The Lipin Family: Mutations and Metabolism

Karen Reue

Departments of Human Genetics and Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095

Abstract

Purpose of review—The family of three lipin proteins act as phosphatidate phosphatase (PAP) enzymes required for glycerolipid biosynthesis, and also as transcriptional coactivators that regulate expression of lipid metabolism genes. The genes for lipin-1, lipin-2 and lipin-3 are expressed in key metabolic tissues, including adipose tissue, skeletal muscle, and liver, but the physiological functions of each member of the family have not been fully elucidated. Here we examine the most recent studies that provide information about the roles of lipin proteins in metabolism and human disease.

Recent findings—Recent studies have identified mutations that cause lipin-1 or lipin-2 deficiency in humans, leading to acute myoglobinuria in childhood or the inflammatory disorder Majeed syndrome, respectively. The effects of lipin-1 deficiency appear to include both the loss of glycerolipid building blocks and the accumulation of lipid intermediates that disrupt cellular function. Several studies have demonstrated that polymorphisms in the *LPIN1* and *LPIN2* genes are associated with metabolic disease traits, including insulin sensitivity, diabetes, blood pressure, and response to thiazolidinedione drugs. Furthermore, lipin-1 expression levels in adipose tissue and/or liver are positively correlated with insulin sensitivity. Studies of lipin-1 in adipocytes have shed some light on its relationship with insulin sensitivity.

Summary—Lipin-1 and lipin-2 are required for normal lipid homeostasis, and have unique physiological roles. Future studies, for example using engineered mouse models, will be required to fully elucidate their specific roles in normal physiology and disease.

Keywords

triglyceride; phosphatidic acid phosphatase; transcriptional coactivator; lipodystrophy; obesity; insulin resistance; myopathy

Introduction

It is clear from diseases such as obesity and lipodystrophy that the regulation of lipid storage is critical for metabolic homeostasis [1,2]. Impaired and excessive triacylglycerol (TAG) storage in adipose tissue are both associated with inappropriate lipid accumulation in tissues such as liver and skeletal muscle, and impaired adipokine production. These, in turn, contribute to insulin resistance and dyslipidemia, key components of the metabolic syndrome [3]. It is therefore of interest to identify factors that influence lipid biosynthesis and storage, and determine how genetic variation in the activity of these factors contributes to metabolic dysfunction. In this review we will highlight recent studies on the mammalian lipin proteins, which function in TAG synthesis and metabolism in tissues such as adipose tissue, liver, and

Correspondence: Karen Reue, Department of Human Genetics, Gonda 6506A, 695 Charles E. Young Drive South, Los Angeles, CA 90095, reuek@ucla.edu, Tel (310) 794-5631, Fax (310) 794-5446.

skeletal muscle. The role of the orthologous yeast protein has recently been described in excellent reviews [4,5] and will not be discussed here.

The lipin protein family

The lipin gene family encodes the proteins lipin-1, lipin-2, and lipin-3. The *Lpin1* gene was first isolated from the fatty liver dystrophy (*fld*) mutant mouse strain [6,7], where lipin-1 deficiency was identified as the cause of lipodystrophy, insulin resistance, peripheral neuropathy, and neonatal fatty liver in these animals [8]. Lipin-1 is expressed as two protein isoforms, lipin-1α and lipin-1β, derived from the *Lpin1* gene by alternative mRNA splicing [8,9]. Lipin-2 and lipin-3 were identified based on 60% amino acid sequence similarity to lipin-1 [8]. Lipin family proteins are present in species ranging from mammals to yeast, and all have highly conserved regions known as the N-LIP and C-LIP domains, which are critical for protein function (see Fig. 1).

The identification of lipin protein molecular function has been reviewed recently [4,10]. The current understanding is that all of the mammalian lipin proteins are phosphatidate phosphatase (PAP) enzymes, which convert phosphatidate to diacylglycerol, and therefore act at a key step in the synthesis of TAG, phosphatidylcholine and phosphatidylethanolamine [11–13]. Additionally, lipin-1 can localize to the nucleus [8,14,15], and is a component of a transcriptional complex with peroxisome proliferator-activated receptor α (PPARα) and PPARγ coactivator 1α (PGC-1α) to regulate fatty acid metabolism in liver [16]. Amino acid motifs required for lipin-1 PAP activity (DIDGT) and transcriptional coactivator activity (LXXIL) reside in the C-LIP domain [12,16] (see Fig. 1).

