J Mol Med (2006) 84: 647–650
DOI 10.1007/s00109-006-0070-4

CASE REPORT

Jeffrey Rhyne · Michael J. Ryan · Charles White · Theodore Chimonas · Michael Miller

The two novel CETP mutations Gln87X and Gln165X in a compound heterozygous state are associated with marked hyperalphalipoproteinemia and absence of significant coronary artery disease

Received: 5 May 2006 / Accepted: 30 May 2006 / Published online: 20 June 2006

Abstract High levels of high-density lipoprotein cholesterol (HDL-C) occur with cholesteryl ester transfer protein (CETP) deficiency. However, the extent to which CETP deficiency states may be associated with protection against coronary artery disease (CAD) has been controversial. We evaluated a Greek pedigree with high levels of HDL-C and no history of premature CAD. The proband, a 45-year-old male with an HDL-C of 194 mg/dl with absent CETP activity, was heterozygous for two novel CETP mutations (Q87X and Q165X). A 64-slice multidetector CT scan revealed minimal (<10%) narrowing of the proximal left anterior descending artery without any other evidence of coronary atherosclerosis. In contrast to previous studies, these data suggest that complete CETP deficiency does not promote premature atherosclerosis. However, it remains unclear as to whether the relative lack of coronary atherosclerosis was the direct consequence of CETP deficiency and/or the lack of traditional CAD risk factors.

Keywords CETP deficiency · Mutations · Multidetector CT scan

JEFFREY RHYNE received his M.S. in Applied Molecular Biology from the University of Maryland, Baltimore, MD, USA and is currently a Research Assistant at the University of Maryland Medical Center, Baltimore, MD, USA. His research interests include mutation discovery and genetic regulation of dyslipidemia with a focus on HDL cholesterol.

MICHAEL MILLER received his M.D. from the University of Medicine and Dentistry of New Jersey, NJ, USA. He is currently an Associate Professor of Medicine, Epidemiology and Preventive Medicine and a Director of Center for Preventive Cardiology at the University of Maryland Medical Center, Baltimore, MD, USA. His research focuses on the molecular basis and clinical implications related to disorders of HDL and triglyceride metabolism.

Introduction

Human plasma cholesteryl ester transfer protein (CETP) is a 476-residue hydrophobic glycoprotein that plays an integral role in reverse cholesterol transport (RCT) by facilitating transfer of cholesteryl esters from high-density lipoprotein (HDL) to lower density lipoproteins en route to hepatobiliary excretion [1]. Decreased or absent activity of CETP results in high (60–99 mg/dl) or very high (>100 mg/dl) levels of HDL-C, and at least ten functional CETP variants have been reported, residing predominantly within the Japanese population [2].

While the traditional view infers that high or very high HDL-C is cardioprotective [3], molecular variation in at least one gene (e.g., hepatic lipase) has been associated with premature coronary artery disease (CAD) [4]. Similarly, an

J. Rhyne · M. J. Ryan · C. White · T. Chimonas · M. Miller
University of Maryland and Veterans Affairs Medical Center, Baltimore, MD, USA

M. Miller (✉)
Division of Cardiology, Room S3B06,
University of Maryland Hospital,
22 South Greene Street,
Baltimore, MD 21201, USA
e-mail: mmiller@heart.umaryland.edu
Tel.: +1-410-3286299
Fax: +1-410-3284382

ongoing debate has emerged as to whether CETP deficiency states may also be associated with atherosclerosis [2, 5].

Recently, multislice CT angiography has been demonstrated to be a highly sensitive and specific tool for the noninvasive evaluation of coronary atherosclerosis [6, 7]. Using this modality, we studied the coronary arteries of a middle-aged male with complete CETP deficiency to evaluate the presence and extent of early coronary atherosclerosis.

Materials and methods

Study subject and pedigree

The proband is a 45-year-old male of Greek descent with no familial history of vascular disease. His biologic relatives reside on the island of Drama, and there is a history of longevity with maternal siblings living into the tenth decade of life. The family pedigree is shown in Fig. 1. The proband reported no history of hypertension, cigarette smoking, or diabetes mellitus. His dietary habits include consumption of red meat and cheese three to four times weekly, and he consumes one to two alcoholic beverages weekly. He is engaged in aerobic activities twice a week and has never complained of chest discomfort, shortness of breath, or other cardiac symptoms. He takes no medications. The range of five fasting lipids and lipoproteins measured during the previous 5 years were: total cholesterol, 240–284 mg/dl; triglycerides, 59–103 mg/dl; HDL-C, 184 to >200 mg/dl; LDL-C, 40–75 mg/dl; and apolipoprotein (apo) B, <50 mg/dl and apo AI, >200 mg/dl. Both the proband and family members signed consent forms, and the protocol was approved by the Institutional Review Board of the University of Maryland Medical Center.

