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Spontaneous Atherosclerosis in Pigeons: A Good Model of Human Disease

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1. Introduction

Avian models of human atherosclerosis such as the chicken, turkey, quail, and pigeon are not currently in widespread use, but have a longer and richer history than most mammalian models of cardiovascular disease. In 1874, the first angioplasty surgery of the aortic wall was performed in birds (Roberts & Strauss, 1965). Spontaneous (non-induced) atherosclerosis in the chicken was first described in 1914 (Roberts & Strauss, 1965), and it has been repeatedly observed that avian lesions bear close resemblance to their human counterparts (Clarkson et al., 1959; Herndon et al., 1962; Cornhill et al., 1980b; Qin & Nishimura, 1998). The pigeon (*Columba livia*) is especially suited for genetic studies of atherosclerosis because susceptible and resistant strains exist in the natural population (Herndon et al., 1962; St. Clair, 1983) eliminating the need to construct an artificial phenotype through genetic or dietary manipulation. In fact, it has been suggested that the White Carneau (WC) pigeon may be one of the most appropriate models of early human lesions (Cornhill et al., 1980b; St. Clair, 1998; Moghadasian et al., 2001). This review is comprised of background information on human atherosclerosis, a description of other animal models and details of the pigeon model.

Atherosclerosis is the most common form of heart disease, a general term encompassing a variety of pathologies affecting the heart and circulatory system. More specifically, atherosclerosis is a disease of the blood vessel itself, and is most likely to develop at branch points and other regions of low shear stress along the arterial tree, such as the celiac bifurcation of the aorta, and in coronary and carotid arteries (Bassiouny et al., 1994; Kjaernes et al., 1981). The disease is a chronic and multifactorial result of both environmental and genetic factors, as well as their interactions (Breslow, 2000; Moghadasian et al., 2001). It remains the number one cause of morbidity and mortality in the United States and other developed countries (Gurr, 1992; Wagner, 1978).

Arterial lesions begin to develop during childhood as lipid-filled foam cells making up "fatty streaks" (Napoli et al., 2002; Stary, 1989), and slowly progress into complex plaques consisting of multiple cell types, intra- and extracellular cholesterol esters, calcium deposits, proteoglycans, and extensive connective tissue. The final and terminating atherosclerotic event is blood vessel occlusion, often caused by plaque rupture, which can lead to a heart attack, stroke, or embolism, depending on the location of the affected artery. However, not all fatty streaks progress to advanced lesions (Getz, 2000), and their progression/regression rate, although well correlated with classical risk factors, is unique to each individual.

Clinical symptoms do not usually appear until later in life (Munro & Cotran, 1988; Stary, 1989). Therefore, research and intervention strategies have focused on delaying the progression of plaque formation rather than preventing the appearance of foam cells or fatty streaks. There is a strong familial component to all forms of heart disease, and many genetic disorders have been identified that contribute to lesion progression and the probability of plaque rupture in the general population. However, little is known about the specific genes that determine predisposition to the disease, nor how these genes interact with each other and the environment to initiate atherosclerotic foam cell formation in any one individual.

2. Human atherogenesis

2.1 The observed beginning: Foam cells and lesion development

In human lesions, early foam cells originate primarily from vascular smooth muscle cells (VSMC) [Wissler et al., 1996]. They are the first cell type to appear in susceptible regions of the aorta (Balis et al., 1964; Ross & Glomset, 1973), and the most abundant cell type in developing fatty streaks (Gabbiani et al., 1984; Katsuda & Okada, 1994; Mosse et al., 1985, 1986; Wissler et al., 1996;). Early electron microscopy studies noted that VSMC were often filled with lipid when there was no lipid in either existing macrophages or in the extracellular space, but the reverse was never observed.

Since those observations, multiple investigators have reported that abnormal VSMC accumulation in susceptible aortic regions precedes the actual lipid accumulation (Mosse et al., 1985; Ross & Glomset, 1973). Atherosclerotic foam cells can be derived from both VSMC and macrophages (Adelman & St. Clair, 1988; Wissler et al., 1996), depending on their physical location (Strong et al., 1999) and the cause of initiation. For example, plaques that develop along the descending thoracic aorta have more macrophages than VSMC, whereas plaques along the abdominal aorta and coronary arteries are comprised mostly of VSMC, with very few macrophages. Human thoracic plaques are very rare, and those that do progress are usually secondary to other chronic conditions such as hypertension and hyperlipidemia (Wissler et al., 1996).

Although VSMC are the first cell type to accumulate lipid and initiate the fatty streak (Doran et al., 2008), much emphasis is placed on macrophage foam cells rather than myogenic foam cells. Macrophage-derived foam cells are quick to develop into lesions and are easy to induce with a high-fat and/or high-cholesterol diet (Knowles & Maeda, 2000; Xu, 2004; Zhang et al., 1992), in common animal models of human atherosclerosis, especially transgenic mice. Unlike VSMC, which can alternate between contractile and synthetic phenotypes, macrophage cells do not change during the disease progression, and so are easier to identify in the laboratory under controlled conditions.

Greater emphasis on macrophage-derived foam cells is problematic because the pathogenic lipid accumulation mechanism appears to be dissimilar for the two cell types. Also, rather than being a primary initiative event in humans, the arrival of macrophages appears to be a secondary response, as they are far more common in advanced plaques than in early lesions (Balis et al., 1964; Nakashima et al., 2007; Stary, 1989; Wissler et al., 1996; Zhang et al., 1992;).

2.2 Atherogenesis risk factors

Major physiological conditions such as high blood cholesterol, high blood pressure, diabetes, a skewed lipoprotein profile, heredity, advanced age, and maleness can increase an

individual's chance of developing atherosclerosis. Collectively, these risk factors, along with lifestyle patterns such as physical inactivity, smoking, obesity, and stress have been statistically correlated with specific stages of lesion development, plaque stability, and overall disease outcome in the general population. Although genotype clearly influences many quantitative traits such as LDL/HDL levels, blood pressure, and adiposity (Gibbons et al., 2004), progress has been made on minimizing the effects of the controllable risk factors in order to disrupt, delay, reverse, or otherwise deter plaque rupture and aortic occlusion in high risk individuals.

Despite moderate success, especially in the realm of cholesterol-lowering drugs, unknown genetic factors continue to influence both the age of onset as well as the frequency/severity of clinical symptoms (Funke & Assmann, 1999). Unfortunately, by the time most people manifest clinical symptoms, it is too late to implement preventative measures because the disease is well into the progressive stage. Early identification of susceptible individuals allows timely therapeutic treatment. Less than 50% of the mortality risk from coronary heart disease can be explained by currently recognized risk factors (Ridker, 2000), even with early diagnosis.

In order to understand events in the at-risk population that remain unidentified under current screening methods, the specific contributions of heredity, diet, and lifestyle influences on atherogenesis and progression must be determined. Towards this end, research emphasis has recently shifted towards identifying cardiovascular disease markers that may be detectable prior to the manifestation of clinical symptoms. Markers are simply variations in alleles that are known to associate with a specific disease phenotype. Markers do not necessarily cause the disease, but can be used to improve diagnosis and risk assessment (Gibbons et al., 2004). Inflammatory markers such as C-reactive protein (CRP) factors [Tsimikas et al., 2006; Ridker, 2000] plus markers of oxidative damage such as myeloperoxidase (Shao et al., 2006) and paraoxanase (Visvikis-Siest & Marteau, 2006) have already increased clinicians' predictive power. As more markers of atherosclerosis are correlated with disease progression and outcome, the genetic variation contributing to predisposition and initial manifestation will become clear.

Until the genetic basis for susceptibility to atherosclerosis is understood, correlation of various risk factors with specific metabolic or pathological features will be difficult to assess, and efforts for prevention will remain equivocal. Understanding the inheritance mechanisms for atherosclerosis is an important step towards reducing the morbidity and mortality from the disease by customizing intervention strategies for individuals based upon unique genotypes and environmental risk exposures.

3. Genetic defects in human atherogenesis

The relative risk for atherosclerosis is clearly higher in individuals with a familial history compared with those having a susceptible lipid profile (Funk & Assmann, 1999; Ordovas & Shen, 2002; Palinski & Napoli, 2002). Many studies have explored the relationship, or concordance, between heredity and atherosclerosis. Heritability for early-onset coronary heart disease has been estimated at 0.63 (Galton & Ferns, 1989). The relationship becomes even clearer after analyzing concordance in twin studies. Twins fertilized from one egg (monozygotic) have a concordance rate of 0.83, whereas twins that arose from two separate fertilizations (dizygotic) demonstrate a concordance rate of 0.22 (Galton & Ferns, 1989).

These concordance values suggest an intimate relationship between the genotype of an individual and the incidence of heart disease. The fact that the concordance rate in monozygotic twins is less than 1.0 (indicating 100% correlation) most likely reflects the attenuating environmental effects on atherosclerosis initiation and progression. This gap in causality underscores the importance of understanding the genetic profile of a client before attempting intervention, because even among those sharing the same set of alleles, the atherosclerosis phenotype will vary depending on individual exposures.

Genetic research on human atherosclerosis has focused primarily on the role of cholesterol metabolism. It is estimated that several hundred genes (Ordovas & Shen, 2002) are involved in the absorption, conversion, transport, deposition, excretion, and biosynthesis, of cholesterol and other lipid substrates in the body (Knowles & Maeda, 2000; Stein et al., 2002). Very few of these genes have been characterized. A defect in any of these pathways may contribute to atherosclerotic susceptibility, because the net result can be a significant increase in plasma lipoprotein concentration, especially LDL, and/or the inappropriate deposition of cholesterol in peripheral tissues such as skin, tendons, and arteries (Garcia et al., 2001).

