

# A quantitative assay measuring the function of lipase maturation factor 1

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**Abstract** Newly synthesized lipoprotein lipase (LPL) and related members of the lipase gene family require an endoplasmic reticulum maturation factor for attainment of enzyme activity. This factor has been identified as lipase maturation factor 1 (Lmf1), and mutations affecting its function and/or expression result in combined lipase deficiency (*clد*) and hypertriglyceridemia. To assess the functional impact of Lmf1 sequence variations, both naturally occurring and induced, we report the development of a cell-based assay using LPL activity as a quantitative reporter of Lmf1 function. The assay uses a cell line homozygous for the *clد* mutation, which renders endogenous Lmf1 nonfunctional. LPL transfected into the mutant *clد* cell line fails to attain activity; however, cotransfection of LPL with wild-type Lmf1 restores its ability to support normal lipase maturation. In this report, we describe optimized conditions that ensure the detection of a complete range of Lmf1 function (full, partial, or complete loss of function) using LPL activity as the quantitative reporter. To illustrate the dynamic range of the assay, we tested several novel mutations in mouse Lmf1. Our results demonstrate the ability of the assay to detect and analyze Lmf1 mutations having a wide range of effects on Lmf1 function and protein expression.— Yin, F., M. H. Doolittle, and M. Péterfy. A quantitative assay measuring the function of lipase maturation factor 1. *J. Lipid Res.* 2009. 50: 2265–2269.

**Supplementary key words** combined lipase deficiency • hypertriglyceridemia • lipoprotein lipase • genetic complementation

Lipase maturation factor 1 (Lmf1) has been identified as the protein affected by the combined lipase deficiency (*clد*) mutation in the mouse (1). Mice homozygous for *clد* exhibit severe chylomicronemia due to the deficiency of lipoprotein lipase (LPL) activity (2). LPL is synthesized and secreted by a wide variety of cell types, and it repre-

sents the rate-limiting step in the tissue influx of circulating triglyceride-derived fatty acids (3). The severity of the LPL deficiency in *clد* mice is similar to homozygous LPL knock-out mice (4), and both exhibit neonatal lethality resulting from chylomicronemia-derived ischemia and the abolishment of triglyceride-derived fatty acid influx (2). Besides LPL deficiency, enzyme activities for several lipase family members are also diminished in *clد* mice, including hepatic lipase (1, 5). Thus, *LMF1* has become a candidate gene for hypertriglyceridemia and related phenotypes associated with *clد* in human populations. In this regard, it is essential to have a quantitative assay to determine the functional consequence of *LMF1* sequence polymorphisms that are currently being identified worldwide in patients exhibiting hypertriglyceridemia. Furthermore, such an assay would be an invaluable tool in uncovering Lmf1 structure/function relationships using site-directed mutagenesis and related strategies that require the ability to assess the impact of induced sequence variation on Lmf1 function.

The *clد* mutation causes a C-terminal truncation of Lmf1 that abolishes its function as a posttranslational maturation factor for LPL (1). Thus, in *clد* mutant cells, LPL protein is synthesized normally, but it never attains activity and remains as an inactive protein trapped in the endoplasmic reticulum, where it is eventually degraded (6). However, when a human or mouse wild-type Lmf1 cDNA expression construct is cotransfected with LPL in *clد* mutant cells, LPL matures normally to an active lipase that is eventually secreted to the medium (1).

The ability to complement lipase maturation by transfection of target Lmf1 constructs in *clد* mutant cells

Abbreviations: *clد*, combined lipase deficiency mutation; hLmf1<sup>wt</sup>, full length human Lmf1 (567 amino acid); hLmf1<sup>Y439X</sup>, human Lmf1 truncated at position 439; Lmf1, lipase maturation factor 1; LPL, lipoprotein lipase; mLmf1<sup>clد</sup>, mouse Lmf1 truncated at position 361; mLmf1<sup>G181E</sup>, mouse Lmf1 containing a G/E substitution at position 181; mLmf1<sup>N430A</sup>, mouse Lmf1 containing a N/A substitution at position 430; mLmf1<sup>wt</sup>, full length mouse Lmf1 (574 amino acid); SEAP, secreted form of alkaline phosphatase; *wt*, wild type.

