Novel mutations of the lipoprotein lipase gene associated with hypertriglyceridemia in members of type 2 diabetic pedigrees[S]

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Abstract **Increased plasma triglyceride and free fatty acid levels are frequently associated with type 2 diabetes mellitus (T2DM). To test the hypothesis that LPL gene mutations contribute to the hypertriglyceridemia observed in members of T2DM pedigrees, we screened the LPL gene in 53 hypertriglyceridemic members of 26 families. Four known and three novel mutations were identified. All three novel mutations, Lys312insC, Thr361insA, and double mutation Lys312insC + Asn291Ser, are clinically associated with hypertriglyceridemia. In vitro mutagenesis and expression studies confirm that these variants are associated with a significant reduction in LPL activity. The modeled structures displaying the Lys312insC and Thr361insA mutations showed loss of the activity-related C-terminal domain in the LPL protein. Another novel double mutation, Lys312insC + Asn291Ser, resulted in the loss of the catalytic ability of LPL attributable to the complete loss of the C-terminal domain and alteration in the heparin association site. Thus, these novel mutations of the LPL gene contribute to the hypertriglyceridemia observed in members of type 2 diabetic pedigrees.**—Hu, Y., Y. Ren, R. Z. Luo, X. Mao, X. Li, X. Cao, L. Guan, X. Chen, J. Li, Y. Long, X. Zhang, and H. Tian. **Novel mutations of the lipoprotein lipase gene associated with hypertriglyceridemia in members of type 2 diabetic pedigrees. *J. Lipid Res.* 2007.** 48: **1681–1688.**

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A high prevalence of dyslipidemias is well recognized in type 2 diabetes mellitus (T2DM) (1, 2). Approximately 77.5% of type 2 diabetic patients exhibit dyslipidemias. Although the dyslipidemias in T2DM, particularly hypertriglyceridemia, are exacerbated by poor glycemic control, lipid abnormalities cannot be explained merely by hyperglycemia (3, 4). Investigators have shown that individuals with impaired glucose regulation and recent-onset T2DM share a similar high prevalence of dyslipidemias with individuals with long-standing diabetes. A high prevalence of dyslipidemias can also be found in normal glucose-tolerant first-degree relatives of type 2 diabetic pedigrees (5, 6). On the other hand, abnormal lipid profiles have also been suggested to predate the onset of T2DM (7, 8), suggesting an inherited component in the association of dyslipidemias with T2DM.

The most common dyslipidemia in individuals with T2DM is increased triglyceride (TG) levels. LPL plays a central role in TG metabolism. LPL, if defective, could predispose to hypertriglyceridemia (9–12). LPL is a 55 kDa glycoprotein and active as a noncovalent dimer, whereas the dissociated monomer enzyme has no activity (13). Activated LPL is bound to the surface of endothelial cells and can be released into blood by heparin. Genetic defects of LPL are responsible for the reduced TG-rich lipoprotein clearance, and mutations in the LPL gene have been shown to play a central role in the development of hypertriglyceridemia in the general population (14–16). Approximately 143 different mutations have been identified to date in the human LPL gene, 90% of which occur in the coding regions and affect LPL functions through catalytic activity, dimerization, secretion, and heparin binding.

To test the hypothesis that LPL gene mutations are responsible for the hypertriglyceridemia observed in members of type 2 diabetic pedigrees, we analyzed the LPL

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Abbreviations: DHPLC, denaturing high-performance liquid chromatography; SSCP, single-strand conformation polymorphism; T2DM, type 2 diabetes mellitus; TG, triglyceride.

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promoter and 10 exons including the adjacent intronic regions from 53 hypertriglyceridemic members of 26 families. Four known and three novel variants and one compound heterozygote were detected in these hypertriglyceridemic members, whereas no mutation was detected among 118 controls. Structural analysis and functional studies suggest that these mutations led to the structural alterations of the LPL protein associated with reduced enzymatic activity and thus appear to contribute to the hypertriglyceridemia in members of type 2 diabetic pedigrees.

MATERIALS AND METHODS

Population study subjects

The study population consisted of 350 members of 45 families of Chinese Han decent who had two or more T2DM siblings. Type 1 diabetes and the other specific diabetes were excluded from our study subjects. All members underwent standard 75 g oral glucose tolerance testing. Diagnosis of T2DM or impaired glucose tolerance was based on the criteria of the World Health Organization (17). Fasting plasma TG concentrations were measured in all members. Members of type 2 diabetic pedigrees with fasting TG levels $>$ 2.25 mmol/l were chosen for analysis of the LPL gene. None of subjects was on lipid-lowering treatment at the time the lipid levels were taken. Fifty-three individuals met the criteria, including 31 individuals with T2DM, 11 individuals with impaired glucose tolerance, and 11 individuals with normal glucose tolerance. When a family member met these criteria, all pedigrees in the family, including the pedigrees with normal plasma TG levels, were also screened for LPL mutation. A total of 87 T2DM family members were subjected to LPL gene mutation screening. The control population consisted of 118 healthy individuals chosen from nondiabetic families without any medication affecting lipid metabolism. Written informed consent was obtained from the subjects who agreed to participate in the study, and the study protocol had the approval of our local ethics committee.