Blood lipid homeostasis and cellular cholesterol metabolism are highly regulated (Attie, 2001). Genetic defects have been found to impact overall cholesterol metabolism at many steps. In humans, most plasma cholesterol is in the form of LDL, having a half-life of about 2.5 days (Goldstein & Brown, 2001). Some of the cholesterol component of LDL is transferred to HDL via the action of cholesterol ester transfer protein (CETP). However, as much as 70% of LDL is removed from the blood by LDL receptors (LDLR) in the liver (Garcia et al., 2001). A variety of single gene defects have been identified that increase the incidence of atherosclerosis by influencing the LDLR activity (Funke & Assmann, 1999).

Probably the most studied of these LDLR defects is familial hypercholesterolemia (FH), an autosomal dominant Mendelian disorder (Brown et al., 1981; Funke & Assmann, 1999; Goldstein & Brown, 2001). This mutation renders the hepatic receptors nonfunctional, so that they are unable to clear circulating LDL from the blood. A second type of hypercholesterolemia, autosomal recessive hypercholesterolemia (ARH), also impacts the LDLR (Garcia et al., 2001; Goldstein & Brown, 2001). ARH is similar to FH, in that both of these hereditary defects result in chronically elevated blood cholesterol. This imbalance has the potential to change the physiology of the arterial wall, making it exceptionally vulnerable to atherogenesis. However, unlike FH, the LDLR in ARH, are believed to be functional, but their altered location in the liver makes them inaccessible to circulating LDL.

Brown and Goldstein also identified a single gene defect known as familial ligand defective apoB-100, the primary human LDL (Fielding et al., 2000) apoprotein. This inherited defect lies in the composition and binding capacity of the apoB-100 to the LDLR, decreasing the ability of the LDL to be picked up by the LDLR (Goldstein & Brown, 2001; Gurr, 1992). In the healthy human aorta, LDL particles are thought to be incorporated into SMC by receptor mediator endocytosis. Chemically modified or oxidized LDL enters via scavenger receptors. Once inside the cell, the LDL cholesterol esters (CE) are transported to the lysosomes where they are hydrolyzed by lysosomal acid lipase (LAL), also known as acid cholesterol ester hydrolase (ACEH). This enzyme breaks each CE into its free fatty acid (usually linoleate), and free cholesterol. There are several known LAL gene mutations that result in the abnormal accumulation of cholesterol esters in the lysosome.

Two of the more common lysosomal storage disease phenotypes of a LAL mutation are Wolman's Disease (Kuriyama et al., 1990; Lohse et al., 1999) and cholesterol ester storage disease (CESD). Both are inherited as an autosomal recessive trait, although Wolman's disease is usually fatal within the first year of life, and so not directly related to atherogenesis in the general population. However, individuals with CESD do demonstrate premature atherosclerosis, in addition to accumulating CE and triglycerides (TG) in the liver, adrenal glands and intestines (Pagani et al., 1996). Niemann-Pick Type C is a third form of lysosomal storage disease that directly impacts cholesterol metabolism at the cellular level (Blanchette-Mackie et al., 1988). In this condition, the CE is successfully hydrolyzed by ACEH, but the released cholesterol component is unable to leave the lysosome to travel to the endoplasmic reticulum, causing the accumulation of free cholesterol in the lysosome.

Lysosomes are also responsible for the degradation of glycosaminoglycan (GAG) chains after the core proteoglycan has been broken down by extracellular proteases such as matrix metallopeptidases (MMP) and disintigrins (ADAMs) [Arndt et al, 2002; Seals & Courtneidge, 2003). There is an extensive repertoire of catalytic lysosomal enzymes, and their functions have been revealed mostly by observing the consequences of their absence (Santamarina-Fojo et al., 2001). Defective enzymes lead to a wide variety of diseased phenotypes known as mucopolysaccharidoses (MP) ranging from the mild Schie Disease to the severe Hurler Disease, which results in childhood mortality. In these two examples, GAGs are not properly degraded, and so will accumulate in the lysosomes and in the extracellular space. GAGs in the ECM will attract LDL that has entered the intima by binding to apoB-100 as previously described, where the cholesterol is most likely endocytosed by macrophages and SMC within the developing plaque.

Once in the cytoplasm, cholesterol that is not needed for routine cellular functions is esterified by acyl CoA: cholesterol acyltransferase (ACAT) and stored in vacuoles. Intracellular CE remains trapped in the cytoplasm until hydrolyzed by neutral cholesterol ester hydrolase (NCEH). This enzyme releases the free cholesterol so it can be removed by HDL and transported to the liver. A pair of ATP binding cassette proteins has been identified that are believed to control this efflux of cellular cholesterol. One of these, ABCP–1 is defective in Tangier Disease (Faber et al., 2002), an inherited condition where cholesterol is unable to exit the cell via reverse cholesterol transport. There is a moderate risk of atherogenesis associated with Tangier Disease, which is increased in the presence of additional risk factors (Tall et al., 2001)

Research is directed towards a range of HDL-associated apoproteins. Genetic factors account for approximately 50% of the variance of HDL composition and plasma concentration in the general population (Tall et al., 2001). The primary apoprotein in HDL is apoA1, followed by apoA2, apoC, and apoE (Fielding, 2000). ApoE is an important ligand for receptor-mediated clearance of HDL from arterial cells (Moghadasian et al., 2001; Stein et al., 2002), whose role is of great interest to investigators of atherosclerotic resistance because most patients with familial dysbetalipoproteinemia (FD) are homozygous for the E2 isoform of apoE (Johns Hopkins University, 2011). Although this defect has been shown to be relevant in some animal models, especially apoE null mice (Smith et al., 2006; Zhang et al., 1992), only 1-4% of humans with the E2/E2 apoE phenotype actually develop FD (Johns Hopkins University, 2011). The pathological influence of apoE dysfunction is important in

these genetically susceptible individuals, but may not be relevant to the more common forms of atherosclerosis in the overall population.

Any of the currently identified monogneic defects that directly or indirectly influence cholesterol metabolism and/or the inflammatory response will increase the likelihood of atherosclerotic events. However, individual genes do not work in a vacuum, and additional genetic and/or environmental factors are often required to determine the overall susceptibility or resistance to disease. Nuclear hormone receptors and other types of transcription factors are under investigation to determine how they exert their regulatory effects (Cohen & Zannis, 2001; Desvergne al., 2006). For example, although the binding capacity of apoB-100 is genetically determined (Goldstein & Brown, 2001), the specific number of hepatic LDLR being expressed at any given time is dependent on dietary and hormonal factors (Gurr, 1992). In a hypothetical situation, the apoB domain of LDL may be functional (non-mutated), but without the adequate expression of the LDLR to bind circulating LDL, the end result could still be high blood cholesterol.

Clinical studies have demonstrated that not all individuals afflicted with FH will develop early onset atherosclerosis. Of those manifesting the heterozygous form of the disease, where circulating LDL levels tend to range between 300-400 mg/dL, only 50% will actually develop cardiovascular disease (Stein et al, 2002). Even though there are both hyper- and hypo- responders to the effects of dietary cholesterol on serum levels, some individuals demonstrate relative resistance to atherogenesis, even in the face of hypercholesterolemia. Equal emphasis should be placed on the search for genes that contribute to individual susceptibility and those that confer resistance.

The ultimate sequence of atherosclerotic events is a result of the combined effects of many genes, regulatory factors, and environmental exposures (Hartman et al., 2001). This synergistic influence on phenotype may give the appearance of a polygenic or multifactorial effect (Funke & Assmann, 1999; Goldstein & Brown, 2001), even when a monogenic abnormality has been clearly implicated. These interactions have made it difficult to establish a universally accepted mechanism of atherogenesis (Peltonen & McKusick, 2001), because the sample sizes needed to test these gene-gene and gene-environment interactions are much larger than those needed for simpler genotype-phenotype associations (Ordovas & Shen, 2002).

Pathways that trigger atherosclerosis in the general population have yet to be elucidated (Visvikis-Siest & Marteau, 2006). Most genomic scale experiments have compared either full-blown plaques against non-affected aortic segments (Archacki et al., 2003; Forcheron et al., 2005; Hiltunen et al., 2002; Shanahan et al., 1997), or they have analyzed differences between ruptured and unruptured plaques (Adams et al., 2006; Faber et al., 2001; Papaspyridonos et al., 2006). In both types of comparisons, differentially expressed genes have been identified that illuminate plaque development and mortality risk. However, genes responsible for initiating foam cell formation could not be discriminated from those involved in later events. This gap is not an oversight by the investigators, but rather reflects the limited availability of human tissue samples at early stages of atherosclerosis for relevant comparative studies. One of the major limitations of elucidating the sequence of events that occur during atherogenesis is that an investigator can "observe and study a single site in the arterial vasculature" only once (Ross & Glomset, 1973). For this and other reasons, most atherogenic research requires animal and in-vitro models of the human disease.

4. Animal models of atherogenesis

4.1 Mammals

No animal model of human disease can fully encompass the unique complexity of molecular machinery and the wide range of expressed clinical phenotypes. However, many important metabolic pathways have been explained by the judicious use of animal models (Hartman et al., 2001). Therefore, the most appropriate choice of a disease model for genetic inquiry will ultimately depend on the specific hypothesis or research question being investigated.

There are some general guidelines to follow when choosing an animal model of human disease. The phenotype should resemble the human physiological condition as closely as possible in both the normal and diseased state (Moghadasian et al., 2001). There are additional practical issues to consider such as the size of the animal and housing requirements, generation times, and the specific cost of overall maintenance, including food, daily care, and experimental treatment (Moghadasian et al., 2001; Suckling, & Jackson, 1993). These concerns become especially important with the development of transgenic models, in that the associated investment costs are much higher than with traditional animal studies.

Several animal models are used currently to investigate various clinical manifestations and genetic mechanisms of human atherosclerosis. Mice (regular laboratory and transgenic), rabbits, and hamsters, are the most common models but miniature swine, primates, rats, dogs, and pigeons are also employed. These models have been used to elucidate the role of specific molecules in atherogenesis, lesion progression, thrombosis, and plaque rupture by direct hypothesis testing. Selected disease characteristics in animal models with their relationship to the human atherosclerosis are presented in **Table 1**.