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provides the basis of an assay to assess Lmf1 function. Theoretically, the ability of target Lmf1 sequences to restore LPL activity in *clld* cells can be used as a measure of Lmf1 function. We provided proof of principle for such an assay by showing that a nonsense mutation (Y439×) in a human Lmf1 expression construct failed to restore LPL activity in *clld* mutant cells (1). However, Y439× represents a complete loss-of-function mutation that is qualitative in nature (all or none) and thus relatively easy to detect. Our original assay did not have the quantitative dynamic range to detect mutations that cause only a limited loss of function, or those that only partially affect Lmf1 protein expression. In this report, we describe an optimized version of our original assay that has the ability to detect Lmf1 mutations that affect function or protein levels over a wide dynamic range. We demonstrate the utility of the assay using several induced mutations in mouse Lmf1.

## MATERIALS AND METHODS

### The *clld* mutant cell line

The *clld* mutant cell line used in the assay has been described previously (7). It represents a hepatocyte-derived cell line derived from 16- to 18-day-old fetal *clld/clld* mice immortalized by transfection with the large SV40 T antigen [pSV3neo (8)], and it is available upon request. The cells lines were maintained at split ratios of 1:20 in DMEM-10% FBS and included passage numbers 25–53. For the Lmf1 assay, a total of  $1.8 \times 10^5$  cells were distributed in a 12-well plate (Falcon), each flat-bottomed well containing 0.8 ml DMEM-10% FBS and having a surface area of 3.8 cm<sup>2</sup>. Transfection was initiated 24 h after plating when cells reached  $\leq 90\%$  confluence.

### Expression constructs and Lmf1 target sequences

The LPL expression construct represents a full-length human LPL cDNA subcloned into the pcDNA6/V5-His expression vector (Invitrogen). The C terminus of the expressed LPL protein is fused with a V5 epitope tag as described (9).

All human and mouse Lmf1 sequences were subcloned into the pcDNA3.1 expression vector (Invitrogen); the expressed Lmf1 proteins contained an N-terminal *c-myc* epitope tag (1). The following human *LMF1* target sequences were used: wild-type Lmf1 (hLmf1<sup>wt</sup>) encoding the full-length 567 amino acid protein, and the Y439× nonsense mutation, removing 127 amino acids from the C terminus of Lmf1 (hLmf1<sup>Y439×</sup>) (1). The following mouse *Lmf1* target sequences were used: wild-type Lmf1 (mLmf1<sup>wt</sup>) encoding the full-length 574 amino acid protein; the naturally occurring *clld* mutation, removing 214 residues from the C terminus of the Lmf1 protein (mLmf1<sup>clld</sup>) (1); an induced missense mutation substituting Ala for Asn at amino acid 430 of Lmf1 that is part of the single Asn-Ala-Ser glycosylation site in wild-type Lmf1 (mLmf1<sup>N430A</sup>); and a missense mutation causing a Glu for Gly substitution at residue 181 of the Lmf1 protein (mLmf1<sup>G181E</sup>). The G181E mutation was identified in a clonal library of ENU-mutagenized ES cells maintained at the University of North Carolina Mouse Mutant Regional Resource Center. The Lmf1 open reading frame was PCR-amplified from ENU-mutagenized ES cell DNA and was scanned for mutations using denaturing HPLC. The G181E mutation is due to a G-to-A substitution in exon 4 of the mouse *Lmf1* gene.

To normalize for transfection efficiency, we used a secreted human placental alkaline-phosphatase (SEAP) reporter gene subcloned into the pM1 expression vector (X-extremeGENE®,

Roche). An LPL/SEAP plasmid master mix was prepared and used throughout all assay experiments performed. The master mix contained LPL and SEAP at a concentration of 0.4 and 0.1 mg/ml, respectively.

