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Original Research

Missense mutation Q384K in the *APOB* gene affecting the large lipid transfer module of apoB reduces the secretion of apoB-100 in the liver without reducing the secretion of apoB-48 in the intestine

Thea Bismo Strøm, PhD*, Emil Asprusten, MD, Jon K. Laerdahl, PhD, Irene Øygard, MD, M. Mahmood Hussain, PhD, Lic, MED, Martin Prøven Bogsrud, MD, PhD, Trond P. Leren, MD, PhD

Unit for Cardiac and Cardiovascular Genetics, Oslo University Hospital, Oslo, Norway (Drs Strøm, Bogsrud and Leren); Lipid Clinic, Oslo University Hospital, Oslo, Norway (Dr Asprusten); Department of Microbiology, Oslo University Hospital, Oslo, Norway (Dr Laerdahl); ELIXIR Norway, Department of Informatics, University of Oslo, Oslo, Norway (Dr Laerdahl); Fagernes Medical Center, Fagernes, Norway (Dr Øygard); Department of Foundations of Medicine, NYU Long Island School of Medicine, Mineola, NY 11501, USA (Dr. Hussain)

KEYWORDS
Apolipoprotein B;
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Background: Molecular genetic testing of patients with hypobetalipoproteinemia may identify a genetic cause that can form the basis for starting proper therapy. Identifying a genetic cause may also provide novel data on the structure-function relationship of the mutant protein.

Objective: To identify a genetic cause of hypobetalipoproteinemia in a patient with levels of low density lipoprotein cholesterol at the detection limit of 0.1 mmol/l.

Methods: DNA sequencing of the translated exons with flanking intron sequences of the genes adenosine triphosphate-binding cassette transporter 1, angiopoietin-like protein 3, apolipoprotein B, apolipoprotein A1, lecithin-cholesterol acyltransferase, microsomal triglyceride transfer protein and proprotein convertase subtilisin/kexin type 9.

Results: The patient was homozygous for mutation Q384K (c.1150C>A) in the apolipoprotein B gene, and this mutation segregated with hypobetalipoproteinemia in the family. Residue Gln_{384} is

Disclosure Statements:

Author Contribution: TPL designed the study in collaboration with TBS and MPB. EA and IØ performed clinical investigations. JKL performed structural and evolutionary conservation analyses of apoB. MMH performed experimental analyses of the Q384K mutation and provided the anti-apoB antibody. TBS performed analysis of apoB from serum. TPL wrote the first draft and the final version of the manuscript. Each author made significant contributions to the manuscript and participated in the revising process.

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* Corresponding author at: Unit for Cardiac and Cardiovascular Genetics, Department of Medical Genetics, Oslo University Hospital, P. Box 4956 Nydalen, NO-0424 Oslo, Norway.

E-mail address: thstro@ous-hf.no (T.B. Strøm).

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located in the large lipid transfer module of apoB that has been suggested to be important for lipidation of apolipoprotein B through interaction with microsomal triglyceride transfer protein. Based on measurements of serum levels of triglycerides and apolipoprotein B-48 after an oral fat load, we conclude that the patient was able to synthesize apolipoprotein B-48 in the intestine in a seemingly normal fashion.

Conclusion: Our data indicate that mutation Q384K severely reduces the secretion of apolipoprotein B-100 in the liver without reducing the secretion of apolipoprotein B-48 in the intestine. Possible mechanisms for the different effects of this and other missense mutations affecting the large lipid transfer module on the two forms of apoB are discussed.

Introduction

Apolipoprotein B (apoB) is an amphipathic glycoprotein that is synthesized in the liver and intestine and is a critical component of lipoproteins.[1] In the liver, a 4536 residues mature protein is synthesized and secreted as a component of very low density lipoprotein (VLDL). In the intestine, the *APOB* transcript is subjected to post-transcriptional mRNA editing, and is translated into a truncated protein of 2152 residues and secreted as a component of chylomicron.[1,2] The latter form of apoB consists of 48% of the residues of the full-length protein, and the two forms are commonly referred to as apoB-48 and apoB-100, respectively. VLDL and its metabolic product low density lipoprotein (LDL) contain apoB-100 and chylomicron contains apoB-48.[1]

During translation, apoB is translocated into the endoplasmic reticulum (ER), where it co-translationally acquires triglycerides and other lipids through interaction with microsomal triglyceride transfer protein (MTP), encoded by the MTP (*MTTP)* gene.[3-5] Proper lipidation is required for apoB to obtain correct folding and to avoid translational and post-translational degradation.[6]

ApoB-100 has been proposed to be comprising five structural segments, NH_2-$\beta\alpha_1$-β_1-α_2-β_2-α_3-COOH.[7] The N-terminal $\beta\alpha_1$ segment (residues 1 to 1000) corresponds to the large lipid transfer (LLT) module which is common to all proteins in the LLT protein superfamily, including egg yolk precursor protein vitellogenin, MTP and apoB.[8] It has been suggested that the apoB LLT module creates a lipid pocket that interacts with MTP and thereby forms the structural basis for recruitment of lipids.[7,9] The N-terminus of the LLT module forms an incomplete β-barrel domain (residues 28 to 320 in human apoB). This is followed by a right-handed alpha solenoid domain comprising 17 α-helices in two layers (residues 321 to 616) with a helix-turn-helix (HTH) repeating unit, in total eight HTH repeats. Finally, at the C-terminus, the LLT module has a domain with two β-sheets, known as the A- and C-sheets (residues 617 to 1017), separated by a lipid-binding cavity.[10-12] Disulfide bonds have been shown to be important for the structure of the LLT module,[13,14] and residues 297-597 within this module, mainly built from the HTH repeats of the alpha solenoid domain, have been shown to be required for optimal binding to MTP.[15]

