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Review

Overview of the Most Popular Currently Used Murine Models of Atherosclerosis

[1]Anastasia Vladimirovna Poznyak, [2]Victoria Alexandrovna Khotina, [2]Alexandra Alexandrovna Melnichenko, [2]Vasily Nikolaevich Sukhorukov, [2]Igor Alexandrovich Sobenin and [1]Alexander Nikolaevich Orekhov

[1]Institute for Atherosclerosis Research, Osennyaya, Moscow, Russia
[2]Laboratory of Angiopathology, Institute of General Pathology and Pathophysiology, Baltiiskaya Street, Moscow, Russia

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Corresponding Author:
Anastasia Vladimirovna Poznyak
Institute for Atherosclerosis Research, Osennyaya, Moscow, Russia
Email: tehhy_85@mail.ru

Abstract: Atherosclerosis is a widespread and serious problem. Every year it causes a huge number of deaths and morbidity. Many aspects of this disease remain not fully understood, which indicates the need to search for the most representative model of atherosclerosis in animal and cell culture models. Animal models resembling the pathophysiology in humans are needed to study the immunometabolic mechanisms and molecular processes mediating the development of the disorder. It's important to point out that no animal model replicates all the attributes of the human disease. Various animal atherosclerosis models have been tested, each of them having its advantages and disadvantages. The use of animal models adheres to ethical guidelines and regulations. Mice are traditionally considered one of the most convenient model objects, including for the study of atherosclerosis. Murine models are relatively cheap, fast-growing, and can be easily manipulated. Nevertheless, there exist numerous limitations when employing murine models. One example of a limitation is that, unlike humans, mice primarily transfer cholesterol in High-Density Lipoprotein (HDL) particles rather than Low-Density Lipoprotein (LDL). There is still no ideal model, even though plenty of them are used for various studies. Within this review, we have compiled pertinent background details concerning the prevailing mouse models employed in atherosclerosis research. We aim to emphasize the benefits and drawbacks associated with their utilization.

Keywords: Mice, Knockout, ApoE, Murine Model, Animal Model

Introduction

Atherosclerosis

Atherosclerosis is a chronic immunometabolic condition that often has no manifestation until the plaque progresses to a point where it obstructs the vessel. The complex nature of this condition is defined by the interplay of lipid metabolism abnormalities, inflammation, oxidative stress, and others. This leads to plaque rupture and results in peripheral artery disease, myocardial infarction, or stroke. The disease starts with the accumulation of cholesterol-rich lipoproteins that contain apolipoprotein B at specific sites like vessel bifurcations. Elevated levels of Low-Density Lipoprotein-Cholesterol (LDL-C) in the bloodstream constitute the primary risk factor for the onset of atherosclerosis (Herrington *et al*., 2016). Lipoproteins gathered in the artery wall are then exposed to different transformations like carboxylation and oxidation. This sets off a number of inflammatory pathways activating the endothelial cells in the vessel.

Activated endothelium expresses adhesion molecules that promote the migration of immune cells, such as T cells and monocytes, into the artery wall, where the monocytes differentiate into macrophages, consume the modified lipoproteins, and turn into lipid-laden foam cells (Lorey *et al*., 2022). Plaque macrophages expressing different scavenger receptors play a crucial role in promoting foam cell formation and macrophage polarization. These receptors aid in the recognition and uptake of modified lipoprotein antigens, such as oxidized lipoproteins. The overage of cholesterol esters causes defects in cholesterol esterification, which leads to free cholesterol building up into cholesterol crystals that injure the cells and trigger apoptosis. This affects efferocytosis clearance of apoptotic cells by phagocytes, which again promotes the aggregation of foam cell remains and sets off further inflammatory pathways, resulting in vessel wall inflammation (Pirahanchi *et al*., 2018). Furthermore, the enzymes expressed by foam cells damage the

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extracellular matrix, which makes the plaque less stable and increases the risk of rupture that would result in platelet accumulation, and blood thickening and eventually lead to thrombus formation. Plaque development and its vulnerability also depend on inflammatory cytokines, like TNF-α and IFN-γ, expressed by immune cells. When cytokines are released, they initiate an immune response within the plaque and result in the death of Vascular Smooth Muscle Cells (VSMCs). This damages the plaque matrix. Furthermore, the atherosclerosis-induced immune and metabolic impairments involve other types of cells and organs, which is why it is recommended to choose an atherosclerotic mouse model that demonstrates pathophysiology as close to human, as possible (Loftus, 2011).

