Transgenic Expression and Genetic Variation of Lmf1 Affect LPL Activity in Mice and Humans

Maryam Hosseini, Nicole Ehrhardt, Daphna Weissglas-Volkov, Ching-Mei Lai, Hui Z. Mao, Jo-Ling Liao, Elina Nikkola, André Bensadoun, Marja-Riitta Taskinen, Mark H. Doolittle, Päivi Pajukanta, Miklós Péterfy

Objective—Lipoprotein lipase (LPL) is a principal enzyme in lipoprotein metabolism, tissue lipid utilization, and energy metabolism. LPL is synthesized by parenchymal cells in adipose, heart, and muscle tissues followed by secretion to extracellular sites, where lipolytic function is exerted. The catalytic activity of LPL is attained during posttranslational maturation, which involves glycosylation, folding, and subunit assembly within the endoplasmic reticulum. A lipase-chaperone, lipase maturation factor 1 (Lmf1), has recently emerged as a critical factor in this process. Previous studies demonstrated that loss-of-function mutations of Lmf1 result in diminished lipase activity and severe hypertriglyceridemia in mice and human subjects. The objective of this study is to investigate whether, beyond its role as a required factor in lipase maturation, variation in Lmf1 expression is sufficient to modulate LPL activity in vivo.

Methods and Results—To assess the effects of Lmf1 overexpression in adipose and muscle tissues, we generated aP2-Lmf1 and Mck-Lmf1 transgenic mice. Characterization of relevant tissues revealed increased LPL activity in both mouse strains. In the omental and subcutaneous adipose depots, Lmf1 overexpression was associated with increased LPL specific activity without changes in LPL mass. In contrast, increased LPL activity was due to elevated LPL protein level in heart and gonadal adipose tissue. To extend these studies to humans, we detected association between Lmf1 gene variants and postheparin LPL activity in a dyslipidemic cohort.

Conclusion—Our results suggest that variation in Lmf1 expression is a posttranslational determinant of LPL activity. (Arterioscler Thromb Vasc Biol. 2012;32:00-00.)

Key Words: Hyperlipoproteinemia • lipases • transgenic models • LPL

Lipoprotein lipase (LPL) is a principal enzyme in plasma lipid metabolism.1 Through the hydrolysis of triglycerides (TG) associated with lipoprotein particles such as chylo-microns and very low-density lipoproteins, LPL releases fatty acids for utilization and storage in various tissues.2 LPL is the rate-limiting enzyme in tissue absorption of dietary and endogenously produced TG, hence its activity is a key determinant of tissue lipid partitioning as well as plasma TG levels.3 Indeed, LPL-deficiency results in elevated plasma TG,2 whereas LPL-transgenic mice exhibit increased tissue lipid content and insulin resistance.4 Consistent with mouse models, genetic variation in the LPL gene is associated with insulin resistance,4 plasma TG concentration, and coronary artery disease5 in human populations. Thus, tissue LPL activity is a critical determinant of metabolic traits in health and disease.

LPL is synthesized in the endoplasmic reticulum (ER) of parenchymal cells in muscle and adipose tissue followed by secretion and transport to the vascular endothelium, the functional site of LPL action. LPL attains catalytic activity within the ER in a multistep process, herein referred to as lipase maturation, which involves glycosylation, glycan processing, folding, and the assembly of homodimers.6 In adipocytes, only about 70% to 80% of newly synthesized LPL is converted into active enzyme, whereas the rest remains permanently inactive and undergoes degradation.7,8 Thus, posttranslational maturation represents a bottleneck in the generation of active LPL in adipocytes.9 Several factors have been implicated in lipase maturation including components of the calnexin/calreticulin and BiP/Grp94 chaperone systems.11 In addition to general chaperones, which are involved in the posttranslational processing of most proteins traveling through the ER, a lipase-specific chaperone, lipase maturation factor 1 (Lmf1), has also been described.12 Lmf1 was identified as the gene affected in a

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mutant mouse strain (cld, combined lipase deficient), which exhibits severe hypertriglyceridemia owing to a lack of LPL activity. Although LPL protein is normally expressed in cld mutant mice, it fails to attain the catalytically active homodimer conformation and is subject to retention in the ER and degradation.\textsuperscript{13} Lmf1 is a polytopic protein of the ER membrane and it physically interacts with LPL through a soluble region exposed to the ER lumen.\textsuperscript{14} Interestingly, LPL is not the only lipase affected by Lmf1, as hepatic lipase and endothelial lipase activities are also diminished in Lmf1 deficiency in both mouse and human subjects.\textsuperscript{12,15,16} Similar to LPL, hepatic lipase, and endothelial lipase also require homodimerization for catalytic activity.\textsuperscript{17,18} In contrast, the enzymatic activity of pancreatic lipase, a related enzyme that is functional as a monomer,\textsuperscript{19} is not dependent on Lmf1 function.\textsuperscript{20} Based on these observations, Lmf1 has been proposed to play a role in the assembly of folded lipase subunits into active dimers, or stabilization of the latter.\textsuperscript{21}

