Case Studies

HDL deficiency due to a new insertion mutation (ApoA-I$_{Nashua}$) and review of the literature

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Abstract: A 61-year-old white man of European ancestry with significant coronary heart disease since age 42 years and marked high-density lipoprotein (HDL) deficiency (HDL cholesterol 1 mg/dL) was evaluated. His fasting low-density lipoprotein cholesterol level was 42 mg/dL, and his triglycerides were 417 mg/dL on therapy with rosuvastatin 40 mg/day, ezetimibe 10 mg/day, fenofibrate 145 mg/day, and extended-release niacin 2 g/day. Further analysis of his plasma revealed an apolipoprotein (apo) A-I level of 23.5 mg/dL (approximately 20% of normal), and the absence of small alpha-4 HDL, medium alpha-3 HDL, and very large alpha-1 HDL, with only very small pre-beta-1 HDL and large alpha-2 HDL being present. APOA-I gene sequencing revealed a novel heterozygous in-frame insertion mutation with duplication of nucleotides 1535 through 1552 inserted at position 1553, causing a new amino acid glycine at codon 157 and a duplication of amino acids alanine, arginine, alanine, histidine, and leucine at codons 158–162. This novel apoA-I mutation results in the formation of apoA-I that appears to have abnormal lipid binding properties, resulting in impaired reverse cholesterol transport, probable enhanced clearance, and premature coronary heart disease.
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Case Report

A 61-year-old white man of European ancestry presented with marked high-density lipoprotein (HDL) deficiency. He had a long-standing history of coronary artery disease that initially was diagnosed at age 42 years, when he developed angina, and at that time he underwent an angioplasty of his left anterior descending coronary artery.

At age 52 years of age, he developed recurrent angina and subsequently underwent coronary artery bypass surgery grafting surgery of his right coronary, circumflex, and left anterior descending arteries. He also had a history of hypertension, past cigarette smoking (quitting at age 41 years), and peripheral arterial disease. His father died at 69 years of age from colon cancer, and his mother was alive at age 88 years, with a history of hypertension and stroke and reportedly normal lipid levels.

His parents were unrelated, so there was no evidence of consanguinity. He had 4 siblings. One sister died at age 49 years of breast cancer. One brother died at age 48 years of a

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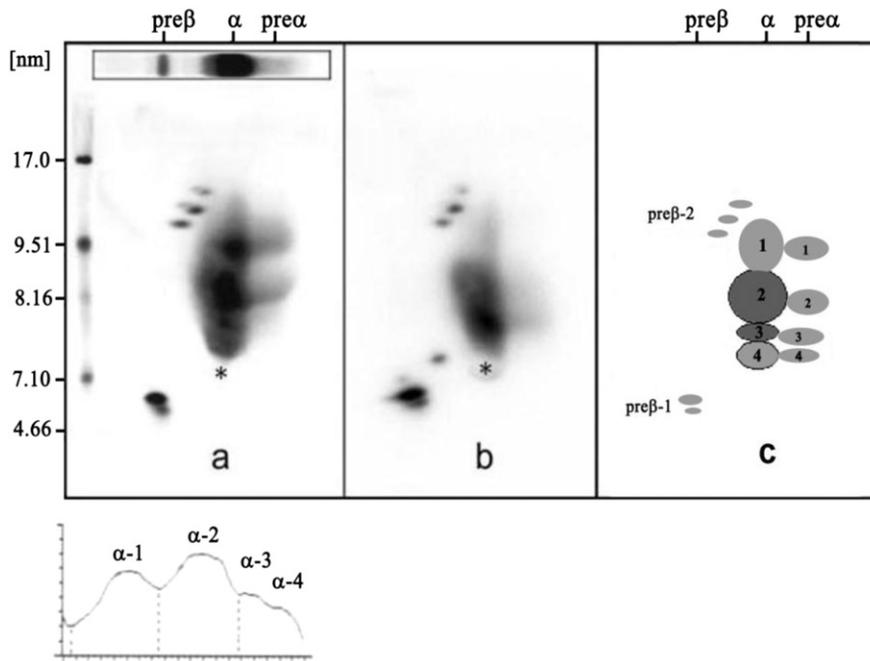E-mail address: ernst.schaefer@tufts.edu

