Animal models of atherosclerosis

Animal models of atherosclerosis

Besa Emini Veseli, Paola Perrotta, Gregory R. A. De Meyer, Lynn Roth, Carole Van der Donckt, Wim Martinet and Guido R.Y. De Meyer*

Laboratories of Physiopharmacology and Pharmacology, University of Antwerp, Belgium

*Correspondence to: University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium. guido.demeyer@uantwerpen.be

Abstract

An ideal animal model of atherosclerosis resembles human anatomy and pathophysiology and has the potential to be used in medical and pharmaceutical research to obtain results that can be extrapolated to human medicine. Moreover, it must be easy to acquire, can be maintained at a reasonable cost, is easy to handle and shares the topography of the lesions with humans. In general, animal models of atherosclerosis are based on accelerated plaque formation due to a cholesterol-rich/Western-type diet, manipulation of genes involved in the cholesterol metabolism, and the introduction of additional risk factors for atherosclerosis. Mouse and rabbit models have been mostly used, followed by pigs and non-human primates. Each of these models has its advantages and limitations. The mouse has become the predominant species to study experimental atherosclerosis because of

its rapid reproduction, ease of genetic manipulation and its ability to monitor atherogenesis in a reasonable time frame. Both Apolipoprotein E deficient ($ApoE^{-/-}$) and LDL-receptor (LDLr) knockout mice have been frequently used, but also ApoE/LDLr double-knockout, ApoE3-Leiden and PCSK9-AAV mice are valuable tools in atherosclerosis research. However, a great challenge was the development of a model in which intra-plaque microvessels, haemorrhages, spontaneous atherosclerotic plaque ruptures, myocardial infarction and sudden death occur consistently. These features are present in $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice, which can be used as a validated model in pre-clinical studies to evaluate novel plaque-stabilizing drugs.

Keywords: animal models, atherosclerosis, plaque rupture, ApoE, LDL receptor, PCSK9

1. Introduction

Atherosclerosis is a progressive inflammatory disease characterized by accumulation of lipids in the arterial vessel wall, which starts early in life. Disease progression leads to build-up of atherosclerotic plaques that cause narrowing of the arterial lumen. Atherosclerotic plaques often remain stable for years, but can rapidly become unstable, rupture and trigger thrombus formation. Accordingly, in addition to restriction of the vessel lumen, the presence of atherosclerotic plaques is linked to an increased risk of acute cardiovascular events such as myocardial infarction (MI) and stroke. The use of animal models of atherosclerosis is an essential tool to improve the understanding of the molecular mechanisms behind

atherosclerotic plaque formation and progression, as well as the occurrence of plaque rupture and its associated cardiovascular events. Moreover, animal models allow to assess novel pharmacological treatments that can prevent or slow down the onset of atherosclerosis. In general, animal models for atherosclerosis are based on accelerated plaque formation due to: (1) a cholesterol-rich/Western-type diet, (2) manipulation of genes involved in the cholesterol metabolism, and (3) the introduction of additional risk factors for atherosclerosis, such as diabetes.

In this review, we will discuss the animal models that have contributed to the understanding of atherosclerosis and its clinical consequences, and that allow significant improvement in treatment.

Numerous studies have shown that high plasma levels of low-density lipoprotein (LDL) represent one of the most prominent risk factors of atherosclerosis. Indeed, LDL tends to accumulate in the sub-endothelial space of the arterial wall and progressively undergoes oxidative modifications to form oxidized LDL (oxLDL). This induces an inflammatory response characterized by overexpression of chemotactic proteins such as monocyte chemoattractant protein-1 (MCP-1), and adhesion molecules (vascular cell adhesion molecule-1 (VCAM-1), E-selectin and P-selectin) by endothelial cells (Fuster et al., 2012; Tabas et al., 2015). Adhesion molecules promote the infiltration of blood-carried monocytes into the inflamed arterial wall. After differentiation into macrophages, these cells engulf oxLDL, transform into foam cells and contribute to plaque development by secreting multiple mediators of the inflammatory process in the vessel wall (Sakakura et al., 2013). The

inflammatory response also promotes recruitment of circulating monocytes and T-cells that stimulate the migration of vascular smooth muscle cells (SMCs) from the tunica media into the sub-endothelial space where they exhibit abnormally high proliferation and secrete extracellular matrix proteins that also contribute to atheroma growth (Fuster et al., 2012). Advanced human plaques are characterized by a large necrotic core, many lipid laden and activated macrophages, few SMCs, intra-plaque neovascularisation and haemorrhages, and a thin fibrous cap that separates the plaque from the blood stream. Rupture of the fibrous cap of such high-risk vulnerable plaques leads to luminal thrombosis, arterial occlusion or embolism in distant vascular beds, resulting in MI, stroke or sudden death (Berliner et al., 1995). In humans, atherosclerotic plaques can usually be found in the aorta, coronary arteries and in the carotid and cerebral arteries (Lusis, 2000).

In 1908, Ignatowski investigated for the first time plaque formation in the aortic wall of rabbits that were fed a cholesterol-rich diet (Konstantinov and Jankovic, 2013). Since then many other animal species such as mice, birds, pigs and non-human primates have been used as an experimental model of atherosclerosis (Fuster et al., 2012; Kapourchali et al., 2014).

An ideal animal model resembles human anatomy and pathophysiology, and has the potential to be used in medical and pharmaceutical research to obtain results that can be extrapolated to human medicine. Moreover, it is important that animals used as models are easy to acquire, can be maintained at a reasonable cost, are easy to handle and have well-defined genetic characteristics. A valuable animal model for atherosclerosis research not only shares the crucial aspects of the disease process

with humans but also the topography of the lesions. In addition, the animals preferably develop lesions in a spontaneous manner after consumption of a diet similar as in humans (Getz and Reardon, 2012). Although several animals develop atherosclerotic plaques after a cholesterol-rich diet, the topography of the lesions is not always similar as compared to humans. Furthermore, it is important to note that in the majority of atherosclerosis models, animals do not spontaneously develop the complications seen in humans such as plaque rupture, MI, stroke and sudden death.

2. Mouse models of atherosclerosis

Over the past decades, the mouse has become the predominant species to study experimental atherosclerosis because of its rapid reproduction, ease of genetic manipulation and its ability to monitor atherogenesis in a reasonable time frame (Bond and Jackson, 2011; Getz and Reardon, 2012; Schwartz et al., 2007; VanderLaan et al., 2004). However, mice are relatively resistant to the development of atherosclerosis due to their significantly different lipid profile as compared to humans. Therefore, genetic manipulation of their lipid metabolism is mandatory (Getz and Reardon, 2012; Meir and Leitersdorf, 2004). In mice, most of the cholesterol is transported in high density lipoprotein (HDL) like particles. Accordingly, mice contain only low concentrations of the atherogenic LDL and very low density lipoprotein (VLDL). Mice deficient in the receptor clearing these LDL particles ($LDLr^{-/-}$ mice) develop significantly higher plasma levels of cholesterol. Apolipoprotein E (ApoE) is a glycoprotein synthesized mainly in the liver and the brain and functions as a ligand for receptors that clear chylomicrons and VLDL

remnants (Meir and Leitersdorf, 2004). Deficiency in this glycoprotein (ApoE$^{-/-}$) leads to increased plasma levels of total cholesterol, mostly in the VLDL and chylomicron fractions (Piedrahita et al., 1992), which are quadrupled by a high-fat or Western-type diet (Plump et al., 1992). Both mouse models have extensively been used to study the mechanisms underlying the initiation and progression of atherosclerosis. Atherosclerotic lesions in mice develop in regions of the vasculature subjected to low and/or oscillatory shear stress (Bond and Jackson, 2011; VanderLaan et al., 2004). Predilection sites in the mouse are the aortic root, lesser curvature of the aortic arch and branch points of the brachiocephalic, left carotid and subclavian arteries. However, on a high-cholesterol diet, ApoE$^{-/-}$ mice develop plaques more rapidly and with a more advanced phenotype as compared to LDLr$^{-/-}$ mice (Silvestre-Roig et al., 2014), making the ApoE$^{-/-}$ model widely used in experimental atherosclerosis studies.

The Apolipoprotein (Apo) E3-Leiden mutation is associated with a genetic form of hyperlipidaemia. Therefore, ApoE3-Leiden transgenic mice can also be used as a model for atherosclerosis, but in comparison with ApoE$^{-/-}$ and LDLr$^{-/-}$ mice, they show rather low levels of total plasma cholesterol and triglycerides when fed a normal diet. Nevertheless, these mice are highly responsive to fat-, sugar-, and cholesterol-containing diets resulting in strongly elevated lipoprotein profiles (Zadelaar et al., 2007). Regardless of lesion development, varying from fatty streaks to mild, moderate, and severe plaques, ApoE3-Leiden mice lack the critical events such as plaque rupture, thrombus formation, and/or haemorrhage, which are of major importance in human atherosclerosis (Lutgens et al., 1999; Ross, 1995).

High plasma levels of lipoprotein (a) [Lp(a)], which is a complex of LDL and a large glycoprotein called Apolipoprotein (a) [Apo(a)], is an independent risk factor for the development of atherosclerosis in humans (Breslow, 1993; Gencer et al., 2017). Virtually all species other than primates lack Apo(a), hampering the use of convenient animal models to study its role in atherosclerotic plaque development. Therefore, transgenic mice that express human Apo(a) are used. When fed a Western-type diet, these mice show the presence of macrophage-like cells in combination with the development of fatty-streak lesions at the base of the aorta (Lawn et al., 1992). In humans, plasma Apo(a) is almost entirely covalently bound to LDL, whereas in mice, Apo(a) circulates as non-lipoprotein associated Apo(a) (Lawn et al., 1992). Therefore, Apo(a) transgenic mice can be used to identify the role of Apo(a) in atherogenesis, independent of human LDL.

The most commonly used mouse models of atherosclerosis are described in detail below and in Figure 1.

2.1 Apolipoprotein E deficient (ApoE$^{-/-}$) mice

ApoE is a glycoprotein with a molecular size of approximately 34 kDa. It is synthesized mainly in the liver and brain, and is a structural component of all lipoprotein particles except low-density lipoproteins. It serves as a ligand for cell-surface lipoprotein receptors whose function is to clear chylomicrons and VLDL remnants. It is also synthesized by monocytes and macrophages (Curtiss and Boisvert, 2000). Other functions include cholesterol homeostasis, local

redistribution of cholesterol within tissues, immunoregulation and dietary absorption and biliary excretion of cholesterol (Mahley, 1988; Sehayek et al., 2000).