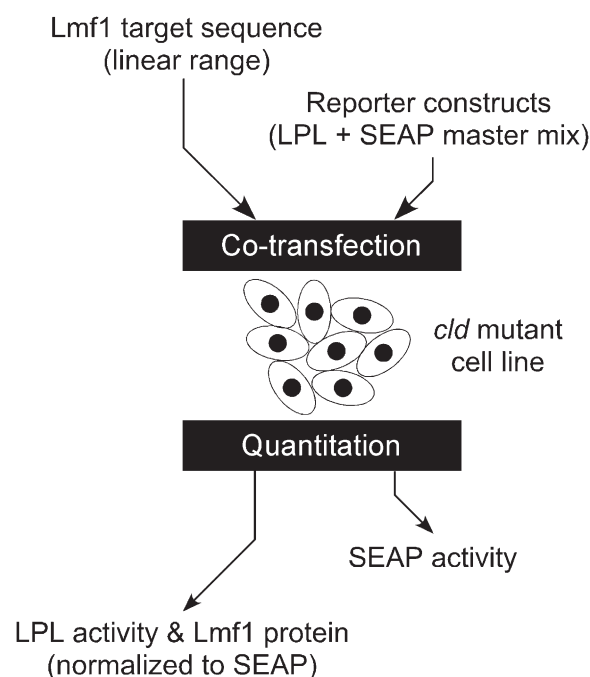
All expression vector plasmids used in these studies were prepared using the EndoFree Maxi kit (Qiagen) according to the manufacturer's instructions. Diluted plasmid solutions were quantitated using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

### Cotransfection and cell harvesting

Each cotransfection experiment contained an Lmf1 target sequence and a mixture of two reporter constructs (LPL/SEAP master mix; see above). Transfection of *clld* mutant cells was achieved using the Effectene® transfection reagent (Qiagen) at a DNA-reagent ratio of 1:10. Each well of a 12-well plate was transfected with 0–20 ng of the Lmf1 target sequence and a mixture of 0.8 µg and 0.2 µg of LPL and SEAP, respectively; transfection was performed according to the manufacturer's instructions. At 24 h posttransfection, a sample of medium was taken for SEAP activity measurement, and then heparin was added to a final concentration of 10 U/ml. At 48 h posttransfection, fresh heparin-containing medium was added ( $t = 0$ ) and at various times later (30 min–8 h), samples were removed for LPL activity measurements. At the end of the experiment, cells were washed twice with PBS and lysed in detergent-containing buffer (0.2% sodium deoxycholate, 10 U/ml heparin, 10 mM Tris-HCl, pH 7.5). After sonication and centrifugation, supernatants from the resulting lysates and medium samples were stored at  $-80^\circ\text{C}$  until use.

### Detection and quantitation

SEAP activity was measured using the SEAP Reporter Assay kit (InvivoGen) according to the manufacturer's instructions. SEAP

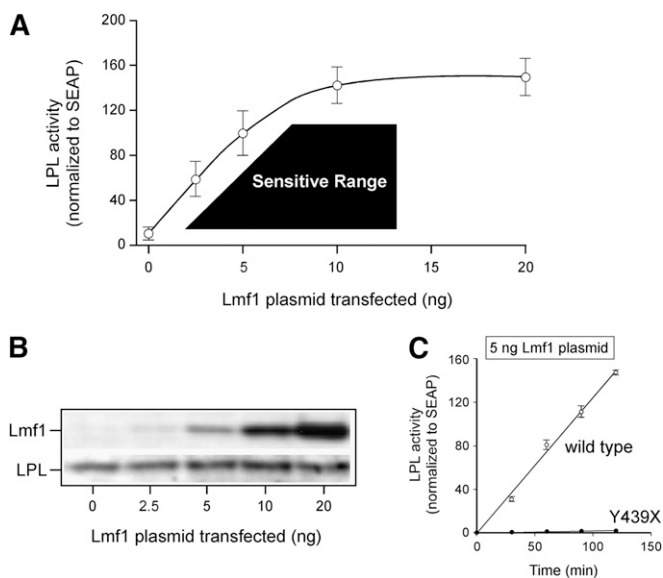


**Fig. 1.** Overview of the Lmf1 functional assay. The first step of the assay is cotransfection of the *clld* mutant cell line with an Lmf1 target sequence and two reporter constructs, LPL and SEAP. The second step quantitates LPL activity, Lmf1 protein levels, and SEAP activity, and the latter used to correct for differences in transfection efficiency. It is essential that the Lmf1 target sequence is expressed at levels that fall within the sensitive range of LPL activity (see text and Figs. 2 and 3).

catalyzes the hydrolysis of *p*Nitrophenyl phosphate producing a yellow end product read spectrophotometrically at 405 nm. For each assay, OD<sub>405nm</sub> readings were taken over 30–240 min to establish the linear range for the assay, which was then used for normalization of LPL activity levels.