Atherosclerosis, a complex condition in humans, can be triggered by various risk factors, with high blood pressure, diabetes mellitus, aging, and hyperlipidemia being among them. All these conditions cause immune and metabolic dysfunctions.

Various Models for Atherosclerosis Studies

When we compare *in vivo* models with *in vitro* models, the latter brings forth noteworthy benefits. *In vitro* models excel in evaluating drug effectiveness and toxicity while also offering a reproducible, cost-effective, high-throughput, and manageable approach to studying biological mechanisms. This makes them highly valuable tools for delving into the underlying mechanisms of atherosclerosis and advancing the development of groundbreaking therapies (Madorran *et al*., 2020).

The utilization of animal models that replicate the pathophysiology observed in humans is needed in order to study the immunometabolic mechanisms and molecular processes mediating the development of the disorder (Katakami, 2018). It is worth mentioning that no animal model resembles all the features of the disease in humans. Various animal (mice, rats, rabbits, pigs, *Primates*, birds, dogs, etc.,) atherosclerosis models have been tested, each of them having their own advantages and disadvantages. Thus, different models suit best for different types of research, depending on their main focus. While non-human *Primates* recapitulate most physiological features of humans and thus are the best atherosclerotic model with the highest clinical relevance, their maintenance is expensive and the disease in *Primates* takes a long time to develop. At the same time, they are more exposed to infections and have high ethical thresholds (Zhang *et al*., 2021). The alternative should be less expensive, easier to maintain, suitable for genetic, interventional, and pharmacological research, and at the same time show a development as close to the human course of the disease as possible.

Mice as Model Animals

Mice are widely preferred as animal models for atherosclerosis research due to their ability to partially fulfill the aforementioned criteria. They are cost-effective, easy to care for, and can be genetically manipulated. Nonetheless, it is crucial to acknowledge significant genetic and physiological disparities between mice and humans, particularly in terms of lipoprotein metabolism (Veseli *et al*., 2017). In contrast to humans, mice predominantly transfer cholesterol through High-Density Lipoprotein (HDL) particles rather than Low-Density Lipoprotein (LDL). Consequently, a majority of blood cholesterol in mice is transported by HDL, resulting in significantly lower overall cholesterol levels compared to humans. These factors result in improved protection against atherosclerosis in mice due to better reverse cholesterol transport pathways (Gordon *et al*., 2015). A contributing factor to this distinction is the reduced presence of Cholesteryl Ester Transfer Protein (CETP) in mice. CETP facilitates the movement of triglycerides from Very Low-Density Lipoproteins (VLDL) to High-Density Lipoprotein (HDL), as well as cholesterol ester from HDL-VLDL. Humans have increased levels of CETP which results in high VLDL and LDL-C concentrations in the blood (Mabuchi *et al*., 2014).

Bile acid composition presents another major difference between humans and mice. Besides the bile acid variations commonly found in humans, mice possess additional hydrophilic α-and β-muricholic acids. These acids play a role in reducing cholesterol absorption in the intestine. Furthermore, the difference in secondary and tertiary bile acid composition (for example, taurine vs glycine conjugation) and higher expression of bile acids in mice contribute to reverse cholesterol transport and fecal cholesterol excretion (Li and Dawson, 2019). These differences make mice less exposed to atherosclerosis than humans.

To overcome the mentioned challenges and establish an appropriate mouse model for atherosclerosis studies, researchers have employed dietary and genetic modifications. Typically, wild-type mice naturally consume low levels of cholesterol (0.02-0.03%) and fats (5-6%) in their food. However, such a low-lipid diet fails to induce thermogenesis. Therefore, researchers opt for "humanized" diets instead. Examples include the "western-type" diet, which contains approximately 21% fat and 0.15% cholesterol, and the "atherogenic diet" with similar fat content but over 1% cholesterol. These modified diets aim to mimic conditions that can facilitate the study of atherosclerosis in mice (Getz and Reardon, 2012). Although a high-fat diet alone is typically insufficient to initiate atherosclerosis in the majority of wild-type mice, it effectively promotes the development of atherogenesis in genetically susceptible mouse models predisposed to the disease (Golforoush *et al*., 2020).

