

## GENETICS AND CORONARY ARTERY DISEASE

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The study of the genetics associated with atherosclerotic coronary artery disease (CAD) is progressing rapidly. Many of the rare, single-gene defects apparent from family pedigrees have been documented. However, the larger issue remains that the disease has multiple potential etiologies (a multifactorial nature). And, the interplay of the environment with the products of hundreds of genes involved in lipid transport, blood clotting, carbohydrate metabolism, and vascular pressure regulation makes it almost impossible to isolate a predominant single defect that accounts for the majority of the CAD in the population.

Review of the current findings and theories in a framework that takes these considerations into account reveals a fragmented picture, but active investigations in all these areas continue, and new revelations that will bring more cohesion to the disparate theories about the origin of CAD should be anticipated.

First, let's look at the mechanisms whose interplay appears to predispose one to developing atherosclerosis and their associated genetic alterations. The abnormalities most clearly related to CAD are the dyslipidemias. The lipid disorders will be the focus of this review with lesser attention paid to other factors such as hypertension, clotting abnormalities, obesity, and other abnormalities of carbohydrate metabolism.

It should be mentioned that finding the DNA variations that result in a multifactorial disease is not as easy as uncovering the role of heredity in a disorder following a simple Mendelian mode of transmission and having a single gene defect. Diseases such as Huntington's or cystic fibrosis allow a phenotype-to-genotype strategy of study that has been very successful in mapping the one-to-one type relationship.<sup>1</sup> Genetic alterations leading to coronary artery disease, on the other hand, must often be inferred using statistical techniques and sophisticated models.

### Lipid metabolism

Before discussing dyslipidemias, it would be beneficial to run through a quick review of blood lipid regulation.<sup>2,3</sup> Lipoproteins are high molecular-weight particles that transport water-insoluble

lipids (cholesterol esters and triglycerides) through the plasma. The lipoproteins can be separated by electrophoresis. They are sometimes referred to by this electrophoretic pattern. Chylomicrons and remnants stay at the origin, very low-density lipoproteins (VLDL) migrate to the pre-beta region, low-density lipoproteins (LDL) migrate to the beta region with the beta-globulins (hence, beta-lipoproteins), as do the intermediate-density lipoproteins (IDL), and the high density lipoproteins migrate to the alpha-globulin region.

A disorder such as hyperalphalipoproteinemia would be expected to be characterized by elevations of HDL cholesterol. The lipoproteins are made up of a non-polar core of cholesterol esters and triglycerides that form an oily droplet. This core is surrounded by a single layer of phospholipid that contains proteins known as apolipoproteins. This outer layer serves as the interface that allows the lipoprotein to be soluble in water and contains the apolipoproteins that interact with enzymes and cell receptors. The apolipoproteins are associated with specific lipoproteins (Table 1).

In the gut, dietary cholesterol is absorbed and synthesized in enterocytes. The water-insoluble, dietary cholesterol esters and triglycerides are then wrapped in the phospholipid layer containing apo B-48 to form chylomicrons. Chylomicrons are then secreted into the intestinal lymph, eventually reaching the general circulation through the thoracic duct. In the circulation, a chylomicron quickly loses apo A-I, A-II, and A-IV and gains apo C-I, C-II, C-III, and E from VLDL and HDL. Lipoprotein lipase acts on the chylomicron in the presence of apo C-II after it binds to the vascular cell wall. This results in the lipolysis of triglycerides into free fatty acids (FFA) and monoglycerides. These FFAs and monoglycerides pass through the endothelium and are taken up by adipocytes and muscle cells for storage or oxidation (use in energy production).

When about 95 percent of the chylomicron's contents are cleared, it detaches from the vascular cell and is removed from the circulation by the liver. The remaining cholesterol in the chylomicron may be excreted by the liver into the bile or repackaged as VLDL and excreted into the circulation. Sixty to 80 percent of cholesterol is synthesized by the body; the remainder is absorbed in the diet. Excessive dietary lipids may result in an increase of chylomicron remnants, which are known to have the effect of

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inhibiting LDL receptors in the liver. This is one hypothesis for the observation that LDL cholesterol levels are increased with a diet high in fats.