To date, the identity of factors limiting the efficiency of LPL maturation in vivo has remained unknown. Based on in vitro studies implicating dimerization as a major bottleneck in lipase maturation\textsuperscript{22} and the suspected role of Lmf1 in this process, we hypothesized that Lmf1 may be a rate-limiting factor in the generation of active LPL.\textsuperscript{23} To test this hypothesis, we overexpressed Lmf1 in adipose, heart, and muscle tissues of transgenic mice. Our results demonstrate that elevated cellular levels of Lmf1 result in increased LPL activity in all tissues tested. Although Lmf1 overexpression increased LPL specific activity, but not LPL mass, in omental and subcutaneous adipose tissues, the converse (ie, higher LPL mass, but no change in specific activity) was observed in heart and the gonadal adipose depot. We also extended these studies to humans by investigating the relationship between Lmf1 and LPL using association analysis and detected studies to humans by investigating the relationship between heart and the gonadal adipose depot. We also extended these studies in the online-only Data Supplement. aP2-Lmf1 and Mck-Lmf1 transgenic mice have similar results were obtained in a second, independent line of online-only Data Supplement) conditions. Furthermore, similar results suggest that endogenous Lmf1 levels limit the expression of LPL activity in adipose tissue. Total tissue lipase activities measured in our experiments include both intracellular (ie, before secretion from adipocytes) and extracellular LPL, the latter representing func-

**Methods**

A detailed description of methods is provided in the online-only Data Supplement. aP2-Lmf1 and Mck-Lmf1 transgenic mice have been generated on the FVB/J genetic background, maintained in a specific pathogen-free facility under 14:10 hour light cycle, and were fed a chow diet. All animal studies were approved by the Institutional Animal Care and Use Committee at the Cedars-Sinai Medical Center. Human study participants were recruited in the Helsinki and Turku University Central Hospitals and gave informed consent. The study design was approved by the ethics committees of the participating centers.

**Results**

**Elevated LPL Activity in aP2-Lmf1 Transgenic Mice**

To investigate the role of Lmf1 in adipose tissue, we generated transgenic mice with elevated Lmf1 expression using the 5.4-kb enhancer/promoter region of the aP2 gene (Figure 1A), which directs expression in terminally differentiated adipocytes.\textsuperscript{24,25} To facilitate the detection of exogenous Lmf1 protein in tissues, we included an N-terminal myc epitope tag, which does not interfere with the lipase maturation function of Lmf1.\textsuperscript{26} As determined by immunoblotting with an anti-myc antibody, the transgene-derived myc-Lmf1 protein was specifically expressed in subcutaneous, omental and, at a lower level, gonadal adipose tissue in aP2-Lmf1 mice (Figure 1B and 1C). To assess the relative expression of endogenous versus exogenous Lmf1, we performed immunoblotting with an antibody recognizing the C-terminus of the protein.\textsuperscript{12} Myc-Lmf1 expression in gonadal adipose was comparable (0.75-fold higher) to that of the endogenous protein, whereas omental (2.1-fold) and subcutaneous (2.9-fold) adipose tissues exhibited higher levels (Figure 1C).

We previously demonstrated that loss-of-function mutations in Lmf1 result in diminished LPL activities.\textsuperscript{12} To test if LPL is also affected by elevated Lmf1 expression, we determined lipase activities in tissues of aP2-Lmf1 mice. LPL activities were significantly higher in all transgenic adipose depots in both fasted (Figure 2A) and fed (Figure 1 in the online-only Data Supplement) conditions. Furthermore, similar results were obtained in a second, independent line of aP2-Lmf1 mice (Figure 2 in the online-only Data Supplement). These results suggest that endogenous Lmf1 levels limit the expression of LPL activity in adipose tissue.

To facilitate the detection of exogenous Lmf1 protein, we included an N-terminal myc epitope tag, which does not interfere with the lipase maturation function of Lmf1.\textsuperscript{26} As determined by immunoblotting with an anti-myc antibody, the transgene-derived myc-Lmf1 protein was specifically expressed in subcutaneous, omental and, at a lower level, gonadal adipose tissue in aP2-Lmf1 mice (Figure 1B and 1C). To assess the relative expression of endogenous versus exogenous Lmf1, we performed immunoblotting with an antibody recognizing the C-terminus of the protein.\textsuperscript{12} Myc-Lmf1 expression in gonadal adipose was comparable (0.75-fold higher) to that of the endogenous protein, whereas omental (2.1-fold) and subcutaneous (2.9-fold) adipose tissues exhibited higher levels (Figure 1C).

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Figure 2. Lipoprotein lipase (LPL) activity, protein expression and specific activity in fasting aP2-lipase maturation factor 1 (Lmf1) mice. 

