Apoe Targeted Mutation Mice

Inactivation of the Apoe gene provides a mouse model for human atherogenesis and diverse applications in studies of lipid transport and metabolism

Applications for the Apoe Targeted Mutation Mouse Model

The lack of a functional *Apoe* gene renders these mice unable to produce a key glycoprotein, apoE (apolipoprotein E), which is essential for the transport and metabolism of lipids. The mice are healthy when born, but have a markedly altered plasma lipid profile compared to normal mice, and rapidly develop atherosclerotic lesions. Numerous potential applications to basic research and drug development include:

- Cholesterol/triglyceride transport mechanisms
- Role of apoE in lipoprotein binding to endothelium and the incorporation of lipids into atherosclerotic plaques
- Cholesterol reverse transport
- Relative contributions of dietary constituents to atherogenesis
- Modeling of human apoE-deficiency
- Impact of lipid oxidation on cellular lipid uptake/metabolism
- Role of apoA-I and apoA-IV in lipoprotein formation and transport
- Apoe function in normal and diseased neural tissue and nerve repair
- Gene therapy to replace specific *Apoe* alleles in Apoe Targeted Mutation Mice
- Evaluation of pharmacologic manipulations of atherosclerotic plaque formation
- Development through crossbreeding of models for specific research programs in atherosclerosis and CNS disease

Features of the Apoe Targeted Mutation Mouse (model APOE) [1,2]

- Homozygous for defective *Apoe* gene
- Complete absence of apoE glycoprotein

Lipoprotein Profiles of Normal and Apoe Targeted Mutation Mice

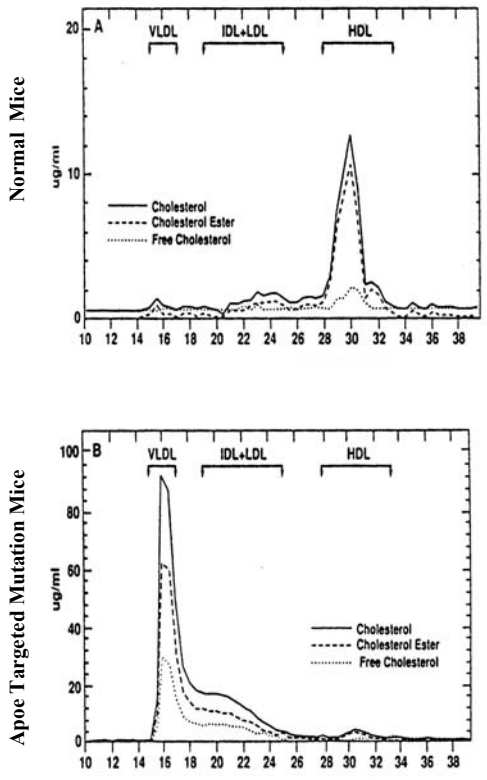

Figure 1. *Reprinted with permission from the Journal of Clinical Investigation.* Kashyap, et al.[4]

- Markedly elevated plasma cholesterol levels (5 times normal)
- Relatively normal plasma triglyceride levels
- Shift from the normal distribution of plasma lipids (cholesterol and triglycerides) from predominantly high density lipoprotein (HDL) complexes to predominately very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) complexes[2]
- Early and severe spontaneous development of atherosclerotic lesions

- Normal reproductive performance in both male and female Apoe Targeted Mutation Mice[2]
- No reported difference in body weights or litter size compared to normal mice
- Enrichment of VLDL and IDL particles with apoA-I, apoA-IV, and apoB-48, similar to their distribution in humans who are apoE deficient, and similar to chylomicron remnants[2]

Scientific Profile of the Apoe Targeted Mutation Mouse Model

Apoe Targeted Mutation Mice are homozygous for a defective *Apoe* gene, and apoE protein is absent from blood of these animals.[1] Plasma lipoproteins in which apoE normally is an integral part (e.g., VLDL, IDL, HDL) are therefore devoid of this apolipoprotein.[2] As a result, Apoe Targeted Mutation Mice show dramatic alterations in lipid metabolism and transport, even on a normal mouse chow diet.

In normal mice, the protein apoE is made predominantly by the liver (as in humans) and becomes incorporated into all lipoprotein particles (except LDL), including those constructed by intestinal cells (chylomicrons) for transport of ingested lipids through the bloodstream, and those constructed by liver cells to distribute lipids via the circulation and fuel cellular metabolism during fasting (VLDLs).[3] apoE is present in other lipoprotein particles that are metabolic products (IDLs, chylomicron remnants), or those involved in acquisition of cholesterol from tissues and reverse cholesterol transport (HDLs).

The most well-documented function of apoE is to facilitate binding of lipoproteins to cell surface receptors, thereby enhancing transfer of components of the particle's lipid moeity (e.g., cholesteryl esters, triacyl-glycerols) to or from cells. Specifically, apoE mediates binding to both LDL and LRP (LDL receptor-related protein) receptors, and possibly the VLDL receptor.[4] Less well understood but intriguing are possible roles for apoE in nerve regeneration and amyloid processing in CNS neurons.

