dystrophy," *American Journal of Human Genetics* 94, no. 1 (2014): 105–112, https://doi.org/10.1016/j.ajhg.2013.11.018.

94. G. L. Yamamoto, W. A. Baratela, T. F. Almeida, et al., "Mutations in PCYT1A Cause Spondylometaphyseal Dysplasia With Cone-Rod Dystrophy," *American Journal of Human Genetics* 94, no. 1 (2014): 113–119, https://doi.org/10.1016/j.ajhg.2013.11.022.

95. F. Payne, K. Lim, A. Girousse, et al., "Mutations Disrupting the Kennedy Phosphatidylcholine Pathway in Humans With Congenital Lipodystrophy and Fatty Liver Disease," *Proceedings of the National Academy of Sciences of the United States of America* 111, no. 24 (2014): 8901–8906, https://doi.org/10.1073/pnas.1408523111.

96. F. Testa, M. Filippelli, R. Brunetti-Pierri, et al., "Mutations in the PCYT1A Gene Are Responsible for Isolated Forms of Retinal Dystrophy," *European Journal of Human Genetics* 25, no. 5 (2017): 651–655, https://doi.org/10.1038/ejhg.2017.23.

97. L. Leonardis, M. Skrjanec Pusenjak, A. Maver, et al., "Axonal Polyneuropathy in 2 Brothers With a Homozygous Missense Variant in the First Catalytic Domain of PCYT2," *Neurology Genetics* 8, no. 2 (2022): e658, https://doi.org/10.1212/NXG.0000000000000658.

98. F. M. Vaz, J. H. McDermott, M. Alders, et al., "Mutations in PCYT2 Disrupt Etherlipid Biosynthesis and Cause a Complex Hereditary Spastic Paraplegia," *Brain* 142, no. 11 (2019): 3382–3397, https://doi.org/10.1093/brain/awz291.

99. V. Vélez-Santamaría, E. Verdura, C. Macmurdo, et al., "Expanding the Clinical and Genetic Spectrum of PCYT2-Related Disorders," *Brain* 143, no. 9 (2020): e76, https://doi.org/10.1093/brain/awaa229.

100. M. Bakovic, M. D. Fullerton, and V. Michel, "Metabolic and Molecular Aspects of Ethanolamine Phospholipid Biosynthesis: The Role of CTP:Phosphoethanolamine Cytidylyltransferase (Pcyt2)," *Biochemistry and Cell Biology* 85, no. 3 (2007): 283–300, https://doi.org/10.1139/o07-006.

101. M. Y. Ahmed, A. Al-Khayat, F. Al-Murshedi, et al., "A Mutation of EPT1 (SELENOI) Underlies a New Disorder of Kennedy Pathway Phospholipid Biosynthesis," *Brain* 140, no. 3 (2017): 547–554, https://doi.org/10.1093/brain/aww318.

102. R. Kaiyrzhanov, S. Wortmann, T. Reid, et al., "Defective Phosphatidylethanolamine Biosynthesis Leads to a Broad Ataxia-Spasticity Spectrum," *Brain* 144, no. 3 (2021): e30, https://doi.org/10.1093/brain/awaa442.

103. M. A. E. Cruz, C. R. Ferreira, C. B. Tovani, et al., "Phosphatidylserine Controls Calcium Phosphate Nucleation and Growth on Lipid Monolayers: A Physicochemical Understanding of Matrix Vesicle-Driven Biomineralization," *Journal of Structural Biology* 212, no. 2 (2020): 107607, https://doi.org/10.1016/j.jsb.2020.107607.

104. B. D. Boyan, N. C. Asmussen, Z. Lin, and Z. Schwartz, "The Role of Matrix-Bound Extracellular Vesicles in the Regulation of Endochondral Bone Formation," *Cells* 11, no. 10 (2022): 1619, https://doi.org/10.3390/cells11101619.

105. A. Merolli and M. Santin, "Role of Phosphatidyl-Serine in Bone Repair and Its Technological Exploitation," *Molecules* 14, no. 12 (2009): 5367–5381, https://doi.org/10.3390/molecules14125367.