Patients who lack functional apoB or MTP due to mutations in the respective genes, are unable to synthesize VLDL and chylomicron. These patients have autosomal dominant familial hypobetalipoproteinemia (OMIM#615558) or autosomal recessive abetalipoproteinemia (OMIM#200100), respectively. The two conditions have similar clinical features.[16,17] As a consequence of the failure to secrete chylomicrons and to absorb fat from the intestine, and the failure to transport fat-soluble vitamins in apoB-containing lipoproteins in plasma, these patients typically present with gastrointestinal and neurological symptoms.[16,17]

The majority of mutations in the apolipoprotein B (*APOB*) gene responsible for familial hypobetalipoproteinemia are nonsense mutations, frame-shift mutations and splicing mutations that generate premature stop codons which lead to truncated apoB.[18] However, a few missense mutations affecting the LLT module of apoB have been identified in patients with hypobetalipoproteinemia.[19-22] By transfection studies in cultured cells these mutations have been found to reduce apoB secretion.[19-21]

We report a patient with hypobetalipoprotenemia who was homozygous for the novel missense mutation Q384K (c.1150C>A, ref. seq.: NM_000384.3) in the *APOB* gene that affects the LLT module of apoB. We also discuss possible mechanisms by which this and other missense mutations that affect the LLT module of apoB, markedly reduce the secretion of apoB-100 in the liver, whereas the secretion of apoB-48 in the intestine appears to be unaffected.

Materials and methods

Informed consent

Written informed consent to publish the data obtained as part of ordinary health care was obtained from all subjects.

Molecular genetic testing for hypobetalipoproteinemia

In order to identify a genetic cause of an abnormal lipid profile, DNA sequencing was performed of the genes: adenosine triphosphate-binding cassette transporter 1,

angiopoietin-like protein 3, *APOB*, apolipoprotein A1, lecithin-cholesterol acyltransferase, *MTTP* and proprotein convertase subtilisin/kexin type 9. Sequence analyses were performed by the use of Sanger di-deoxy sequencing of polymerase chain reaction (PCR) products spanning the translated exons with 20 bp of flanking intron sequences. A 3730XL DNA Analyzer (ThermoFisher Scientific, Waltham, MA) was used for analyses of the sequencing reactions. The primer sequences used for the PCRs are available upon request. Numbering of the codons and nucleotides of genes in this study was based upon the ATG initiation codon being codon #1 and A of the ATG initiation codon being nucleotide #1.

Immunoprecipitation of apoB from serum and Western blot analysis

ApoB was immunoprecipitated from 250 µl serum using Dynabeads Protein G-MAG Sepharose (Cytiva, Marlborough, MA) coated with 2 µg of the monoclonal anti-apoB antibody 1D1 directed at an epitope in the LLT module,[23] or a polyclonal anti-apoB antibody (ab98132, Abcam, Cambridge, UK) directed against the full length native human apoB isolated from human plasma. The immunoprecipitate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 4-20% Tris-HCl Criterion™ TGX™ Precast Gels (BioRad Laboratories, Inc., Hercules, CA) and blotted onto Immuno-Blot PVDF Membranes (BioRad Laboratories, Inc., Hercules, CA) using the Trans-Blot Turbo Transfer System (BioRad Laboratories, Inc., Hercules, CA). ApoB-48 and apoB-100 were detected using the monoclonal anti-apoB antibodies 1D1 and ab98132 and the bands were visualized as chemiluminescence on a ChemiDoc XRS system (BioRad Laboratories, Inc., Hercules, CA). Lipoprotein-deficient serum (LPDS) (density > 1.25 g/ml) and LDL (density 1.019-1.063 g/ml) were isolated by ultracentrifugation of human serum and used as negative and positive controls, respectively.

Results

Clinical characteristics of the proband

The proband was a 43-year old female who was referred for molecular genetic testing because of low levels of total serum cholesterol, HDL cholesterol and LDL cholesterol. For several years she had experienced tiredness and symptoms of generalized muscle pain. A diagnosis of fibromyalgia had been considered. She had been diagnosed with low levels of vitamin D and put on cholecalciferol (vitamin D_3) 2000 IU/day with little or no effects on vitamin D levels or symptoms. The proband has not had any symptoms of fat malabsorption, oral fat intolerance, steatorrhea or diarrhea. Her height was 159 cm and her weight was 72 kg which gives a body mass index of 28.5.

The levels of total serum cholesterol in the proband were approximately 1 mmol/l and the levels of LDL cholesterol were around or below the detection limit of 0.1 mmol/l (Table 1). HDL cholesterol levels were low with values ranging from 0.4 mmol/l to 0.8 mmol/l. On some occasions turbid serum had been observed in non-fasting blood samples with levels of triglycerides of 6.6 mmol/l and 10.4 mmol/l (Table 1). Fasting triglyceride levels, however, were in the normal range with values ranging from 0.4 mmol/l to 0.6 mmol/l (Table 1). Lp(a) lipoprotein levels were below the detection limit of 7 nmol/l (Table 1). This lipid profile indicates that the patient had hypobetalipoproteinemia. The indicated normal range for serum parameters are from the laboratory that has performed the respective analyses.

