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MINIREVIEW

Major Apolipoprotein B-100 Mutations in Lipoprotein Metabolism and Atherosclerosis

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Summary

Apolipoprotein (apo) B-100 is a key protein compound of plasma lipid metabolism. This protein, as a sole component of LDL particles, to a great extent controls the homeostasis of LDL cholesterol in the plasma. Therefore, this protein and its structural variants play an important role in development of hyperlipidemia and atherosclerosis. Intensive research into the structure and biological functions of apoB-100 has led to identification of its complete structure as well as the responsible binding sites. With the development of the methods of molecular biology, some structural variants of the apoB-100 protein that directly affect its binding properties have been described. These are mutations leading to amino acid substitution at positions 3500 (R3500Q and R3500W) and 3531 (R3531C) that have been shown to decrease the binding affinity of apoB-100 *in vitro*. However, only the former mutations have been unequivocally demonstrated to cause hyperlipidemia *in vivo*. This minireview is aimed to discuss the impact of apoB-100 and its structural variants on plasma lipid metabolism and development of hyperlipidemia.

Key words

Apolipoprotein B-100 • Mutations • Hyperlipidemia • Atherosclerosis

Introduction

Over the last 50 years there has been a growing incidence of cardiovascular diseases in highly developed countries. Despite the positive trend observed in the last decades when the incidence of cardiovascular diseases ceased to increase, it still remains a leading cause of morbidity and mortality in western societies. Thus, it is not surprising that research of the underlying mechanisms of cardiovascular diseases, especially of the development of atherosclerosis which is involved in most of them, has attracted much attention.

Atherosclerosis as a degenerative disease of the vessel wall has a complex and multiple etiology which has not been fully explained despite all efforts and progress in the field over the last few years. Theories concerning the onset and course of the atherosclerotic changes have been changing recently, ranging from the lipid hypothesis and theory of the endothelial response to injury to the nowadays most accepted theory of atherosclerosis which takes into account both the lipid and endothelial factors that are involved in the atherosclerotic process. More and more is being discovered about the involvement of endothelial

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dysfunction and hemostatic factors in this process. Numerous studies performed up to now have identified a number of risk factors of atherogenesis including the genetic background, disorders of lipid metabolism, hypertension and diabetes mellitus.

Hyperlipoproteinemias and their genetic basis have been studied extensively and some of these studies have several candidate genes the polymorphism or mutations of which affect the metabolic pathways of plasma lipoproteins. Apart from apolipoprotein E and its polymorphism, the genes for lipoprotein lipase, LDL receptor and many others, the gene for apolipoprotein B-100 has been identified as a key component influencing the metabolism of plasma lipids and lipoproteins. This mini-review is aimed to present up-to-date knowledge of this interesting protein molecule and its gene.

Structure of apolipoprotein B-100

Apolipoprotein B-100 (apoB-100) is a huge protein molecule consisting of 4560 amino acid residues (including the 24-amino-acid-long signal peptide) with a molecular weight exceeding 500 000 Da (Elovson *et al.* 1985). It is secreted mainly by the liver, but also other sites of its secretion have been proposed (Veniant *et al.* 1999). The complete primary structure of this protein has been identified and its secondary and tertiary structure have been proposed. These studies also led to the identification of the LDL receptor binding domain of the protein which was mapped between residues 3359 through 3367 (Cladaras *et al.* 1986). It is formed by positively charged residues that can interact with negatively charged regions of the LDL receptor. Moreover, it is closely homologous with the LDL receptor binding domain of apolipoprotein E (Rall *et al.* 1981). Further research and especially the investigations in patients with a significantly reduced binding affinity of apoB-100 proved the importance of amino acid substitutions within other parts of the protein outside the originally proposed binding region. This is most likely caused by alteration in the tertiary structure of the protein or by changing the binding site of apoB-100 for cell proteoglycans (Dunning *et al.* 1991, Olsson *et al.* 1997). Cell-surface proteoglycans contribute to the cell association with LDL particles and to their internalization, probably by creating a pericellular compartment that concentrates the particles in the immediate neighborhood of the LDL receptor (Lund-Katz *et al.* 1991).