106. S. Sugahara, Y. Ishino, K. Sawada, et al., "Disease-Related PSS1 Mutant Impedes the Formation and Function of Osteoclasts," *Journal of Lipid Research* 64, no. 11 (2023): 100443, https://doi.org/10.1016/j.jlr.2023.10044.

107. S. A. Lieffrig, G. Gyimesi, Y. Mao, and S. C. Finnemann, "Clearance Phagocytosis by the Retinal Pigment Epithelial During Photoreceptor Outer Segment Renewal: Molecular Mechanisms and Relation to Retinal Inflammation," *Immunological Reviews* 319, no. 1 (2023): 81–99, https://doi.org/10.1111/imr.13264.

108. S. B. Sousa, D. Jenkins, E. Chanudet, et al., "Gain-of-Function Mutations in the Phosphatidylserine Synthase 1 (PTDSS1) Gene Cause Lenz-Majewski Syndrome," *Nature Genetics* 46, no. 1 (2014): 70–76, https://doi.org/10.1038/ng.2829.

109. J. Piard, J. Lespinasse, M. Vlckova, et al., "Cutis Laxa and Excessive Bone Growth due to de Novo Mutations in PTDSS1," *American Journal of Medical Genetics. Part A* 176, no. 3 (2018): 668–675, https://doi.org/10.1002/ajmg.a.38604.

110. H. H. Afifi, M. S. Abdel-Hamid, M. I. Mehrez, G. el-Kamah, and G. M. H. Abdel-Salam, "Lenz-Majewski Syndrome in a Patient From Egypt," *American Journal of Medical Genetics. Part A* 179, no. 10 (2019): 2039–2042, https://doi.org/10.1002/ajmg.a.61327.

111. S. Gracie, N. Sengupta, C. Ferreira, et al., "De Novo Loss-of-Function Variant in PTDSS1 Is Associated With Developmental Delay," *American Journal of Medical Genetics. Part A* 188, no. 6 (2022): 1739–1745, https://doi.org/10.1002/ajmg.a.62695.

112. M. Sohn and T. Balla, "Lenz-Majewski Syndrome: How a Single Mutation Leads to Complex Changes in Lipid Metabolism," *Journal of Rare Diseases Research & Treatment* 2, no. 1 (2016): 47–51, https://doi.org/10.29245/2572-9411/2017/1.1080.

113. K. M. Girisha, L. von Elsner, K. Neethukrishna, et al., "The Homozygous Variant c.797G>A/p.(Cys266Tyr) in PISD Is Associated With a Spondyloepimetaphyseal Dysplasia With Large Epiphyses and Disturbed Mitochondrial Function," *Human Mutation* 40, no. 3 (2019): 299–309, https://doi.org/10.1002/humu.23693.

114. V. G. Peter, M. Quinodoz, J. Pinto-Basto, et al., "The Liberfarb Syndrome, a Multisystem Disorder Affecting Eye, Ear, Bone, and Brain Development, Is Caused by a Founder Pathogenic Variant in the PISD Gene," *Genetics in Medicine* 21, no. 12 (2019): 2734–2743, https://doi.org/10.1038/s41436-019-0595-x.

115. L. Aagaard Nolting, T. Holling, G. Nishimura, et al., "Novel Biallelic PISD Missense Variants Cause Spondyloepimetaphyseal Dysplasia With Disproportionate Short Stature and Fragmented Mitochondrial Morphology," *Clinical Genetics* 106, no. 3 (2024): 360–366, https://doi.org/10.1111/cge.14549.

116. T. Zhao, C. M. Goedhart, P. N. Sam, et al., "PISD Is a Mitochondrial Disease Gene Causing Skeletal Dysplasia, Cataracts, and White Matter Changes," *Life Science Alliance* 2, no. 2 (2019): e201900353, https://doi.org/10.26508/lsa.201900353.

117. M. A. Frohman, "Role of Mitochondrial Lipids in Guiding Fission and Fusion," *Journal of Molecular Medicine (Berlin, Germany)* 93, no. 3 (2015): 263–269, https://doi.org/10.1007/s00109-014-1237-z.

