FAMILIAL DISORDERS OF LOW HDL

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Internal Medicine Grand Rounds

March 23, 1989

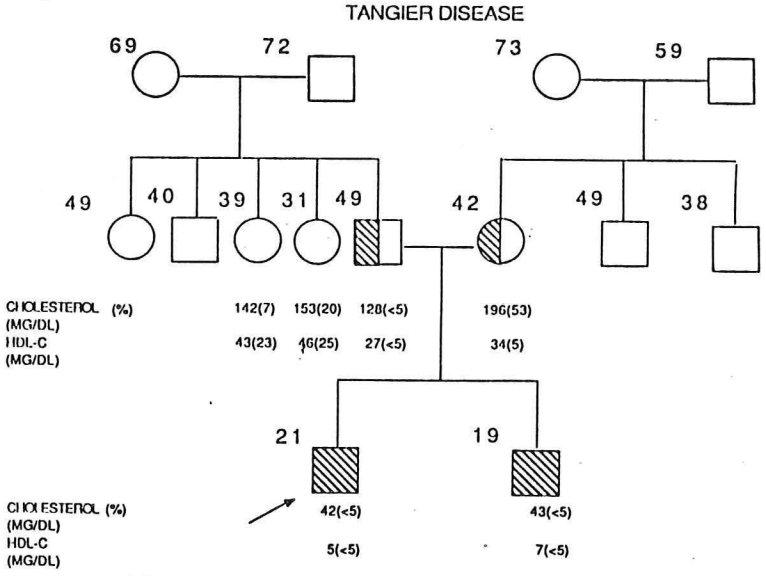
Case History

T.M. is an 19 year old white man referred for evaluation of a 6 month history of lower extremity weakness associated with a plasma cholesterol of 64 mg%. The patient had been well until 6 months prior to presentation when he noted weakness in his left leg. He was seen by a neurologist who found evidence of weakness in both lower extremities (L>R). He had absent ankle and knee jerks bilaterally. On EMG studies there was evidence of motor neuron disease with denervation and renervation. Lumbar puncture, myelogram, and CAT scan of his head and spine were all unrevealing. He was noted to have a plasma cholesterol of 64 mg% and was referred here. The patient denied any history of paresthesias or loss of sensation in any of his extremities. He denied any history of gastrointestinal or visual problems. His past medical history was unremarkable excluding a history of acne for which he was given acutane for 6 months 1 year prior to presentation. He had a history of a tonsillectomy which was done in Columbia, South America at age 5.

On physical exam he was a thin, pleasant, well-developed, well-nourished, white man in no acute distress. His vital signs were unremarkable and his physical exam was remarkable for the presence of yellow-orange tonsillar tags. He had no corneal opacifications, lymphadenopathy, hepatosplenomegaly or skin lesions. On neurological exam, his cranial nerves were all tested and intact. There were no sensory deficits. He had 3+/4+ strength in his left deltoid and biceps and 4+/4+ strength in his left triceps and hand flexors and right upper extremity. In his lower extremities he had 3+/4+ strength in all muscle groups of left lower extremities and right foot. His reflexes were absent in his lower extremities, trace in his left upper extremity and 1+ in his upper right extremity. The rest of his neurological exam was unremarkable.

Laboratory evaluation including SMA12, CBC, TFT's, and urinalysis was within normal limits excluding a slightly depressed platelet count of 120,000 and a slightly elevated alkaline phosphatase of 142mg/dl. Heavy metal screen on a 24 hour urine was negative. His total cholesterol was 42 (<5%) with a triglyceride of 92 (77%), VLDL-C of 18 (78%), LDL-C of 16 (<5%), and HDL-C of 5 (<5%).

The diagnosis of Tangier disease was made and a pedigree of his family is shown below.



Introduction

Several epidemiological studies have demonstrated that low levels of high density lipoproteins (HDL) are associated with increased risk for the development of coronary artery disease. However, it remains unclear if low HDL-cholesterol (HDL-C) is a primary risk factor in atherosclerosis or an epiphenomomen. Today I would like to briefly review normal HDL metabolism and discuss several familial disorders in which HDL metabolism is clearly disrupted with the goal of providing some insights into the relationship of HDL levels with atherosclerosis.

The inverse correlation between levels of HDL-C and coronary artery disease has been noted repeatedly over the past forty years (1-4). The significance of this association has been difficult to estimate for a variety of reasons. Many of the known cardiac risk factors lower HDL-C, including smoking (5), diabetes (6), and obesity (7). Another risk factor, hypertension, does not significantly modify HDL-C levels but many antihypertensive medications used in its treatment lower HDL-C (8). It is thus difficult to assess the independent effect of HDL levels on the development of atherosclerosis. Examination of Mendelian disorders of HDL metabolism provide the opportunity to identify single protein defects and assess their clinical effects. If low HDL-C is a primary risk factor for the development of cardiovascular disease, it would be expected that low HDL levels would segregate with ischemic heart disease in families with a genetic form of hypoalphalipoproteinemia. I will focus on the following disorders which are all associated with very low levels of HDL: 1) Tangier Disease, 2) Apoprotein A-I/C-III deficiency, 3) Apoprotein A-I Milano, 4) Fish Eye Disease, and 5) Familial Hypoalphalipoproteinemia. I will also briefly discuss Apo A-I variants, Diffuse Planar Xanthomas, and LCAT deficiency. For each disease, emphasis will be placed on the effects of low HDL levels on lipoprotein metabolism in an effort to better understand the various clinical manifestations.

HDL Metabolism

A brief review of the normal metabolism of HDL lipoproteins will be presented prior to a discussion of the familial disorders. The plasma lipoproteins have been classified according to their density and the HDL lipoproteins have a density between 1.063 and 1.210 gm/ml. On agarose gel electrophoresis, HDL particles migrate with alpha mobility due to their small size (9-12 nm) and thus are referred to as the alpha-lipoproteins (Fig 1.). In general, HDL particles have the following composition: 50% protein, 25% phospholipids, 20% cholesterol, and 5% triglyceride (Figure 2). The core of the HDL particle is comprised of cholesterol esters, triglyceride and the fatty acids of phospholipids, while the surface is made up of free cholesterol, apoproteins and the polar head groups of phospholipids. The major protein moieties in HDL

are apoprotein A-I (apo A-I), and apoprotein A-II (apo A-II) which comprise 65% and 30% of the total protein mass of HDL, respectively. The ratio of apo A-I to apo A-II is higher in HDL_2 than HDL_3 (9). The minor components of HDL are Apo A-IV, B, C-1, C-II, C-III, D, E, and F, which vary in amount in the different subfractions.

Figure 1.
Agarose Gel Electrophoresis

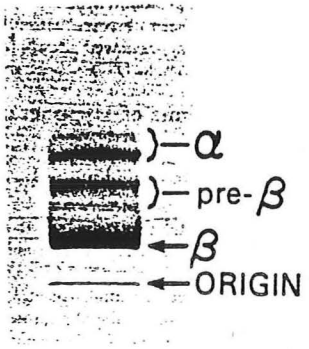
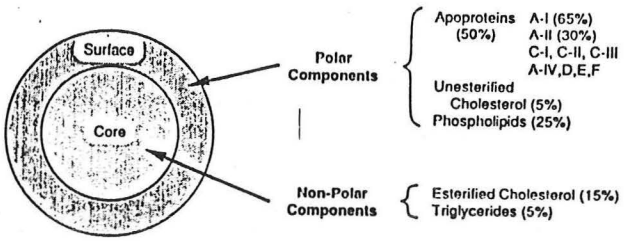


Figure 2
General Structure of HDL



The HDL lipoproteins are subclassified into three types of particles that differ in size, protein content, and lipid composition; they are HDL_1 (d=1.05-1.063 g/ml), HDL_2 (d=1.063-1.125 g/ml) and HDL_3 (d=1.125-1.210 g/ml). The predominant HDL species found in man are HDL_2 and HDL_3, and only between 5-15% of HDL protein is in HDL_1. HDL_2 is made up of 40% protein and 60% lipid and HDL_3, a denser particle, is 55% protein and 45% lipid (Fig. 3). The difference in HDL levels between individuals is almost exclusively due to different amounts of HDL_2 (10). In Figure 4 the normal ranges and mean levels of total HDL-C in men and women are shown (11). Beginning in adolescence, women have higher levels of HDL-C than men due to higher levels of HDL_2 (12).

Figure 3

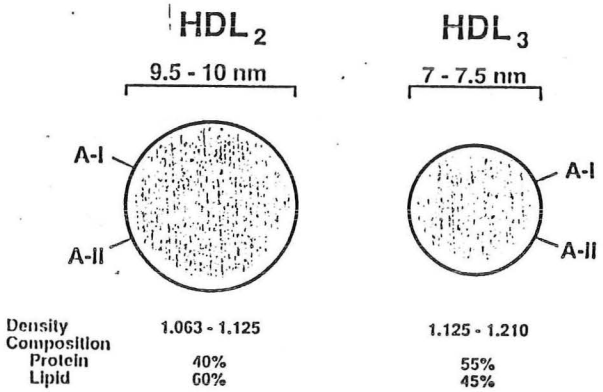


Figure 4

Normal Range of HDL-C

Age, Years	WOMEN Mean	WOMEN 5th to 95th Percentile	MEN Mean	MEN 5th to 95th Percentile
<10	55	33-75	50	40-75
10-19	50	35-75	50	35-75
20-29	55	35-80	45	30-65
30-39	55	35-80	45	30-65
40-49	50	35-90	45	30-65
50-59	60	35-95	45	30-65
60-69	65	35-95	50	30-80
70+	60	35-95	50	30-80

Apo A-I is a protein of known amino acid sequence that is synthesized in the liver and intestines and is secreted as part of chylomicrons and VLDL (13). Soon after it is secreted, Apo A-I becomes associated with HDL particles. Apo A-I is a potent activator of lecithin:cholesterol acyltransferase (LCAT)(14), a plasma enzyme that catalyzes the transfer of an unsaturated fatty acids from the sn-2 position of phosphotidylcholine to the hydroxyl group of unesterified cholesterol to form cholesterol esters (Fig. 5). LCAT activity can be found in all the major lipoprotein but in normal individuals it is preferentially associated with HDL (15). There is a rare autosomal recessive disorder, LCAT deficiency, in which this enzyme is defective or absent (16).

LCAT and apo A-I play crucial roles in the formation of the HDL particle. Nascent HDL particles are synthesized in the liver and small intestines and they have been visualized in liver (17) and intestinal (18) perfusates of various animals. The particles are comprised largely of phospholipids and free cholesterol and have a bilaminar disc-like appearance. They have a different protein composition than mature HDL, and contain only small amounts of apo A-I and apoproteins of the C series. Apoprotein E is the major apoprotein associated with the particles secreted by the liver (17). These nascent HDL particles are not normally seen in plasma because there is a rapid change in their composition and configuration after being secreted. Apo A-I and A-II are transferred (probably from chylomicrons) to the disc-like particles (19) and LCAT is activated. The free cholesterol on the surface of the particle is esterified. The cholesterol esters produced form an apolar core and the particle becomes spherical (Fig. 6). In patients with LCAT Deficiency who have little or no LCAT activity these disc-like particles remain in the plasma without undergoing spherical transformation (20).