Serum levels of fat-soluble vitamins A, D, E and K during treatment with cholecalciferol (vitamin D_3) 2000 IU/day were: vitamin A: 2.0 µmol/l (Normal: > 0.7 µmol/l), vitamin D: 14 nmol/l (Normal range: 50 nmol/l-150 nmol/l), vitamin E: 8 µmol/l (Normal range: 12–42 µmol/l) and vitamin K: 0.5 ng/ml (Normal range: 0.1–2.2 ng/ml). Thus, the patient had lower than normal levels of vitamins D and E in serum.

Slightly increased levels of aspartate aminotransferase (ASAT) were observed, whereas levels of alanine aminotransferase (ALAT) were in the upper normal range (Table 1). Values for lactate dehydrogenase, gamma-glutamyl transpeptidase and bilirubin were normal with values of 162 U/l (Normal range: 105 U/l-205 U/l), 60 U/l (Normal range: 10 U/l-75 U/l) and 8 µmol/l (Normal range: 5 µmol/l-25 µmol/l), respectively. Ultrasonography of abdomen revealed that the liver was of normal size with a normal contour and without focal lesions. However, there was increased echogenicity of the liver consistent with hepatic steatosis.

Molecular genetic testing

DNA sequencing revealed that the patient was homozygous for mutation Q384K (c.1150C>A: NM_000384.3) in exon 10 of the *APOB* gene. To our knowledge, this mutation has not previously been reported as a cause of hypobetalipoproteinemia. *In silico* predictions programs Sorting Intolerant from Tolerant, MutationTaster and Polymorphism Phenotyping v2 gave inconsistent predictions of pathogenicity by predicting mutation Q384K to be Deleterious, Benign and Probably Damaging, respectively. Mutation Q384K is not predicted to affect RNA splicing by the use of *in silico* prediction software and it has not been reported in the databases Genome Aggregation Database (https://gnomad.broadinstitute.org/), ClinVar (https://ncbi.nlm.nih.gov/vlinvar/) or Human Gene Mutation Database.[24]

Segregation analysis

Fasting serum lipid values were obtained and molecular genetic testing for mutation Q384K were performed in the proband, her spouse and in their eight children aged 12-25 years (Fig. 1). Whereas, the spouse had values for LDL cholesterol and apoB in the upper normal range, all eight children had low levels of LDL cholesterol and apoB with

Table 1 Values for serum lipids and liver function tests in the 43-year old proband. Non-fasting serum lipid values obtained in March and April of 2021 as well as fasting serum lipid values obtained in July 2021 and January 2022 in the proband, are shown. Also shown are values for aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT). The normal range for these parameters are given in parenthesis.

	Non-fasting		Fasting	
	March 2021	April 2021	July 2021	January 2022
Total serum cholesterol (mmol/l, NR: 3.3–6.9)	1.1	1.3	0.7	0.9
HDL cholesterol (mmol/l, NR: 1.0–2.7)	0.4	0.5	0.6	0.8
Triglycerides (mmol/l, NR: 0.5–2.6)	6.6	10.4	0.6	0.4
LDL cholesterol (mmol/l, NR: 1.9–4.8)[#]	0.2	0.1	<0.1	<0.1
ApoA1 (g/l, NR: 1.1–2.3)	ND	ND	0.7	0.8
ApoB (g/l, NR: 0.5–1.3)[§]	ND	ND	<0.2	<0.2
Lp(a) lipoprotein (nmol/l, NR: <75)[&]	ND	ND	<7	<7
ASAT (U/l, NR: 15–35)	ND	ND	41	36
ALAT (U/l, NR: 10–45)	ND	ND	48	37

ND: Not determined, NR: Normal range, [#]Detection limit 0.1 mmol/l, [§]Detection limit 0.2 g/l, [&]Detection limit 7 nmol/l.