118. R. C. Sengers, J. M. Trijbels, J. L. Willems, O. Daniels, and A. M. Stadhouders, "Congenital Cataract and Mitochondrial Myopathy of Skeletal and Heart Muscle Associated With Lactic Acidosis After Exercise," *Journal of Pediatrics* 86, no. 6 (1975): 873–880, https://doi.org/10.1016/s0022-3476(75)80217-4.

119. J. A. Mayr, T. B. Haack, E. Graf, et al., "Lack of the Mitochondrial Protein Acylglycerol Kinase Causes Sengers Syndrome," *American Journal of Human Genetics* 90, no. 2 (2012): 314–320, https://doi.org/10.1016/j.ajhg.2011.12.005.

120. A. Haghighi, T. B. Haack, M. Atiq, et al., "Sengers Syndrome: Six Novel AGK Mutations in Seven New Families and Review of the Phenotypic and Mutational Spectrum of 29 Patients," *Orphanet Journal of Rare Diseases* 9 (2014): 119, https://doi.org/10.1186/s13023-014-0119-3.

121. D. B. Beck, K. Cusmano-Ozog, N. Andescavage, and E. Leon, "Extending the Phenotypic Spectrum of Sengers Syndrome: Congenital Lactic Acidosis With Synthetic Liver Dysfunction," *Translational Science of Rare Diseases* 3, no. 1 (2018): 45–48, https://doi.org/10.3233/TRD-180020.

122. S. M. Houten, "Protein Moonlighting in Inborn Errors of Metabolism: The Case of the Mitochondrial Acylglycerol Kinase," *Journal of Inherited Metabolic Disease* 40, no. 6 (2017): 755–756, https://doi.org/10.1007/s10545-017-0090-y.

123. M. Falabella, C. Pizzamiglio, L. C. Tabara, et al., "Biallelic PTPMT1 Variants Disrupt Cardiolipin Metabolism and Lead to a Neurodevelopmental Syndrome," *Brain* 148, no. 2 (2024): 647–662, https://doi.org/10.1093/brain/awae268.

124. D. J. Pagliarini, C. A. Worby, and J. E. Dixon, "A PTEN-Like Phosphatase With a Novel Substrate Specificity," *Journal of Biological Chemistry* 279, no. 37 (2004): 38590–38596, https://doi.org/10.1074/jbc.M404959200.

125. H. Zheng, Q. Li, S. Li, et al., "Loss of Ptpmt1 Limits Mitochondrial Utilization of Carbohydrates and Leads to Muscle Atrophy and Heart Failure in Tissue-Specific Knockout Mice," *eLife* 12 (2023): RP86944, https://doi.org/10.7554/eLife.86944.

126. S. B. Wortmann, M. Espeel, L. Almeida, et al., "Inborn Errors of Metabolism in the Biosynthesis and Remodelling of Phospholipids," *Journal of Inherited Metabolic Disease* 38 (2015): 99–110, https://doi.org/10.1007/s10545-014-9759-7.

127. O. Sarig, D. Goldsher, J. Nousbeck, et al., "Infantile Mitochondrial Hepatopathy Is a Cardinal Feature of MEGDEL Syndrome (3-Methylglutaconic Aciduria Type IV With Sensorineural Deafness, Encephalopathy and Leigh-Like Syndrome) Caused by Novel Mutations in SERAC1," *American Journal of Medical Genetics. Part A* 161A, no. 9 (2013): 2204–2215, https://doi.org/10.1002/ajmg.a.36059.

128. R. R. Maas, K. Iwanicka-Pronicka, S. Kalkan Ucar, et al., "Progressive Deafness-Dystonia due to SERAC1 Mutations: A Study of 67 Cases," *Annals of Neurology* 82, no. 6 (2017): 1004–1015, https://doi.org/10.1002/ana.25110.

129. S. B. Wortmann, F. M. Vaz, T. Gardeitchik, et al., "Mutations in the Phospholipid Remodeling Gene SERAC1 Impair Mitochondrial Function and Intracellular Cholesterol Trafficking and Cause Dystonia and Deafness," *Nature Genetics* 44, no. 7 (2012): 797–802, https://doi.org/10.1038/ng.2325.