Table 1: Advantages and disadvantages of the use of various murine models

Model	Advantages	Disadvantages	References
Dietary model	No engineering interventions needed	could not maintain the chronic inflammation process associated with atherosclerosis in humans; small lesions very different. From human plaques; no fibrous atheroma's development	Poznyak *et al*. (2020); with Getz and Reardon (2006); Vinué *et al*. (2018); Stylianou *et al*. (2012); Kappel *et al*. (2020)
Apolipoprotein E deficient (ApoE-/-) mice	Develops all types of atherosclerotic lesions	Thrombosis and plaque rupture are rarely observed in mice	Getz and Reardon (2009); Aguilar-Ballester *et al*. (2020); Getz and Reardon (2016b); Mak *et al*. (2014); Mushenkova *et al*. (2019); Muthuramu *et al*. (2022); Feingold (2021); Bond and Jackson (2010); Wang *et al*. (2019); Linton *et al*. (2019); Baetta *et al*. (2007); Sasaki *et al*. (2006); Xu *et al*. (2015); Leong *et al*. (2015)
LDL receptor-deficient (LDLr-/-) mice	The lipid profile in mice bears greater resemblance to that of humans, as a significant portion of cholesterol is carried by LDL particles; unlike ApoE knockout, LDLr deficiency does not induce inflammatory processes that would contribute to the atherogenesis; LDLr-/-mice demonstrate some features typical for familial hypercholesterolemia phenotype in humans which is also based on lack of functional LDL receptors	Requires a cholesterol-rich diet to produce complex of atherosclerotic lesions	Zhang *et al*. (2016); Miyajima *et al*. (2018); Berneis and Krauss (2002); Whitman (2004); Young *et al*. (2008); Getz and Reardon (2016a); Al-Allaf *et al*. (2010)
ApoE/LDL receptor double-knockout mice	Appropriate for trials of anti-atherosclerotic treatments that do not require an atherogenic diet	The higher mortality rate in animals	Véniant *et al*. (2001); Gisterå *et al*. (2022);
ApoE3-Leiden mice	The lipoprotein profile of ApoE3-Leiden mice share striking resemblance to that of humans, as a predominant fraction of cholesterol is conveyed through LDL and VLDL particles; unlike the ApoE-/-mice, ApoE3-Leiden mice can express functional ApoE, so that the effect of high lipid concentrations in plasma can be studied independently and atherogenesis is not affected by inflammation	Expensive; A relatively small number of individuals are affected by the specific condition known as APOE3-Leiden familial dysbetalipoproteinemia	Paalvast *et al*. (2017); Parma *et al*. (2020); Mohanta *et al*. (2016); Groot *et al*. (1996); Pouwer *et al*. (2019); Row *et al*. (2017); Lardenoye *et al*. (2002)
PCSK9-AAV mice	Created without genetic modifications, cost-efficient, versatile, and quick the introduction of mutant human PCSK9 through a single injection has demonstrated a consistent the atherogenic effect, with minimal concerns regarding biosafety	Require long study periods and a cholesterol-rich diet to produce complex atherosclerotic lesions	Roche-Molina *et al*. (2015); Seidah (2017); Barale *et al*. (2021); Louloudis *et al*. (2021); Sun *et al*. (2018); Tavori *et al*. (2016); Goettsch *et al*. (2016)

Murine Models

In order to create mouse models predisposed to atherosclerosis to a degree comparable to humans, the targeted modification of various genes resulted in an alteration of the lipoprotein profile, specifically leading to increased concentrations of VLDL and LDL-C (Lo Sasso *et al*., 2016).

There are notable differences in lesion development between mice and humans. In humans, most lesions predominantly occur in the carotid and coronary arteries and advance into larger atheromas. On the other hand, mice tend to develop plaques in the brachiocephalic artery, proximal aorta, aortic arch, and aortic sinus, but these plaques do not typically progress into fibrous atheromas. Plaque rupture, infarction, or myocardial ischemia is not revealed even in transgenic mouse models predisposed to atherosclerosis (Oppi *et al*., 2019).

Below various genetically altered mouse models will be presented as well as some guidelines on how to choose the most appropriate model depending on the focus of the study Table (1).

