ELSEVIER

Structure–function analysis of D9N and N291S mutations in human lipoprotein lipase using molecular modelling

Hamid Razzaghi,* Billy W. Day,† Richard J. McClure,‡ and M. Ilyas Kamboh*

**Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261, USA*
†Department of Environmental & Occupational Health and Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA 15238, USA
‡Neurophysics Laboratory, Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261, USA

Lipoprotein lipase (LPL) plays a central role in lipid metabolism. The D9N and N291S mutations in the LPL gene are associated with elevated triglyceride and decreased HDL-cholesterol levels. Published in vitro expression studies suggest that these two mutations are associated with reduced LPL enzymatic activity. We sought to gain further insight on the impact of these two mutations on the LPL structure and function by molecular modelling techniques. Homology modelling was used to develop a three-dimensional (3D) structure of LPL from human pancreatic lipase. Two separate LPL models for the D9N and N291S substitutions were constructed and compared with the wild type LPL for differences in hydrophobicity, atomic burial, hydrogen bond pattern, and atomic mobility. In comparison to the wild type model, the 9N model was associated with significantly increased atomic mobility of its neighboring residues, but the catalytic site was not affected. The region near residue 9 in the upper part of the N-domain was considered a candidate site for protein–protein interaction. In the N291S model, alterations in H-bonds and constrained atomic mobility were among conformational changes in the region where the substitution had occurred. These are hypothesized to cause an increase in the rate of dissociation in LPL dimerization, subsequently affecting the LPL enzymatic activity. We also modelled the C-domain of apoCII, the obligatory cofactor of LPL, from 2D NMR data and docked the model with LPL to explore their interaction site. These docking experiments suggest that the C-domain of apoCII interacts with the interface of N- and C-domains of LPL and part of the lid structure that covers the catalytic site. In summary, we provide molecular modelling data on two well-known mutations in the LPL gene to help explain the published in vitro expression findings and propose a possible LPL-apoCII interaction site. Our data indicate that molecular modelling of LPL mutations could provide a valuable tool to understand the effects of a mutation on the structure–function of this important enzyme. © *2001 by Elsevier Science Inc.*

Keywords: mutation, lipoprotein lipase, molecular modelling, lipid metabolism, LPL, structure–function

INTRODUCTION

Lipoprotein lipase (LPL) plays a central role in triglyceride metabolism by regulating the catabolism of triglyceride-rich lipoprotein particles.[1] It is responsible for the influx of free fatty acids into peripheral tissues for storage or as a source of energy. The LPL dimer, attached to the luminal surface of capillary endothelial cells through heparan sulfate proteoglycans, and in the presence of its obligatory cofactor, apolipoprotein CII (apoCII), is widely believed to be the active form of the enzyme responsible for its hydrolytic action.[2,3] Because of its physiological importance in lipid-related pathologies, LPL has been extensively examined in both in vivo and in vitro studies (for review see Fisher et al.[4] and Hokenson[5]).

LPL, pancreatic lipase (PL), and hepatic lipase (HL) make up the mammalian lipase family. Of the three human lipases, the three-dimensional (3D) X-ray crystallographic structure of PL has been determined,[6,7,8] which has served as the model for molecular studies of other lipase family members. Structural features of LPL have been described elsewhere.[9,10]

Color Plates for this article are on pages 587–590.

Corresponding author: M. Ilyas Kamboh, 624 Parran Hall, GSPH, Department of Human Genetics, University of Pittsburgh, 130 DeSoto Street, Pittsburgh, PA 15261, USA. Tel.: 412-624-3066; fax: 412-383-7844. *E-mail address:* ikamboh@helix.hgen.pitt.edu (M.I. Kamboh)

Journal of Molecular Graphics and Modelling 19, 487–494, 2001

655 Avenue of the Americas, New York, NY 10010

1093-3263/01/$–see front matter
PII S1093-3263(00)00096-6

The D9N and N291S mutations in the LPL gene are associated with increased levels of triglyceride and decreased levels of high density lipoproteins-cholesterol (HDL-C), two major risk factors for coronary heart disease.[11] In vitro mutagenesis and expression studies indicate that both mutations are associated with reduced LPL catalytic activity.[12–18] The goal of this study was to use molecular modelling techniques to examine the effect of these two mutations on the structure and function of LPL and to provide structural explanations for the observed expression studies.

Two structural features near the LPL residue 9 are notable. N43, one of two putative N-linked glycosylation sites in LPL, is in close proximity to residue 9. Glycosylation at the conserved N43 site in the N-domain of LPL is required for the synthesis of a fully active and secreted lipase.[19] Also, a pocket containing a tryptophan residue is in close proximity to residue 9, which makes it a potential site for protein–protein interaction. We hypothesized that apoCII, the cofactor for LPL, could participate in similar interaction, involving residue 9. ApoCII consists of two functionally distinctive domains: the N-domain (residues 1 to 49) acts as an anchor to the lipoprotein particles, and the C-domain (residues 50 to 79) activates LPL.[20] The interaction site between LPL and apoCII and the nature of LPL activation are not known. We developed a 3D molecular model for the C-domain of apoCII and performed protein docking (molecular recognition) to determine the interaction site between LPL and apoCII and whether residue 9 is part of this modelled interaction.