Molecular screening of the LPL gene and analysis of mutant alleles in families

Single-strand conformation polymorphism (SSCP), denaturing high-performance liquid chromatography (DHPLC), and direct DNA sequencing were applied for analysis of the LPL gene. We screened potential mutations of the LPL promoter and 1–10 exons including the adjacent intronic regions from 87 individuals of 26 families, including 53 members with hypertriglyceridemia ($\geq$2.25 mmol/l) (see supplementary Table I) and their normal plasma TG family members in the pedigrees.

The PCR products with abnormal SSCP patterns or abnormal DHPLC waveforms were further confirmed by DNA sequencing. Genomic DNA was extracted from whole blood. The LPL promoter and 10 exons including the adjacent intronic regions were amplified by PCR using the described primers (see supplementary Table II). SSCP was performed as follows. The PCR product was mixed with one-fourth volume of loading buffer containing 95% formamide, 10 mM EDTA, pH 8.0, 0.05% (w/v) bromophenol blue, and 0.05% (w/v) xylene cyanol FF. The mixture was denatured at 95°C for 5 min and then snap-cooled on ice. Electrophoresis was performed on a 6% polyacrylamide gel with 0.5 $\times$ TBE (Tris-Borate_EDTA) buffer under various conditions: acrylamide-bis-acrylamide 37:1 or 49:1 containing 5% or 10% glycerol at 4°C or 20°C. DNA was visualized by ethidium bromide staining. DHPLC was performed on an automated HPLC instrument (Hewlett-Packard Instrument). PCR products were eluted with a linear acetonitrile gradient of 1.8%/min at a flow rate of 0.8%/ml/min. The start and end points of the gradient were adjusted according to the size of the PCR products. The temperature for successful resolution of the heteroduplex molecules was predicted by the DHPLC algorithm available at http://insertion.stanford.edu/melt.html. PCR products that demonstrated variant mobility on SSCP analysis or variant waveforms on DHPLC analysis were examined by direct sequencing. The heterozygous and homozygous samples were cloned in T-Easy vector (Promega, Madison, WI) and then sequenced.

In vitro site-directed mutagenesis and expression

Total RNA was isolated from human epiploon adipose tissue. RT-PCR primers were designed and synthesized according to the reported cDNA sequence of the LPL. The LPL cDNA was cloned into the pcDNA3.1Zeo(+) vector. The recombinant pcDNA3.1Zeo(+)/LPL cDNA was identified by DNA sequencing. Desired mutant LPL constructs were created using the Site-Directed Kit (Takara) with pcDNA3.1Zeo(+)/LPL as template. LPL constructs were transfected to COS-1 cells using Lipofectamine 2000™ (Invitrogen, Carlsbad, CA). The LPL mass in cells and the culture medium was determined with a Markit-M LPL Kit (Dainippon Pharmaceutical). LPL activity in cell lysates and the culture medium was measured using the LPL activity kit according to the protocol provided by the manufacturer (JianChen Institute of Biologic Engineering, Nanjing, China).

Structural modeling for mutant LPL

The three-dimensional model structure of the human LPL was reconstructed using the UCSF Chimera package (http://www.cgl.ucsf.edu/chimera) from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (18, 19), based on the coordinates of the human LPL models kindly provided by Dr. I. Inoue (19). The LPL dimer

TABLE 1. LPL gene mutations in hypertriglyceridemic members of type 2 diabetic pedigrees

Region	Nucleotide Change	Amino Acid Change	Type of Mutation	Affected Pedigree No.	TG Level
Exon 3	GCC→ACC	Ala71Thr	Missense mutation	3	↑↑
Exon 3	GTG→GTA	Val108Val	Silent mutation	27	Normal
Exon 6	CTG→CCG	Leu286Pro	Missense mutation	1	↑↑
Exon 6	AAT→AGT	Asn291Ser	Missense mutation	8, 20	↑ or normal
Exon 6	AAA→AACA	Lys312insC	Insertion mutation	8	? ?
Exon 8	ACC→ACAC	Thr361insA	Insertion mutation	14, 28	? ?
Exon 8	CTC→CTG	Leu376Leu	Silent mutation	5, 10, 18, 24	? ?

TG, triglyceride.