Animal lipid metabolism studies become complicated because the majority of circulating cholesterol is in HDL (Suckling & Jackson, 1993) for most species except humans who utilize LDL (Garcia et al., 2001). For example, a decrease in plasma HDL has been associated with a reduced risk of atherosclerosis in mice (Breslow, 2000). It does make sense that relatively low levels of HDL decreased the clinical atherosclerosis incidence because HDL (Moghadasian et al., 2001) is 70% of mouse total cholesterol.

However, in humans, decreased HDL levels are associated with an increased risk of atherosclerosis. Despite this marked inconsistency, the successful extrapolation of animal studies to human atherosclerosis is exemplified by the fact that it was impossible to raise circulating LDL levels, and thus increase atherosclerosis risk in experimental models, without LDLR that were compromised, either genetically or in response to dietary overload (Brown et al., 1981; Goldstein & Brown, 2001). Subsequently, over 600 human LDLR gene mutations similar to FH that trigger varying degrees of hypercholesterolemia have been identified (Goldstein & Brown, 2001). In addition, hamsters, rabbits and primates have repeatedly shown reduced functional capacity of hepatic receptors in response to a high fat (Suckling & Jackson, 1993) diet. Individual LDLR activity varied in response to dietary fat and cholesterol because primates, like humans, dogs, and rabbits can be hypo- or hyper-responsive to diet (Goldstein & Brown, 2001; Moghadasian et al., 2001; Overturf et al., 1990; Stein et al., 2002), with some individuals demonstrating unique resistance.

In newborn humans and many animal species, hepatic LDLR have a maximum operative capacity when circulating LDL levels are approximately 0.25 mg/dL (Khosla & Sundram, 1996). Approximately 60% of plasma LDL in hamsters is removed by hepatic receptors. The clearance rate in hamsters is much faster than that of humans, with the hamster LDLR taking up 3.1 mg/hr whereas the companion human LDLR only removes 0.6 mg/hour

(Suckling & Jackson, 1993). However, the fact that hamsters and humans share a common LDL clearance mechanism makes the hamster a suitable model for this aspect of cholesterol metabolism.

Hamsters and humans also share CETP molecules (Suckling & Jackson, 1993) that transfer the cholesterol component of LDL to HDL, a key step in reverse cholesterol transport. These homologous features are in direct contrast to the mouse, which, despite being fed a high-fat high-cholesterol diet (Pitman et al., 1998) and its evolutionary relationship to hamsters, does not develop advanced atherosclerotic plaques resembling those in humans unless animals with sensitized genetic backgrounds (Xu, 2004) are used.

	Hamster	**Mouse**		**Pig**	**Rabbit**		**Pigeon**	**Human**
		Normal	Transgenic		Normal	WHHL/ MI		
Lipoprotein Profiles								
Predominant	LDL	HDL	HDL	LDL	HDL	HDL	HDL	LDL
CETP	+	-	-	-	+	+	+	+
LDLR	+	+	-	+	+	-	-	+
ApoE	+	+	-	+	+	+	-	+
ApoB100	+	+	+	+	+	+	+	+
ApoB-48	+	+	+	+	+	+	-	+
Lesions/Foam Cells								
Primary Location	Arch	Root	Root	Arch	Arch, Thoracic	Arch, Thoracic	Celiac branch	Coronary , Celiac branch
Primary Cell								
Macrophage	+	+	+	-	+	+	-	-
SMC	-	-	-	+	+	+	+	+
Characteristics								
Spontaneous	-	-	-	+	-	-	+	+
Diet-induced	+	+	+	+	+	+	+	+
Thrombosis	-	-	+	+	-	+	+	+
Myocardial infarction	-	-	+	-	-	+/-	+	+
Genome size (Gbp)	3.55	3.45	3.45	3.10	3.47	3.47	1.47	3.40
Wild-type diet								
Omnivore	+	+	+	+	-	-	+	+
Herbivore	-	-	-	-	+	+	-	-

Table 1. Comparison of selected characteristics of atherosclerosis between animal models and humans.

The mouse is technically advantageous because of its small size, short generation time, large litters, and the availability of many inbred strains (Breslow, 2000). However, laboratory mice fed on a chow diet do not develop spontaneous atherosclerotic lesions. Atherosclerosis must be experimentally induced by feeding a diet containing 15% fat, 1.25% cholesterol, and 0.5% cholic acid. These non-physiological conditions create serious limitations for comparison with human studies. The most important factor may be the presence of cholate in the diet. Cholate is enough, in and of itself, to induce a chronic inflammatory state in mice (Breslow, 2000; Shi et al., 2003) confounding the true atherogenic role of inflammation. This is further exacerbated by the fact that some mice are more sensitive to inflammatory cues (Rader & Pure, 2000) so that some genetic differences between susceptible and resistant mouse strains pertain to the diet used, rather than the atherogenic process as it is observed on Western diets (Breslow, 2000).

These and other genetic differences that exist between mouse strains can cause significant problems when interpreting and comparing the results of gene expression studies (Sigmund, 2000). For example, just because a specific inflammatory marker was identified in an atherosclerotic plaque and not in a healthy aorta does not mean that inflammation is causing the disease. Indeed, the molecule could be there to accelerate the cascade; but it could also be there in an attempt to reverse the pathology, or may even be responding to a cellular signal not specific to plaque progression (Knowles & Maeda, 2000) such as cholate. This is true even with transgenic mice because the foundation stock may be different. Also, because gene insertion is random, knock-in models do not by definition contain the gene of interest at the same locus. Therefore, simple transgenics may not be sufficient to prove the role of any given trait because of positional insertion effects on both absolute gene expression and copy number variation (Warden & Fisler, 1997). Delineating the specific function of a candidate gene is difficult, if not impossible, without being able to precisely correlate the phenotype to the initiating mechanism of foam cell formation. The heterogenic background of the mice combined with the variable responses to the atherogenic diet confound the interpretation.

Despite these often overlooked limitations of extrapolating mouse studies to the human disease, research using transgenic mice has enhanced the concept that atherosclerosis is not a simple lipid disorder. New atherogenic theories must be explored to explain the occurrence of atherosclerotic heart disease in individuals displaying no dyslipidemia. Most of the more than twenty unique quantitative trait loci (QTL) identified in mice (Smith et al., 2006) do not influence plasma lipid levels or blood pressure (Allayee et al., 2003; Colinayo et al., 2003). This finding has been especially interesting because these QTL were identified in hypothesis-driven experiments exploring cholesterol metabolism in LDLR and/or apoE knockout mice. Many of these studies have demonstrated the strong genetic influence in the arterial wall on the susceptible and resistant phenotypic differences between mouse strains (Lusis et al., 2004). For example the major mouse QTL, Ath29 on chromosome 9, in the BXH ApoE(-/-) cross fed a chow diet was associated with early lesion development but not with risk factors including circulating lipids (Wang et al., 2007).

Knockout models theoretically mirror homozygous recessive forms of inherited disease because of the loss of gene function (Knowles & Maeda, 2000). As in familial hypercholesterolemia (FH), LDLR null mice experience a 2X increase in plasma cholesterol levels, even on a regular diet, that is further exacerbated on the high-fat, high-cholesterol atherogenic diet (Knowles & Maeda, 2000). The same is true for apoE null mice, although

the mutation's impact on plasma cholesterol is greater than in the LDLR negative mice, with 4-5 times the normal amount of circulating lipoproteins (Knowles & Maeda, 2000; Zhang et al., 1992;). However, preliminary studies revealed no relationship between these elevated lipid levels and lesion size in apoE null mice (Zhang et al., 1994). Only 2% of the homozygous apoE2 null mice developed aortic lesions at all, and the contribution of this mutation to the overall human disease burden has been questioned (Visvikis-Siest & Marteau, 2006). Subsequent studies have shown contradictory results, as the nature of the lesion appears to be dependent on the parental strain used in the experiment rather than the particular knockout gene (Allayee et al., 2003; Getz, 2000; Sigmund, 2000; Smith et al., 2006).
The largest effect in these hyper-cholesterolemic models resulted from the macrophage colony stimulation factor (MCSF) impact on lesion progression (Knowles & Maeda, 2000). MCSF has been reported in advanced human atheromas, and this finding in mice lends experimental support to the role of the inflammatory response in atherosclerosis. However, the role of this molecule in atherogenesis per se is difficult to elucidate in the mouse, because of its chronically inflamed state.
Although not yet yielding consistent results applicable to human therapeutics (Yutzey & Robbins, 2007), transgenic mouse research has reinforced the importance of genetic background in determining atherosclerotic susceptibility or resistance in an individual. These studies have also suggested that the mechanism of foam cell formation varied among individuals under discrete experimental and/or environmental stimuli. The importance of the specific initiating mechanisms on the developing phenotype has been further demonstrated in rabbit models of atherosclerosis.
Rabbits, like hamsters, have CETP and do develop atherosclerotic foam cells when induced by an unnatural diet (Suckling & Jackson, 1993). Unlike the other animal models described in Table 1, rabbits are vegetarian, and so cholesterol is not a normal component of their wild-type diet. The Watanabe Heritable Hyperlipidemic (WHHL) rabbit was developed through selective breeding, and does not have LDLR (Watanabe et al., 1985;). WHHL rabbits get lesions along the aortic arch within six months, but do not experience thrombosis or myocardial infarction. However, these advanced atherosclerotic phenomena are observed in a sub-strain, the WHHLMI rabbit. This rabbit does get a heart attack similar to one of the human atherosclerotic (Shiomi et al., 2003) endpoints.
One of the important contributions of the rabbit model to understanding human disease was the observation that rabbit foam cells can be derived from smooth muscle cells (SMC) or macrophages, depending on the specific dietary perturbation (Weigensberg et al, 1985). This is in direct contrast to the mouse, where the predominant cell type in early lesions is always the macrophage, regardless of diet and genetic strain (Lusis, 2000). Rabbit myogenic foam cells are biochemically and morphologically distinct from macrophage derived foam cells, and both types of early lesions are structurally different from those produced by catheter injury (Weigensberg et al, 1985). Recognizing that different types of foam cells develop in response to different initiating mechanisms should help unravel the controversy of foam cell origin. In all probability, the predominant cell type in early atherogenesis is dependent on the pathological stimulus, and the specific model under study.
A second revelation from rabbit research has been that both macrophages and SMC express receptors for the MCSF protein (Inaba et al., 1992). The proto oncogene c-fms3 induces SMC migration and proliferation, as well as macrophage recruitment to the atherosclerosis-prone regions of the aorta (Mozes et al., 1998). This is important for atherogenesis investigations

because the ratio of SMC to macrophages, both found in human lesions, changes as the disease progresses. The fact that both cell types share an activation mechanism means that the presence of MCSF in an experimental sample does not by definition mean that only macrophages will be recruited. This simple fact is not evident from the plethora of mouse studies, and is further evidence that multiple models are needed to grasp the complexity of human atherosclerosis, especially at the initiation stage.