Figure 5

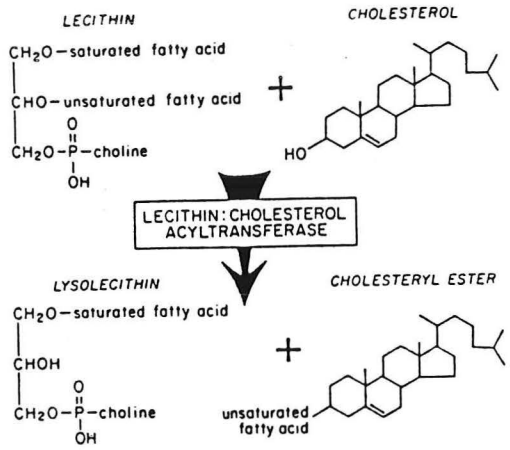
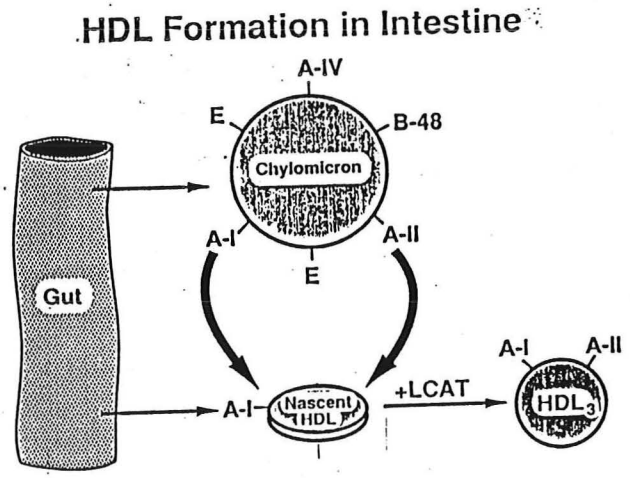


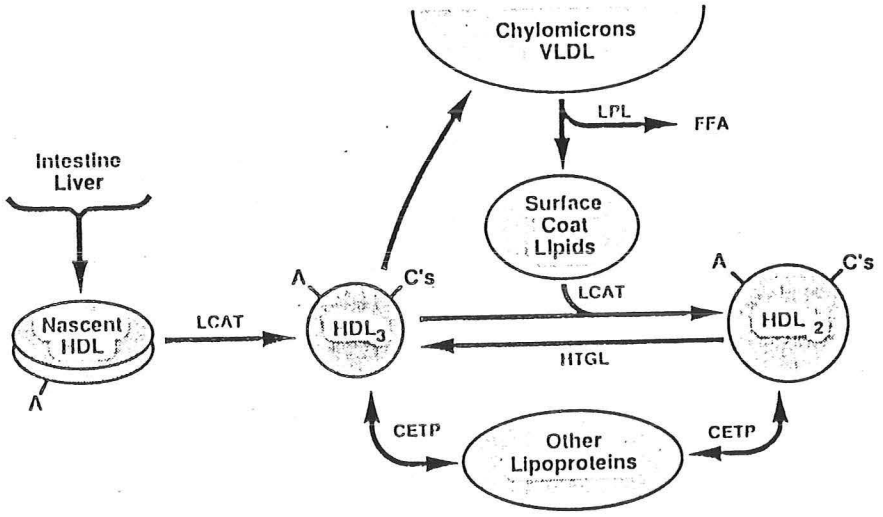
Figure 6



Once the mature, spherical, HDL particle is formed, it exchanges both apoproteins and lipid components with other classes of lipoproteins (as well as cells), making the exact fate of HDL particle difficult to pinpoint. One clear role of HDL is in the lipolysis of the triglyceride-rich lipoproteins, VLDL and chylomicrons, which are produced by the liver and intestines, respectively. Soon after these triglyceride-rich lipoproteins are secreted they acquire apoproteins from HDL, including apo C-I, C-II and C-III (21) (Fig. 7). In order for lipoprotein lipase to hydrolyze the triglycerides of chylomicrons, apo C-II, a potent activator of lipoprotein lipase (22), must be present. There is an autosomal recessive disorder in which apo C-II is absent called Apo C-II Deficiency, and in this disorder chylomicrons accumulate in the plasma (23). When the triglyceride-rich lipoproteins circulate through the capillaries, especially of adipose tissue and muscle, the triglycerides are hydrolyzed by lipoprotein lipase (Fig. 7). Lipoprotein lipase is made in adjacent adipocytes and muscle cells, and secreted from the cell. It diffuses across the vascular endothelium and attaches to proteoglycan "trees" that extend from the capillary endothelium into the lumen. When chylomicrons and VLDL interact with lipoprotein lipase, the triglycerides are hydrolyzed and glycerol and fatty acids are released. During lipolysis phospholipids, free cholesterol, triglyceride, and apoproteins of the C series are transferred to the smaller and denser HDL particles, HDL_3. The HDL_3 particle is thought to be transformed into a larger and lighter particle called HDL_2. Supportive of this notion is the finding that if VLDL, HDL_3, and lipoprotein lipase are co-incubated in vitro, HDL_2 is produced (24). Also, during lipolysis surface components of chylomicrons and VLDL can form nascent HDL particles but how much these contribute to the HDL mass in vivo has not been determined (25).

Figure 7

HDL Metabolism in Plasma



The free cholesterol transferred to the HDL particle is rapidly esterified by LCAT to form cholesterol ester. The cholesterol ester can then be exchanged for triglyceride with the other lipoproteins (see Fig. 7). This exchange is facilitated by a protein called cholesterol ester transfer protein (CETP)(26). The net effect of the exchange is to transfer cholesterol esters from HDL to the apo E and B containing lipoprotein particles. Interestingly, most animals do not have cholesterol ester exchange activity and carry most of their cholesterol in HDL, as do neonatal humans. It has been proposed that the transfer of cholesterol esters from HDL to VLDL, IDL, and LDL in humans contributes to their increased susceptibility to the development of atherosclerosis (26).

After interacting with lipoprotein lipase and donating surface lipids and apoproteins of the C series to HDL, chylomicron remnants are rapidly taken up by the liver (Fig. 8). This uptake is only possible after the apo C's leave these particles since they inhibit hepatic uptake. VLDL remnants continue to exchange lipids with HDL and become increasingly cholesterol ester-rich and triglyceride-poor and are termed intermediate density lipoproteins (IDL). The IDL particles are either cleared by the liver or further metabolized by another lipase, hepatic triglyceride lipase located on the vascular endothelium of the liver (27). This enzyme has both triglyceride hydrolase and phospholipase activity. IDL loses its triglycerides, phospholipids, and apo E, upon interaction with hepatic triglyceride lipase and matures into LDL. Hepatic triglyceride lipase also removes phospholipids and triglycerides from the HDL_2, forming HDL_3 (see Fig. 9). The importance of hepatic triglyceride lipase in the maturation of IDL and the regeneration of HDL_3 is supported by the observed effects of its absence. In Hepatic Lipase deficiency, an autosomal recessive disorder in which there is no hepatic lipase activity, both IDL and HDL_2 particles accumulate in the plasma (28).

Figure 8

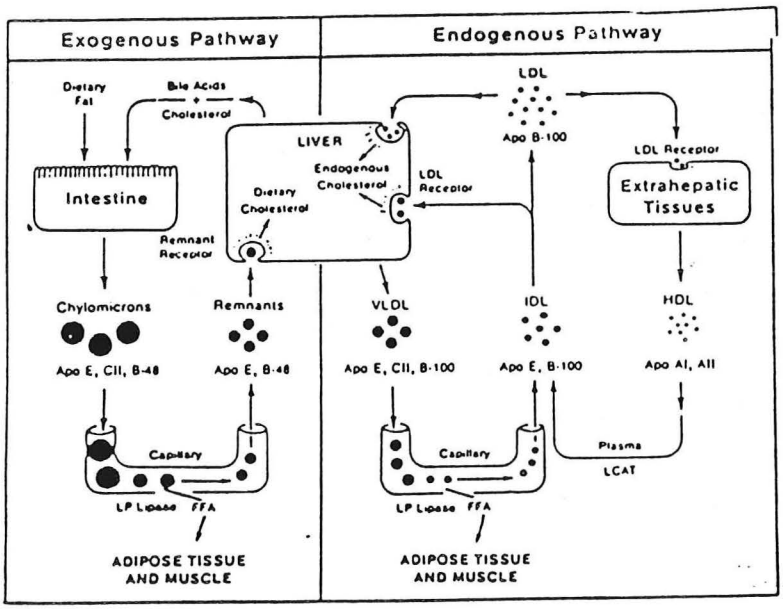
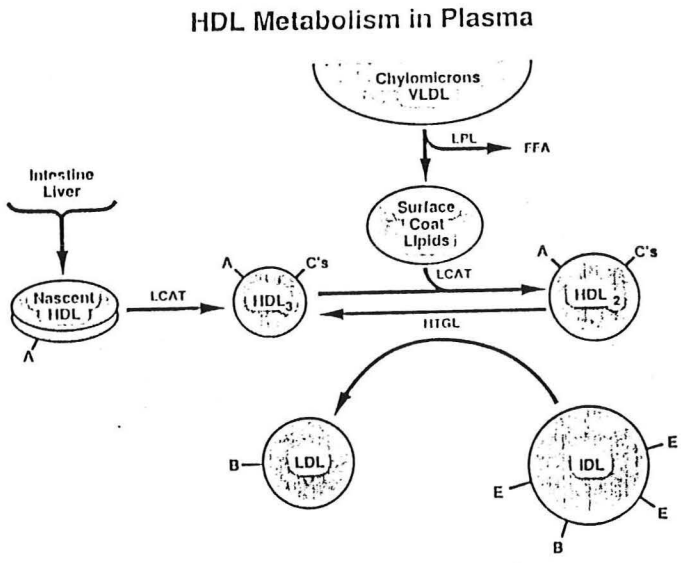


Figure 9



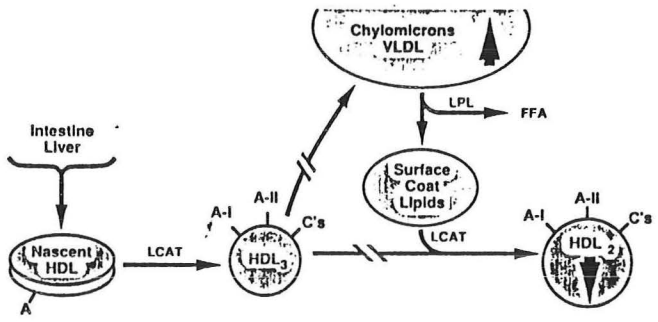
Numerous studies have demonstrated an inverse correlation between VLDL and HDL-C (29). Many factors that increase VLDL lower the level of HDL such as obesity (7), diabetes (Type II) (6), nephrotic syndrome (30), uremia (31), diuretics (8), B-blockers (8), and a high carbohydrate diet (32). Interventions which lower VLDL-C frequently result in reciprocal changes in the HDL-C level, suggesting that the metabolism of these two particles is inter-related (Fig. 10). The exact cause of this interdependence is not clear but two possible explanations are:

1) If HDL levels are low, there is no recipient for the free cholesterol, apoproteins, and phospholipids generated during the lipolysis of VLDL and chylomicrons. Therefore, the triglyceride-rich particles cannot be catabolized efficiently, and accumulate.

2) Conversely, if there is a primary problem of lipolysis there will be no transfer of surface lipid and protein components from chylomicrons and VLDL to HDL_3 and so the levels of HDL_2 will fall. In both Lipoprotein Lipase deficiency and Apo C-II deficiency (23) there are very low levels of HDL_2. In the disease Multiple Symmetric Lipomatosis, where there are increased levels of lipoprotein lipase activity in the involved adipose tissue, the HDL_2 levels are high (33).

Figure 10

Inverse relationship between HDL-C and VLDL-C



Probably both explanations are correct in different situations. Nikkila demonstrated that HDL-C concentrations are positively correlated with lipoprotein lipase levels in adipose tissue (34). However, in general, efforts to correlate HDL-C levels with lipoprotein lipase activity have failed (35).