A, Tissue LPL activity in wild-type (open bars, n=8–10) and transgenic (Tg) (filled bars, n=6–7) adipose depots. B, LPL protein mass in the same tissue samples shown in panel A. C, LPL specific activity calculated as the ratio of tissue LPL activity and LPL mass. Wt indicates wild type; Tg, transgenic.
tionally relevant enzyme. To determine if LPL activity is increased in the functional compartment of transgenic adipose tissue, fat pads were incubated with heparin ex vivo and lipase activity released into the medium was measured. Heparin-released LPL activity from transgenic adipose was 3-fold elevated compared to wild-type tissue (Figure IIIA in the online-only Data Supplement) indicating that Lmf1 overexpression results in increased secreted enzyme activity.

Next, we investigated potential molecular mechanisms responsible for increased LPL activity in transgenic tissues. Consistent with the posttranslational role of Lmf1 in LPL maturation, LPL mRNA expression was unchanged in tissues of aP2-Lmf1 mice (Figure IVA in the online-only Data Supplement). However, the analysis of LPL protein expression by ELISA revealed distinct differences among adipose depots. In gonadal adipose, LPL mass was significantly increased in female aP2-Lmf1 mice and showed a trend toward that effect in males, which explains the higher LPL activities detected in this tissue (Figure 2B). In contrast, LPL mass was largely unaffected in omental and subcutaneous depots, with the exception of omental adipose in male mice (Figure 2B). These data suggest that in omental and subcutaneous adipose, a larger proportion of total LPL mass was present in a catalytically active form in aP2-Lmf1 mice. Indeed, calculation of LPL specific activity (ie, LPL activity per LPL mass) demonstrated that increased LPL activities were due to higher specific activity of the enzyme in these tissues (Figure 2C).

To assess the potential metabolic consequences of elevated adipose tissue LPL activity, we characterized metabolic parameters in aP2-Lmf1 mice. No changes were detected in body weight or composition, plasma parameters including lipid, glucose, and insulin levels or postheparin LPL activity (Table I in the online-only Data Supplement).

**Elevated LPL Activity in Mck-Lmf1 Transgenic Mice**

The results obtained in aP2-Lmf1 mice prompted us to investigate whether the effects of Lmf1 overexpression on LPL extend to other major LPL-expressing tissues, such as heart and skeletal muscle. We used a 6.5-kb genomic DNA fragment encompassing the promoter, enhancer 1, exon 1, and intron 2 of the Mck gene to drive myc-Lmf1 expression (Figure 3A). As expected, myc-Lmf1 was specifically expressed in these tissues (Figure 3B). Comparison of exogenous and endogenous Lmf1 levels demonstrated several fold overexpression of myc-Lmf1 protein in transgenic muscle (12-fold) and heart (21-fold) tissues (Figure 3C).

Consistent with aP2-Lmf1 mice, Lmf1 overexpression also increased tissue LPL activities in heart and muscle of Mck-Lmf1 mice under both fasted (Figure 4A) and fed (Figure V in the online-only Data Supplement) conditions. These results were confirmed in a second Mck-Lmf1 mouse line representing an independent transgene integration event (Figure VI in the online-only Data Supplement). Similar to aP2-Lmf1 adipose tissue, ex vivo heparin-release experiments demonstrated increased secreted LPL activity in the extracellular compartment in Mck-Lmf1 muscle (Figure IIIB in the online-only Data Supplement).

Consistent with a posttranslational mechanism, elevated LPL activities in Mck-Lmf1 tissues were not associated with increased LPL mRNA expression (Figure IVB in the online-only Data Supplement). However, in contrast to aP2-Lmf1 adipose tissue, increased LPL activity was primarily due to higher LPL protein levels (Figure 4B). The only exception to this is female muscle tissue, which exhibited elevated LPL specific activity in transgenic animals (Figure 4C).

Similar to aP2-Lmf1 mice, postheparin LPL activity and metabolic parameters were indistinguishable between Mck-Lmf1 and wild-type littermates with the exception of slightly lower HDL-cholesterol levels in transgenic males (Table II in the online-only Data Supplement).

**Lmf1 Is Associated With Postheparin LPL Activities in Humans**

Although tissue-specific overexpression of Lmf1 did not result in elevated plasma LPL activities in our transgenic mouse models, we hypothesized that genetic variation resulting in altered Lmf1 expression in all tissues may affect postheparin plasma LPL activity in human subjects. To investigate whether common genetic variation influences LPL activity in human samples, we performed a tag-SNP approach in the Lmf1 gene region (130kb). We genotyped 20 SNPs capturing >90% of the SNPs with minor allele frequency ≥10% in Lmf1 in 1100 individuals from 92 Finnish dyslipidemic families. We did not attempt to capture genetic
variation with minor allele frequency <10%, as our study sample is not sufficiently powered to detect the effects of such variants. The SNPs were tested with continuous post-heparin LPL activity levels in family-based association analyses using quantitative transmission disequilibrium test (QTDT) analyses for quantitative lipoprotein lipase (LPL) activity (range 77–487 μmol/mL) are shown. The dashed line indicates the Bonferroni-corrected significance threshold [log₁₀(2.50×10⁻³)]. The location of the SNPs is shown in relation to the gene structure of Lmf1.