In 1992, the first line of ApoE$^{-/-}$ mice was developed almost contemporaneously in two laboratories (Piedrahita et al., 1992; Plump et al., 1992). The deletion of the ApoE gene was done in mouse embryonic stem cells by homologous recombination. ApoE$^{-/-}$ mice were healthy, had a similar body weight as wild-type mice, and were born at the expected frequency (Jawien et al., 2004; Plump et al., 1992). However, their lipoprotein profile disclosed significant differences with the wild-type mates. The ability of ApoE$^{-/-}$ mice to clear plasma lipoproteins is severely impaired resulting in plasma cholesterol levels of 400-600 mg/dl when fed a normal diet, whereas wild-type mice have levels of 75-110 mg/dl (Nakashima et al., 1994; Plump and Breslow, 1995). This drastic change is due to an increase in VLDL-sized particles. The development of significant hypercholesterolaemia, even when fed a normal diet, suggests that in the absence of an environmental stimulus, deficiency of ApoE is sufficient to cause massive changes in lipoprotein metabolism. Furthermore, the lack of ApoE boosts the sensitivity to dietary fat and cholesterol. After several weeks of feeding a Western-type diet (consisting of 21% fat and 0.15% cholesterol, which is similar to the everyday diet of Western countries), plasma cholesterol levels double in wild-type mice, whereas in ApoE-deficient mice a fourfold increase in total plasma cholesterol is observed (Plump et al., 1992). Extensive atherosclerosis is seen in mice on both types of diet by 2 to 3 months of age (Reddick et al., 1994). On the other hand, heterozygous ApoE-deficient mice do not show an increase in plasma cholesterol levels even when fed a Western-type diet,

presuming that a 50% decrease in ApoE is not sufficient to increase plasma lipids. Of note, plasma cholesterol levels in mice are not affected by age or sex of the animal (Nakashima et al., 1994).

The entire spectrum of atherosclerotic lesions is present in ApoE$^{-/-}$ mice (Jawien et al., 2004). Monocyte attachment to endothelial cells is noticed from 6 weeks of age, and after 8 weeks foam cell lesion development is detectable. After 15 to 20 weeks, intermediate lesions are present containing mostly SMCs as well as fibrous plaques consisting of SMCs, extracellular matrix and a necrotic core covered with a fibrous cap (Nakashima et al., 1994). In more advanced lesions, fibro-fatty nodules are a nidus for calcification and plaques become more calcified with time (Rattazzi et al., 2005). When fed a Western-type diet, the time course for lesion formation is tremendously accelerated (Jawien et al., 2004). Compared to mice fed a low-fat diet, lesions are 3-4 times larger within the same period of time. This response implies a diet-dependent mechanism, i.e. increased fat leads to increased plasma cholesterol, which in turn leads to increased atherosclerosis, which resembles the diet-dependency of atherosclerotic heart disease observed in humans (Plump et al., 1992).

ApoE$^{-/-}$ mice tend to develop atherosclerotic plaques at vascular branch points, with predilection for the aortic root, the lesser curvature of the aortic arch, the principal branches of the aorta as well as the pulmonary and carotid arteries (Nakashima et al., 1994). Sequential events of plaque formation in ApoE$^{-/-}$ mice are considerably similar to those in well-established larger animal models of atherosclerosis and in humans (Nakashima et al., 1994). Although this mouse model is used by many

research groups, it has some limitations. For instance, ApoE is a multifunctional protein that has an impact on inflammation, oxidation, reverse cholesterol transport by macrophages, and smooth muscle proliferation and migration. These functions might affect atherosclerotic plaque development in ApoE$^{-/-}$ mice, independent of plasma lipid levels (Getz and Reardon, 2009). Furthermore, not LDL, which is characteristic of human atherosclerosis, but VLDL is the most abundant lipoprotein in ApoE$^{-/-}$ mice (Plump et al., 1992). However, the major limitation of the 'classical' mouse models of atherosclerosis is the rarity of plaque rupture and thrombosis (Plump and Lum, 2009; Smith and Breslow, 1997), whereas these events are fairly common in humans and can lead to MI and stroke (Jawien et al., 2004). It has been suggested that this might be due to the tiny diameter of the mouse vessels; as the vessel diameter decreases, the surface tension increases exponentially, impeding the likelihood of plaque rupture (Jawien et al., 2004). However, also other explanations have to be taken into account as discussed below in the section 'mouse models of atherosclerotic plaque rupture'.

A method for the induction of accelerated atherogenesis and plaque rupture is the placement of a perivascular collar or cuff, mainly in ApoE$^{-/-}$ mice. In their study, Sasaki et al., claim that cuff placement around the left carotid artery results in an animal model of plaque rupture. By using the ligation technique to induce neo-intimal hyperplasia, they observed lipid- and collagen-rich lesions accompanied with intra-plaque haemorrhage and plaque rupture. Furthermore, a decrease in collagen content, and formation of fibrinogen-positive thrombi were detected, analogous to plaque rupture in humans (Sasaki et al., 2006). Along with this

observation, perivascular carotid collar placement also reproduces the induction of rapid and site-controlled atherosclerosis (von der Thusen et al., 2001), while maintaining the structural integrity of the endothelium. Formed plaques are located primarily in the area proximal to the collar. The advantages of this model over the conventional animal models of mechanically induced atherosclerosis include the closer resemblance to human plaque morphology and endothelial expression pattern (von der Thusen et al., 2001).

2.2 LDL receptor-deficient ($LDLr^{-/-}$) mice

The LDL receptor is a membrane receptor with a molecular weight of 160 kDa, which mediates the endocytosis of cholesterol-rich LDL and thus maintains the plasma level of LDL. It also facilitates the cellular uptake of apolipoprotein B- and E-containing lipoproteins. LDL receptor deficiency along with mutations in the gene encoding for the LDL receptor count for the phenotypic events described in familial hypercholesterolaemia (Defesche, 2004; Marais, 2004). Mice with a targeted inactivation of the LDL receptor were created in 1993 (Ishibashi et al., 1993; Ishibashi et al., 1994a). Compared with wild-type, $LDLr^{-/-}$ mice display modestly elevated plasma cholesterol levels and develop no or only mild atherosclerosis when fed a normal diet (Ishibashi et al., 1994a). In terms of lipoprotein particles, the increase is higher among IDL and LDL sized particles, whereas HDL and triglycerides remain unaffected (Ishibashi et al., 1993; Ishibashi et al., 1994a). It is worth to note that this is different from $ApoE^{-/-}$ mice, in which cholesterol is

primarily accumulated in large lipoprotein particles such as chylomicron remnants, VLDL and IDL particles (*vide supra*)(Plump et al., 1992; Zhang et al., 1992). The response to high-fat/high cholesterol Western-type diets shows a remarkable change in lipoprotein profile of these mice with a high probability for atherosclerotic lesion development.

The plaques that develop in LDLr$^{-/-}$ mice are generally the same as those seen in ApoE$^{-/-}$ mice (Knowles and Maeda, 2000). A Western-type diet induces larger and more advanced lesions with a collagen-rich fibrous cap, a necrotic core containing cholesterol clefts and cellular enrichment adjacent to the lumen (Hartvigsen et al., 2007). The plaque development occurs in a time-dependent manner, initially in the proximal aorta, and spreading toward the distal aorta. Similar to humans, the locations where the blood flow is disturbed are more prone to atherosclerotic lesions (Knowles and Maeda, 2000). By making LDLr$^{-/-}$ and ApoE$^{-/-}$ mice homozygous for the ApoB-100 allele, total plasma cholesterol levels of approximately 300 mg/dl were obtained on a normal diet. LDLr$^{-/-}$ ApoB$^{100/100}$ mice developed more atherosclerotic lesions than the ApoE$^{-/-}$ ApoB$^{100/100}$ mice, even with a normal diet (Véniant et al., 2000; Veniant et al., 2001).

The LDLr$^{-/-}$ mouse model has some advantages in comparison with ApoE$^{-/-}$ mice. Firstly, plasma cholesterol is mostly carried by LDL particles, which generates a more human-like lipid profile. Secondly, the absence of the LDL receptor does not have an impact on inflammation as compared to ApoE deficiency. Thus, atherosclerotic plaque development in this mouse model is based on elevated plasma lipid levels and not caused by other functions linked to the LDL receptor

(Getz and Reardon, 2012). Thirdly, the LDLr$^{-/-}$ mouse model shares the characteristics observed in human familial hypercholesterolaemia, which is caused by the absence of functional LDL receptors (Hobbs et al., 1990; Lee et al., 2017).

2.3 ApoE/LDL receptor double-knockout mice

Introduced shortly after ApoE$^{-/-}$ and LDLr$^{-/-}$ mice, ApoE/LDL receptor double knock out mice represent a model that develops more severe hyperlipidaemia and atherosclerosis than the former ones (Bonthu et al., 1997). It is an animal model with spontaneous atherosclerotic plaque development and it has been reported that even on regular chow diet, the progression of atherosclerosis is usually more marked in ApoE/LDL receptor double knock out mice than in mice deficient for ApoE alone (Witting et al., 1999). There is no significant difference in the lipoprotein profile of the double knockouts compared to ApoE$^{-/-}$ mice, they both have high levels of VLDL and LDL (Caligiuri et al., 1999), except the marked elevations in B48 and B100 apolipoproteins (Ishibashi et al., 1994b). This mouse model is considered suitable to study the anti-atherosclerotic effects of possible treatments, without the need of an atherogenic diet (Jawien et al., 2004).

2.4 ApoE3-Leiden mice

Although ApoE$^{-/-}$ mice and LDLr$^{-/-}$ mice are the two most frequently used mouse models for atherosclerosis, also ApoE3-Leiden mice are utilized in many studies.

Apolipoprotein (Apo)E3-Leiden is associated with a genetic form of hyperlipidaemia and is particularly expressed in a Dutch family. Transgenic mice have been generated using a genomic 27-kilobase DNA construct (containing the ApoE gene, ApoC1 gene and all regulatory elements) isolated from the APOE3-Leiden proband, to study the effect of the ApoE3-Leiden mutation *in vivo* (Lutgens et al., 1999; van den Maagdenberg et al., 1993).

Remarkably, although these mice are less susceptible for atherosclerosis than the ApoE deficient mice, they also show dramatically elevated total plasma cholesterol and triglyceride levels when fed a Western-type diet. This is mainly attributed to an increase in VLDL/LDL particles, which demonstrates that ApoE3-Leiden mice have a human-like lipoprotein profile (van Vlijmen et al., 1994). Another advantage is that ApoE3-Leiden mice have the ability to synthesize functional ApoE. This offers the possibility to study the effect of elevated plasma lipid levels without disturbing inflammatory processes, which is an important limitation of the 'classical' ApoE$^{-/-}$ mouse model (Gijbels et al., 1999).