The role of HDL in the reverse transport of cholesterol from tissues and other lipoproteins to the liver is not completely understood, though many observations suggest that HDL is an important constituent in the reverse cholesterol pathway. Almost all cells of the body are unable to breakdown or excrete cholesterol. Indirect evidence from studies of cultured cells

suggest that cholesterol can be transferred efficiently to HDL particles (36-38). A number of investigators have identified high affinity binding sites for HDL on culture cell surfaces (39-40). Schmitz, et. al. claim peritoneal macrophages have an HDL receptor and that HDL binds to the receptor, is endocytosed, and transported to endosomes which acquire cholesterol from intracellular cytoplasmic stores prior to being resecreted (41). Whether or not such an HDL receptor exists remains highly controversial, and despite efforts by numerous groups to purify and clone the receptor, it has remained elusive.

Where does HDL deliver the cholesterol it takes up from tissues? It has been demonstrated that some HDL-C is taken up by rat adrenal cortex and ovaries to be used in steroidogenesis (42). However, most of the cholesterol is delivered back to the liver either indirectly, by transfer to chylomicron remnants, IDL, or LDL particles or, directly, as part of an HDL_1 particle. This particle contains apo E and is a ligand for the LDL receptor (43).

With respect to mass balance, it has been difficult to determine the synthetic and catabolic rates of HDL. Kinetic studies of HDL are fraught with problems since the composition of HDL is in constant flux and different components are exchanged and catabolized at different rates (44,45). Also, as stressed previously, HDL is comprised of a heterogenous group of particles and they each are metabolized at different rates. Despite the difficulties in studying HDL metabolism it is clear that HDL has multiple diverse functions in lipoprotein metabolism which include the following (Fig. 11):

1) It transfers apoproteins to other particles (i.e. apo C's to chylomicrons and VLDL) and it accepts free cholesterol, phospholipids, triglycerides, and apoproteins generated during lipolysis.

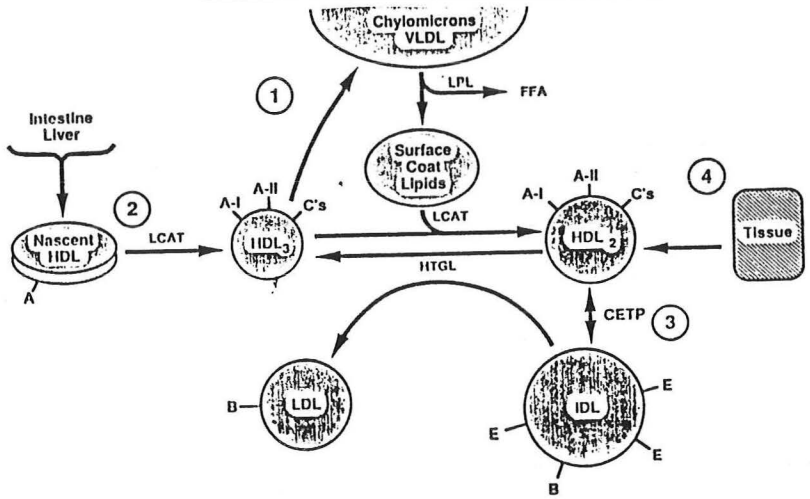
2) It acquires and esterifies free cholesterol from other lipoproteins and cells.

3) It exchanges cholesterol esters for triglyceride with other lipoproteins.

4) It delivers cholesterol from cells and other lipoproteins to the liver (and selective other tissues), the so-called "reverse cholesterol transport" pathway.

Figure 11

HDL Metabolism in Plasma

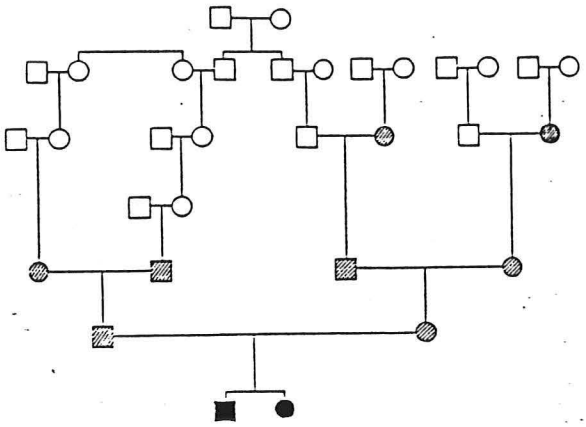


Abnormalities in HDL, whether primary or secondary, can have very diverse effects on lipoprotein metabolism, and depending on the nature of the defect will have a variety of different clinical sequelae.

Tangier Disease

Tangier Disease was the first genetic disease described to be associated with hypoalphalipoproteinemia (46). The disease was named after the home of the first two patients identified with this disorder. Tangier Island is in Chesapeake Bay and has a population of approximately 900. Many of its present day population are descended from the original settler, John Crockett, who moved to the island in 1686. The proband was a 5 year old boy who underwent a routine tonsillectomy for massively enlarged tonsils. Microscopic evaluation of the tonsillar tissue revealed numerous lipid-laden foam cells. He was also noted to have hepatosplenomegaly as well as mild diffuse lymphadenopathy. His first degree relatives were examined and his sister was found to have massively enlarged orange-yellow tonsils. Her tonsils were excised, analyzed chemically, and compared to a normal control (a fisherman from Tangier Island). The esterified cholesterol content of her tonsils was found to be 50-100 times higher than the fisherman's (46). Both children were found to have HDL-C levels that were dramatically reduced. The family pedigree of these two sibs is shown in Figure 12 and the family history is compatible with an autosomal recessive pattern of inheritance (47).

Figure 12



The propositi are shown in solid symbols. The crosshatched symbols indicate relatives examined, none of whom had tonsillar hypertrophy.

Over 30 additional patients with Tangier disease have been reported since the description of these two patients. Many have been asymptomatic and have only been identified by screening of families of affected individuals. The mean age at the time of diagnosis is 24.6 years (48). The most frequent presentation is with large, orange-yellow lobular tonsils and the next most frequent is with neurological deficits (48). Occasionally patients present with lymphadenopathy, hepatomegaly or hypersplenism (49). A few patients have been identified by routine chemical studies disclosing a very low cholesterol level.

On physical examination all (except two patients) had large, discolored tonsils (48). Corneal opacifications can be seen by slit lamp examination in older patients but rarely is there any associated visual impairment (50). A "dot-like" hazy appearance is present throughout the stroma of the cornea (51). Sometimes patients have generalized lymphadenopathy and hepatosplenomegaly. Occasionally they have tendinous xanthomas or hyperpigmented cutaneous macules. On sigmoidoscopic examination, brown-orange lipid laden cells invariably are seen speckling the rectal mucosa (49). Some have characterized the rectal mucosa as having a "cheetah fur" appearance.

Nineteen out of 33 cases reported prior to 1984 had neurological involvement with an average age of onset of 26 years (48). There are two main patterns of involvement and any single patient will have only one constellation of neurological findings. First, they can have a multifocal peripheral neuropathy. The neuropathy can be either symmetrical or asymmetrical and can involve sensory neurons, motor neurons, or both (52-54). EMG studies typically show signs of muscle denervation and the conduction velocities are usually normal. Biopsies of the involved nerves in patients with this fluctuating sensorimotor neuropathy sometimes show no abnormalities although membrane bound or free lipid deposits are frequently seen in Schwann cells. Evidence of demyelination and remyelination of peripheral nerve fibers can be

seen and the average diameter of myelinated neurons is oftentimes reduced. Typically, there is loss of unmyelinated nerves and increased endoneurial fibrosis in involved tissues (54). The clinical course of the peripheral neuropathy is typically fluctuating. It can wax and wane in severity and spontaneously remit and recur for no obvious reason.

The other pattern of neurological involvement seen in patients with Tangier disease resembles sphingomyelia (55-58). These Tangier patients usually present later in life than those with the multifocal peripheral neuropathy. They develop facial diplegia, atrophy of intrinsic hand muscles, and loss of pain and temperature sensation over the trunk with sparing of the distal extremities. EMG studies can show conduction velocity delay as well as the findings seen with the multifocal peripheral neuropathy described above. On biopsies of involved nerves, there is greater nerve fiber loss, especially among nerves of smaller diameter, than seen in the other form. Vacuoles are often seen in Schwann cells associated with unmyelinated and small myelinated fibers but only after there is evidence of prior nerve fiber degeneration (56). Therefore, it has been suggested that the vacuoles are secondary to the nerve fiber degeneration and not the primary cause of the nerve loss. In cases of Wallerian or axonal degeneration, the myelin is broken down and taken up by Schwann cells and then it is usually removed within weeks or months. However, in this disorder there seems to be a defect in the removal of myelin lipids from Schwann cells. This sphingomyelia-like polyneuropathy, unlike the multifocal peripheral neuropathy rarely remits and is usually inexorably progressive (Fig. 13).

Figure 13

CLINICAL CHARACTERISTICS AND PATHOLOGICAL FEATURES OF THE TWO COMMON PERIPHERAL NERVE SYNDROMES IN TANGIER DISEASE

	Pseudosyringomyelic neuropathy	Multiple Mononeuropathy
Onset (decades)	3-6	1-2
Pathology	Axonal degeneration	Dymelination-remyelination
Early fiber loss	Small MF + UF	UF
Lipid inclusions	All cells	Schwann cells
Initial weakness	Facio-brachial	Isolated peripheral nerve(s)
Sensory loss		
Early	Dissociated	Not dissociated
Late	All modalities:centrally	All modalities:distally
EMG		
Denervation	+++	+
Latencies	N	↑
Course	Progressive	Relapsing-remitting
Prognosis	Disabiilty	Benign

The lipoprotein profile of Tangier Disease is unique in dyslipoproteinemias. The plasma cholesterol levels are depressed and the triglyceride levels tend to be elevated. The VLDL levels can be normal or slightly increased (59). The LDL levels are markedly depressed and the LDL particles are strikingly abnormal in their composition, though most are normal in their spherical appearance. They have a dramatically increased proportion of triglyceride. Normally, only 15% of LDL is triglyceride, but in Tangier disease the proportion is 59% (59). CETP activity is normal or even high in these patients so can not be implicated directly for the triglyceride-rich particles seen in this disease (60,61).

The HDL-C level is markedly depressed and invariably less than 10% of normal. On agarose gel electrophoresis no normal alphalipoproteins are seen. It is the only dyslipoproteinemia where there are very low levels of both HDL and LDL. The apo A-I level is less than 1% of normal and the apo A-II level is approximately 6% of normal. The particles with a density of HDL have almost no apo A-I. The apo A-I to apo A-II ratio in Tangier HDL is much lower than normal; the normal ratio of apo A-I to A-II in HDL is 3:1 but in Tangier it is 1:12 (62). Most of the HDL particles in this disease are small and spherical and contain only apo A-II. There is a small population of larger apo A-I containing particles that have a pre-B migration (63).

Despite the very low levels of apo A-I, the total plasma LCAT activity in these patients has been found to be normal or only moderately reduced (70%), which is reflected in a somewhat decreased cholesterol ester to free cholesterol ratio in total plasma (64, 65). The LCAT activity does not fractionate with apo A-I though it is still associated with the high density fraction and for this reason it is thought that either apo C-I (which in vitro can activate LCAT in vitro) or apo A-II (in the absence of apo A-I) replaces A-I as an activator of LCAT (66,67).

Multiple tissues from Tangier patients have been examined microscopically and many are found to contain lipid-laden foam cells. Reticuloendothelial cells of the liver, spleen, lymph nodes, tonsils, bone marrow, skin, jejunal and rectal mucosa as well as Schwann cells and nonvascular smooth muscle cells contain large amounts of lipid. In biopsies of normal appearing skin, lipid deposits can be seen in both histiocytes and fibroblasts, as well as in the extracellular matrix. Some lipid droplets are bound by membranes and others are not (68). Importantly, no lipid-laden cells are seen in the endothelium or in vascular smooth muscle. The deposits do not deform or disrupt the surrounding tissues architecture in sharp contrast to the lipid deposits of atherosclerotic lesions. It is thus not surprising that the original patients were thought to have a lysosomal storage disease. It has been proposed by many investigators that the reason macrophages are particularly involved in this disorder is because,

unlike other tissues that regulate their uptake of cholesterol by the LDL receptor, these cells have unregulated uptake of lipids associated with their phagocytic function (69). They must be able to donate excess cholesterol to recipient particles to get rid of the cholesterol they obligately acquire during phagocytosis.