We identified a common SNP (minor allele frequency = 0.4), rs3751666, in intron 1 of Lmf1 to be associated with LPL activity (P = 3×10⁻⁴) (Figure 5 and Table III in the online-only Data Supplement). This result is gene-wide significant, as it surpasses the Bonferroni correction for 20 SNPs tested (Bonferroni-adjusted P = 0.006). There was one other SNP (rs3829491) surpassing the Bonferroni correction (Bonferroni-adjusted P = 0.038), however, this SNP does not represent an independent association signal as it is in high linkage disequilibrium with rs3751666 (r² = 0.96) (Table III in the online-only Data Supplement). The association of rs3751666 was only nominally significant with plasma TGs and HDL-cholesterol (P = 0.03 and 0.01, respectively).

Next, we used an imputation method to extend our association analysis to tagged and nontagged SNPs in the genomic region of Lmf1 (57 additional SNPs). We imputed genotype dosage (expected allele counts 0–2) using the MACH program and analysis of dosage data were performed with quantitative transmission disequilibrium test. Overall, we obtained the strongest evidence of association for the genotyped rs3751666 SNP even though we tested for association with many more common SNPs (minor allele frequency >5%) in the region (Figure VII in the online-only Data Supplement). Furthermore, the strength of the association signal of the regional SNPs was highly correlated with the strength of their pairwise linkage disequilibrium with rs3751666 (r² = 0.96) (Table III in the online-only Data Supplement) suggesting that rs3751666 is the only independent common association signal at this locus.

The genotypic means (±SEM) of LPL activity adjusted for age, sex, and kinship were 225 ± 1.07 μmol FA/h/mL for the A/A common homozygotes, 217 ± 1.06 μmol FA/h/mL for the A/G heterozygotes, and 210 ± 1.1 μmol FA/h/mL for the G/G rare homozygotes. In agreement with Lmf1 transgenic mouse data, these results suggest that variation in Lmf1 expression and/or activity may modulate LPL activity in humans. We did not observe significant associations between the 20 SNPs and postheparin hepatic lipase activity, consis-
tent with this lipase being less dependent on Lmf1 for maturation than LPL.12

Discussion

Previous characterization of loss-of-function mutations identified Lmf1 as a critical factor in the posttranslational maturation of lipases in mice and human subjects.12,15 The goal of the present study was to investigate whether altered Lmf1 expression also affects this process. We pursued 2 approaches to address this issue. First, we generated transgenic mouse lines overexpressing Lmf1 in various tissues. Characterization of these mice demonstrated that LPL activity was elevated in all transgenic tissues tested. Second, we sought genetic evidence for an effect of variable Lmf1 expression on LPL in human subjects. Consistent with the mouse studies, we detected significant association between Lmf1 SNPs and LPL activity in a dyslipidemic cohort. Taken together, our results extend the previously established role of Lmf1 as a required factor for lipase maturation and suggest that the expression level of this chaperone is a determinant of LPL activity.

Although LPL activity was uniformly increased in all Lmf1-transgenic tissues, initially distinct mechanisms seemed to be operating in different tissues. In some (gonadal adipose, heart, and muscle), elevated LPL mass was clearly responsible for increased LPL activity. In others (omental and subcutaneous adipose), higher specific activity of LPL without changes in LPL mass explained elevated tissue LPL activity. Despite the apparent differences, the direct effects of Lmf1 overexpression are likely to be the same in all tissues, namely enhanced LPL dimer assembly in the ER and increased rate of secretion of the active enzyme, as demonstrated in our study. However, the rate of extracellular LPL is tissue context-dependent and determined in large part by the expression of angiopoietin-like proteins 3 and 4.1 One dissociates and inactivates the LPL dimer,32 and the turnover rate of inactive LPL as a function of tissue perfusion and hepatic degradation.33 Consistent with such tissue-specific differences, LPL specific activity was found to be highly variable between human adipose depots and adipose versus heart tissue in the mouse.34,35 In conclusion, LPL mass and specific activity in a given tissue are subject to the effects of intra- and extracellular biosynthetic and degradation pathways and the apparent variability in our transgenic models is a likely reflection of this complexity.

The aP2 promoter-driven transgenic construct resulted in variable expression in different adipose depots allowing us to assess the relationship between Lmf1 levels and LPL activity. Consistent with previous reports, subcutaneous and gonadal depots exhibited the highest and lowest level of Lmf1 transgene expression, respectively.36 Accordingly, LPL activities showed a similar overall pattern across adipose depots and in general seemed to be commensurate with the degree of Lmf1 overexpression. An important caveat to the study on Mck-Lmf1 mice is that the transgene was expressed at supraphysiological levels, which limits the interpretation of this model. Nonetheless, in general agreement with the aP2-Lmf1 transgenics, LPL activity was elevated in the heart and muscle of Mck-Lmf1 mice, although several-fold over-expression of Lmf1 resulted in only 30% to 50% increases in LPL activity. It is conceivable that the relatively modest impact on LPL is due to a saturation effect. Alternatively, the differences between aP2- and Mck-Lmf1 transgenics may reflect distinct mechanisms involved in the regulation of LPL activity in adipose and muscle tissues.37,38