LPL activity was measured using a lecithin-stabilized radiolabeled triolein (glycerol tri[9,10(n)-<sup>3</sup>H]oleate) substrate as described (10). Each mU of LPL activity is equivalent to 1 nmol free fatty acid released per minute. Secreted LPL activity is given as mU/ml of medium, normalized to SEAP activity per ml (i.e., mU/SEAP). Because SEAP activity is based on OD<sub>405nm</sub> measurements, normalized LPL activity is a relative value and thus given as an arbitrary number (see Figs. 2 and 3).

For detection of Lmf1 protein, a biotinylated rabbit anti-*c-myc* polyclonal antibody (Gene Tex) was used at a dilution of 1:2,500; LPL protein was detected using a biotinylated rabbit anti-LPL antibody (a kind gift from André Bensadoun) at 1:2,000. Biotinylated antibodies were detected using horseradish peroxidase-conjugated Streptavidin (Invitrogen) at 1:50,000 and visualized using a chemiluminescent substrate (ECL+®, GE Healthcare). Quantitation of Lmf1 band intensity was performed using the software NIH Image 1.63.



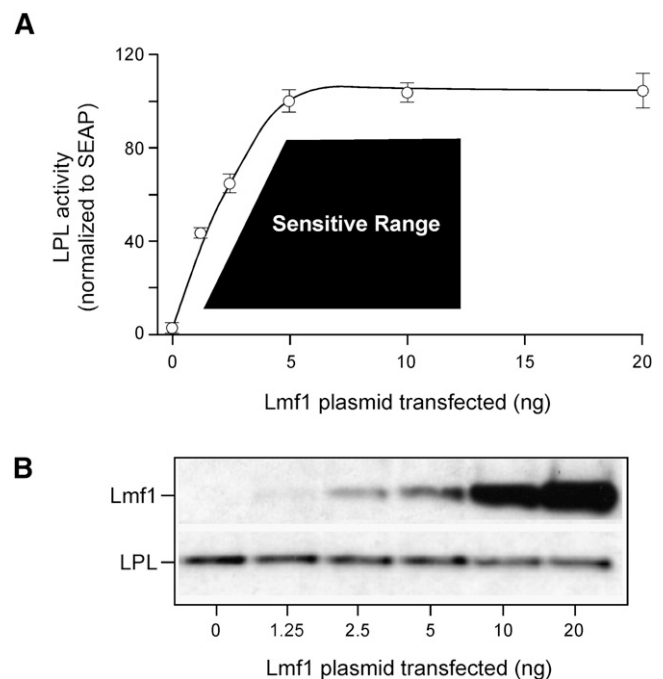
**Fig. 2.** Quantitative assay measuring function of human Lmf1. A: The *clد* mutant cell line was transfected with increasing amounts of a human wild-type Lmf1 expression plasmid (hLmf1<sup>wt</sup>; see Methods and Materials); at 24 h posttransfection, SEAP activity was measured and heparin added to medium. Eight hours postheparin, secreted LPL activity (mU/ml) was measured in the medium. Initially, changes in LPL activity (normalized to SEAP) were dependent on the amount of Lmf1 plasmid transfected but then plateaued at ~10 ng; the sensitive range of the assay is illustrated. The normalized LPL activity value at 5 ng Lmf1 plasmid was arbitrarily set to 100. B: Mutant *clد* cells transfected with increasing amounts of Lmf1 plasmid (shown in A) were subjected to Western blot analysis to assess Lmf1 and LPL protein levels. The signals shown were obtained from equal loads of SEAP activity. C: Mutant *clد* cells were transfected with either an hLmf1<sup>wt</sup> or hLmf1<sup>Y439X</sup> expression construct within the sensitive range of the assay (5 ng Lmf1 plasmid). SEAP was measured 24 h posttransfection and heparin added immediately after (t = 0). Medium was collected through 120 min and assayed for LPL activity, which was then normalized to SEAP activity. Normalized LPL activity levels in A and C represent the average of three individual transfections (n = 3) ± SEM.