Figure 1 Two-dimensional gel electrophoresis patterns followed by immunoblotting with monospecific apoA-I antibody of a normal subject (a) and of a patient with premature heart disease (b) in the center, and with a schematic of the individual HDL particles (c). The electrophoresis is performed in the horizontal dimension to separate pre-beta, alpha, and pre-alpha particles and in the vertical dimension using a 4%–30% gradient gel (particles of 5 to 12 nm in diameter, followed by immunoblotting with specific apoA-I antibody. The pattern from the patient with premature CHD in the center has decreased large HDL. All of the particles shown contain apoA-I, but only the alpha-3 and alpha-2 HDL particles contain appreciable amounts of apoA-II.

cerebral artery aneurysm. One brother died at age 51 years of a drug overdose. One brother was alive and well at age 52 years, with no history of coronary heart disease (CHD), with normal lipids, but with an HDL cholesterol (HDL-C) value in the 30 mg/dL range. The patient's wife was in good health. He had 2 offspring, a daughter age 31 years in good health with normal lipids and an HDL-C of 62 mg/dL, and a son, age 36 years in good health, with unknown lipid levels. The patient declined to have his family members studied.

At the time of his examination in our clinic he was on the following medications: aspirin 81 mg/day, extended-release diltiazem 120 mg/day, extended-release niacin 2 grams/day, ezetimibe 10 mg/day, fenofibrate 145 mg/day, fish oil 1 g/day, isosorbide 10 mg 3 times daily, metoprolol 25 mg 3 times/day, and rosuvastatin 40 mg/day. A physical examination revealed the following: height 71 inches, weight 231 pounds, blood pressure 140/99 mmHg, and heart rate 66 beats/minute. His eye examination revealed significant circular arcus senilis. However, in contrast to the arcus observed with aging or familial hypercholesterolemia, in this patient there was no space between the arcus and the limbus. The arcus was previously observed for some time by his referring physician, but it is unclear precisely at what age it developed. He had normal heart and lung examinations. The pulses in his legs (popliteals, dorsalis pedis, and posterior tibial) were present but were significantly diminished bilaterally. He had no evidence of hepatosplenomegaly or xanthomas. Blood was drawn after an overnight fast for laboratory analysis.

Laboratory analysis

Laboratory results on blood obtained after an overnight fast as analyzed by Quest Laboratories (Cambridge, MA) revealed total cholesterol 112 mg/dL (normal), triglycerides 447 mg/dL (elevated), HDL-C <5 mg/dL (very low), and
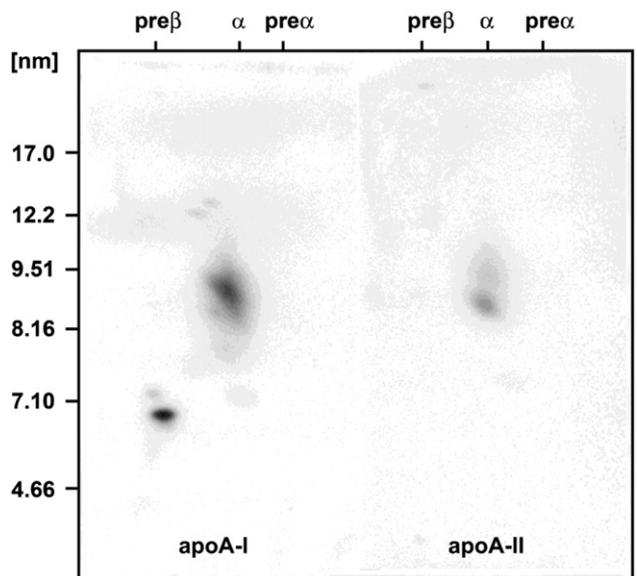

Figure 2 The two-dimensional gel electrophoretic pattern of the proband's plasma followed by immunoblotting with specific immunopurified apoA-I antibody is shown. Here we document the absence of alpha-4, alpha-3, and alpha-1 HDL particles.

Figure 3   Structure of apoA-I.

LDL-C 77 mg/dL (close to optimal), and lipoprotein (a) 25 nmol/L (normal). His other values were: liver alanine transaminase 24 U/L (normal), aspartate transaminases 39 U/L (slightly increased), alkaline phosphatase 65 U/L (normal), blood urea nitrogen 22 mg/dL (normal), creatinine 0.82 mg/dL (normal), thyroid-stimulating hormone 1.53 uIU/mL (normal), C-reactive protein 1.5 mg/L (normal), glycosylated hemoglobin 5.4% (normal), and fasting glucose 107 mg/dL, albumin 4.7 g/dL, bilirubin 0.9 mg/dL, and calcium 10.8 mg/dL (elevated). His electrolytes were all normal.