The metabolic effects of elevated tissue LPL activities remained undetectable in our transgenic mouse models. This is not unexpected and is consistent with previous studies on LPL-transgenic mice. For example, LPL overexpression in aP2-LPL mice, which exhibit similarly elevated LPL activities in adipose tissue as aP2-Lmf1 mice, had no effect on plasma lipids, postheparin LPL activity, or other metabolic traits.39 Likewise, Mck-LPL transgenic mice with muscle-specific LPL overexpression exhibited wild-type levels of plasma lipids, glucose, and insulin.3 However, in contrast to tissue-specific models, transgenic mice overexpressing LPL in all tissues showed elevated plasma LPL activity and altered lipid traits.40–42 Taken together, these results suggest that the metabolic effects of increased LPL activity limited to individual tissues may be too small to be detected or masked by compensatory changes. Potential compensatory mechanisms may involve reduced LPL expression in nontransgenic tissues or changes in the activities of posttranslational regulators of LPL, such as apolipoproteins C2, C3, and A5, and angiopoietin-like proteins 3 and 4.1

Our transgenic mouse studies suggested that Lmf1 expression level is a determinant of LPL activity. To extend this conclusion to humans, we tested the effects of Lmf1 variation on LPL by performing association analyses between common genetic variants in the Lmf1 region and postheparin plasma LPL activity in a dyslipidemic cohort. To the best of our knowledge, this is the first study to investigate the effects of common variants on LPL activity in human dyslipidemia. We detected genome-wide significant association between LPL activity and a tag-SNP (rs3751666) within the first intron of Lmf1 suggesting that variation in Lmf1 also influences LPL activity in humans. The association signal may be due to linkage disequilibrium with functional polymorphism(s) affecting the expression or function of Lmf1. Alternatively, as rs3751666 occurs within a short distance (28 bp) from exon 2, it may directly affect splicing of the Lmf1 message. Additional larger cohorts characterized for the lipase phenotype will be required to replicate the association with rs3751666 and fine-map the functional variant.

The apparent lack of associations between Lmf1 and plasma lipid levels in our study and a previous genome-wide association study5 is likely due to insufficient power to detect small effects. Consistent with the generally small effects of common variants, the impact of rs3751666 on LPL activity is relatively modest. Furthermore, LPL is only one of many factors in the determination of plasma lipid levels. Indeed, LPL is responsible for only 8.8% and 5.6% of the variation in plasma TG and HDL-C levels, respectively, in our cohort. In summary, we provided genetic evidence indicating that common variants of Lmf1 affect plasma LPL activity in humans, which is consistent with the quantitative effects of Lmf1 expression on tissue LPL in mouse models. Although association with lipids could not be detected in the present study,
the role of Lmf1 in plasma lipid metabolism has been previously established. In particular, rare Lmf1 variants, which were not interrogated in the current analysis or other genome-wide association studies, have been demonstrated to exert major effects on plasma TG levels.12,15,43

The main conclusion of the present study is that variation in Lmf1 expression is associated with changes in LPL activities. Nonetheless, important questions remain to be addressed in future studies. For example, the molecular mechanisms responsible for increased LPL specific activity in some transgenic tissues, but elevated LPL mass in others is not well understood. It is also unclear whether hepatic lipase and endothelial lipase are similarly affected by Lmf1 overexpression. Finally, our results raise the interesting possibility that lipase regulation through the modulation of Lmf1 expression and/or activity. Further studies are warranted to investigate the physiological contexts and molecular mechanisms affecting Lmf1 expression and function.

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Disclosures
None.

References


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Transgenic expression and genetic variation of LMF1 affect LPL activity in mice and humans

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Supplementary Methods

3 Supplementary Tables

7 Supplementary Figures
SUPPLEMENTARY METHODS

Generation and maintenance of transgenic mice.

For the generation of aP2-Lmf1 transgene construct, mouse Lmf1 cDNA with an amino-terminal myc epitope tag\(^1\) and including EcoRV and NotI restriction sites at the 5' and 3' end, respectively, was generated using PCR. The EcoRV/NotI-restricted Lmf1 fragment was inserted between the Smal and NotI sites into the pBS aP2 promoter plasmid (Addgene plasmid #11424) retrofitted with a SfiI site 3' of the polyA cassette. The aP2 promoter-Lmf1-pA fragment was released by KpnI/SfiI restriction. For the generation of Mck-Lmf1 transgene construct, the myc-Lmf1 cDNA was generated with NsiI and NotI restriction sites at the 5' and 3' end, respectively, using PCR and inserted into the pBS MCK promoter plasmid (Addgene #12528) between the PstI and NotI sites. The Mck promoter-myc-Lmf1-pA fragment was released by BssHII restriction. Gel-purified and concentrated fragments were microinjected into fertilized FVB/J oocytes at the University of California, Irvine, Transgenic Mouse Facility. Founder mice were identified by PCR analysis and mated with FVB/J mice to produce hemizygous offspring. Two independent lines each of the aP2-Lmf1 (lines 27.3 and 21.8) and Mck-Lmf1 (lines 99.3 and 9.4) transgenics were characterized in these studies. Data in Figures 1-2, I, III, IV and Table I correspond to aP2-Lmf1(27.3), whereas Figure II represents line aP2-Lmf1(21.8). Similarly, Figures 3-4, III, IV, V and Table II correspond to line Mck-Lmf1(99.3), whereas Figure VI illustrates Mck-Lmf1(9.4). Animals were housed under controlled temperature (23°C) and a
14:10 hour light/dark cycle with free access to water and mouse chow (LabDiet 5015). Mice in these studies were either fasted for 16 hours (from 1800-1000 h), or fasted and then re-fed for 2 h. All protocols for animal use and euthanasia were reviewed and approved by the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center.

