

My name is Charlie Lowenstein, and I work at Johns Hopkins in the Division of Cardiology. I'd like to talk to you today about the genetics of atherosclerosis. In order to do that, first I'm going to discuss how family history is a risk factor for coronary artery disease. Then, I'm going to briefly examine the stages of atherogenesis. And next I've selected three of those stages, and I'm going to look at each one of those to see how scientists are studying the genetics of atherosclerosis. First, family history. I'm going to look briefly at the epidemiology and then discuss risk factors and studies that show that family history is a risk factor for atherosclerosis.

There are over eleven million Americans that have coronary artery disease and, each year, 1.5 million Americans have a myocardial infarction, or MI. About one-half a million Americans die annually from coronary artery disease, either acutely from a myocardial infarction or from the chronic complications of MI.

When we think about the risk factors for atherosclerosis, we often divide them into the traditional ones that are well established and the novel risk factors. Traditional ones are age, smoking and diabetes, and each year it appears that a novel risk factor comes along, like homocysteine, infections with *Chlamydia pneumoniae*, or C-reactive protein. But we can also think of risk factors for atherosclerosis in another way — those that are environmental and those that have a component of heritability. Those genetic risk factors include cholesterol levels, like a high LDL or low HDL, hypertension, a genetic predisposition for diabetes, and even unusual risk factors like C-reactive protein or Progeria syndromes.

I'd like briefly to mention environmental risks for atherosclerosis and why we think the environment is important before I focus on genetic risks for atherosclerosis. It's clear that environment is important. For example, there are population migration studies of the Japanese showing that the Japanese in Japan have a low incidence of coronary artery disease, but when they move to America, they have a higher incidence of coronary artery disease.

There are also environmental modification studies, for example, studies of the Pima Indians in Arizona. When they have the native American lifestyle, they have a low incidence of obesity and diabetes but, over the last few decades, as they've adopted the Western lifestyle, they have a much higher incidence of obesity and diabetes. So, clearly, the environment is important as a risk factor for atherosclerosis. But I'm not going to discuss the environment. I'm going to focus on genetic risks for atherosclerosis.

So, how important are genes as a risk factor for coronary artery disease? This is a complex question and, for many reasons, it's difficult to measure. Since coronary artery disease is very common as a population ages, one approach is to calculate the increased risk of premature coronary artery disease, for example, looking at relatives of affected individuals and comparing them to the general population.

There are about five to ten studies that have all given the same answer. The relative risk is five to seven-fold increased for people with first degree relatives with premature coronary artery disease.

How, then, have scientists discovered genes that can cause, or modify, atherosclerosis. Well, one way is to concentrate on monogenic disorders. These are rare but often have a large impact so it's easy to identify affected individuals. On the other hand, there are polygenic disorders, more common, with a smaller impact and, therefore, harder to identify.

Scientists often go to animal models with either candidate gene approaches or screening to identify genes which then will be studied in humans and also to confirm human results. Other scientists go directly to human studies, performing association studies with candidate or screening the whole genome, and I'll be discussing these human studies as we go along.

Here is one example of several recent studies looking at a linkage analysis for myocardial infarction. This was performed as a collaboration of United States and German scientists where they identified 500 European families with several members who had premature myocardial infarction, or coronary artery disease. They analyzed known risk factors, adjusted for those risk factors, and then did a total genome scan with about 400 markers and a linkage analysis.

As you can see on the diagram, even though the scale is too small to see clearly, you can see that there are several chromosomes, several regional chromosomes, which had a high LOD score. One of those regions in chromosome 14 had a LOD score of almost 4, and there are a set of unknown genes in this region which are actively being studied. The same group also looked for linkage with known loci which are known to be associated with coronary artery disease taken from the validity of their approach. And serum concentrations of Lp(a) in this cohort of about 500 families showed a strong linkage to the locus for apolipoprotein A. So, this is an approach which is validated for known genes and someday will prove of great potential into the discovery of new genes that are important for myocardial infarction.

So, there are many studies that show family history is a risk for coronary artery disease. Now, I'd like to discuss stages of atherogenesis. The reason I want to discuss these stages individually is because these provide ideal pathways in which we can search for candidates of genes that might predispose one to atherosclerosis.