Dietary Models

Wissler and colleagues created the first murine atherosclerosis model in 1960. The utilization of a dietary regimen comprising 30% fat, 5% cholesterol, and 2% colic acid proved to be effective in inducing hypercholesterolemia while facilitating the deposition of lipid sediments in various vascular regions. Although the high-fat diet did set off acute inflammation, it could not maintain the chronic smoldering inflammation process associated with atherosclerosis in humans. In addition, the high toxicity of such a diet resulted in mice losing weight and becoming more exposed to infections (Poznyak *et al*., 2020).

The inclusion of cholate in dietary studies on atherosclerosis has been widely employed, even in contemporary research. Nonetheless, cholate exhibits diverse effects and its utilization can potentially complicate the interpretation of both lipoprotein balance and the chronic inflammation crucial to atherosclerotic lesions. Being a bile salt, it assists in cholesterol absorption and, via hepatobiliary circulation, modulates the conversion of cholesterol into bile acid (Zhao *et al*., 2020). Consequently, its inclusion in the diet leads to increased cholesterol loading and subsequent hypercholesterolemia. Furthermore, it has been noted that cholate has the ability to increase the activation of genes linked to hepatic fibrosis. Moreover, cholate serves as a ligand for the nuclear hormone receptor FXR, which regulates the expression of various genes involved in lipoprotein metabolism. Among these genes, apoCII and apoCIII could potentially account for the reduced levels of plasma triglycerides observed in diets that incorporate cholate, in comparison to diets lacking cholate (Voisin *et al*., 2020).

Subsequently, the researchers devised a safer diet composition consisting of a mere 15% fat, 1.25% cholesterol, and 0.5% cholic acid. As a result, different types of atherosclerosis were observed in various strains of mice administered this diet. Strain C57BL/6 turned out to be most predisposed to atherosclerosis (Getz and Reardon, 2006). The mice developed low-grade hypercholesterolemia with cholesterol at around 200 mg/mL and after three to nine months of such feeding, lipid lesions were revealed in the aortic root. The small lesions predominantly consisting of macrophages were still very different from human plaques and did not develop into fibrous atheromas (Vinué *et al*., 2018). This difference between atherosclerosis development in mice and humans is a limitation that makes data extrapolation less precise. However, through dietary models, significant progress was made in pinpointing specific genetic factors related to atherosclerosis susceptibility. One such gene, Ath-1, was identified to be located near Alp-2 on chromosome 1 (Stylianou *et al*., 2012).

Recently there have been carried out studies involving germ-free mice administered antibiotic cocktails. A metabolomics approach allowed scientists to identify dietary lipid phosphatidylcholine (lecithin) in animals, which has been linked to the onset of atherosclerosis in humans. This proves how important is the microbiome in the intestine for regulating plasma lipoprotein levels (Kappel *et al*., 2020).

Apolipoprotein E Deficient (ApoE-/-) Mice

ApoE, weighing approximately 34 kDa, is a protein primarily synthesized in the brain and liver. Additionally, macrophages and monocytes also contribute to its production. This glycoprotein is present in all types of lipoprotein particles apart from LDLs and is a ligand for lipoprotein receptors on the surface of the cell that are responsible for the clearance of VLDL remnants and chylomicrons (Getz and Reardon, 2009). The protein is also involved in cholesterol metabolism, homeostasis and transportation in tissues, immunoregulation, dietary uptake of cholesterol, and its excretion (Aguilar-Ballester *et al*., 2020).

In 1992, two separate laboratories independently produced the first ApoE-/-mice. The ApoE gene knockout was achieved through the process of homologous recombination in mouse embryonic stem cells (Getz and Reardon, 2016a) the transgenic mice were born healthy and within the expected timeframe, with body weight similar to that of wild-type mice. However, they exhibited a notable difference in lipoprotein metabolism when compared to the wild-type mice. The absence of the ApoE gene caused significant impairment in their ability to process plasma lipoproteins, resulting in elevated cholesterol levels in the blood (400-600 mg/dL even when fed a normal diet. In contrast, wild-type mice had cholesterol concentrations of 75-110 mg/dL (Mak *et al*., 2014). The reason for this difference is an overage of VLDL-sized particles. Based on the findings, it can be inferred that the absence of ApoE alone is sufficient to bring about significant

alterations in the lipoprotein profile, irrespective of external influences. Furthermore, the deficiency of ApoE heightened the mice's susceptibility to dietary fats and cholesterol. After being fed a Western-type diet for a few weeks, ApoE knockout mice experienced a fourfold surge in plasma cholesterol levels, whereas the total plasma cholesterol in wild-type mice only doubled under the same diet (Mushenkova *et al*., 2019). Within two to three months of age transgenic mice develop extensive atherosclerosis, independently of the diet. Nevertheless, when mice with heterozygous ApoE deficiency were subjected to a Western-type diet, there was no notable rise in plasma cholesterol levels. This suggests that a 50% decrease in ApoE is inadequate to trigger an elevation in plasma cholesterol. No difference was observed between mice of different age or sex (Muthuramu *et al*., 2022).