130. B. Roeben, R. Schule, S. Ruf, et al., "SERAC1 Deficiency Causes Complicated HSP: Evidence From a Novel Splice Mutation in a Large Family," *Journal of Medical Genetics* 55, no. 1 (2018): 39–47, https://doi.org/10.1136/jmedgenet-2017-104622.

131. R. G. Lee, S. Balasubramaniam, M. Stentenbach, et al., "Deleterious Variants in CRLS1 Lead to Cardiolipin Deficiency and Cause an Autosomal Recessive Multi-System Mitochondrial Disease," *Human Molecular Genetics* 31, no. 21 (2022): 3597–3612, https://doi.org/10.1093/hmg/ddac040.

132. K. Koh, Y. Ichinose, H. Ishiura, et al., "PLA2G6-Associated Neurodegeneration Presenting as a Complicated Form of Hereditary Spastic Paraplegia," *Journal of Human Genetics* 64, no. 1 (2019): 55–59, https://doi.org/10.1038/s10038-018-0519-7.

133. B. Ozes, N. Karagoz, R. Schüle, et al., "PLA2G6 Mutations Associated With a Continuous Clinical Spectrum From Neuroaxonal Dystrophy to Hereditary Spastic Paraplegia," *Clinical Genetics* 92, no. 5 (2017): 534–539, https://doi.org/10.1111/cge.13008.

134. P. S. Atwal, M. Midei, D. Adams, A. Fay, F. Heerinckx, and P. Milner, "The Infantile Neuroaxonal Dystrophy Rating Scale (INAD-RS)," *Orphanet Journal of Rare Diseases* 15, no. 1 (2020): 195, https://doi.org/10.1186/s13023-020-01479-5.

135. J. Aicardi and P. Castelein, "Infantile Neuroaxonal Dystrophy," *Brain* 102, no. 4 (1979): 727–748, https://doi.org/10.1093/brain/102.4.727.

136. N. Nardocci, G. Zorzi, L. Farina, et al., "Infantile Neuroaxonal Dystrophy: Clinical Spectrum and Diagnostic Criteria," *Neurology* 52, no. 7 (1999): 1472–1478, https://doi.org/10.1212/wnl.52.7.1472.

137. C. Paisán-Ruiz, A. Li, S. A. Schneider, et al., "Widespread Lewy Body and Tau Accumulation in Childhood and Adult Onset Dystonia-Parkinsonism Cases With PLA2G6 Mutations," *Neurobiology of Aging* 33, no. 4 (2012): 814–823, https://doi.org/10.1016/j.neurobiolaging.2010.05.009.

138. N. V. Morgan, S. K. Westaway, J. E. Morton, et al., "PLA2G6, Encoding a Phospholipase A2, Is Mutated in Neurodegenerative Disorders With High Brain Iron," *Nature Genetics* 38, no. 7 (2006): 752–754, https://doi.org/10.1038/ng1826.

139. C. C. Chiu, C. S. Lu, Y. H. Weng, et al., "PARK14 (D331Y) PLA2G6 Causes Early-Onset Degeneration of Substantia Nigra Dopaminergic Neurons by Inducing Mitochondrial Dysfunction, ER Stress, Mitophagy Impairment and Transcriptional Dysregulation in a Knockin Mouse Model," *Molecular Neurobiology* 56, no. 6 (2019): 3835–3853, https://doi.org/10.1007/s12035-018-1118-5.

140. M. Gafner, M. Michelson, K. Yosovich, L. Blumkin, T. Lerman-Sagie, and D. Lev, "Infantile Onset Progressive Cerebellar Atrophy and Anterior Horn Cell Degeneration-A Novel Phenotype Associated With Mutations in the PLA2G6 Gene," *European Journal of Medical Genetics* 63, no. 4 (2020): 103801, https://doi.org/10.1016/j.ejmg.2019.103801.

141. S. Khateeb, H. Flusser, R. Ofir, et al., "PLA2G6 Mutation Underlies Infantile Neuroaxonal Dystrophy," *American Journal of Human Genetics* 79, no. 5 (2006): 942–948, https://doi.org/10.1086/508572.