Specialized laboratory testing

Because of these abnormal findings, a sample of his plasma was analyzed in our laboratory (Lipid Metabolism Laboratory) at Tufts University. In this laboratory all lipid analyses have been standardized by the Lipid Standardization Program of the Centers for Disease Control, Atlanta, GA. His lipid values were: total cholesterol 116 mg/dL (decreased), triglycerides 368 mg/dL (elevated), HDL-C 1.2 mg/dL (markedly decreased), and direct LDL-C 42 mg/dL (decreased), and very low density lipoprotein-C 29.3 mg/dL (elevated), all measured as previously described.[1–5] His apolipoprotein A-I (apoA-I) level as measured by immunoassay as previously described was 23.5 mg/dL (markedly decreased, about 20% of normal).[2,3] His insulin as measured by immunoassay as previously described was 19.0 uU/ml (normal range, 5–15 uU/ml).[4] The percentage of his total cholesterol that was in the unesterified form was normal at approximately 30%.

Two-dimensional gel electrophoresis of his plasma followed by immunoblotting with antibody specific for apoA-I as previously described revealed normal levels of apoA-I in very small pre-beta-1 HDL of 7.5 mg/dL and large pre-beta-2 HDL of 1.2 mg/dL, significantly decreased levels of apoA-I in large alpha-2 HDL of 14.8 mg/dL (29% of normal), and undetectable apoA-I in small alpha-4 HDL, medium alpha-3 HDL, and very large alpha-1 HDL (see Figs. 1 and 2).[2,3]

Because of these abnormal findings, a sample of his DNA was sent to Dr. Robert Hegele of the Robarts Research Institute, London, Ontario, Canada, for sequencing of his APOA-I gene. This analysis revealed a novel heterozygous apoA-1 in-frame insertion mutation with a duplication of nucleotides 1535 through 1552 inserted at position 1553, causing a new amino acid glycine at codon 157 and a duplication of amino acids alanine, arginine, alanine, histidine, and leucine at codons 158–162 (Figs. 3 and 4). This novel apoA-I mutation results in the formation of apoA-I that appears to have abnormal lipid-binding properties, resulting in impaired reverse cholesterol transport and premature CHD.

Comment and discussion

Decreased HDL-C <40 mg/dL has been identified as an independent risk factor for CHD by the National Cholesterol Education Program.[6,7] Severe HDL deficiency (<10 mg/d) can be caused by to severe liver disease, familial apoA-I deficiency states, apoA-I variants, Tangier disease (defective ATP-binding cassette A1 transporter function), and lecithin:cholesterol acyltransferase (LCAT deficiency).[5] Liver disease was ruled out on the basis of the patient's history and normal liver function tests. The patient did have elevated triglyceride levels, but not enough to account for the severe HDL deficiency. apoA-I deficiency was ruled out by the presence of apoA-I in plasma at a concentration of 23.5 mg/dL. Tangier disease was ruled out by the finding of HDL particles other than pre-beta-1 HDL.[4]

In-frame Insertion at nucleotide 1553, GCGCGCGCGCCCATGTGG. It is a duplication of nucleotides 1535-1552, as bolded and underlined in the figure. The insertion causes a new amino acid, Gly, at codon 157, and a duplication of amino acids Ala, Arg, Ala, His, Leu at codon 158 through 162.

Figure 4   New insertion mutation (apoA-I_Nashua).