The three lipin genes each exhibit a unique pattern of tissue expression, suggesting independent physiological roles. Lipin-1 is expressed at highest levels in adipose tissue, skeletal muscle, and testis, and is also detected in liver, heart, brain, kidney, and other tissues [8]. Using tissues from lipin-1 deficient *fld* mice, it was shown that lipin-1 accounts for virtually all of the PAP activity in adipose tissue, skeletal muscle, and heart, but that other proteins may contribute to activity in liver [11,13]. The role of lipin-1 in adipose tissue has been studied extensively and found to be required both for expression of key adipogenic genes during adipocyte differentiation, and for TAG accumulation [15,17]. A role for lipin-1 in skeletal muscle has also been demonstrated through studies of muscle-specific lipin-1 transgenic mice, which exhibit reduced fatty acid oxidation in muscle and reduced energy expenditure, becoming obese [18]. Lipin-2 is expressed in many tissues including liver, kidney, brain, and lung, whereas lipin-3 is detected at low levels in liver and other visceral tissues that have been tested [11]. Recent studies have made use of naturally occurring mutations in lipin-1 and lipin-2 to further elucidate the roles of these proteins in vivo.

Lipin-1 gene mutations and disease

It was previously shown that two naturally occurring mutations in the mouse *Lpin1* gene (gene rearrangement leading to a null allele, and a Gly84Arg substitution in the N-LIP domain) cause the fatty liver dystrophy phenotype (Fig. 1) [8]. Recently, the first mutations causing lipin-1 deficiency in humans have been documented (Fig. 1). Homozygous or compound heterozygous *LPIN1* mutations cause recurrent muscle pain, weakness, and myoglobinuria in childhood [19]. Nonsense mutations that lead to lipin-1 deficiency were detected in subjects of various ethnic backgrounds. All patients presented before age 7 with episodes of myoglobinuria. Curiously, unlike lipin-1 deficient mice, patients with *LPIN1* mutations do not appear to have lipodystrophy, although they have thus far been examined only in childhood. The basis for the species difference in symptoms resulting from lipin-1 deficiency is not clear. It has been observed that lipin-2 is expressed in human adipose tissue raising the possibility of

compensation for lipin-1 deficiency [11]; however, studies in cultured mouse adipocytes indicate that lipin-2 cannot substitute for lipin-1 in adipocyte differentiation [17,20].

In the same study reporting *LPIN1* nonsense mutations, the authors identified a missense mutation (Pro610Ser) in the C-LIP domain in an individual with myopathy occurring after treatment with statin drugs [19]. Although it must be expanded to larger numbers of individuals, this observation raises interesting questions. Statin drugs are used worldwide to treat hypercholesterolemia, and a proportion of individuals develop mild (2–7%) or severe (0.5%) muscle pain and myopathy [21]. Statin drugs inhibit synthesis of the cholesterol precursor mevalonate, which is also a precursor of ubiquinone, a critical component of the mitochondrial electron transport chain. In rat muscle, lipin-1 expression is induced by acute exercise and may contribute to exercise-induced mitochondrial enzyme induction [22]. Further investigation will be necessary to establish whether reduced lipin-1 PAP activity sensitizes muscle to mitochondrial toxicity in response to statin treatment.

Lipin-2 mutations and disease

Homozygous and compound heterozygous *LPIN2* mutations cause Majeed syndrome, a rare disorder characterized by recurrent osteomyelitis, cutaneous inflammation, and anemia [23–25]. Several independent *LPIN2* mutations have been described, including nonsense mutations and a missense mutation (Ser734Leu) in the C-LIP domain (Fig. 1). Several additional missense mutations in *LPIN2* have been associated with psoriasis [26], suggesting that these lead to impaired, but not absent, lipin-2 function. Since lipin-2 expression has not been characterized in tissues such as bone, skin, and blood cells, the etiology of Majeed syndrome and psoriasis symptoms in these tissues is unclear at present.