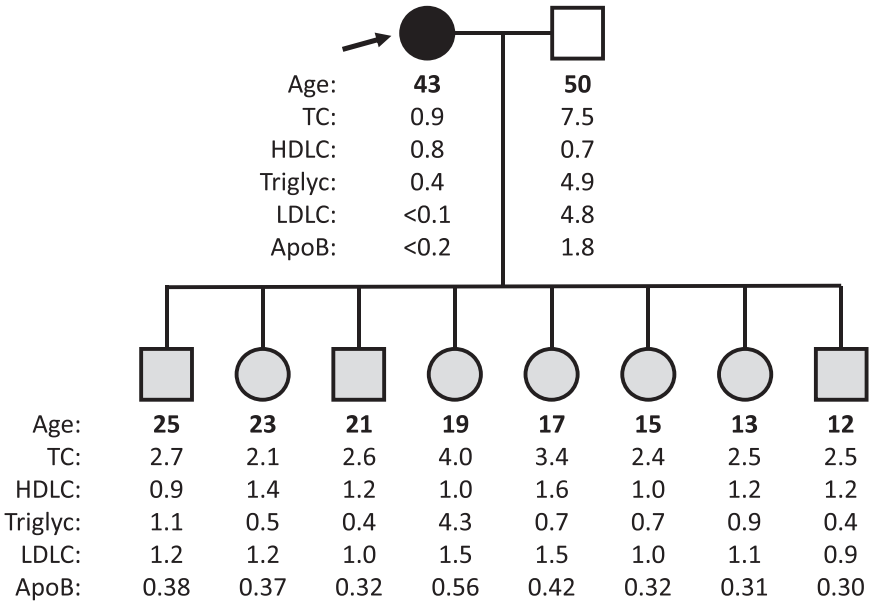

Fig. 1 Pedigree with lipid levels and mutation status in family members. Age (years) and fasting values for total serum cholesterol (TC), HDL cholesterol (HDLC), triglycerides (Triglyc) and LDL cholesterol (LDLC), all in mmol/l, as well as fasting values for apolipoprotein B (ApoB) in g/l in the proband, her spouse and in their eight children. The proband is indicated by an arrow. Homozygosity for mutation Q384K is indicated by a black symbol, whereas heterozygosity for mutation Q384K is indicated by a grayish symbol.

mean values of 1.2 mmol/l and 0.37 g/l, respectively. The spouse was not heterozygous for mutation Q384K, whereas all the eight children were heterozygous for this mutation. These data indicate that mutation Q384K segregates with hypobetalipoproteinemia in the family, and that hypobetalipoproteinemia is inherited in an autosomal dominant fashion.

Effect of an oral fat load on the serum lipid levels

The lipid profile in the proband with non-fasting values for triglycerides in the range of 6-10 mmol/l and values for LDL cholesterol around or below the detection limit of 0.1 mmol/l, could indicate that apoB-48 is made normally in the intestine, whereas apoB-100 is made in markedly reduced amounts, if at all, in the liver.

To test this hypothesis, a standardized oral fat load was given to the proband and three ostensibly healthy, unrelated controls after an overnight fast. The healthy controls were two female and one male laboratory personnel. The fat load consisted of 145 ml Calogen (N.V. Nutrica, Zoetemeer, The Netherlands) containing 72.5 g of fat. A blood sample was drawn for serum lipid measurements before and 2 h 15 min after the oral fat load (Table 2). In the proband, a four-fold increase in the triglyceride level from 0.4 mmol/l before the fat load, to 1.6 mmol/l after the fat load, was observed. In the three healthy controls, a mean increase in the triglyceride levels from 0.7 mmol/l before the fat load, to 1.2 mmol/l after the fat load was observed (Table 2). Thus, the proband responded to the oral fat load by an increase in triglyceride levels that was even larger than that observed for the healthy controls.

Table 2 Serum lipids were measured before and 2 h 15 min after a fatty meal consisting of 72.5 g fat in the proband and in three healthy controls.

	Proband		Control 1		Control 2		Control 3	
	Before	After	Before	After	Before	After	Before	After
Total serum cholesterol (mmol/l)	0.9	0.9	4.3	4.4	3.9	3.2	4.9	4.5
HDL cholesterol (mmol/l)	0.8	0.7	1.8	1.6	2.1	2.0	1.2	1.2
Triglycerides (mmol/l)	0.4	1.6	0.9	1.8	0.5	1.0	0.7	0.9
LDL cholesterol (mmol/l)	<0.1	<0.1	2.8	2.6	1.7	1.6	3.1	2.9
ApoB (g/l)	<0.2	<0.2	0.8	0.8	0.5	0.5	1.0	0.9
ApoA1 (g/l)	0.8	0.7	1.8	1.8	1.8	1.5	1.4	1.3

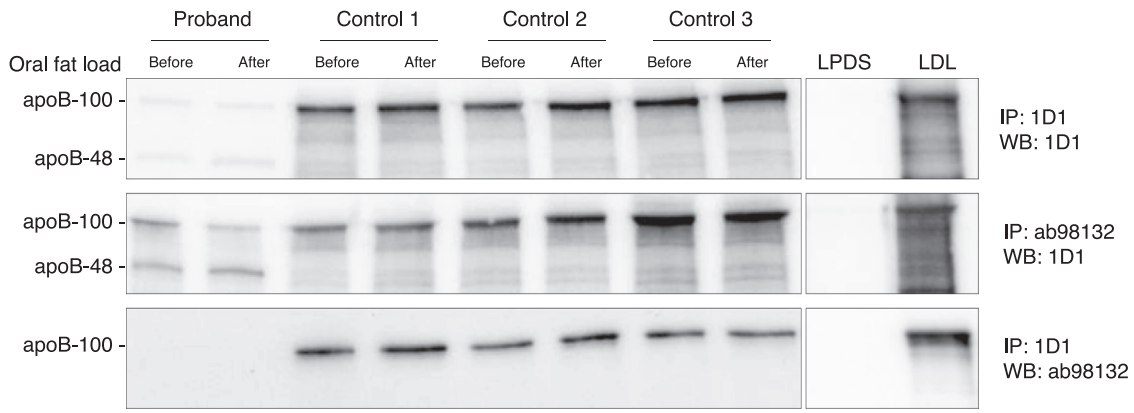

Fig. 2 Western blot of apoB in serum before and after an oral fat load. After an overnight fast, serum was obtained before and after an oral fat load of 72.5 g of fat from the proband homozygous for mutation Q384K in the *APOB* gene and from three healthy controls (Controls 1-3). Western blot analysis (WB) was performed of apoB immunoprecipitated (IP) from serum before and after the fat load using two different anti-apoB antibodies (ID1 and ab98132). Lipoprotein-deficient serum (LPDS) and LDL were used as negative and positive controls for apoB-100, respectively but exposed for a shorter time than the patient samples due to the high apoB chemiluminescence of the LDL sample compared to that of the serum samples. ApoB-48 in the three healthy controls is partly masked by the degradation products of apoB-100 when anti-apoB ID1 is used for detection. ApoB-48 is immunoprecipitated, but not detected by the ab98132 antibody. Representative Western blots from three separate experiments is shown. Full-length blots is shown in Suppl. Fig. S1.

