Characterization of High Density Lipoprotein Particles in Familial Apolipoprotein A-I Deficiency With Premature Coronary Atherosclerosis, Tuboeruptive and Planar Xanthomas

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Abstract

Our aim was to characterize high density lipoprotein (HDL) subspecies and fat soluble vitamin levels in a kindred with familial apolipoprotein (apo) A-I deficiency. Sequencing of the APOA1 gene revealed a nonsense mutation at codon -2, Q(-2)X, with two documented homozygotes, eight heterozygotes, and two normal subjects in the kindred. Homozygotes presented markedly decreased HDL cholesterol levels, undetectable plasma apo A-1, tubo-eruptive and planar xanthomas, mild corneal arcus and opacification, and severe premature coronary artery disease. In both homozygotes, analysis of HDL particles by two dimensional gel electrophoresis revealed undetectable apo A-I, decreased amounts of small α-3 migrating apo A-II particles, and only modestly decreased normal amounts of slow α migrating apo A-IV and apo E-containing HDL, while in the 8 heterozygotes there was loss of large α-1 HDL particles. There were no significant decreases in plasma fat soluble vitamin levels noted in either homozygotes or heterozygotes, as compared to normal control subjects. Our data indicate that isolated apo A-I deficiency results in marked HDL deficiency with very low apoA-II α-3 HDL particles, modest reductions in the separate and distinct plasma apoA-IV and apo E HDL particles, tubo-eruptive xanthomas, premature coronary atherosclerosis, and no evidence of fat malabsorption.
Introduction

Decreased plasma high density lipoprotein (HDL) cholesterol levels (<40 mg/dl in men and <50 mg/dl in women) have been associated with an increased risk of coronary heart disease (CHD) (1). Marked HDL deficiency states (HDL cholesterol < 5 mg/dl) and undetectable plasma apolipoprotein (apo) A-I levels, have been reported in humans due to mutations at the APOA1/C3/A4 gene locus (2-18). Such patients lack apo A-I containing HDL in plasma, with normal or decreased triglyceride levels, normal low density lipoprotein (LDL) cholesterol levels, and often strikingly premature CHD (2-18). Other patients with marked HDL deficiency have mutations affecting the apo A-I sequence that can affect the activity of lecithin: cholesterol acyl transferase activity (19-26). In this regard they differ from patients with homozygous Tangier disease, due to mutations in the ATP binding cassette A1 gene (ABCA1), who have defective cellular cholesterol efflux, detectable plasma apo A-I in preβ-1 HDL only, hypertriglyceridemia, and decreased LDL cholesterol (27-29). Previously defects involving the APOA1/C3/A4 gene cluster, the contiguous APOA1 and APOC3 genes, and the APOA1 gene in isolation have been described (2-26). Here we report a kindred with isolated apo A-I deficiency, with precise lipoprotein and clinical characterization, characterization of fat soluble vitamin levels, and document differences between this type of apo A-I deficiency and those combined with other apolipoprotein deficiencies in humans. These data provide us with important insights about the function of these apolipoproteins in human health and disease, as well as about HDL particle subspecies.

Methods

Kindred

The index case presented to the Lipid Clinic at the Heart Institute (InCor) of the University of Sao Paulo Hospital, Sao Paulo, Brazil. He was a 39 year-old male with striking tubo-
eruptive xanthomas on his buttocks and lower back, and biopsy of these lesions confirmed lipid laden macrophages. He also had palmar and planar xanthomas as evidenced by yellow creases on his palms and wrist creases, as well as corneal arcus and corneal opacification detected on slit lamp examination. Examination of his retina was normal, as was his neurological examination. On physical examination he had: a blood pressure of 120/80 mmHg, height 1.80 m, weight 94.0 kg, body mass index 29.0 kg/m², and waist circumference 105 cm. He had no evidence of hepatosplenomegaly, or enlarged orange tonsils. On laboratory testing he had normal liver, renal, and thyroid function, and a normal complete blood count. His fasting glucose was 92 mg/dl. His most striking laboratory finding was an HDL cholesterol level of 4 mg/dl. He had no history of chest pain, heart disease, hypertension, diabetes, or cigarette smoking. He was asymptomatic, but on coronary stress testing had evidence of ischemia, and on coronary angiography had a complete obstruction of his right coronary artery and a 90% narrowing of his left anterior descending coronary artery, and underwent successful coronary artery bypass surgery.