The ApoE3-Leiden mice develop atherosclerotic lesions in the aorta and large vessels when fed a Western-type diet. Lesions are also observed in the proximal coronary arteries, the aortic root, the aortic arch and its main branch points, the thoracic aorta, the abdominal aorta, the renal artery branch points, the abdominal aorta bifurcation, and the iliac artery bifurcations. It is interesting to note that this strain develops early foam cell lesions on normal chow diet. However, more complex and advanced lesions are observed after 1, 3 and 6 months of Western-type diet feeding (Lutgens et al., 1999).

ApoE3-Leiden mice are used as a model to elucidate factors involved in the metabolism of ApoE and the aetiology of familial dyslipidaemia in particular. Furthermore, ApoE3-Leiden mice are utilized to study complications of venous bypass grafting, a clinical procedure that bypasses an atherosclerotic obstruction in an artery. Similar to humans, the grafted vein in this model undergoes remodelling, which is a consequence of exposure to higher blood pressure and shear stress but also vessel injury due to surgery. This process results in the formation of intimal hyperplasia and accelerated atherosclerosis, which may lead to obstruction of the graft (de Vries et al., 2016; Karper et al., 2011; Lutgens et al., 1999).

Because similarities were found between lesions in vein grafts and native atherosclerosis, a murine model of vein graft disease has been established (Zou et al., 1998). In this model, the thoracic caval vein of a donor mouse is grafted in the carotid artery of a receiver mouse. This procedure has been used in mice that are susceptible to atherosclerosis (ApoE$^{-/-}$ or ApoE3-Leiden mice) so that vein grafts with accelerated atherosclerosis could be studied (Dietrich et al., 2000; Lardenoye et al., 2002a; Lardenoye et al., 2002b). It has been show that vein grafts in these transgenic mice are morphologically similar to rupture-prone plaques in humans. The lesions in this model have the typical characteristics of late stage atherosclerosis, including the presence of foam cells, a large necrotic core, intra-plaque neovascularization, calcification and cholesterol clefts (Lardenoye et al., 2000).

2.5 PCSK9-AAV mice

Besides the abovementioned models, a new line of mouse model without germline genetic engineering is emerging in the research field of atherosclerosis. The so called pro-protein convertase subtilisin/kexin type 9 (PCSK9) - adeno associated virus (AAV) mice were described independently by two research groups in 2014 as a rapid, versatile and cost-effective animal model for atherosclerosis (Bjorklund et al., 2014; Roche-Molina et al., 2015). PCSK9, a newly identified human subtilase, is a serine protease with plasma concentrations of ≈100 to 200 ng/mL and it is highly expressed in the liver (Akram et al., 2010; Denis et al., 2012). Several studies have shown that PCSK9 reduces hepatic uptake of LDL by increasing the endosomal and lysosomal degradation of LDL receptors (Li et al., 2007). In brief, after protein maturation and secretion, circulating PCSK9 binds the LDL receptors on the cell surface and is subsequently co-internalized together with the receptor. This distracts the normal recycling process of the receptor to the plasma membrane and promotes degradation in the lysosome (Akram et al., 2010).

Recombinant AAV vectors support long-term transgene expression in many animal models (Cerrone et al., 2012; Kaspar et al., 2005; Suhy et al., 2012) and humans (Zsebo et al., 2014). Following single intravenous injection with human D374Y (Roche-Molina et al., 2015) or murine D377Y (Bjorklund et al., 2014) gain-of-function mutant PCSK9, mice were stably expressing $PCSK9^{DY}$ mRNA in the liver. AAV viral infection does not elicit any adverse effects in the animals and no signs of liver damage or immunologic response were observed following infection. At 30 days after injection, total serum cholesterol in $PCSK9^{DY}$-AAV transgenes was doubled compared to control mice. These differences remained the same even after

1 year post-infection, confirming a chronic effect of a single AAV injection (Roche-Molina et al., 2015). Western-type diet exacerbated hyperlipidaemia in PCSK9^{DY}-AAV mice, leading to plasma cholesterol levels of up to 1165 mg/dl, while chow diet fed mice barely reached 316 mg/dl. The lipoprotein profile of Western-type diet fed PCSK9^{DY}-AAV mice showed an equal distribution between VLDL and LDL particles (Roche-Molina et al., 2015). PCSK9^{DY} transgenic mice develop atherosclerosis in a dose-dependent manner. Hyperlipidaemia provokes the build-up of lesions throughout the vasculature resembling those of LDLr$^{-/-}$ mice, which is exacerbated by HFD feeding (Bjorklund et al., 2014; Roche-Molina et al., 2015). Aortic root lesions show advanced plaque development with foam cells, smooth muscle cells, macrophage infiltration and fibrous tissue, but importantly, lesions progress to the fibro-atheromatous stage (Bjorklund et al., 2014; Roche-Molina et al., 2015) and within the time frame of 15-20 weeks, vascular calcification occurs (Goettsch et al., 2016). When Roche-Molina et al. combined PCSK9^{DY} expression and ApoE deficiency, they revealed an expected synergistic effect: the lesions doubled in size with no significant differences in lipoprotein profile as compared to single mutants on the same diet (Roche-Molina et al., 2015).

Overall, the induction of hyperlipidaemia and atherosclerosis in animals with different genetic backgrounds, the robust stability after single administration of mutant human PCSK9 and the fact that there are no major biosafety concerns in using AAVs as vectors, makes the PCSK9-AAV model a valuable tool in atherosclerosis research (Roche-Molina et al., 2015).

3. Mouse models of atherosclerotic plaque rupture

Despite major advances in cardio- and cerebrovascular research, plaque rupture remains the leading cause of acute events (Ylä-Herttuala et al., 2011). Therefore, the need for the development of plaque-stabilizing therapies is high. Several research groups have tried to develop suitable models of plaque rupture for the last 15 years but in these models rupture occurs only sporadically, after a long period of time, or depends on mechanical injury (Chen et al., 2013; Ni et al., 2009; Schwartz et al., 2007). Moreover, the reproducibility is low and events as seen in humans are rarely observed.

As discussed earlier, atherosclerotic plaques in mice develop in specific sites such as the aortic root, the lesser curvature of the aortic arch and the branch points of the brachiocephalic, the left carotid and the subclavian arteries. However, mice show only minor plaque development in the coronary and carotid arteries, which are the main sites of atherosclerotic plaque development in humans (Bond and Jackson, 2011; Getz and Reardon, 2012; VanderLaan et al., 2004). To induce plaque rupture in mice, several approaches based on surgical (such as arterial ligation or the positioning of a cuff around an artery) or genetic manipulation have been proposed (Table 1). Although these models have been useful in understanding the concepts of plaque rupture, none of them exhibit the full combination of the characteristics seen in human vulnerable/ruptured plaques. Moreover, plaque rupture with a superimposed occlusive thrombus, the most common complication of human atherosclerosis, is rarely observed (Bentzon and Falk, 2010). Consequently, clinical

events such as MI or ischemic stroke are almost never seen in these models (Bond and Jackson, 2011; Ylä-Herttuala et al., 2011). Furthermore, most of these models do not show 'spontaneous' plaque ruptures. When spontaneous ruptures are observed, they only occur sporadically and after a long period of time. However, recently a model of consistent, spontaneous atherosclerotic plaque ruptures in mice has been described, as discussed below.

3.1 Apolipoprotein E-deficient Fibrillin-1 mutant (ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$) mice

The extracellular matrix is a complex network of predominantly elastin and collagen, which is essential to provide structural, adhesive and biochemical signalling support to the vessel wall. In elastic arteries, elastin is the most abundant protein. The elastic fibres comprise the elastin core, which is surrounded by a mantle of fibrillin-rich microfibrils (Kielty et al., 2002). The elastic-fibre-associated microfibrils have as the main structural component fibrillin-1, a large glycoprotein of about 350 kDa, whose major role is in binding and sequestering growth factors, such as transforming growth factor-β (TGF-β), as well as providing the scaffold for the deposition and the cross-linking of elastin (Judge and Dietz, 2005; Van Herck et al., 2009).

Recently, we reported the effect of an impaired elastin structure of the vessel wall on the progression of atherosclerosis by cross-breeding ApoE$^{-/-}$ mice with mice containing a heterozygous mutation (C1039G$^{+/-}$) in the fibrillin-1 (Fbn1) gene (Van

Herck et al., 2009). Mutations in the Fbn1 gene lead to the Marfan syndrome, a genetic disorder characterized by fragmentation of elastic fibres (Judge et al., 2004). This results in increased arterial stiffening, elevated pulse pressure and progressive aortic dilatation (Mariko et al., 2011; Medley et al., 2002; Van Herck et al., 2009). Moreover, the mutation leads to the development of highly unstable plaques in ApoE$^{-/-}$ mice, resulting in spontaneous plaque rupture with end-points including MI and sudden death (Van der Donckt et al., 2015b; Van Herck et al., 2009). Importantly, these events do not – or only very occasionally – occur in ApoE$^{-/-}$ mice on a Western-type diet or in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice fed a normal diet (Van der Donckt et al., 2015a; Van der Donckt et al., 2015b). These findings underscore the importance of elastin fragmentation in combination with a Western-type diet as prerequisites for atherosclerotic plaque rupture in mice.

ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice have significantly larger plaques with a highly unstable phenotype, characterized by a large necrotic core (occupying about 30% of total plaque area), and a strongly diminished collagen content. Accelerated atherogenesis in these mice is likely the result of enhanced vascular inflammation, leading to increased monocyte attraction, oxidation and accumulation of lipids (Fulop et al., 2005). Inducible nitric oxide synthase (iNOS), a marker for activated macrophages and inflammation, is significantly more expressed in plaques of ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on either Western-type diet or normal diet as compared to ApoE$^{-/-}$ mice on Western-type diet. Accordingly, inflammatory cytokines tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) are highly increased. In addition, a higher infiltration of T-cells and their activation marker interferon-γ

(IFN-γ) is present, the latter playing an important role in collagen turn-over by inhibiting SMCs to synthesise collagen, required to repair and maintain fibrous cap integrity (Koenig and Khuseyinova, 2007; Libby, 2013). Moreover, matrix metalloproteinase (MMP)-2, -9, -12 and -13 expression or activity is increased in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice. MMP-2 and MMP-9 are implicated in both atherosclerosis and angiogenesis (Raffetto and Khalil, 2008). For example, ApoE$^{-/-}$ mice lacking MMP-2 develop smaller and more stable plaques, whereas macrophages overexpressing active MMP-9 promote neovascularisation, intra-plaque haemorrhage (de Nooijer et al., 2005; Gough et al., 2006) and features of plaque rupture in ApoE$^{-/-}$ mice (Gough et al., 2006). In the latter case, those features were attributed to elastin degradation, underscoring its role in plaque destabilisation and rupture. MMP-12 and MMP-13 additionally contribute to elastin and (type I) collagen degradation, respectively. Taken together, in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on Western-type diet enhanced collagen/extracellular matrix breakdown together with decreased synthesis and repair are likely responsible for weakening of the fibrous cap and rendering it more rupture-prone (Libby, 2013; Raffetto and Khalil, 2008).

