APOE Targeted Replacement Mice

Replacement of the mouse Apoe gene with one of the three human APOE alleles provides mouse models for exploring the differential influences of these alleles on lipid transport and metabolism, and on diseases associated with dysfunctions in these processes

Applications for APOE Targeted Replacement Mouse Models

Taconic's APOE Targeted Replacement Mice express the human apoE protein at physiological levels and retain the endogenous regulatory sequences required for modulating apoE expression. All three APOE Targeted Replacement Mouse lines were created by gene targeting and carry one of the three human alleles *(APOE2, APOE3,* or *APOE4*) in place of the endogenous murine *apoE* gene. Therefore the mice have identical levels of apoE expression in all normally apoE-occurring mouse cell types. This provides a complete *in vivo* system that allows for direct comparison studies to measure any apoE isoform-specific effects. Several potential applications for the APOE Targeted Replacement Mouse Models, include:

- Refinement of the differential roles of apoE in lipid-associated disease processes
- Use as an animal model to discover the underlying mechanisms and treatments of Alzheimer's disease, hyperlipidemia and atherosclerosis, stroke and head injury
- Characterization of apoE-mediated cholesterol/triglyceride transport and metabolism
- Correlation of specific apoE isoforms with normal lipid physiology and with pathological conditions or diseases
- Discovery of lipoprotein particle functions and properties, both with and without normal apoE content
- Clarification of apoE function in general, and of its specific isoforms, in recovery from brain or nerve injury
- Characterization of the level of expression of apoE in specific cells, such as in glial cells versus neurons
- Exploration of the function of transgenic promoter sequences versus host promoters in gene expression

Diagram of the Apoe Region in APOE Targeted Replacement Mice

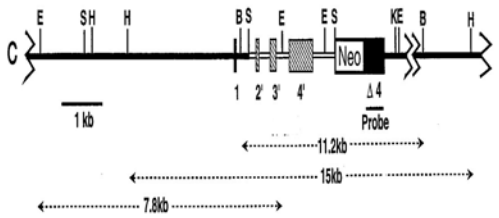

Figure 1. Each ApoE Targeted Replacement Model contains mouse regulatory and flanking sequences (solid lines) and the murine exon 1of the *Apoe* gene, surrounding the inserted human exons (2', 3', 4', hatched boxes and clear lines). A partial murine exon 4 (Probe) is retained to detect the targeted allele. E, *Eco*RI; S, *Sac*I; H, *Hin*dIII; P, *Pvu*I; K, *Kpn*I; X, *Xba*I; B, *Bam*HI; Neo, neomycin-resistance cassette; huE, human *APOE* exons. Reprinted with permission from the *Journal of Biological Chemistry*.[2]

Features of APOE Targeted Replacement Mice

APOE2 Targeted Replacement Mice (Model 001547)[1]

- Physiological expression of the human *APOE2* allele
- Elevated plasma lipid profiles (cholesterol and triglycerides)
- Impaired plasma clearance of cholesterol-rich lipoprotein particles (VLDL)
- Essentially normal HDL level
- Spontaneous atherogenesis that is exacerbated with a high-fat diet
- Features of human type III hyperlipoproteinemia, including plasma lipid patterns, response to corrective medication, and development of xanthomas

APOE3 Targeted Replacement Mice (Model 001548)[1,2]

- Expression of the human *APOE3* allele

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- Normal plasma cholesterol and triglyceride levels
- Delayed plasma clearance of cholesterol-rich lipoprotein particles (VLDL) to about an eighth the rate in wild-type mice
- Normal HDL levels
- Marked hyperlipidemia in response to a high-fat diet
- No spontaneous atherogenesis, but high susceptibility on a high-fat diet

APOE4 Targeted Replacement Mice (Model 001549)[3]

- Expression of the human *APOE4* allele
- Normal plasma cholesterol and triglyceride levels
- Delayed plasma clearance of cholesterol-rich lipoprotein particles (VLDL) to about a sixteenth the rate in wild-type mice
- Increased size and number of VLDL particles compared to other ApoE Targeted Replacement and wild-type mice
- Normal HDL levels
- Marked hyperlipidemia in response to a high-fat diet
- No spontaneous atherogenesis, but high susceptibility on a high-fat diet

Clearance of Injected Lipoprotein Particles (VLDL) from Plasma of APOE2 (2/2), APOE3 (3/3) and APOE4 Targeted Replacement Mice (4/4)