A family tree is shown in figure 1. The proband had three children who were healthy at ages 3, 6, and 8 years with HDL cholesterol levels of 14, 21, and 34 mg/dl, respectively. The proband’s 41 year-old brother had previously sustained a myocardial infarction followed by coronary artery bypass graft surgery at the age of 38 years. His brother was also noted to have tubero-eruptive xanthomas, as well as corneal arcus, and his HDL cholesterol was found to be 2 mg/dl. His daughter was in good health at age 12 years, and was presumably a heterozygote, but did not consent for to having her blood drawn. Two other siblings of the index case were examined, and were found not to have xanthomas, with HDL cholesterol levels of 15 and 22 mg/dl, consistent with heterozygosity. They were in good health at ages 33 and 35 years. Their children were in good health at ages 3, 8, and 10 with HDL
cholesterol levels of 21, 45, and 42 mg/dl, respectively. The parents of the index case were alive and well at ages 65 and 66 years, respectively, with HDL cholesterol levels that were both 24 mg/dl. They were first cousins, and their fathers were non-identical twin brothers. Therefore the index case, his homozygous brother, and his two heterozygous siblings were products of a consanguineous marriage. Control subjects matched for the age and gender of affected family members were also selected for study. Informed consent was obtained from all study subjects or their relatives.

Biochemical Measurements

Blood was collected in all subjects after an overnight fast, immediately placed on ice, and plasma was separated in a refrigerated centrifuge. Plasma cholesterol, triglyceride, HDL cholesterol, and calculated LDL cholesterol were assessed by standardized automated enzymatic methods in whole plasma and after precipitation of apo B containing lipoproteins for HDL cholesterol in the clinical chemistry laboratory at the Heart Institute (InCor) of the University of Sao Paulo Hospital. All subjects had been off lipid lowering medication for at least two months at the time of assessment. Plasma levels of the fat soluble vitamins (A as retinol, D as 1,25-dihydroxy vitamin D, and E as α-tocopherol) were measured by high performance liquid chromatography in the Raul Dias dos Santos Laboratory in Sao Paulo.

Samples of plasma which had been stored at -80°C were shipped to the Lipid Metabolism Laboratory at Tufts University in Boston, and the results of the analyses from Sao Paulo were confirmed. This laboratory also performed automated enzymatic lipid analyses, standardized by the Centers of Disease Control Lipid Standardization program as previously described (27-35). Free cholesterol was also assessed in whole plasma and in the HDL fraction. Plasma levels of apo A-I, A-II, B, C-III, and E were measured by immunoassay utilizing kits obtained from Wako, Inc (Richmond, VA), and apo A-I-, A-II-, A-IV-, C-III- and E-containing
HDL subpopulations were assessed by two dimensional gel electrophoresis as previously described (27-35). Absolute plasma concentrations were calculated only for apo A-I-containing particles by multiplying the plasma total apo A-I concentration (mg/dl) by the percentile value of each subpopulation. HDL subpopulations were characterized by charge (pre-β, α, preα) based on their relative mobility to albumin (first dimension); and size determined from molecular weight standards (second dimension, please see figures 2 and 3). Each membrane was first probed for the apolipoprotein of primary interest and percent distributions of the particles were calculated. Subsequently, membranes were re-probed for apo A-I to co-localize each apolipoprotein with apo A-I and for human albumin as a reference point for the α-front. One representative subject of each group was selected for illustration of the various apolipoprotein-containing HDL profiles (figures 3-6). All lipid and apolipoprotein data reported are from this latter laboratory, and are shown in tables 1 and 2. Coefficients of variation for all assays within and between runs were less than 5%.

DNA Sequencing

DNA was isolated from blood cells at InCor, and shipped to Tufts University. An aliquot of DNA from the proband was then sent to Dr. Robert Hegele at the London Regional Genomics Center in Canada for sequencing of the APOA1 gene using genomic DNA. The proband was found to be homozygous for a mutation at APOA1 codon -2, namely Q[-2]X, identical to a mutation in a previously reported Canadian family with apo A-I deficiency (17). This mutation results in the generation of a termination codon, and the lack of any mature apo A-I being expressed in homozygotes. DNA from the proband, his homozygous brother, and all other available family members (n=10) were then submitted for APOA1 gene sequencing to the core sequencing facility of Tufts University School of Medicine. Molecular analysis confirmed the prior results and revealed 2 homozygotes, 8 heterozygotes, and 2
normal subjects, the latter of whom were the offspring of a heterozygote and had HDL cholesterol levels of 42 mg/dl and 45 mg/dl, respectively. These two subjects were included in the control group in subsequent analysis. APOE genotyping and APOE gene sequencing was suggested by Dr. Jean Davignon of Montreal, because of the proband’s striking planar xanthomas. APO E genotyping in all family members was either the E3/3 or E4/3 genotype; moreover the proband’s APOE gene was sequenced in the core facility at Tufts University, and was found to be normal.