Swine are unique among the other mammals depicted in Table 1 because, although they are LDL carriers like the hamster (Julien et al., 1981), and most lesions develop in the aortic arch, they also develop spontaneous lesions in the abdominal aorta. The initial foam cells are derived from intimal SMC (Scott et al., 1985), and appear similar to those found in early stages of the human disease. Unfortunately, these lesions do not progress to advanced atheromas without being induced by a 4% (w/w) cholesterol diet (Moghadasian et al., 2001). Even after 90 days on a hyperlipidemic diet, less than 5% of the cells are monocytes (Scott et al., 1985). Swine could adequately model the gradual transition from a myogenic fatty streak to an advanced lesion with activated macrophage cells, reflecting the inflammatory response in humans over time.

4.2 Pigeons

The WC pigeon is unique among non-primate models in that it develops naturally occurring (spontaneous) atherosclerosis at both the celiac bifurcation of the aorta and in the coronary arteries (Clarkson et al., 1959; Prichard et al., 1964). Foam cells develop into fatty streaks which progress into mature plaques in the absence of elevated plasma cholesterol and other traditional risk factors (Wagner, 1978; Wagner et al., 1979). These non-induced atherosclerotic lesions are morphologically and ultrastructurally similar to those seen in humans and occur at parallel anatomical sites along the arterial tree (Cornhill et al., 1980a, 1980b; Hadjiisky, et al., 1991; Kjaernes et al., 1981). Multiple studies have clearly demonstrated that susceptibility in the WC resides at the level of the arterial wall (St. Clair et al., 1986; Wagner et al., 1973, 1979;). Lesion site specificity, severity, and disease progression as a function of age are also highly predictable (Cooke & Smith, 1968; Santerre et al., 1972).

Show Racer pigeons (SR) are resistant to atherosclerosis, while consuming the same cholesterol-free diet. This susceptibility difference occurs despite similar plasma cholesterol and lipoprotein concentrations in both WC and SR (Barakat & St. Clair, 1985). WC pigeons are one of the few animal models to develop severe atherosclerosis while consuming a cholesterol free diet, and comparing results with the resistant SR enables pathological changes associated with the disease to be distinguished from changes due to the natural pigeon aging process. Virtually all WC and SR differences occur at the arterial tissue level as there are few system level differences (Fronek & Alexander, 1981).

Both pigeon breeds are hypercholesterolemic compared to humans, and, like mice and rabbits, they are primarily HDL carriers. However, pigeons are unique in that for the first three days of life, cholesterol is circulated in the form of LDL, after which time the lipoprotein profile switches to HDL (unpublished data) for the remainder of the pigeon's life. Neither breed has apoE (Randolph et al., 1984) or LDLR (Randolph & St. Clair, 1984; St. Clair et al., 1986), so the effect of these variables in other models of the human disease is not a factor in the pigeon pathology. Combined unpublished data gathered from several hundred birds aged 6 months to 3 years over a twenty-year period shows that the average

plasma cholesterol concentration in pigeons ranges from 201 mg/dL in the SR to 242 mg/dL in the WC (+/- 16 mg/dL in both groups). Although these values are borderline significant, they do not change during disease progression, nor does it appear that blood cholesterol induces WC foam cell development. This fact is further supported in wild mourning doves, a close relative of the pigeon, that have 258 mg/dL average plasma cholesterol but do not get atherosclerosis (Schulz et al., 2000). Sterol balance studies have revealed that the WC excretes less neutral sterols than the SR breed (Siekert et al., 1975; Subbiah & Connelly, 1976), but this difference had much greater impact in diet-induced atherosclerosis than in the susceptible phenotype of the WC to the naturally occurring form of the disease (Hulcher & Margolis, 1982).

The most widely studied spontaneous atherosclerotic lesion in susceptible pigeons occurs at the celiac bifurcation of the aorta, and by three years of age reaches a size to be easily visible on gross examination (Nicolosi et al., 1972; Santerre et al., 1972). Early pathological and metabolic changes are apparent microscopically in this site by six months of age (Cooke & Smith, 1968). In contrast, diet-induced lesions in the WC aorta occur at various and unpredictable sites along the descending (Gosselin, 1979; Jerome & Lewis, 1985; Wagner, 1978) and abdominal aortas, and are pathologically very different from non-induced lesions. Foam cells in spontaneous lesions consist primarily of modified SMC (Cooke & Smith, 1968; St. Clair, 1983) while cholesterol-induced foam cells are mostly composed of macrophages (Denholm & Lewis, 1987; Gosselin, 1979; Jerome & Lewis, 1984; St. Clair, 1983).

As with mice, diet-induced lesions develop more rapidly in the pigeon than their spontaneous counterparts (Jerome & Lewis, 1984; Xu, 2004), but different atherogenic mechanisms appear to be involved (Santerre et al., 1972; St. Clair, 1983). One of the primary diet induction effects is to shift the physiological lipoprotein profile from HDL to LDL (Jones et al., 1991; Langelier et al., 1976). In fact, 1% diet supplementation with cholesterol causes such a rapid onset of atherosclerotic foam cells in both breeds that it becomes unfeasible to detect the influence of intrinsic factors (Lofland, 1966) contributing to either WC susceptibility or SR resistance. Therefore, the spontaneous lesion model is best suited for genetic studies to identify candidate genes for susceptibility or resistance as the introduction of an artificial diet confounds the interpretation of the earliest events occurring in atherogenesis.

Since 1959, many studies have been performed to systematically characterize the initiating factor in lesion development in the susceptible WC pigeon. However, the mechanism(s) leading to WC foam cell development is not known, and few studies have been conducted in the spontaneous model to identify the gene(s) or gene product(s) that are specific to initiation. Clarkson and associates (1959) observed that age and heredity were the biggest factors in atherosclerotic susceptibility. Diet, exercise, and gender were not primary factors in the WC pathology.

Further studies of age and heredity effects demonstrated that genetics play a larger role in lesion development than the normal aging process (Goodman & Herndon; 1963). The authors hypothesized that inheritance was a polygenic trait. Wagner and co-workers (1973) compared susceptibility to lesion development between the WC and SR celiac bifurcation of the aorta. The authors found a greater number of advanced lesions in the WC than in the SR, and concluded that the genetic control conferring susceptibility or resistance in the pigeon appeared to be at the level of the artery. Supplementary experiments by that group showed that blood cholesterol, triacylglycerol, and glucose levels were not different between the two

breeds (Wagner, 1978), and that elevated blood pressure is actually a consequence of pigeon atherosclerosis, rather than being an initiating factor (Wagner et al., 1979). The latter study provided initial indications that although diet is not the primary factor contributing to atherosclerotic susceptibility in the pigeon, it can impact the severity of a lesion once formed; thus indicating a role in progression.

A range of metabolic differences between the arterial wall of WC and SR pigeons have been identified. In vivo, differences in the WC susceptible foci include increased glycosaminoglycans, especially chondroitin-6-sulfate (Curwen & Smith, 1977), greater lipid content, predominantly in the form of cholesterol esters (Hajjar et al., 1980b; Nicolosi et al., 1972), lower oxidative metabolism(Hajjar et al., 1980a; Santerre et al., 1974), relative hypoxia (Hajjar et al., 1988), decreased acid cholesterol hydrolase (Sweetland et al., 1999), and neutral cholesterol ester hydrolase (Fastnacht, 1993) activities, increased glycolysis (Zemplenyi & Rosenstein, 1975), decreased tricarboxylic acid cycle activity (Zemplenyi & Rosenstein, 1975), and the increased synthesis of prostaglandin E2, which also decreased cholesterol ester hydrolase activity (Subbiah et al., 1980). Although these studies did not distinguish the primary or underlying problem from secondary effects, increases in non-esterified fatty acids (NEFA) and in chondroitin-6-sulfate (C6S) seem to precede many of the other observed differences. The role of excess NEFA and C6S in pigeon atherogenesis is not yet clear, although the presence of C6S in the susceptible pigeon by six weeks of age does support the response to retention theory. Both human and pigeon smooth muscle cells synthesize C6S as part of the ECM (Edwards et al., 1995; Wight, 1985), where it has been observed to complex with plasma LDL entering the vascular wall (Nakashima et al., 2007; Tovar et al., 1998; Wagner et al., 1989; Wight, 1980).