142. L. A. Engel, Z. Jing, D. E. O'Brien, M. Sun, and P. T. Kotzbauer, "Catalytic Function of PLA2G6 Is Impaired by Mutations Associated With Infantile Neuroaxonal Dystrophy but Not Dystonia-Parkinsonism," *PLoS One* 5, no. 9 (2010): e12897, https://doi.org/10.1371/journal.pone.0012897.

143. J. Xue, D. X. Ding, G. Y. Xu, et al., "A Systematic Analysis of Genotype-Phenotype Associations With PLA2G6," *Parkinsonism & Related Disorders* 112 (2023): 105477, https://doi.org/10.1016/j.parkreldis.2023.

144. F. Magrinelli, S. Mehta, G. Di Lazzaro, et al., "Dissecting the Phenotype and Genotype of PLA2G6-Related Parkinsonism," *Movement Disorders* 37, no. 1 (2022): 148–161, https://doi.org/10.1002/mds.28807.

145. S. Rainier, M. Bui, E. Mark, et al., "Neuropathy Target Esterase Gene Mutations Cause Motor Neuron Disease," *American Journal of Human Genetics* 82, no. 3 (2008): 780–785, https://doi.org/10.1016/j.ajhg.2007.12.018.

146. M. Synofzik, M. A. Gonzalez, C. M. Lourenco, et al., "PNPLA6 Mutations Cause Boucher-Neuhauser and Gordon Holmes Syndromes as Part of a Broad Neurodegenerative Spectrum," *Brain* 137, no. 1 (2013): 69–77, https://doi.org/10.1093/brain/awt326.

147. R. B. Hufnagel, G. Arno, N. D. Hein, et al., "Neuropathy Target Esterase Impairments Cause Oliver-McFarlane and Laurence-Moon Syndromes," *Journal of Medical Genetics* 52, no. 2 (2015): 85–94, https://doi.org/10.1136/jmedgenet-2014-102856.

148. P. A. Chang and Y. J. Wu, "Neuropathy Target Esterase: An Essential Enzyme for Neural Development and Axonal Maintenance," *International Journal of Biochemistry & Cell Biology* 42, no. 5 (2010): 573–575, https://doi.org/10.1016/j.biocel.2009.

149. J. P. Fernández-Murray and C. R. McMaster, "Phosphatidylcholine Synthesis and Its Catabolism by Yeast Neuropathy Target Esterase 1," *Biochimica et Biophysica Acta* 1771, no. 3 (2007): 331–336, https://doi.org/10.1016/j.bbalip.2006.04.004.

150. C. Tesson, M. Nawara, M. A. Salih, et al., "Alteration of Fatty-Acid-Metabolizing Enzymes Affects Mitochondrial Form and Function in Hereditary Spastic Paraplegia," *American Journal of Human Genetics* 91, no. 6 (2012): 1051–1064, https://doi.org/10.1016/j.ajhg.2012.11.001.

151. R. Liguori, M. P. Giannoccaro, A. Arnoldi, et al., "Impairment of Brain and Muscle Energy Metabolism Detected by Magnetic Resonance Spectroscopy in Hereditary Spastic Paraparesis Type 28 Patients With DDHD1 Mutations," *Journal of Neurology* 261, no. 9 (2014): 1789–1793, https://doi.org/10.1007/s00415-014-7418-4.

152. R. Dard, C. Meyniel, V. Touitou, et al., "Mutations in DDHD1, Encoding a Phospholipase A1, Is a Novel Cause of Retinopathy and Neurodegeneration With Brain Iron Accumulation," *European Journal of Medical Genetics* 60, no. 12 (2017): 639–642, https://doi.org/10.1016/j.ejmg.2017.08.015.

153. J. M. Inloes, H. Jing, and B. F. Cravatt, "The Spastic Paraplegia-Associated Phospholipase DDHD1 Is a Primary Brain Phosphatidylinositol Lipase," *Biochemistry* 57, no. 39 (2018): 5759–5767, https://doi.org/10.1021/acs.biochem.8b00810.

154. A. Mignarri, A. Rubegni, A. Tessa, et al., "Mitochondrial Dysfunction in Hereditary Spastic Paraparesis With Mutations in DDHD1/SPG28," *Journal of the Neurological Sciences* 362 (2016): 287–291, https://doi.org/10.1016/j.jns.2016.02.007.