These findings indicate that the proband is able to synthesize apoB-48 in the intestine as a component of chylomicrons, in a seemingly normal fashion.

Western blot analysis of apoB-48 and apoB-100 in serum

To further study the amounts of apoB-48 and apoB-100 in serum, Western blot analysis of serum from the proband and the three healthy controls, obtained before and after the oral fat load, was performed. As can be seen from Fig. 2, only minimal amounts of apoB-100 were detected in serum from the proband, in comparison to that of the healthy controls. However, apoB-48 was identified in the serum obtained from the proband before the oral fat load, and a slightly increased amount of apoB-48 was observed after the oral fat load (Fig. 2). This finding confirms that the proband is able to synthesize apoB-48 in the intestine in a normal fashion. The smear observed on the Western blot for the three healthy controls and LDL fraction represents degradation products of apoB-100 (Suppl. Fig. S1).

Effect of mutation Q384K on the structure of apoB

Mutation Q384K will affect the alpha solenoid domain (residues 321 to 616) of the LLT module. To study the conservation of Gln_{384}, 409 vertebrate apoB orthologs from mammals, birds, reptiles, amphibians, teleost fish, cartilaginous fish and the lamprey were collected by standard sequence searching[25] in the NCBI RefSeq database.[26] The analyses revealed that the motif QxxxQCGxxxC (residues 380 to 390), including Gln_{384}, was conserved in all 409 vertebrate orthologs (See subset of 43 orthologs in Suppl. Fig. S2). The conservation of this motif strongly suggests that it is important for apoB function. The AlphaFold structural model of apoB (Suppl. Fig. S3) was used in an attempt to understand the location and function of Gln_{384} in the folded apoB protein structure.

A disulfide bridge between the absolutely conserved residues Cys_{385} and Cys_{390} of apoB is necessary for the correct folding and stability of the alpha solenoid domain, contributing to the packing of HTH repeats 2 and 3 (Fig. 3).[27] It is possible that Gln_{384} facilitates and helps the formation of this disulfide bridge (Fig. 3). Gln_{384} also contributes to

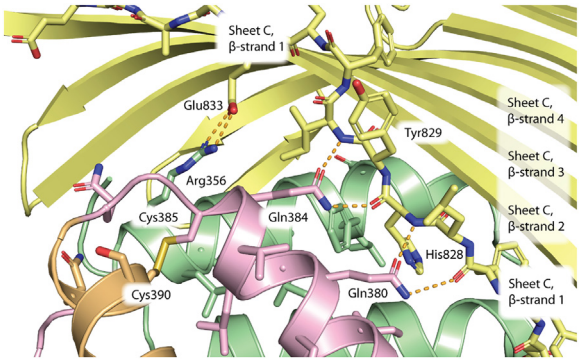

Fig. 3 Gln_{384} contributes to the conserved and strong interaction between the alpha solenoid domain and the C-sheet. The AlphaFold structural model of the LLT module of apoB (Suppl. Fig. S3) shows a network of interactions between the first β-strand ("Sheet C, β-strand 1", in sticks rendering, with light yellow carbons) of a major lipid-binding structure, the C-sheet, and the two first HTH repeats of the right-handed alpha solenoid domain (HTH repeats 1, 2, and 3, in forest green, pink, and gold, respectively). The C-sheet, strands 2 to 8 (light yellow), and the HTH repeats are shown in cartoon rendering, with some selected side chains as sticks. See also Suppl. Fig. S3B for an explanation of the different domains and segments of the LLT module. Arg_{356} is absolutely conserved in all vertebrate apoB, as well as in vitellogenin and MTP. This residue forms a strong salt bridge to Glu_{833} of the C-sheet. Gln_{380} and Gln_{384} are absolutely conserved in all vertebrate apoB homologs (Suppl. Fig. S2) and contribute to four H-bonds involving protein backbone atoms at Phe_{826}, His_{828}, and Ile_{830}. The structure of the right-handed alpha solenoid domain is stabilized by a disulfide bridge between Cys_{385} and Cys_{390}.

the strong interactions between the alpha solenoid domain, mainly HTH repeats 1 and 2, and the second lipid-binding β-sheet, the C-sheet, through a network of H-bond and salt bridge interactions, together with other conserved residues such as Phe_{348}, Arg_{356}, and Gln_{380} (Fig. 3). It is unlikely that replacement of Gln_{384} with the larger and charged Lys is compatible with the strong interactions illustrated in Fig. 3. The mutant Q384K-apoB is likely to have a weaker interaction between the C-sheet and alpha solenoid domain, and an overall less stable LLT module.

ApoB and MTP are the only two proteins in the LLT protein superfamily found in humans. Mutation D361Y in the *MTTP* gene has been found to cause abetalipoproteinemia.[28] Structural alignment of the MTP protein structure and the apoB model clearly demonstrate that MTP Asp_{361} is homologous to apoB Gln_{380}, the first residue in the conserved QxxxQCGxxxC motif (Fig. 3 and Suppl. Fig. S2). Together, these data indicate a critical role for Gln_{384}, as well as Gln_{380}, for the normal structure and lipidation of apoB-100.