Quantitative RT-PCR.
Tissues dissected for RNA analysis were snap frozen in liquid nitrogen and stored at –80°C until use. Total RNA was isolated using the Trizol reagent (Invitrogen) followed by further purification using an RNA miniprep kit (Bioland Scientific). RNA was quantified by OD_{260} measurement (Nanodrop 2000, Thermo Scientific) and its quality was checked with an Agilent Bioanalyzer (Agilent Technologies). cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and quantitative RT-PCR was performed with TaqMan assays using an Applied Biosystems 7500 real-time PCR system. The LPL (Mm00434770_m1) and Rodent GAPDH Control assays were validated for similar amplification efficiencies and the ΔΔCt method was used for quantitative analysis.

Western blot analysis.
Dissected tissues were homogenized in homogenization buffer (25 mM Tris-HCl, pH 7.5, 0.2% deoxycholic acid, 10% glycerol) with a Polytron homogenizer. Particulate matter (pellet) and lipids (top layer) were removed by centrifugation at
20,000xg for 10 min at 4°C. Protein content was determined using the bicinchoninic (BCA) assay (Pierce) and equal total protein amounts were separated by 3-8% Tris-acetate (Invitrogen) or 7% Tris-glycine SDS-PAGE. After electrotransfer, PVDF membranes were blocked with 5% dried milk in PBST buffer for 1 hour, followed by overnight incubations with primary antibodies at 4°C and secondary antibodies for 1 hour. Myc-Lmf1 was detected with horseradish peroxidase (HRP)-conjugated anti-myc antibody (1:5,000, Invitrogen). Endogenous Lmf1 was detected with a polyclonal rabbit antibody (1:12,000) raised against a C-terminal peptide of Lmf1 and goat anti-rabbit IgG-HRP (1:40,000, sc-2054, Santa Cruz Biotechnology). Filter-bound HRP was detected using a chemiluminescent substrate (ECL+, GE Healthcare).

**LPL assays.**

For the measurement of total tissue LPL activity, tissue homogenates were prepared as described above except that 10 u/ml heparin was also added to the homogenization buffer. Protein was assayed using the BCA reagent (Pierce). To obtain post-heparin plasma, mice were tail vein-injected with 50 units of heparin-sodium in 0.9% NaCl solution and blood was obtained from the retro-orbital plexus under anesthesia 10 min later. For the determination of heparin-released LPL activity, fresh tissue was minced, mixed with 1% BSA in PBS and a ‘pre-heparin’ aliquot was taken. After the addition of 20 u/ml heparin, tissue pieces were agitated at 4°C for 30 min and a ‘post-heparin’ aliquot was collected. Heparin-released activity was calculated as the difference between post- and
pre-heparin aliquots. LPL activities in various samples were measured using a lecithin-stabilized radiolabeled triolein (glycerol tri[9,10(n)-\(^3\)H]oleate) substrate as described.\(^3\) In a typical experiment, 4 \(\mu\)l tissue lysates, 2 \(\mu\)l post-heparin plasma and 40 \(\mu\)l \textit{in vitro} tissue supernatants were used to assay LPL activity. Lipase assays were performed in the presence and absence of 1M NaCl and LPL activity was calculated as the salt-inhibited fraction. LPL protein levels in tissue lysates were determined with ELISA as described.\(^4\)

**Body composition and plasma parameters.**

Whole-body lean and fat mass was determined with a nuclear magnetic resonance system (EchoMRI). To obtain plasma, mice were anesthetized by isoflurane followed by retroorbital puncture with a heparinized glass capillary and microtainer tube (Becton Dickinson). Red blood cells were sedimented by centrifugation (6,000xg for 2 min) and plasma was collected and stored at –80°C. Plasma lipid levels were determined as previously described.\(^5\) Insulin levels were assayed by ELISA (Alpco Diagnostics).

**Human study participants**

A total of 92 Finnish dyslipidemic families were included in this study. The families were recruited in the Helsinki and Turku University Central Hospitals, as described previously.\(^6-8\) All study subjects gave their informed consent. The study design was approved by the ethics committees of the participating centers. Fasting serum lipid measurements were performed with standardized methods
as described earlier.\textsuperscript{8} Patients who used lipid-lowering drugs were studied after their lipid-lowering treatment was withdrawn for 4 weeks. Post-heparin activity of LPL was measured as previously described,\textsuperscript{9} and the measurements were available for 580 subjects.