In contrast to residue 9, residue 291 is located on the back of the molecule that is rich with positively charged residues, a region suspected to participate in heparan sulfate binding. LPL binds avidly to heparan sulfate and heparin, which are sulfated glycosaminoglycans with very high negative charge density.[3] The interaction of LPL with heparan sulfate proteoglycans is critical for localizing LPL at its site of action. This interaction probably stabilizes the dimer form of LPL, which is known to dissociate rapidly into inactive LPL monomers when free in solution.[2] We examined the effect of the N291S mutation on the structural conformation of the putative heparan sulfate binding site on LPL.

METHODOLOGY

Homology Modelling of LPL from PL

The segment match modelling (SMM) algorithm,[21] incorporated in LOOK software (Molecular Application Group, San Diego, CA) implemented on a Silicon Graphics O_2 computer was used for molecular homology modelling. The software uses a database of highly refined, known protein X-ray structures to build an unknown target structure from its amino-acid sequence and the atomic coordinates of a few of its atoms (generally only the Cα atoms). SMM first models missing main-chain atoms (N, H, Cα, C, O), and then models missing sidechain atoms. The final model was obtained by limiting energy minimization, which enforces good stereochemistry.[22] Utilizing all Cα atoms, the SMM method is able to calculate positions for main-chain atoms with an accuracy of 0.4 Å and sidechain atoms with an accuracy of 1.8 Å.[21] A model of LPL was constructed based on its homology with human PL, whose 3D structure is known. Atomic coordinates for human PL structure (1LPA and 1LPB) were obtained from the Protein Data Bank (http://www.rcsb.org/pdb).[7,8] There is a 31% sequence identity and 52% sequence similarity between LPL and PL. Both LPL and PL are approximately the same length, 448 and 449 amino acids, respectively. However, compared to the PL protein sequence, LPL has a shorter N-terminal by 25 residues and a C-terminal extended by 10 residues (Color Plate 1a). To maximize the sequence alignment between LPL and PL, the last 15 residues of the C-terminal of LPL (435 to 448) were left unaligned and were excluded from the modelled structure since there was no counterpart in PL to model. This segment presumably is an extension of the β-sheet structure of the C-domain. The LPL model was superimposed on its corresponding PL structure and both showed the same overall fold (Color Plate 1b). Two separate LPL structure were homology modelled with the open form (1LPA) and closed form (1LPB) of PL. The two models were superimposed and checked for subtle differences between them (Color Plate 1c). The closed form of the LPL model was used for subsequent studies. There are substantial differences between the active forms of PL and LPL. The active form of PL is a monomer and only requires the presence of its cofactor, colipase. PL also has no affinity for heparan sulfate. In contrast, three factors must be present to activate LPL: formation of a homodimer; presence of heparan sulfate; and binding to its cofactor, apoCII. Therefore, neither the closed nor open models of LPL monomer would be active.

Mutant Modelling of LPL

The LPL model was mutated on the computer at position 9 by substituting asparagine (N), an uncharged polar residue, for aspartate (D), an acidic residue. A detailed stereochemical refinement was automatically performed on the D9N mutant model to minimize conformational strain. The same procedure was applied to the N291S mutation, substituting serine (S), an uncharged polar residue, at position 291 for asparagine, another uncharged polar residue.

Structural Analysis of D9N and N291S

The two LPL mutant models for the D9N and N291S substitutions were compared with the wild type LPL model for their structural properties, including differences in hydrophobicity, atomic burial (bulkiness), hydrogen bond pattern, atomic mobility (flexible region), and sidechain conformation. These analyses are explained in the following sections.

Burial/Accessibility Analysis

Macromolecules fold to produce highly compacted globular structures containing large regions partially or completely inaccessible to solvent. The pattern of burial strongly reflects both tertiary fold and secondary structure, and has tremendous importance for biological interactions. Burial/accessibility analysis quantifies the degree of hydrophobic burial of selected residues. LOOK measures the surface area accessible to solvent for each atom in a residue, averages the individual values, and returns the overall accessibility as a fraction of the residue's surface. Colorizing this data presents 'hot' regions as the most buried, and 'cold' regions as the most accessible. Corey, Pauling, and Koltun (CPK) models provide space-filling representations of a molecule's atomic surface, useful for visualizing binding pockets and other instances where packing is

critical. Using the LOOK CPK representation, larger spheres represent more buried regions.[23]

Hydrophobicity Analysis

Hydrophobicity analysis examines hydrophobicity patterns within selected residues. Colorizing this data also presents 'hot' regions as the most hydrophobic, and 'cold' regions as least hydrophobic. Using the LOOK CPK representation, sphere size increases as hydrophobicity increases.[23]

Atomic Mobility Analysis

A crystallographically determined molecular structure is actually the average of many individual structures in a crystal lattice. Atomic positions fluctuate, either because they are moving or because individual molecules in the lattice differ slightly. B-values quantify this individual atom fluctuation and provide insight into constrained and flexible regions of a molecule. CPK size and colorizing were used to illustrate this analysis. LOOK also generates B-values for the modelled structure, which are not directly related to experimental crystallographic B-values provided in PDB files. The B-values generated within LOOK are standard deviations of the coordinates of ten independent models produced within LOOK.[23]