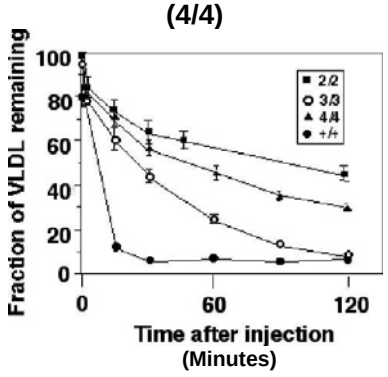

Figure 2. Clearance of VLDL is greatly impaired in mice bearing the human *APOE2* allele (2/2), and delayed in targeted replacement mice carrying the human *APOE3* allele (3/3) or *APOE4* allele (4/4). For comparison, wild-type mice expressing murine *Apoe* require about 15 minutes to remove the injected particles, and mice with the *APOE3*(3/3) or *APOE4* (4/4)allele require about two or four hours respectively. Supplied by Dr. N. Maeda, modified from Sullivan, P. M., et al.[1]

Scientific Profiles of the APOE Targeted Replacement Mouse Models

Each of Taconic's APOE Targeted Replacement Mouse Models express one of the human ApoE isoforms in place of the endogenous mouse apoE protein. Absence of the endogenous apoE protein, without replacement by a human protein, causes multiple alterations in lipid metabolism and transport (as evidenced by the characteristics of Taconic's APOE Targeted Mutation Mice.)[4] These abnormalities are, to differing degrees, corrected in each of the APOE Targeted Replacement Mouse Models, depending on which human allele is present. These models replace only the coding region of the mouse endogenous *ApoE* gene with one of the human *APOE* alleles, thereby retaining those sequences necessary for response to normal environmental and biofeedback signals.[1,2] These models therefore provide an excellent opportunity to scrutinize the roles of each allele in normal lipid physiology and in lipid-associated disease processes.

The *APOE* gene encodes one of many liver-derived proteins (apoE), an integral component of blood-borne lipoprotein particles (except for LDLs.) The protein is amphipathic, making it an important lipid-transport molecule in the bloodstream. In addition, apoE facilitates the binding of lipoprotein particles to specific cell surface receptors, such as the low-density lipoprotein receptor (LDLR), thereby enhancing transfer of the particles' lipid moeity (e.g., cholesteryl esters, triacylglycerols) to or from cells. The clearance of very low-density lipoprotein (VLDL) particles from the bloodstream by just such a mechanism is one specific action that depends on apoE.

Each of the APOE Targeted Replacement Mouse Models carries one of the three human alleles of the *APOE* gene. The alleles, designated *APOE2, APOE3,* and *APOE4*, encode isoforms of the apoE protein that differ from each other by only one amino acid, but have markedly differing affinities for the LDLR. This may in part explain an observed association between specific isoforms and certain human diseases. For example, Alzheimer patients have a much higher frequency of apoE4, and other brain pathologies have been associated with this allele.[5] A specific form of hyperlipidemia and

premature atherosclerosis are associated with apoE2.[1]

APOE2 Targeted Replacement Mice carry the human *APOE2* allele, and exhibit markedly abnormal plasma lipid profiles, beginning at 2 months of age, even on a normal diet.[1] Circulating levels of total cholesterol and triglycerides are two to three times that of normal, and remnants of both very low density lipoproteins (VLDL) and chylomicrons are abnormally elevated due to a deficit in clearance. This results in high levels of cholesterol-laden β-VLDL particles in plasma. Atherogenesis occurs spontaneously and is exacerbated by a diet high in fats and cholesterol (which induces even more dramatic elevations in plasma lipids). Thus, APOE2 Targeted Replacement Mice are especially appropriate as a model for atherosclerosis and hyperlipidemic disorders. These mice parallel the human condition of type III hyperlipoproteinemia by the preceding characteristics, and also by an inordinately high expression of the apoE2 protein (which becomes particularly sequestered in remnant lipoprotein particles), an accumulation of lipophilic material in the skin (xanthomas) on a high-fat diet, and an improvement of lipid profiles with clofibrate treatment. Interestingly, liver histology reveals healthy cells even on a high-fat diet, contrasting with the conspicuous hepatocyte damage seen among wild-type mice on the same diet.