ApoE-/-mice exhibit the formation of various types of atherosclerotic lesions. From the age of six weeks onwards, there is observable adherence of monocytes to the endothelium and after eight weeks, the development of foam cell lesions begins. By 15-20 weeks, medium-sized lesions and fibrous plaques primarily composed of smooth muscle cells, extracellular matrix, as well as plaques with a necrotic core and a fibrous cap can be identified (Muthuramu *et al*., 2022). In later stages, fibrous niduses containing lipids are formed which soon become calcified. Western-type diet accelerates the plaque formation process remarkably and within the same time span mice can develop three to four times bigger lesions compared to animals fed with a normal diet. This diet-dependent mechanism resembles the diet-dependent course of human atherosclerotic heart disease where increased fat content in the diet increases plasma cholesterol levels (Feingold, 2021).

Typically, atherosclerotic lesions tend to form in ApoE knockout mice at sites where blood vessels bifurcate. These sites are particularly prominent in the carotid and pulmonary arteries, the main branches of the aorta, the aortic root, and the aortic arch. The process of plaque formation in transgenic mice resembles the disease course in well-established larger animal models and humans (Bond and Jackson, 2010). Being used by many scientists, the ApoE-/-mouse model still has some limitations. ApoE plays a crucial role in various biological processes, including inflammatory pathways, the proliferation and migration of Smooth Muscle Cells (SMCs), reverse cholesterol transport mechanisms, and oxidation. Even in the absence of significant changes in blood cholesterol levels, these factors can potentially contribute to the progression of atherosclerosis in mice (Wang *et al*., 2019). Also, the most prevalent lipoprotein in ApoE-/-mice is VLDL, while human atherosclerosis is mostly associated with LDL excess. Still, the main limitation of such models is that thrombosis and plaque rupture are rarely observed in mice, while these events are very common in the course of atherosclerosis in humans, resulting in stroke and MI. The reason behind this disparity lies in the contrasting thickness of mouse and human blood vessels. Mouse vessels are considerably thinner, thus exhibiting lower surface tension. This characteristic reduces the likelihood of plaque rupture occurring. However, one should also take other explanations presented below into consideration (Linton *et al*., 2019).

Perivascular collar placement is a technique utilized to induce site-specific atherosclerosis and plaque rupture in ApoE knockout mice, as documented by Baetta *et al*. (2007). Additionally, the research conducted by Sasaki *et al*. (2006). Demonstrates that ligating the left carotid artery in the animal model leads to plaque rupture (Sasaki *et al*., 2006). Cuff placement caused marked intimal hyperplasia, resulting in lesions containing collagen and lipids and eventually leading to intraplaque hemorrhage and plaque rupture. Furthermore, the study unveiled a reduction in collagen levels, along with an escalation in apoptotic cells and the development of luminal thrombi positive for fibrin (ogen), mirroring the process of plaque rupture observed in humans. Perivascular collar placement in the carotid artery can also model rapid and localized atherosclerosis without damaging the endothelial structure (Xu *et al*., 2015). Lesions mostly appear close to the collar. The endothelial expression mechanisms and plaque composition in these models closely resemble human atherosclerosis, setting this model apart from traditional animal models (Leong *et al*., 2015).

LDL Receptor-Deficient (LDLr-/-) Mice

The LDL receptor, a 160 kDa mosaic cell surface protein, plays a vital role in the uptake of cholesterol-rich Low-Density Lipoprotein (LDL) and helps regulate LDL levels in the bloodstream. Additionally, it governs the uptake of lipoproteins containing apolipoproteins B and E into cells (Zhang *et al*., 2016). When there is a deficiency in LDL receptors or mutations in the corresponding gene, it leads to the manifestation of familial hypercholesterolemia phenotype. In 1993 mice with genetically deactivated LDLr were created when these mice were provided with a regular diet, they demonstrate mild hypercholesterolemia and develop no or only low-grade atherosclerosis, compared to wild-type animals (Miyajima *et al*., 2018). There was observed a significant increase of IDL and LDL sized lipoprotein particles, while no change was noticed in the number of triglycerides and HDL. It is worth mentioning that LDLr-/-mice differ from ApoE-/-mice predominantly accumulated cholesterol in large lipoprotein particles like VLDL, IDL, or chylomicron remnants. Nevertheless, when given a Western-style diet rich in fat and cholesterol, LDLr-/-mice exhibited a profoundly distinct and highly atherogenic lipoprotein profile (Berneis and Krauss, 2002).