Data Analysis

Mean values and standard deviations were calculated for all study groups. Data obtained from heterozygotes were compared to data from controls using ANOVA analyses. A two tailed p value of <0.05 was considered as statistically significant.

Results

Table 1 shows data on plasma lipids, lipoprotein cholesterol, and apolipoproteins in controls (n=10), heterozygotes (n=8) and homozygotes (n=2) for this kindred with familial apo A-I deficiency. Homozygotes (n=2) had mean values of HDL-C, apo A-I, apo A-II, and apo E that were 6.7%, 0%, 28.9%, and 55.9% of normal, respectively, and free cholesterol represented 30.2% of total cholesterol, and about the same percentage of HDL cholesterol as free cholesterol, ruling out lecithin:cholesterol acyltransferase (LCAT) deficiency. Heterozygotes had HDL cholesterol, apo A-I, and apo A-II values that were 42.6%, 46.4%, and 68.4% of control values (all p<0.05), with relatively normal amounts of apo B, C-III, and E in plasma.

A schematic diagram of 2-dimensional electrophoresis of normal apoA-I containing HDL particles is shown in figure 2, and control values are provided in Table 2. These data are expressed both in terms of the concentration of apo A-I in the various HDL subspecies, as
well as in terms of percentages of total plasma apo A-I. In normals ~12 mg/dl of apo A-I (~10% of the total) is found in two small, discoidal preβ-1 HDL particles, ~22 mg/dl or (~20% of the total) is found in either large, spherical α-1 HDL or in the adjacent large, spherical preα-1 HDL (please see figures 2 and 3). All these HDL particles contain apo A-I without A-II. In contrast intermediate sized spherical α-2 and α-3 HDL contain both apo A-I and A-II, and have a combined apo A-I concentration of ~60 mg/dl or ~50% of the total plasma apo A-I and ~100% of apo A-II in normal plasma (please see figures 2 – 4). Serum amyloid A protein can also be found in α-2 HDL. Adjacent to α-2 and α-3 HDL are the intermediate spherical preα-2 and preα-3 HDL, which contain apo A-I without A-II, and together have an apo A-I concentration of ~10 mg/dl or ~8% of total plasma apo A-I. The smallest α migrating HDL particles are α-4 HDL and the adjacent preα-4 HDL, which both contain apo A-I without A-II, and are discoidal particles, which together have an apo A-I concentration of about 2 mg/dl, and represent about 1.5% of total plasma apo A-I. Finally there are three large preβ-2 HDL particles which altogether have an apo A-I concentration of about 2 mg/dl or approximately 1.5% of the total. These particles do not contain apo A-II (see figures 2 – 4).

Table 2 also summarizes data on apo A-I-containing HDL subpopulations in heterozygous subjects. Homozygotes had undetectable levels of apo A-I-containing HDL, as shown in figure 3. They had HDL particles in the alpha-3 region containing apoA-II, but no apo A-I (figures 3 and 4), as well as relatively normal amounts of the separate apo A-IV- and E-containing HDL particles, which have slow α mobility (figures 5 and 6). Apo C-III in the homozygotes was entirely in the free form, and not associated with other apolipoproteins (data not shown). Heterozygotes had markedly decreased mean apo A-I concentration in large α-1 HDL (19.6% of normal), some decrease in α-2 and α-3 HDL (61.8% and 71.3% of normal), and a more marked decrease in α-4 HDL (30.4% of normal). They also had preβ-1
HDL values that were 51.6% of normal, but their preβ-2 HDL levels were 2.24 fold higher than normal. Gel photographs of apo A-I-, A-II-, A-IV-, and E-containing HDL in heterozygotes are shown in figures 3-6. As previously mentioned heterozygotes had marked decreases in α-1 and α-4 HDL, and a marked increases in preβ-2 HDL, with a relatively normal distribution and amount of apo A-II-, A-IV- and E-containing HDL. Both heterozygotes and homozygotes had reductions in the amount of apoA-IV and apo E staining in HDL by about 50% as compared to controls (see figures 5 and 6).