## RESULTS AND DISCUSSION

Our assay is based on the ability of transfected Lmf1 to restore LPL activity in *clد* mutant cells; thus, LPL activity becomes a reporter of Lmf1 function. Because these cells do not endogenously synthesize LPL, they are cotransfected with LPL along with a second reporter construct, SEAP (Fig. 1). LPL restoration by an Lmf1 target sequence is compared with a range of values whose maximum is defined by wild-type Lmf1 expressing full function as an LPL maturation factor and a minimum defined by Lmf1 carrying a loss-of-function mutation. Thus, an Lmf1 target sequence to be tested requires comparison to both wild-type and mutant Lmf1 controls, each representing separate cotransfection experiments. To account for differences in transfection efficiency occurring in separate experiments, LPL activity is normalized to SEAP activity, and this value is used as the readout of Lmf1 function. In a similar manner, Lmf1 protein levels are compared between the Lmf1 target sequence and the wild-type control (Fig. 1). In such a way, the normalized LPL activity for any given Lmf1 target sequence can be assessed relative to wild-type Lmf1 function and protein level, as shown below.

### Determining the sensitive range of the assay

The sensitive range of the assay ensures the detection of a wide range of Lmf1 function (full, partial, or complete loss of function) using LPL activity as a quantitative reporter.



**Fig. 3.** Quantitative assay measuring function of mouse Lmf1. A: The *clد* mutant cell line was transfected with increasing amounts of a mouse wild-type Lmf1 expression plasmid (mLmf1<sup>wt</sup>). LPL and SEAP activity were measured as described in the legend to Fig. 2. The normalized LPL activity value at 5 ng Lmf1 plasmid was arbitrarily set to 100; the values are the average of three individual transfections ± SEM. B: Mutant *clد* cells transfected with increasing amounts of Lmf1 plasmid (shown in A) were subjected to Western blot analysis to assess Lmf1 and LPL protein levels. The signals were obtained from equal loads of SEAP activity.



In this range, the quantitative reporter (LPL activity) responds proportionally to changes in Lmfl protein levels (or function), as shown in **Fig. 2A**. When increasing amounts (0–20 ng) of wild-type human Lmfl (hLmfl<sup>wt</sup>) plasmid are cotransfected with a constant amount (0.8 μg) of LPL plasmid, levels of LPL activity are directly dependent on transfected Lmfl plasmid levels until they become saturating at ~10 ng (Fig. 2A). Notably, increasing the amount of hLmfl<sup>wt</sup> plasmid in the transfection increased Lmfl protein levels proportionally (Fig. 2B). The assay is most sensitive when expressed Lmfl protein levels fall within the linear portion of the LPL activity curve (highlighted in Fig. 2A). A similar sensitive range was determined for mouse Lmfl (**Fig. 3**), showing that both human and mouse Lmfl target sequences can be tested successfully in the assay. As expected, LPL protein levels are relatively unchanged, because the amount of LPL plasmid used in the transfection is held constant (Fig. 3B).