The types of plaques in LDLr knockout mice and ApoE knockout mice are mostly similar. Upon consuming a Western-style diet, the mice undergo a progression of advanced atheromas characterized by the presence of fibrous caps composed of collagen, a necrotic core abundant in lipids, and an increased cellular concentration nearer to the vessel lumen. The lesion initially manifests in the proximal aorta and gradually advances towards the distal aorta as time elapses (Whitman, 2004). Vessel sites where the blood flow is hindered are more susceptible to plaque formation. When mice with homozygosity for the ApoB-100 allele, specifically LDLr-/-and ApoE-/-strains, were examined, it was observed that their cholesterol levels reached approximately 300 mg/dL under normal dietary conditions. Surprisingly, even on a normal diet, atherosclerotic plaques were more commonly observed in LDLr-/-ApoB100/100 mice compared to ApoE-/-ApoB100/100 mice (Young *et al*., 2008).

Compared to ApoE-/-mice, the LDLr-/-model has a number of advantages. To begin with, it is noteworthy that their lipid profile bears a closer resemblance to that of humans. This is primarily due to the fact that a significant portion of their cholesterol is transported within LDL particles. Secondly, unlike ApoE knockout, LDLr deficiency does not induce inflammatory processes that would contribute to atherogenesis (Getz and Reardon, 2016b). Therefore, the progression of atherosclerosis in LDLr knockout mice is solely attributed to elevated cholesterol levels in their plasma and remains unaffected by any other deficiencies resulting from the absence of LDLr. Thirdly, LDLr-/-mice demonstrate some features typical for familial hypercholesterolemia phenotype in humans which is also based on a lack of functional LDL receptors (Al-Allaf *et al.*, 2010).

ApoE/LDL Receptor Double-Knockout Mice

Following the establishment of ApoE-/-and LDLr-/-mouse models, ApoE/LDL receptor double knockout mice were promptly created. In this particular model, the presence of hypercholesterolemia and atherosclerosis is more pronounced compared to ApoE-/-and LDLr-/-mice (Véniant *et al*., 2001). Even on a normal diet, these animals develop spontaneous atherosclerotic plaques and generally demonstrate a more severe progression of the disease than ApoE knockout mice. However, the lipoprotein profile of these two models is quite similar, both demonstrating increased VLDL and LDL concentrations, although the levels of B48 and B100 Apo lipoproteins are higher in ApoE/LDL receptor double knockout mice. The utilization of ApoE/LDL receptor double knockout mice is highly advantageous for conducting trials on anti-atherosclerotic treatments, particularly those that do not necessitate an atherogenic diet (Gisterå *et al*., 2022).

ApoE3-Leiden Mice

While ApoE-/-mice and LDLr-/-mice are the most commonly used murine models, ApoE3-Leiden models also see frequent utilization. The Apolipoprotein (Apo) E3-Leiden mutation, originally discovered in a large Dutch family, is associated with familial hyperlipidemia. *In vivo* studies of this mutation have been conducted using transgenic mice. A genomic 27-kilobase DNA construct containing the APOE gene, APOC1 gene, and all known regulatory elements was isolated from the APOE3-Leiden proband (Paalvast *et al*., 2017).

The presence of the E3-Leiden mutation results in the production of a malfunctioning protein with reduced affinity for the Low-Density Lipoprotein receptor (LDLr), impairing the clearance of triglyceride and cholesterol-rich lipoproteins (such as chylomicron and VLDL remnants). This mutation mirrors the slow clearance observed in individuals, particularly those with familial dyslipidemia (Wardell *et al*., 1989). When E3L mice are fed a Western-type diet high in saturated fat and cholesterol, they exhibit susceptibility to developing hyperlipidemia and atherosclerosis. Similar to humans, these mice respond similarly to interventions used in clinical practice to modulate lipid levels, such as statins, fibrates, niacin, and PCSK9 inhibitors (Pouwer *et al*., 2019).