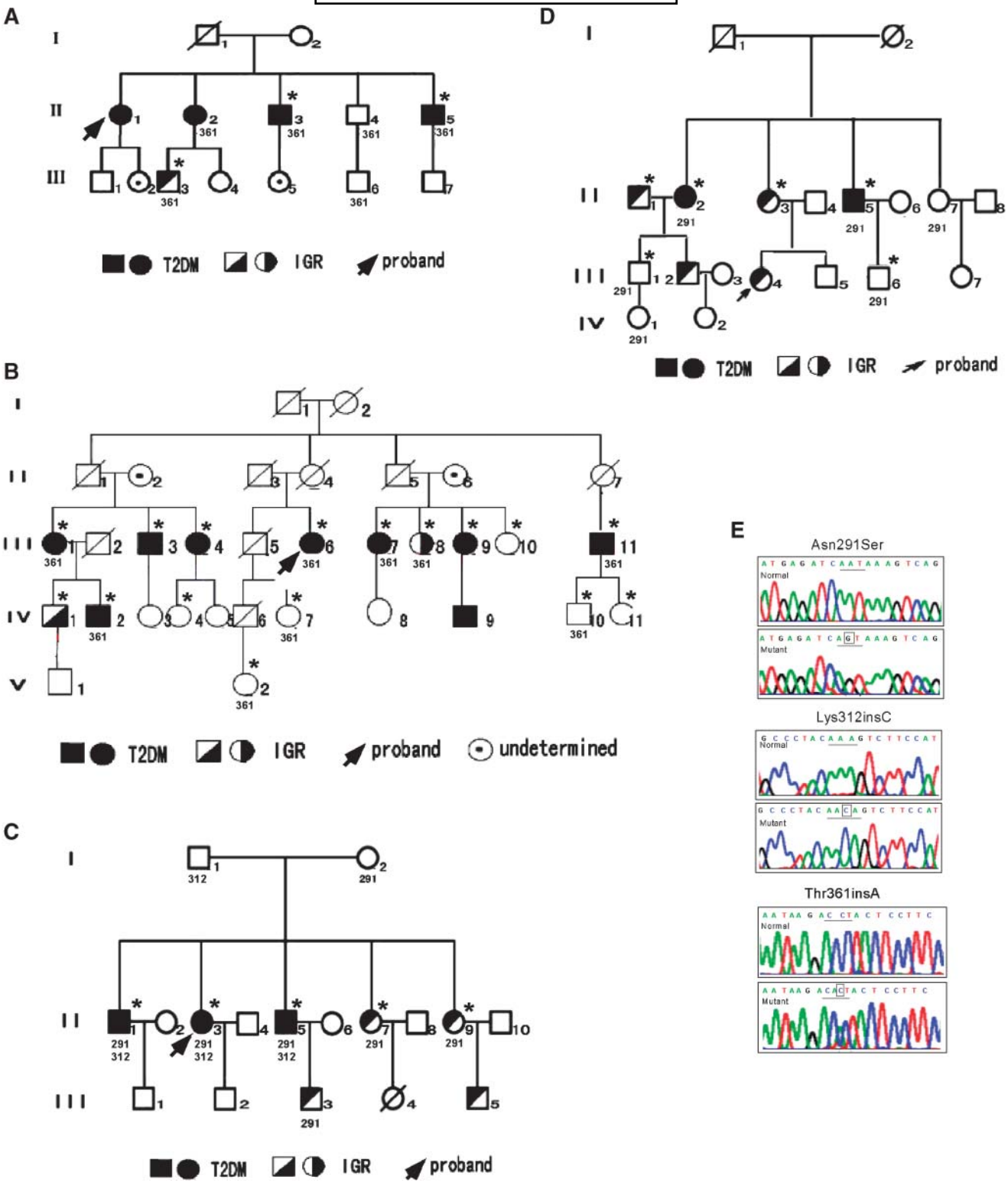

Fig. 1. LPL mutations detected in pedigrees of families 8, 14, 20, and 28. Pedigrees are shown for families 14 (A), 28 (B), 8 (C), and 20 (D) in which LPL mutations were detected. Arrows indicate probands, and asterisks indicate subjects with hypertriglyceridemia (plasma triglyceride > 1.7 mmol/l). Numerals 291, 312, and 361 indicate locations of the residue in LPL with the detected mutation. IGR (impaired glucose regulation) indicates all three minor glucose metabolism defects: impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and combined IGT + IFG. Panel E shows DNA sequencing plots of the cloned mutant LPL plasmids with mutations Asn291Ser, Lys312insC, and Thr361insA.

TABLE 2. Characteristics of pedigree members with or without the Thr361insA mutation in families 14 and 28

Characteristic	Noncarriers (Thr361insA)	Carriers (Thr361insA)	*t*	*P*
No.	17	15		
Sex (female/male)	10/7	7/8	0.473(Chi-square)	0.723
Age (years)	36.1 ± 15.5	50.3 ± 17.4	2.44	0.02
Body mass index (kg/m^2)	21.8 ± 3.36	25.4 ± 5.74	2.22	0.04
TG (mmol/l)	1.6 ± 0.4	2.5 ± 0.86	3.63	0.001
HDL-cholesterol (mmol/l)	1.7 ± 0.48	1.5 ± 0.47	0.72	0.48
Total cholesterol (mmol/l)	4.9 ± 0.87	5.6 ± 1.01	1.91	0.07
Fasting plasma glucose (mmol/l)	6.5 ± 2.51	7.02 ± 2.32	0.64	0.53
Fasting insulin (mU/l)	14.2 ± 7.78	15 ± 7.81	0.28	0.78

Values for TG were log-transformed for analysis, and results are presented as the antilog for comparison.

structure is represented using a ribbon model in the figures. Three residues (serine-132, aspartate-156, and histidine-241) that play important roles in catalysis are shown in a space-fill model, and the deleted regions caused by insertion mutants are represented using different colors.