The pathology of atherosclerosis is well-defined. A fatty streak leads to a fibrous plaque. A fatty streak often appears grossly in pathology as yellow, and histology shows foam cells. This can be seen in infants. A fibrous plaque is white because there's a smooth muscle cell cap surrounding macrophages and is often found in adolescents. If the plaque becomes unstable, it ruptures, either due to erosion or fissure, and ruptured plaques can be seen in adults.

While the first three stages — fatty streak, fibrous plaque, and ruptured plaque — can take years, decades, to develop, once a plaque ruptures, a thrombus forms immediately. Acutely, it appears red; chronically, when the red blood cells are lysed and the fibrin mesh is complete, it appears white and can be seen in adults.

Atherosclerosis involves changes in the phenotypes of cells, and we'll discuss this in more detail in a moment. Endothelial cells, which formerly were functional and protect the vessel, become dysfunctional. Smooth muscle cells, which are contractile, instead become synthetic. And macrophages, which are resting, become activated.

Inflammation is often the first step in the pathways to atherogenesis. Endothelial cells become dysfunctional, monocytes are recruited and differentiate, smooth muscle cells then migrate and proliferate and, finally, when the plaque ruptures, platelets are activated, they adhere to the vessel wall and aggravate with each other, forming a thrombus.

Here is a cartoon that illustrates all of these pathways. You can see a cross-section of an artery with a lumen on the top, lining of endothelial cells in the intima, and smooth muscle cells in the media below. Some form of inflammation activates these endothelial cells. The form of inflammation that is most studied is LDL. Particles of LDL can be taken up by the vessel wall. If they are oxidized in the oxidized LDL, they become even more atherogenic.

Endothelial cells damaged by inflammation, for example, oxidized LDL, express adhesion molecules. They also express chemoattractants, which attract leukocytes. Monocytes adhere to the vessel wall by interacting with endothelial adhesion markers. Then they migrate into the vessel wall, and the monocytes become macrophages. The macrophages can take up LDL, or oxidized LDL, and become foam cells.

These foam cells, differentiated macrophages, are highly active and release multiple factors, like TNF alpha, IL6, all inflammatory factors, and PDGF, and basic fibroblast growth factor. These factors, in turn, stimulate smooth muscle cells to migrate and proliferate and cause further endothelial dysfunction. Once the smooth muscle cells are activated, they secrete metalloproteinases, they alter the amounts and types of collagen that they produce, and the plaque becomes unstable. The plaque can then erode, or a fissure can form in the plaque. Once the sub-basement collagen is exposed, platelets adhere to the vessel wall, and then they can aggregate. Platelets, once they've aggregated, can release other factors, causing even more inflammation and more atherosclerosis.

So, there are discrete pathways which contribute to atherogenesis. Genes and the environment can contribute to each stage of atherogenesis. So, I've picked three pathways that are involved in atherosclerosis, and I'd like to speak about the genetics of each one of these pathways — lipids, endothelial cell function, and inflammation.

First, lipids, and I'll be discussing lipid transport, diagnosis of hyperlipoproteinemias and, finally, familial syndromes. I just want to review the process we're studying is LDL entering a vessel wall and becoming oxidized.

It turns out that there are genetic diseases which are autosomal dominant or recessive. And there are examples of both of these in the hypercholesterolemic syndromes that run in families. For recessive diseases, as you know, both genes must be abnormal for a phenotype. For autosomal dominant diseases, one abnormal gene can cause the phenotype. The classic example of a familial hyperlipidemia syndrome is familial hypercholesterolemia, or FH. This is characterized by elevated cholesterol and premature coronary artery disease and heart attacks. Even though it's autosomal dominant, and even though most people show up as heterozygotes, very, very rarely one can find homozygotes, one in a million, which have extremely high cholesterol and heart attacks as children.

The discovery and characterization of FH is an amazingly interesting story. In 1966, Brown and Goldstein were interns at the MGH in Boston, Massachusetts, and they first met each other. They both post-doc'd in separate labs at the NIH, and they became interested in familial hypercholesterolemia when they saw two children with severe FH. Several years later, they both ended up at the University of Texas in Dallas, UT Southwestern, and they both began studying cholesterol metabolism in cultured cells.

In 1973, a young girl with FH came to their attention. It turns out, as luck would have it, that this was a patient with a very rare homozygous form of FH. When Brown and Goldstein added LDL cholesterol to normal cells, normally, LDL cholesterol would shut down cholesterol synthesis in normal cells. However, when they added LDL cholesterol to the cells from this patient, JP, cholesterol synthesis did not shut down.