C-Domain Modelling of ApoCII

MacroModel v4.5 molecular modelling software[24] implanted on a Silicon Graphics Indigo computer was used to develop a 3D model for the C-domain of apoCII based on secondary structural elements from a 2D NMR structure described by Ohman et al.[25] The 2D NMR structure of the C-domain of apoCII was determined in an aqueous solvent with 35% 1,1,1,3,3,3,-hexafluoro-2-propanol, 20mM DOAC, and a final peptide concentration of approximately 3mM.[26] The model incorporated α-helices between residues 67–74 and 56–59 and a backbone conformation approximating the refined apoCII fragment. Using MacroModel, the peptide fragment (aa 50–79), corresponding to aa 1–30 in the model was constructed, based on secondary structural elements provided by Ohman et al.[25] The sidechains were adjusted to the 3D structure by torsion angle rotation. The model was subjected to energy minimization (PRCG minimization), using the AMBER* force field implemented in MacroModel v4.5. The solvent (H_2O) was treated as a fully equilibrated analytical continnum starting near the Vander Waals surface of apoCII using the GB/SA model implemented in MacroModel v4.5.[24]

Protein Docking

Knowledge of the 3D structure of protein–protein complexes provides a valuable understanding of the function of molecular systems. The 3D structures of most protein complexes reveal a close geometric match between the surfaces of the protein and the ligand that are in contact. Global Range Molecular Matching (GRAMM, v1.03)[27] is a program for protein docking that requires the atomic coordinates of the two molecules to predict the structure of a complex. The simulation of molecular docking involves an exhaustive search through three translations and three rotations (X, Y, Z) of the molecule. GRAMM methodology is an empirical approach to smoothing the intermolecular energy function by changing the range of the atom–atom potentials. The technique can be applied to structures of different accuracy to locate the global minimum of intermolecular energy.[28] GRAMM can be applied to either high- or low-resolution docking, depending on the accuracy of the interacting molecules. Docking the C-domain of apoCII to LPL utilized the low-resolution molecular recognition GRAMM,[29] a suitable procedure (with scanning at 7 Å) for NMR or modelled structures, since possible inaccuracies in the models should not affect the overall LPL-apoCII recognition (Color Plate 3e).

RESULTS

3D Structure of LPL

The final model of the LPL molecule, in ribbon format, is illustrated in Color Plate 1d. Similar to PL, LPL is also organized into two distinct structural domains, amino terminal (residues 1–312) and carboxy terminal (residues 313–448). The lid structure (residues 216–239) that covers the catalytic site is located in the N-domain. Exons 1–4 and exons 5–6 make up the upper and lower parts of the N-domain, respectively, whereas exons 7–9 make up the C-domain. In a survey of all reported LPL mutations, we found that more than 60% of the point mutations occurred in exons 5 and 6, which contribute to the catalytic domain and the lid structure. The most significant aspects of this structure include the high degree of hydrophobicity, for interaction with the lipid layers of chylomicrons (CM) and very low density lipoproteins (VLDL), and the high atomic mobility at the shaft of the lid to cover the catalytic site (data not shown). Known biochemical features of the LPL molecule are also highlighted on the structure (Color Plate 1d).

D9N Mutational Analysis

The LPL model was mutated on the computer by substituting asparagine at position 9 for aspartate. The resulting D9N mutant model was then subjected to energy minimization for structural refinement. Since residue 9 is located in the coil region outside the foldcore, no substantial change was observed in the backbone of the molecule compared with wild type LPL when the two models were superimposed. Therefore, subsequent analyses were performed on the sidechains. D9 interacts with five neighboring residues: D6, F7, I8, I10, and S88 (Color Plate 2a). The D9N substitution did not disturb the two hydrogen bonds between residue 9 and its structural neighbors in the wild type model. The D9N substitution also did not affect the hydrophobicity and atomic burial of residue 9 and its structural neighbors. On the other hand, the D9N mutation resulted in an approximate 13-fold increase in the atomic mobility of N9 and its structural neighbor residues in the mutant model, while this region of the molecule was very rigid in the wild type (Color Plate 2b). We also observed a pocket containing tryptophan, a potential binding site groove near residue 9, and a possible site for LPL-apoCII interaction. ApoCII is an obligatory cofactor for the LPL activity, whose binding site to LPL has not been determined. The LPL–HL chimeric studies are not conclusive regarding the site(s) of the LPL–apoCII interaction.[30–32] Based on the above structural analysis of the upper part of the LPL N-domain, we hypothesized that residue 9 might participate in apoCII binding and the D9N mutation would decrease such interactions by introducing

atomic flexibility in that region. In the model proposed here, the apoCII cofactor would interact with the upper region of the LPL N-domain (Color Plate 3a). We tested this hypothesis by docking experiments.

Molecular modelling of LPL-apoCII Interaction

ApoCII is a necessary cofactor to activate LPL. The C-domain of apoCII (residues 50 to 79) is essential for activation of LPL[20]; however, the interaction site on LPL and the nature of the activation are not known. Protein docking was used as a method of molecular recognition to determine whether the LPL N-domain near residue 9 was involved in binding apoCII. Since the C-domain of apoCII activates LPL independent of the N-domain,[26] we modelled only the C-domain and subjected to structural analyses and validation. This model of apoCII was used in the docking experiments (Color Plate 3b–e).