Statistical analysis

Data are expressed as means ± SEM. Statistical comparisons between groups were performed using Student's *t*-test. Differences in proportions were evaluated by the Chi-square test and Fisher's exact test of the Spearman correlation. $P < 0.05$ was considered statistically significant.

Data from the two families in which the Thr361insA mutation segregated were pooled for analysis. TG levels were log-transformed before analysis, and all values were compared between carriers and wild-type individuals by Student's two-tailed *t*-test. Fisher's exact test of the Spearman correlation was applied to explore the correlation between high TG levels and the Thr361insA mutation in families 14 and 28. A univariate analysis of covariance between hypertriglyceridemia and the Thr361insA mutation within the pedigrees was applied to eliminate the effects of differences in age and body mass index on TG. All statistical analyses were performed on SPSS 10.0 (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Molecular screening of the LPL gene in hypertriglyceridemic members of type 2 diabetic pedigrees

Seven different LPL variants were detected (**Table 1**). Four of the variants were previously detected in exon 3 (Ala71Thr and Val108Val) and exon 6 (Leu286Pro and Asn291Ser) (9, 15, 16, 20–22). Three novel variants were detected in exon 6 (Lys312insC) and exon 8 (Thr361insA and Leu376Leu), including the compound heterozygotes Asn291Ser + Lys312insC.

In pedigree 1, the proband (TG = 11.9 mmol/l) was found carrying the Leu286Pro mutation; in pedigree 3, the proband (TG = 13.0 mmol/l) was found carrying the Ala71Thr mutation. When reported previously, the mutations Leu286Pro and Ala71Thr showed significant associations with hypertriglyceridemia (15, 22). The mutations in these two pedigrees seem to contribute to their hypertriglyceridemia (see supplementary Table IIIa, b). One silent mutation, Val108Val, which should not affect LPL activity, was detected. Two pedigrees showed the most common missense mutation, Asn291Ser (see supplementary Table IIIc, d). Similar to previous reports, this mutation in our subjects also appeared not to be consistently connected to hyperlipidemia (9, 16, 20, 21).

Three novel mutations of LPL were associated with hyperglycemia in members of type 2 diabetic pedigrees

Three novel mutations of LPL, Lys312insC, Thr361insA, and Lys312insC + Asn291Ser compound heterozygote, were detected in 37 members from seven families. Results showed that all three novel mutations were associated with hypertriglyceridemia. All subjects with known or novel LPL mutations were either heterozygotes or compound heterozygotes.

The Thr361insA mutation was detected in families 14 and 28 (**Fig. 1A**, **B**; see also supplementary Table IIIe, f). Fifteen individuals carried the mutation, and 17 individuals had wild-type alleles. Comparison of the clinical and laboratory findings in the 15 carriers with those in noncarriers by two-tailed *t*-test showed that the individuals with the mutation had significantly higher TG levels (**Table 2**). Adjustment for age and body mass index did not change the statistical significance of the TG level between the two groups ($R^2 = 0.56$, $P < 0.001$) using a univariate analysis of covariates. To further explore the potential relationship between hypertriglyceridemia and the Thr361insA mutation, analysis of correlation within the pedigrees (**Table 3**) showed that the Thr361insA mu-

TABLE 3. Correlation between hypertriglyceridemia and the Thr361insA mutation within the pedigrees

Pedigree	Noncarriers (Thr361insA)	Carriers (Thr361insA)	Total	Chi-Square	*P*
14					
HTG	7	9	16		
NTG	5	0	5		
Total	12	9	21	4.922	0.027
28					
HTG	0	4	4		
NTG	5	2	7		
Total	5	6	11	5.238	0.022
14 + 28					
HTG	7	13	20		
NTG	10	2	12		
Total	17	15	32	7.036	0.008

HTG, high triglyceride; NTG, normal triglyceride.