ApoB-100 is secreted from the hepatocytes as a protein compound of VLDL particles. These undergo intravascular processing during which all other types of apoproteins are removed so that apoB-100 remains the sole apoprotein of LDL particles. Each LDL particle contains one apoB-100 molecule which becomes of special importance in cases when apoB-100 is defective in its binding to the receptors (Havel and Kane 1989).

Function of apoB-100

The function of apoB-100 is closely related to its structure. Its serves both to maintain the integrity of LDL particles and to control the plasma levels of LDL-C through binding to the receptors (Brown and Goldstein 1986). The apoB-100 molecule is modeled as a belt which surrounds the LDL particle (Schumaker *et al.* 1994). Therefore, the diameter of LDL particle and thus apoB-100 has implications for the binding affinity of apoB-100 to the receptors (Miserez and Keller 1995). A central role in this respect seems to be exerted by the arginine residue at position 3500 of the apoB-100 protein which stabilizes two clusters of basic amino acids (regions 3147 to 3157 and 3359 to 3367) that have been assumed to ensure the binding of the apoB to the LDL receptors (März *et al.* 1993).

ApoB-100 gene

ApoB-100 is encoded by a gene that has been localized to the short arm of chromosome 2. Its complete nucleotide sequence has been described. The gene consists of 28 introns and 29 exons. The longest coding sequence within the gene is exon 26, which is also the longest exon detected in the entire human genome. More than one half of the apoB-100 protein molecule is coded by this exon (Blackhart *et al.* 1986). There have been several mutations identified in the apoB-100 gene leading to premature truncation of protein synthesis or to amino acid substitution within the protein. Such changes can influence the metabolism of plasma lipoproteins and may therefore be important in the development of hyperlipidemia. Using the molecular genetic approach, three mutations in the apoB-100 gene leading to decreased binding affinity of apoB-100 to the receptors have been identified.

R3500Q mutation

First of the mutations was identified by Innerarity *et al.* (1987). It is a G to A nucleotide transition at position 10 708 of the apoB gene which results in

amino acid substitution of glutamine for arginine at position 3500 (R3500Q mutation) (Innerarity *et al.* 1987). The autosomal co-dominant trait associated with this genetic condition was designated as familial defective apolipoprotein B-100 (FDB). FDB is a disorder which is clinically and biochemically indistinguishable from familial hypercholesterolemia (FH), a disease caused by LDL receptor gene mutation. This was demonstrated by the fact that approximately 3-5 % of FDB patients are incorrectly diagnosed as FH (Weisgraber *et al.* 1988). However, reviews dealing with the comparison between FH and FDB homozygotes and heterozygotes showed that hypercholesterolemia, which arises from the genetic condition, is generally milder and more variable in FDB (Miserez and Keller 1995). Furthermore, the development of atherosclerosis is delayed in comparison with FH patients (Brousseau *et al.* 1995, Tybjaerg-Hansen *et al.* 1998, Češka *et al.* 2000). The observed differences between FDB and FH become even more pronounced when the homozygotes for these diseases are compared (Funke *et al.* 1992). Three possible explanations of the lower plasma lipid levels found in FDB individuals have been offered.

Firstly, studies in FDB homozygotes showed that residual binding affinity of LDL in homozygous FDB was 10-20 % of normal values required for significant catabolism of LDL particles through the LDL receptor pathway (Gallagher and Myant 1995).

Secondly, lower plasma lipoprotein levels in FDB can partially be caused by an increased uptake of VLDL remnants, as evidenced by *in vivo* studies in which FDB probands and healthy controls have been compared. This finding, together with observations in patients with type III hyperlipoproteinemia, supports the idea that apolipoprotein E plays a more important role in the metabolism of remnant particles than apoB-100 (Maher *et al.* 1993, Hořejší and Češka 2000). The uptake of LDL precursors *via* apo E is enhanced by the up-regulation of LDL receptors (Miserez and Keller 1995). This becomes even more pronounced when statin therapy is introduced in FDB patients (Illingworth *et al.* 1992). The up-regulation of LDL receptors in FDB arises from the decreased flux of LDL-derived cholesterol into hepatocytes (Schaefer *et al.* 1997).