In collaboration with other workers at UT Southwestern, when they added radiolabeled LDL, they found that the LDL bound to normal cells, but not to the cells from JP. This led to the discovery of the LDL receptor. LDL, it turns out, binds to an LDL receptor on the cell surface, but patients with homozygous FH lack both copies of the LDLR gene, don't make any LDL receptor, and can't bind LDL.

Brown and Goldstein had discovered an example of receptor mediated endocytosis. In this schematic, LDL is binding to the LDL receptor, it is endocytosed, cholesterol is released from the endosome, and then the receptor recycles to the cell surface.

In 1982, in collaboration with Anderson, Brown and Goldstein isolated the LDL receptor gene and, two years later, characterized the first of what turned out to be hundreds of different mutations in the LDL receptor. And in 1985, they received the Nobel Prize for Physiology or Medicine for their discoveries.

Since then, it's turned out that we've characterized other genetic defects which can cause elevated cholesterol in coronary artery disease. Many of these diseases affect

lipoprotein metabolism. Lipoproteins are particles that carry cholesterol and triglyceride in the plasma. They have a non-polar lipid core made of non-polar lipids like triglyceride and cholesterol esters, and they're surrounded by apolipoproteins and non-esterified cholesterol.

Cholesterol transport in the body is made up of, in simplified terms, two separate pathways — an exogenous and an endogenous pathway. In the exogenous pathway, we eat dietary fat, it's packaged and sent to the liver, and then can recycle and be excreted in the small intestine. In the endogenous pathway, the liver synthesizes lipoprotein, it's delivered in the circulation to peripheral cells, and then recycles back to the liver. These pathways have been well characterized, and I'm not going to spend a lot of detail on them. I will show you, however, that this pathway involves many steps.

On the left is the exogenous pathway where dietary fat is packaged into chylomicrons. Chylomicrons are taken up by the liver, and the liver then secretes sterols and cholesterol back into the small intestine. And in the endogenous pathway, the liver packages cholesterol and triglycerides in the LDL particles, which then eventually turn into LDL particles which deliver cholesterol to peripheral cells and then are returned to the liver. When there is vast excess of LDL, as you can see in the red cell up in the top, LDL is delivered inappropriately to the arterial wall.

Certain proteins on the surface of all of these lipoproteins, for example, ApoB, regulate the metabolism, biosynthesis, and the clearance of all of these lipoproteins. And I've only shown ApoB, one of many apolipoproteins as an example.

Over the last 20 to 30 years, lipoprotein diseases have been classified. This is well-known, and I'm not going to spend a lot of time on it. They are divided into different types, Type 1 through 5, which are characterized by different elevations in LDL, VLDL, or chylomicrons. There are now classifications of diseases of hyperlipoproteinemia. For example, there are monogenic diseases that elevate cholesterol, that are hypercholesterolemic, or that have elevated triglyceride, or that have both elevated cholesterol and triglycerides. For example, autosomal dominant diseases include familial hypercholesterolemia, characterized by a defect in LDL receptor, or familial defective ApoB, characterized by a deficiency in ApoB.

There are also monogenic primary hyperlipoproteinemias that are inherited in an autosomal recessive pattern, for example, familial LPL deficiency, and familial apoC2 deficiency. One of the most interesting of these is sitosterolemia where patients can't excrete large amounts of ingested plant sterols. Recently, Helen Hobbs, at the University of Texas Southwestern, discovered the molecular cause of this disease. Mutations in two ABC binding cassette, or ABC proteins, which are responsible for mediating efflux of plant sterols out of the liver into the intestine and then excreted.

Another disease that Dr. Hobbs discovered the basis for is autosomal recessive hypercholesterolemia, or ARH. The ARH gene encodes an adapter molecule that binds in the inside of the cell to the cytoplasmic domain of the LDL receptor and helps facilitate

internalization of the LDL receptor and the LDL particle. Thus, a deficiency in ARH is, in a sense, a phenocopy of a defect in the LDL receptor. So, there are also a set of monogenic primary hyperlipoproteinemias that are inherited in an autosomal recessive pattern.

There are also primary hyperlipoproteinemias of unknown etiology. Among the five percent of the population with the highest LDL, about one in twenty have FH, and about two in twenty have familial combined hypercholesterolemia. But the remainder, 17 out of 20 of those with the highest LDL have a polygenic syndrome. Polygenic hypercholesterolemia is not well-defined. Patients with high LDL, but those with few affected individuals and the lack of peripheral findings, such as tendon xanthomas. It is thought that this is a disease due to multiple genetic and environmental factors. Perhaps many polymorphisms in proteins regulating lipoprotein metabolism and clearance, are involved.