Levels of fat soluble vitamins were generally normal in this kindred in both homozygotes and heterozygotes with mean values (standard deviation) being in the normal range for retinol at 41.3 (15.3) mcg/ml (normal, 30-80 mcg/ml); 1,25 hydroxy vitamin D at 36.7 (46.4) pg/ml (normal,16-60 pg/ml), and α-tocopherol at 0.7 (0.4) mg/dl (normal, 0.5-1.8 mg/dl). In the two homozygotes, retinol was 33.9 and 37.6 mcg/ml, 1,25 dihydroxy vitamin D was 19.4 and 30.2 pg/ml and α-tocopherol was 0.4 and 1.1 mg/dl. Only α- tocopherol was somewhat below the normal range in one homozygote at 0.4 mg/dl.

Discussion

Three forms of familial apo A-I deficiency have been recognized, lack of apo A-I, lack of apo A-I and apo C-III, and lack of apo A-I, C-III, and A-IV. Schaefer and colleagues in January 1982 described one homozygote and multiple heterozygotes in a kindred of English origin, residing in northern Alabama, United States (2). The index case had no xanthomas, marked HDL deficiency, low triglyceride, normal LDL cholesterol levels, and severe premature coronary artery disease. She had no history of diabetes, smoking, or hypertension, and was pre-menopausal. She died at the time of bypass surgery at age 43 years (2-6). At autopsy severe diffuse coronary atherosclerosis was documented (2-4). The defect in this kindred was subsequently found be a large deletion of the entire APOA1/C3/A4 gene cluster.
Decreased plasma levels of the fat soluble vitamins A, D, and E (<50% of normal) and a moderately prolonged prothrombin time, consistent with malabsorption of fat and fat-soluble vitamins in the homozygote were noted (5). Heterozygotes were found to have plasma HDL cholesterol, apo A-I, apo C-III, and apo A-IV levels that were about 50% of normal (5). Apo A-I gene transfection studies indicated that apo A-I was essential for HDL formation, similar to what was noted in this initial kindred (6). With molecular characterization, the family was denoted as having familial APOA1/C3/A4 deficiency (5).

In June of 1982 a second kindred with apo A-I deficiency was described by Norum and colleagues in two sisters with marked HDL deficiency and planar xanthomas. They had premature CHD and underwent coronary artery bypass grafting surgery at ages 29 and 30 years (7). They had no history of smoking, hypertension, or diabetes, and no fat malabsorption was reported. Their triglyceride levels were reduced, and their LDL cholesterol levels were normal. The genetic defect was subsequently found to be a DNA rearrangement affecting the adjacent APOA1 and APOC3 genes, resulting in a lack of production of these two apolipoproteins, and their absence from plasma (8,9). It was also subsequently reported that these homozygotes had small amounts of apo A-II-containing HDL, and enhanced clearance of very low density lipoprotein apo B, presumably because there was no apo C-III present in plasma to inhibit lipolysis (10-14). This kindred was decribed as having familial APOA1/C3 deficiency. A second kindred with premature CHD, marked HDL deficiency, and absence of apo A-I and C-III in plasma has also been described (15).