Discussion

In this study we have shown that mutation Q384K in the *APOB* gene causes familial hypobetalipoproteinemia. However, only secretion of apoB-100 in the liver was affected with, apoB-48 being secreted in an apparently normal fashion in the intestine. Thus, the homozygous patient synthesized chylomicrons in a normal fashion, whereas VLDL and its metabolic product LDL were synthesized in minimal amounts. The low levels of vitamins D and E in plasma of the homozygote are most likely caused by reduced transport capacity in VLDL and LDL. It is conceivable that the low plasma levels of these vitamins may contribute to the neuromuscular symptoms and symptoms of fatigue in the proband. The failure to synthesize and secrete VLDL normally explains the finding of hepatic steatosis and the failure to detect Lp(a) lipoprotein in plasma. Apo(a) of Lp(a) lipoprotein binds to Cys_{4353} in the carboxy terminal part of apoB-100.[29]

The fasting lipid profile in the proband with total serum cholesterol levels of ~1 mmol/l, undetectable levels of LDL cholesterol, approximately half-normal levels of HDL cholesterol and normal levels of triglycerides, is similar to that of two patients homozygous for the R490W (c.1468C>T) mutation in the *APOB* gene which causes familial hypobetalipoproteinemia.[30] The lipid profile is also similar to that of a patient homozygous for the R532W (c.1594C>T) mutation in the *APOB* gene which also causes familial hypobetalipoproteinemia.[21] In that study, data on the fasting status of the 11 months old R532W homozygote was not presented. Regarding the synthesis of apoB-48, a patient homozygous for the R490W mutation was reported to have a three-fold postprandial increase in triglyceride levels.[31] This response to fat intake is similar to the four-fold postprandial increase in triglyceride levels observed in the Q384K homozygote. Together, these data indicate that mutations Q384K, R490W and R532W, that all modify the alpha solenoid domain known to be required for optimal binding of apoB to MTP,[15] affect secretion of VLDL without affecting secretion of chylomicrons.

Analysis of protein structure shows that Gln_{384} is essential in a strong and evolutionary conserved network of interactions between two important domains in the apoB LLT module (Fig. 3), and the mutation Q384K will very likely weaken the packing of these domains. It is also possible that mutation Q384K will disrupt the disulfide bond between Cys_{385} and Cys_{390} and thereby lead to abnormal folding of the LLT module. As a consequence, recruitment of lipids to apoB in the ER will be affected, which again will result in reduced secretion.[32] Similar findings of abnormal folding of the LLT module have been found for mutations L370V and R490W.[19] ApoB Leu_{370} and Arg_{490} are located in HTH repeats 2 and 5 in the alpha solenoid domain, respectively. The missense mutation L370V is very likely destroying the strong packing of HTH repeats 1 and 2 and destabilizes the alpha solenoid domain, while R490W disrupts the interaction between the alpha solenoid domain and the A-sheet. These data therefore underscore the role of proper folding of the LLT module for assembly and lipidation of apoB.[7,13,14]

According to the current model for lipidation of apoB, where the N-terminal 1000 residues form a lipid-binding pocket that interacts with MTP,[7,9] one could expect mutation

Q384K to affect the secretion of apoB-48 and apoB-100 in a similar fashion. The normal secretion of apoB-48 indicates that apoB-48 and apoB-100 have different requirements for lipidation through interaction with MTP. As apoB is elongated to generate apoB-100, more hydrophobic residues will be exposed which could make apoB-100 more vulnerable to defective lipidation than the shorter apoB-48. This notion is supported by the data of Benoist and Grand-Perret.[33] They found that by the use of an MTP inhibitor, apoBs larger than apoB-65 were more susceptible to co-translational degradation than the shorter apoBs. Similar data were obtained by Nicodeme et al.[34] who found that apoB peptides larger than apoB-53 were susceptible to degradation in the presence of an MTP inhibitor. Moreover, by using MTP inhibitors in Caco2 cells that synthesize both apoB-48 and apoB-100, it has been shown that secretion of apoB-48 was unaffected, whereas secretion of apoB-100 was reduced.[34,35] It therefore seems that secretion of apoB-100 is more dependent on the interaction with MTP than that of apoB-48. It has also been shown that translocation of apoB-48 and apoB peptides shorter than apoB-48 into the ER, does not require MTP.[36,37] Thus, reduced translocation of apoB-100 into the ER due to mutations affecting the LLT module, could contribute to the reduced secretion of apoB-100.

The notion that the synthesis of apoB-100 has a higher requirement for MTP than that of apoB-48, is also supported by the findings in patients with abetalipoproteinemia who are compound heterozygotes for mutations in the *MTTP* gene, and where one allele is having some residual activity. One such patient with abetalipoproteinemia who was compound heterozygous for a deletion of exon 10 and mutation R623L (c.1868G>T) in the *MTTP* gene, had normal levels of apoB-48 in serum.[38] While the former mutation abolishes MTP activity, the latter mutation makes the mutant retain 65% of normal activity. Another patient with abetalipoproteinemia who had normal levels of apoB-48 in serum was compound heterozygous for mutations Q272X (c.814C>T) and G709R (c.2125G>A) in the *MTTP* gene.[39] It was suggested that the allele carrying mutation G709R could encode a protein with some residual activity that allowed the synthesis of apoB-48, but not of apoB-100.[39]

ApoB mutants caused by mutations L370V and R490W in the *APOB* gene found in familial hypobetalipoproteinemia show increased binding to MTP.[19,30] One could therefore speculate that a mechanism by which mutations affecting the LLT module cause reduced secretion of apoB-100, is increased binding of these mutants to MTP. This could increase the retention time of apoB-MTP complexes in the ER and reduce the availability of MTP to assist in translocation and lipidation of apoB-100.