The absence of apoE has multiple effects on lipid metabolism and transport. In normal mice, the profile of cholesterol-containing plasma lipoproteins includes an abundance of high density lipoprotein (HDL) and only trace amounts of lower-density lipoproteins (VLDL, IDL, LDL).[2,3] In contrast, Apoe Targeted Mutation Mice have the reverse distribution: 80% of serum cholesterol is sequestered in lower density lipoproteins, while HDL-cholesterol is half the normal level.[2] In addition, total cholesterol levels in plasma of Apoe Targeted Mutation mice are up to five times that of normal mice (e.g., 434 mg/dl versus 86 mg/dl, respectively).[2] Thus, both the quantity of cholesterol and its distribution among lipoprotein fractions are shifted in the mutant mice to a pattern known to be associated in humans with risk of atherosclerosis.

The alterations generated in the Apoe Targeted Mutation Mice confer a spontaneous susceptibility to early and extensive development of atheromas regardless of diet. Unlike normal mice, which do not develop atherosclerotic lesions (except for some strains on high fat/high cholesterol diets), Apoe Targeted Mutation Mice fed normal mouse chow develop foam cell accumulations on their aortic walls by 3 months of age, which progress to extensive atherosclerotic lesions and severe vessel occlusion by 8 months.[2]

Other than lipid alterations and propensity to develop atherosclerosis, Apoe Targeted Mutation Mice are of similar weight to normal mice and appear otherwise healthy.[2] This, combined with early atherosclerosis and underlying changes in plasma lipid profile that mimic the human atherogenic profile, make Apoe Targeted Mutation Mice an ideal model for human atherogenesis. They also can serve as a model for human apoE deficiency, a rare genetic disorder.

The apoE deficiency has been corrected experimentally in Apoe Targeted Mutation Mice, with a return to normal lipoprotein distribution and cholesterol levels (650 mg/dl in apoE deficient mice versus 100-150 mg/dl in apoE corrected mice).[4] This was accomplished by gene replacement through infusion of an

adenovirus vector containing the human *Apoe* gene, and resulted in mice with markedly reduced incidence of atherosclerosis.

Origin of the Model

The Apoe Targeted Mutation Mouse Model was developed by Nobuyo Maeda and colleagues at the University of North Carolina in Chapel Hill.[1] Inactivation of the *Apoe* gene was accomplished by homologous recombination, by electroporation of cultured E14TG2a cells with a plasmid that contains a modification of the murine *Apoe* locus, in which a portion of the normal *Apoe* gene sequence had been replaced by neomycin-resistance and thymidine kinase marker genes.

Electroporated cells that had undergone successful recombination of wild-type and plasmid-derived *Apoe* gene sequences were identified by positive neomycin resistance, and hence, presence of the disrupted *Apoe* gene. Chimeric mice then were generated by injecting such cells into blastocysts of C57BL/6J mice.

Of the mice that developed from the blastocysts, a male chimera (JH126.1) was found to transmit the embryonic cell genome to 100% of his offspring in a backcross to a C57BL/6 female. The resulting pups inherited the *Apoe* gene in expected Mendelian proportion (50%), indicating integration of the mutation into the genome and no effect of the mutation on embryo viability.

The mice were transferred to Taconic in May 1998 after ten backcrosses (N10) to C57BL/6J from the (129 X C57BL/6) chimera and intracrossing to homozygosity. The line was then further backcrossed at Taconic to C57BL/6N (N11) and derived by embryo transfer. The colony is maintained at N11 through intercrossing of homozygous mice.

Ready for Your Experiments

Taconic's Apoe targeted mutation mice are produced in Isolator Barrier Unit (IBU™) facilities. Mice are shipped in Taconic Transport Cages (TTC™) and come with an up-to-date health report documenting their Murine Pathogen Free (MPF™) health status. Barrier housing conditions are recommended for maintenance of Apoe Targeted Mutation homozygous mice.

Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to lipoprotein metabolism and atherogenesis. Call, fax, or visit Taconic's website for information about these additional models:

- **ApoB100 Microinjected Mouse (model 001004) –** develops atherosclerosis on a high fat/high cholesterol diet, due to expression of high levels of human apolipoprotein B100 and a resultant elevated plasma cholesterol (total and LDL-associated).
- **APOE2 Targeted Replacement Mouse (model 001547) –** expressing the human apoE2 protein instead of murine apoE, with several abnormalities of lipid physiology, including elevated serum levels, altered lipoprotein profiles, and early development of atherosclerosis, all of which parallel features of human type III lipoproteinemia.
- **APOE3 Targeted Replacement Mouse (model 001548) –** expressing the human apoE3 protein instead of murine apoE, with normal serum cholesterol and triglyceride levels, but certain abnormalities of lipid physiology, including delayed clearance of lipoprotein particles (VLDL) and propensity to develop atherosclerosis on a high-fat diet.
- **APOE4 Targeted Replacement Mouse (model 001549) –** expressing the human apoE4 protein instead of murine apoE, with normal serum cholesterol and triglyceride levels but certain abnormalities of lipid physiology that are similar to those of ApoE3 Targeted Replacement Mice; impairment in clearance of lipoprotein particles (VLDL) and development of atherosclerosis on a high-fat diet are more pronounced**.**
- **CETP Microinjected Mouse (model 001003) –** expressing human cholesteryl ester transfer protein, a plasma enzyme normally absent in mice, which in humans mediates HDL-cholesterol transfer to VLDL

and LDL and enhances cholesterol uptake into cells; transgenic mice have dramatically reduced HDL-cholesterol.

- **CETP/ApoB100 Double Microinjected Mouse (model 001007)** – develops atherosclerosis rapidly on a high-fat/high-cholesterol diet or normal mouse chow after six months, due to expression of both human CETP and apoB100 and consequent alterations in plasmalipid profile (elevated LDL-cholesterol, reduced HDL-cholesterol)

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