What is the origin of these lipids and why do they deposit in these tissues? It has been noted in numerous Tangier patients that the chylomicrons formed after ingestion of a meal are cleared very slowly, resulting in prolonged and pronounced hypertriglyceridemia after meals (70) (Figure 14). Bizarre appearing chylomicron remnants remain in circulation for long periods of time. Lipoprotein lipase activity is normal (or low normal) (64,65) in these patients but the absence of any normal HDL results in defective lipolysis of triglyceride-rich particles. Normally, HDL shuttles apo C-II (which is needed to activate lipoprotein lipase) to chylomicrons and VLDL prior to lipolysis and in Tangier disease there is evidence that VLDL is apo-C poor suggesting that this transfer does not proceed normally (59). Also, during lipolysis, HDL accepts surface coat lipids and apoproteins from the triglyceride-rich particles but in Tangier disease there is no normal HDL to accept the by-products of lipolysis. On high fat diets, Tangier patients not only accumulate chylomicron remnants, but also a large number of flattened translucent particles appear in the HDL fraction (70,71). These abnormal particles may be comprised of surface lipids generated during the lipolysis of chylomicrons and VLDL. Presumably, both the abnormal chylomicron remnants and HDL particles are taken up by reticuloendothelial cells. Why exactly they are taken up preferentially by certain tissues (such as the tonsils) and not others is not clear.

Figure 14

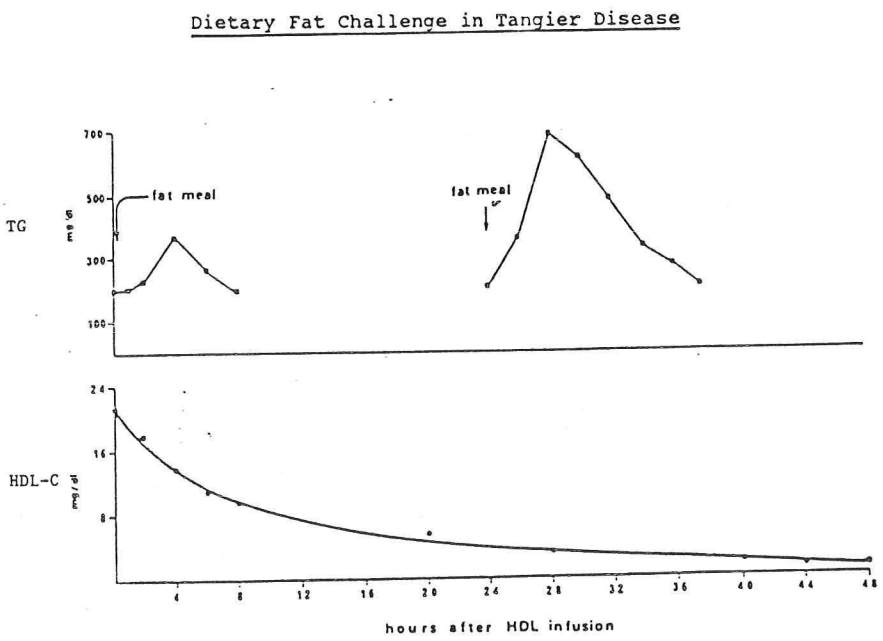
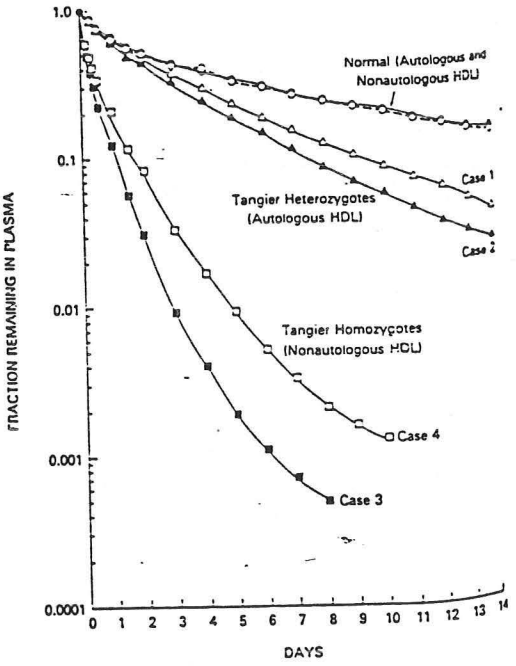


Figure 15

^{125}I-HDL Turnover Study in Tangier Heterozygotes and Homozygotes



One patient with Tangier disease had a particular dramatic accumulation of lipids in his reticuloendothelial cells and his clinical course was unusual. He developed secondary hypersplenism in his 40's and underwent a splenectomy. After splenectomy, his cholesterol rose from 30-45 mg% to 100-155 mg% and he developed a generalize papular eruption which, upon biopsy, revealed dense deposits of cholesterol esters intracellularly and extracellularly (72). EM studies of his plasma HDL showed large numbers of bizarre shaped particles similar to those seen after fat ingestion in other Tangier patients. He presented again at age 63 with an abdominal mass and multiple gastrointestinal complaints. At surgery he was found to have a grossly enlarged and deformed omentum. On histological examination the omental tissue was loaded with lipid-laden histiocytes (73).

Therefore, in this patient there was a hierarchy of sites for cholesterol deposition. After his spleen was removed, the abnormal lipoproteins were deposited in other cells which are not the usually prominent sites of cholesterol deposition in Tangier disease. Another Tangier patient who demonstrated abnormal catabolism of his lipoproteins normalized his lipoproteins after being given a total plasma exchange while on cardiopulmonary bypass. However, within 64 hours his HDL and LDL levels returned to baseline levels, suggesting that there was increased catabolism of the infused particles (74). HDL turnover studies have been done in a number of Tangier patients (74-76). In these studies radiolabeled HDL from a normal individual is infused into a Tangier patient and its decay is monitored. When compared to normal controls, HDL synthesis was calculated to be decreased by 50% in Tangier patients, but the striking finding of these studies was the rapid turnover of HDL (Fig. 15). In this study, the mean residence time of HDL was 5.21 days in normals, 3.51 days in heterozygotes and 1.8 days in Tangier patients. Therefore, the infused HDL in the Tangier patient was being degraded or removed almost 3 times as rapidly as in normal patients. It is remarkable that the level of LDL as well as HDL is dramatically depressed in this disorder. An LDL turnover has not been done in a Tangier patient, but LDL, like HDL, may be abnormally catabolized. The finding of increased catabolism of normal HDL in Tangier patients suggests that the primary defect in this disease resides in the reticuloendothelial cells accumulating HDL and remnant particles, and not in the lipoproteins themselves.

These studies do not entirely rule out the possibility that the primary defect is in the lipoprotein particles. When normal HDL is infused into a Tangier patient it may exchange components with resident abnormal particles and then get targeted for phagocytosis by macrophages. Efforts have been directed at defining a specific abnormality in the lipoprotein particles of Tangier patients. There has been a careful analysis of each component of the lipoproteins, including sequencing the Apo A-I gene from a Tangier patient (77), but no abnormalities have been

identified. It has been found that there is a delay in the cleavage of 6 amino acids at the N-terminal end of the protein (the pro-Apo A-I) (78). However, the accumulation of pro-apo A-I in this disease probably reflects an increase in apo A-I synthesis due to its rapid catabolism.

In support of the theory that the primary defect resides in the macrophage and not in the lipoprotein particle are the studies of Schmitz and Assmann (79-81). They have isolated monocytes from the blood of Tangier patients and controls and compared their binding and uptake of HDL. They find that in normal monocytes, HDL binds to the surface, is internalized, and then acquires cholesterol within the cell prior to being secreted. They report that in Tangier cells the HDL gets taken up by the monocytes but is then found in secondary lysosomes and never gets exocytosed. They believe the primary defect in this disease is in the intracellular trafficking of HDL so that it gets degraded rather than being resecreted.

Heterozygotes for Tangier are asymptomatic and do not have enlarged tonsils. Some demonstrate lipid laden cells in their bone marrow and rectal mucosa. In HDL turnover studies they have an HDL residence time intermediate between normal subjects and Tangier homozygotes (74). They have moderately increased (or normal) VLDL levels, and normal LDL levels. The HDL-C, apo A-1, and apo A-II levels are all reduced by 50% (82,83). Their HDL particles, however, are completely normal in appearance and composition so they can not be diagnosed without a family history of an affected individual. Despite their lower levels of HDL-C, heterozygotes have no increased risk for ischemic heart disease (48).

Schaefer, et. al. reviewed 27 cases of Tangier disease to see if homozygotes have an increased incidence of cardiovascular disease (48). There was no evidence of ischemic heart disease in any of the 31 individuals under 40 years of age. Of the nine patients over 40, six had evidence of vascular disease. Two brothers developed angina at age 59 and 42, respectively. The 42 year old also was hypertensive, obese, and a smoker. Two other patients developed angina in their early 40's. The remaining two had a history of past cerebrovascular accidents with an onset in their 50's or late 60's. Therefore, there does seem to be some increased risk of vascular disease in these patients compared to the normals although it is certainly not as dramatic as in patients with homozygous familial hypercholesterolemia. It can be argued that the incidence of cardiovascular disease in these patients is so low because they also have very low levels of LDL-C.

There is no clearly effective treatment for Tangier disease. It has been suggested (but not proven) that a low fat diet can improve neurological function by decreasing the formation of the abnormal HDL and of chylomicron remnant particles.

In summary, Tangier disease is characterized by the formation of HDL that can not perform its normal functions. Following are the effects on lipoprotein metabolism (Figure 16 and 17):

1) HDL-Tangier fails to transfer apo C's to chylomicrons and VLDL resulting in decreased lipolysis of these particles. HDL-Tangier fails to be a normal recipient of surface lipids and apoproteins during lipolysis of triglyceride-rich particles resulting in the formation of abnormal particles that get recognized and taken up by macrophages.

2) HDL-Tangier has decreased ability to esterify cholesterol resulting in accumulation of free cholesterol in tissues, including the cornea.

3) Though cholesterol ester exchange activity in Tangier is normal, HDL-Tangier has decreased ability to coordinate the esterification of cholesterol and its exchange with other lipoproteins resulting in the formation of abnormal triglyceride-rich LDL.

4) HDL-Tangier is not able to transport cholesterol out of macrophages and other tissues. This may contribute to the relative increase in atherosclerosis seen in these patients.

Figure 16

Tangier disease

Effects on lipoprotein metabolism

1. ↓ lipolysis → ↑ VLDL and chylomicron remnants

   ↓ transport Apo C's to TG-rich particles (?)