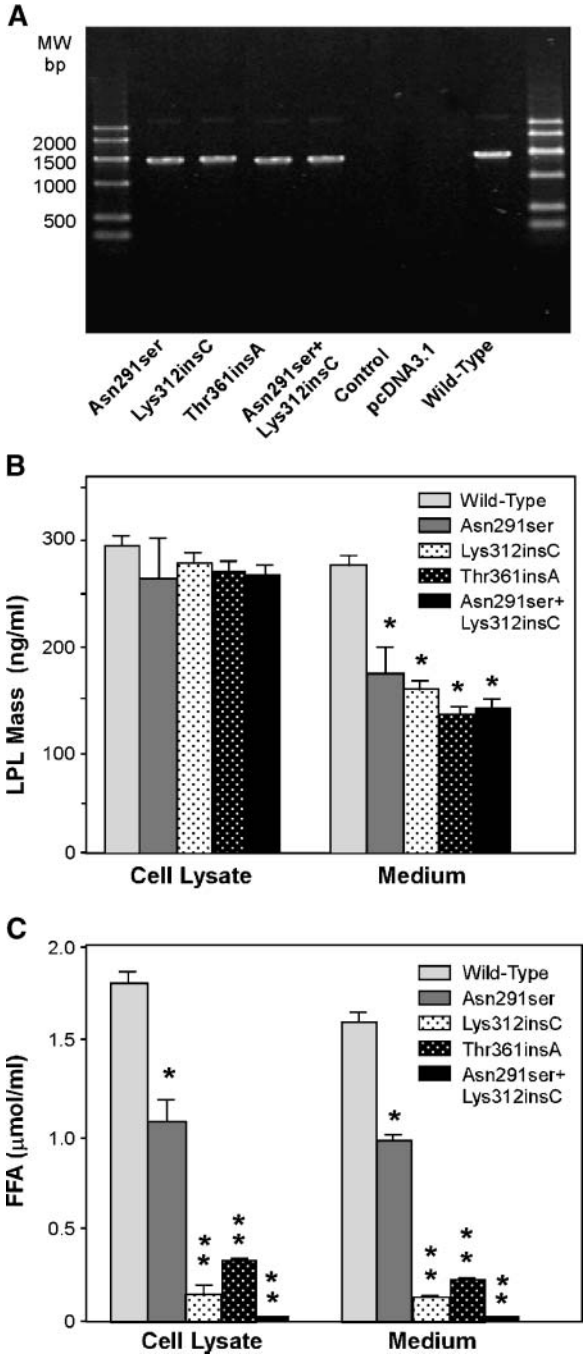

Fig. 2. Novel LPL mutations reduce LPL secretion and activity. A: Expression of LPL in COS-1 cells. COS-1 cells were cultured to 65% confluence and transfected with wild-type or mutant LPL plasmids. Semiquantitative RT-PCR was used to evaluate LPL expression in transfected cells. MW, molecular mass. B: LPL mass in cell lysates and cultured medium of the transfected COS-1 cells. Decreased LPL mass was detected in the cultured medium of mutant LPL cells. * $P < 0.05$. C: LPL activities in cell lysates and cultured medium of the transfected COS-1 cells. Decreased LPL activities were detected in both cell lysates and cultured medium of mutant LPL cells. * $P < 0.05$, ** $P < 0.01$. Data in B and C are expressed as means ± SEM.

tation of LPL was significantly correlated with hypertriglyceridemia ($P < 0.022 \sim 0.008$).

The Lys312insC mutation also appeared to be associated with hypertriglyceridemia. In family 8 (Fig. 1C), all six individuals who carried the Lys312insC mutation had hypertriglyceridemia, whereas other members who did not carry the mutation had normal TG levels. Thus, Lys312insC seemed to have a pathogenic correlation with hypertriglyceridemia. Three individuals who carried the Lys312insC mutation also carried the Asn291Ser mutation on their second allele and had a much higher hypertriglyceridemia, indicating that the compound heterozygote mutation Lys312insC + Asn291Ser exacerbates the defect of LPL function in these carriers. No individual was detected to carry the Lys312insC, Asn291Ser, or Thr361insA mutation in the control group.

In vitro analysis of LPL mutations

To further examine the significance of the Asn291Ser, Thr361insA, Lys312insC, and Lys312insC + Asn291Ser mutations, constructs containing these mutations were generated from pcDNA3.1Zeo(+)/LPL cDNA and transformed into COS-1 cells. LPL mass and enzyme activity in both culture medium and cell lysate were measured. COS-1 cells transfected with wild-type LPL exhibited consistent levels of LPL mass and activity, whereas all mutant construct-transfected COS-1 cells produced relatively consistent LPL mass (**Fig. 2A**, **B**) but significantly decreased LPL activities in both cell lysate and medium (Fig. 2C) ($P < 0.01$). No LPL activity was detected in either cell lysate or medium of the Asn291Ser + Lys312insC transfected cells, even though sufficient LPL mass was detected in these samples (Fig. 2B, C). Although similar LPL masses were found in the cell lysates of both wild-type and mutant LPL-expressed cells, much lower LPL masses (~48–61% of the wild type) were detected in the culture medium of mutant LPL-expressed cells (Fig. 2B) ($P < 0.05$), suggesting that the mutations Asn291Ser, Lys312insC, Thr361insA, and Asn291Ser + Lys312insC affect the secretion of LPL from these cells as well as their activity. Statistical analysis showed that the compound heterozygote Asn291Ser + Lys312insC in LPL further reduced the expression of LPL mass compared with either the Asn291Ser or Lys312insC single mutation ($P < 0.05$) and almost completely abolished LPL activity (**Table 4**).