155. J. H. Schuurs-Hoeijmakers, A. T. van Vulto- Silfhout, L. E. L. M. Vissers, et al., "Identification of Pathogenic Gene Variants in Small Families With Intellectually Disabled Siblings by Exome Sequencing," *Journal of Medical Genetics* 50, no. 12 (2013): 802–811, https://doi.org/10.1136/jmedgenet-2013-101644.

156. F. Nicita, F. Stregapede, A. Tessa, et al., "Defining the Clinical-Genetic and Neuroradiological Features in SPG54: Description of Eight Additional Cases and Nine Novel DDHD2 Variants," *Journal of Neurology* 266, no. 11 (2019): 2657–2664, https://doi.org/10.1007/s00415-019-09466-y.

157. H. Doi, M. Ushiyama, T. Baba, et al., "Late-Onset Spastic Ataxia Phenotype in a Patient With a Homozygous DDHD2 Mutation," *Scientific Reports* 4 (2014): 7132, https://doi.org/10.1038/srep07132.

158. P. Hofer, G. F. Grabner, M. König, et al., "Cooperative Lipolytic Control of Neuronal Triacylglycerol by Spastic Paraplegia-Associated Enzyme DDHD2 and ATGL," *Journal of Lipid Research* 64, no. 11 (2023): 100457, https://doi.org/10.1016/j.jlr.2023.100457.

159. J. M. Inloes, K. L. Hsu, M. M. Dix, et al., "The Hereditary Spastic Paraplegia-Related Enzyme DDHD2 Is a Principal Brain Triglyceride Lipase," *Proceedings of the National Academy of Sciences of the United States of America* 111, no. 41 (2014): 14924–14929, https://doi.org/10.1073/pnas.1413706111.

160. F. Jia, X. Wang, Y. Fu, S. M. Zhao, B. Lu, and C. Wang, "DDHD2, Whose Mutations Cause Spastic Paraplegia Type 54, Enhances Lipophagy via Engaging ATG8 Family Proteins," *Cell Death and Differentiation* 31, no. 3 (2024): 348–359, https://doi.org/10.1038/s41418-024-01261-1.

161. L. Leonardi, L. Ziccardi, C. Marcotulli, et al., "Pigmentary Degenerative Maculopathy as Prominent Phenotype in an Italian SPG56/CYP2U1 Family," *Journal of Neurology* 263, no. 4 (2016): 781–783, https://doi.org/10.1007/s00415-016-8066-7.

162. G. Minase, S. Miyatake, S. Nabatame, et al., "An Atypical Case of SPG56/CYP2U1-Related Spastic Paraplegia Presenting With Delayed Myelination," *Journal of Human Genetics* 62, no. 11 (2017): 997–1000, https://doi.org/10.1038/jhg.2017.77.

163. M. Masciullo, A. Tessa, S. Perazza, F. M. Santorelli, A. Perna, and G. Silvestri, "Hereditary Spastic Paraplegia: Novel Mutations and Expansion of the Phenotype Variability in SPG56," *European Journal of Paediatric Neurology* 20, no. 3 (2016): 444–448, https://doi.org/10.1016/j.ejpn.2016.02.001.

164. A. Legrand, C. Pujol, C. M. Durand, et al., "Pseudoxanthoma Elasticum Overlaps Hereditary Spastic Paraplegia Type 56," *Journal of Internal Medicine* 289, no. 5 (2021): 709–725, https://doi.org/10.1111/joim.13193.

165. L. Ducassou, L. Dhers, G. Jonasson, et al., "Membrane-Bound Human Orphan Cytochrome P450 2U1: Sequence Singularities, Construction of a Full 3D Model, and Substrate Docking," *Biochimie* 140 (2017): 166–175, https://doi.org/10.1016/j.biochi.2017.07.007.

166. A. Bowron, J. Honeychurch, M. Williams, et al., "Barth Syndrome Without Tetralinoleoyl Cardiolipin Deficiency: A Possible Ameliorated Phenotype," *Journal of Inherited Metabolic Disease* 38, no. 2 (2015): 279–286, https://doi.org/10.1007/s10545-014-9747-y.