The GRAMM algorithm was used to examine the interaction site between LPL (wild type model) and the C-domain of apoCII. The ten lowest energy complexes showed a potentially important cluster of interactions of apoCII with LPL residues at the interface between the C- and N-domains of LPL and with the LPL lid structure. These results suggested that the upper part of the LPL N-domain was not involved in the LPL-apoCII interaction. Consequently, conformational changes of D9N and its neighboring residues would not affect the predicted complex (Color Plate 3e). The structure of this complex suggests that the C-domain of apoCII interacts with the interface of LPL C- and N-domains and the LPL lid in a way that could result in drastic LPL conformational changes, exposing its catalytic site to the lipoprotein particle core for hydrolyzing triglyceride substrates. Study of the kinetics of LPL activation and assessment of such an assumption, however, requires molecular dynamics experiments. Although the docking experiment did not support the initial hypothesis, it did identify the most probable region of the LPL structure for interaction with apoCII. Further functional studies targeting specific residues of the interaction sites at the C-and N-domains of LPL will help to identify the residues critical for apoCII-LPL binding. The ideal docking experiment would have been with the homodimer LPL bound to heparan sulfate, the active form. However, this was not feasible due to computational limitations of present docking software with large molecules, including GRAMM. Nevertheless, our findings using monomer LPL are compatible with the current head-to-tail, back-to-back model of homodimer LPL.[10,33] The structural significance that these modelling studies attribute to residue 9 strongly suggest interaction of the tip of the N-domain of LPL with another molecule from the lipid metabolism repertoire that could contribute to the observed phenotypes for D9N mutation. One possible target is the N-domain of apoB.[34,35]

N291S Mutational Analysis The LPL model was mutated on the computer by substituting serine at position 291 for asparagine and then it was subjected to energy minimization for structural refinement. Since residue 291 is located in the coil region of a loop, no substantial change was observed in the backbone of the molecule compared with the wild type LPL when the two models were superimposed. Subsequent analyses were therefore performed on the sidechains. Residue 291 was in close vicinity to N281, R282, C283, N284, E289, I290, K292, V293, and R294 and showed interaction with these eight neighboring residues (Color Plate 4a). The N291S substitution had a major impact on the hydrogen bond pattern between residue 291 and its structural neighbors (Color Plate 4d-e). In the wild type model, N291 and its structural neighbors formed six H-bonds with an average length of 3.2 Å; N291 participated in four of the six bonds. In the mutant model, S291 and its structural neighbors formed three H-bonds with an average length of 3.4 Å. The reduction in H-bonding in this region of the molecule may affect heparin binding and LPL dimerization by altering the conformation of the side chains in that region of the molecule. Furthermore, the atomic mobility of S291 and its neighbors decreased by about 3-fold and the pattern of structural flexibility was changed (Color Plate 4b). Another result of this substitution was that the upper structural neighbors of S291 (N281, R282, C283, N284) became more buried in the mutant structure (Color Plate 4c). The conformation of the sidechains in this region of the molecule was also drastically altered as a result of the substitution. No change in the hydrophobicity of this region of the molecule was observed as a result of the mutation. Residues 279–282, 292–304 from the heparin binding sites in the backside of the molecule.[12] For dimer formation, heparan sulfate could play an important role since positively charged clusters of the dimer could form a single heparin binding site. Results observed from heparin-Sepharose chromatography support this view.[36] Our structural analyses suggest that residue 291 could participate both in heparin binding and LPL dimerization. Therefore, the N291S mutation could alter the heparin binding sites to favor monomers rather than dimer formation. A hypothetical LPL–heparin–LPL complex is presented in Color Plate 4f–4g. Due to computational limitations, no attempt was made to optimize the interactions between heparin and the LPL dimer.

DISCUSSION

Structural Features of the LPL Molecule

Important structure–functional properties of LPL are highlighted on our model (Color Plate 1d). Two of these structural sites relevant to our D9N and N291S mutational analyses are the N-linked glycosylation site, on the top portion of the N-domain, and the heparin binding site, on the back of the molecule. These motifs will be discussed in the following sections. Furthermore, van Tilbeurgh et al.[10] reported a homology-based model of LPL and its structural analysis. Our LPL model is very similar to the reported one, except for minor changes in the C-domain due to differences in sequence alignment of the C-domain. Both models confirm the involvement of the backside of the molecule in heparin binding and head-to-tail dimer formation. To avoid redundancy, we have focused on structural analysis related to D9N and N291S mutations and their implications for the enzyme function.

D9N Mutation

The carrier frequency of the N9 allele has been reported to vary between 1.6 and 4.1% in different populations and the N9 allele is associated with low levels of HDL-C and high levels of triglycerides.[11,13,37] The in vitro expression functional studies of D9N indicate that the N9 allele is associated with 8–34% reduction in LPL activity and 11–30% reduction in LPL mass.[13–15] These data suggest that in addition to a moderate

impairment of LPL catalytic function, LPL secretion may also be partially defective in individuals carrying the N9 allele.