   ↓ transfer of surface coat lipids to HDL_3 → ↓ HDL_2

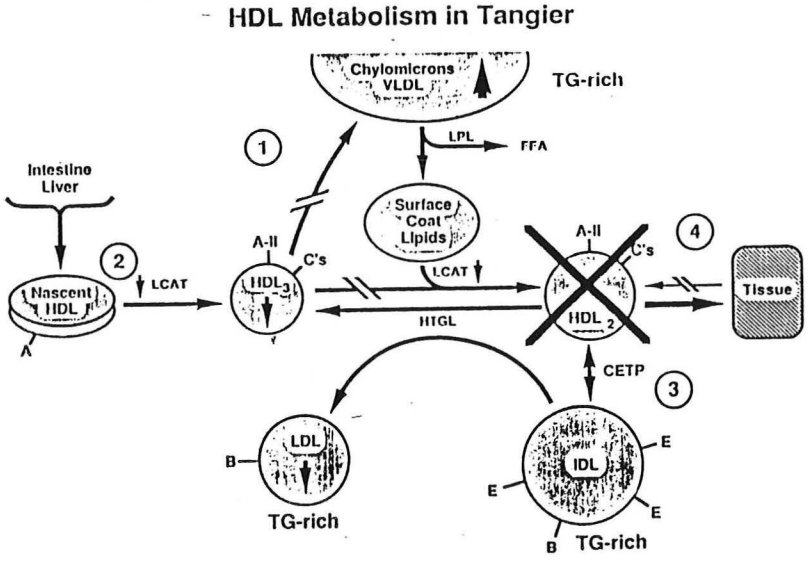
2. ↓ esterification of free cholesterol

   ↓ LCAT activity

3. ↓ cholesterol ester exchange

4. ↓ reverse cholesterol transport

Figure 17

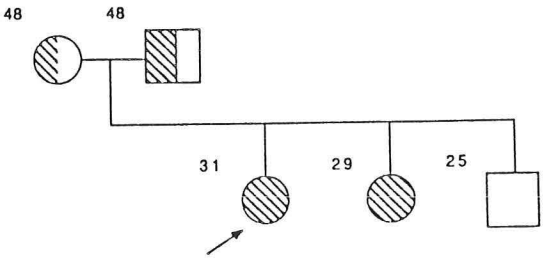


Apoprotein A-I/C-III deficiency

Unlike Tangier Disease, hypoalphalipoproteinemia due to Apo A-I/C-III deficiency is always associated with premature coronary as well as peripheral vascular disease. The disease is very rare but coincidentally two pedigrees, one from Alabama (84, 85) and one from Detroit (86,87), were recognized by two different investigators. The Detroit pedigree is shown in Figure 18. The proband was a 31 year old woman who presented with congestive heart failure. At presentation, she was noted to have multiple yellow-orange, indurated, raised plaques on her trunk, neck, eyelids, chest, arms, and back, which she reportedly had developed when she was a teenager. She also was noted to have mild corneal opacifications in a peripheral distribution. Coronary angiography revealed severe three vessel disease and she underwent coronary bypass surgery. Her 29 year old sister gave a history of congestive heart failure, associated with a pregnancy at age 25. She had developed yellowish plaques on the extensor surfaces of her arms and her eyelids by age 9. She previously had a xanthoma removed from a tendon in her left foot, and pathological examination of that specimen revealed lipid-filled histiocytes in the dermis in a perivascular distribution. She also had mild corneal opacifications. Their brothers were asymptomatic and their mother had no signs or symptoms of ischemic heart disease but was found to have a yellowish plaque on one eyelid that had developed at age 50. None of the proband's children had any evidence of coronary artery disease. This family had no history of consanguinity.

Figure 18

APOPROTEIN A-1 / C-111 DEFICIENCY



(mg/dl) CHOLESTEROL(%)			
	180 (24th)	130 (<5th)	122 (<5th)
TRIGLYCERIDE	70 (24th)	31 (<5th)	51 (18th)
VLDL-C	25 (79th)	6 (25th)	10 (44th)
LDL-C	120 (42nd)	124 (70th)	112 (56th)
HDL-C	35(6th)	0	0
APO-A1	80	0	0

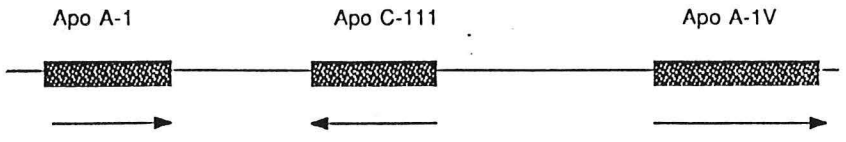
Lipoprotein analysis of plasma from these two women disclosed similar findings. The cholesterol and triglyceride levels were normal or low. The plasma cholesterol levels were 130 and 122

mg/dl and the triglycerides were 31 and 51 mg/dl. The levels of VLDL and IDL were low and the levels of LDL-C were normal. There was no measurable HDL-C by ultracentrifugation in either patient, but there were lipoproteins in the HDL fraction noted on polyacrylamide gel electrophoresis (level was 3-5% of normal). There were two populations of HDL particles seen on EM studies. Most of the HDL was small and spherical (resembling HDL_3), but some was large and found to be very rich in apo E and apo A-II (87). Quantification of the plasma apoproteins disclosed no immunoreactive apo A-I or apo C-III. Levels of apo A-II, E, C-I and C-III were decreased by about 50%, while the level of apo C-II was only 5-16% of normal.

The HDL particles were spherical despite the absence of apo A-I, so once again there was evidence of LCAT activity without Apo A-I being present. When measured, LCAT activity was found to be reduced by 40% (85), and though the ratio of free cholesterol to esterified cholesterol levels was normal in total plasma, it was decreased in HDL. Fat feeding was associated with the transient appearance of discoidal HDL particles. These particles probably represent surface lipid components, released from chylomicrons during lipolysis, that do not undergo cholesterol esterification as rapidly as usual due to the lower level of LCAT (88).

Unlike Tangier disease, in this disease the defect is known. The genes encoding apo A-I and apo C-III are situated next to each other (2.6 kb apart) on chromosome 11 and the apo A-IV gene is immediately downstream of the apo C-III gene (Fig. 19). These two patients were found to be homozygous for an inversion involving both the apo A-I and apo C-III gene. There is no normal mRNA encoding apo A-I or apo C-III; therefore, no apo A-I or C-III protein is produced in these patients. The patient with apo A-I/C-III deficiency from Alabama (referred to as variant II) had a similar clinical picture, though she did not have any dermatological manifestations, and she presented at age 45 (i.e. 20 years later) with ischemic heart disease. She also was found to have a low plasma level of vitamin E and linoleic acid. She died at age 45 after bypass surgery, and at autopsy was found to have generalized severe atherosclerosis. When her apo A-I/C-III locus was examined, she was found to have a large deletion involving all three genes, apo A-I, C-III and A-IV (personal communication, E. Schaefer). Recently another patient with combined apo A-I and C-III deficiency has been described from Japan, but his apo A-I and C-III gene has not been evaluated (90).

Figure 19 APOPROTEIN A-1/ C-111/ A-1V LOCUS



Although both Tangier disease and Apo A-I/C-III deficiency are characterized by an absence of HDL, premature atherosclerosis is a dominant feature of only the latter. The reason for this difference is not known, but may be related to the fact that in Tangier Disease apo A-I is made normally (unlike in A-I/C-III deficiency) and even though the HDL is rapidly turned over, a subset of HDL may be able to perform some of its "anti-atherogenic" functions. The lower level of LDL-C in Tangier may also be protective, as mentioned previously. Another factor that distinguishes Tangier disease from Apo A-I/C-III deficiency is the complete absence of apo C-III seen in the latter disease.

The exact role of apo C-III in lipoprotein metabolism is not clear. In vitro experiments show that apo C-III inhibits both lipoprotein lipase and hepatic triglyceride lipase (91,92). Liver perfusion studies suggest it also inhibits the uptake of remnant particles by the liver (93). If it acts as an inhibitor of lipoprotein lipase, in its absence it would be expected that the hydrolysis of the triglycerides in VLDL and chylomicrons would be accelerated. And, if it inhibits the uptake of remnants by the liver, in its absence the clearance of these particles would be increased. ^{125}I-LDL and VLDL turnover studies in two patients with Apo A-I/C-III deficiency demonstrated the anticipated results (94). The fractional catabolic rate of ^{125}I-VLDL was 3-5 times normal. There was rapid hydrolysis of the VLDL-triglyceride and rapid formation of LDL, so almost all the apo E was found in the HDL fraction, and there was little VLDL or IDL identified. The LDL produced was protein and cholesterol ester-rich, and triglyceride-poor. It is paradoxical in this disorder that the triglyceride-rich particles undergo such rapid hydrolysis, since there is no normal apo A-I containing HDL, and only small amounts of apo C-II (5-16% normal). It can be concluded that if apo C-III is absent, defects in apo A-I do not impact importantly on the lipolysis of triglyceride-rich particles.

Thus, in this disease there is a duel defect. First, there is increased production of cholesterol ester-rich particles due to the absence of apo C-III, and second, there is no normal HDL due to the absence of Apo A-I. Taken together these two factors lead to a dramatic acceleration in the development of atherosclerosis. More atherogenic particles are produced, and there is no normal HDL to facilitate the efflux of cholesterol out of the tissue in which the particles are deposited (Figure 20 and 21).

Figure 20

Apo A-1/C-III deficiency

Effects on lipoprotein metabolism

1. ↑ lipolysis → ↓ VLDL

   despite no apo A-I, TG-rich particles undergo accelerated lipolysis

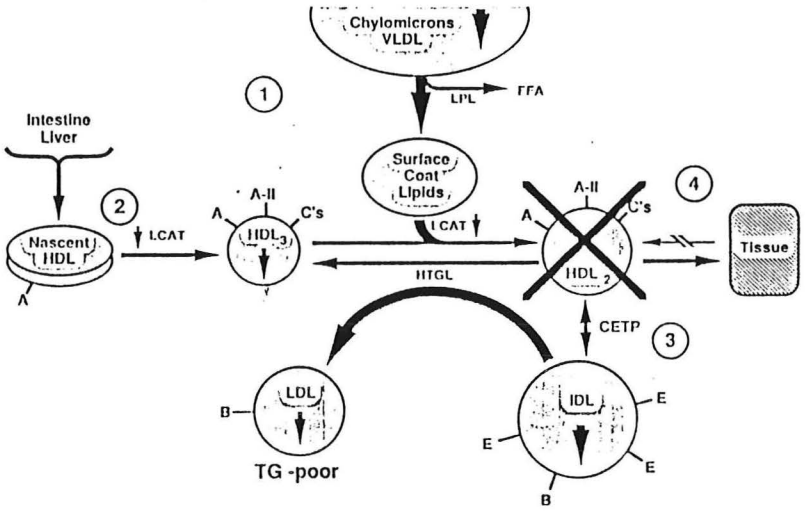
2. ↓ esterification of free cholesterol

   LCAT ↓ 40% → ↓ CE/C in HDL

3. - cholesterol ester exchange

4. ↓ reverse cholesterol transport

Figure 21 HDL Metabolism in Apo A-I/C-III Deficiency



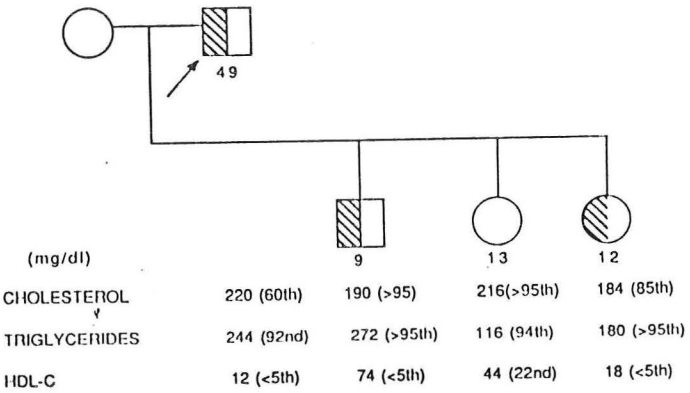
It is difficult to assess the individual contributions of a deficiency of apo A-I or apo C-III to the development of premature atherosclerosis in this disease. There has never been a case of isolated apo C-III deficiency found but recently a case of complete apo A-I deficiency was reported in abstract form. The patient was a 5 yr old Turkish girl who had a total absence of apo A-I due to a nonsense codon in her apo A-I gene (95). She had corneal opacifications, diffuse planar xanthomas, negligible amounts of HDL-C, and no detectable apo A-I. She had no evidence of coronary insufficiency, but is still very young and it is not known if any of her relatives had premature atherosclerosis. It will be important to follow her and her family to see if this mutation is associated with accelerated atherosclerosis.