Human atherosclerosis is considered to be a multifactorial disease, with many genes and environmental factors contributing to the specific phenotype and ultimate endpoint. In pigeons, where individual lifestyle choices are not a factor, the numbers and types of genes contributing to baseline susceptibility and resistance may be easier to elucidate. Preliminary crossbreeding studies indicated a polygenic mechanism of inheritance (Goodman & Herndon, 1963) with resistance being the dominant trait. However, the authors noted that each breed responded differently to dietary manipulation (Herndon et al., 1962), so it is possible that the genetic differences observed may have reflected the confounding influence of diet, rather than the spontaneous expression profile.

Pigeons are not as well suited for traditional inheritance studies as mammalian species because the birds mate for life, and do not reach sexual maturity until seven months of age (Brannigan, 1973). Although excess cholesterol esters can be detected biochemically at 12 weeks, three years are required in order to definitively characterize the complete atherosclerotic phenotype. However, the pigeon genome is approximately half the size of its counterpart mammalian models, and comparative genomic studies are facilitated by the published chicken (Gallus gallus) genome, which is similar in size (Hillier et al., 2004) to the pigeon.

A 15-year cross breeding study at the University of New Hampshire examined grossly visible lesions (or lack thereof) at three years of age in the celiac foci of susceptible WC, resistant SR, and in F1, F2, and backcross progeny. The results supported autosomal recessive inheritance of susceptibility to spontaneous atherosclerosis in the pigeon (Smith et al., 2001). This finding contrasted earlier results (Herndon et al., 1962) that indicated a polygenic mechanism based only on the F1 progeny, but the latter researchers carried the

experiments through the backcross generations and did not use a cholesterol-supplemented diet (Smith et al., 2001). In addition, and probably of greater importance to the experimental results, all pigeons consumed the same cholesterol-free diet. Parallel investigation of the smooth muscle cells cultured from several tissues of the WC, SR, and F1 pigeons demonstrated that lipid accumulation observed at the celiac bifurcation is a constitutive property of WC (Smith et al., 2001).

The finding that spontaneous atherosclerosis in the susceptible WC appears to be the result of a single gene, and not the net result of many interacting genes, as is thought to be the case in humans, makes the pigeon model a simplest case system. Identification of the gene responsible for predisposition, and an understanding of how this gene influences the described metabolic and morphological changes could reveal an initiating mechanistic pathway that remains undetected in more confounded models of atherogenesis.

Experiments have demonstrated that the SMC monolayers grown in vitro accumulate lipid and synthesize proteoglycans in the same manner as aortic cells in vivo (Cooke & Smith, 1968; Smith et al., 1965; Wight, 1980; Wight et al., 1977) but at an accelerated rate. A comparison of the maturation and degeneration of pigeon aortic cells in vivo and in vitro is presented in **Table 2**. In culture, foam cell development is evident in WC SMC by 8-10 days, where several weeks are needed in order to observe the same phenomena in vivo. Other differences in the WC SMC include more esterified cholesterol present in lipid vacuoles, less arachidonate, and decreased mitochondrial metabolism. Although it has been demonstrated that the act of culturing aortic cells can change the SMC phenotype from contractile to synthetic (Worth et al., 2001), this has not been observed in primary cultures, where the lack of sub-culture minimizes potential genetic alterations. WC aortic cells obtained in vitro demonstrate a similar degenerative progression as those cells observed in the celiac bifurcation (Cooke & Smith, 1968; Wight et al., 1977), offering further evidence that the gene expression profile is comparable between the two model systems.

In vitro, there is no signal communication between SMC and endothelial cells, monocytes, hormones, neurotransmitters, other humoral factors, and whole body feedback systems (Shanahan & Weissberg, 1998; Thyberg et al., 1990). The only source of interaction is between the SMC and the media components, resulting in cell growth and ECM synthesis. This makes it possible to observe the intrinsic characteristics of WC and SR aortic cell development in a controlled, time-compressed setting, while limiting the number of genes under investigation to those specific to aortic SMC. Interestingly, although only the SMC of the WC celiac and coronary bifurcations are susceptible to atherogenesis in vivo, SMC taken from other WC tissue such as the gizzard or small intestine exhibit features in vitro similar to atherogenesis in aortic cells. This is not the case in the SR, where neither SMC from the celiac foci, nor SMC from any other tissue undergo phenotype modification when cultured under identical conditions.

The aforementioned experiments provide additional evidence that the genetic defect predisposing the WC to atherosclerosis is conditionally expressed in SMC. Factors that stimulate the expression of atherogenic genes at the celiac bifurcation in vivo appear to be present in vitro, as the cultured WC cells undergo degeneration parallel to their counterparts in aortic tissue (Cooke & Smith, 1968; Wight et al., 1977). Genetic factors denoting resistance in the SR remain expressed in both experimental environments.

Approximate Age *(in vivo)* variable starting at 6 weeks	Salient Morphological Features		Approximate Age *(in vitro)*
1-10 days	Myoblasts ↓		1-2 days
10-18 days	Myofilaments increase Organelles increase Myofilaments align ↓ Formation of myofibrils Dilation of ER ↓		2-4 days
	Show Racer	White Carneau	
1 day–6 months	Myofibrils enlarge ↓	Extension & dilation of ER Modified smooth muscle cell ↓	4-6 days
2 weeks–2 years	Myofibrils increase Organelles decrease ↓	Pinched off cisternae of ER Loss of ribosomes Mitochondrial abnormalities Lipid inclusions, vacuoles Cell rounding ↓	6-8 days
	Mature smooth muscle cell	Foam cell	8-10 days

Table 2. Maturation and degeneration of pigeon aortic cells.

Anderson (2008) analyzed differential gene expression in vitro at day seven of cellular growth. Ninety-one genes were uniquely expressed in the susceptible WC cells compared to 101 genes exclusive to the resistant SR. There was a marked difference in energy metabolism between the two breeds. The SR VSMC expressed genes related to oxidative phosphorylation such as cytochrome B, cyctochrome C oxidase subunit I, NADH dehydrogenase subunit 4, ubiquinone, and ATP synthase subunit 4B. This was in direct contrast to the glycolytic genes expressed by the WC which included enolase, glucose phosphate isomerase, and lactate dehydrogenase subunit B.

In addition, genes expressed by the SR were indicative of a contractile VSMC phenotype whereas susceptible WC pigeons expressed genes that reflected a synthetic phenotype. Spondin, decorin, vimentin and beta actin were upregulated in the WC. Myosin heavy chain, myosin light chain kinase, tropomyosin, and alpha actin were expressed in the SR. The resistant SR appeared to develop and maintain an extracellular matrix with structural integrity, whereas the susceptible WC was already expressing proteases and immune signals.

Although many genes were different between the two breeds, the compressed time frame made it difficult to determine what happens first: changes in energy metabolism or changes in cellular phenoptye. Future in vivo studies are necessary to elucidate the chronological

sequence of events and determine the single gene responsible for atherogenesis in the WC pigeon.

Analysis of SMC soluble proteins from WC and SR pigeons revealed differential expression between the two breeds. Eight discrete zones of molecular weight versus pI were identified, five which included only proteins unique to susceptible cells and three which included proteins unique to resistant cells. Eighty-eight differentially-expressed proteins were found in susceptible cells with 41 located in unique zones. Resistant cells had 29 of 82 differentially-expressed proteins in unique zones. Some annotated proteins, including smooth muscle myosin phosphatase, myosin heavy chain, fatty acid binding protein, ribophorin, heat shock protein, TNF alpha-inducing factor, and lumican, corresponded to genes identified previously or to current hypotheses to explain atherogenesis (Smith et al., 2008).

Additional research to identify the causative gene for spontaneous atherosclerosis will be facilitated by pigeon genome sequencing. Comparative studies between the resistant versus susceptible breeds may reveal sequence variation contributing to the disease. The pigeon remains an important model to study the genetic role at the site of lesion development that is associated with human atherosclerosis.

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6. References

Adams, L.D., Geary, R.L., Li, J., Rossini, A., & Schwartz, S.M. (2006). Expression profiling identifies smooth muscle cell diversity within human intima and plaque fibrous cap: loss of RGS5 distinguishes the cap. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 26, 319-325

Adelman, S., & St. Clair, R. (1988). Lipoprotein metabolism by macrophages from atherosclerosis-susceptible White Carneau and resistant Show Racer pigeons. *Journal of Lipid Research,* Vol. 29, 643-656

Allayee, H., Ghazalpour, A., & Lusis, A.J. (2003). Using mice to dissect genetic factors in atherosclerosis. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 23, 1501-1509

Anderson, J. L. (2008). Differentially expressed genes in aortic cells from atherosclerosis-resistant and atherosclerosis-susceptible pigeons. *Ph. D. Dissertation*, University of New Hampshire, Durham

Archacki, S.R., Angheloiu, G., Tian, X.L., Tan, F.L., DiPaola, N., Shen, G.Q., Moravec, C., Ellis, S., Topol, E.J., & Wang, Q. (2003). Identification of new genes differentially expressed in coronary artery disease by expression profiling. *Physiological Genomics*, Vol. 15, 65-74

Arndt, M., Lendeckel, U., Röcken, C., Nepple, K., Wolke, C., Spiess, A., Huth, C., Ansorge, S., Klein, H.U. & Goette, A. (2002). Altered Expression of ADAMs (a disintegrin and metalloproteinase) in fibrillating human atria. *Circulation*, Vol. 105, 720-725

Attie, A.D. (2001). *Atherosclerosis modified*. Circulation Research, Vol. 89, 102-104

Balis, J.U., Haust, M.D., & More, R.H. (1964). Electron-microscopic studies in human atherosclerosis: cellular elements on aortic fatty streaks. *Experimental and Molecular Pathology,* Vol. 3, 511-525

Barakat, H., & St. Clair, R. (1985). Characterization of plasma lipoproteins of grain- and cholesterol-fed White Carneau and Show Racer pigeons. *Journal of Lipid Research*, Vol. 26, 1252-1268

Bassiouny, H., Zarins, C., Kadowaki, M., & Glagov, S. (1994). Hemodynamic stress and experimental aortoiliac atherosclerosis. *Journal of Vascular Surgery,* Vol. 19, 426-434