Thirdly, the increased uptake of apoE containing particles from the plasma results in a decrease of apoB-100 production rate as well as of LDL particles. This has been clearly demonstrated by *in vivo* studies in FDB homozygotes (Schaefer *et al.* 1997).

In the plasma of FDB heterozygotes, two subpopulations of LDL particles can be identified – one bearing the mutant and the second with the normal apoB-100 molecule. The two subpopulations can be distinguished by binding assays using monoclonal antibody called MB19 (Innerarity *et al.* 1988) or by direct assessment of the affinity of apoB-100 for the LDL receptor on human cultured fibroblasts (Innerarity *et al.* 1987). Another method is the U937 monocyte proliferation assay which is based on the critical dependence of the cell line on cholesterol supply *via* the apoB, apoE receptors (van den Broek *et al.* 1994). The detection of the amino acid substitution by monoclonal antibody MB47, which binds to the mutant LDLs with approximately 60 % higher affinity than to the normal LDL particles, can also be used (Weisgraber *et al.* 1988). The particles bearing mutant apoB-100 molecule are highly prevalent in the plasma of FDB heterozygous individuals because their binding affinity for the LDL receptor is only about 5 % of normal (Innerarity *et al.* 1988). The estimated ratio of normal to defective LDL particles in affected patients is 30:70 (Friedl *et al.* 1991).

It was demonstrated that the binding affinity of apoB-100 to the LDL receptor differs according to the size of the LDL particle, being the highest in intermediate density LDLs. The FDB mutation distorts the structure of the apoB-100 binding domain preferentially to particles with either lowest or highest densities. While the large, buoyant LDLs are cleared at a normal rate *via* their apoE moiety, the small dense LDL particles accumulate in the plasma of FDB individuals and therefore form the prevalent LDL subfraction found in FDB patients (Nigon *et al.* 1991, März *et al.* 1993).

The longer presence of mutant LDLs in the plasma, their increased susceptibility to oxidation as well as the changes in their affinity to the receptors, arterial wall and proteoglycans due to conformational changes in the mutant apoB-100 molecule have led to a suggestion that LDLs in FDB might be more atherogenic. This notion, however, was not confirmed by a comparison of atherosclerosis in FDB patients with closely matched FH patients (Maher *et al.* 1995).

This mutation the frequency of which in different general population samples ranges from 1:500 to 1:700 (Tybjaerg-Hansen 1990, Schuster *et al.* 1990, Innerarity *et al.* 1990), was detected in hyper-cholesterolemic populations with a frequency of 1-5 %. The mutation was not identified in a genetically isolated Finnish population (Hämälainen *et al.* 1990), but the highest frequency of this mutation was found in a

Swiss study (Miserez *et al.* 1993). The geographical distribution and knowledge of probable routes by which the early humans spread into Europe and Asia allowed Myant *et al.* (1997) to postulate a hypothesis that the mutation originated within the Central European region approximately 7000 years ago. The fact that the same, rare apoB haplotype designated 194 has been found in all white carriers of the mutation identified to date, further supports the idea of a common European ancestor in whom the mutation originated (Rauh *et al.* 1991).

R3500W mutation

Besides the amino acid substitution of arginine for glutamine yet another mutation affecting codon 3500 of the apoB-100 protein has also been identified. This concerns the substitution of arginine for tryptophan (R3500W mutation) which leads to the same biochemical and clinical picture as the previously described mutation. The higher incidence of the mutation in Asian populations as well as its association with a unique haplotype indicate that the mutation is of Asian descent (Tai *et al.* 1998, Choong *et al.* 1997). So far the mutation has only been identified in two hyperlipidemic probands of Caucasian origin (Gaffney *et al.* 1995).