Table 1	Reported insertion mutations in the human APOA-I gene

Insertion mutations	Codon changes
apoA-I_{Tsukuba}: A C insertion in codons 3-5 which resulted in a frame-shift and the synthesis of an abnormal polypeptide containing only 33 instead of 243 amino acids	
A C insertion in codon 17 which resulted in a frame-shift and the synthesis of an abnormal polypeptide containing only 25 instead of 243 amino acids	
apoA-I_{Sasebo}: A gene duplication of 23 nucleotides which resulted in a frame-shift and the synthesis of an abnormal polypeptide containing only 207 amino acids	

apoA-I_{Tsukuba}:

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        1    2    3    4    5    6      32   33   34
Normal  Asp  Glu  Pro  Pro  Gln  Ser---- Gln  Phe  Glu
        GAT  GAA  CCC  CCC  CAG  AGC--   CAG  TTT  GAA

Mutant  GAT  GAA  CCC CCC CCA  GAG---    CCA  GTT  TGA
        Asp  Glu  Pro Pro Pro  Glu       Pro  Val  Term
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Nakata K, Kobayashi K, Yanagi H, Shimakura Y, Tsuchiya S, Arinami T, Hamaguchi H. Autosomal dominant hypoalphalipoproteinemia due to A completely defective apolipoprotein A-1 gene. Biochem. Biophys. Res. Commun. 1993;196:950-955.

Codon 17 mutation:

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        16   17   18   19   20   21   22   23   24   25   26
Normal  Ser  Gln  Ala  Arg  His  Phe  Trp  Gln  Gln  Asp  Glu
        AGC  CAG  GCT  CGG  CAT  TTC  TGG  CAG  CAA  GAT  GAA

Mutant  AGC  CCA  GGC  TCG  GCA  TTT  CTG  GCA  GCA  AGA  TGA
        Ser  Pro  Gly  Ser  Ala  Phe  Leu  Ala  Ala  Arg  Term
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Pisciotta L, Fasano T, Calabresi L, Bellocchio A, Fresa R, Borrini C, Calandra S, Bertolini S. A novel mutation of the apolipoprotein A-I gene in a family with familial combined hyperlipidemia. Atherosclerosis 2008; 198:145-151.

apoA-I_{Sasebo}:

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161           170            180           190           200          208
acgcccatgtggacgcgctgcgcacgcatctggccccctacagcgacgagctgcgccagcgcttggccgcgcgccttgaggctctcaaggagaacggcggcgccagactggccgagtaccacgccaaggccaccgagcatctga
T P M W T R C A R I W P P T A T S C A S A W P R A L R L S R R T A A P D W P S T T P R P P S I End

THLAPYS D E L R Q R L A A R L E A L K E N G G A R L A E Y H A K A T E H L S T L S E K A K   Q End
acgcatctggcccccctacagcgacgagctgcgccagcgcttggccgcgcgccttgaggctctcaaggagaacggcggcgccagactggccgagtaccacgccaaggccaccgagcatctgagcacgctcagcgagaaggccaag···cagtga
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Moriyama K, Sasaki J, Takada Y, Matsunaga A, Fukui J, Albers JJ, Arakawa K. A cysteine-containing truncated apoA-I variant associated with HDL Deficiency. Arterioscler Thromb Vasc Biol 1996; 16: 1416-23.

LCAT deficiency was ruled by the finding of HDL particles other than pre-beta-1 HDL and alpha-4 HDL, and by lack of significant corneal opacification.[5]

apoA-I is a single polypeptide chain composed of 243 amino acids in the mature sequence. It is composed of 4 exons located on chromosome 11q23. It plays an important role as the major protein of HDL and a cofactor for LCAT. Based on the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/all.php) queried on February 15, 2012, the following types of mutations in the APOA-I gene have been reported: 29 missense/nonsense, 1 splicing, 4 regulatory, 13 small deletions, 3 small insertions, 2 gross deletions, 1 gross insertion, and 3 complex mutations. With regard to the insertions, the first of these was reported by Nakata et al[8] in a healthy 10-year-old Japanese girl whose HDL-C was 27 mg/dL and her apoA-1 levels were 76 mg/dL, both <1% percentile for her age and gender-specific Japanese population. She was found to be heterozygous for a single nucleotide insertion of a C in a region of a seven C run in exon 3 between codons 3 and 5, resulting in a premature stop codon at codon 34. The mutation was designated as apoA-I_{Tsukuba} and resulted in a frameshift mutation with the synthesis of an abnormal polypeptide containing only the N terminal 33 amino acids (of 243) of apoA-I amino acid sequence. Her mother and her maternal aunt had similarly low HDL-C and apoA-I levels and were also heterozygous for this mutation (Table 1).