Finally, there are other genetic disorders which don't directly affect lipoprotein metabolism but involve other systems which indirectly affect it. These are secondary hyperlipoproteinemias that include endocrine diseases, drugs, renal diseases, and other diseases as well.

So, in summary, there are a set of hyperlipoproteinemias which involve abnormal lipid transport caused by increased synthesis, or decreased degradation of lipoproteins. The etiology of some of these diseases is well-defined — primary defects in lipoprotein synthesis or degradation.

Clinically, most of these disorders are characterized either by premature atherosclerosis or pancreatitis, and treatment with diet and drugs can lower cholesterol and reduce the risk of myocardial infarction in these patients.

So, I've spoken about the genetics of atherosclerosis and lipid pathways. Now, I'm going to turn to more recent discoveries, some of which are speculative, but which emphasize the idea that there are other pathways which are important in the process of atherogenesis.

First, endothelial cells and endothelial function. Going back to our stages of atherosclerosis, one of the first problems in a vessel wall can be traced to endothelial cell dysfunction, or endothelial cell activation, with the endothelial cell expressing markers and releasing mediators which perpetuate vascular inflammation.

Endothelial cells maintain the vessel integrity. Not only do they cause vasodilatation, but they block inflammation and inhibit vessel thrombosis. Endothelial cells do this by releasing a wide variety of factors, factors that promote vasodilatation, inhibit vasoconstriction, block platelet function, promote anticoagulation and fibrinolysis, and inhibit inflammation.

And it makes sense that if there are genetic defects in any one of these individual pathways, a patient will be at risk or be predisposed to atherosclerosis. One potential

pathway is a pathway of endothelial cell inflammation. This pathway involves nitric oxide and endothelial cell exocytosis.

Nitric oxide is one of the major defining products of an endothelial cell. Nitric oxide acts as a vasodilator, relaxing smooth muscle, and antithrombotic, inhibiting platelet adhesion and aggregation, and also has mysterious anti-inflammatory properties. Conversely, during vessel inflammation, endothelial cells can't make NO. There's less vascular protection, and lack of NO causes vasoconstriction, it permits platelets to form thrombi, and it is also pro-inflammatory.

We recently discovered that the lack of nitric oxide permits endothelial cells to release inflammatory granules. As you can see on the left, wild-type mice have lots of endothelial granules shown in brown. However, on the right, nitric oxide synthase null mice that can't make nitric oxide lack these normal little brown dots and, instead, have a wide sweeping release of those brown dots in the sub-endothelial space, where you can see them as a large brown swath. Those brown granules, containing von Willebrand's factor, are inflammatory granules inside endothelial cells, also called Weibel-Palade bodies. Normal resting endothelial cells have lots of these Weibel-Palade bodies inside them but, during inflammation, when inflammatory mediators like thrombin, complement, leukotrienes, any of a vast set of inflammatory stimuli that can start the process of atherosclerosis. When these inflammatory mediators activate endothelial cells, the endothelial cells become inflamed and the hallmark of inflamed endothelial cells is that they instantly release these granules in a process called exocytosis. These granules can be released into the vessel lumen above, releasing P selectin, IL8, and Von Willebrand's Factor, or VWF, factors that promote thrombosis and leukocyte activation. It can also be released into the vessel wall which you can see as the red smear below, further activating leukocytes.

We recently discovered that nitric oxide can block this process, that one of the reasons nitric oxide is beneficial is because nitric oxide can block vascular inflammation. Here's how. One way we've shown this is by taking a movie of platelets as they roll along a vessel wall. We took a mouse and fluorescently labeled platelets and injected these platelets. When we treated a mouse with histamine, as we'll see in just a moment, you can see these platelets rolling along. These platelets show up as a white streak going from the top right to the bottom left of your screen. The endothelial cells have become activated by histamine, the platelets are sticking to them, forming microthrombi. However, if we pre-treat the animals with nitroarginine and block nitric oxide synthesis, there is a dramatic change. Now you can see that platelets are much more sticky. The endothelial cells have been activated, but they lack the protective effects of nitric oxide. Now, platelets that you can see are rolling along at a higher frequency, they're much slower, and there are many more of them sticking to the vessel wall. This shows that nitric oxide, endogenous nitric oxide, made in the vessel wall, can regulate platelet adhesion in mice. The same phenomenon is true for leukocyte adhesion. Nitric oxide, made by your vessels, blocks thrombus and blocks vascular inflammation.