R3531C mutation

Screening for new mutations within the putative receptor binding domain of apoB-100 in patients with hypercholesterolemia revealed two mutations affecting the codon 3500 and one mutation leading to an amino acid interchange at position 3531 of the apoB-100 protein (R3531C mutation). The original findings in patients with this mutation suggested that it might have been another cause of hypercholesterolemia and premature atherosclerosis. An extensive study with 2570 individuals screened for the R3531C mutation performed by Pullinger *et al.* (1999) indicated that the mutation is sufficient to cause hypercholesterolemia because of the lower binding affinity of the defective apoB molecule to the LDL receptors. This results in accumulation of the LDL particles bearing the mutant apoB molecule in the plasma. However, the binding affinity of R3531C apoB is almost three times greater than that of R3500Q apoB (27 % vs. 10 % of the binding affinity of the wild type apoB-100). This difference explains the various mass ratios of the mutant and the wild type apoB bearing particles in the plasma of heterozygous carriers of either mutation (27:73 in R3500Q carriers and 58:42 in R3531C carriers). These results suggest that the impact of the R3531C mutation on lipoprotein metabolism is much weaker in comparison to the R3500Q mutation. In Pullinger's study, the association of hypercholesterolemia with the R3531C mutation was reported. However, when the affected individuals were compared with their non-affected relatives only a statistically insignificant increase in plasma total and LDL cholesterol levels was observed. The increase became significant after evaluating all R3531C mutation carriers identified up to now in several studies together (Rabés *et al.* 1997, Wenham *et al.* 1997, Ludwig *et al.* 1997, Pullinger *et al.* 1999). This result is plausible because only hypercholesterolemic mutation carriers, irrespective of the cause of hypercholesterolemia, were included in the meta-analysis. Moreover, in another study performed by Wenham *et al.* (1997) a clear co-segregation of hypercholesterolemia was shown for one but not for the other of the two identified R3531C pedigrees. Recent extensive studies did not find the R3531C mutation sufficiently large to cause hypercholesterolemia. Tybjaerg-Hansen *et al.* (1998) reported only one carrier of the mutation among 987 patients with coronary artery disease and hypercholesterolemia, while another 7 normocholesterolemic probands were identified among 9255 subjects from the general population. These authors concluded that other genetic and environmental factors were necessary for the development of hypercholesterolaemia in R3531C carriers.

The frequencies of R3531C mutation in selected populations of patients examined at lipid clinics vary from 1:206 to 1:987 which is about half the frequency of the R3500Q mutation which was found in different studies with a frequency from 1:46 to 1:208 (Rabés *et al.* 1997, Wenham *et al.* 1997, Ludwig *et al.* 1997, Tybjaerg-Hansen *et al.* 1998, Pullinger *et al.* 1999). The incidence of the disorder in patients is similar as in the general population which is in accordance with the notion that R3531C does not cause hypercholesterolemia.

Most authors have identified the R3531C carriers with a common haplotype suggesting that the mutation originated from a common Celtic ancestor (Ludwig and McCarthy 1990).

Conclusions

The mechanisms underlying the process of atherosclerotic changes of the vessel wall have been extensively studied both clinically and experimentally. Up to now, numerous risk factors have been identified

among which hyperlipidemia plays an important role. Studying factors leading to disorders of metabolism of plasma lipids revealed the importance of structural variants of apoproteins which play a key role in homeostasis of plasma lipoproteins. One of the most intensively studied apoproteins is apolipoprotein B-100, a sole component of LDL particles in the plasma. Searching the apoB gene for new mutations has disclosed three mutations leading to a decreased binding affinity of the protein to the receptors, but only the mutations affecting the codon 3500 have been proved to be associated with hyperlipidemia *in vivo*. Moreover, studies of the structural variants of apoB-100 have helped to improve our understanding of its physiological functions as well as the structure-function relationship.

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References

BLACKHART BD, LUDWIG EH, PIEROTTI VR, CIAIATI L, ONASCH MA, WALLIS SC, POWELL L, PEASE R, KNOTT TJ, CHU ML, MAHLEY RW, SCOTT J, McCARTHY BJ, LEVY-WILSON B: Structure of the human apolipoprotein B gene. *J Biol Chem* **261:** 15364-15367, 1986.

BROUSSEAU T, ARVEILER D, CAMBOU JP, EVANS AE, LUC G, FRUCHART JCH, CAMBIEN F: Familial defective apolipoprotein B-100 and myocardial infarction. The ECTIM study. *Atherosclerosis* **116:** 267-271, 1995.