Stable expression of the mutants L370V-apoB-100 and R490W-apoB-100 in McA-RH7777 cells, has been shown to reduce secretion of the two apoB-100 mutants by ~50%.[19] When stably transfected McA-RH7777 cells were used to study secretion of the mutants L370V-apoB-48 and R490W-apoB-48, secretion of the two apoB-48 mutants was reduced by ~25% and 50%, respectively.[19] A similar transfection study with the R532W *APOB* mutation, has been shown to reduce the synthesis of the mutant R532W-apoB-48 by 40%.[21] These findings may appear to be at variance with the clinical findings of a virtual lack of apoB-100 secretion and normal apoB-48 secretion in subjects homozygous for mutations Q384K or R490W. The explanation for this discrepancy is not readily apparent but could be secondary to overexpression of mutant apoBs in the transfection studies. An alternative strategy to using transfection studies could be the use of CRISPR/Cas9 technology which has been employed to study mutation L351R in the *APOB* gene.[22]

In conclusion, missense mutation Q384K affecting the N-terminal LLT module of apoB, markedly reduces the synthesis and secretion of the full-length apoB-100 in the liver, whereas the synthesis of the shorter apoB-48 in the intestine appears to be unaffected. Further studies are needed to identify the exact mechanisms by which the two forms of apoB are affected differently.

Ethical Approval

The study was conducted as part of a diagnostic investigation of the family to establish the underlying cause for the lipid disorder. Such diagnostic investigations, regularly performed in our laboratory, does not require individual approval from an ethics committee. The patients signed written consent for the diagnostic investigation and publication of results. The inclusion of a healthy control group material was considered by the Norwegian Regional Committees for Medical and Health Research Ethics - South East to not need a formal approval (ref. 469809).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jacl.2023.08.009.

References

1. Chan L. Apolipoprotein B, the major protein component of triglyceride-rich and low density lipoproteins. *J Biol Chem*. 1992;267:25621–25624.
2. Davidson NO, Shelness GS. Apolipoprotein B: mRNA editing, lipoprotein assembly, and presecretory degradation. *Annu Rev Nutr*. 2000;20:169–193.
3. Hussain MM, Shi J, Dreizen P. Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *J Lipid Res*. 2003;44:22–32.
4. Wetterau JR, Lin MC, Jamil H. Microsomal triglyceride transfer protein. *Biochim Biophys Acta*. 1997;1345:136–150.
5. Rustaeus S, Stillemark P, Lindberg K, Gordon D, Olofsson SO. The microsomal triglyceride transfer protein catalyzes the post-translational assembly of apolipoprotein B-100 very low density lipoprotein in McA-RH7777 cells. *J Biol Chem*. 1998;273:5196–5203.
6. Yao Z, Tran K, McLeod RS. Intracellular degradation of newly synthesized apolipoprotein B. *J Lipid Res*. 1997;38:1937–1953.
7. Segrest JP, Jones MK, De Loof H, Dashiti N. Structure of apolipoprotein B-100 in low density lipoproteins. *J Lipid Res*. 2001;42:1346–1367.