Triglycerides and cholesterol esters synthesized in the liver are packaged as VLDL and released into the circulation. This synthesis is stimulated by the ingestion of excess carbohydrates. Apo B-100 is the principal apolipoprotein of VLDL. VLDL meets the same fate as chylomicrons in the peripheral capillaries. After lipolysis the VLDL is reduced to IDL, which can be bound by the liver or further metabolized (triglycerides removed) resulting in conversion to LDL. There are LDL receptors on many cells, and the cholesterol removed from LDL is used to synthesize cell membranes and steroid hormones. Excess LDL in the circulation is thought to be removed by the scavenger cell pathway. Chemical changes in LDL takes place in conditions of excess LDL that lead to its accumulation in macrophages and smooth muscle cells. This leads to the formation of foam cells, the precursors to atherosclerotic plaques.

HDL particles are secreted into the circulation devoid of cholesterol esters. Associated with the nascent HDL is lecithin: cholesterol acyltransferase (LCAT). LCAT acts to transfer cholesterol into HDL, resulting first in a mature HDL<sub>3</sub> and later a larger HDL<sub>2</sub>. HDL<sub>2</sub> returns cholesterol from peripheral tissues to the liver (known as reverse cholesterol transport) for subsequent excretion in the bile. Cholesterol ester transfer protein (CETP) catalyzes the exchange of insoluble cholesterol esters from HDL for triglyceride molecules from VLDL and chylomicrons in the circulation. HDL<sub>2</sub> transports these triglycerides to the liver for lipolysis, resulting in the conversion of HDL<sub>2</sub> back to the smaller HDL<sub>3</sub>.

### Dyslipidemias

The Frederickson classification of hyperlipidemias is a method of categorizing observed, phenotypic patterns of lipoprotein abnormalities (Table 2). As more information is discovered about the underlying defects in lipid metabolism, it is apparent that the lipoprotein patterns can result from many different molecular defects. Continued research may result in tests capable of defining the underlying defect in an individual having a particular pattern as a guide to prognosis and therapy.

### Total cholesterol, LDL, Apo B, Apo E, and the LDL receptor gene

While some controversy exists, the majority of twin studies have revealed a significant genetic influence on total cholesterol levels, with one estimate that heredity accounted for as high as 55 percent of the variations.<sup>4</sup> However (possibly due to the multifactorial nature of CAD), the frequency distribution of total cholesterol levels of those patients with CAD does not vary dramatically from that of the general population. In other words, very high total cholesterol or LDL levels correlate well with disease, but the absence of high levels does not preclude it.

A great amount of lipid research focuses on the lipoprotein whose elevation seems most closely correlated to atherosclerosis: the one involved in transport of cholesterol and binding to peripheral tissues, LDL. LDL particles are complex, being made up of cholesterol, phospholipids, triglyceride, and an outer shell of apolipoprotein B. Seven subclasses of LDL have been identified, with most individuals showing a predominance of one or two subclasses. The density of subclasses ranges from large and buoyant (more lipid, less apo B) to small and dense (less lipid content, more apo B).

Patients with CAD are found to have a preponderance of small, dense particles in their circulation. This type of LDL pattern typically has increased triglycerides, lower HDL cholesterol, and increased apo B. While some would claim that the LDL subclasses are strongly affected by genetics (with distributions in Mormon nuclear families approaching that seen in a single-gene, dominantly inherited trait), there is other evidence that hormonal and dietary influences can affect LDL particle size, making it an unreliable predictor of CAD risk. Response to dietary lipids affects LDL levels. It has been shown that there can be as much as a consistent 2.5-fold difference in the blood level response to a dietary cholesterol load. And this effect (hypo or hyperresponse) will persist in an individual over a period of years.<sup>5</sup>

Ten to 15 percent of individuals with premature (before age 60) coronary disease will have a common genetic disorder known as familial combined hyperlipidemia (FCHL). This disorder is associated with elevated levels of total cholesterol, triglycerides, apoprotein B, and dense LDL. It is characterized by multiple lipoprotein phenotypes (Fredrickson type IIa, IIb, IV) in the same family. The inheritance pattern suggests an autosomal trait with either a dominant or an additive pattern. Penetrance is reduced in males under the age of 20 to 25 and premenopausal women. The defect is not well defined, but may be related to increased production of apo B. In general, studies of Apo B levels in families with history of early CAD are inconclusive as to whether this is a major gene effect or a polygenic model with nontransmitted "types." It is quite possible that the genetic etiology of the elevation of Apo B is heterogeneous.<sup>6</sup>

Many geneticists feel that the "genetic architecture" of CAD involves many common alleles, each contributing small effects that combine additively. At least 200 genes may be involved in the regulation of lipid uptake in the gut, metabolism in the plasma, liver and peripheral cells, and removal from the body. In addition, there are a few loci with rare alleles that result in very large phenotypic effects.<sup>7</sup> Some examples of the latter, that are known to be associated with inter-individual variation of total plasma cholesterol and triglycerides, would be the single gene mutation that determines defective cell surface receptors for LDL particles, and the inherited isoforms of apo E, and the apo(a) molecule.