Lipin gene expression levels and polymorphisms associated with metabolic traits

Previous studies in adipose tissue-specific lipin-1 transgenic mice suggested a positive relationship between lipin-1 levels in adipose tissue and whole body glucose tolerance, irrespective of body fat mass [18]. The improved glucose homeostasis with enhanced lipin-1 expression may reflect more efficient fatty acid trapping in adipose tissue due to increased PAP activity, and protection of other tissues from inappropriate lipid accumulation. Several recent studies have confirmed the relationship between adipose tissue lipin-1 levels and insulin sensitivity in humans. These include studies in lean and obese subjects with normal or impaired glucose tolerance [27–29], in HIV-associated lipodystrophic subjects [27,30], and in healthy young men [31]. In healthy young men, lipin-1 levels were also positively correlated with insulin-stimulated respiratory quotient, oxygen consumption during exercise, and the expression of genes involved in fatty acid oxidation, including PPARα [31].

In a unique study in which lipin-1 levels were monitored before and after gastric bypass surgery of extremely obese subjects, lipin-1β mRNA levels in liver and adipose tissue were increased in parallel with improved insulin sensitivity following marked weight loss [32]. Hepatic lipin-1β mRNA levels were also correlated with PGC-1α expression, suggesting that the downregulation of these proteins may contribute to reduced insulin sensitivity in obesity. Patients with polycystic ovary syndrome (PCOS), a condition associated with insulin resistance, were shown to have reduced lipin-1β expression in both visceral and subcutaneous adipose tissue, which was independent of body mass index [33]. Greater levels of lipin-1β were detected in subcutaneous compared to visceral fat, and may be a determinant of the greater capacity for expansion and TAG storage in this depot.

LPIN1 polymorphisms and haplotypes may confer interindividual variation in lipin-1 action and have been associated with several components of the metabolic syndrome. *LPIN1* polymorphisms have been associated with body mass index [28,34,35], insulin levels [28, 36], resting metabolic rate [36], and responsiveness to thiazolidinediones drugs [37]. A common *LPIN1* haplotype was associated with risk for metabolic syndrome, while two less common haplotypes appeared to have a protective effect and to associate with low systolic blood pressure and hemoglobin A1C levels [35,38]. However, in a study of UK populations, no associations were detected between common *LPIN1* variants and insulin levels, nor were *LPIN1* mutations detected in 23 lipodystrophic patients [34]. Overall, the studies to date indicate that *LPIN1* gene polymorphisms may influence several traits related to the metabolic syndrome, although this may differ among populations. In the pig, *Lpin1* polymorphisms have been associated with percent leaf fat and intramuscular fat [39]. Although not widely studied thus far, a *LPIN2* gene polymorphism has been associated with diabetes risk [40].

Mechanisms of disease in lipin-1 dysfunction

Recent studies in both lipin-1 deficient mice and humans suggest that deleterious effects of PAP deficiency in tissues such as peripheral nerve, adipose tissue and muscle may be attributable to both the lack of PAP products and the accumulation of phosphatidate substrate. In an elegant study designed to evaluate the biochemical basis for the demyelination of peripheral nerves in *fld* mice, Nadra and colleagues demonstrated that lipin-1 PAP deficiency causes phosphatidate to accumulate in adipose tissue and peripheral nerve [41]. This, in turn, leads to activation of the MEK-Erk signaling pathway in Schwann cells and demyelination. Along the same lines, muscle tissue from one lipin-1 deficient human subject was shown to have elevated levels of lysophosphatidate, phosphatidate, and lysophospholipids [19]. Normally, intracellular levels of phosphatidate are tightly controlled, and disturbances may lead to inappropriate modulation of signaling cascades, oxidative processes, cAMP degradation, protein and lipid phosphorylation, and membrane function [42].