8. Smolenaars MMW, Madsen O, Rodenburg KW, van der Horst DJ. Molecular diversity and evolution of the large lipid transfer protein superfamily. *J Lipid Res*. 2007;48:489–502.
9. Dashti N, Gandhi M, Liu X, Lin X, Segrest JP. The N-terminal 1000 residues of apolipoprotein B associate with microsomal triglyceride transfer protein to create a lipid transfer pocket required for lipoprotein assembly. *Biochemistry*. 2002;41:6978–6987.
10. Thompson JR, Banaszak LJ. Lipid-protein interactions in lipovitellin. *Biochemistry*. 2002;41:9398–9409.
11. Biterova EI, Isupov MN, Keegan RM, et al. The crystal structure of human microsomal triglyceride transfer protein. *Proc Natl Acad Sci USA*. 2019;116:17251–17260.
12. Jeiran K, Gordon SM, Sviridov DO, et al. A new structural model of apolipoprotein B100 based on computational modeling and cross linking. *Int J Mol Sci*. 2022;23:11480.
13. Shelness GS, Thornburg JT. Role of intramolecular disulfide bond formation in the assembly and secretion of apolipoprotein B-100-containing lipoproteins. *J Biol Chem*. 1996;37:408–419.
14. Tran K, Borén J, Macri J, et al. Functional analysis of disulfide linkages clustered within the amino terminus of human apolipoprotein B. *J Biol Chem*. 1996;273:7244–7251.
15. Hussain MM, Bakillah A, Nayak N, Shelness GS. Amino acids 430-570 in apolipoprotein B are critical for its binding to microsomal triglyceride transfer protein. *J Biol Chem*. 1998;273:25612–25615.
16. Kane JP, Havel RJ. Disorders of the biogenesis and secretion of lipoproteins containing the apoB apolipoproteins. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw Hill; 2001:2717–2752.
17. Bredefeld C, Hussain MM, Averna M, et al. Guidance for the diagnosis and treatment of hypolipidemia disorders. *J Clin Lipidol*. 2022;16:797–812.
18. Tarugi P, Averna M, Di Leo E, et al. Molecular diagnosis of hypobetalipoproteinemia: an ENID review. *Atherosclerosis*. 2007;195:e19–e27.
19. Burnett JR, Zhong S, Jiang ZG, et al. Missense mutations in *APOB* within $\beta\alpha 1$ domain of human apoB-100 result in impaired secretion of apoB and apoB-containing lipoproteins in familial hypobetalipoproteinemia. *J Biol Chem*. 2007;282:24270–24283.
20. Zhong S, Magnolo AL, Sundaram M, et al. Nonsynonymous mutations within *APOB* in human familial hypobetalipoproteinemia: evidence for feedback inhibition of lipogenesis and postendoplasmic reticulum degradation of apolipoprotein B. *J Biol Chem*. 2010;285:6453–6464.
21. Yilmaz BS, Mungan NO, Di Leo E, et al. Homozygous familial hypobetalipoproteinemia: a Turkish case carrying a missense mutation in apolipoprotein B. *Clin Chim Acta*. 2016;452:185–190.
22. Vanhoye X, Janin A, Caillaud A, et al. *APOB* CRISPR/Cas9 engineering in hypobetalipoproteinemia: a promising tool for functional studies of novel variants. *Int J Mol Sci*. 2022;23:4281.
23. Mcleod RS, Zhao Y, Shelby SL, Westerlund J, Yao Z. Carboxy-terminal truncation impairs lipid recruitment by apolipoprotein B100 but does not affect secretion of the truncated apolipoprotein B-containing lipoproteins. *J Biol Chem*. 1994;269:2852–2862.
24. Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database (HGMD®): optimizing its use in a clinical diagnostic or research setting. *Hum Genet*. 2020;139:1197–1207.
25. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. NCBI BLAST: a better web interface. *Nucleic Acids Res*. 2008;36:W5–W9.
26. O'Leary NA, Wright MW, Brister JR, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res*. 2016;44:D733–D745.
27. Yang CY, Kim TW, Weng SA, Lee BR, Yang ML, Gotto jr AM. Isolation and characterization of sulfhydryl and disulfide peptides of human apolipoprotein B-100. *Proc Natl Acad Sci USA*. 1990;87:5523–5527.
28. Walsh MT, Di Leo E, Okur I, Tarugi P, Hussain MM. Structure-function analyses of microsomal triglyceride transfer protein missense mutations in abetalipoproteinemia and hypobetalipoproteinemia subjects. *Biochim Biophys Acta*. 2016;1861:1623–1633.
29. McCormick SP, Ng JK, Taylor S, Flynn LM, Hammer RE, Young SG. Mutagenesis of the human apolipoprotein B gene in a yeast artificial chromosome reveals the site of attachment for apolipoprotein(a). *Proc Natl Acad Sci USA*. 1995;92:10147–10151.
30. Burnett JR, Shan J, Miskie BA, et al. A novel nontruncating *APOB* gene mutation, R463W, causes familial hypobetalipoproteinemia. *J Biol Chem*. 2003;278:13442–13452.
31. Burnett JR, Proos AL, Koutts J, Burnett L. Familial hypobetalipoproteinemia: a rare presentation to the lipid clinic. *Med J Aust*. 1993;159:272–274.
32. Huang XF, Shelness GS. Identification of cysteine pairs within the amino-terminal 5% of apolipoprotein B essential for hepatic lipoprotein assembly and secretion. *J Biol Chem*. 1997;272:31872–31876.
33. Benoist F, Grand-Perret T. Co-translational degradation of apolipoprotein B100 by the proteasome is prevented by microsomal triglyceride transfer protein. *J Biol Chem*. 1997;272:20435–20442.
34. Nicodeme E, Benoist F, McLeod R, et al. Identification of domains in apolipoprotein B100 that confer a high requirement for the microsomal triglyceride transfer protein. *J Biol Chem*. 1999;274:1986–1993.
35. Haghpassand M, Wilder D, Moberly JB. Inhibition of apolipoprotein B and triglyceride secretion in human hepatoma cells (HepG2). *J Lipid Res*. 1996;37:1468–1480.
36. Wang S, Mcleod RS, Gordon DA, Yao Z. The microsomal triglyceride transfer protein facilitates assembly and secretion of apolipoprotein B-containing lipoproteins and decreases cotranslational degradation of apolipoprotein B in transfected COS-7 cells. *J Biol Chem*. 1996;271:14124–14133.
37. Rusiñol AE, Jamil H, Vance JE. In vitro reconstitution of assembly of apolipoprotein B48-containing lipoproteins. *J Biol Chem*. 1997;272:8019–8025.
38. Di Filippo M, Frachon SC, Janin A, et al. Normal serum APOB48 and red cells vitamin E concentrations after supplementation in a novel compound heterozygous case of abetalipoproteinemia. *Atherosclerosis*. 2019;284:75–82.
39. Takahashi M, Ozaki N, Nagashima S, Wakabayashi T, Iwamoto S, Ishibashi S. Normal plasma apoB48 despite the virtual absence of apoB100 in a compound heterozygote with novel mutations in the *MTTP* gene. *J Clin Lipidol*. 2021;15:569–573.