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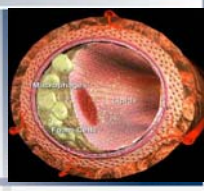

# A Porcine Model for the Study of Atherosclerosis


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## Abstract

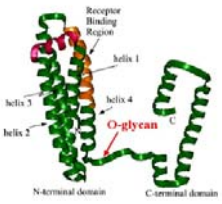

Atherosclerotic cardiovascular disease is the leading cause of death for both men and women among all racial and ethnic backgrounds in Western populations, accounting for nearly 1 million deaths in the United States annually. The development of atherosclerosis involves initial injury to the endothelial cell lining, followed by accumulation of macrophages, adherence of LDL, and accumulation of cholesterol. Apolipoprotein E (apoE) plays a major role in the metabolisms of cholesterol and triglyceride by serving as a ligand for receptors that clear remnants of chylomicrons and very low density lipoprotein (VLDL) from plasma. The link between APOE, serum cholesterol levels, and the development of atherosclerosis has been well established in humans. Numerous animal models have been used to study the pathogenesis and potential treatment of the lesions of atherosclerosis. Mice are highly resistant to atherosclerosis and the vascular lesions differ in location and histology when compared to humans. Rats and dogs are not good models for atherosclerosis because they do not develop spontaneous lesions and require heavy modifications of diet to develop vascular lesions. Rabbits are highly responsive to cholesterol manipulation, but the lesions they develop are much more fatty and macrophage-rich than the human and their plasma cholesterol levels are extraordinarily high. Pigs, however, are very good models when fed high cholesterol diets because they reach plasma cholesterol levels and atherosclerotic lesions similar to humans. In addition, pig models of the disease initially revealed that monocyte infiltration was one of the primary cellular events in the atherogenic process. Therefore, we are investigating the potential of the pig as a model for atherosclerosis by utilizing sequence information to compare human APOE to pig APOE. We are comparing the coding region SNPs in exon 4 of human APOE to pig APOE, which account for the phenotypic differences. We are also comparing SNP frequencies of APOE in twelve animals from eight different pig breeds. Ultimately, we aim to develop a pig model of atherosclerosis that will be used for therapeutic intervention and prevention. We will accomplish this by making a point mutation in the pig at the location of the SNP that accounts for the high cholesterol phenotype in humans as well as create an apoE-knockout pig.


## ApoE Gene

The apoE gene is located on the long arm of chromosome 19. Unlike other apolipoproteins, apoE is synthesized not only in the liver, but in the brain, spleen, lungs, kidneys, smooth muscle cells, and ovaries, but not in the intestinal epithelium. Due to the genetic polymorphism, there are several apoE isoforms coded by alleles of the human genome. Three major isoforms (E2, E3, and E4) are encoded by three alleles (epsilon 2, epsilon 3, and epsilon 4) of the apoE gene. Thus, there is a possibility of six genotypes: E2/2, E2/3, E3/3, E3/4, E4/4 and E2/4. The amino acid changes in positions 112 and 158 confer different lipid binding properties and are responsible for the association of each isoform to different diseases.

**Table 1. ApoE Polymorphism**

| | ApoE2 | ApoE3 | ApoE4 |
|---|---|---|---|
| Residue 112 | Cysteine | Cysteine | Arginine |
| Residue 158 | Cysteine | Arginine | Arginine |
| Carrier population | 8% | 77% | 15% |
| Associated disease | type III hyperlipoproteinemia | | Alzheimer's Disease Atherosclerosis |



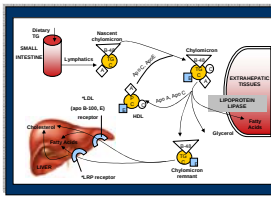

Model of human apolipoprotein E

## ApoE Structure

Apolipoprotein E is a polypeptide that is composed of 299 amino acids which binds to various types of lipoproteins. It is mostly bound with VLDL particles and chylomicrons, and less frequently with HDL cholesterol. The domain for the binding of the LDL receptor is located on the N-terminal between amino acids 130 and 150. ApoE is a ligand of both an LDL receptor (LDLR) and LDL receptor-related protein (LRP). There are three distinguished apoE isoforms, which differ in the substitution of one amino acid. Amino acids on positions 112 and 158 are essential for distinguishing the apoE type. An amino acid substitution in the apoE2 molecule results in a decrease of LDL receptor affinity compared to the other apoE isoforms. Even with a change in position 158, which is not directly in the domain for LDL binding, the alpha helix structure of the apoE molecule is altered and so is its binding ability.

## ApoE Metabolism

ApoE has an effect on the degradation of lipoproteins and consequently on plasma lipid levels. ApoE is a ligand of the LDL receptor, also called the apoB/E receptor. The LDL receptor plays a role in the degradation of lipoprotein particles. Another specific receptor for apoE binding is the receptor for chylomicron remnants, also called the LDL receptor-related protein. ApoE binds chylomicron remnants rich in triglycerides with the help of this receptor. While the LDL receptor is able to bind apoE and apoB-100 binding terminals, the LRP receptor can only bind apoE. After synthesis in the liver, apolipoprotein E is bound in VLDL particles. The nascent synthesized VLDL contain one molecule of apoB, large amounts of apoC and a small amount of apoE. During degradation mediated by lipoprotein lipase, the lipoprotein content in the particle changes. The apoE proportion in IDL particles rises and the proportion of apoC decreases. The final LDL particles contain apoB-100 only. Chylomicrons from ingested food are transported to the liver. During this transport in the circulation they are subjected to lipoprotein lipase which degrades them to chylomicron remnants. Before entering the liver, chylomicron remnants are enriched with apolipoprotein E, which mediates the binding to the receptors.