A hallmark feature of lipin-1 deficiency in the *fld* mouse is the occurrence of a fatty liver and hypertriglyceridemia during the neonatal period [6], suggesting that lipin-1 PAP activity is not required for hepatic TAG synthesis and secretion. In support of this, a recent study demonstrates that lipin-1β overexpression decreases hepatic very low density lipoprotein (VLDL) TAG secretion [43]. Further, using mutant recombinant lipin-1β proteins, it was shown that PAP activity is not required for the suppression of TAG synthesis, whereas the LXXIL motif conferring transcriptional coactivator activity and PPARα binding is required. A separate study, performed in hepatocytes, demonstrated that lipin-1α or lipin-1β overexpression increases glycerol-labeled lipid secretion, and decreases the degradation of the predominant VLDL protein, apolipoprotein B [14]. The lipin-1 nuclear localization signal was shown to be required for protein localization to microsomal membranes and PAP activity. Together, the two studies indicate a role for lipin-1 in the regulation of VLDL-TAG synthesis and/or secretion, but suggest that lipin-1 PAP and coactivator activities may have distinct roles. An additional complication is the presence of substantial lipin-2 in liver, which likely affects hepatic TAG synthesis ([11,44]; discussed in a later section).

Regulation of lipin levels and activity

It was previously shown that lipin-1 is phosphorylated at several sites in response to insulin and amino acids, and dephosphorylated in response to oleic acid or epinephrine [9,13,45]. Phosphorylation appears to influence lipin-1 activity by modulating subcellular localization [13]. In the past year, several studies have focused on delineating the regulation of lipin-1 gene expression. All three lipin genes are expressed in liver, and it has been known for decades that PAP activity in liver is induced by glucocorticoid treatment, which increases its capacity to

store TAG for subsequent assembly into lipoproteins or use in beta-oxidation (reviewed in [10]). It was recently shown that dexamethasone increases lipin-1, but not lipin-2 or lipin-3, mRNA, and this resulted in increased lipin-1 protein synthesis and PAP activity [44]. The glucocorticoid stimulatory effect was enhanced by cAMP or glucagon, and diminished by insulin. Dexamethasone also induces lipin-1 expression and PAP activity during adipocyte differentiation, which is mediated by glucocorticoid receptor binding to a DNA sequence upstream of *Lpin1* [46]. Lipin-1 gene transcription during adipocyte differentiation is also regulated by binding of CAAT/enhancer binding protein α in the *Lpin1* upstream region [47]. Lipin-1 then acts in combination with PPARγ to promote expression of adipocyte genes, including glucose transporter 4 (Glut4) [47].

Consistent with the correlation between lipin-1 levels and insulin sensitivity, lipin-1 expression is induced in adipose tissue by the insulin-sensitizing thiazolidinediones and harmine [29,48, 49]. Thiazolidinediones also increase PAP activity, with greatest effect on subcutaneous compared to visceral adipose tissue [48]. This may contribute to the fat redistribution that is observed in conjunction with insulin sensitization in response to thiazolidinediones. Several studies have shown that lipin-1 expression is correlated with Glut4 expression [33,47,50], providing a plausible mechanism for the relationship between thiazolidinediones, lipin-1 expression, and insulin sensitivity.

Other stimuli repress lipin-1 expression. Lipin-1 expression is inhibited by estrogen in the uterus and liver, suggesting a potential role for lipin-1 in reproductive biology [51]. Consistent with this, elevated estrogen levels and impaired fertility in non-obese diabetic mice are associated with depletion of lipin-1 in the uterus and liver, a state that can be reversed by insulin administration [51]. Lipin-1 expression is repressed in adipocytes by activation of toll-like receptors TLR4 and TLR2 by lipopolysaccharide and zymosan, respectively [52]. These effects appear to be mediated by inflammatory cytokines such as TNFα and IL-1, and it is proposed that lipin-1 repression may contribute to the reduced fat storage that accompanies infection and inflammation.