Extensive neovascularisation and intra-plaque haemorrhages consistently occur in the brachiocephalic and common carotid arteries of ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on Western-type diet. These features are rarely seen in murine atherosclerosis models but are known to highly affect plaque progression and vulnerability in humans (Virmani et al., 2005). In ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on a Western-type diet, intra-plaque neo-vessels, likely arising from adventitial vasa vasorum, clearly sprout out of the media (Moulton et al., 2004; Rademakers et al., 2013). Neo-vessels are not

only present at the base of the plaque but are also frequently observed in its centre, similar to human pathology (Rademakers et al., 2013; Virmani et al., 2005). Angiogenesis requires extracellular matrix degradation by proteases, including MMPs, to enable endothelial cell migration into the surrounding tissue (Raffetto and Khalil, 2008). In addition, degradation of the extracellular matrix induces release of sequestered angiogenic factors such as vascular endothelial growth factor (VEGF) and TGF-β (de Nooijer et al., 2005; Raffetto and Khalil, 2008), also observed in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on Western-type diet. The extent of neovascularisation in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice correlates with the degree of elastin fragmentation in the vessel wall. However, degradation of the extracellular matrix alone is not sufficient to induce neovascularisation in atherosclerotic plaques, because microvessels are not present in plaques of ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on a normal diet. This observation indicates that an additional factor is needed to trigger plaque neovascularisation. Hypoxia, a well-known angiogenesis trigger (Sluimer et al., 2009), is strongly increased in plaques of brachiocephalic and carotid arteries in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on Western-type diet. By contrast, hypoxia in the ascending aorta is minor, which likely explains the absence of neo-vessels at that site. Thus, the highly permeable arterial wall, due to degradation of the extracellular matrix, combined with intra-plaque hypoxia seems required for neo-vessel formation in atherosclerotic plaques of ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on Western-type diet. Importantly, those neo-vessels are highly leaky. Moreover, the presence of intra-plaque erythrocytes near neo-vessels at the base of the plaque points to intra-plaque haemorrhages, substantiating ruptured neo-vessels as source of intra-plaque

bleeding (de Nooijer et al., 2005; Kockx et al., 2003; Virmani et al., 2005). Erythrocytes are important sources of free cholesterol, thereby increasing necrotic core size. Hence, neovascularisation, besides supplying plaques with leukocytes and lipoproteins, can promote focal plaque expansion when microvessels rupture or become thrombotic (Kockx et al., 2003; Sluimer et al., 2009; Virmani et al., 2005). Taken together, these observations in this mouse model are in line with current concepts of human vulnerable plaques.

In addition to enhanced plaque vulnerability, plaque rupture is consistently present in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on a Western-type diet, but only very rarely in ApoE$^{-/-}$ mice on a Western-type diet. Moreover, fibrin-rich mural thrombi are present in brachiocephalic, carotid and coronary arteries and ascending aortas. Both intrinsic (i.e. a highly unstable plaque phenotype) and extrinsic factors (i.e. forces acting on the plaque) are elementary for plaque rupture (Slager et al., 2005). In general, rupture occurs when the mechanical stress applied on the fibrous cap exceeds its tensile strength. The latter is mainly determined by the collagen content of the plaque, which is significantly decreased in plaques of ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on Western-type diet (Libby, 2013; Slager et al., 2005). Elevated pulse pressure (as a consequence of arterial stiffening) (Van Herck et al., 2009) leads to repetitive plaque deformation, increasing the tensile stress on the cap (Huang et al., 2013; Medley et al., 2002). When applied chronically, this can lead to plaque fatigue, making it prone to rupture (Huang et al., 2013; Slager et al., 2005). Moreover, due to the progressive aortic dilatation and outward remodelling (as a result of the large plaques), the collagen and elastin fibres of the cap are stretched and become more rigid,

increasing the susceptibility to mechanical stress. Aortic dilatation is highly pronounced in the ascending aorta of ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on a Western-type diet, suggesting that this mechanism is responsible for rupture of unstable plaques at this site. In brachiocephalic and carotid arteries, intra-plaque neovascularisation and haemorrhage are frequently present, further increasing plaque size and vulnerability to rupture.

In addition, sudden death is observed in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on a Western-type diet, mainly between 16 and 23 weeks, with 50% mortality after 20 weeks. Moreover, ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on a Western-type diet that died suddenly show a significantly higher frequency of coronary stenosis compared to survivors, suggesting that the presence of coronary artery plaque plays an important role in cardiac death. The majority of ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on Western-type diet show infarcted areas, which compromise cardiac function even more. Although it is not known whether the increased infarcted area is the result of plaque rupture or due to pronounced plaque formation and coronary artery stenosis, these findings are remarkable because coronary artery plaque and spontaneous MIs almost never develop in ApoE$^{-/-}$ mice on a Western-type diet. Also in humans, differences in fibrillin-1 genotype have shown to greatly affect plaque progression and severity of coronary artery disease, underscoring the pathophysiological relevance of fibrillin-1 mutations in cardiovascular disease (Medley et al., 2002).

Thus, elastin fragmentation in combination with a Western-type diet leads to plaque destabilisation and rupture in ApoE$^{-/-}$ mice. ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice show many features of human end-stage atherosclerosis, such as an enlarged necrotic core, a

thin fibrous cap with an important loss of collagen fibres, outward remodelling and the presence of intra-plaque microvessels and haemorrhage, resulting in plaque rupture, MI and sudden death. Therefore, ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice on a Western-type diet offer the opportunity to investigate the role of key factors involved in plaque destabilisation, including intra-plaque neovascularisation, which will provide more insight into the mechanisms of plaque disruption and potential targets for therapeutic interventions (Roth et al., 2015a; Roth et al., 2016; Roth et al., 2015b; Van der Donckt et al., 2015b).

4. Rabbits

The rabbit has been one of the most frequently used animals in atherosclerosis research because of their easy handling and relatively inexpensive maintenance (Getz and Reardon, 2012). However, there has been a reduced trend of using this animal model since 2000, probably due to the availability of ApoE and LDL receptor knock-out mice (Fan et al., 2015). Multiple approaches and models have been used to study atherosclerosis and its complication in rabbits, including genetically hypercholesterolaemic rabbits such as Watanabe heritable hyperlipidaemic rabbits (WHHL) (Watanabe, 1980), New Zeeland White rabbits fed a cholesterol-rich diet (Baumgartner et al., 2016), and very recently ApoE$^{-/-}$ rabbits (Niimi et al., 2016).

Rabbits have a lipoprotein metabolism that is similar to humans (except for their hepatic lipase deficiency) and show significant differences with mice. Unlike mice, in which HDL is the predominant plasma lipoprotein, rabbits transport significant

amounts of cholesterol via ApoB-containing particles (VLDL and LDL) (Fan and Watanabe, 2000). Consequently, rabbits have been useful to point out the role of elevated plasma cholesterol as a critical factor in the initiation of atherosclerosis. Limitations of rabbit models include a highly abnormal diet required for the development of hypercholesterolaemia, massive inflammation and hepatic toxicity due to the long term high cholesterol feeding (Fuster et al., 2012).

4.1 Watanabe heritable hyperlipidaemic (WHHL) rabbits

WHHL rabbits are a mutant strain that shows spontaneous hypercholesterolaemia and atherosclerosis due to a defect in the LDL receptor (Watanabe, 1980). Homozygous WHHL rabbits fed a normal diet are hypercholesterolaemic from birth with LDL as the predominant lipoprotein. They exhibit various types of atherosclerotic lesions ranging from early fatty streaks to advanced lesions in the aorta, coronary arteries and cerebral artery (Atkinson et al., 1989; Baumgartner et al., 2016). These rabbits also show an increased risk of MI. The WHHL rabbit was one of the first rabbit models in which the effect of statins to suppress plaque destabilization and to reduce thrombogenicity was investigated. High-fructose and high fat–diet fed WHHL rabbits develop early insulin resistance and glucose tolerance and show aortic lesions with a lipid core and calcifications. This model has allowed researchers to investigate the effect of insulin resistance on atherosclerosis lesion formation (Ning et al., 2015).

4.2 New Zealand White (NZW) rabbits

NZW rabbits are commonly used to study atherosclerosis. NZW rabbits that are fed a normal diet have low plasma cholesterol levels (mostly < 50 mg/dl) and consequently do not develop spontaneous atherosclerosis. However, supplementing the diet with 0.3-0.5% cholesterol increases the plasma cholesterol level up to 1000 mg/dl. The plaque composition is determined by the level of dietary cholesterol and the duration of cholesterol feeding. One protocol uses adult rabbits that are fed a cholesterol-rich diet (1.0-1.5% cholesterol) for a short period of time (about 8 weeks). By using such a diet, rabbits develop severe hypercholesterolaemia with plasma cholesterol levels between 1500 and 3000 mg/dl, which are never seen in humans, resulting in atherosclerotic plaques primarily composed of macrophage-derived foam cells. For this reason, the most used protocol lasts 20 to 26 weeks and consists of a diet containing 0.3% cholesterol. On average, cholesterol levels rise to about 800 mg/dl. This protocol develops atherosclerotic plaques in the aortic arch and thoracic aorta, rather than in the abdominal aorta (less pronounced plaque formation) (Baumgartner et al., 2016; Fan and Watanabe, 2000), whereas in humans, plaques are commonly found in the abdominal aorta. Coronary atherosclerosis is also observed in cholesterol-fed rabbits but is usually restricted to the left coronary arterial trunks. Depending on the length of the cholesterol feeding, also plaque calcification occurs. However, there is no evidence for spontaneous plaque rupture.