Determination of levels of plasma lipids and lipoproteins

Blood samples were collected after a 12-h overnight fast. Samples drawn in Drama, Greece were placed on wet ice and shipped by Federal Express to Baltimore, Maryland (U.S.A.). Levels of plasma total cholesterol and triglyceride were measured using enzymatic/colorimetric methods with the Vitros 950 Chemistry Analyzer (Johnson & Johnson, New Brunswick, NJ, USA). HDL-C was determined by the heparin–manganese precipitation method, and apolipoproteins AI and B were measured using radial immunodiffusion as previously described [8]. LDL-C was calculated using the formula of Friedewald et al [9].

CETP activity assay

Cholesterol ester transfer activity was determined using the Roar CETP Activity Assay Kit (NYC, NY, USA). Briefly, 4 μl of donor particle and 4 μl of acceptor particle were combined with the desired CETP source (0.2 to 0.8 μl of undiluted plasma or serum, fresh or frozen) in 200 μl total volume with reconstituted assay buffer. Samples were incubated for 3 h at 37°C. The increase in fluorescence of samples was measured using a fluorimeter (excitation: 465 nm; emission: 535 nm). The fluorescence intensity transferred in the plasma or serum sample was determined by subtracting the blank fluorescence intensity from each sample.

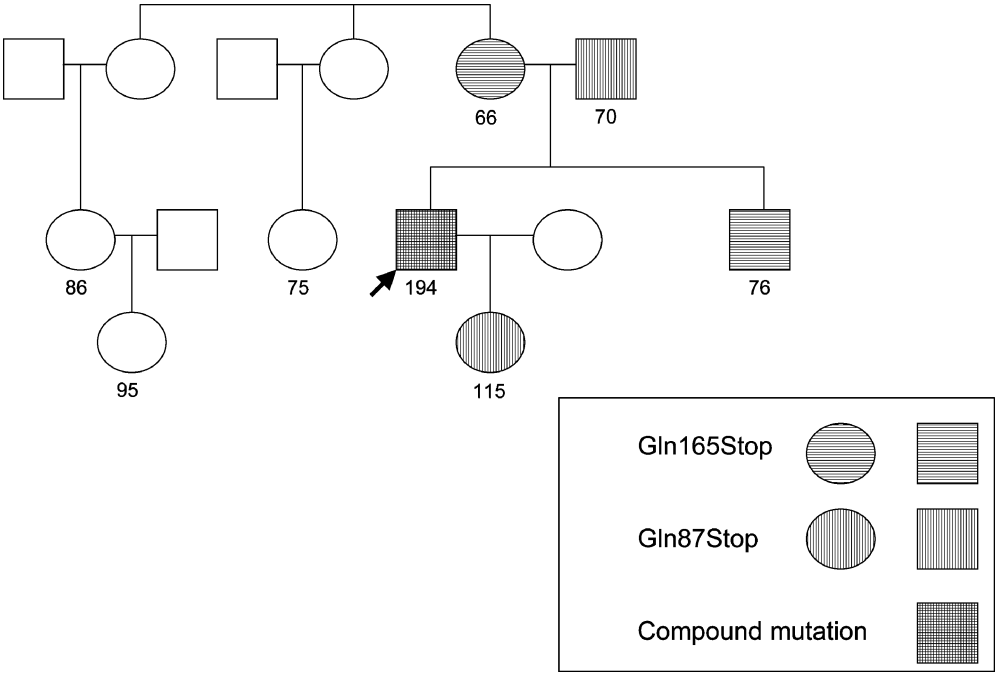

Fig. 1 Greek pedigree with CETP deficiency. Numerical values listed below each subject indicate HDL-C (mg/dl). *Arrow* denotes the proband

Table 1 Lipids, lipoproteins, and apolipoproteins AI and B (mg/dl) in the pedigree with CETP deficiency

Subject ID	Gender	Age	Total Cholesterol	Triglyceride	HDL	LDL	Apo A	Apo B
I-1	F	71	223	180	66	121	164	98
I-2	M	87	222	116	70	129	116	95
II-1	F	47	245	93	86	140	93	100
II-2	F	51	257	95	75	163	95	111
II-3	M	45	246	59	194	40	>200	<50
II-4	M	43	209	78	76	117	78	94
III-1	F	23	234	124	95	114	124	85
III-2	F	14			115			