BROWN MS, GOLDSTEIN JL: A receptor mediated pathway for cholesterol homeostasis. *Science* **232:** 34-47, 1986.

CHOONG ML, KOAY ES, KHOO KL, KHAW MC, SETHI SK: Denaturing gradient gel electrophoresis screening of familial defective apolipoprotein B-100 in a mixed Asian cohort: two cases of arginine3500→tryptophan mutation associated with a unique haplotype. *Clin Chem* **43:** 916-923, 1997.

CLADARAS C, HADZOUPOULOU-CLADARAS M, NOLTE RT, ATKINSON D, ZANNIS VL: The complete sequence and structural analysis of human apolipoprotein B-100: relationship between apoB-100 and apoB-48. *EMBO J* **5:** 3495-3507, 1986.

ČEŠKA R, VRABLÍK M, HOŘÍNEK A: Familial defective apolipoprotein B-100: a lesson from homozygous and heterozygous patients. *Physiol Res* **49** (Suppl 1): S125-S130, 2000.

DUNNING AM, HOULSTON R, FROSTEGARD J, REVILL J, NILSSON J, HAMSTEN A, TALMUD P, HUMPHRIES S: Genetic evidence that the putative binding domain of apolipoprotein B (residues 3130-3630) is not the only region of the protein involved in interaction with the low density lipoprotein receptor. *Biochim Biophys Acta* **1096:** 231-237, 1991.

ELOVSON J, JACOBS JC, SCHUMAKER VN, PUPPIONE DL: Molecular weights of apoprotein B obtained from human low-density lipoprotein (apoprotein B-PI) and from rat very low density lipoprotein (apoprotein B-PIII). *Biochemistry* **24**: 1569-1578, 1985.

FRIEDL W, LUDWIG EH, BALESTRA ME, ARNOLD KS, PAULWEBER B, SANDHOFER F, McCARTHY BJ, INNERARITY TL: Apolipoprotein B gene mutations in Austrian subjects with heart disease and their kindred. *Arterioscler Thromb* **11:** 371-378, 1991.

FUNKE H, RUST S, SEEDORF J, BRENNHAUSEN B, CHIRAZI A, MOTTI C, ASSMAN G: Homozygosity for familial defective apolipoprotein B-100 is associated with lower plasma cholesterol than homozygosity for familial hypercholesterolemia. *Circulation* **86** (Suppl I): 691, 1992.

GAFFNEY D, REID JM, CAMERON IM, VASS K, CASLAKE MJ, SHEPHERD J, PACKARD CJ: Independent mutations at codon 3500 of the apolipoprotein B gene are associated with hyperlipidemia. *Arterioscler Thromb Vasc Biol* **15:** 1025-1029, 1995.

GALLAGHER JJ, MYANT NB: The affinity of LDL and VLDL remnants for the LDL receptor in homozygous familial defective apolipoprotein B-100. *Atherosclerosis* **115:** 263-272, 1995.

HÄMÄLAINEN T, PALOTIE A, AALTO-SETALA K, KONTULA K, TILKANEN MJ: Absence of familial defective apolpoprotein B-100 in Finnish patients with elevated serum cholesterol. *Atherosclerosis* **82:** 177-183, 1990.

HAVEL RJ, KANE JP. Structure and metabolism of plasma lipoproteins. In: *The Metabolic Basis of Inherited Disease*, CR SCRIVER (ed), McGraw-Hill, New York, 1989, pp 1129-1138.

HOŘEJŠÍ B, ČEŠKA R: Apolipoproteins and atherosclerosis. Apolipoprotein E and apolipoprotein (a) as candidate genes of premature development of atherosclerosis. *Physiol Res* **49** (Suppl 1): S63-S69, 2000.

ILLINGWORTH DR, VAKAR F, MAHLEY RW, WEISGRABER KH: Hypocholesterolemic effects of lovastatin in familial defective apolipoprotein B-100. *Lancet* **339:** 598-600, 1992.

INNERARITY TL, WEISGRABER KH, ARNOLD KS, MAHLEY RW, KRAUSS RM, VEGA GL, GRUNDY SM: Familial defective apolipoprotein B100: low density lipoproteins with abnormal receptor binding. *Proc Natl Acad Sci USA* **84:** 6919-6923, 1987.