4.3 Apolipoprotein E knockout (ApoE$^{-/-}$) rabbits

Recently, ApoE$^{-/-}$ rabbits have been reported as a model to study the relationship between atherosclerosis and human hyperlipidaemia (Niimi et al., 2016). ApoE$^{-/-}$ rabbits can be generated using genome editing enzymes such as zinc finger nucleases, transcription activator-like effector nucleases (TALENs) or RNA-guided CRISPR-associated protein 9 (Cas9) endonucleases. Because the rabbit lipoprotein profile is similar to humans (Brousseau and Hoeg, 1999), the ApoE$^{-/-}$ rabbit represents an attractive alternative to the ApoE$^{-/-}$ mouse. Even on a normal diet, ApoE$^{-/-}$ rabbits show mild hyperlipidaemia with plasma total cholesterol levels around 200 mg/dl. However, when fed a cholesterol-rich diet (0.3% cholesterol and 3% soybean oil for 2 weeks) their plasma total cholesterol levels increase to about 1000 mg/dl (vs. about 170 mg/dl in cholesterol-fed wild-type rabbits). ApoE$^{-/-}$ rabbits develop more pronounced aortic atherosclerosis than wild-type rabbits when fed with a cholesterol diet for 10 weeks (Niimi et al., 2016). Because both ApoE and the LDL receptor play an important role in mediating cholesterol metabolism, ApoE$^{-/-}$ rabbits together with LDL receptor-deficient WHHL rabbits may be valuable models for the study of human hyperlipidaemia: ApoE$^{-/-}$ rabbits show elevation of remnant lipoproteins, whereas WHHL rabbits have high levels of LDL accompanied by low HDL (Niimi et al., 2016).

5. Large animal models (pigs and non-human primates)

Although small animal models have provided insight into the mechanisms that drive atherosclerosis, additional strategies are required to translate these findings into improved prevention and treatment of symptomatic atherosclerosis in humans. Efficient large animal models of atherosclerosis may be useful to deal with these challenges. Indeed, the translation of the knowledge obtained from studies in mice to the development of drugs for human atherosclerosis can benefit from a bridging tool such as porcine models of atherosclerosis. Not only the effects of pharmacological treatments on atherosclerosis can be studied in such models but also clinical imaging end-points can be evaluated as guiding tool for subsequent phase II clinical trials (Shim et al., 2016). Gene-editing tools for large animals have made it possible to create gene-modified minipigs that develop atherosclerosis with many similarities to humans in terms of predilection for lesion sites and histopathology. For instance, minipigs with liver-specific expression of human D374Y-PCSK9 show severe hypercholesterolaemia and development of progressive atherosclerotic lesions (Al-Mashhadi et al., 2013). Together with existing porcine models of atherosclerosis that are based on spontaneous mutations or severe diabetes, such models may provide new approaches for translational research in atherosclerosis (Shim et al., 2016).

Non-human primates show hypercholesterolaemia when fed a high fat/high cholesterol diet and develop coronary fibro-fatty atherosclerotic plaques, similar to humans (Getz and Reardon, 2012). Yet, working with monkeys is expensive, highly regulated, and requires very specialized laboratory animal science skills. Therefore, these models are not frequently used. A few years ago, however, also knockout non-

human primates have been created, which may reinforce the interest in large animal models with accelerated atherosclerosis (Niu et al., 2014; Shim et al., 2016).

In conclusion, many efforts have been made to develop animal models that resemble human atherosclerosis as good as possible. However, each of the current animal models has its advantages and limitations, as summarized in Table 2. A great challenge was the development of an animal model of spontaneous (i.e. without mechanical interventions) plaque rupture with human-like endpoints such as MI, stroke and sudden death. These features are present in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice, making it a promising model to evaluate potential plaque stabilizing therapies.

Acknowledgements:

Besa Emini Veseli and Paola Perrotta are PhD fellows of the Horizon 2020 program of the European Union - Marie Skłodowska-Curie Actions, Innovative Training Networks (ITN), Call: H2020-MSCA-ITN-2015, NUMBER — 675527 — MOGLYNET

References

Akram, O.N., Bernier, A., Petrides, F., Wong, G., Lambert, G., 2010. Beyond LDL cholesterol, a new role for PCSK9. Arterioscler. Thromb. Vasc. Biol. 30, 1279-1281.

Al-Mashhadi, R.H., Sorensen, C.B., Kragh, P.M., Christoffersen, C., Mortensen, M.B., Tolbod, L.P., Thim, T., Du, Y., Li, J., Liu, Y., Moldt, B., Schmidt, M., Vajta, G., Larsen, T., Purup, S., Bolund, L., Nielsen, L.B., Callesen, H., Falk, E., Mikkelsen, J.G., Bentzon, J.F., 2013. Familial hypercholesterolemia and atherosclerosis in cloned minipigs created by DNA transposition of a human PCSK9 gain-of-function mutant. Sci. Transl. Med. 5, 166ra161.

Atkinson, J.B., Hoover, R.L., Berry, K.K., Swift, L.L., 1989. Cholesterol-fed heterozygous Watanabe heritable hyperlipidemic rabbits: a new model for atherosclerosis. Atherosclerosis 78, 123-136.

Baumgartner, C., Brandl, J., Münch, G., Ungerer, M., 2016. Rabbit models to study atherosclerosis and its complications – Transgenic vascular protein expression in vivo. Prog. Biophys. Mol. Biol. 121, 131-141.

Bentzon, J.F., Falk, E., 2010. Atherosclerotic lesions in mouse and man: is it the same disease? Curr. Opin. Lipidol. 21, 434-440.

Berliner, J.A., Navab, M., Fogelman, A.M., Frank, J.S., Demer, L.L., Edwards, P.A., Watson, A.D., Lusis, A.J., 1995. Atherosclerosis: Basic Mechanisms : Oxidation, Inflammation, and Genetics. Circulation 91, 2488-2496.

Bjorklund, M.M., Hollensen, A.K., Hagensen, M.K., Dagnaes-Hansen, F., Christoffersen, C., Mikkelsen, J.G., Bentzon, J.F., 2014. Induction of atherosclerosis in mice and hamsters without germline genetic engineering. Circ. Res. 114, 1684-1689.

Bond, A.R., Jackson, C.L., 2011. The Fat-Fed Apolipoprotein E Knockout Mouse Brachiocephalic Artery in the Study of Atherosclerotic Plaque Rupture. J. Biomed. Biotechnol. 2011, 1-10.

Bonthu, S., Heistad, D.D., Chappell, D.A., Lamping, K.G., Faraci, F.M., 1997. Atherosclerosis, vascular remodeling, and impairment of endothelium-dependent relaxation in genetically altered hyperlipidemic mice. Arterioscler. Thromb. Vasc. Biol. 17, 2333-2340.

Borissoff, J.I., Otten, J.J.T., Heeneman, S., Leenders, P., van Oerle, R., Soehnlein, O., Loubele, S.T.B.G., Hamulyák, K., Hackeng, T.M., Daemen, M.J.A.P., Degen, J.L., Weiler, H., Esmon, C.T., van Ryn, J., Biessen, E.A.L., Spronk, H.M.H., ten Cate, H., 2013. Genetic and Pharmacological Modifications of Thrombin Formation in Apolipoprotein E-deficient Mice Determine Atherosclerosis Severity and Atherothrombosis Onset in a Neutrophil-Dependent Manner. PLoS One 8, e55784.

Breslow, J.L., 1993. Transgenic mouse models of lipoprotein metabolism and atherosclerosis. Proc. Natl. Acad. Sci. U. S. A. 90, 8314-8318.

Brousseau, M.E., Hoeg, J.M., 1999. Transgenic rabbits as models for atherosclerosis research. J. Lipid Res. 40, 365-375.

Calara, F., Silvestre, M., Casanada, F., Yuan, N., Napoli, C., Palinski, W., 2001. Spontaneous plaque rupture and secondary thrombosis in apolipoprotein E-deficient and LDL receptor-deficient mice. J. Pathol. 195, 257-263.

Caligiuri, G., Levy, B., Pernow, J., Thoren, P., Hansson, G.K., 1999. Myocardial infarction mediated by endothelin receptor signaling in hypercholesterolemic mice. Proc. Natl. Acad. Sci. U. S. A. 96, 6920-6924.

Cerrone, M., Noorman, M., Lin, X., Chkourko, H., Liang, F.X., van der Nagel, R., Hund, T., Birchmeier, W., Mohler, P., van Veen, T.A., van Rijen, H.V., Delmar, M., 2012. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. Cardiovasc. Res. 95, 460-468.

Chen, Y.C., Bui, A.V., Diesch, J., Manasseh, R., Hausding, C., Rivera, J., Haviv, I., Agrotis, A., Htun, N.M., Jowett, J., Hagemeyer, C.E., Hannan, R.D., Bobik, A., Peter, K., 2013. A Novel Mouse Model of Atherosclerotic Plaque Instability for Drug Testing and

Mechanistic/Therapeutic Discoveries Using Gene and MicroRNA Expression Profiling. Circ. Res. 113, 252-265.

Curtiss, L.K., Boisvert, W.A., 2000. Apolipoprotein E and atherosclerosis. Curr. Opin. Lipidol. 11, 243-251.

de Nooijer, R., Verkleij, C.J., von der Thüsen, J.H., Jukema, J.W., van der Wall, E.E., van Berkel, T.J., Baker, A.H., Biessen, E.A., 2005. Lesional Overexpression of Matrix Metalloproteinase-9 Promotes Intraplaque Hemorrhage in Advanced Lesions But Not at Earlier Stages of Atherogenesis. Arterioscler. Thromb. Vasc. Biol. 26, 340-346.

de Vries, M.R., Simons, K.H., Jukema, J.W., Braun, J., Quax, P.H.A., 2016. Vein graft failure: from pathophysiology to clinical outcomes. Nat. Rev. Cardiol. 13, 451-470.

Defesche, J.C., 2004. Low-density lipoprotein receptor--its structure, function, and mutations. Semin. Vasc. Med. 4, 5-11.

Denis, M., Marcinkiewicz, J., Zaid, A., Gauthier, D., Poirier, S., Lazure, C., Seidah, N.G., Prat, A., 2012. Gene inactivation of proprotein convertase subtilisin/kexin type 9 reduces atherosclerosis in mice. Circulation 125, 894-901.

Dietrich, H., Hu, Y., Zou, Y., Huemer, U., Metzler, B., Li, C., Mayr, M., Xu, Q., 2000. Rapid Development of Vein Graft Atheroma in ApoE-Deficient Mice. Am. J. Pathol. 157, 659-669.

Fan, J., Kitajima, S., Watanabe, T., Xu, J., Zhang, J., Liu, E., Chen, Y.E., 2015. Rabbit models for the study of human atherosclerosis: from pathophysiological mechanisms to translational medicine. Pharmacol. Ther. 146, 104-119.

Fan, J., Watanabe, T., 2000. Cholesterol-fed and Transgenic Rabbit Models for the Study of Atherosclerosis. J. Atheroscler. Thromb. 7, 26-32.