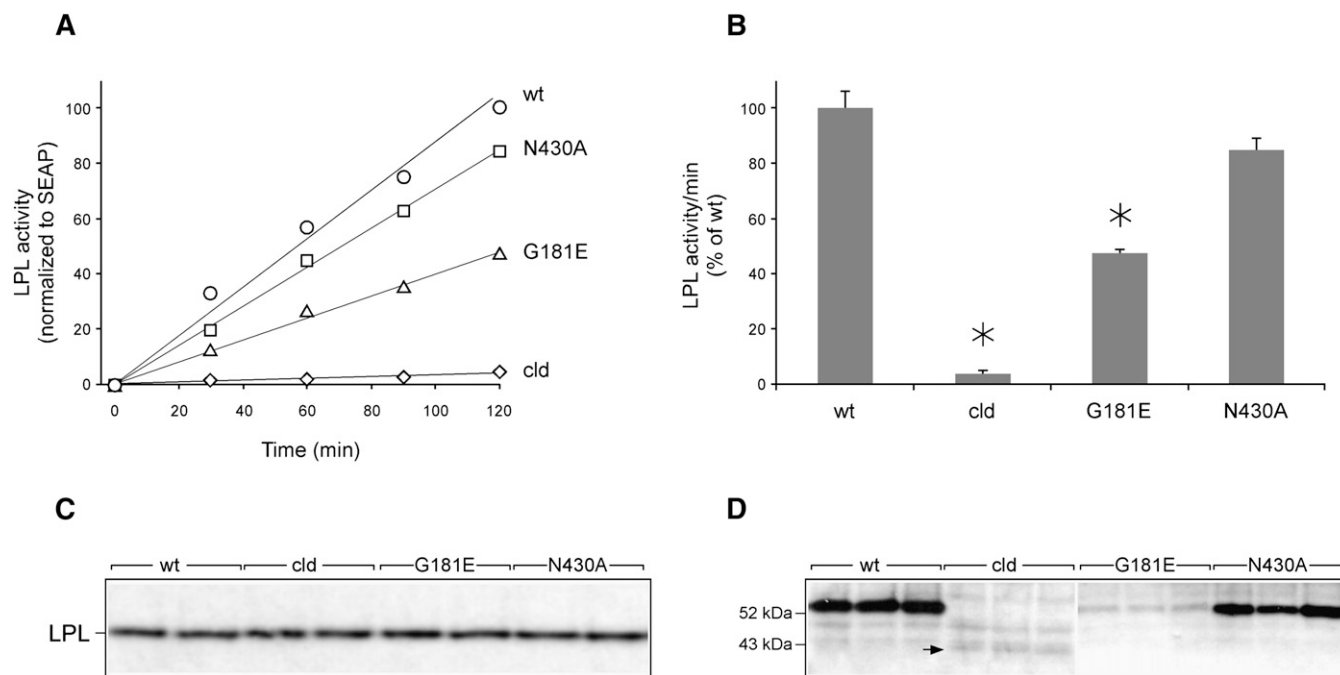
Figures 2A and 3A indicate that the ratio of target Lmfl to LPL plasmid should be chosen within the sensitive range of LPL activity, ensuring that the full dynamic range of the assay is utilized. Such a ratio was used to compare hLmfl<sup>wt</sup> to hLmfl<sup>Y439X</sup>, the latter containing a C-terminal deletion causing *clld* and hypertriglyceridemia in a human patient (1). In this case, 5 ng of both Lmfl plasmids were used while the amount of LPL plasmid remained constant (0.8 μg), representing an hLmfl<sup>wt</sup>-LPL ratio falling within the predetermined sensitive range (Fig. 2A). As shown in

Fig. 2C, the secretion curves of LPL activity arising from hLmfl<sup>wt</sup> and hLmfl<sup>Y439X</sup> target sequences confirms Y439X as a mutation causing loss of function. In general, we recommend that the protein level of an Lmfl target sequence should be less than or equal to a wild-type Lmfl control whose sensitive range has been predetermined (see below).

#### Analyzing the function of Lmfl mutant proteins

To illustrate the dynamic range of the assay, we tested several novel mutations in mouse Lmfl. These included two induced missense mutations, G181E and N430A, and the naturally occurring *clld* truncation (1). N430A removes the single glycosylation site in mouse Lmfl, while G181E is adjacent to a putative transmembrane-spanning region. Both Asn and Gly at positions 430 and 181 are conserved amino acids; however, glycosylation occurs only in mouse Lmfl (data not shown), suggesting that loss of N-linked glycosylation by N430A may be unimportant. The effect of G181E on Lmfl function is unknown, but its juxtaposition near a transmembrane domain could disrupt proper membrane insertion, affecting Lmfl membrane topology.