APOE3 Targeted Replacement Mice carry the human *APOE3* allele, which is the most common allele (78.3% occurrence).[2] Although only one amino acid difference exists between this and the apoE2 isoform, plasma total cholesterol and triglycerides, and their pattern of distribution among various lipoprotein particles, are normal in ApoE3 Targeted Replacement Mice that are fed a standard diet. Clearance of remnant VLDL particles does occur, but is hindered, requiring about eight times longer than in wild-type mice. Differences in serum lipid transport compared to wild-type mice include a higher proportion of VLDL/IDL remnants and few LDL particles in the plasma of fasted animals, and greatly reduced apoB100 but higher apoB48. In addition, most of the APOE is found in VLDL and IDL particles, not in HDL as is typical in wild-type mice. Although atherosclerosis does not occur spontaneously, ApoE3 Targeted Replacement Mice are notably more susceptible to diet-induced hypercholesterolemia and atherosclerosis than are wild-type mice.

APOE4 Targeted Replacement Mice share many characteristics of APOE3 Targeted Replacement Mice.[3] When fed a normal diet, the two lines have equivalent levels of total plasma cholesterol, triglycerides, HDL-cholesterol, and apoE protein, which are within the normal range. However, ApoE4 Targeted Replacement Mice are more prone to diet-induced atherosclerosis (produce larger plaques), due to a clearance of VLDL that is half as efficient as that of APOE3 Targeted Replacement Mice (a sixteenth the clearance rate in wild-type mice). There are also slight differences between these two targeted replacement models in the distribution of cholesterol, triglycerides, and apoE among different types of lipoprotein particles in plasma.

Together, the characteristics of the APOE Targeted Replacement Mouse Models indicate a key role for apoE in lipid physiology that relates to several human conditions. APOE2 Targeted Replacement Mice provide a model for human type III hyperlipoproteinemia with premature atherosclerosis, whereas the APOE3 and APOE4 Targeted Replacement Mice are models for diet-induced atherosclerosis. APOE genotype impacts the risk of developing Alzheimer's disease in humans. Additional roles for apoE, such as in normal brain physiology, injury and healing, and disease, can be explored using any of these models.

Origins of the Models

The APOE Targeted Replacement Mouse Models each were developed by Dr. Nobuyo Maeda and her colleagues at the University of North Carolina at Chapel Hill.[1-3] In earlier work, this group created several strains of transgenic mice by microinjection of each of the human *APOE* alleles and their promoters into zygotes of Apoe Targeted Mutation Mice. However the resulting mice did not express tissue specific or temporal-specific regulation making comparisons between the transgenic lines expressing different isotypes of the *APOE* gene difficult.

To ensure that tissue-specific protein expression would be similar to that of normal mice, a different strategy was employed to generate the models

described here. Each model was created by electroporating cultured embryonic stem cells of a 129 mouse strain with a targeting construct that contained the human coding sequences (exons 2-4) for either *APOE2*, *APOE3*, or *APOE4*, in combination with flanking sequences from a 129 mouse. Homologous recombination produced loci that retained all normal mouse regulatory sequences (plus non-coding exon one) together with the human protein-encoding exons.

Embryonic stem cells containing the recombined locus were introduced into C57BL/6J embryos, and the resultant chimeric mice were bred with C57BL/6J mice. Offspring carrying the modified locus in the germ-line were interbred to generate the homozygous transgenic genome. All F1 matings produced normal litter sizes with a Mendelian distribution of the locus.

Ready for Your Experiments

Taconic's APOE Targeted Replacement Models 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 Replacement Mice.

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Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to lipoprotein metabolism and atherogenesis. Call or fax 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).
- **Apoe Targeted Mutation Mouse (model APOE) –** lacking the ApoE gene, with highly altered lipid transport and metabolism, causing elevated serum lipids and spontaneous atherosclerosis that is further exacerbated on a high-fat diet.
- **APPSWE Microinjected Mouse (models 001349 and 002789)** – carrying the APPSWE (2576) transgene coding for the 695-amino acid isoform of human Alzheimer β-amyloid precursor protein derived from a Swedish family with early-onset Alzheimer's disease; useful for study of the development and treatment of Alzheimer's disease.
- **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 plasma lipid profile (elevated LDL-cholesterol, reduced HDL-cholesterol)

Taconic Transgenic Models
Publication Reference List
APOE2, APOE3, APOE4 Targeted Replacement Mice

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