Fulop, T., Larbi, A., Fortun, A., Robert, L., Khalil, A., 2005. Elastin peptides induced oxidation of LDL by phagocytic cells. Pathol. Biol. (Paris) 53, 416-423.

Fuster, J.J., Castillo, A.I., Zaragoza, C., Ibáñez, B., Andrés, V., 2012. Animal Models of Atherosclerosis, Prog. Mol. Biol. Transl. Sci. Elsevier BV, pp. 1-23.

Gencer, B., Kronenberg, F., Stroes, E.S., Mach, F., 2017. Lipoprotein(a): the revenant. Eur. Heart J.

Getz, G.S., Reardon, C.A., 2009. Apoprotein E as a lipid transport and signaling protein in the blood, liver, and artery wall. J. Lipid Res. 50 Suppl, S156-161.

Getz, G.S., Reardon, C.A., 2012. Animal Models of Atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 32, 1104-1115.

Gijbels, M.J., van der Cammen, M., van der Laan, L.J., Emeis, J.J., Havekes, L.M., Hofker, M.H., Kraal, G., 1999. Progression and regression of atherosclerosis in APOE3-Leiden transgenic mice: an immunohistochemical study. Atherosclerosis 143, 15-25.

Goettsch, C., Hutcheson, J.D., Hagita, S., Rogers, M.A., Creager, M.D., Pham, T., Choi, J., Mlynarchik, A.K., Pieper, B., Kjolby, M., Aikawa, M., Aikawa, E., 2016. A single injection of gain-of-function mutant PCSK9 adeno-associated virus vector induces cardiovascular calcification in mice with no genetic modification. Atherosclerosis 251, 109-118.

Gough, P.J., Gomez, I.G., Wille, P.T., Raines, E.W., 2006. Macrophage expression of active MMP-9 induces acute plaque disruption in apoE-deficient mice. J. Clin. Invest. 116, 59-69.

Hartvigsen, K., Binder, C.J., Hansen, L.F., Rafia, A., Juliano, J., Horkko, S., Steinberg, D., Palinski, W., Witztum, J.L., Li, A.C., 2007. A Diet-Induced Hypercholesterolemic Murine Model to Study Atherogenesis Without Obesity and Metabolic Syndrome. Arterioscler. Thromb. Vasc. Biol. 27, 878-885.

Hobbs, H.H., Russell, D.W., Brown, M.S., Goldstein, J.L., 1990. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. Annu. Rev. Genet. 24, 133-170.

Hu, J.H., Du, L., Chu, T., Otsuka, G., Dronadula, N., Jaffe, M., Gill, S.E., Parks, W.C., Dichek, D.A., 2010. Overexpression of Urokinase by Plaque Macrophages Causes Histological Features of Plaque Rupture and Increases Vascular Matrix Metalloproteinase Activity in Aged Apolipoprotein E-Null Mice. Circulation 121, 1637-1644.

Huang, Y., Teng, Z., Sadat, U., He, J., Graves, M.J., Gillard, J.H., 2013. In vivo MRI-based simulation of fatigue process: a possible trigger for human carotid atherosclerotic plaque rupture. BioMed. Eng. OnLine 12, 36.

Ishibashi, S., Brown, M.S., Goldstein, J.L., Gerard, R.D., Hammer, R.E., Herz, J., 1993. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J. Clin. Invest. 92, 883-893.

Ishibashi, S., Goldstein, J.L., Brown, M.S., Herz, J., Burns, D.K., 1994a. Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. J. Clin. Invest. 93, 1885-1893.

Ishibashi, S., Herz, J., Maeda, N., Goldstein, J.L., Brown, M.S., 1994b. The two-receptor model of lipoprotein clearance: tests of the hypothesis in "knockout" mice lacking the low density lipoprotein receptor, apolipoprotein E, or both proteins. Proc. Natl. Acad. Sci. U. S. A. 91, 4431-4435.

Jawien, J., Nastalek, P., Korbut, R., 2004. Mouse models of experimental atherosclerosis. J. Physiol. Pharmacol. 55, 503-517.

Jin, S.x., Shen, L.h., Nie, P., Yuan, W., Hu, L.h., Li, D.d., Chen, X.j., Zhang, X.k., He, B., 2012. Endogenous Renovascular Hypertension Combined With Low Shear Stress Induces Plaque Rupture in Apolipoprotein E-Deficient Mice. Arterioscler. Thromb. Vasc. Biol. 32, 2372-2379.

Judge, D.P., Biery, N.J., Keene, D.R., Geubtner, J., Myers, L., Huso, D.L., Sakai, L.Y., Dietz, H.C., 2004. Evidence for a critical contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. J. Clin. Invest. 114, 172-181.

Judge, D.P., Dietz, H.C., 2005. Marfan's syndrome. Lancet 366, 1965-1976.

Kapourchali, F.R., Surendiran, G., Chen, L., Uitz, E., Bahadori, B., Moghadasian, M.H., 2014. Animal models of atherosclerosis. World J. Clin. Cases 2, 126-132.

Karper, J.C., de Vries, M.R., van den Brand, B.T., Hoefer, I.E., Fischer, J.W., Jukema, J.W., Niessen, H.W.M., Quax, P.H.A., 2011. Toll-Like Receptor 4 Is Involved in Human and Mouse Vein Graft Remodeling, and Local Gene Silencing Reduces Vein Graft Disease in Hypercholesterolemic APOE*3Leiden Mice. Arterioscler. Thromb. Vasc. Biol. 31, 1033-1040.

Kaspar, B.K., Roth, D.M., Lai, N.C., Drumm, J.D., Erickson, D.A., McKirnan, M.D., Hammond, H.K., 2005. Myocardial gene transfer and long-term expression following intracoronary delivery of adeno-associated virus. J. Gene Med. 7, 316-324.

Kielty, C.M., Sherratt, M.J., Shuttleworth, C.A., 2002. Elastic fibres. J. Cell Sci. 115, 2817-2828.

Knowles, J.W., Maeda, N., 2000. Genetic Modifiers of Atherosclerosis in Mice. Arterioscler. Thromb. Vasc. Biol. 20, 2336-2345.

Kockx, M.M., Cromheeke, K.M., Knaapen, M.W., Bosmans, J.M., De Meyer, G.R.Y., Herman, A.G., Bult, H., 2003. Phagocytosis and macrophage activation associated with hemorrhagic microvessels in human atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 23, 440-446.

Koenig, W., Khuseyinova, N., 2007. Biomarkers of Atherosclerotic Plaque Instability and Rupture. Arterioscler. Thromb. Vasc. Biol. 27, 15-26.

Konstantinov, I.E., Jankovic, G.M., 2013. Alexander I. Ignatowski: a pioneer in the study of atherosclerosis. Tex. Heart Inst. J. 40, 246-249.

Lardenoye, J.H., Delsing, D.J., de Vries, M.R., Deckers, M.M., Princen, H.M., Havekes, L.M., van Hinsbergh, V.W., van Bockel, J.H., Quax, P.H., 2000. Accelerated atherosclerosis by placement of a perivascular cuff and a cholesterol-rich diet in ApoE*3Leiden transgenic mice. Circ. Res. 87, 248-253.

Lardenoye, J.H.P., De Vries, M.R., Grimbergen, J.M., Havekes, L.M., Knaapen, M.W., Kockx, M.M., van Hinsbergh, V.W., van Bockel, J.H., Quax, P.H., 2002a. Inhibition of Accelerated Atherosclerosis in Vein Grafts by Placement of External Stent in ApoE*3-Leiden Transgenic Mice. Arterioscler. Thromb. Vasc. Biol. 22, 1433-1438.

Lardenoye, J.H.P., de Vries, M.R., Löwik, C.W., Xu, Q., Dhore, C.R., Cleutjens, J.P., van Hinsbergh, V.W., van Bockel, J.H., Quax, P.H., 2002b. Accelerated Atherosclerosis and Calcification in Vein Grafts: A Study in APOE*3 Leiden Transgenic Mice. Circ. Res. 91, 577-584.

Lawn, R.M., Wade, D.P., Hammer, R.E., Chiesa, G., Verstuyft, J.G., Rubin, E.M., 1992. Atherogenesis in transgenic mice expressing human apolipoprotein(a). Nature 360, 670-672.

Lee, Y.T., Lin, H.Y., Chan, Y.W., Li, K.H., To, O.T., Yan, B.P., Liu, T., Li, G., Wong, W.T., Keung, W., Tse, G., 2017. Mouse models of atherosclerosis: a historical perspective and recent advances. Lipids Health Dis. 16, 12.

Li, J., Tumanut, C., Gavigan, J.A., Huang, W.J., Hampton, E.N., Tumanut, R., Suen, K.F., Trauger, J.W., Spraggon, G., Lesley, S.A., Liau, G., Yowe, D., Harris, J.L., 2007. Secreted PCSK9 promotes LDL receptor degradation independently of proteolytic activity. Biochem. J. 406, 203-207.

Libby, P., 2013. Mechanisms of Acute Coronary Syndromes and Their Implications for Therapy. N. Engl. J. Med. 368, 2004-2013.

Lusis, A.J., 2000. Atherosclerosis. Nature 407, 233-241.

Lutgens, E., Daemen, M., Kockx, M., Doevendans, P., Hofker, M., Havekes, L., Wellens, H., de Muinck, E.D., 1999. Atherosclerosis in APOE*3-Leiden transgenic mice : from proliferative to atheromatous stage. Circulation 99, 276-283.

Mahley, R., 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240, 622-630.

Marais, A.D., 2004. Familial hypercholesterolaemia. Clin. Biochem. Rev. 25, 49-68.

Mariko, B., Pezet, M., Escoubet, B., Bouillot, S., Andrieu, J.-P., Starcher, B., Quaglino, D., Jacob, M.-P., Huber, P., Ramirez, F., Faury, G., 2011. Fibrillin-1 genetic deficiency leads to pathological ageing of arteries in mice. J. Pathol. 224, 33-44.

Medley, T.L., Cole, T.J., Gatzka, C.D., Wang, W.Y., Dart, A.M., Kingwell, B.A., 2002. Fibrillin-1 Genotype Is Associated With Aortic Stiffness and Disease Severity in Patients With Coronary Artery Disease. Circulation 105, 810-815.

Meir, K.S., Leitersdorf, E., 2004. Atherosclerosis in the Apolipoprotein E-Deficient Mouse: A Decade of Progress. Arterioscler. Thromb. Vasc. Biol. 24, 1006-1014.

Moulton, K.S., Olsen, B.R., Sonn, S., Fukai, N., Zurakowski, D., Zeng, X., 2004. Loss of Collagen XVIII Enhances Neovascularization and Vascular Permeability in Atherosclerosis. Circulation 110, 1330-1336.