Blanchette-Mackie, E.J., Dwyer, N.K., Amende, L.M., Kruth, H.S., Butler, J.D., Sokol, J., Comly, M.E., Vanier, M.T., August, J.T., Brady, R.O., & Pentchev, P.G. (1988). Type-C Niemann-Pick disease: low density lipoprotein uptake is associated with premature cholesterol accumulation in the golgi complex and excessive cholesterol storage in lysosomes. *Proccedings of the National Academy of Sciences USA*, Vol. 85, 8022-8026

Brannigan, D. (1973). Reproductive behavior and squab development in atherosclerosis-susceptible White Carneau and atherosclerosis-resistant show racer pigeons. *Ph. D. Dissertation*, University of New Hampshire, Durham

Breslow, J.L. (2000). Genetic differences in endothelial cells may determine atherosclerosis susceptibility. *Circulation*, Vol. 102, 5-6

Brown, M., Kovanen, P., & Goldstein, J. (1981). Regulation of plasma cholesterol by lipoprotein receptors. Science, Vol. 212, 628-635

Clarkson, T.B., Prichard, R.W., Netsky, M.G., & Lofland, H.B. (1959). Atherosclerosis in pigeons: its spontaneous occurrence and resemblance to human atherosclerosis. *American Medical Association Archives of Pathology*, Vol. 68, 143-147

Cohen, J.C., & Zannis, V.I. (2001). Genes affecting atherosclerosis. *Current Opinion in Lipidology*, Vol. 12, 93-95

Colinayo, V., Qiao, J.H., Wang, X., Krass, K.L., Schadt, E., Lusis, A.J., & Drake, T.A. (2003). Genetic loci for diet-induced atherosclerotic lesions and plasma lipids in mice. *Mammalian Genome*, Vol. 14, 464-471

Cooke, P., & Smith, S. C. (1968). Smooth muscle cells: source of foam cells in atherosclerotic White Carneau pigeons. *Experimental and Molecular of Pathology*, Vol. 8, 171-189

Cornhill, J., Akins, D., Hutson, M., & Chandler, A. (1980a). Localization of atherosclerotic lesions in the human basilar artery. *Atherosclerosis*, Vol. 35, 77-86

Cornhill, J., Levesque, M., & Nerem, R. (1980b). Quantitative study of the localization of sudanophilic coeliac lesions in the White Carneau pigeon. *Atherosclerosis*, Vol. 35, 103-10

Curwen, K., & Smith, S. (1977). Aortic glycosaminoglycans in atherosclerosis-susceptible and -resistant pigeons. *Experimental and Molecular Pathology,* Vol. 27, 121-133

Denholm, E., & Lewis, J. (1987). Monocyte chemoattractants in pigeon aortic atherosclerosis. *American Journal of Pathology*, Vol. 126, 464-475

Desvergne, B., Michalik, L., & Wahli, W. (2006). Transcriptional regulation of metabolism. *Physiological Reviews*, Vol. 86, 465-514

Edwards, I. J., Xu, H., Obunike, J. C., Goldberg, I. J., & Wagner, W. D. (1995). Differentiated macrophages synthesize a heparan sulfate proteoglycan and an oversulfated chondroitin sulfate proteoglycan that bind lipoprotein lipase. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 15, 400-409

Faber, B., Heeneman, S., Daemen, M., & Cleutjens, K. (2002). Genes potentially involved in plaque rupture. *Current Opinion in Lipidology*, Vol. 13, 545-552

Faber, B.C., Cleutjens, K., Niessen, R.L., Aarts, P., Boon, W., Greenberg, A.S., Kitslaar, P., Tordoir, J. & Daemen, M. (2001). Identification of genes potentially involved in rupture of human atherosclerotic plaques. *Circulation Research*, Vol. 89, 547-554

Fastnacht, C. (1993). Egasyn-esterase activity in atherosclerosis-susceptible White Carneau and atherosclerosis-resistant Show Racer pigeon aortas. *M.S. Thesis*, University of New Hampshire, Durham

Fielding, C. (2000). Lipoprotein synthesis, transport, and metabolism, In: *Genetic Biochemical and Physiological Aspects of Human Nutrition,* M. Stipanuk, (Ed). pp 351-364, W.B. Saunders, ISBN 072164452X, Philadelphia

Forcheron, F., Legedz, L., Chinetti, G., Feugier, P., Letexier, D., Bricca, G., & Beylot, M. (2005). Genes of cholesterol metabolism in human atheroma: overexpression of perilipin and genes promoting cholesterol storage and repression of ABCA1 expression. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 25, 1711-1717

Fronek, K. & Alexander, N. (1981). Genetic difference in the sympathetic nervous activity and susceptibility to atherosclerosis in pigeon. *Atherosclerosis*, Vol. 39, 25-33

Funke, H. & Assmann, G. (1999). Strategies for the assessment of genetic coronary artery disease risk. *Current Opinion in Lipidology*, Vol. 10, 286-291

Galton, D.J., & Ferns, G.A. (1989). Candidate genes for atherosclerosis, In: *Genetic Factors in Atherosclerosis: Approaches and Model Systems,* R.S. Sparkes & A.J. Lusis, (Eds). pp 95-109, Karger, ISBN 3805548907, Basel

Garcia, C.K., Wilund, K., Arca, M., Zuliani, G., Fellin, R., Maioli, M., Calandra, S., Bertolini, S., Cossu, F., Grishin, N., Barnes, R., Cohen, J.C., & Hobbs, H.H. (2001). Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. *Science*, Vol. 292, 1394-1398

Getz, G.S. (2000). When is atherosclerosis not atherosclerosis? *Arteriosclerosis Thrombosis and Vascular Biology*, Vol. 20, 1694

Gibbons, G.H., Liew, C.C., Goodarzi, M.O., Rotter, J.I., Hsueh, W.A., Siragy, H.M., Pratt, R., & Dzau, V.J. (2004). Genetic markers: progress and potential for cardiovascular disease. *Circulation*, Vol. 109, 47-58

Goldstein, J.L., & Brown, M.S. (2001). The cholesterol quartet. Science, Vol. 292, 1310-1312

Goodman, H. O. & Herndon, C. N. (1963). Genetic aspects of atherosclerosis in pigeons. Federation Proceedings Vol. 22, Abstract 1336, Atlantic City, April 1963

Gosselin, E. J. (1979). A morphological and ultrastructural study of spontaneous and cholesterol-aggravated atherosclerosis in susceptible and resistant pigeons. *M.S. Thesis*, University of New Hampshire, Durham

Gurr, M.I. (1992). Dietary lipids and coronary heart disease: old evidence, new perspective. *Progress in Lipid Research*, Vol. 31, 195-243

Hadjiisky, P., Bourdillon, M., & Grosgogeat, Y. (1991). Experimental models of atherosclerosis. Contribution, limits and trends. *Archive Mal Coeur Vaiss*, Vol. 84, 1593-1603

Hajjar, D., & Smith, S. (1980a). Focal differences in bioenergetic metabolism of atherosclerosis-susceptible and -resistant pigeon aortas. *Atherosclerosis*, Vol. 36, 209-22

Hajjar, D., Farber, I., & Smith, S. (1988). Oxygen tension within the arterial wall: relationship to altered bioenergetic metabolism and lipid accumulation. *Archives of Biochemistry and Biophysics*, Vol. 262, 375-380

Hajjar, D., Wight, T., & Smith, S. (1980b). Lipid accumulation and ultrastructural change within the aortic wall during early spontaneous atherogenesis. *American Journal of Pathology*, Vol. 100, 683-706

Hartman, J.L., IV, Garvik, B., & Hartwell, L. (2001). Principles for the buffering of genetic variation. *Science*, Vol. 291, 1001-1004

Herndon, C. N., Goodman, H. O., Clarkson, T. B., Lofland, H. B., & Prichard, R. W. (1962). Atherosclerosis resistance and susceptibility in two breeds of pigeons. Genetics, Vol. 47, 958

Hillier, L. W. 171 co-authors., (2004). International Chicken Genome Sequencing Consortium. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature, Vol. 432, 695-716

Hiltunen, M., Tuomisto, T.T., Niemi, M., Brasen, J.H., Rissanen, T.T., P Toronen, P., Vajanto, I., & Yla-Herttuala, S. Changes in gene expression in atherosclerotic plaques analyzed using DNA array. *Atherosclerosis*, Vol. 165, 23-32 (2002).