INNERARITY TL, BALESTRA ME, ARNOLD KS, MAHLEY RW, VEGA GL, GRUNDY SM, YOUNG SG: Isolation of defective receptor-binding low density lipoproteins from subjects with familial defective apolipoprotein B-100. *Arteriosclerosis* **8:** 551a, 1988.

INNERARITY TL, MAHLEY RW, WEISGRABER KH, BERSOT TP, KRAUSS RM, VEGA GL, GRUNDY SM, FRIEDL W, DAVIGNON J, MCCARTHY BJ: Familial defective apolipoprotein B-100: a mutation of apolipoprotein B that causes hypercholesterolemia. *J Lipid Res* **31:** 1337-1349, 1990.

LUDWIG EH, MCCARTHY BJ: Haplotype analysis of the human apolipoprotein B mutation associated with familial defective apolipoprotein B 100. *Am J Hum Genet* **47:** 712-720, 1990.

LUDWIG EH, HOPKINS PN, ALLEN A, WU LL, WILLIAMS RR, ANDERSON JL, WARD RH, LALOUEL JM, INNERARITY TL: Association of genetic variations in apolipoprotein B with hypercholesterolemia, coronary artery disease and receptor binding of low density lipoproteins. *J Lipid Res* **38:** 1361-1373, 1997.

LUND-KATZ S, INNERARITY TL, ARNOLD KS, CURTISS LK, PHILIPS MC: ^{13}C NMR evidence that substitution of Gln for Arg 3500 in FDB-100 disrupts the conformation of the receptor binding domain. *J Biol Chem* **266:** 2701-2709, 1991.

MAHER VMG, GALLAGHER JJ, MYANT NB: The binding affinity of very low density lipoprotein remnants to the low density receptor in FDB-100. *Atherosclerosis* **102:** 51-61, 1993.

MAHER VMG, GALLAGHER JJ, THOMPSON GR, MYANT NB: Does the presence of the 3500 mutant apolipoprotein B-100 in low density lipoprotein particles affect their atherogenicity? *Atherosclerosis* **118:** 105-110, 1995.

MÄRZ W, BAUMSTARK MW, SCHNARGL H, RUZICKA V, BUXBAUM S, HERWIG J, POHL T, RUSS A, SHAAF L, BERG A, BÖHLES H-J, USADEL KH, GROSS W: Accumulation of „small dense“ low density lipoproteins (LDL) in a homozygous patient with familial defective apolipoprotein B-100 results from heterogeneous interaction of LDL subfractions with the LDL receptor. *J Clin Invest* **92:** 2922-2933, 1993.

MISEREZ AR, KELLER N: Differences in the phenotypic characteristics of subjects with familial defective apolipoprotein B100 and familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* **15:** 1719-1729, 1995.

MISEREZ AR, LAAGER R, CHIODETTI N, KELLER U: High prevalence of familial defective apolipoprotein B-100 in Switzerland. *J Lipid Res* **34:** 799-805, 1993.

MYANT NB, FORBES SA, DAY INM, GALLAGHER J: Estimation of the age of the ancestral arginine 3500→glutamine mutation in human apoB-100. *Genomics* **45:** 78-87, 1997.

NIGON F, LESNIK P, ROUIS M, CHAPMAN MJ: Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. *J Lipid Res* **32:** 1741-1753, 1991.

OLSSON U, CAMEJO G, HURT-CAMEJO E, ELFSBER K, WIKLUND O, BONDJERS G: Possible functional interactions of apolipoprotein B-100 segments that associate with cell proteoglycans and the apoB/E receptor. *Arterioscler Thromb Vasc Biol* 17: 149-155, 1997.

PULLINGER CR, GAFFNEY D, GUTIRREZ M, MALLOY MJ, SCHUMAKER VN, PACKARD CJ, KANE JP: The apolipoprotein B R3531C mutation: characteristics of 24 subjects from 9 kindreds. *J Lipid Res* **40:** 318-327, 1999.