Since 1991 ten kindreds with isolated apo A-I deficiency have been described (16-26). However, only the homozygous probands in the two kindreds described by Matsunaga et al in 1991 (codon 84 nonsense mutation) and Ng et al in 1994 (nonsense mutation at codon -2), had undetectable plasma apo A-I levels, marked HDL deficiency, and premature CHD (16).
In the Matsunaga kindred from Japan the female proband had normal triglyceride and LDL cholesterol levels, and yellow-orange planar xanthomas. The APOA1 Q[-2]X mutation described in this report, was first reported by Ng et al in a Canadian kindred of mixed European ancestry, including Portuguese ancestry, living in Toronto (17,18). The index case was a 34 year old female who presented with marked HDL deficiency, mildly thickened Achilles tendons, xanthelasmas, mild midline cerebellar ataxia, and asymmetric bilateral neurosensory hearing loss. She also had bilateral cataracts, and bilateral sub-retinal lipid deposition with exudative proliferative retinopathy, with resultant bilateral retinal detachments requiring surgical repair. Her apo A-I levels were undetectable and her HDL cholesterol was 2 mg/dl, her triglycerides and LDL cholesterol levels were elevated. Four other homozygotes from this pedigree also had marked HDL deficiency (mean 4 mg/dl), normal triglycerides (mean 123 mg/dl) and elevated LDL cholesterol (mean 175 mg/dl). One homozygous sister at age 38, had xanthelasma, Achilles tendon xanthomas, planar xanthomas in the web spaces of the hands and the cubital and popliteal fossae. She had sustained a myocardial infarction at age 34 years, and had coronary artery bypass grafting surgery at age 37. A second homozygous sister had angina and documented reversible myocardial ischemia on stress testing, as well as cerebellar ataxia. The two other homozygotes at ages 26 and 28, as well as four heterozygotes (ages 14-39) were asymptomatic, and had no evidence of CHD, neuropathy, or visual impairment. In the discussion the authors concluded that the combined hyperlipidemia in that kindred was probably not related to the APOA1 gene mutation. The findings in the present kindred would tend to support this speculation, since we observed neither combined hyperlipidemia nor Achilles tendon xanthomas. While the Canadian kindred with APOA1 Q[-2]X did not have independently segregating classical familial
hypercholesterolemia (FH), the authors speculated that some other unmeasured defect of cholesterol metabolism resulted in the elevated total and LDL cholesterol.

Other apo A-I deficient cases reported had apo A-I present in their plasma, had evidence of LCAT deficiency, no evidence of premature CHD or xanthomas, and had corneal opacification (19-25). Therefore LCAT deficiency can occur when there are mutations in the LCAT gene or in the apo A-I gene causing the formation of abnormal apo A-I, which does not allow for the normal activation of LCAT, and interferes with the activation of LCAT by other apolipoproteins.

In our APOA1 Q[-2]X kindred, in contrast to the Canadian kindred, there was no evidence of Achilles tendon xanthomas. Moreover we did not note the combined hyperlipidemia, ataxia, cataracts or proliferative retinopathy in our kindred in contrast to the kindred reported by Ng and colleagues (17,18). That pedigree, as well as our own, had consanguinity documented, raising the possibility of other homozygous mutations being present, contributing to retinal disease, ataxia, combined hyperlipidemia, and tendinous xanthomas. The common features of the two kindreds are the APOA1 Q[-2]X mutation itself, marked HDL deficiency, planar xanthomas, and premature CHD. Members of our kindred had striking tubo-eruptive xanthomas that were not observed in the kindred described by Ng and colleagues. In addition we searched for APOE deficiency, APOE mutations and E2/2 homozygosity, but did not find these features.

These data indicate that apo A-I is essential for normal HDL formation, and its absence results in severe HDL deficiency, xanthomas, and premature CHD. The additional presence of apo C-III deficiency as observed in APOA1/C3 deficiency results in the same phenotype except for the presence of very low triglyceride levels, consistent with the concept that apo C-III can impair lipolysis, thus its absence is associated with lower triglyceride concentrations.
The more complex and truly polygenic APOA1/C3/A4 deficiency results in the same phenotype, except that there are no xanthomas, while fat malabsorption is present, consistent with the concept that apo A-IV plays a role in the intestinal absorption of fat and fat-soluble vitamins.

Research on HDL particle metabolism and function indicates that coronary heart disease patients have increases in preβ-1 and small α-4 and α-3 HDL and decreases in larger α-2 and α-1 HDL (30-32). Moreover it is mainly the preβ-1 HDL that picks up free cholesterol from cells via ABCA1, while it is mainly the large α-2 and α-1 HDL that interacts with SRB1 in hepatocytes and other cells to promote bi-directional cholesterol flux (33).

Studies in this kindred with apo A-I deficiency and in kindreds with Tangier disease indicate that the production of apo A-I is critical for HDL formation and the existence of preβ-1 HDL, while the presence of normal ABCA1 function and the addition of free cholesterol and phospholipids is critical for the conversion of this particle to small discoidal α-4 HDL (27-29).