Hulcher, F., & Margolis, R. (1982). Rate-limiting, diurnal activity of hepatic microsomal cholesterol-7 alpha-hydroxylase in pigeons with high serum cholesterol. *Biochimica et Biophysica Acta*, Vol. 712, 242-249

Inaba, T., Yamada, N., Gotoda, T., Shimano, H., Shimada, M., Momomura, K., Kadowaki, T., Motoyoshi, K., Tsukada, T., & Morisaki, N. (1992). Expression of M-CSF receptor encoded by c-fms on smooth muscle cells derived from arteriosclerotic lesion. *Journal of Biological Chemistry*, Vol. 267, 5693-5699

Jerome, W., & Lewis, J. (1984). Early atherogenesis in White Carneau pigeons. I. leukocyte margination and endothelial alterations at the celiac bifurcation. *American Journal of Pathology*, Vol. 116, 56-68

Jerome, W., & Lewis, J. (1985). Early atherogenesis in White Carneau pigeons. II. ultrastructural and cytochemical observations. *American Journal of Pathology* 119, 210-222

Johns Hopkins University, (2011). Apolipoprotein E, MIM ID +107741, In: Online Mendelian Inheritance in Man (OMIM), 5/23/2011, Available from: http://www.ncbi.nlm.nih.gov/omim

Jones, N., Jerome, W., & Lewis, J. (1991). Pigeon monocyte/macrophage lysosomes during beta VLDL uptake. induction of acid phosphatase activity. a model for complex arterial lysosomes. *American Journal of Pathology*, Vol. 139, 383-392

Julien P, Downar E, & Angel A. (1981). Lipoprotein composition and transport in the pig and dog cardiac lymphatic system. Circulation Research, Vol. 49, 248-54

Katsuda, S., & Okada, Y. (1994). Vascular smooth muscle cell migration and extracellular matrix. *Journal of Atherosclerosis and Thrombosis*, Vol. 1 Suppl 1, S34-38

Khosla, P., & Sundram, K. (1996). Effects of dietary fatty acid composition on plasma cholesterol. *Progress in Lipid Research*, Vol. 35, 93-132

Kjaernes, M., Svindland, A., Walloe, L., & Wille, S. (1981). Localization of early atherosclerotic lesions in an arterial bifurcation in humans. *Acta Pathologica Microbiologica Scandanavia [A]*, Vol. 89, 35-40

Knowles, J.W., & Maeda, N. (2000). Genetic modifiers of atherosclerosis in mice. *Arteriosclerosis, Thrombosis and Vascular Biology,* Vol. 20, 2336-2345

Kuriyama, M., Yoshida, H., Suzuki, M., Fujiyama, J., & Igata, A. (1990). Lysosomal acid lipase deficiency in rats: lipid analyses and lipase activities in liver and spleen. *Journal of Lipid Research*, Vol. 31, 1605-1642

Langelier, M., Connelly, P., & Subbiah, M. (1976). Plasma lipoprotein profile and composition in White Carneau and Show Racer breeds of pigeons. *Canadian Journal of Biochemistry,* Vol. 54, 27-31

Lofland, H. B., Jr. (1966). Irradiation and atherosclerosis in pigeons. Nutrition Reviews, Vol. 24, 178-179

Lohse, P., Maas, S., Lohse, P., Sewell, A.C., van Diggelen, O.P., & Seidel, D. (1999). Molecular defects underlying Wolman disease appear to be more heterogeneous than those resulting in cholesteryl ester storage disease. *Journal of Lipid Research*, Vol. Res 40, 221-228

Lusis, A.J. (2000). Atherosclerosis. *Nature*, Vol. 407, 233-241

Lusis, A.J., Fogelman, A.M., & Fonarow, G.C. (2004). Genetic basis of atherosclerosis: part I: new genes and pathways. *Circulation*, Vol. 110, 1868-1873

Moghadasian, M.H., Frohlich, J.J., & McManus, B. M. (2001). Advances in experimental dyslipidemia and atherosclerosis. *Laboratory Investigation*, Vol. 81, 1173-1183

Mosse, P., Campbell, G., & Campbell, J. (1986). Smooth muscle phenotypic expression in human carotid arteries. II. Atherosclerosis-free diffuse intimal thickenings compared with the media. *Arteriosclerosis, Thrombosis and Vascular Biology,* Vol. 6, 664-669

Mosse, P., Campbell, G., Wang, Z., & Campbell, J. (1985). Smooth muscle phenotypic expression in human carotid arteries. I. Comparison of cells from diffuse intimal thickenings adjacent to atheromatous plaques with those of the media. *Laboratory Investigation,* Vol. 53, 556-562

Mozes, G., Mohacsi, T., Gloviczki, P., Menawat, S., Kullo, I., Spector, D., Taylor, J., Crotty, T.B., & O'Brien, T. (1998). Adenovirus-mediated gene transfer of macrophage colony stimulating factor to the arterial wall in vivo. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 18, 1157-1163

Munro, J., & Cotran, R. (1988). The pathogenesis of atherosclerosis: atherogenesis and inflammation. *Laboratory Investigation,* Vol. 58, 249-261

Nakashima, Y., Fujii, H., Sumiyoshi, S., Wight, T.N., & Sueishi, K. (2007). Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 27, 1159-1165

Napoli, C., deNigris, F., Welch, J.S., Calaria, F.B., Stuart, R.O., Glass C.K., & Palinski, W. (2002). Maternal hypercholesterolemia during pregnancy promotes early atherogenesis in LDL receptor-deficient mice and alters aortic gene expression determined by microarray. *Circulation,* Vol. 105, 1360-1367

Nicolosi, R. J., Santerre, R. F., & Smith, S. C. (1972). Lipid accumulation in muscular foci in White Carneau and Show Racer pigeon aortas. Experimental and Molecular of Pathology, Vol. 17, 29-37

Ordovas, J., & Shen, A. (2002). Genetics, the environment, and lipid abnormalities. *Current Cardiology Reports*, Vol. 4, 508-513

Overturf, M., Smith, S.A., Gotto, A.M., Jr., Morrisett, J.D., Tewson, T., Poorman, J., & Loose-Mitchell, D.S. (1990). Dietary cholesterol absorption, and sterol and bile acid excretion in hypercholesterolemia-resistant white rabbits. *Journal of Lipid Research*, Vol. 31, 2019-2027

Pagani, F., Garcia, R., Pariyarath, R., Stuani, C., Gridelli, B., Paone, G., & Baralle, F.E. (1996). Expression of lysosomal acid lipase mutants detected in three patients with cholesteryl ester storage disease. *Human Molecular Genetics*, Vol. 5, 1611-1617

Palinski, W., & Napoli, C. (2002). The fetal origins of atherosclerosis: maternal hypercholesterolemia and cholesterol-lowering or antioxidant treatment during pregnancy influence in utero programming and postnatal susceptibility to atherogenesis. *FASEB Journal*, Vol. 16, 1348-1360

Papaspyridonos, M., Smith, A., Burnand, K.G., Taylor, P., Padayachee, S., Suckling, K.E., James, C.H., Greaves, D.R., & Patel, L. (2006). Novel candidate genes in unstable areas of human atherosclerotic plaques. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 26, 1837-1844

Peltonen, L., & McKusick, V.A. (2001). Genomics and medicine: dissecting human disease in the postgenomic era. *Science*, Vol. 291, 1224-1229

Pitman, W.A., Hunt, M.H., McFarland, C., & Paigen, B. (1998). Genetic analysis of the difference in diet-induced atherosclerosis between the inbred mouse strains SM/J and NZB/BlNJ. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 18, 615-620

Prichard, R., Clarkson, T., Goodman, H., & Lofland, H. (1964). Aortic atherosclerosis in pigeons and its complications. *Archives of Pathology*, Vol. 77, 244-257

Qin, Z., & Nishimura, H. (1998). Ca2+ signaling in fowl aortic smooth muscle increases during maturation but is impaired in neointimal plaques. *Journal of Experimental Biology*, Vol. 201, 1695-1705

Rader, D.J., & Pure, E. (2000). Genetic susceptibility to atherosclerosis: insights from mice. Circulation Research, Vol. 86, 1013-1015

Randolph, R. & St. Clair, R. (1984). Pigeon aortic smooth muscle cells lack a functional low density lipoprotein receptor pathway. *Journal of Lipid Research*, Vol. 25, 888-902

Randolph, R., Smith, B., & St. Clair, R. (1984). Cholesterol metabolism in pigeon aortic smooth muscle cells lacking a functional low density lipoprotein receptor pathway. *Journal of Lipid Research*, Vol. 25, 903-912

Ridker, P. (2000). New clue to an old killer: inflammation and heart disease. *Nutrition Action* Vol. 27, 3-5

Roberts, J.C., Jr., & Straus, R., (Eds). (1965). *Comparative Atherosclerosis; the Morphology of Spontaneous and Induced Atherosclerotic Lesions in Animals and its Relation to Human Disease,* Harper & Row, New York

Ross, R., & Glomset, J.A. (1973). Atherosclerosis and the arterial smooth muscle cell. *Science,* Vol. 180, 1332-1339

Santamarina-Fojo, S., Remaley, A. T., Neufeld, E. B. & Brewer, H. B., Jr. (2001). Regulation and intracellular trafficking of the ABCA1 transporter. *Journal of Lipid Research*, Vol. 42, 1339-1345

Santerre, R., Nicolosi, R., & Smith, S. (1974). Respiratory control in preatherosclerotic susceptible and resistant pigeon aortas. *Experimental and Molecular of Pathology*, Vol. 20, 397-406

Santerre, R., Wight, T., Smith, S., & Brannigan, D. (1972). Spontaneous atherosclerosis in pigeons. A model system for studying metabolic parameters associated with atherogenesis. *American Journal of Pathology*, Vol. 67, 1-22

Schulz, J., Bermudez, A., Tomlinson, J., Firman, J., & He, Z. (2000). Blood plasma chemistries from wild mourning doves held in captivity. *Journal of Wildlife Diseases,* Vol. 36, 541-545

Scott, R., Kim, D., & Schmee, J. (1985). Endothelial and lesion cell growth patterns of early smooth-muscle cell atherosclerotic lesions in swine. *Archives of Pathology and Laboratory Medicine*, Vol. 109, 450-453

Seals, D.F., & Courtneidge, S.A. (2003). The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes and Development*, Vol., 17, 7-30

Shanahan, C.M., & Weissberg, P.L. (1998). Smooth muscle cell heterogeneity: patterns of gene expression in vascular smooth muscle cells in vitro and in vivo. *Arteriosclerosis, Thrombosis and Vascular Biology,* Vol. 18, 333-338

Shanahan, C.M., Carey, N.R., Osbourn, J.K., & Weissberg, P.L. (1997). Identification of osteoglycan as a component of the vascular matrix. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 17, 2437-2447

Shao, B., Oda, M., Oram, J., & Heinecke, J. (2006). Myeloperoxidase: an inflammatory enzyme for generating dysfunctional high density lipoprotein. *Current Opinion in Cardiology*, Vol. 21, 322-328

Shi, W., Brown, M.D., Wang, X., Wong, J., Kallmes, D.F, Matsumoto, A.H., Helm, G.A., Drake, T.A., & Lusis, A.J. (2003). Genetic backgrounds but not sizes of atherosclerotic lesions determine medial destruction in the aortic root of apolipoprotein E-deficient mice. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 23, 1901-1906