It is important to mention that the ApoE3-Leiden model, although less susceptible to atherosclerosis compared to ApoE knockout mice, exhibits a notable increase in triglyceride and total plasma cholesterol levels when subjected to a Western-type diet. The lipoprotein profile of ApoE3-Leiden mice closely resembles that of humans, as a majority of cholesterol is transported in LDL and VLDL particles. Another advantage of this model is that, unlike the ApoE-/-mice, ApoE3-Leiden mice can express functional ApoE, so that the effect of high lipid concentrations in plasma can be studied independently and the development of atherosclerosis is not affected by inflammation (Parma *et al*., 2020).

When ApoE3-Leiden mice are fed a Western-type diet, they develop lesions in various areas including the aorta and large vessels. Specifically, these lesions are observed in the aortic root, arch, major bifurcation points, proximal coronary arteries, renal artery branch points, thoracic aorta, abdominal aorta and its bifurcation and iliac artery bifurcations (Mohanta *et al*., 2016; Groot *et al*., 1996). Remarkably, early foam cell formations are revealed in ApoE3-Leiden mice on a normal diet. After several months of consuming a Western-type diet, ApoE3-leiden mice develop more advanced and intricate lesions (Pouwer *et al*., 2019). These mice are commonly used in researching ApoE metabolism and investigating factors related to the development of familial dyslipidemia. Additionally, they are valuable for studying

potential complications associated with venous bypass surgery. This surgical procedure involves connecting a graft to a blood vessel to bypass an atherosclerotic blockage (Pouwer *et al*., 2019). Just like in humans, the grafted vessel in the model animal is exposed to vessel injury, increased blood pressure, and shear stress as a result of the surgery and remodeling. The presence of these factors leads to intimal hyperplasia and contributes to the progression of atherosclerosis, potentially resulting in graft obstruction (Row *et al*., 2017). To mimic native atherosclerosis, a mouse model of vein graft disease has been developed. This model involves using ApoE-/-or ApoE3-Leiden mice and grafting the thoracic caval vein from a donor mouse onto the carotid artery of a recipient mouse. By utilizing this model, researchers can investigate the accelerated development of atherosclerosis in vein grafts, which closely resemble human atherosclerotic plaques that are prone to rupture (Lardenoye *et al*., 2002). This model develops complex lesions typical for advanced atherosclerosis characterized by a necrotic core with cholesterol clefts, the presence of foam cells, neovascularization, and calcification.

PCSK9-AAV Mice

The pro-protein convertase subtilisin/kexin type 9 (PCSK9) -Adeno Associated Virus (AAV) mice are a new strain generated independently in two different laboratories in 2014 for the research of atherosclerosis (Roche-Molina *et al*., 2015). This model is created without genetic modifications and is described as cost-efficient, versatile, and quick. PCSK9 is a recently identified subtilisin-like serine protease primarily produced in the human liver. Normal PCSK9 plasma levels are approximately ≈100-200 ng/mL. The enzyme affects LDL uptake in the liver. PCSK9 has a crucial function of binding to the LDLr found on hepatocytes and facilitating their endosomal and lysosomal degradation. It plays a vital role in regulating cholesterol levels. By interacting with hepatic LDLr, PCSK9 promotes its degradation, leading to reduced uptake of LDL-C and the subsequent increase in LDL-C levels in the bloodstream (Seidah, 2017). Recombinant AAV vectors are commonly utilized in animal models and human studies to ensure long-term transgene expression. A single intravenous injection of murine D377Y or human D374Y gain-of-function mutant PCSK9 can lead to stable hepatic expression of PCSK9DYmRNA in mice. No immune response, signs of liver damage, or adverse effects were identified in the animals following the AAV injection (Barale *et al*., 2021). One month after the infection total plasma cholesterol concentrations became two times higher in PCSK9DY-AAV models compared to wild-type controls. The same difference was observed a year later, proving that a single AAV infection has chronic consequences. The hyperlipidemia impact is greatly intensified when PCSK9DY-AAV mice are fed a Western-type diet, resulting in total plasma cholesterol levels reaching as high as 1165 mg/dL (Louloudis *et al*., 2021). Meanwhile, the PCSK9DY-AAV mice fed a normal chow diet only reached a cholesterol level of 316 mg/dL. When it comes to lipoprotein particles, those on a Western-type diet exhibited an even distribution between VLDL and LDL. The severity of atherosclerosis in this model is dependent on the dosage of PCSK9. The resulting lesions in PCSK9DY-AAV mice due to hyperlipidemia closely resemble those in LDLr-/-mice fed a high-fat diet. The lesions progress in the aortic root, advancing to fibrous atheromas with the presence of Smooth Muscle Cells (SMCs), macrophage infiltration, and foam cells. Calcification of the blood vessels typically occurs after approximately 4-5 months. Roche-Molina conducted a study on the combination of ApoE deficiency and PCSK9DY expression, observing a synergistic effect. These mice developed lesions twice the size compared to individual mutants, despite similar lipoprotein profiles between the models. The PCSK9-AAV mice are a valuable tool in atherosclerosis research due to the sustained atherogenic effect of a single injection of mutant human PCSK9, low concerns regarding biosafety and its efficacy across various genetic profiles in animals (Goettsch *et al*., 2016).