167. F. M. Vaz, H. van Lenthe, M. A. T. Vervaart, et al., "An Improved Functional Assay in Blood Spot to Diagnose Barth Syndrome Using the Monolysocardiolipin/Cardiolipin Ratio," *Journal of Inherited Metabolic Disease* 45, no. 1 (2022): 29–37, https://doi.org/10.1002/jimd.12425.

168. T. Morikawa, M. Takahashi, Y. Izumi, et al., "Oleic Acid-Containing Phosphatidylinositol Is a Blood Biomarker Candidate for SPG28," *Biomedicine* 11, no. 4 (2023): 1092, https://doi.org/10.3390/biomedicines11041092.

169. A. Meyyazhagan and A. Orlacchio, "Hereditary Spastic Paraplegia: An Update," *International Journal of Molecular Sciences* 23, no. 3 (2022): 1697, https://doi.org/10.3390/ijms23031697.

170. E. Panza, A. Meyyazhagan, and A. Orlacchio, "Hereditary Spastic Paraplegia: Genetic Heterogeneity and Common Pathways," *Experimental Neurology* 357 (2022): 114203, https://doi.org/10.1016/j.expneurol.2022.

171. O. J. Rickman, E. L. Baple, and A. H. Crosby, "Lipid Metabolic Pathways Converge in Motor Neuron Degenerative Diseases," *Brain* 143, no. 4 (2020): 1073–1087, https://doi.org/10.1093/brain/awz382.

172. J. Wang, X. Sun, L. Jiao, et al., "Clinical Characteristics and Variant Analyses of Transient Infantile Hypertriglyceridemia Related to GPD1 Gene," *Frontiers in Genetics* 13 (2022): 916672, https://doi.org/10.3389/fgene.2022.916672.

173. I. Lamiquiz-Moneo, R. Mateo-Gallego, J. Fernández-Pardo, et al., "Glycerol Kinase Deficiency in Adults: Description of 4 Novel Cases, Systematic Review and Development of a Clinical Diagnostic Score," *Atherosclerosis* 315 (2020): 24–32, https://doi.org/10.1016/j.atherosclerosis.2020.10.897.

174. E. I. Ginns, J. A. Barranger, S. W. McClean, et al., "A Juvenile Form of Glycerol Kinase Deficiency With Episodic Vomiting, Acidemia, and Stupor," *Journal of Pediatrics* 104, no. 5 (1984): 736–739, https://doi.org/10.1016/s0022-3476(84)80956-7.

175. A. K. Agarwal, E. Arioglu, S. De Almeida, et al., "AGPAT2 Is Mutated in Congenital Generalized Lipodystrophy Linked to Chromosome 9q34," *Nature Genetics* 31, no. 1 (2002): 21–23, https://doi.org/10.1038/ng880.

176. Y. Li, X. Yang, L. Peng, et al., "Role of Seipin in Human Diseases and Experimental Animal Models," *Biomolecules* 12, no. 6 (2022): 840, https://doi.org/10.3390/biom12060840.

177. C. Michot, L. Hubert, N. B. Romero, et al., "Study of LPIN1, LPIN2 and LPIN3 in Rhabdomyolysis and Exercise-Induced Myalgia," *Journal of Inherited Metabolic Disease* 35, no. 6 (2012): 1119–1128, https://doi.org/10.1007/s10545-012-9461-6.

178. D. L. Narayanan, K. S. Gogineni, and V. A. Badiger, "*LPIN2*-Related Majeed Syndrome," in *GeneReviews®*, ed. M. P. Adam, J. Feldman, G. M. Mirzaa, et al. (University of Washington, 2023).

179. K. Azukaitis, E. Simkova, M. A. Majid, et al., "The Phenotypic Spectrum of Nephropathies Associated With Mutations in Diacylglycerol Kinase ε," *Journal of the American Society of Nephrology* 28, no. 10 (2017): 3066–3075, https://doi.org/10.1681/ASN.2017010031.

180. S. Sánchez-Iglesias, A. Fernández-Pombo, S. Cobelo-Gómez, et al., "Celia's Encephalopathy (*BSCL2*-Gene-Related): Current Understanding," *Journal of Clinical Medicine* 10, no. 7 (2021): 1435, https://doi.org/10.3390/jcm10071435.