Apo A-I Milano

Apo A-I Milano is a disease that is also due to a mutation in the apo A-I gene but in this case it is a missense mutation leading to production of an abnormal apo A-I protein. It was first described molecular variant of a human apoprotein (96,97). The proband was an asymptomatic 49 year old Italian father of three, who had hypertriglyceridemia which was resistant to diet and medical therapy (Fig. 22). He had a normal physical examination with no corneal opacifications, skin lesions, tonsillar enlargement, lymphadenopathy, or organomegaly. He had a normal cholesterol level (220 mg%), an elevated triglyceride (244 mg%), and an HDL-C of 12 mg% (20-30% of normal). Two of his three children had lipoprotein values similar to their father. They both had normal cholesterol levels, mild elevations in their triglycerides, VLDL-C, and LDL-C, and a markedly decreased HDL-C. The remaining sib had a normal HDL-C. The mother was never analyzed. The inheritance pattern was consistent with an autosomal dominant pattern.

Figure 22

APOPROTEIN A-I MILANO



Since this family was identified, a number of other affected individuals with apo A-I Milano have been found in the same region of Italy (98). 94% of the population (~1000) of Limone sul Garda, a town in northern Italy, were sampled, and 33 were found with this disorder. In a survey of 29 affected adults, none were found to have corneal opacifications or other physical stigmata of hypoalphalipoproteinemia. Most, but not all, had low total plasma cholesterol levels and elevated triglyceride (and VLDL) levels. The LDL levels were normal but both VLDL and LDL were slightly triglyceride-rich (96). Irrespective of whether the triglyceride level was elevated, the level of HDL was depressed to about 20% of normal, and the composition of the HDL particles was distinctly abnormal. The HDL was triglyceride, phospholipid and protein-rich, and cholesterol ester poor. Analysis of the HDL by zonal ultracentrifugation showed little or no HDL_2 and a decreased amount of HDL_3 (Fig. 23) (99). Total plasma LCAT activity was either normal or decreased and was directly related to the HDL mass (96,98). Some patients had a decrease in the ratio of cholesterol esters to free cholesterol in their plasma, but none had corneal opacifications (98). Those individuals with the lowest levels of HDL had the smallest HDL particles and the highest triglyceride levels (99,100), which is consistent with some impairment in lipolysis, despite normal (or slightly decreased) lipoprotein lipase activity (96).

Figure 23

Rate Zonal Ultracentrifugation Profiles of HDL

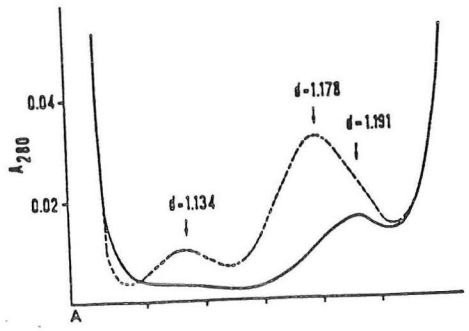
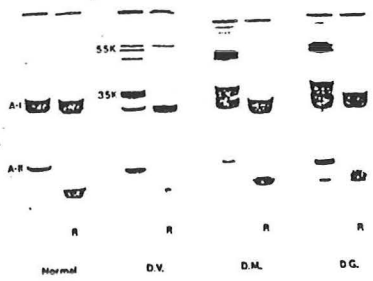


Figure 24

SDS Gels of Control and Reduced HDL from Apo A_1 Milano and Normal Subjects



In Apo A-I Milano all apoprotein levels are normal except apo A-I, which is less than 50% of normal. The mutation responsible for this disease is a base change that results in an arginine to cysteine substitution at amino acid 173 of apo A-I (101). All patients found with this disorder are heterozygous for this mutation. The mutant apo A-1 protein forms homodimers, as well as heterodimers, with apo A-II via the cysteine residue. Figure 24 shows an SDS gel electrophoresis of native and reduced HDL proteins from a normal subject and three individuals with Apo A-I Milano. The Apo A-I Milano HDL has some normal apo A-I (16.1-25.7%) since they have one normal apo A-I allele. They also have some larger bands that decrease in amount or disappear after reduction. These higher molecular weight bands represent homo- and heterodimers of apo A-I. The complexes of apo A-I Milano with apo A-I and with apo A-II probably interfere with the normal structure and function of HDL, and have the following observed effects on lipoprotein metabolism (Figure 25 and 26).

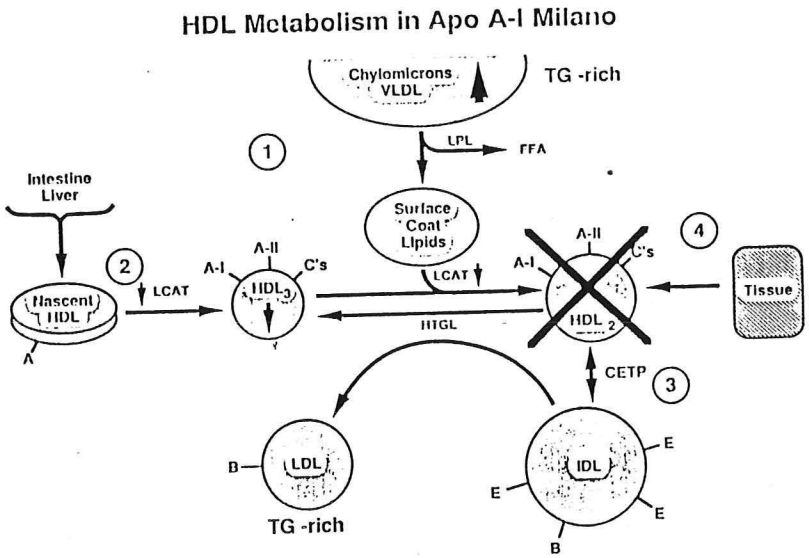
Figure 25

Apo A-I Milano

Effects on lipoprotein metabolism

1. ↓ lipolysis → ↑ VLDL
   ↓ transport Apo C's to TG-rich particles (?)
   ↓ transfer of surface coat lipids to HDL_3 → ↓ HDL_2
2. - or ↓ esterification of free cholesterol
   - or ↓ LCAT activity
3. - or ↓ cholesterol ester exchange (?)
   ↓ esterification/exchange (?)
4. - reverse cholesterol transport

Figure 26



1) Despite very small amounts of HDL, there is enough to transfer apoproteins of the C series to the triglyceride rich particles. But, the very low levels of HDL_2 seen in this disorder suggest that there is defective transfer of surface coat lipids to HDL during lipolysis. The apoprotein complexes probably alter the configuration of the HDL particles, and hinder the conversion from HDL_3 to HDL_2. Some patients with Apo A-I Milano have normal levels of triglyceride, and these patients probably have a larger population of HDL particles with monomeric apo A-I.

2) Probably LCAT activity is decreased in this disorder because most of the normal apo A-I is complexed with the apo A-I Milano and the structure of HDL is deformed. Though some patients have a modest decrease in the plasma cholesterol ester to free cholesterol ratio, there is no deposition of free cholesterol in the cornea or other peripheral tissues.

3) The effect on cholesterol ester exchange has not been examined but probably is negligible.

4) Reverse cholesterol transport is probably not affected by the mutation since there is no evidence of tissue accumulation of cholesterol.

Despite the near absence of HDL_2 and dramatically increased total cholesterol/HDL-C ratio, (both of which have been associated with increased coronary risk), these patients show no evidence of increased incidence of coronary artery disease (96,102). Of the 33 affected individuals identified with this disease, only 1 had evidence of any vascular disease. That patient required aorto-femoral bypass surgery at age 69, and ironically, he happened to have the highest HDL-C in the group (= 25mg/dl) (102). Therefore, the correlation between a low HDL-C and an increased risk of cardiovascular disease is not a simple or universal one.

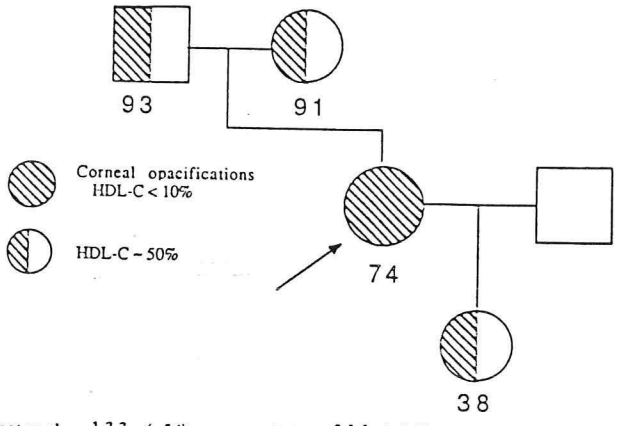
Fish Eye Disease

Apo A-I Milano is not the only familial disorder where strikingly low levels of HDL-C are not associated with an increased incidence of coronary artery disease. Another example is Fish Eye disease, a disorder first described in 1981 by Lawrence A. Carlson, et. al., in Sweden (103). The name of the disease is derived from the fact that the people in the Swedish village, where the index family lived, thought some of the individuals in this family had eyes that resembled boiled fish. The family was referred to as the one with "fish eyes". Three families with this disease have been identified, two from Sweden and one from Canada. A pedigree of one of the Swedish families is shown in Figure 27a (104).

The proband was a 74 year old woman who developed clouding of her cornea at age 20. She had no tonsillar enlargement, lymphadenopathy or organomegaly. Her father was 93, and her mother

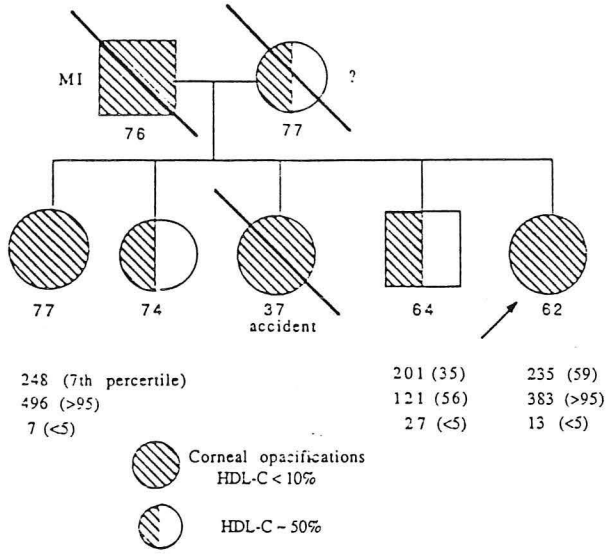
had died at age 91. The father and the proband's daughter (age 38) were both analyzed and they were found to have HDL-C levels that were about half the normal level. The proband had an HDL-C level of just 7 mg% (Nl = 60 mg%). No one in the family showed evidence of coronary artery disease and her daughter, who was 38 years old, was asymptomatic.

Figure 27a



Cholesterol (mg/dl)	133	(<5th percentile)	311	(>95)
TG (mg/dl)	45	(<5)	538	(>95)
VLDL-C	6	(21)	73	(>95)
-TG	11		292	
LDL-C	102	(<5)	227	(>95)
-TG	31		211	
HDL-C	28	(<5)	7	(<5)
-TG	9		6	

Figure 27b



(mg/dl)				
Chol	248	(7th percentile)	201 (35)	235 (59)
TG	496	(>95)	121 (56)	383 (>95)
HDL-C	7	(<5)	27 (<5)	13 (<5)

The pedigree of the second Swedish family is shown in Figure 27b (103). The proband, a 62 year-old woman, her father, and two of her 5 siblings all had "fish eyes". One affected sib died at age 37 in an accident. The two sisters with the corneal opacifications were available for evaluation. They both reported that they started to develop clouding of their cornea when they were teenagers. The opacifications increased in severity and started to interfere with their vision in their 50's and 60's. The proband had undergone corneal transplantation at age 64 and the cornea was evaluated microscopically and was found to have lipid filled vacuoles in the stroma and Bowman's layer. Chemical analysis of the excised tissue disclosed a 100% increase in free cholesterol. The patient responded well to the corneal transplant and did not develop any recurrent opacification over the ensuing few years. She denied having symptomatic evidence of ischemic heart disease but a year after presentation she had a positive exercise test. Her sister had a myocardial infarction at age 77, and died suddenly six months later. These two affected sibs had markedly low HDL-C. Unfortunately, their father and the other affected sib died prior to analysis. Both Swedish families were

from small towns and their ancestry could be traced to the early 18th century. There was no history of consanguinity found in either family, and no evidence that the two families were related, though most certainly they were distantly connected.