Future Perspectives

Current animal models used for studying atherosclerosis involve Causing rapid plaque development by implementing a diet rich in cholesterol or manipulating genes associated with cholesterol metabolism. However, there is a need for further development of models that accurately represent the advanced disease stage, including the rupture of plaques and subsequent atherothrombosis. It is of utmost importance to thoroughly assess experimental findings within the framework of the particular model utilized and comprehensive experimental procedures are vital for promoting reproducibility. Although sophisticated *in vitro* systems are valuable for examining mechanisms, the intricate interaction of multiple organ systems in atherosclerosis requires the utilization of *in vivo* models (Mushenkova *et al*., 2019).

Animal models offer several advantages, including the ability to induce disease states similar to those in humans, shorter lifespans, authority over environmental variables, ethical considerations, availability of suitable littermate controls, and predetermined genetic backgrounds. Nonetheless, there are also drawbacks to consider, including the expenses related to housing and breeding, extended generation periods, and variations in metabolic, physiological, and anatomical attributes

(Khorramizadeh and Saadat, 2020). For the effective application of findings in human medicine, it is vital to have a thorough comprehension of the fundamental pathophysiology. Recent progressions, including microbiota control, thermoneutral housing, and the integration of human-like lipoproteins and immune systems, have enhanced the possibilities of achieving successful translation. Anichkov once emphasized the importance of conducting parallel research on both humans and experimental animals to gain accurate insights into the morph dynamics of diseases. Verifying disease mechanisms through the study of human patient samples and cohorts continues to be crucial in bridging the divide between preclinical and clinical medicine (Gisterå *et al*., 2022).

Moving forward, it is crucial to appreciate the benefits of vascular disease models while also acknowledging their limitations. This understanding has brought attention to two key obstacles in the advancement of cardiovascular biology. Firstly, effectively assimilating the extensive existing knowledge to create innovative and well-defined hypotheses and testing them within the most suitable models poses a challenge. Secondly, there is a necessity to enhance and refine the models used for studying atherosclerosis and its various manifestations. It is imperative to employ rigorous approaches that promote swift progress in the field, enabling comprehensive exploration of hypothesis-generating aspects that arise from data-driven high-throughput research (Harper *et al*., 2022).

Conclusion

New murine models will be introduced in the future for more specific studies around the etiology of atherosclerosis aimed at revealing and describing other mechanisms behind the development of the disease. Tissue-specific overexpression murine models or knockout mice are being used by most scientists today. Moreover, ongoing studies are investigating the impact of particular mutations and posttranslational protein modifications, such as simulation, on the progression of atherosclerosis and cardiovascular ailments. It is evident that further comprehensive research is warranted to understand these mechanisms in greater detail. Additionally, the remarkable and swift implementation of the CRISPR/Cas9 system has facilitated the introduction of novel murine models and larger animal models to examine the underlying causes of atherosclerosis. This breakthrough undoubtedly paves the way for expediting both fundamental and practical investigations pertaining to cardiovascular diseases.

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Author's Contributions

Anastasia Vladimirovna Poznyak: Drafted written.

Victoria Alexandrovna Khotina, Alexandra Alexandrovna Melnichenko, Vasily Nikolaevich Sukhorukov, Igor Alexandrovich Sobenin and Alexander Nikolaevich Orekhov: Drafted reviewed, and edited.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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