RABÉS JP, VARRET M, SAINT-JORE B, ERLICH D, JONDEAU G, KREMPF M, GIRAUDET P, JUNIEN C, BOILEAU C: Familial ligand-defective apolipoprotein B100: simultaneous detection of the Arg3500Gln and Arg3531Cys mutations in a French population. *Hum Mutat* **10:** 160-163, 1997.

RALL SC, WEISGRABER KH, MAHLEY RW: Human apolipoprotein E. The complete amino acid sequence. *J Biol Chem* **257:** 4171-4178, 1981.

RAUH G, SCHUSTER H, FISCHER J, KELLER C, WOLFRAM G, ZÖLLNER N. Familal defective apolipoprotein B-100: haplotype analysis of the arginine 3500→ glutamine mutation. *Atherosclerosis* **88:** 219-226, 1991.

SCHAEFER JR, SCHNARGL H, BAUMSTARK MW, SCHWEER H, ZECH LA, SEYBERTH H, WINKLER K, STEINMETZ A, MÄRZ W: Homozygous familial defective apolipoprotein B-100. Enhanced removal of apolipoprotein E-containing VLDLs and decreased production of LDLs. *Arterioscler Thromb Vasc Biol* **17:** 348-353, 1997.

SCHUMAKER VN, PHILLIPS ML, CHATTERTON JE: Apolipoprotein B and low-density lipoprotein structure: implications for biosynthesis of triglyceride-rich lipoproteins. In: *Advances in Protein Chemistry.* CB ANFINSEN, JT EDSALL, FM RICHARDS, DS EISENBERG (eds), Calif. Academic Press, San Diego, 1994, pp 205-248.

SCHUSTER H, RAUH G, KORMANN B, HEPP T, HUMPHRIES SE, KELLER C, WOLFRAM G, ZÖLLNER N: Familial defective apolipoprotein B-100. Comparison with familial hypercholesterolemia in eighteen cases detected in Munich. *Arteriosclerosis* **10:** 577-581, 1990.

TAI DY, PAN JP, LEE-CHEN GJ: Identification and haplotype analysis of apolipoprotein B-100 Arg3500→Trp mutation in hyperlipidemic Chinese. *Clin Chem* **44:** 1659-1665, 1998.

TYBJAERG-HANSEN A, GALLAGHER J, VINCENT J, HOULSTON R, TALMUD P, DUNNING AM, SEED M, HAMSTEN A, HUMPHRIES SE, MYANT NB: Familial defective apolipoprotein B-100: detection in the United Kingdom and Scandinavia, and clinical characteristics of ten cases. *Atherosclerosis* **80:** 235-242, 1990.

TYBJAERG-HANSEN A, STEFFENSEN R, MEINERTZ H, SCHNOHR P, NORDESTGAARD BG: Association of mutations in the apolipoprotein B gene with hypercholesterolemia and the risk of ischemic heart disease. *N Engl J Med* 338: 1577-1584, 1998.

VAN DEN BROEK AJCM, HOLLAAR L, HERMAN I, SCHAEFER MB, VAN DER LAARSE A, SCHUSTER H, DEFESCHE JC, KASTELEIN JJP, VAN'T HOOFT FM. Screening for familial defective apoliporotein B-100 with improved U937 monocyte proliferation assay. *Clin Chem* 40: 395-399, 1994.

VENIANT MM, NIELSEN LB, BOREN J, YOUNG SG. Lipoproteins containing apolipoprotein B-100 are secreted by the heart. *Trends Cardiovasc Med* **9:** 103-107, 1999.

WEISGRABER KH, INNERARITY TL, NEWHOUSE YM, YOUNG SG, ARNOLD KS, KRAUSS RM, VEGA GL, GRUNDY SM, MAHLEY RW. Familial defective appolipoprotein B-100: enhanced binding of monoclonal antibody MB47 to abnormal low density lipoproteins. *Proc Natl Acad Sci USA* **85:** 9758-9762, 1988.

WENHAM PR, HENDERSON BG, PENNEY MD, ASHBY JP, RAE PW, WALTER SW. Familial ligand-defective apolipoprotein B-100: detection, biochemical features and haplotype analysis of the R3531C mutation in the UK. *Atherosclerosis* **129:** 185-192, 1997.

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