Nakashima, Y., Plump, A.S., Raines, E.W., Breslow, J.L., Ross, R., 1994. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler. Thromb. Vasc. Biol. 14, 133-140.

Ni, M., Chen, W.Q., Zhang, Y., 2009. Animal models and potential mechanisms of plaque destabilisation and disruption. Heart 95, 1393-1398.

Niimi, M., Yang, D., Kitajima, S., Ning, B., Wang, C., Li, S., Liu, E., Zhang, J., Eugene Chen, Y., Fan, J., 2016. ApoE knockout rabbits: A novel model for the study of human hyperlipidemia. Atherosclerosis 245, 187-193.

Ning, B., Wang, X., Yu, Y., Waqar, A.B., Yu, Q., Koike, T., Shiomi, M., Liu, E., Wang, Y., Fan, J., 2015. High-fructose and high-fat diet-induced insulin resistance enhances atherosclerosis in Watanabe heritable hyperlipidemic rabbits. Nutr. Metab. (Lond.) 12.

Niu, Y., Shen, B., Cui, Y., Chen, Y., Wang, J., Wang, L., Kang, Y., Zhao, X., Si, W., Li, W., Xiang, Andy P., Zhou, J., Guo, X., Bi, Y., Si, C., Hu, B., Dong, G., Wang, H., Zhou, Z., Li, T., Tan, T., Pu,

X., Wang, F., Ji, S., Zhou, Q., Huang, X., Ji, W., Sha, J., 2014. Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos. Cell 156, 836-843.

Piedrahita, J.A., Zhang, S.H., Hagaman, J.R., Oliver, P.M., Maeda, N., 1992. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. PNAS 89, 4471-4475.

Plump, A.S., Breslow, J.L., 1995. Apolipoprotein E and the Apolipoprotein E-Deficient Mouse. Annu. Rev. Nutr. 15, 495-518.

Plump, A.S., Lum, P.Y., 2009. Genomics and Cardiovascular Drug Development. J. Am. Coll. Cardiol. 53, 1089-1100.

Plump, A.S., Smith, J.D., Hayek, T., Aalto-Setälä, K., Walsh, A., Verstuyft, J.G., Rubin, E.M., Breslow, J.L., 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 71, 343-353.

Rademakers, T., Douma, K., Hackeng, T.M., Post, M.J., Sluimer, J.C., Daemen, M.J.A.P., Biessen, E.A.L., Heeneman, S., van Zandvoort, M.A.M.J., 2013. Plaque-Associated Vasa Vasorum in Aged Apolipoprotein E-Deficient Mice Exhibit Proatherogenic Functional Features In Vivo. Arterioscler. Thromb. Vasc. Biol. 33, 249-256.

Raffetto, J.D., Khalil, R.A., 2008. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. Biochem. Pharmacol. 75, 346-359.

Rattazzi, M., Bennett, B.J., Bea, F., Kirk, E.A., Ricks, J.L., Speer, M., Schwartz, S.M., Giachelli, C.M., Rosenfeld, M.E., 2005. Calcification of Advanced Atherosclerotic Lesions in the Innominate Arteries of ApoE-Deficient Mice: Potential Role of Chondrocyte-Like Cells. Arterioscler. Thromb. Vasc. Biol. 25, 1420-1425.

Reddick, R.L., Zhang, S.H., Maeda, N., 1994. Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression [published erratum appears in Arterioscler Thromb 1994 May;14(5):839]. Arterioscler. Thromb. Vasc. Biol. 14, 141-147.

Roche-Molina, M., Sanz-Rosa, D., Cruz, F.M., Garcia-Prieto, J., Lopez, S., Abia, R., Muriana, F.J., Fuster, V., Ibanez, B., Bernal, J.A., 2015. Induction of sustained hypercholesterolemia by single adeno-associated virus-mediated gene transfer of mutant hPCSK9. Arterioscler. Thromb. Vasc. Biol. 35, 50-59.

Ross, R., 1995. Cell biology of atherosclerosis. Annu Rev Physiol 57, 791-804.

Roth, L., Rombouts, M., Schrijvers, D.M., Lemmens, K., De Keulenaer, G.W., Martinet, W., De Meyer, G.R.Y., 2015a. Chronic intermittent mental stress promotes atherosclerotic plaque vulnerability, myocardial infarction and sudden death in mice. Atherosclerosis 242, 288-294.

Roth, L., Rombouts, M., Schrijvers, D.M., Martinet, W., De Meyer, G.R.Y., 2016. Cholesterol-independent effects of atorvastatin prevent cardiovascular morbidity and mortality in a mouse model of atherosclerotic plaque rupture. Vascul. Pharmacol. 80, 50-58.

Roth, L., Van Dam, D., Van der Donckt, C., Schrijvers, D.M., Lemmens, K., Van Brussel, I., De Deyn, P.P., Martinet, W., De Meyer, G.R.Y., 2015b. Impaired gait pattern as a sensitive tool to assess hypoxic brain damage in a novel mouse model of atherosclerotic plaque rupture. Physiol. Behav. 139, 397-402.

Sakakura, K., Nakano, M., Otsuka, F., Ladich, E., Kolodgie, F.D., Virmani, R., 2013. Pathophysiology of Atherosclerosis Plaque Progression. Heart Lung Circ. 22, 399-411.

Sasaki, T., Kuzuya, M., Nakamura, K., Cheng, X.W., Shibata, T., Sato, K., Iguchi, A., 2006. A Simple Method of Plaque Rupture Induction in Apolipoprotein E-Deficient Mice. Arterioscler. Thromb. Vasc. Biol. 26, 1304-1309.

Schwartz, S.M., Galis, Z.S., Rosenfeld, M.E., Falk, E., 2007. Plaque Rupture in Humans and Mice. Arterioscler. Thromb. Vasc. Biol. 27, 705-713.

Sehayek, E., Shefer, S., Nguyen, L.B., Ono, J.G., Merkel, M., Breslow, J.L., 2000. Apolipoprotein E regulates dietary cholesterol absorption and biliary cholesterol excretion: Studies in C57BL/6 apolipoprotein E knockout mice. PNAS 97, 3433-3437.

Shim, J., Al-Mashhadi, R.H., Sorensen, C.B., Bentzon, J.F., 2016. Large animal models of atherosclerosis--new tools for persistent problems in cardiovascular medicine. J. Pathol. 238, 257-266.

Silvestre-Roig, C., de Winther, M.P., Weber, C., Daemen, M.J., Lutgens, E., Soehnlein, O., 2014. Atherosclerotic Plaque Destabilization. Circ. Res. 114, 214-226.

Slager, C.J., Wentzel, J.J., Gijsen, F.J.H., Thury, A., van der Wal, A.C., Schaar, J.A., Serruys, P.W., 2005. The role of shear stress in the destabilization of vulnerable plaques and related therapeutic implications. Nat. Clin. Pract. Cardiovasc. Med. 2, 456-464.

Sluimer, J.C., Kolodgie, F.D., Bijnens, A.P.J.J., Maxfield, K., Pacheco, E., Kutys, B., Duimel, H., Frederik, P.M., van Hinsbergh, V.W.M., Virmani, R., Daemen, M.J.A.P., 2009. Thin-Walled

Microvessels in Human Coronary Atherosclerotic Plaques Show Incomplete Endothelial Junctions. J. Am. Coll. Cardiol. 53, 1517-1527.

Smith, J.D., Breslow, J.L., 1997. The emergence of mouse models of atherosclerosis and their relevance to clinical research. J. Intern. Med. 242, 99-109.

Suhy, D.A., Kao, S.C., Mao, T., Whiteley, L., Denise, H., Souberbielle, B., Burdick, A.D., Hayes, K., Wright, J.F., Lavender, H., Roelvink, P., Kolykhalov, A., Brady, K., Moschos, S.A., Hauck, B., Zelenaia, O., Zhou, S., Scribner, C., High, K.A., Renison, S.H., Corbau, R., 2012. Safe, long-term hepatic expression of anti-HCV shRNA in a nonhuman primate model. Mol. Ther. 20, 1737-1749.

Tabas, I., García-Cardeña, G., Owens, G.K., 2015. Recent insights into the cellular biology of atherosclerosis. J. Cell Biol. 209, 13-22.

van den Maagdenberg, A.M., Hofker, M.H., Krimpenfort, P.J., de Bruijn, I., van Vlijmen, B., van der Boom, H., Havekes, L.M., Frants, R.R., 1993. Transgenic mice carrying the apolipoprotein E3-Leiden gene exhibit hyperlipoproteinemia. J Biol Chem 268, 10540-10545.

Van der Donckt, C., Roth, L., Vanhoutte, G., Blockx, I., Bink, D.I., Ritz, K., Pintelon, I., Timmermans, J.P., Bauters, D., Martinet, W., Daemen, M.J., Verhoye, M., De Meyer, G.R.Y., 2015a. Fibrillin-1 impairment enhances blood–brain barrier permeability and xanthoma formation in brains of apolipoprotein E-deficient mice. Neuroscience 295, 11-22.

Van der Donckt, C., Van Herck, J.L., Schrijvers, D.M., Vanhoutte, G., Verhoye, M., Blockx, I., Van Der Linden, A., Bauters, D., Lijnen, H.R., Sluimer, J.C., Roth, L., Van Hove, C.E., Fransen, P., Knaapen, M.W., Hervent, A.S., De Keulenaer, G.W., Bult, H., Martinet, W., Herman, A.G., De Meyer, G.R.Y., 2015b. Elastin fragmentation in atherosclerotic mice leads to intraplaque neovascularization, plaque rupture, myocardial infarction, stroke, and sudden death. Eur. Heart J. 36, 1049-1058.

Van Herck, J.L., De Meyer, G.R.Y., Martinet, W., Van Hove, C.E., Foubert, K., Theunis, M.H., Apers, S., Bult, H., Vrints, C.J., Herman, A.G., 2009. Impaired Fibrillin-1 Function Promotes Features of Plaque Instability in Apolipoprotein E-Deficient Mice. Circulation 120, 2478-2487.

van Vlijmen, B.J., van den Maagdenberg, A.M., Gijbels, M.J., van der Boom, H., HogenEsch, H., Frants, R.R., Hofker, M.H., Havekes, L.M., 1994. Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3-Leiden transgenic mice. J Clin Invest 93, 1403-1410.

VanderLaan, P.A., Reardon, C.A., Getz, G.S., 2004. Site Specificity of Atherosclerosis: Site-Selective Responses to Atherosclerotic Modulators. Arterioscler. Thromb. Vasc. Biol. 24, 12-22.