Lipoprotein analysis of the three available affected individuals disclosed the following findings (105). The cholesterol levels were normal (except in the first patient who was hypothyroid), but all three individuals had markedly elevated triglyceride levels. The level of VLDL was increased, though normal in composition. The LDL-C level was also normal (except in the hypothyroid patient), but the composition of the LDL was strikingly abnormal. On agarose gel electrophoresis, the pre-beta and β-bands were continuous, reflecting an increase in IDL, and the triglyceride content of the LDL (which was five times normal). On EM, the average size of the LDL particles was 10% smaller than normal, but there was also a small population of abnormally large particles (106). The HDL-C levels in these three affected individuals were dramatically decreased. As in Apo A-I-Milano, most of the HDL was in the HDL_3 fraction. On agarose gel electrophoresis, the HDL had normal mobility (unlike Tangier disease). The HDL particles were rich in phospholipids, free cholesterol, and triglyceride, while poor in cholesterol ester (106) and predictably, on EM studies, disc-like particles were seen. On average, the HDL particles were 30% smaller than normal, but some large vesicular particles were seen that resembled the abnormal HDL particles seen in Tangier disease. The apo A-I and apo A-II levels were decreased proportionately to approximately 10% of normal.

Measurements of total plasma LCAT actively were normal, and the cholesterol ester to free cholesterol ratio in plasma was normal or slightly decreased, at 60% (nl=70%) (105). Analysis of LCAT activity in individual lipoprotein fractions disclosed normal activity in VLDL and LDL and markedly decreased activity in HDL (107,108). Normally, 80% of HDL is comprised of cholesterol esters, but in these patients only 20% of the cholesterol was esterified (107). In normal individuals, most of the plasma LCAT activity is concentrated in the HDL fraction, but there is some activity in VLDL and LDL. Fish Eye disease is the first lipoprotein disorder where there has been absence of LCAT activity in the α-lipoproteins but normal activity in the B-lipoproteins. The cause of this selective reduction in LCAT activity in the α-lipoproteins is not known.

Measured levels of cholesterol ester transfer from solid phase bound HDL to VLDL, IDL and LDL in one patient were decreased by almost 40% (60), but other measurements by other investigators have been normal. The decrease in CETP activity may be indirectly caused by the lower LCAT activity in HDL. If less cholesterol ester is formed, then there is less to exchange with other lipoproteins. The failure of this exchange results in an increase

in the amount of IDL and triglyceride-rich LDL. Radiolabeled VLDL and LDL turnover studies have been done in two patients with Fish Eye disease, and a 30% decrease in conversion of VLDL to IDL and of IDL to LDL was found (110), despite the fact that in-vitro measurements of lipoprotein lipase and hepatic triglyceride lipase were normal (105, 111). This is probably related to the failure of cholesterol esterification in HDL, and its exchange with other lipoproteins.

A summary of the defects in lipoprotein metabolism seen in Fish Eye disease are summarized in Figures 28 and 29.

Figure 28

Fish Eye disease

Effects on lipoprotein metabolism

1. ↓ lipolysis → ↑ VLDL

   ↓ transport Apo C's to TG-rich particles (?)

   ↓ transfer of surface coat lipids to HDL_3 → ↓ HDL_2

2. ↓ esterification of free cholesterol

   - β-LCAT activity

   ↓ α-LCAT activity → ↓ CE/C in HDL

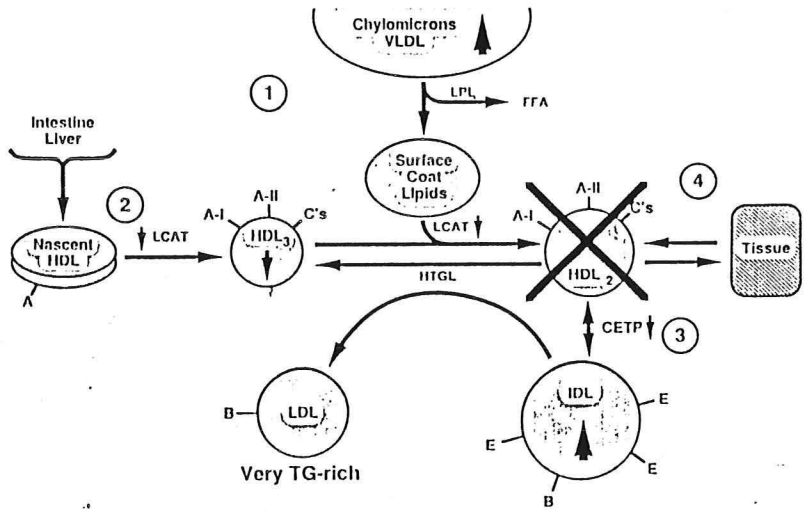
3. ↓ Cholesterol ester exchange (?)

   ↓ esterification/exchange → TG-rich LDL

4. - reverse cholesterol transport

Figure 29

HDL Metabolism in Fish Eye Disease



Many of the first degree relatives of these two patients were found to have HDL-C levels that were reduced by 50%. There was no history of early coronary artery disease in either the obligate heterozygotes or homozygotes with this disease. One additional

person with Fish Eye Disease from Vancouver has been identified (112) Once again, despite dramatically lower levels of HDL_2, there is no evidence of premature cardiovascular disease in the proband or his family.

Other Disorders

There are several other single gene disorders that are associated with low levels of HDL-C, which will be described briefly including: 1) Apo A-I variants, 2) Familial Apo A Deficiency or Diffuse Planar Xanthomas, and 3) LCAT Deficiency. There are a number of mutant Apo A-I proteins that have been identified: Apo A-I Marburg, Apo A-I Giessen, Apo A-I Munster (1-7) and Apo A-I Norway (113,114). Each mutations results in one or two amino acid substitutions or deletions in the apo A-I protein. No homozygous individuals for any of these mutations have been identified. In general the patients are clinically normal though some have a mild elevation in their triglyceride or mild depressions in their level of HDL-C. It is likely that numerous other mutations will be identified at the apo A-I locus and it can be expected that they will vary as to their effect upon HDL level and function. To date, there have been no clear association between any of these apo A-I variants and premature cardiovascular disease.

Familial Apo A deficiency or Diffuse Planar Xanthomas is a disease described by Lindeskog and Gustafson (115,116). The index case was a 43 year old woman who had diffuse symmetrical patches of yellowish discoloration, which were particularly pronounced around her eyes and in her intertriginous areas. Her mucous membranes were also noted to have a yellowish hue. She had corneal opacifications as well as hepatomegaly. Her tonsils were not enlarged. One of the skin lesions were excised and showed changes consistent with a xanthelasma. It contained large amounts of both free and esterified cholesterol. Her mother, who died at age 72 after a cholecystectomy, reportedly also had the same skin changes.

On lipoprotein analysis, the cholesterol was normal but the triglycerides on multiple samplings were elevated. The concentration of HDL-C was markedly depressed, being less the 1% of the normal level. VLDL-C and IDL-C levels were five and ten times normal, respectively, and both had a lower percentage of triglyceride than normal. The LDL level was normal, but, as in the other familial low HDL disorders, the particles were rich in triglycerides (27.4% compared to normal = 4.3%) and low in cholesterol ester and phospholipid.

Quantification of the apoproteins were normal (including apo C-III) except apo A-I (1%) A-II (20%) and D (50%). Almost all the apo A-I present was in VLDL and most of the apo A-II was in LDL, (unlike in Tangier disease where the apo A-II is still associated with HDL). The total plasma cholesterol to cholesterol ester ratio

was normal as was lipoprotein lipase activity (when compared to similarly hypertriglyceridemia individuals). The patient with this disease was not studied in detail, so it is difficult to discern the nature of the primary defect. She was noted to have developed angina between ages 48 and 53 years but her mother, who presumably had the same disease, died at 77 after surgery and had no prior history of ischemic heart disease. Therefore, it is unclear whether this disease is associated with accelerated atherosclerosis.

Familial LCAT Deficiency is also a rare disorder. but one important to consider. Defects in LCAT activity profoundly disrupt HDL metabolism and it shares some clinical features with other familial low HDL syndromes (23). LCAT deficiency is an autosomal recessive disease where the ability to esterify cholesterol is severely impaired. As noted previously, cholesterol esters comprise the core of most lipoproteins and, thus, are important determinants of the shape of the lipoprotein particle. Without cholesterol esters, the particles can not form a central core and thus remain discoidal in shape. As mentioned previously, in LCAT deficiency the HDL particle resemble the nascent disc-like particles seen in liver perfusates of animals and are similarly rich in apo E. The LDL particles are bizarre in size, shape, and composition and are rich in free cholesterol and phospholipids. All the lipoproteins are triglyceride-rich since cholesterol ester formation is absent and thus the exchange of cholesterol ester for triglyceride can not proceed normally.

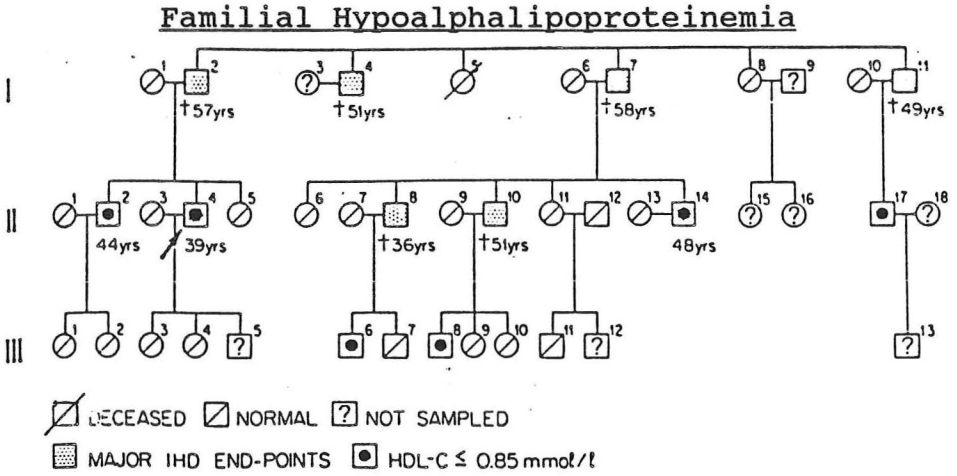
The clinical hallmarks of this disorder are corneal opacifications, mild hemolytic anemia, peripheral neuropathy, and renal defects. There is generalized corneal opacification associated with peripheral arcus. Microscopic examination of the cornea reveals extracellular cholesterol and phospholipid deposits. The hemolytic anemia is due to the abnormal composition of the red blood cell membranes. The renal disease can be mild or severe but presents clinically with proteinuria and can progress to renal failure. The most striking clinical feature that Familial LCAT Deficiency shares with other low HDL syndromes are the corneal opacification. It is not clear why the cornea is so susceptible to the deposition of cholesterol, but it is a shared feature of Tangier Disease, Apo A-I/C-III Deficiency, Fish Eye Disease, and Diffuse Planar Xanthoma and LCAT deficiency. It has been noted that the opacifications are central in Tangier disease, LCAT deficiency, and Fish Eye disease but peripheral in Apo A-I/C-III deficiency. LCAT deficiency is associated with early atherosclerosis especially involving the aorta and renal, and iliac vessels.