Studies in patients with familial LCAT deficiency indicate that cholesterol esterification via LCAT is critical for the maturation of α-4 HDL to larger spherical HDL particles, and the presence of CETP is critical for the formation of normal spherical α-1 HDL that contain apo A-I, but no apo A-II (34,35). Surprising human subjects lacking plasma apo A-II have no HDL levels and no evidence of premature CHD (36). In our studies in patients with homozygous CETP deficiency and lack of CHD, we noted the presence of very large spherical α migrating HDL which contain apo A-I, apo A-II, and apo E (35). It is precisely these type of particles that we have observed in C57BL6 mice, which naturally lack CETP (B. Asztalos, unpublished observations). Mice may not therefore be an ideal model for human lipoprotein metabolism, and apo A-I knockout mice do not have increased atherosclerosis unless they also lack the LDL receptor (37-40).
A critical element of our data is the clear documentation of slow α migrating HDL particles containing only apo E and only apo A-IV, which exist in normal plasma as well as in the plasma of patients lacking apo A-I. These particles have a distinct metabolism and probably distinct functions separate from apo A-I containing HDL which require further elucidation. Apo A-I is known to activate LCAT, but other apolipoproteins can also do this, and therefore in this kindred there is no evidence of LCAT deficiency. In contrast kindreds with apo A-I mutations affecting LCAT activity have been well described, and while they have HDL deficiency, apo A-I is present in their plasma usually in excess of 10 mg/dl and they do not appear to have premature CHD (19,21,22,23,25). Apo A-IV in HDL is clearly on its own HDL particle and its residence time in plasma is about 3 days, considerably different from that of HDL apo A-I, which is about 5 days, and HDL apo A-II which is about 5.5 days (41,42). Moreover apo A-IV, like apo A-I, is a significant protein component of intestinal apo B-48 containing triglyceride-rich lipoproteins (41). Apo A-IV appears to play a role in fat absorption, and hence its absence there is a modest degree of malabsorption of fat and fat soluble vitamins, not observed in this kindred with isolated apo A-I deficiency. Both apo A-I and apo A-IV are proteins are transferred from TRL to HDL during TRL lipolysis, then reassociate with newly formed TRL in the extra-plasma space, and they can recycle multiple time in this manner, similar to the C and E apolipoproteins (42-45). Apo E also has its own HDL particle, and its residence time within HDL is substantially lower than that of other apolipoproteins in HDL at about 1 day (46,47). Only apo B-100 and apo A-I have production rates into the plasma space in humans in excess of 10 mg/kg/day, while apo E has a plasma production rate in humans of about 3.5 mg/kg/day, and a somewhat lower HDL apo E production or transport rate. Apo E in TRL clearly plays a critical role in the fractional clearance of apo B containing particles of both liver and intestinal origin, since it is absence there is a marked
increase in remnant apo B-100 and apo B-48 particles with markedly delayed clearance, but HDL levels are normal (48). Apo E HDL could clearly play an important role in reverse cholesterol transport, since it can bind to hepatic LDL receptors. In humans, familial apo A-I deficiency results in much more severe and premature atherosclerosis than familial apo E deficiency, while in mice the converse is true, underscoring the concept that the best model for human lipoprotein metabolism remains the human, and not the mouse.

The overall data in the present kindred indicate that isolated familial apo A-I deficiency results in marked HDL deficiency, xanthomas, premature CHD, and no evidence of fat or fat soluble vitamin malabsorption. Moreover the evidence indicates that in the absence of apo A-I, small amounts of A-II particles can be found in the α-migrating region of HDL at the α-3 position, and levels of apo A-IV- and E-containing HDL are only moderately reduced, and have normal electrophoretic mobility and particle size. The data also indicate that there are at least three separate and distinct type of HDL that exist in normal plasma: 1) multiple α, pre α, and preβ-1 particles containing apo A-I (the predominant HDL type), 2) two small and intermediate sized slow alpha migrating particles containing mainly apo A-IV (a relatively minor HDL type), and 3) large slow α migrating HDL containing mainly apo E (also a relatively minor HDL type). These latter two types of HDL are only moderately reduced in homozygous familial apoA-I deficiency and their role in health and disease requires further exploration.
References


Figure legends:

Figure 1: The family tree is shown with two homozygotes (in black), two heterozygous siblings and their four heterozygous offspring (shaded), and two normal offspring, as well as their two heterozygous parents (shaded), who were first cousins (their fathers were non-identical twins). The designation of normals, heterozygotes, and homozygotes was confirmed by direct sequencing of the apoA-I gene in all individuals, except the presumably heterozygous daughter of one of the homozygotes who did not consent to having blood drawn.