Structural properties of mutant LPLs

To explore the molecular mechanism underlying the correlation between LPL mutations and their activity, we reconstructed the three-dimensional structure of the human LPL. Three residues (serine-132, aspartate-156, and histidine-241) that play important roles in catalysis are shown in a space-fill model. The deleted regions caused by insertion mutations are represented using different colors. Compared with the wild-type structure, the insertion mutation Lys312insC or Thr361insA resulted in loss of the whole (green plus red) or most (green) of the C-terminal domain (**Fig. 3**), which plays a

TABLE 4. LPL mass and enzyme activity in transfected COS-1 cells

LPL Source	Cell Lysate	Growth Medium				
	Mass	Activity	Specific Activity	Mass	Activity	Specific Activity
	ng/ml	*μmol/ml/h*	*nmol/ng/min*	*ng/ml*	*μmol/ml/h*	*nmol/ng/min*
COS-1	ND	ND	ND	ND	ND	ND
PCDNA3.1Zeo(+)	ND	ND	ND	ND	ND	ND
Wild type	298 ± 14	1.69 ± 0.03	0.057 ± 0.003	277 ± 7	1.47 ± 0.02	0.0053 ± 0.0003
Asn291Ser	267 ± 11.2	1.02 ± 0.02[a]	0.004 ± 0.00003[a]	171 ± 6.8[a]	0.91 ± 0.01[a]	0.0052 ± 0.0002[a]
Lys312insC	277 ± 9.2	0.19 ± 0.01[a]	0.00067 ± 0.00002[a]	155 ± 7.9[a]	0.17 ± 0.01[a]	0.0011 ± 0.0002[a]
Thr361insA	269 ± 12	0.34 ± 0.01[a]	0.0017 ± 0.0002[a]	131 ± 4.9[a]	0.27 ± 0.01[a]	0.002 ± 0.0001[a]
Asn291Ser + Lys312insC	274 ± 3.9	ND	ND	135 ± 7[abc]	ND	ND

Specific activity is calculated by dividing the LPL activity by the LPL mass. ND, undetectable.
[a] $P < 0.05$, compared with the wild type.
[b] $P < 0.05$, compared with Asn291Ser.
[c] $P < 0.05$, compared with Lys312insC.

critical role in the formation and stabilization of the LPL dimer (19, 23). Dimerization of the LPL monomer has been shown to be crucial for executing LPL activity (19, 23). Thus, the markedly reduced levels of LPL activity in COS-1 cells expressing the LPL mutants Lys312insC and Thr361insA may result from impaired LPL homodimerization.

The Asn291Ser mutation is a known mutant in LPL and is thought to participate significantly in both heparin binding and LPL dimerization (24, 25). The three-dimensional model of LPL shows that Asn291 is located in the back of the molecule on the region for heparin binding and homodimer formation and is also close to the activation center of the LPL protein. This important

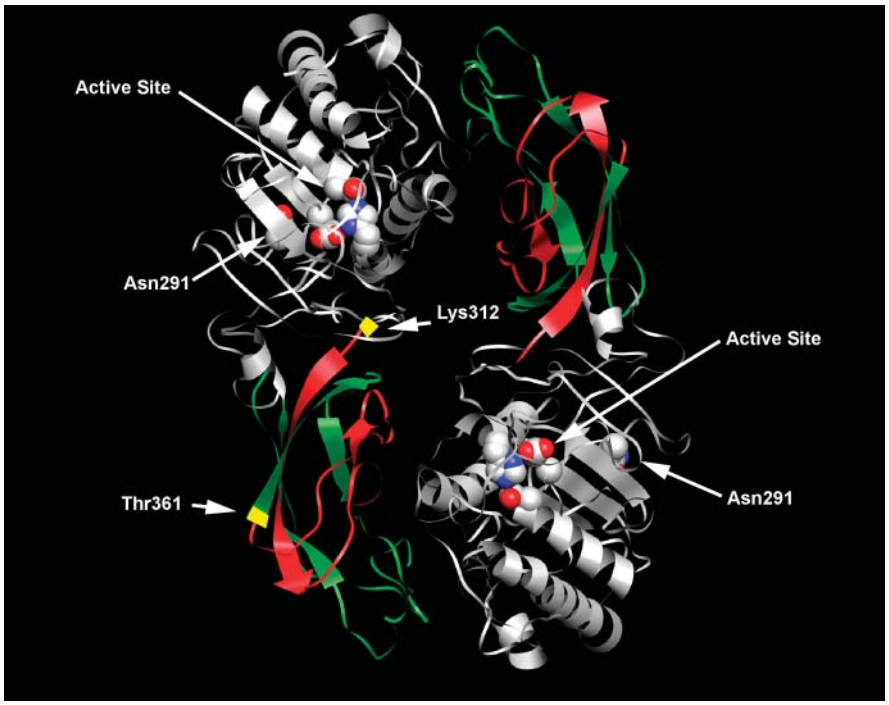

Fig. 3. Three-dimensional model structure of the human LPL proteins in a noncovalent homodimer with a head-to-tail configuration. The monomer LPL exhibited two domains: a large N-terminal domain (amino acid residues 1–315) and a small C-terminal domain (amino acid residues 316–448) that are essential for the formation and/or stability of the LPL homodimer. The catalytic triad of serine-132, aspartate-156, and histidine-241 as well as asparagine-291 are represented in a space-fill model. The red region represents residues 312–360, whereas the green region represents residues 362–437. Arrows indicate the active center and the Asn291Ser, Lys312insC, and Thr361insA mutation sites. The Lys312insC mutation (started with green) and the Thr361insA mutation site (started with red) led to a truncation of the whole or part of the LPL protein (green and red parts of the LPL structure) and would jeopardize the stability of the LPL dimer and cause subsequent significant reduction of LPL activity. The Lys312insC + Asn291Ser compound heterozygote contained a point mutation of Asn291Ser and a loss of the whole C-terminal domain (all green and red residues).

location of Asn291 (Fig. 3) could explain its critical role in LPL activity.