A well studied example of a single mutation is the familial defective apolipoprotein B-100 (FDB).<sup>8</sup> These individuals display mild to moderate elevation of LDL cholesterol. The LDL binding ac-

tivity is reduced in affected individuals, probably due to an altered apo B-100. It appears to be transmitted in a codominant fashion, resulting (in heterozygotes) in two populations of LDL particles. Those LDL particles containing the defective apo B are thought to be ineffectively cleared, resulting in moderate cholesterol elevations. The human apo B gene is located on chromosome 2p24 and spans 43 kilobases.<sup>9</sup> The defect is thought to be caused by a G to A mutation in exon 26 of the apolipoprotein B gene, which results in a substitution of glutamine for arginine (CGG to CAG mutation) at amino acid 3500 in apo B-100.<sup>8</sup>

To date, most of these cases have been found in those with elevated cholesterol, but it is estimated that the incidence of FDB in the general population may be between one in 500 to 700. Preliminary studies indicate that FDB may represent between three and six percent of familial hypercholesterolemia and Type IIa hyperlipidemia.<sup>8</sup> Apo B-100 connected to apo(a) by a disulfide bond is also referred to as lipoprotein(a)(Lp[a]).<sup>10</sup> This moiety is suspected of atherogenic properties due to a resemblance to plasminogen and will be discussed later in this paper.

Another influence on LDL levels is genetic variation in the apo E gene. The human apo E gene is located on chromosome 19q13. Apo E plays a role as the ligand on the surface of intermediate density lipoprotein (IDL) and chylomicron remnants that governs receptor mediated clearance of these particles. The three most common apo E alleles are E2, E3, and E4. These combine to result in six common apo E phenotypes. Approximately 90 percent of individuals having Fredrickson's type III hyperlipidemia will be homozygous with the E2/E2 isoform. However, only two percent of individuals with the E2/E2 isoform will have type III hyperlipidemia, so there must be another defect required for phenotypic expression. It has been shown that the E2/E2 isoform results in reduced rates of binding in the liver, thereby reducing the clearance of dietary fat, which affects the levels of LDL in the plasma.

And finally, mutations in the LDL receptor gene can cause dramatic effects on the plasma levels of LDL. Alterations in this gene have been shown to underlie the impairment familial hypercholesterolemia (FH). This gene is located on chromosome 19p13, and it is estimated that 100 different mutations of this gene exist. And it has been shown in some families that individuals heterozygous for the mutated gene have LDL cholesterol levels 140mg/dl higher than those with two normal alleles.<sup>9</sup> FH has an autosomal dominant transmission with severe disease in the homozygous individual. It is the second most common form of inherited hyperlipidemia, affecting one out of every 500 people.

### **HDL, apo A, apo C**

Turning to the HDL lipid fraction, you'll recall that HDL is believed to be beneficial through a mechanism of reverse transport of cholesterol. Genetic influence on HDL blood levels has been estimated to be from 35 percent to 50 percent. This HDL

contains mostly cholesterol and apolipoprotein A-1 (apo A-1). It is widely accepted that binding of specific HDL apolipoproteins to recognition sites on the cell surface of peripheral adipocytes, macrophages, and fibroblasts facilitates the mobilization and transfer of cholesterol pools to the HDL particle.<sup>11</sup> Other compounds crucial to this process, which have been found to be deficient or ineffective on the basis of genetic alterations, include:

- Lecithin cholesterol acyltransferase (LCAT), which esterifies the cell-derived cholesterol for transport;
- Cholesterol ester-transfer protein (CETP), which plays a role in transferring the cholesterol esters into and from IDL, LDL, and VLDL particles, which can then be taken up by hepatocytes via hepatic apo B and apo E receptors; and
- Apo C-III, which is encoded from a region closely clustered on a gene on chromosome 11q23 with the apo A-I and apo A-IV genes. This peptide has three isoforms and variation in percentage of total can be associated with hyperlipidemias.

More than 20 amino acid substitutions have been identified in apo A-I in studies of families. Interestingly, a few of these mutations result in severely reduced plasma levels of HDL but did not appear to be correlated with increased risk of CAD, while others did cause reduced HDL and increased risk of CAD.<sup>11</sup>

Studies of families with histories of premature CAD have identified certain transmission patterns and disorders (Table Three). Again, some of these patterns and disorders may be found to be associated with linked genes and not a single-gene defect.