A, apolipoprotein A; B-48, apolipoprotein B-48; C apolipoprotein C; E, apolipoprotein E; HDL, high-density lipoprotein; TG, triglyceride; C, cholesterol; P, phospholipid; LRP, LDL receptor-related protein.

## ApoE and Associated Diseases

The occurrence of apoE2 is associated with type III hyperlipoproteinemia, characterized by elevated levels of chylomicron remnants and accumulation of β-VLDL particles. The low binding apoE2 activity to receptors decelerates the catabolic change of chylomicrons, VLDL, and remnant particles thus increasing their content in the plasma. In addition, the enhanced activity of hepatic LDL receptors lowers LDL cholesterol concentrations and increases HDL cholesterol concentrations in the plasma. Type III hyperlipoproteinemia occurs most commonly in homozygous carriers of the E2 allele, but only about 1% of these predisposed individuals develop the disorder. Therefore, other genetic and environmental factors are necessary for the expression of hyperlipoproteinemia in those with the E2/2 genotype. This type of hyperlipoproteinemia may occur in patients with allele E3 or E4, however, the association is substantially lower. Several studies have linked the E4 allele with a greater risk of coronary heart disease and an increased risk and decreased age of onset for Alzheimer's disease. The occurrence of apoE4 is associated with increased total and LDL cholesterol and increased apoB. LDL receptor downregulation is a common explanation for the increased cholesterol levels.

## ApoE and Atherosclerosis

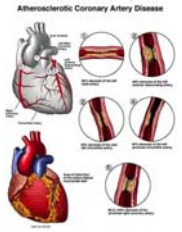
Generally, apoE protects against the development of atherosclerosis, but this depends on the apoE isoform, the total plasma apoE level, and the cell type responsible for the synthesis and secretion of apoE. The protective effects of apoE are apparent from its roles in normal lipoprotein metabolism, especially in targeting remnant lipoproteins for removal from circulation and its role in reverse cholesterol transport. With the exception of the association with type III hyperlipoproteinemia, the presence of the E2 allele is considered to be a protective factor against premature atherosclerosis, compared with the presence of allele E3 and particularly E4.

**Table 2. Animal Models of Atherosclerosis**

| | |
|---|---|
| Mouse | Mice are highly resistant to atherosclerosis. However, the C57BL6 strain is an exception when fed a very high cholesterol diet containing cholic acid. Still, the vascular lesions differ from humans in histology and location. |
| Rat | Do not develop spontaneous lesions and require heavy dietary modifications to produce vascular lesions. |
| Rabbit | Do not develop spontaneous lesions, however, rabbits are highly responsive to dietary cholesterol manipulation. The lesions that they develop are much more fatty and macrophage rich than humans and plasma cholesterol levels are extraordinarily high. |
| Dog | Do not develop spontaneous lesions and require heavy dietary modifications to produce vascular lesions. |
| Pig | The development of atherosclerosis occurs both spontaneously and by experimental induction in swine fed high levels of fat and cholesterol. The use of pig models of the disease initially revealed that monocyte infiltration was one of the primary cellular events in the atherogenic process. |

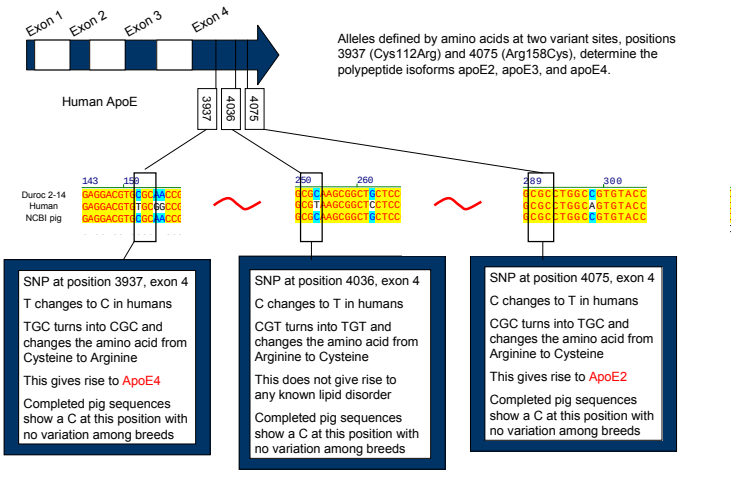
## Objectives

- ❑ To investigate the potential of the pig as a model for atherosclerosis by utilizing sequence information
  - -Sequence porcine APOE, LDLR, and LRP genomic DNA
  - -Make sequence comparisons among humans and other species
  - -Identify SNPs present among twelve pigs from eight breeds
- ❑ Develop a transgenic pig that is a suitable model




## Comparison

We have pulled BACs from the CHORI-242 porcine library containing the APOE, LDLR, and LRP genes. We have sequenced 1 kb spanning exon 4 of the APOE genomic region and compared it to the human and NCBI pig sequences. We are comparing the sequence data to detect relative SNP frequency among selected pig breeds. We are using the same strategy to characterize SNPs in the LDLR and LRP genes.




Selected pig breeds:

| | |
|---|---|
| Yorkshire | Duroc |
| Large White | Hampshire |
| Landrace | Meishan |
| Berkshire | Pietrain |

We are in the process of sequencing the APOE, LDLR, and LRP genes in twelve animals from eight pig breeds. This information is essential in the process of genetically manipulating the porcine apoE gene.

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