Shiomi, M., Ito, T., Yamada, S., Kawashima, S., & Fan, J. (2003). Development of an animal model for spontaneous myocardial infarction (WHHLMI Rabbit). *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 23, 1239-1244

Siekert, R., Dicke, B., Subbiah, M., & Kottke, B. (1975). Cholesterol balance in atherosclerosis-susceptible and atherosclerosis-resistant pigeons. *Research Communications in Chemical Pathology and Pharmacology*, Vol. 10, 181-184

Sigmund, C.D. (2000). Viewpoint: are studies in genetically altered mice out of control? *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 20, 1425-1429

Smith, J.D., Bhasin, J.M., Baglione, J., Settle, M., Xu, Y., & Barnard, J. (2006). Atherosclerosis susceptibility loci identified from a strain intercross of apolipoprotein E-deficient mice via a high-density genome scan. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 26, 597-603

Smith, S. C., Smith, E. C. Gilman, M. L. Anderson, J. L., and Taylor, R. L., Jr. (2008). Differentially expressed soluble proteins in aortic cells from atherosclerosis-susceptible and resistant pigeons. *Poultry Science*, Vol. 87, 1328-1334

Smith, S.C., Smith, E.C., & Taylor, R.L., Jr. (2001). Susceptibility to spontaneous atherosclerosis in pigeons: an autosomal recessive trait. *Journal of Heredity*, Vol. 92, 439-442

Smith, S.C., Strout, R.G., Dunlop, W.R., & Smith, E.C. (1965). Fatty acid composition of cultured aortic smooth muscle cells from White Carneau and Show Racer pigeons. *Journal of Atherosclerosis Research*, Vol. 5, 379-387

St Clair, R. (1983). Metabolic changes in the arterial wall associated with atherosclerosis in the pigeon. *Federation Proceedings*, Vol. 42, 2480-2485

St Clair, R. (1998). The contribution of avian models to our understanding of atherosclerosis and their promise for the future. *Lab Animal Science*, Vol. 48, 565-568

St. Clair, R., Leight, M., & Barakat, H. (1986). Metabolism of low density lipoproteins by pigeon skin fibroblasts and aortic smooth muscle cells. comparison of cells from atherosclerosis- susceptible and atherosclerosis-resistant pigeons. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 6, 170-177

Stary, H. (1989). Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. *Arteriosclerosis, Thrombosis and Vascular Biology* Vol. 9, 19-32

Stein, O., Thiery, J., & Stein, Y. (2002). Is there a genetic basis for resistance to atherosclerosis? *Atherosclerosis*, Vol. 160, 1-10

Strong, J.P., Malcom, G.T., McMahon, C.A., Tracy, R.E., Newman, W.P., Herderick, E.E., & Cornhill, J.F. (1999). Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the pathobiological determinants of atherosclerosis in youth study. *Journal of the American Medical Association*, Vol. 281, 727-735

Subbiah, M., & Connelly, P. (1976). Effect of dietary restriction on plasma cholesterol and cholesterol excretion in the White Carneau pigeon. *Atherosclerosis*, Vol. 24, 509-13

Subbiah, M., Schweiger, E., Deitmeyer, D., Gallon, L. & Sinzinger, H. (1980). Prostaglandin synthesis in aorta of atherosclerosis susceptible and atherosclerosis resistant pigeons. *Artery*, Vol. 8, 50-55

Suckling, K.E., & Jackson, B. (1993). Animal models of human lipid metabolism. *Progress in Lipid Research*, Vol. 32, 1-24

Sweetland, R. (1999). Lysosomal acid lipase activity in atherosclerosis susceptible and resistant pigeon aortas. *M.S. Thesis*, University of New Hampshire, Durham

Tall, A., Breslow, J.L., & Rubin, E.M. (2001). Genetic disorders affecting plasma high-density lipoproteins, In: *The Metabolic and Molecular Bases of Inherited Disease,* C.R. Scriver, A.L. Beaudet, W.S. Sly, & D. Valle, (Eds). pp 2915-2936, McGraw-Hill, ISBN 0079130356, New York

Thyberg, J., Hedin, U., Sjolund, M., Palmberg, L., & Bottger, B. (1990). Regulation of differentiated properties and proliferation of arterial smooth muscle cells. *Arteriosclerosis, Thrombosis and Vascular Biology,* Vol. 10, 966-990

Tovar, A. M. F., Cesar, D. C. F., Leta, G. C., & Mourao, P. A. S. (1998). Age-related changes in populations of aortic glycosaminoglycans : species with low affinity for plasma low-density lipoproteins, and not species with high affinity, are preferentially affected. *Arteriosclerosis Thrombosis and Vascular Biology,* Vol. 18, 604-614

Tsimikas, S., Willerson, J.T., & Ridker, P. M. (2006). C-Reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. *Journal of the American College of Cardiology*, Vol. 47, 19-31

Visvikis-Siest, S. & Marteau, J. (2006). Genetic variants predisposing to cardiovascular disease. *Current Opinion in Lipidology*, Vol. 17, 139-151

Wagner, W. D., Clarkson, T. B., Feldner, H. B., Lofland, H. B., & Prichard, R. W. (1973). The development of pigeon strains with selected atherosclerosis characteristics. *Experimental and Molecular Pathology*, Vol. 19, 304-319

Wagner, W. D., Conner, J., & Labutta, T. (1979). Blood pressure in atherosclerosis-susceptible and -resistant pigeons. *Proceedings of the Society for Experimental Biology & Medicine*, Vol. 162, 101-104

Wagner, W.D. (1978). Risk factors in pigeons genetically selected for increased atherosclerosis susceptibility. *Atherosclerosis*, Vol. 31, 453-463

Wang, S.S., Shi, W., Wang, X., Velky, L., Greenlee, S., Wang, M.T., Drake. T.A., & Lusis, A.J. (2007). Mapping, genetic isolation, and characterization of genetic loci that determine resistance to atherosclerosis in C3H mice. *Arteriosclerosis Thrombosis and Vascular Biology, V*ol. 27, 2671-2676

Warden, C.H., & Fisler, J.S. (1997). Integrated methods to solve the biological basis of common disease. *Methods in Enzymology*, Vol. 13, 347-357

Watanabe, Y., Ito, T., & Shiomi, M. (1985). The effect of selective breeding on the development of coronary atherosclerosis in WHHL rabbits. An animal model for familial hypercholesterolemia. *Atherosclerosis*, Vol. 56, 71-9

Weigensberg, B., Lough, J., & More, R. (1985). Modification of two types of cholesterol atherosclerosis in rabbits by blocking lipoprotein lysine epsilon-amino groups. *Atherosclerosis*, Vol. 57, 87-98

Wight, T. (1980). Differences in the synthesis and secretion of sulfated glycosaminoglycans by aorta explant monolayers cultured from atherosclerosis-susceptible and -resistant pigeons. *American Journal of Pathology*, Vol. 101, 127-142

Wight, T. (1985). Proteoglycans in pathological conditions: atherosclerosis. *Federation Proceedings*, Vol. 44, 381-385

Wight, T., Cooke, P., & Smith, S. (1977). An electron microscopic study of pigeon aorta cell cultures: cytodifferentiation and intracellular lipid accumulation. *Experimental and Molecular Pathology*, Vol. 27, 1-18

Wissler, R.W., Hiltscher, L., Oinuma, T., and the PDAY Research Group. (1996). The lesions of atherosclerosis in the young: from fatty streaks to intermediate lesions, In: *Atherosclerosis and Coronary Artery Disease,* V. Fuster, R. Ross, & E.J. Topol, (Eds). pp 475–489, Lippincott-Raven Press, ISBN 978-078-1702-66-9, New York

Worth, N., Rolfe, B., Song, J.& Campbell, G. (2001). Vascular smooth muscle cell phenotypic modulation in culture is associated with reorganisation of contractile and cytoskeletal proteins. *Cell Motility and Cytoskeleton*, Vol. 49, 130-145

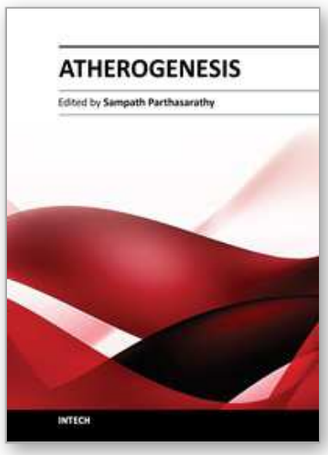
Xu, Q. (2004). Mouse models of arteriosclerosis: from arterial injuries to vascular grafts. *American Journal of Pathology,* Vol. 165, 1-10

Yutzey, K.E., & Robbins, J. (2007). Principles of genetic murine models for cardiac disease. *Circulation*, Vol. 115, 792-799

Zemplenyi, T., & Rosenstein, A. (1975). Arterial enzymes and their relation to atherosclerosis in pigeons. *Experimental and Molecular of Pathology*, Vol. 22, 225-41

Zhang, S., Reddick, R., Burkey, B., & Maeda, N. (1994). Diet-induced atherosclerosis in mice heterozygous and homozygous for apolipoprotein E gene disruption. *Journal of Clinical Investigation*, Vol. 94, 937-945

Zhang, S., Reddick, R., Piedrahita, J., & Maeda, N. (1992). Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science,* Vol. 258, 468-471



Atherogenesis

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This monograph will bring out the state-of-the-art advances in the dynamics of cholesterol transport and will address several important issues that pertain to oxidative stress and inflammation. The book is divided into three major sections. The book will offer insights into the roles of specific cytokines, inflammation, and oxidative stress in atherosclerosis and is intended for new researchers who are curious about atherosclerosis as well as for established senior researchers and clinicians who would be interested in novel findings that may link various aspects of the disease.

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