The second insertion mutation was reported in 2007 by Kiss et al[9] and is an insertion of a C in codon 33 in exon 3, resulting in a premature stop with only the N terminal 32 amino acids of apoA-I, and low HDL-C. No other information about the case was provided. The third insertion was reported in 2008 by Pisciotta et al[10] in a 39-year-old man with a positive family history of CHD and an HDL-C value of 28 mg/dL and an apoA-I level of 72 mg/dL, with no corneal opacification or xanthomas. The patient was noted to have a C insertion at codon 17 in exon 3 resulting in a frameshift and a premature stop at position 26, resulting in only the N terminal 25 amino acids being present in the apoA-I sequence (Table 1).

A gross insertion mutation was also reported in 1996 by Moriyama et al[11] in a 50-year-old Japanese woman who presented with xanthomas involving the eyelids, elbows, neck, knees, and Achilles tendons, as well as corneal opacities. She had an HDL-C value of 1.2 mg/dL and an apoA-I

level of 0.8 mg/dL and was found to an apoA-I molecular weight of 24,000 instead of the normal 28,000.[11] She was found be homozygous for a mutation with a partial gene re-duplication encompassing 23 nucleotides leading to a tandem repeat of bases 333 to 355 from the 5′ end of exon 4, causing a frameshift mutation with a premature stop codon resulting in a truncated apoA-I after residue 207 (Table 1). This mutant apoA-I was designated as apoA-I$_{Sasebo}$.

Our case is most similar to the last case except that our patient was heterozygous for a gross insertion in apoA-I of a novel heterozygous in-frame insertion mutation (a duplication of nucleotides 1535 through 1552 inserted at position 1553) causing a new amino acid glycine at codon 157 and a duplication of amino acids alanine, arginine, alanine, histidine, and leucine at codons 158–162 (Fig. 4). This novel apoA-I mutation results in the formation of apoA-I that appears to have abnormal lipid binding properties, resulting in impaired reverse cholesterol transport, probable enhanced clearance, and premature CHD. We have designated this new apoA-I mutation as apoA-I$_{Nashua}$. In homozygous apoA-I deficiency, there is clearly premature CHD, but in heterozygotes it is not as clear and premature CHD probably depends on the presence or absence of other risk factors such as cigarette smoking.

Financial disclosures

References

1. Ai M, Otokozawa S, Asztalos BF, et al. Small dense low density lipoprotein cholesterol and coronary heart disease: results from the Framingham Offspring Study. *Clin Chem.* 2010;56:967–976.

2. Asztalos BF, Cupples LA, Demissie S, Horvath KV, Batista MC, Schaefer EJ. High-density lipoprotein subpopulation profile and coronary heart disease prevalence in male participants in the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol.* 2004;24:2181–2187.

3. Asztalos BF, Schaefer EJ, Horvath KV, et al. Role of LCAT in HDL remodeling: an investigation in LCAT deficiency states. *J Lipid Res.* 2007;48:592–599.

4. Ai M, Otokozawa S, Schaefer EJ, et al. Glycated albumin and direct low density lipoprotein cholesterol levels in type 2 diabetes mellitus. *Clin Chim Acta.* 2009;406:71–74.

5. Schaefer EJ, Santos RD, Asztalos BF. Marked HDL deficiency and premature coronary heart disease. *Curr Opin Lipidol.* 2010;21:289–297.

6. Expert Panel. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *J Am Med Assoc.* 2001;285:2486–2497.

7. Grundy SM, Cleeman JI, Merz CN, et al, Coordinating Committee of the National Cholesterol Education Program. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. *J Am Coll Cardiol.* 2004;44(3): 720–732.

8. Nakata K, Kobayashi K, Yanagi H, et al. Autosomal dominant hypo-alphalipoproteinemia due to A completely defective apolipoprotein A-1 gene. *Biochem Biophys Res Commun.* 1993;196:950–955.

9. Kiss RS, Kavaslar N, Okuhira K, et al. Genetic etiology of isolated low HDL syndrome. *Arterioscler Thromb Vasc Biol.* 2007;27: 1139–1145.

10. Pisciotta L, Fasano T, Calabresi L, et al. A novel mutation of the apolipoprotein A-I gene in a family with familial combined hyperlipidemia. *Atherosclerosis.* 2008;198:145–151.

11. Moriyama K, Sasaki J, Takada Y, et al. A cysteine-containing truncated apoA-I variant associated with HDL Deficiency. *Arterioscler Thromb Vasc Biol.* 1996;16:1416–1423.