The modelling of D9N suggests that this mutation results in a significant increase in the atomic mobility of N9 and its neighboring residues in the mutant model; this region of the molecule had very little atomic mobility in the wild type model (Color Plate 2b). However, differences in atomic mobilities of the N9 model did not affect the architecture of the LPL catalytic site. This region of the N-domain of LPL is completely conserved across several species[38] and includes a structural pocket containing tryptophan, which is located close to residue 9. Tryptophan is well suited to mediate interaction of proteins with lipid/water interfaces due to its amphipathic properties; this residue contains aromatic character and a nitrogen atom and thus can have both hydrophobic and hydrophilic interactions. For example, tryptophans play an important role in the stabilization and conformation of transmembrane helices in several integral membrane proteins.[39] Furthermore, the cluster of three tryptophan residues in the C-domain of LPL has been shown to contribute to the binding of LPL to the lipid–water interface. Based on these structural properties associated with the upper part of the N-domain, we suspected this region to be involved in a protein–protein interaction, possibly with binding to apoCII-containing lipoprotein particles. The docking experiments reported here, however, do not support the involvement of residue 9 and its neighbors in the apoCII–LPL interaction. These experiments suggest that the C-domain of apoCII most likely interacts at the interface between N- and C-domains of LPL and the lid of LPL. An alternative to the apoCII binding hypothesis is the potential binding of apoB to the upper region of the LPL N-domain. It has been shown that LPL has a protein–protein interaction with the N-terminus of apoB.[34,35] The upper region of the N-domain of LPL is an excellent candidate for interaction with apoB. It would be interesting to test whether this region participates in the apoB-LPL interaction and to determine the role D9N would play in such an interaction. Nevertheless, modelling of apoB would be a challenging task. It is unclear how the observed structural anomalies could result in defective LPL secretion. However, N43, which is one of two glycosylation sites in the LPL molecule and is important for LPL enzymatic activity and secretion,[19] is located in this region. We suspect that the replacement of N9 with D9 introduces a potential glycosylation site near the N43 glycosylation site. This may have an adverse effect on glycosylation of LPL and hence its secretion. A 30–50% reduction in LPL mass in vitro expression studies supports our hypothesis.

N291S Mutation

The carrier frequency of the S291 allele has been reported to vary between 0.0–6.7% in different populations and this mutation is associated with decreased levels of HDL-C and increased levels of triglycerides.[4] Reymer et al.[40] first reported that the N291S mutation resulted in significantly lower catalytic function of LPL. They also found that this mutation was associated with significantly increased LPL monomer compared to wild type protein as well as a significant decreased LPL dimer to monomer ratio in LPL containing the mutation.[40] This indicates a more rapid dissociation of the active LPL dimer to the inactive monomer in the carriers of N291S. Additional expression studies by other groups confirmed that this mutation was associated with a 32–50% reduction in LPL activity.[12,14,16,17,18] The affinity of the N291S mutant for heparin appears to be reduced in comparison with the wild type LPL.[41]

The molecular modelling of N291S suggests structural differences between the wild and mutant types and this may provide a structural explanation for the results observed in functional in vitro studies. The N291S substitution changes an amide group to a hydroxyl group, resulting in decreased H-bonding. This leads to both local decreases in atomic mobility and changes in movement patterns, which could cause the upper structural neighbors of S291 to become more buried in the molecule. Consequently, such conformational changes alter the backside of the molecule where the heparin binding site is located, which in turn may result in reduced interaction between heparin and LPL. These conformational changes most likely result in a more rapid dissociation of the active LPL dimer to the inactive monomer, increasing the ratio of LPL monomers to dimers.

LPL is enzymatically active only as a dimer and in the presence of heparin.[9] Thus, a mutant protein that dimerizes less avidly will result in reduced catalytic activity. This is likely to be of particular importance when the lipolytic system becomes challenged by environmental factors, such as pregnancy, obesity, and increasing age.[12,42,43] Other environmental factors, such as diet, can influence the expression of the N291S mutation and unmask a significant partial lipolytic defect that may not be evident in the fasting state. Evidence of such a factor in

Table 1. Summary of the genetic, expression and structural studies of the D9N and N291S mutations

	D9N	N291S
Carrier Frequency	1.6–4.1%[11,13,37]	0.0–6.7%[4]
Association Studies	↓ HDL-C, ↑ TG[11,13,37]	↓ HDL-C, ↑ TG[4,40]
Expression Studies	↓ enzymatic activity, ↓ mass[13–15]	↓ enzymatic activity, ↑ monomer/dimer ratio[12,14,16–18,40,41]
Structural Studies	↑ Atomic mobility •may reduce protein–protein interaction (a putative site) •not part of LPL-apoCII interaction site •D9N may introduce another glycosylation site	↓ Hydrogen bonds ↓ Atomic mobility ↑ Atomic burial •may reduce the affinity for heparin binding, which results in reduced LPL dimerization

↑ increased; ↓ decreased; • major findings/conclusions

vivo was provided by Pimstone et al.,[44] who showed that normolipidemic N291S carriers had a significantly greater CM postprandial response to a fat load than normolipidemic non-N291S carriers.