We discover that the mechanism for this is that nitric oxide interrupts the cycle of granule transport where granules are budded, they dock, and then they release their contents by fusing with the vessel wall, and nitric oxide blocks the molecular machinery which causes granule transport infusion.

So, exocytosis of endothelial cell granules can activate vascular inflammation, but nitric oxide can block that process. Nitric oxide blocks inflammation by regulating exocytosis. And it's quite reasonable to expect that mutations in these genes might influence atherosclerosis. For example, there are several studies out now looking at mutations in the proteins that make nitric oxide. There are also studies looking at SNPs in proteins that regulate exocytosis. But I'm going to focus instead on a recent study that examines the process of endothelial cell development in transcription.

Wang, Topol and Wang recently showed that a critical endothelial cell transcription factor is related to coronary artery disease. They studied a large family of which 13 had premature coronary artery disease, and 9 of those 13 had premature myocardial infarctions, and it appeared that this was inherited in an autosomal dominant pattern. A genome-wide linkage analysis, with about 400 markers, showed positive linkage for one of these markers for a LOD score of 4 in chromosome 15q26.

You can see the pedigree on the bottom right. In their Science article on the bottom left, they showed an area of atherosclerosis marked by the arrow and, on the far bottom right, is a picture of chromosome 15.

This identified region, they discovered, had about 95 genes, and one of those was a candidate, MEF2A or MEF2A. When they sequenced MEF2A, they identified a 21-base pair deletion, causing a deletion in 7 amino acids. This mutation was present in all 10 of the living affected family members and absent from all other family members. Furthermore, it was absent from another set of controls, 119 with normal coronary angiograms.

It turned out that MEF2A, MEF2A, studied by many investigators, including Eric Olson, encodes myocyte enhancer factor 2A, expressed in endothelial cells, a transcription factor that enters the nucleus and regulates gene expression. The mutated region in these affected patients encodes a nuclear localization domain. Thus, this mutant protein can't enter the nucleus.

So, in the confocal microscopy shown below, the top three bars show wild-type MEF, the blue dots show a normal nucleus, and the green dots in the center on the top show normal MEF2A entering the nucleus. And on the far right on the top, you can see that the green and blue dots perfectly overlay with each other. The MEF2A is entering the nucleus. In contrast, if you look at the mutated MEF2A on the bottom three panels, on the left you can see nuclei, and on the bottom middle pattern, on the bottom middle panel, you can see that MEF2A is not in the nuclei; it's sticking in the cytoplasm of the cells. So, there's no co-localization in the overlay on the bottom right. The green MEF2A does not appear in the nucleus that's colored

blue. So, the mutated MEF2A can't enter the nucleus and, in other figures in their paper, they show that mutated MEF2A doesn't activate transcription the way it's supposed to.

So, mutations in MEF2A may play a role in atherogenesis in some patients. Now this general approach might identify mutations in other genes that regulate endothelial cells. For example, in the same study, Topol et al. found three large kindreds, three large other kindreds with premature myocardial infarction, and this was not linked to chromosome 15q26.

Now, I would like to turn to inflammation, a critical and interesting component of atherogenesis. I'll focus on innate immunity and look at the receptors and effectors of the innate immune system. Over the last ten to twenty years, more and more investigators have come to the realization that atherosclerosis is an inflammatory disease, and inflammation plays a role in every stage of atherosclerosis. Selected inflammatory pathways might be activated during atherosclerosis and, therefore, mutations in genes in these selected pathways might influence atherosclerosis.

As I described before, there are several stages of atherosclerosis in which inflammation is important, for example, macrophages entering a vessel, differentiating into foam cells, and releasing factors which can activate endothelial cells and smooth muscle cells.

So, which pathways are activated in atherogenesis? In general, inflammation is a response to injury. Injury engenders an inflammatory response designed to kill pathogens, limit damage, and repair tissue. Inflammation and the immune response can be divided into two large categories. On one hand, there's the innate immune system and, on the other hand, there is the adaptive immune system. Innate immunity is rapid and not very specific. Adaptive immunity, in contrast, is slow and much more specific. And there's an interesting interaction between the innate and the adaptive immune systems.