**SNP selection and genotyping**

Single nucleotide polymorphisms (SNPs) in the LMF1 gene and flanking 5 kb regions (130kb) with minor allele frequency (MAF) > 10% and $r^2$ threshold of 0.8 were selected using the HapMap CEU population with Europe ancestry,\textsuperscript{10} and our previous Caucasian resequencing data.\textsuperscript{2} Accordingly, 20 SNPs were genotyped in 1,100 family members using SNPlex genotyping technology and TaqMan Allelic Discrimination Assays (Applied Biosystems, Foster City, California). The average genotype call rate was >90% and all SNPs were in Hardy-Weinberg equilibrium in the unrelated founders (p-value >0.05). We found <1 Mendelian errors per SNP using the PEDCHECK program\textsuperscript{11} and these were excluded from subsequent analyses.

**Association analysis**

We tested a total of 20 SNPs for association with LPL activity levels. Association analysis was performed utilizing the quantitative transmission disequilibrium test (QTDT) implemented in the genetic analysis package SOLAR using an additive model with age and sex as covariates.\textsuperscript{12, 13} LPL activity measurements were log transformed to approach a normal distribution. Furthermore, the t-distribution
rather than the normal distribution option of SOLAR was used to allow for robust estimation of the mean and variance even if the trait distribution deviates from normality.\textsuperscript{14} To correct for the multiple tests performed, we adjusted for the 20 SNPs tested (Bonferroni-adjusted p-value ≤ 0.0025). To test for associations with untyped SNPs (i.e. imputed SNPs) we used the Markov Chain based haplotype (MACH) program.\textsuperscript{15} We utilized the phased chromosomes of 60 HapMap CEU founders to impute genotypes of SNPs with MAF > 5% in the genomic region of LMF1 (Chr16: 838329-976641, Build 36.1). For association analyses, we used genotype dosage (expected allele counts 0-2) of only those SNPs (n = 57) that were imputed with high confidence, i.e. SNPs for which the predicted $r^2$ between true and imputed genotypes was greater than 0.3,\textsuperscript{15, 16} and with average quality scores (i.e. posterior probability for calling a genotype) greater than 0.9. Analysis of dosage data was performed with QTDT using the same procedures as described for the genotype data.

**Statistical analysis**

Results are shown as means ± S.E. Statistical comparisons between two groups of data were made using two-tailed unpaired Student’s t-test. A p-value <0.05 was considered statistically significant.
References


## Supplementary Table I
Metabolic characteristics of fed aP2-Lmf1 mice (8-12 weeks of age, n = 4-10)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>non-Tg</td>
<td>Tg</td>
<td>non-Tg</td>
<td>Tg</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>26.7 ± 0.6</td>
<td>26.1 ± 0.7</td>
<td>19.7 ± 0.9</td>
<td>20.0 ± 0.5</td>
</tr>
<tr>
<td>Fat weight (g)</td>
<td>3.22 ± 0.36</td>
<td>3.16 ± 0.92</td>
<td>1.23 ± 0.26</td>
<td>1.28 ± 0.51</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>15.2 ± 1.3</td>
<td>15.3 ± 1.6</td>
<td>7.9 ± 1.5</td>
<td>8.1 ± 3.03</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>274 ± 41</td>
<td>247 ± 20</td>
<td>243 ± 18</td>
<td>239 ± 18</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>218 ± 10</td>
<td>223 ± 10</td>
<td>176 ± 12</td>
<td>172 ± 5</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>168 ± 5</td>
<td>169 ± 3</td>
<td>118 ± 3</td>
<td>124 ± 4</td>
</tr>
<tr>
<td>Free fatty acids (mg/dl)</td>
<td>26.1 ± 1.6</td>
<td>25.8 ± 1.4</td>
<td>24.4 ± 1.7</td>
<td>24.0 ± 1.6</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>252 ± 13</td>
<td>233 ± 13</td>
<td>224 ± 7</td>
<td>233 ± 8</td>
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<tr>
<td>Insulin (ng/ml)</td>
<td>1.74 ± 0.051</td>
<td>1.69 ± 0.069</td>
<td>1.65 ± 0.090</td>
<td>1.39 ± 0.158</td>
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<tr>
<td>ph LPL activity (mU/ml)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>426 ± 23</td>
<td>447 ± 51</td>
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</tbody>
</table>
**Supplementary Table II**  
Metabolic characteristics of fasted Mck-Lmf1 mice (8-14 weeks of age, n = 4-8)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>non-Tg</td>
<td>Tg</td>
<td>non-Tg</td>
<td>Tg</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>28.8 ± 0.6</td>
<td>29.5 ± 0.9</td>
<td>22.3 ± 0.7</td>
<td>22.3 ± 0.6</td>
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<tr>
<td>Fat weight (g)</td>
<td>3.79 ± 0.46</td>
<td>4.65 ± 0.65</td>
<td>3.42 ± 0.54</td>
<td>2.76 ± 0.37</td>
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<tr>
<td>Fat content (%)</td>
<td>13.4 ± 1.4</td>
<td>15.5 ± 1.9</td>
<td>15.2 ± 2.0</td>
<td>12.1 ± 1.4</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>139 ± 22</td>
<td>174 ± 21.5</td>
<td>160 ± 19</td>
<td>156 ± 14</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>246 ± 12</td>
<td>223 ± 11</td>
<td>202 ± 12</td>
<td>192 ± 5</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>193 ± 5</td>
<td>178 ± 3*</td>
<td>149 ± 10</td>
<td>148 ± 5</td>
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<tr>
<td>Free fatty acids (mg/dl)</td>
<td>39.3 ± 1.7</td>
<td>40.6 ± 1.9</td>
<td>56.3 ± 3.1</td>
<td>56.0 ± 3.6</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>140 ± 6</td>
<td>156 ± 10</td>
<td>139 ± 10</td>
<td>135 ± 12</td>
</tr>
<tr>
<td>Insulin (pg/ml)</td>
<td>374 ± 76</td>
<td>442 ± 224</td>
<td>98 ± 14</td>
<td>67 ± 11</td>
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<tr>
<td>ph LPL activity (mU/ml)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>735 ± 6</td>
<td>678 ± 26</td>
</tr>
</tbody>
</table>