181. K. Thompson, L. Bianchi, F. Rastelli, et al., "Biallelic Variants in *TAMM41* Are Associated With Low Muscle Cardiolipin Levels, Leading to Neonatal Mitochondrial Disease," *Human Genetics and Genomics Advances* 3, no. 2 (2022): 100097, https://doi.org/10.1016/j.xhgg.2022.10009.

182. T. Fiskerstrand, D. H'mida-Ben Brahim, S. Johansson, et al., "Mutations in ABHD12 Cause the Neurodegenerative Disease PHARC: An Inborn Error of Endocannabinoid Metabolism," *American Journal of Human Genetics* 87, no. 3 (2010): 410–417, https://doi.org/10.1016/j.ajhg.2010.08.002.

183. M. S. Abdel-Hamid, G. M. H. Abdel-Salam, S. F. Abdel-Ghafar, and M. S. Zaki, "Delineating the Phenotype of PNPLA8-Related Mitochondriopathies," *Clinical Genetics* 105, no. 1 (2024): 92–98, https://doi.org/10.1111/cge.14421.

184. B. Burnyte, R. Vilimiene, K. Grigalioniene, I. Adomaitiene, and A. Utkus, "Cerebellar Ataxia and Peripheral Neuropathy in a Family With *PNPLA8*-Associated Disease," *Neurology Genetics* 9, no. 3 (2023): e200068, https://doi.org/10.1212/NXG.0000000000200068.

185. A. Johansen, R. O. Rosti, D. Musaev, et al., "Mutations in MBOAT7, Encoding Lysophosphatidylinositol Acyltransferase I, Lead to Intellectual Disability Accompanied by Epilepsy and Autistic Features," *American Journal of Human Genetics* 99, no. 4 (2016): 912–916, https://doi.org/10.1016/j.ajhg.2016.07.019.

186. N. Arashiki, K. Niitsuma, M. Seki, Y. Takakuwa, and F. Nakamura, "ATP11C T418N, a Gene Mutation Causing Congenital Hemolytic Anemia, Reduces Flippase Activity due to Improper Membrane Trafficking," *Biochemical and Biophysical Research Communications* 516, no. 3 (2019): 705–712, https://doi.org/10.1016/j.bbrc.2019.06.092.

187. J. Suzuki, M. Umeda, P. J. Sims, and S. Nagata, "Calcium-Dependent Phospholipid Scrambling by TMEM16F," *Nature* 468, no. 7325 (2010): 834–838, https://doi.org/10.1038/nature09583.

188. J. A. Pater, C. Penney, D. D. O'Rielly, et al., "Autosomal Dominant Non-Syndromic Hearing Loss Maps to DFNA33 (13q34) and Co-Segregates With Splice and Frameshift Variants in ATP11A, a Phospholipid Flippase Gene," *Human Genetics* 141, no. 3–4 (2022): 431–444, https://doi.org/10.1007/s00439-022-02444-x.

189. K. Segawa, A. Kikuchi, T. Noji, et al., "A Sublethal ATP11A Mutation Associated With Neurological Deterioration Causes Aberrant Phosphatidylcholine Flipping in Plasma Membranes," *Journal of Clinical Investigation* 131, no. 18 (2021): e148005, https://doi.org/10.1172/JCI148005.

190. M. Kurban, M. Wajid, Y. Shimomura, and A. M. Christiano, "Mutations in LPAR6/P2RY5 and LIPH Are Associated With Woolly Hair and/or Hypotrichosis," *Journal of the European Academy of Dermatology and Venereology* 27, no. 5 (2013): 545–549, https://doi.org/10.1111/j.1468-3083.2012.04472.x.

191. O. E. Onat, S. Gulsuner, K. Bilguvar, et al., "Missense Mutation in the ATPase, Amino-Phospholipid Transporter Protein ATP8A2 Is Associated With Cerebellar Atrophy and Quadrupedal Locomotion," *European Journal of Human Genetics* 21, no. 3 (2013): 281–285, https://doi.org/10.1038/ejhg.2012.170.

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