So, which of these pathways is activated in atherogenesis? Well, there's some evidence for adaptive immunity, but there's much more evidence that innate immunity is activated during the process of atherosclerosis. Now, innate immune cells use a limited, a very limited, group of receptors to recognize pathogen targets. If you're going to contrast adaptive and innate immunity, innate immunity is more rapid, it is more general and, for recognition, it uses special receptors. These receptors are encoded in the germ line. In contrast, in adaptive immunity, the receptors are rearranged throughout development, for example, B-cell rearrangement of antibody genes and T-cell rearrangement of T-cell receptor genes.

So, how does the innate immune system recognize antigens? Well, the antigens that it's designed to recognize are pathogen-associated molecular patterns, invariant patterns that are expressed by microbes, normally not found in the host. These targets are normally recognized by the innate immune system using pattern recognition receptors. These are invariant receptors encoded in the germ line; they're not rearranged. They can involve proteins that are secreted, chemoproteins, receptors that engulf antigens, or signaling receptors. One of the classic pattern recognition receptors is a set of toll-like receptors. I'm going to give one

example of the toll-like receptors. It turns out that gram negative rods have lipopolysaccharide, or LPS, also called endotoxin, on their surface. LPS is recognized by an LBP, or lipopolysaccharide binding protein, which delivers LPS to two molecules — CD14 and a toll-like receptor number 4. TLR4, once it engages this complex, activates a downstream signaling cascade. In vertebrates, it looks like this. A pathogen interacts with a toll-like receptor, the toll-like receptor then interacts with accessory molecules, like myD88 and IRAK, and then a signaling cascade is activated which results in transcription and an anti-pathogen response. This is how, this is one of the many ways in which the body fights infections.

So, are there any aspects of innate immunity which is designed to attack pathogens? Are there any aspects of innate immunity which are related to coronary artery disease? Well, a collaboration between Austrian and American scientists studied subjects enrolled in Italy in the Bruneck study, a prospective survey of atherosclerosis. In this study, 800 subjects were followed for ten years, and the researchers measured, among other things, carotid artery disease, including intimal media thickness and atherosclerotic plaque diameter in the carotid arteries. Then, they assessed two toll-like receptor for polymorphisms which had been described previously and which decrease signaling.

Of the 800 patients that they tested, 53 were heterozygous for an ASP 299 glycine polymorphism, and 2 of the 810 were homozygous. The presence of this polymorphism was associated with several very interesting clinical findings. This polymorphism was associated with lower plasma levels of inflammatory cytokines, as if there was a malfunction in innate immune signaling. If there's an innate immune system malfunction, you would expect that there would be an increased risk of infection and, indeed, this polymorphism was associated with an increased risk of infection, a large increase, 33 percent versus only 6 percent in the wild-type patients.

Most interesting, there was a reduced risk of atherosclerosis in patients with this polymorphism. This was measured in two ways. One is intima media thickness was decreased, 937 with those who had polymorphisms versus 1,006 microns for those who were wild-type, and the plaque diameter was also decreased about 3.0 versus 4.3 millimeters.

So, this is an incredibly interesting and provocative study. This suggests, first of all, a functional innate immune system can protect against infections. After all, mutations reduce your protection from infection. Furthermore, it suggests that the innate immune pathways normally mediate a low-grade inflammation in healthy subjects, perhaps we are always being bombarded by pathogens, and/or innate immune system has a low level of inflammation geared to suppress these infections.

So, the innate immune system is beneficial. However, an active immune system which fights against lots of infections can also damage the vasculature. An absence of the innate immune system, mutations in the toll-like receptor, decreased atherosclerosis. This is an

important and interesting study. It suggests that mutations and other innate genes can also influence atherogenesis.

So, in summary, the pathology of atherogenesis involves discrete stages. The pathophysiology includes overlapping pathways, and inflammation plays a prominent role throughout atherogenesis. Genetic alterations can influence atherogenesis. For example, there are genetic defects in pathways of lipid transport, endothelial cell function, and inflammation, all associated with changes in atherosclerosis. Many monogenic diseases have been identified which alter the risk of atherosclerosis. And, in the future, our challenge is to identify polygenic diseases to understand them better to identify combinations of polymorphisms and mutations which predispose large populations to atherosclerosis. And, to explore the interaction of the environment and genes.

Thank you.