Véniant, M.M., Sullivan, M.A., Kim, S.K., Ambroziak, P., Chu, A., Wilson, M.D., Hellerstein, M.K., Rudel, L.L., Walzem, R.L., Young, S.G., 2000. Defining the atherogenicity of large and small lipoproteins containing apolipoprotein B100. J. Clin. Invest. 106, 1501-1510.

Veniant, M.M., Withycombe, S., Young, S.G., 2001. Lipoprotein Size and Atherosclerosis Susceptibility in Apoe-/- and Ldlr-/- Mice. Arterioscler. Thromb. Vasc. Biol. 21, 1567-1570.

Virmani, R., Kolodgie, F.D., Burke, A.P., Finn, A.V., Gold, H.K., Tulenko, T.N., Wrenn, S.P., Narula, J., 2005. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscler. Thromb. Vasc. Biol. 25, 2054-2061.

von der Thusen, J.H., van Berkel, T.J., Biessen, E.A., 2001. Induction of rapid atherogenesis by perivascular carotid collar placement in apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice. Circulation 103, 1164-1170.

von der Thusen, J.H., van Vlijmen, B.J.M., Hoeben, R.C., Kockx, M.M., Havekes, L.M., van Berkel, T.J.C., Biessen, E.A.L., 2002. Induction of atherosclerotic plaque rupture in apolipoprotein E-/- mice after adenovirus-mediated transfer of p53. Circulation 105, 2064-2070.

Watanabe, Y., 1980. Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit) *1Incidence and development of atherosclerosis and xanthoma. Atherosclerosis 36, 261-268.

Williams, H., 2002. Characteristics of Intact and Ruptured Atherosclerotic Plaques in Brachiocephalic Arteries of Apolipoprotein E Knockout Mice. Arterioscler. Thromb. Vasc. Biol. 22, 788-792.

Witting, P.K., Pettersson, K., Ostlund-Lindqvist, A.M., Westerlund, C., Eriksson, A.W., Stocker, R., 1999. Inhibition by a coantioxidant of aortic lipoprotein lipid peroxidation and atherosclerosis in apolipoprotein E and low density lipoprotein receptor gene double knockout mice. FASEB J. 13, 667-675.

Ylä-Herttuala, S., Bentzon, J.F., Daemen, M., Falk, E., Garcia-Garcia, H.M., Herrmann, J., Hoefer, I., Jukema, J.W., Krams, R., Kwak, B.R., Marx, N., Naruszewicz, M., Newby, A., Pasterkamp, G., Serruys, P.W.J.C., Waltenberger, J., Weber, C., Tokgözoglu, L., 2011. Stabilisation of atherosclerotic plaques. Thromb. Haemost. 106, 1-19.

Zadelaar, S., Kleemann, R., Verschuren, L., de Vries-Van der Weij, J., van der Hoorn, J., Princen, H.M., Kooistra, T., 2007. Mouse models for atherosclerosis and pharmaceutical modifiers. Arterioscler. Thromb. Vasc. Biol. 27, 1706-1721.

Zhang, S.H., Reddick, R.L., Piedrahita, J.A., Maeda, N., 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 258, 468-471.

Zou, Y., Dietrich, H., Hu, Y., Metzler, B., Wick, G., Xu, Q., 1998. Mouse Model of Venous Bypass Graft Arteriosclerosis. Am. J. Pathol. 153, 1301-1310.

Zsebo, K., Yaroshinsky, A., Rudy, J.J., Wagner, K., Greenberg, B., Jessup, M., Hajjar, R.J., 2014. Long-term effects of AAV1/SERCA2a gene transfer in patients with severe heart failure: analysis of recurrent cardiovascular events and mortality. Circ. Res. 114, 101-108.

Figure 1. Overview of current mouse models of atherosclerosis. This figure describes the different models with their total plasma cholesterol levels on normal (ND) and Western-type diet (WD), lipoprotein profile, plaque characteristics, advantages and limitations. The distribution of the plaques in the thoracic aorta and the complexity is shown for mice fed a WD for 20 weeks. The composition of the most complex lesions at that time point is displayed (usually found in the aortic root or brachiocephalic artery).

Table 1. Mouse models of atherosclerotic plaque rupture

Strain	mechanism	Duration (weeks)	Plaque disruption	Luminal thrombus	Intra-plaque neo-vessels	IPH	Outward remodelling	'Human-like' complications	Comments	Ref.
ApoE$^{-/-}$	'ageing'	60	12%	3%	N.D.	N.D.	N.D.	Coronary thrombosis	Long term experiment Low rate of plaque rupture and thrombosis	(Calara et al., 2001)

ApoE$^{-/-}$	Mixed C57BL/6-129SvJ background	30-65	52%	73%	N.D.	N.D.	N.D.	MI ('some cases') sudden death	Long term experiment mixed background	(Williams, 2002)
ApoE$^{-/-}$	Collar: Ad-p53 in SMC + phenylephrine	15-17	40%	5%	N.D.	35%	N.D.	N.D.	Plaque rupture not spontaneous, complicated manipulation	(von der Thusen et al., 2002)
ApoE$^{-/-}$	Active MMP-9 overexpression in Mϕ	41	40%	Fibrin deposition (100%)	N.D.	90%	N.D.	Sudden death (20%)	Long term experiment, complicated manipulation	(Gough et al., 2006)
ApoE$^{-/-}$	Cuff (+ ligation)	13-14	29-63%	17-42%	N.D.	31-47%	N.D.	N.D.	Plaque rupture not spontaneous	(Sasaki et al., 2006)
ApoE$^{-/-}$: TM$^{Pro/Pro}$	Collar, genetic hypercoagulability	16-17	+	+	N.D.	+	+	N.D.	Plaque rupture not spontaneous	(Borissoff et al., 2013)
ApoE$^{-/-}$	Partial ligation carotid + renal arteries	16	+	50%	N.D.	80%	N.D.	N.D.	Plaque rupture not spontaneous	(Jin et al., 2012)
ApoE$^{-/-}$	uPA overexpression in Mϕ	43-48	78%	Fibrin deposition (67%)	N.D.	61%	N.D.	N.D.	Long term experiment, complicated manipulation	(Hu et al., 2010)
ApoE$^{-/-}$	Tandem stenosis	14-22	32%	+	+	50%	+	N.D.	Plaque rupture not spontaneous	(Chen et al., 2013)
ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$	Elastin fragmentation	20-35	50-70%	carotid	+	90%	+	MI, stroke, sudden death	Spontaneous plaque rupture	(Van der Donckt et al., 2015b)

IPH, intra-plaque haemorrhage; Ref., reference; +, present; MI, myocardial infarction N.D., not determined; Ad, adenovirus; Mφ, macrophages; uPA, Urokinase-type plasminogen activator

Table 2. Most important advantages and limitations of commonly used models of atherosclerosis

	Advantages	Limitations
Mice	• Relatively cheap • Efficient • Easy crossbreeding • Low cost for pharmacological intervention studies	• Differences with human plaques: – Less coronary plaque formation – No intra-plaque neovascularisation and haemorrhage – Rare plaque rupture and thrombosis However, these limitations are overcome in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice.

Rabbits	• Allows translation research such as catheterisation of the aorta (coronary arteries are too small) • Relatively cheap • Easy to breed and handle	• Differences in the localisation of the plaques as compared to humans: – Plaques are most pronounced in the aortic arch and descending thoracic aorta (in contrast to the abdominal aorta in humans)
Pigs	• Similarities with human plaques: – Localisation: coronary arteries, abdominal aorta, ileo-femoral – Neovascularisation towards the plaque	• Plaque development mostly ends in the foam cell stage (this can be overcome with the selection of natural mutants, mechanical damage, introduction of other risk factors, genetic engineering with minipigs) • Thrombosis due to plaque rupture is rare (in contrast to humans) • Relatively expensive and more difficult to handle
Non-human primates	• Very similar plaque formation as compared to humans (both micro- and macroscopic) • Plaque formation in the coronary arteries	• Non-human primates are expensive and highly regulated • Specialized training is needed

	Model	Lipid profile	Plaque distribution and characteristics (20 weeks WD)		Advantages & limitations
ApoE$^{-/-}$	Disruption of the ApoE gene 	**Plasma cholesterol:** 400-600 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ⇑⇑ VLDL ⇑ LDL ⇓ HDL		**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ Develops atherosclerosis on ND ⊖ No human-like lipid profile ⊖ ApoE plays a role in inflammation → influence plaque development ⊖ No spontaneous plaque rupture, thrombosis and complications
LDLr$^{-/-}$	Disruption of the LDL receptor gene 	**Plasma cholesterol:** 200-300 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ⇑ VLDL ⇑⇑ LDL = HDL		**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ Human-like lipid profile (LDL) ⊕ Functional ApoE → no impact on inflammation ⊖ Complex lesion development requires a WD ⊖ No spontaneous plaque rupture, thrombosis and complications
ApoE$^{-/-}$ LDLr$^{-/-}$	Disruption of the ApoE and the LDL receptor gene 	**Plasma cholesterol:** 400-600 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ⇑⇑ VLDL ⇑⇑ LDL ⇓ HDL		**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ Develops atherosclerosis on ND ⊖ No spontaneous plaque rupture, thrombosis and complications
ApoE3-Leiden	ApoE3-Leiden mutation via DNA construct (ApoE, ApoC1) from the ApoE3-Leiden proband 	**Plasma cholesterol:** 100-200 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ⇑⇑ VLDL ⇑ LDL ⇑ HDL (only on WD)		**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ Functional ApoE → no impact on inflammation ⊖ Complex lesion development requires a WD ⊖ No spontaneous plaque rupture, thrombosis and complications
PCSK9-AAV	Single Adeno-Associated Virus–mediated gene transfer of mutant PCSK9 	**Plasma cholesterol:** 300 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ⇑ VLDL (only on WD) ⇑ LDL = HDL		**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ No genetic modification needed ⊖ Lesion development requires a WD ⊖ No spontaneous plaque rupture, thrombosis and complications
ApoE$^{-/-}$Fbn1$^{C1039G+/-}$	ApoE$^{-/-}$ mice X mice with a mutation in the Fbn1 gene (C1039G) 	**Plasma cholesterol:** 400-600 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ⇑⇑ VLDL ⇑ LDL ⇓ HDL		**Rupture-prone plaques:** ⇓ Smooth muscle cells ⇓ Extracellular matrix ⇑ Inflammatory cells ⇑ Necrotic core Neovascularisation Haemorrhage	⊕ Accelerated plaque development ⊕ Spontaneous plaque rupture with complications (MI, stroke) and sudden death ⊕ Intra-plaque neovascularisation and haemorrhage present ⊖ Male mice on WD die prematurely due to aneurysm rupture