PCR amplification and dHPLC of CETP

The promoter and all coding regions of the CETP gene were screened using one of the two methods. The promoter and the majority of exons were directly sequenced. The remaining exons were screened using denaturing high-performance liquid chromatography (dHPLC) (Transgenomics Wave, Omaha, NE, USA), and altered patterns were then subjected to direct sequencing. Sequencing reactions were run on an ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA). PCR cycling conditions included initial denaturation of 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s to 1.5 min (depending upon the length of the amplicon) and additional extension of 72°C for 5 min. Amplifications were performed in a Techne Genius thermocycler (Techne, Burlington, NJ, USA). Samples were amplified using 50 μl reaction volumes consisting of 20 to 100 ng genomic DNA, 10 pmol each primer, 0.2 mM each dNTP, 1× Gold Buffer, 2.0 mM $MgCl_2$, and 1.25 U AmpliTaq Gold.

Gated multislice CT angiography

Images were acquired using a 64-slice multidetector CT scanner (Brilliance, Phillips Medical Systems, Best, Netherlands). Retrospective ECG-gated images were obtained through the heart during a single breathhold beginning in a craniocaudal direction. Scanning protocol included collimation of 16×0.625 mm with section thickness of 0.9 mm. Scanning technique was performed at 140 kVp and 600 mA s. A pitch of 0.24 was utilized with a scanner rotation time of 0.4 s. One-hunded twenty cubic centimeters of iodinated contrast was injected through a 20-gauge angiocatheter into an antecubital vein at 5 cm^3/s. Automated bolus timing was performed using a threshold value of 150 HU and a region of interest placed over the ascending aorta.

Table 2 Assessment of CETP activity in the proband (II-3) and family members with the CETP mutations Q87X and/or Q165X

Subject ID	CETP activity (pmol/3 h)	Activity of controls (%)	Mutation
I-1	59.25	70.75	Q165X
I-2	54.61	65.21	Q87X
II-1	83.66	99.90	Normal
II-2	83.82	100	Normal
II-3	0.00	0.00	Q87X/ Q165X
II-4	54.90	65.56	Q165X
III-2	91.07	108.75	Q87X

Results

Lipid and lipoprotein levels of the proband and associated family members are shown in Table 1. Both paternal and

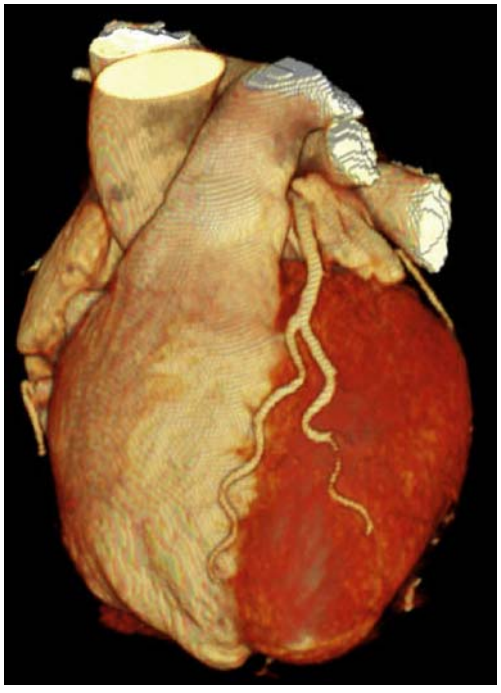

Fig. 2 Curved planar reconstructed image of the left coronary artery using a 64-multidetector CT scan demonstrating minimal CAD in a 45-year-old male with complete CETP deficiency

maternal family members displayed elevated HDL-C with the most striking levels observed in the proband. After the identification of absent CETP activity in the proband (Table 2), mutational analysis of the CETP gene disclosed two novel variants in the proband. Each mutation was caused by a C-to-T substitution, resulting in conversion of glutamine with a stop codon (CAG→TAG) and premature protein truncation (Q87X and Q165X). All first-degree relatives manifested either of the two mutations. To the best of our knowledge, this represents the first pedigree of Greek ancestry identified to date with CETP deficiency. The proband also underwent 64-multidetector CT scan imaging, and Fig. 2 illustrates a curved planar reconstructed view of the left coronary artery. There was no evidence of coronary calcification present and only minimal disease (<10%) estimated in the proximal left anterior descending coronary artery. The remainder of the coronary vascular bed was free of atherosclerosis.