Figure 2: Schematic representation of apoA-I-containing HDL subpopulations in human plasma separated by non-denaturing agarose-polyacrylamide gel electrophoresis. The nomenclature is based on HDL particle separation by electrophoretic charge relative to albumin (preβ, α, and preα) in the first dimension, and by size relative to the molecular weight standards in the second dimension.

Figure 3: Distribution of apoA-I containing HDL subpopulations of a homozygote (left), a heterozygote (middle) and a normal control subject (right) separated by 2-dimensional, non-denaturing agarose-PAGE. Subpopulations were characterized by charge (pre-β, α, preα) based on their relative mobility to albumin (first dimension); and size determined from molecular weight standards (Pharmacia high molecular weight standard proteins [7.1 nm-17.0 nm] supplemented with LDH 4.66 nm) (second dimension). The asterisk indicates the endogenous human serum albumin marking the α-mobility front. The images indicate undetectable apoA-I containing HDL in the homozygote and decreases in large α-1 HDL in heterozygote. Images were virtually identical in both homozygotes, and very similar in heterozygotes.
Figure 4: ApoA-II-containing HDL subpopulations of a homozygote, a heterozygote, and a representative control subject, showing decreased amounts of apoA-II in the α-3 position only, whereas in the heterozygote and the control subjects apoA-II is found in both the α-3 alpha 3 and the α-4 position. Images were virtually identical in both homozygotes, and very similar heterozygotes and controls.

Figure 5: ApoA-IV-containing HDL subpopulations of a homozygote, a heterozygote, and a control subject, showing normal amounts and distribution of apoA-IV HDL in the slowly migrating α position in all three subjects. Images were virtually identical in both homozygotes, and very similar in heterozygotes and controls.

Figure 6: Apo E-containing HDL subpopulations of a homozygote, a heterozygote, and a control subject, showing relatively normal amounts and distribution of apo E HDL in the slowly migrating α position in all three subjects. Please note that apo E HDL particles are larger than apoA-IV HDL particles. Images were virtually identical in both homozygotes, and very similar in heterozygotes and controls.
Table 1: Characteristics of Study Participants.

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<td>7.6 ± 3.6</td>
<td>4.1 ± 1.2</td>
<td>4.7, 3.8</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

* Significantly different (p<0.05) from controls.
Table 2: Apo-A-I Concentrations in HDL Subpopulations (mg/dl)*

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>Controls (n=10)</th>
<th>Heterozygotes (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preβ-1</td>
<td>12.2 ± 3.2 (9.9%)</td>
<td>6.3 ± 2.0** (9.8%)</td>
</tr>
<tr>
<td>Preβ-2</td>
<td>1.7 ± 0.9 (1.4%)</td>
<td>3.8 ± 1.5** (6.0%)**</td>
</tr>
<tr>
<td>α-1</td>
<td>16.8 ± 8.9 (13.6%)</td>
<td>3.3 ± 2.4** (4.8%)**</td>
</tr>
<tr>
<td>α-2</td>
<td>39.3 ± 9.7 (31.8%)</td>
<td>24.3 ± 6.2** (37.1%)</td>
</tr>
<tr>
<td>α-3</td>
<td>24.0 ± 5.7 (19.4%)</td>
<td>17.1 ± 3.5** (26.5%)</td>
</tr>
<tr>
<td>α-4</td>
<td>13.5 ± 3.6 (10.9%)</td>
<td>4.1 ± 1.2** (6.4%)</td>
</tr>
<tr>
<td>Preα-1</td>
<td>5.3 ± 3.4 (4.3%)</td>
<td>0.3 ± 0.4** (0.5%)**</td>
</tr>
<tr>
<td>Preα-2</td>
<td>6.2 ± 2.4 (5.0%)</td>
<td>2.8 ± 0.6** (4.3%)</td>
</tr>
<tr>
<td>Preα-3</td>
<td>3.5 ± 1.4 (2.8%)</td>
<td>2.0 ± 0.7** (3.1%)</td>
</tr>
<tr>
<td>Preα-4</td>
<td>1.0 ± 0.4 (0.8%)</td>
<td>0.8 ± 0.3 (1.2%)</td>
</tr>
</tbody>
</table>

*Data are mean (mg/dl) ± standard deviations, with the percentage of the total value in parentheses; **significantly different (p<0.05) from controls. No values are reported for homozygotes because of undetectable plasma apoA-I levels.
Figure 2
Figure 4
Figure 6