The high TG/low HDL-C phenotype is a predisposing factor for atherosclerosis in at least two ways. Slower conversion of TG-rich lipoprotein (CM and VLDL) particles due to reduced TG hydrolysis could lead to a longer half-life of intermediate-sized lipoproteins in plasma (CM-R, LDL). Such particles could be retained in vessel walls by the bridging function of LPL.[45] Also, the surface coat of TG-rich lipoproteins is an important source for HDL precursors, since excess surface molecules of their remnants are transferred to HDL particles. Defective lipolysis of both CM and VLDL particles could play an important role in defective HDL production. Therefore, fewer CM and VLDL particles would be available for reverse cholesterol transport (the removal of cholesterol from peripheral cells and from the arterial wall). As a result, both ways may promote atherogenesis.[46–48]

CONCLUSION

Our results suggest that the mechanisms by which the D9N and N291S mutations cause reduction in lypolytic activity of LPL are different due to distinct structural changes introduced by amino-acid substitutions in different functional domains. Structural anomalies resulting from the D9N substitution do not affect the catalytic activity of the enzyme or the interaction of LPL with apoCII. The D9N mutation could affect the lypolytic activity by reducing the secretion of the enzyme as a result of the presence of another glycosylation site at positions 9 and presence of substantial atomic mobility near a putative site for protein–protein interaction. The N291S substitution results in structural alterations in the back of the molecule, the site of interactions with heparin and another LPL molecule to form the active dimer. In addition, our docking experiments suggest the interface between N- and C- domains of LPL and part of the lid compose the most likely site for interaction with its cofactor apoCII. We have summarized our structural findings in relation with what is known about these two mutations in Table 1.

ACKNOWLEDGMENT

This work was supported by HL #44672 grant from the National Heart, Lung and Blood Institute.

The Look software application has recently been integrated to another program called “GeneMine” and now is available to academia free of charge at the following web site: http://www.bioinformatics.ucla.edu/genemine

REFERENCES

1 Goldberg, I.J. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* 1996, **37**, 693–707

2 Osborne, J.C. Jr., Bengtsson-Olivecrona, G., Lee, N.S., and Olivecrona, T. Studies on inactivation of lipoprotein lipase: Role of the dimer to monomer dissociation. *Biochemistry.* 1985, **24**, 5606–5611

3 Sehayek., E., Olivecrona, T., Bengtsson-Olivecrona, G., Vlodavsky, I., Levkovitz, H., Avner. R., and Eisenberg, S. Binding to heparan sulfate is a major event during catabolism of lipoprotein lipase by HepG2 and other cell cultures. *Atherosclerosis* 1995, **114**, 1–8

4 Fisher, R.M., Humphries, S.E., and Talmud, P.J. Common variation in the lipoprotein lipase gene: Effects on plasma lipids and risk of atherosclerosis. *Atherosclerosis* 1997, **135**, 145–159

5 Hokanson, J.E. Functional variants in the lipoprotein lipase gene and risk cardiovascular disease. *Curr. Opin. Lipidol.* 1999, **10**, 393–399

6 Winkler, F.K., D’Arcy, A., and Hunziker, W. Structure of human pancreatic lipase. *Nature* 1990, **343**, 771–774

7 van Tilbeurgh, H., Egloff, M.P., Martinez, C., Rugani, N., Verger, R., and Cambillau, C. Interfacial activation of the lipase-procolipase complex by mixed micelles revealed by X-ray crystallography. *Nature* 1993, **29**, 814–820

8 Egloff, M.P., Marguet, F., Buono, G., Verger, R., Cambillau, C., and van Tilbeurgh, H. The 2.46 A resolution structure of the pancreatic lipase-colipase complex inhibited by a C11 alkyl phosphonate. *Biochemistry* 1995, **7**, 2751–2762

9 Santamarina-Fojo, S., and Dugi, K.A. Structure, function and role of lipoprotein lipase in lipoprotein metabolism. *Curr. Opin. Lipidol.* 1994, **5**, 117–125

10 van Tilbeurgh, H., Roussel, A., Lalouel, J.M., and Cambillau, C. Lipoprotein lipase. Molecular model based on the pancreatic lipase X-ray structure: Consequences for heparin binding and catalysis. *J. Biol. Chem.* 1994, **269**, 4626–4633

11 Gerdes, C., Fisher, R.M., Nicaud, V., Boer, J., Humphries, S.E., Talmud, P.J., and Faergeman, O. Lipoprotein lipase variants D9N and N291S are associated with increased plasma triglyceride and lower high-density lipoprotein cholesterol concentrations: studies in the fasting and postprandial states: the European Atherosclerosis Research Studies. *Circulation* 1997, **96**, 733–740

12 Ma, Y., Henderson, H.E., Liu, M.S., Zhang, H., Forsythe, I.J., Clarke-Lewis, I., Hayden, M.R., and Brunzell, J.D. Mutagenesis in four candidate heparin binding regions (residues 279–282, 291–304, 390–393, and 439–448) and identification of residues affecting heparin binding of human lipoprotein lipase. *J. Lipid Res.* 1994, **35**, 2049–2059