The regulation of lipin-2 has recently been examined in adipocytes and liver. During differentiation of 3T3-L1 adipocytes, lipin-1 and lipin-2 expression occurs in a reciprocal manner, with lipin-2 protein detected in preadipocytes, but falling dramatically after 24 hours of differentiation, after which lipin-1 protein is detectable [20]. Lipin-2 cannot substitute for lipin-1 in adipocyte differentiation, indicating that the two proteins do not have redundant functions in adipocytes [20]. Lipin-2 is expressed at highest levels in liver, suggesting a role as a key PAP in this tissue [11]. In the liver, lipin-2 protein content was increased in neonatal lipin-1 deficient *fld* mice, as well as in response to food deprivation or obesity [53]. Inhibition of lipin-2 in hepatocytes by RNAi reduced PAP activity, consistent with a role for this lipin as an important PAP enzyme in liver [53].

Conclusion

The lipin proteins modulate intracellular lipid levels through roles in lipid synthesis and in fatty acid metabolism. The study of mouse and human mutations has established that lipin-1 and lipin-2 each have a unique physiological function that cannot be substituted by the other family members. Future studies utilizing engineered mouse mutations may be a useful strategy to better define normal physiological function of each lipin protein, disease mechanisms, and the roles of enzymatic and transcriptional coactivator activities.
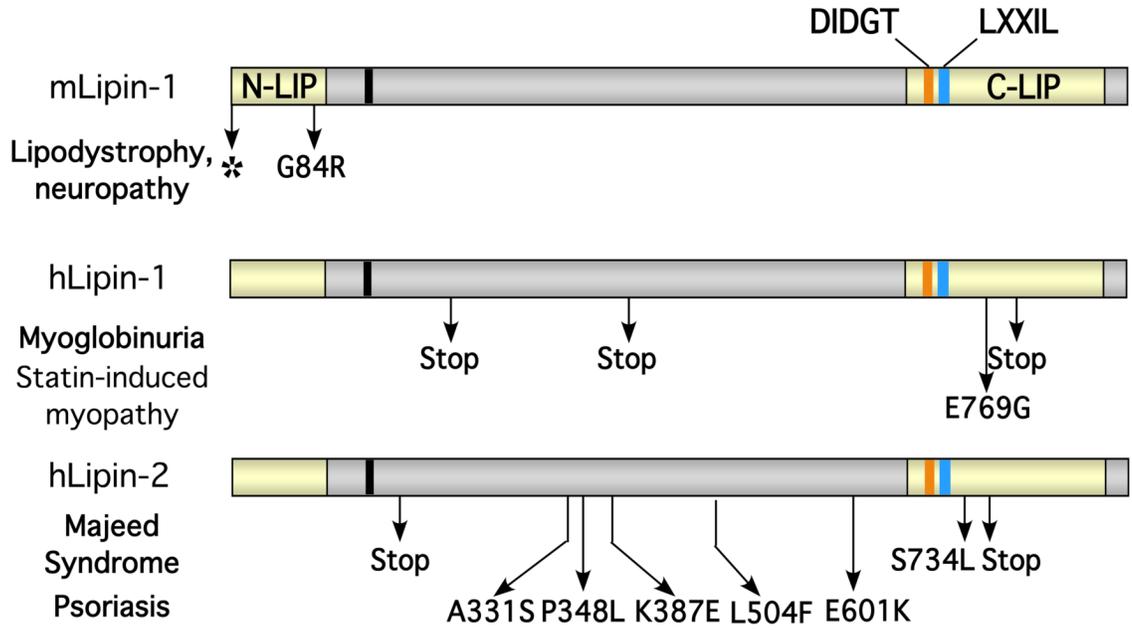
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Fig. 1. Position of lipin protein functional motifs and naturally occurring mutations
Schematic diagrams of mouse (m) and human (h) lipin-1 and lipin-2 proteins, with conserved domains (N-LIP, C-LIP), nuclear localization signal (vertical black bar), PAP enzyme (DIDGT) and coactivator motifs (LXXIL) indicated. Positions and types of mutations are shown along the length of each protein; the associated disease phenotypes are indicated in the corresponding color at left. *, mutation resulting in a gene rearrangement and lack of lipin-1 mRNA; Stop, single nucleotide changes that introduce a premature stop codon; fs-Stop, frameshift mutation that introduces a premature stop codon.