### **Central obesity, impaired carbohydrate metabolism, and hypertension**

The other risk factors of CAD are well-recognized. There is also a well-recognized association among these risk factors, such that they often occur together in those with CAD. Body mass index (BMI) has been studied in monozygotic and dizygotic twins who were raised separately, with the conclusion that heredity accounted for an estimated 70 percent of the variation in BMI. Waist-to-hip ratio (WHR) has been shown to be an accurate measure that corresponds to visceral fat content and is found to aggregate in families. An increase in WHR has been associated to an increase in coronary morbidity, with the highest risk in that subgroup with a male-type upper-body obesity.

Diabetes has also been shown to have a hereditary component. In 58 percent of monozygotic co-twins of diabetic twins will develop diabetes between the ages of 52 and 65. And the cumulative prevalence rate of diabetes in offspring of two diabetic parents has been estimated at 36 percent by age 60.<sup>4</sup>

Hypertension has also been found to have a genetic component in twin studies of patients aged 42 to 56 years. And a hereditary disease called familial dyslipidemic hypertension has been described. This condition may be found in as many as 21 to 54 percent of families with premature coronary artery disease.<sup>4</sup>

There appears to be a connection between diabetes and hypertension because offspring of a parent with essential hypertension have been found to have increased insulin levels and decreased insulin sensitivity. Coronary heart disease incidence is known to rise progressively with an increase in blood pressure. Hypertension is also present in 26 to 50 percent of individuals with non-insulin dependent diabetes mellitus (NIDDM), which is two to three times the rate in the general population.<sup>4</sup>

### Clotting abnormalities

As mentioned earlier, the apo(a) gene is located on the long arm of chromosome six, adjacent to the plasminogen gene. Apo(a) and plasminogen have a homologous structure, and it is suspected that apo(a) can compete for the plasminogen cell receptors. Studies have shown that apo(a) can interfere with the processes involved in plasmin generation and clot lysis. It is also known to have the ability to bind to and immobilize fibrin in the vascular intima, thereby contributing to atherosclerotic plaque.<sup>10</sup>

### Cumulative mitochondrial DNA damage

The mutation of mitochondrial DNA (mtDNA) has been associated with a number of degenerative neuromuscular diseases. A general hypothesis of aging and degenerative disease would rely on the fact that oxidative phosphorylation (OXPHOS) is the process by which the cell generates ATP, the source of the cell's energy. The OXPHOS process relies on complexes encoded by nuclear DNA (nDNA) and mtDNA. Deprivation of nutrients and oxygen from ischemia results in oxidative damage to OXPHOS, inhibits mitochondrial biogenesis, and increases replication errors and mtDNA deletions.

Measurement of mtDNA deletions reveals that their accumulation accelerates in CAD. With aging, there is a gradual increase in the number of mtDNA deletions, but ischemic heart disease dramatically increases the number of deletions with the left ventricle showing the highest accumulation. The percentage of deletions seems to correlate well with the damage to the heart muscle.<sup>12</sup>

### Discussion

The model of the genetic basis of CAD is clearly complex and needs to be further defined. On the lowest level there are mutations and alterations in the myriad of genes that play a role in the coding of apolipoproteins, lipoproteins, receptors, clotting factors, pressure regulators, pathways of carbohydrate metabolism, determinates of obesity, and possibly others. These mutations may or may not be significant in the ultimate development of CAD. In the individual with significant genetic alterations there is a middle layer of abnormal effectors that may be additive or cancel one another out. These effectors are then acting in the milieu of external factors such as diet, exercise, smoking, and exposure to pollution, chemicals, infectious agents, etc. The final level is the phenotypic expression of disease or the absence

of disease that results from the interplay of the intermediate effectors and the environment. If you accept that this disease is multifactorial and that no major gene will be identified that causes the predominance of pathology, then the usefulness of the research will lie in those situations where further definition of the problem may result in better treatment or better determination of prognosis. For example, an individual with a significant elevation of LDL may be screened for receptor deficiencies or alterations of apo B to further define the defect for targeted therapy. It is unlikely that new screening tests, beyond the current lipid screens, will be utilized unless the test is found to be specific, sensitive, and tests for a prevalent cause of the disorder.

The risk selection of patients with elevated lipids has been discussed in other works.<sup>14,15</sup> Patients known to have molecular defects of lipid metabolism should be assessed on an individual basis with consideration given to family history and success of treatment. Also an appropriate additional rating for abnormal lipids should be considered when the individual with the known metabolic defect has already developed CAD.

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