DISCUSSION

We show here that genetic variants of the LPL gene occur commonly in hypertriglyceridemic members of type 2 diabetic pedigrees in a Chinese population. Similar to those previously reported, three known mutations of the LPL gene, Leu286Pro, Ala71Thr, and Asn291Ser, were associated with variable degrees of hyperlipidemia (15, 21), supporting the hypothesis that Leu286Pro and Ala71Thr contribute to hypertriglyceridemia in these two pedigrees (pedigrees 1 and 3 in supplementary Table IIIa, IIIb). We now report three novel mutations of LPL, all of which negatively affect LPL activity and contribute to hypertriglyceridemia.

To elucidate structure-function relationships of the LPL molecule with the novel mutants that we detected, we performed in vitro expression of mutant proteins and molecular modeling analysis to examine their functional alterations. The crystal structure of human LPL is currently not available. However, the molecular model structure of LPL has been constructed and used successfully to analyze LPL function based on the known crystal structure of PL as a template (19, 23, 25). The model structure of the wild-type LPL protein exhibited two domains: a large N-terminal domain (amino acid residues 1–315) and a small C-terminal domain (amino acid residues 316–448). The N-terminal domain contains the heparin binding sites and the active site region, including the substrate binding site and the catalytic center (25). The C-terminal domain is also involved in heparin and substrate binding and, more importantly, has been considered essential for the formation and/or stability of the LPL head-to-tail noncovalent homodimer, a configuration essential for the activity of the enzyme (19, 23–26).

The novel mutations Lys312insC and Thr361insA, both located at the beginning of the C terminus of LPL, appeared to contribute to the hypertriglyceridemia in members of type 2 diabetic pedigrees, based on both the clinical findings and the results of in vitro experiments (Fig. 2). From our molecular model, the Lys312insC mutation generated a stop codon at amino acid position 326 within exon 7 and thus resulted in premature termination. This mutation causes the loss of the whole C-terminal domain, which plays a critical role in forming a functional LPL homodimer (19, 23). Consistently, our in vitro experiments showed that the mutant Lys312insC LPL exhibited both decreased catalytic activity (~12% of wild-type activity) and secretion ability (approximately half that of the wild type) (Fig. 2), supporting the hypothesis that the Lys312insC mutation is a pathogenic cause of LPL deficiency and the observed hypertriglyceridemia in T2DM pedigrees. Similarly, the insertion mutation Thr361insA detected in two families, which shorten LPL by 74 amino acids or about half of the C-terminal domain of LPL, resulted in a significant correlation with hypertriglyceridemia (Table 4). In vitro expression of Thr361insA mutant LPL showed reductions of both secretion (~20% of wild-type activity) and catalytic activity (approximately half that of the wild type) (Fig. 2). No association between this mutation and T2DM was observed.

The three compound heterozygote individuals with the Lys312insC and Asn291Ser mutations had some of the highest plasma TG levels (up to 8.8 mmol/l) in our family cohort of patients, and all three individuals had T2DM. The results from the in vitro expression studies showed that LPL protein containing both of these mutations was virtually inactive. The Asn291Ser mutation alone is the most common mutation observed in Caucasians, but this mutation usually only results in mild deficiency in LPL activity and variable hyperlipidemia (26). Three-dimensional structural models showed that Asn291 is located in the region of heparin binding and may participate in LPL dimerization. Thus, LPL mutants with both impaired heparin binding and homodimerization may explain the severe hypertriglyceridemia observed in the three compound heterozygote individuals.