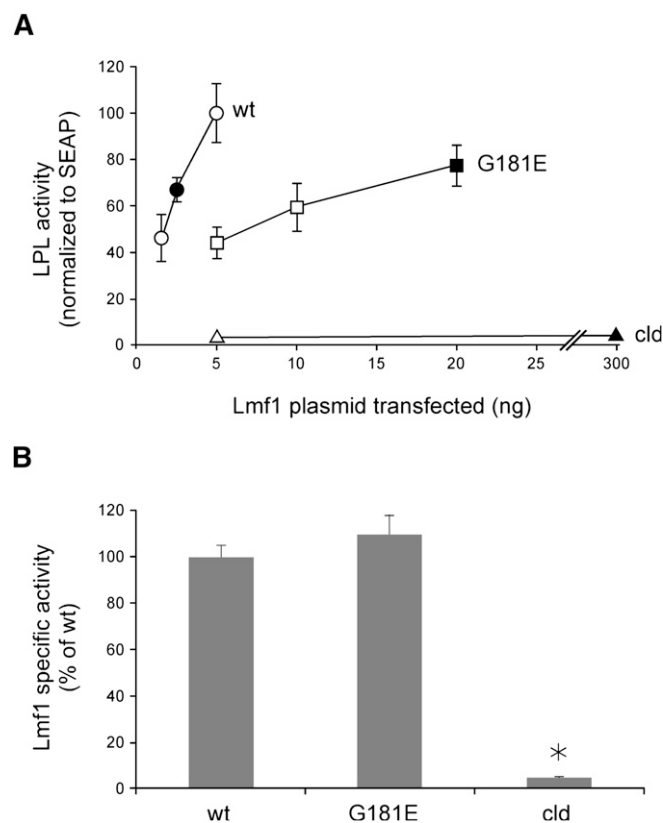
In an initial comparison, equivalent plasmid amounts were used for all transfections (5 ng) based on a wild-type Lmfl control predetermined to be within the sensitive range of the assay (Fig. 3A). The secretion curves of the three Lmfl mutant plasmids compared with the wild-type control are shown in **Fig. 4A**, whose slopes are replotted in Fig. 4B as LPL activity secreted per minute. While the



**Fig. 4.** Dynamic range of the Lmfl assay. **A:** LPL activity, normalized to SEAP, was determined in the medium after addition of heparin as described in the legend to Fig. 2C. Shown are the secretion curves for two induced missense mutations in mouse Lmfl (N430A, G181E), and the naturally occurring *clld* truncation (*clld*), as compared with the *wt* control. **B:** The slopes of the secretion curves (i.e., LPL activity/min) are replotted as a percentage of the *wt* control. Each histogram represents the average of three individual transfections  $\pm$  SEM. Asterisks denote a significant difference in the *clld* ( $P = 0.004$ ) and G181E ( $P = 0.011$ ) values as compared with the *wt* control. **C:** Shown are Western blots of LPL protein levels for the four Lmfl plasmids tested. An equivalent amount of SEAP activity was loaded in each lane. Shown are two of three separate transfections carried out for each Lmfl plasmid. **D:** Shown are Western blots of Lmfl protein levels for all four Lmfl plasmid constructs. For each construct, the results of all three transfections are shown; an equivalent amount of SEAP activity was loaded in each lane. The arrowhead points to the position of the *clld*-truncated Lmfl protein.

N430A mutation is comparable to the wild-type control, Lmfl carrying either G181E (mLmfl<sup>G181E</sup>) or *cld* (mLmfl<sup>cld</sup>) exhibit a significant decrease in their ability to restore LPL activity levels (Fig. 4B). Notably, the assay is able to detect a distinct difference between all three mutations by showing that G181E is intermediate in its severity when compared with N430A and *cld*. Regardless of the Lmfl sequence tested, LPL protein expression remained relatively unchanged (Fig. 4C), indicating that G181E and *cld* affect LPL activity in a posttranslational manner.

Decreased Lmfl protein levels and loss of Lmfl function could account for the observed decrease in the ability of mLmfl<sup>G181E</sup> and mLmfl<sup>cld</sup> to restore LPL activity (Fig. 4B). Indeed, the G181E and *cld* mutations cause markedly reduced Lmfl protein levels when compared with N430A and the *wt* control (Fig. 4D). Thus, both mutations likely



**Fig. 5.** Functional analysis of Lmfl mutants. A: Shown are LPL activity curves using an extended range of plasmid amounts for the two mutants, G181E and *cld*; plasmid amounts for the *wt* control were identical to those used in Fig. 3A. B: Densitometric scans of Lmfl band intensities after Western blot analysis were used as a measure of Lmfl protein mass. Lmfl mass was measured from transfected Lmfl plasmid amounts of 2.5 ng (*wt*), 20 ng (G181E), and 300 ng (*cld*) as denoted by solid symbols in A, as they gave rise to similar Lmfl band intensities of 38.8 (*wt*), 41.1 (G181E), and 60.4 (*cld*). These values lie within the sensitive range of the assay, whose upper limit is defined by the *wt* construct (5 ng), giving an Lmfl mass of 100 arbitrary units. For each construct, the corresponding LPL activity levels (denoted by solid symbols) were used to calculate Lmfl specific activity, defined as LPL activity per unit Lmfl protein mass. The *wt* control was arbitrarily set to 100, and each value represents the average  $\pm$  SEM of three separate transfections. The asterisk denotes a significant difference between *cld* and *wt* ( $P < 0.001$ ).