*, p < 0.05 (vs non-Tg)
**Supplementary Table III.**

Association results of LMF1 polymorphisms with LPL activity levels in Finnish dyslipidemic families.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Pos*(bp)</th>
<th>Major, minor allele†</th>
<th>MAF‡</th>
<th>Z-value§</th>
<th>P-value</th>
<th>LD (r²) with rs3751666</th>
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<tr>
<td>rs4984943</td>
<td>838329</td>
<td>G/A</td>
<td>0.36</td>
<td>0.55</td>
<td>0.58</td>
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<tr>
<td>rs126680</td>
<td>863866</td>
<td>G/C</td>
<td>0.35</td>
<td>-0.38</td>
<td>0.70</td>
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<td>rs9939901</td>
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<td>T/C</td>
<td>0.09</td>
<td>0.14</td>
<td>0.89</td>
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<tr>
<td>rs7196136</td>
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<td>1.62</td>
<td>0.11</td>
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<td>rs4984705</td>
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<td>rs2277893</td>
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<td>-0.09</td>
<td>0.93</td>
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<td>rs2277892</td>
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<td>rs4984982</td>
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<td>rs4984719</td>
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<td>0.19</td>
<td>0.85</td>
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<td>rs1544799</td>
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<td>-2.02</td>
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<td>rs12448994</td>
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<td>rs17146060</td>
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<td>-0.81</td>
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<tr>
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<tr>
<td><strong>rs3829491</strong></td>
<td>944835</td>
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<td>1.90E-03</td>
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<tr>
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<td>-2.00</td>
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<tr>
<td>rs2382948</td>
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<td>-1.92</td>
<td>0.05</td>
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<tr>
<td>rs1978480</td>
<td>966399</td>
<td>G/A</td>
<td>0.46</td>
<td>-1.87</td>
<td>0.06</td>
<td>0.74</td>
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</table>

*Base pair position in kb according to human reference sequence Build 36.1. †Alleles are in + strand relative to the human reference sequence. ‡Minor allele frequency. §Z score for the minor allele. SNPs surpassing the significance threshold are in boldface.
Supplementary Figure I. Total adipose tissue LPL activities in wild-type (open bars, n = 6-8) and aP2-Lmf1 (filled bars, n = 6-10) mice in the fed state. *p<0.05
**Supplementary Figure II.** Total adipose tissue LPL activities in a second, independent line of aP2-Lmf1 transgenic (line 21.8; filled bars, n = 5-8) and wild-type (open bars, n = 7-8) mice in the fasted state. *p<0.05
Supplementary Figure III. In vitro heparin-released LPL activity from subcutaneous adipose tissue of fed aP2-Lmf1 (A) and quadriceps muscle of fasted Mck-Lmf1 (B) mice (n = 4-5). *p<0.05
Supplementary Figure IV. Lmf1 mRNA expression as determined by qRT-PCR analysis in tissues of (A) aP2-Lmf1 and (B) Mck-Lmf1 mice. Male wild-type (open bars, n = 2-5) and Tg (filled bars, n = 3-4) mice were used.
**Supplementary Figure V.** Total tissue LPL activity in the heart and muscle of wild-type (n = 4-6) and Mck-Lmf1 (n = 4-7) mice in the fed state. *p<0.05
Supplementary Figure VI. Total tissue LPL activity in the heart and muscle of a second, independent line of Mck-Lmf1 transgenic (line 9.4; n = 8) and wild-type (n = 8-9) mice in the fasted state. *p<0.05
Supplementary Figure VII. Association results and LD of genotyped and imputed polymorphisms across the LMF1 gene with LPL activity. The –log10 of the P-values obtained from the QTDT analyses for genotyped (solid symbol) and imputed (open symbol) SNPs are shown. The dashed gray lines indicate the pairwise LD in $r^2$ with rs3751666. The location of the SNPs is shown in relation to the gene structure of LMF1.