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REFERENCES

1. Krauss, R. M., and P. W. Siri. 2004. Dyslipidemia in type 2 diabetes. *Med. Clin. North Am.* **88:** 897–909.
2. Steinmetz, A. 2003. Treatment of diabetic dyslipoproteinemia. *Exp. Clin. Endocrinol. Diabetes.* **111:** 239–245.
3. Dunn, F. L. 1990. Hyperlipidemia in diabetes mellitus. *Diabetes Metab. Rev.* **6:** 47–61.
4. Stern, M. P., and S. M. Haffner. 1991. Dyslipidemia in type 2 diabetes: implications for therapeutic intervention. *Diabetes Care.* **14:** 1144–1159.
5. Haffner, S. M., M. P. Stern, H. P. Hazuda, B. D. Mitchell, J. K. Patterson, and E. Ferannini. 1989. Parental history of diabetes is associated with increased cardiovascular risk factors. *Arteriosclerosis.* **9:** 928–933.
6. Schumacher, M. C., T. Maxwell, L. L. Wu, S. C. Hunt, R. R. Williams, and S. C. Elbein. 1992. Dyslipidemias among normoglycemic members of familial NIDDM pedigrees. *Diabetes Care.* **15:** 1285–1289.
7. Haffner, S. M., M. P. Stern, H. P. Hazuda, B. D. Mitchell, and J. K. Patterson. 1990. Cardiovascular risk factors in confirmed prediabetic individuals: does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *J. Am. Med. Assoc.* **263:** 2893–2898.
8. Haffner, S. M., and H. Miettinen. 1997. Insulin resistance implications for type II diabetes mellitus and coronary heart disease. *Am. J. Med.* **103:** 152–162.
9. Goldberg, U., and M. Merkel. 2001. Lipoprotein lipase: physiology, biochemistry, and molecular biology. *Front. Biosci.* **6:** d388–d405.
10. Deeb, S., and R. Peng. 1989. Structure of the human lipoprotein lipase gene. *Biochemistry.* **28:** 4131–4135.
11. Hide, W., L. Chan, and W. H. Li. 1992. Structure and evolution of the lipase superfamily. *J. Lipid Res.* **33:** 167–178.

12. Olivecrona, T., O. Hernell, and T. Egelrud. 1975. Lipoprotein lipase. *Adv. Exp. Med. Biol.* **52:** 269–279.
13. Jap, T. S., S. F. Jenq, Y. C. Wu, C. Y. Chiu, and H. M. Cheng. 2003. Mutations in the lipoprotein lipase gene as a cause of hypertriglyceridemia and pancreatitis in Taiwan. *Pancreas.* **27:** 122–126.
14. Benlian, P., J. L. De Gennes, L. Foubert, H. Zhang, S. E. Gagne, and M. Hayden. 1996. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. *N. Engl. J. Med.* **335:** 848–854.
15. Razzaghi, H., C. E. Aston, R. F. Hamman, and M. I. Kamboh. 2000. Genetic screening of the lipoprotein lipase gene for mutations associated with high triglyceride/low HDL-cholesterol levels. *Hum. Genet.* **107:** 257–267.
16. Yang, T., C. P. Pang, M. W. Tsang, C. W. Lam, P. M. Poon, L. Y. Chan, X. Q. Wu, B. Tomlinson, and L. Baum. 2003. Pathogenic mutations of the lipoprotein lipase gene in Chinese patients with hypertriglyceridemic type 2 diabetes. *Hum. Mutat.* **21:** 453–463.
17. Alberti, K. G., and P. L. Zimmet. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part I. Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabet. Med.* **15:** 539–553.
18. Pettersen, E. F., T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, and T. E. Ferrin. 2004. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* **25:** 1605–1612.
19. Kobayashi, Y., T. Nakajima, and I. Inoue. 2002. Molecular modeling of the dimeric structure of human lipoprotein lipase and functional studies of the carboxyl-terminal domain. *Eur. J. Biochem.* **269:** 4701–4710.
20. Pimstone, S. N., S. M. Clee, S. E. Gagne, L. Miao, H. Zhang, E. A. Stein, and M. R. Hayden. 1996. A frequently occurring mutation in the lipoprotein lipase gene (Asn291Ser) results in altered postprandial chylomicron triglyceride and retinyl palmitate response in normolipidemic carriers. *J. Lipid Res.* **37:** 1675–1684.
21. Razzaghi, H., B. W. Day, R. J. McClure, and M. I. Kamboh. 2001. Structure-function analysis of D9N and N291S mutations in human lipoprotein lipase using molecular modelling. *J. Mol. Graph. Model.* **19:** 487–490.
22. Zhang, Q., E. Cavallero, M. M. Hoffmann, J. Cavanna, A. Kay, A. Charles, S. Braschi, W. Marz, L. Perlemuter, B. Jacotot, et al. 1997. Mutations at the lipoprotein lipase gene locus in subjects with diabetes mellitus, obesity and lipemia. *Clin. Sci.* **93:** 335–341.
23. Lookene, A., L. Zhang, M. Hultin, and G. Olivecrona. 2004. Rapid subunit exchange in dimeric lipoprotein lipase and properties of the inactive monomer. *J. Biol. Chem.* **279:** 49964–49972.
24. Evans, V., and J. J. Kastelein. 2002. Lipoprotein lipase deficiency—rare or common? *Cardiovasc. Drugs Ther.* **16:** 283–287.
25. van Tilbeurgh, H., A. Roussel, J. M. Lalouel, and C. Cambillau. 1994. Lipoprotein lipase. Molecular model based on the pancreatic lipase X-ray structure: consequences for heparin binding and catalysis. *J. Biol. Chem.* **269:** 4626–4633.
26. Keiper, T., J. G. Schneider, and K. A. Duji. 2001. Novel site in lipoprotein lipase (LPL415–438) essential for substrate interaction and dimer stability. *J. Lipid Res.* **42:** 1180–1186.