Familial Hypoalphalipoproteinemia

There have been multiple reports of a distinct genetic disease that has been called familial hypoalphalipoproteinemia (117-125).

Numerous investigators have noted that there seems to be familial clustering of hypoalphalipoproteinemia (defined as an HDL-C <10%), and that there is co-segregation of low HDL-C with ischemic heart disease in these families. Attempts have been made to implicate a single gene effect. One such family is shown in Figure 30 (117). In the first generation, four brothers died by age 58 of presumed myocardial infarctions. In the second generation, of 11 offspring, 3 had previously had a myocardial infarction and all three had very low HDL-C levels. Two had died of a sudden death and the lipoprotein levels had not been obtained. However, there is not sufficient clinical information given on each individual to rule out possible secondary causes of low HDL-C. Later studies of this family gave conflicting information about the pedigree, making genetic analysis difficult (119). However, in this family there is a striking preponderance of ischemic heart disease and depressed levels of HDL-C (note: no women in this family had low HDL-C).

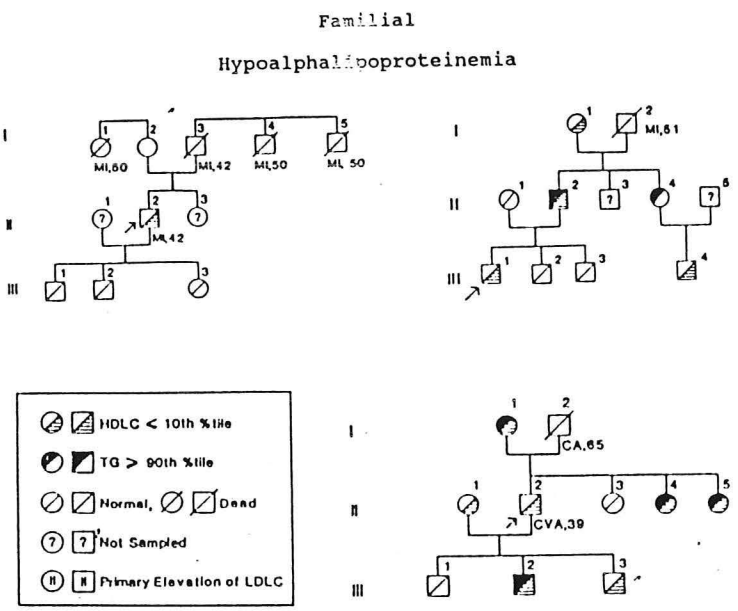
Figure 30



Another study suggesting that familial hypoalphalipoproteinemia was a distinct clinical due to a major gene effect was reported by Glueck, et al. (120). He selected 16 probands (15 males and 1 female) who had HDL-C levels less than the 10th percentile without any associated hypertriglyceridemia or other lipoprotein abnormalities. Many of the patients were referred for evaluation of premature ischemic heart disease or cerebrovascular disease. The relatives of these 16 patients were evaluated and at least one relative was identified as having either a low HDL-C or premature cardiovascular disease. It was suggested that these 16 families had an autosomal dominant disorder, though genetic heterogeneity or a polygenic etiology could not be ruled out. Examples of some of the pedigrees identified in this study are shown in Figure 31. In reviewing these pedigrees, two things are clear. In some cases, the inheritance pattern is not typical of an autosomal dominant disorder; the low level of HDL-C skips generations. In many of the families, there is not enough lipoprotein information to assess the inheritance pattern. In some families there are individuals with elevated triglyceride or LDL-

C levels. Some of these pedigrees probably have either familial hypertriglyceridemia or familial combined hyperlipidemia, both autosomal dominant disorders associated with low HDL-C. Familial combined hyperlipidemia is also associated with an increased risk for coronary artery disease. The lack of good genetic evidence of a common major gene effect does not negate the fact that early vascular disease is frequently associated with low levels of HDL-C in these families. It does not necessarily mean, however, that the low HDL-C levels and increased vascular disease are causally related.

Figure 31



The HDL-C is a quantitative trait and it is difficult to convincingly demonstrate a bimodal distribution of HDL-C levels in these families. Perhaps if a lower cut-off of HDL-C was used (i.e. < 5th percentile) a subset of pedigrees with a monogenic disorder might have been identified. As with elevations in LDL-C, it is probable that most cases of low HDL-C have a polygenic etiology. Multiple genes, or genetic plus environment factors contribute to the lowering of HDL-C. However, there does seem to exist a subset of patients with strikingly low levels of HDL-C that are found frequently in association with premature atherosclerosis in families.

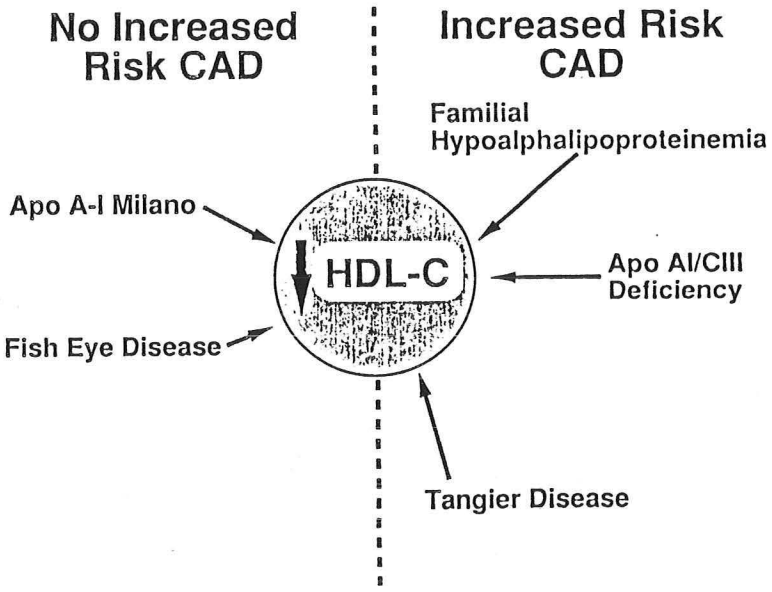
In summary, there are both monogenic and polygenic familial disorders of low HDL-C. Although they all share a low HDL-C level, the clinical manifestations of each disorder are distinctly

different (for review, see Figure 32). Some disorders are associated with increased cardiovascular disease and others are not (Figure 33).

Figure 32

	Tangier	AI/CIII Deficiency	Apo-A_1 Milano	Fish Eye
HDL	↓↓↓	↓↓↓	↓↓	↓↓
Apo A_1	1%	-	50%	10%
LDL	↓↓ TG-rich	N TG-poor	N TG-rich	N - ↑ TG-rich
VLDL	↑	↓	N - ↑	↑
Organomegaly	+++	-	-	-
Neuropathy	+++	-	-	-
Skin lesions	+	+++ or -	-	-
Corneal opacif.	+	++	-	+++
Atherosclerosis	+	+++	-	-

Figure 33



Overview

How can we apply the analysis of genetic disorders associated with low levels of HDL to the management of patients? Should we direct our diagnostic studies to the detection of low HDL-C levels and should we be treating low HDL-C levels? Recently, the Expert

Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults in the National Cholesterol Education Program has been criticized for ignoring HDL-C in its treatment protocols (121). Specifically, they did not recommend that the HDL-C level be determined in individuals with a total cholesterol under 200 mg/dl, or in individuals without cardiovascular disease or risk factors and a total cholesterol between 200 and 240 mg%. They did not recommend directing therapy specifically at a low HDL-C if the LDL-C was normal. The controversy surrounding these recommendations stem largely from the results of two sets of studies. Some studies have shown that a low HDL-C is a risk factor for ischemic heart disease even if the LDL-C is normal (123,124). Other studies have suggested that administration of medications that raise HDL-C is associated with a decrease in ischemic cardiac events and/or a decrease in the development of atherosclerotic lesions. In both the Helsinki Gemfibrozil Study (125,126) and the Cholesterol-Lowering Atherosclerotic Study (127), there were dramatic increases in HDL-C (as well as decreases in VLDL-C and LDL-C) in patients given either gemfibrozil, or nicotinic acid and a bile acid resin, respectively. Investigators have attributed some of the beneficial outcome of the treatment group to the increase in HDL-C.

Once again, the question is raised whether or not a low HDL-C alone should be treated with measures aimed specifically at raising its level. In favor of treating HDL-C directly are the following factors:

1) Numerous studies (though oftentimes flawed by not addressing secondary causes of a low HDL-C) have shown an inverse correlation between HDL-C and coronary artery disease.

2) Many factors that are independent risk factors for atherosclerosis also lower the HDL-C: smoking, obesity, diabetes.

3) The Helsinki Gemfibrozil study and Cholesterol Lowering Atherosclerosis Study both demonstrated an improvement in cardiac risk associated with an elevation in HDL-C.

4) In families with hypoalphalipoproteinemia, low levels of HDL-C levels are associated with increased coronary artery disease. Conversely, families with hyperalphalipoproteinemia may have a decreased risk of coronary artery disease (128).

5) In Apo A-I/C-III deficiency, there is almost no HDL and normal (but not high) levels of LDL-C. This disease is clearly associated with ravaging coronary and peripheral vascular disease.

Against HDL-C being a primary risk factor for atherosclerosis are the following considerations:

1) Comparisons between high and low risk populations do not

correlate with differences in HDL-C levels as they do with LDL-C levels (129,130). In populations where the LDL-C levels are low, such as the Masai, Tarhaumura Indians of Mexico, and vegetarians low HDL-C does not impart increased risk for the development of atherosclerosis (131-133).

2) Both gemfibrozil and nicotinic acid, the two pharmacological agents thought to lower cardiac risk above and beyond their LDL lowering effects, also impact importantly on the composition of other lipoproteins. The beneficial effect of these drugs may not be due to the elevation of HDL-C, but rather to the decrease in VLDL, IDL or perhaps Lp(a) (for review of Lp(a), see ref. 134). No study has been done where HDL-C alone has been raised and demonstrated to have a beneficial effect on cardiac risk.

3) Recent animal studies using probucol (Lorelco), a drug associated with dramatic lowering of HDL-C, have shown a marked decrease in progression of atherosclerosis in association with a depression in HDL-C (135).

4) In Apo A-I Milano and Fish Eye Disease there is little to no HDL_2, and no evidence of an increased risk for the development of atherosclerosis. In Tangier disease, where there is no normal HDL, there is only a moderately increased incidence of coronary artery disease. Only in Apo A-I/C-III deficiency is there clearly an elevated incidence of atherosclerosis, and there is not just a low HDL-C, but also increased production of cholesterol-rich particles that may be more atherogenic.

Therefore, based on the limited information available, it is still advisable to direct therapy to lowering the level of LDL-C level. However, the HDL-C levels should be measured and monitored during treatment and any possible secondary causes of low HDL should be identified. In individuals where the HDL-C is low in concert with a high LDL-C (and oftentimes high VLDL-C) a medication should be selected that both lowers LDL-C and VLDL-C and raises HDL-C, such as nicotinic acid or gemfibrozil. In individuals with normal lipoprotein levels excluding a depressed HDL-C, or in patients with a normal LDL-C, increased VLDL-C, and low HDL-C, there is no evidence yet that treating the low HDL-C is beneficial. However, if a patient with low HDL-C has established coronary artery disease or a strong family history of cardiovascular disease, it may be advisable to direct therapy at further lowering the LDL below the target level of 130 mg%. At the present time, based on the information available, it is not clear how to optimally treat such patients.

Prospective clinical trials need to be done in the treatment of hypoalphalipoproteinemia in patients with a normal level of LDL-C with or without elevations in VLDL-C. Clearly, there are many different etiologies of low levels of HDL-C, and not all are

associated with increased cardiovascular disease. Hopefully, a better understanding of the molecular defects responsible for the genetic diseases I discussed today will help clarify which subset of patients with low HDL-C levels are at increased risk for the development of ischemic heart disease.

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For review:

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