13 Mailly, F., Tugrul, Y., Reymer, P.W., Bruin, T., Seed, M., Groenemeyer, B.F., Asplund-Carlson, A., Vallance, D., Winder, A.F., and Miller, G.J. A common variant in the gene for lipoprotein lipase (Asp9→Asn). Functional implications and prevalence in normal and hyperlipidemic subjects. *Arterioscler. Thromb. Vasc. Biol.* 1995, **15**, 468–478

14 Zhang, H., Henderson, H., Gagne, S.E., Clee, S.M., Miao, L., Liu, G., and Hayden, M.R. Common sequence variants of lipoprotein lipase: standardized studies of in vitro expression and catalytic function. *Biochem. Biophys. Acta.* 1996, **1302**, 159–166

15 Gagne, E., Genest, J. Jr., Zhang, H., Clarke, L.A., and Hayden, M.R. Analysis of DNA changes in the LPL gene in patients with familial combined hyperlipidemia. *Arterioscler. Thromb.* 1994, **14**, 1250–1257

16 Zhang, H., Reymer, P.W., Liu, M.S., Forsythe, I.J., Groenemeyer, B.E., Frohlich, J., Brunzell, J.D., Kastelein, J.J., Hayden, M.R., and Ma, Y. Patients with apoE3 deficiency (E2/2, E3/2, and E4/2) who manifest with