cause increased Lmfl protein turnover, possibly due to misfolding and/or improper membrane topology. To offset these low Lmfl protein levels in the assay, increased amounts of plasmid for mLmfl<sup>G181E</sup> and mLmfl<sup>cld</sup> were used in the transfection while maintaining the preestablished plasmid levels for the mLmfl<sup>wt</sup> control. As shown in Fig. 5A, LPL activity was restored by mLmfl<sup>G181E</sup> in a dose-dependent manner; in contrast, mLmfl<sup>cld</sup> remained non-functional even when using highly elevated plasmid amounts in the assay. Based on these results, G181E clearly causes reduced Lmfl protein levels, but it is difficult to assess whether it also affects Lmfl function. To eliminate Lmfl protein level as a variable, the readout of the assay was changed from LPL activity to Lmfl-specific activity, defined as LPL activity restored per unit Lmfl protein. As shown in Fig. 5B, Lmfl-specific activity clearly shows that G181E does not affect Lmfl function, which is in stark contrast to Lmfl truncated by *cld*. Thus, G181E causes reduced Lmfl protein expression without affecting function, whereas *cld* clearly represents a loss-of-function mutation. We hypothesize that G181E may decrease the efficiency of proper Lmfl membrane insertion, resulting in increased protein turnover. However, some fraction of mLmfl<sup>G181E</sup> attains a native state, and these molecules remain stable and function normally as a lipase maturation factor. Importantly, the N430A, G181E, and *cld* mutations illustrate the ability of the assay to detect and analyze mutations having a wide range of effects on Lmfl function and protein expression.

## REFERENCES

- Peterfy, M., O. Ben-Zeev, H. Z. Mao, D. Weissglas-Volkov, B. E. Aouizerat, C. R. Pullinger, P. H. Frost, J. P. Kane, M. J. Malloy, K. Reue, et al. 2007. Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. *Nat. Genet.* **39**: 1483–1487.
- Reue, K., and M. H. Doolittle. 1996. Naturally occurring mutations in mice affecting lipid transport and metabolism. *J. Lipid Res.* **37**: 1387–1405.
- Merkel, M., R. H. Eckel, and I. J. Goldberg. 2002. Lipoprotein lipase: genetics, lipid uptake, and regulation. *J. Lipid Res.* **43**: 1997–2006.
- Coleman, T., R. L. Seip, J. M. Gimble, D. Lee, N. Maeda, and C. F. Semenkovich. 1995. COOH-terminal disruption of lipoprotein lipase in mice is lethal in homozygotes, but heterozygotes have elevated triglycerides and impaired enzyme activity. *J. Biol. Chem.* **270**: 12518–12525.
- Paterniti, J. R., Jr., W. V. Brown, H. N. Ginsberg, and K. Artzt. 1983. Combined lipase deficiency (*cld*): a lethal mutation on chromosome 17 of the mouse. *Science*. **221**: 167–169.
- Briquet-Laugier, V., O. Ben-Zeev, A. White, and M. H. Doolittle. 1999. *cld* and *lec23* are disparate mutations that affect maturation of lipoprotein lipase in the endoplasmic reticulum. *J. Lipid Res.* **40**: 2044–2058.
- Boedeker, J. C., M. H. Doolittle, and A. L. White. 2001. Differential effect of combined lipase deficiency (*cld/cld*) on human hepatic lipase and lipoprotein lipase secretion. *J. Lipid Res.* **42**: 1858–1864.
- Southern, P. J., and P. Berg. 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Mol. Appl. Genet.* **1**: 327–341.
- Ben-Zeev, O., H. Z. Mao, and M. H. Doolittle. 2002. Maturation of lipoprotein lipase in the endoplasmic reticulum. Concurrent formation of functional dimers and inactive aggregates. *J. Biol. Chem.* **277**: 10727–10738.
- Briquet-Laugier, V., O. Ben-Zeev, and M. H. Doolittle. 1999. Determining lipoprotein lipase and hepatic lipase activity using radiolabeled substrates. *Methods Mol. Biol.* **109**: 81–94.