Discussion

In the present study, two novel CETP mutations were identified (Gln87X and Gln165X), each predicts a considerably truncated product and the composite results in complete loss of CETP activity as compared with the normal translated protein of 476 amino acids. Under normal circumstances, CETP mediates the transfer of cholesteryl ester from HDL to lower density lipoproteins in exchange for triglycerides. With complete absence of CETP activity, HDL-C levels rise, although associated alterations in particle size and apolipoprotein composition may render them less efficient in RCT [10]. These unfavorable characteristics may be offset by associated low LDL-C due to lack of cholesteryl ester exchange from HDL to lower density lipoproteins as evident in the present case. Therefore, the biochemical impact of CETP deficiency increased, but functionally impaired HDL may translate into an overall reduced CAD risk if associated with low LDL-C. Indeed, a history of familial longevity was reported in ten homozygous CETP-deficient subjects [11]. However, what is more likely to be meaningful from a clinical standpoint is whether CETP deficiency negates the proatherogenic state amplified by CAD risk factors. To this end, CETP deficiency states have not been shown to be cardioprotective if elevated LDL-C and/or traditional risk factors are present [5, 12]. Thus, while the present case demonstrates minimal coronary atherosclerosis in a CETP-deficient patient, it cannot be determined whether the low rate of atherosclerosis resulted in part from lipoprotein alterations and/or the absence of CAD risk factors. Ongoing clinical trials with CETP inhibitors should help to resolve this issue and determine whether this therapeutic modality effectively impacts CAD event rates.

Note added in proof

Following submission of this manuscript, Matsuura et al., found that cholesterol efflux from CETP-deficient patients was enhanced, rather than decreased as previously believed due to reduced efficiency of ABCA1 to interact with enlarged HDL particles. Rather, ABCG1 mediates cholesterol efflux with enhanced CE formation resulting from LCAT activation by apoE enriched HDL.

Matsuura F, Wang N, Chen W, Jiang XC, Tall AR. (2006) HDL from CETP-deficient subjects shows enhanced ability to promote cholesterol efflux from macrophages in an apoE- and ABCG1-dependent pathway. J Clin Invest. 116:1435–1442.

References

1. Tall A (1995) Plasma lipid transfer proteins. Annu Rev Biochem 64:235–257
2. Barter PJ, Brewer HB Jr, Chapman MJ, Hennekens CH, Rader DJ, Tall AR (2003) Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol 23:160–167
3. Glueck CJ, Gartside PS, Steiner PM, Miller M, Todhunter T, Haaf J, Pucke M, Terrana M, Fallat RW, Kashyap ML (1977) Hyperalpha- and hypobeta-lipoproteinemia in octogenarian kindreds. Atherosclerosis 27:387–406
4. Hegele RA, Little JA, Vezina C, Maguire GF, Tu L, Wolever TS, Jenkins DJ, Connelly PW (1993) Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. Arterioscler Thromb 13:720–728
5. Nagano M, Nakamura M, Kobayashi N, Kamata J, Hiramori K (2005) Effort angina in a middle-aged woman with abnormally high levels of serum high-density lipoprotein cholesterol: a case of cholesteryl-ester transfer protein deficiency. Circ J 69:609–612
6. Leber AW, Knez A, von Ziegler F, Becker A, Nikolaou K, Paul S, Wintersperger B, Reiser M, Becker CR, Steinbeck G, Boekstegers P (2005) Quantification of obstructive and nonobstructive coronary lesions by 64-slice computed tomography: a comparative study with quantitative coronary angiography and intravascular ultrasound. J Am Coll Cardiol 46: 147–154
7. Hoffmann MH, Shi H, Schmitz BL, Schmid FT, Lieberknecht M, Schulze R, Ludwig B, Kroschel U, Jahnke N, Haerer W, Brambs HJ, Aschoff AJ (2005) Noninvasive coronary angiography with multislice computed tomography. JAMA 293:2471–2478
8. Miller M, Bachorik PS, McCrindle B, Kwiterovich PO (1993) Effect of gemfibrozil in men with primary isolated hypoalphalipoproteinemia: a randomized, double-blind, placebo-controlled, crossover study. Am J Med 94:7–12
9. Friedewald WT, Levy RI, Fredrikson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
10. Asztalos BF, Horvath KV, Kajinami K, Nartsupha C, Cox CE, Batista M, Schaefer EJ, Inazu A, Mabuchi H (2004) Apolipoprotein composition of HDL in cholesteryl ester transfer protein deficiency. J Lipid Res 45:448–455
11. Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Maruhama Y, Mabuchi H, Tall AR (1990) Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. N Engl J Med 323:1234–1238
12. Haraki T, Inazu A, Yagi K, Kajinami K, Koizumi J, Mabuchi H (1997) Clinical characteristics of double heterozygotes with familial hypercholesterolemia and cholesteryl ester transfer protein deficiency. Atherosclerosis 132:229–236