hyperlipidemia have increased frequency of an Asn 291→Ser mutation in the human LPL gene. *Arterioscler. Thromb. Vas. Biol.* 1995, **15**, 1695–1703
17 Syvanne, M., Antikainen, M., Ehnholm, S., Tenkanen, H., Lahdenpera, S., Ehnholm, C., and Taskinen, M.R. Heterozygosity for Asn291→Ser mutation in the lipoprotein lipase gene in two Finnish pedigrees: effect of hyperinsulinemia on the expression of hypertriglyceridemia. *J. Lipid Res.*. 1996, **37**, 727–738
18 Krapp, A., Zhang, H., Ginzinger, D., Liu, M.S., Lindberg, A., Olivecrona, G., Hayden, M.R., and Beisiegel, U. Structural features in lipoprotein lipase necessary for the mediation of lipoprotein uptake into cells. *J. Lipid Res.* 1995, **36**, 2362–2373
19 Ben-Zeev, O., Stahnke, G., Liu, G., Davis, R.C, and Doolittle, M.H. Lipoprotein lipase and hepatic lipase: The role of asparagine-linked glycosylation in the expression of a functional enzyme. *J. Lipid Res.* 1994, **35**, 1511–1523.
20 Wang, C.S. Structure and functional properties of apolipoprotein C-II. *Prog. Lipid Res.* 1991, **30**, 253–258
21 Levitt, M. Accurate modeling of protein conformation by automatic segment matching. *J. Mol. Biol.* 1992, **226**, 507–533
22 Levitt M. Protein folding by restrained energy minimization and molecular dynamics. *J. Mol. Biol.* 1983, **170**: 723–64.
23 Molecular Application Group. *Look User's Guide.* Stanford University and Yeda. 9.5–9.9, 1995
24 Mohamadi, F., Richards, N.G.J., Guida W.C., Liskamp R., Lipton, M., Caufield, C., Chang G., Hendrickson, T., and Still, W.C. MacroModel—an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* 1990, **11**, 440–467
25 Ohman, A., Lycksell, P.O., and Graslund, A. A refined three-dimensional solution structure of a carboxy terminal fragment of apolipoprotein CII. *Eur. Biophys. J.* 1993, **22**, 351–357
26 Lycksell, P.O., Ohman, A., Bengtsson-Olivecrona, G., Johansson, L.B., Wijmenga, S.S., Wernic, D., and Graslund, A. Sequence specific 1H-NMR assignments and secondary structure of a carboxy-terminal functional fragment of apolipoprotein CII. *Eur. J. Biochem.* 1992, **205**, 223–231
27 Vakser, I.A. Protein docking for low-resolution structures. *Protein Eng.* 1995, **8**, 371–377
28 Vakser, I.A. Long-distance potentials: an approach to the multiple-minimal problem in ligand–receptor interaction. *Protein Eng.* 1996, **9**, 37–41
29 Vakser, I.A. Main-chain complementarity in protein–protein recognition. *Protein Eng.* 1996, **9**, 741–744
30 Wong, H., Davis, R.C., Nikazy, J., Seebart, K.E., and Schotz, M.C. Domain exchange: characterization of a chimeric lipase of hepatic lipase and lipoprotein lipase. *Proc. Natl. Acad. Sci. USA* 1991, **88**, 11290–11294
31 Davis, R.C., Wong, H., Nikazy, J., Wang, K., Han, Q., and Schotz, M.C. Chimeras of hepatic lipase and lipoprotein lipase. Domain localization of enzyme-specific properties. *J. Biol. Chem.* 1992, **267**, 21499–21504
32 Dichek, H.L., Parrott, C., Ronan, R., Brunzell, J.D., Brewer, H.B., Jr, and Santamarina-Fojo, S. Functional characterization of a chimeric lipase genetically engineered from human lipoprotein lipase and human hepatic lipase. *J. Lipid Res.* 1993, **34**, 1393–1340
33 Wong H., Yang D., Hill, J.S., Davis, R.C., Nikazy, J., and Schotz, M.C. A molecular biology-based approach to resolve the subunit orientation of lipoprotein lipase. *Proc. Natl. Acad. Sci. USA.* 1997, **94**, 5594–5598
34 Choi, S.Y., Sivaram, P., Walker, D.E., Curtiss, L.K., Gretch, D.G., Sturley, S.L., Attie, A.D., Deckelbaum, R.J., and Goldberg, I.J. Lipoprotein lipase association with lipoproteins involves protein–protein interaction with apolipoprotein B. *J. Biol. Chem.* 1995, **270**, 8081–8086
35 Sivaram, P., Choi, S.Y., Curtiss, L.K., and Goldberg, I.J. An amino-terminal fragment of apolipoprotein B binds to lipoprotein lipase and may facilitate its binding to endothelial cells. *J. Biol. Chem.* 1994, **269**, 9409–9412
36 Hata A., Ridinger, D.N., Sutherland, S., Emi, M., Shuhua, Z., Myers, R.L., Ren, K., Cheng, T., Inoue, I., and Wilson, D.E. Binding of lipoprotein lipase to heparin. Identification of five critical residues in two distinct segments of the amino-terminal domain. *J. Biol. Chem.* 1993, **268**, 8447–8457
37 Mailly, F., Fisher, R.M., Nicaud, V., Luong, L.A., Evans, A.E., Marques-Vidal, P., Luc. G., Arveiler, D., Bard, J.M., Poirier, O., Talmud, P.J., and Humphries, S.E. Association between the LPL-D9N mutation in the lipoprotein lipase gene and plasma lipid traits in myocardial infarction survivors from the ECTIM Study. *Atherosclerosis* 1996, **122**, 21–28
38 Raisonnier, A., Etienne, J., Arnault, F., Brault, D., Noe, L., Chuat, J.C., and Galibert, F. Comparison of the cDNA and amino acid sequences of lipoprotein lipase in eight species. *Biochem. Mol. Biol.* 1995, **111**, 385–398
39 Lookene, A., Groot, N.B., Kastelein, J.J.P., Olivecrona, G, and Bruin, T. Mutation of tryptophan residues in lipoprotein lipase. Effects on stability, immunoreactivity, and catalytic properties. *J. Biol. Chem.* 1997, **272**, 766–772
40 Reymer, P.W., Gagne, E., Groenemeyer, B.E., Zhang, H., Forsyth, I., Jansen, H., Seidell, J.C., Kromhout, D., Lie, K.E., and Kastelein, J. A lipoprotein lipase mutation (Asn291Ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis. *Nat. Genet.* 1995, **10**, 28–34
41 Fisher, R.M., Mailly, F., Peacock, R.E., Hamsten, A., Seed, M., Yudkin, J.S., Beisiegel, U., Feussner, G., Miller, G., Humphries, S.E., et al. Interaction of the lipoprotein lipase asparagine 291→serine mutation with body mass index determines elevated plasma triacylglycerol concentrations: A study in hyperlipidemic subjects, myocardial infarction survivors, and healthy adults. *J. Lipid Res.* 1995, **36**, 2104–2112
42 Glueck, C.J., Christopher, C., Mishkel, M.A., Tsang, R.C., and Mellies, M.J. Pancreatitis, familial hypertriglyceridemia, and pregnancy. *Am. J. Obstet. Gynecol.* 1980, **136**, 755–761
43 Wilson, D.E., Emi, M., Iverius, P.H., Hata, A., Wu, L.L., Hillas, E., Williams, R.R., and Lalouel, J.M. Phenotypic expression of heterozygous lipoprotein lipase deficiency in the extended pedigree of a proband homozygous for a missense mutation. *J. Clin. Invest.* 1990, **86**, 735–750
44 Pimstone, S.N., Clee, S.M., Gagne, S.E., Miao, L., Zhang, H., Stein, E.A., and Hayden, M.R. 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.* 1996, **37**, 1675–1684
45 Pentikäinen, M.O., Öörni, K., and Kovanen, P.T. Lipoprotein lipase (LPL) strongly links native and oxidized low density lipoprotein particles to decorin-coated collagen. *J. Biol. Chem.* 2000, **275**, 5694–5701
46 Barter, P.J., and Rye, K.A. Molecular mechanisms of reverse cholesterol transport. *Curr. Opin. Lipidol.* 1996, **7**, 82–87
47 Pimstone, S.N., Gagne, S.E., Gagne, C., Lupien, P.J., Gaudet, D., Williams, R.R., Kotze, M., Reymer, P.W., Defesche, J.C., and Kastelein, J.J. Mutations in the gene for lipoprotein lipase. A cause for low HDL cholesterol levels in individuals heterozygous for familial hypercholesterolemia. *Arterscler. Thromb. Vas. Biol.* 1995, **15**, 1704–1712
48 Nordestgaard, B.G., Wootton, R., and Lewis, B. Selective retention of VLDL, IDL, and LDL in the arterial intima of genetically hyperlipidemic rabbits in vivo. Molecular size as a determinant of fractional loss from the intima-inner media. *Arterioscler. Thromb. Vas. Biol.* 1995, **15**, 534–542