JB Review

J. Biochem. **140**, 23–38 (2006)
doi:10.1093/jb/mvj137

Overexpression of Lysosomal Acid Lipase and Other Proteins in Atherosclerosis

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Received May 6, 2006; accepted June 4, 2006

Atherosclerosis is one of the major causes of morbidity and mortality in the western world. The existing data of elevated expression levels of proteins like DNA damage and DNA repair enzymes in human atherosclerotic plaques are reviewed. From the literature, the effect of overexpression of different proteins using adenoviral vectors or the model of transgenic mice on the development of atherosclerosis will be discussed. Special focus is placed on the lysosomal acid lipase (LAL), because LAL connects extra-cellular with intra-cellular lipid metabolism and is the only hydrolase for cleavage of cholesteryl esters delivered to the lysosomes. Patients with a deficiency of LAL show an accumulation of lipids in the cells and develop pre-mature atherosclerosis. To answer the question of the influence of LAL in atherosclerosis if overexpressed, we show for the first time data of transgenic mice overexpressing LAL and the effect on the lipid level.

Key words: adenovirus, atherosclerosis, DNA damage, lysosomal acid lipase, protein expression, transgenic mice.

Complications of atherosclerosis are the leading cause of death in the western world. The disease is characterized by thickening, hardening, and loss of elasticity of the blood vessel walls. On the cellular level, atherosclerosis involves the infiltration of several cell types, including monocytes, differentiating to macrophages in the vessel wall. After take-up and accumulation of lipids, macrophages transform to foam cells and secrete both growth factors as well as metalloproteinases, leading to matrix degeneration and lesions in the intima.

The causes for this disease are manifold and different etiologies have been discussed. The primary risk factors are diabetes, obesity, male gender, family history of coronary heart disease at an early stage, smoking, elevated blood pressure (hypertension), low HDL cholesterol, and high LDL cholesterol. However, these factors are not fully responsible for the whole atherosclerotic risk, since some patients without any of the above mentioned risk factors may develop heart attacks. For example, chronic infections with certain viruses, such as CMV, and bacteria, such as *Chlamydia pneumoniae* and *Porphyromonas gingivalis*, elevated homocysteine levels in the blood or elevated blood levels of lipoprotein A, Lp(a), are additional risk factors which may cause atherosclerosis. Some scientists believe that emotional stress is also a risk factor, but the evidence for this is not clear-cut yet.

This review will summarize the existing data of elevated expression levels of proteins like DNA damage and DNA repair enzymes in human atherosclerotic plaques. From the literature, the effect of overexpression of different proteins using adenoviral vectors and the model of transgenic mice on the development of atherosclerosis will be discussed. Special focus is placed on the lysosomal acid lipase (LAL), because LAL connects extra- cellular with intracellular lipid metabolism and is the only hydrolase for cleavage of cholesteryl esters delivered to the lysosomes. Patients with a deficiency of LAL show an accumulation of lipids in the cells and develop pre-mature atherosclerosis. Thus, overexpression of LAL may be one target for clinical trials in atherosclerosis. To prove this assumption, we produced mice stably overexpressing LAL and show for the first time data of the effect on the lipid level in those mice.

I. Risk factors for atherosclerosis

Several factors are well known to be involved in the development of atherosclerosis. There are some factors which cannot be altered therapeutically. These include male gender, high age and positive familial anamnesis. Clinically more interesting are the factors which can be altered. Probably, the most frequent disorders are elevated blood pressure, diabetes mellitus and obesity, often combined with the metabolic syndrome.

Obesity. It is out of the question that increasing degrees of obesity are accompanied by higher rates of cardiovascular disease (*1*, *2*). Leptin, a circulating hormone produced by adipose tissue, regulates body weight, food intake and metabolism. An increase of leptin, which is pronounced in obesity, could contribute to cardiovascular events. Leptin increases oxidative stress, leading to oxidation of lipoproteins and damage in the vessel wall (*3*, *4*).

Lipids. In atherosclerosis, lipid accumulation in the artery wall plays a major role. LDL oxidation is the main cause of endothelial injury and induces the expression of proinflammatory molecules in endothelial cells. Other lipids show comparable effects; notably, high

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plasma levels of VLDL are associated with increased risk of atherosclerosis (*5*). Hydroxymethylglutaryl coenzyme A (HMG-Co A)-reductase inhibitors (statins) reduce cardiovascular disease events and improve outcomes by lowering LDL (and perhaps elevating HDL) plasma levels. However, serial trials have suggested that the clinical benefit of statins is greater than expected from the lowering effect on lipids (*6*). In addition, the statins show pleiotropic effect by improving endothelial function, enhancing the stability of atherosclerotic plaques, decreasing oxidative stress, inflammation, and inhibiting the thrombogenic response (*7–9*).

Smoking. Smoking is a major risk factor in the development of atherosclerosis. The pathogenesis includes the formation of free radicals, and this is also true for passive smoking or environmental tobacco smoke. A 30 min passive smoking exposure was found to affect coronary flow velocity reserve in nonsmokers (*10*, *11*).

Hypertension. High blood pressure shows a strong correlation with the development of atherosclerosis. Moderately elevated blood pressure levels can be treated with changes in life-style, for example by doing more physical training, and by reduced sodium intake and alcohol consumption. Angiotensin II exhibit an effect on hypertension on the molecular level and can also promote long-term changes in vascular smooth muscle cell function by its ability to induce cellular hypertrophy and extracellular matrix production (*12*). Thus, angiotensin-converting enzyme inhibitors (ACEI) are important medications for people with hypertension. Besides lowering blood pressure, ACEI show additional protective effects on atherosclerosis by having anti-inflammatory activity (*13*).

Diabetes. Data suggest that diabetic patients have already developed vascular disease by the time of clinical diagnosis (*14*). Cytokine release and processes contribute to the development of athero-inflammatory complexes (*15*). It was shown for example that the blood level of c-reactive protein is in average higher in diabetic patients than in normal people indicating that inflammation contributes to the development of the disease (*16*). Although type 2 diabetes is the state of increased plasma coagulability, endothelial dysfunction is the most important factor in thrombotic complications. It is present more frequently in type 2 diabetes than type 1 (*17*).

Chronic infections causing atherosclerosis. Inflammatory processes are involved in the development of atherosclerosis and its complications. Mediators of inflammation can be found at all stages of the life cycle of the atherosclerotic plaques. These include macrophages and lymphocytes, cytokines, growth factors, matrix degenerating proteinases, and tissue factors (*18*). Risk factors such as hypertension, smoking or elevated levels of LDL result in injury to the endothelial cell of the artery, and this injury initiates the inflammatory process. However, many patients with vascular disease do not show these risk factors. For this reason viral and bacterial pathogens have been determined as possible causative factors in the pathogenesis of coronary artery disease (CAD). It was shown in many publications that infectious agents like herpes simplex virus (HSV), cytomegalovirus (CMV), *Chlamydia pneumoniae*, and *H. pylori* play a role in the development of atherosclerosis and its manifestations, especially in relationship to CAD (*19*), but *C. pneumoniae* is thought to be the most important pathogen related to the development of atherosclerosis (*20*, *21*).

Burnett *et al*. infected apoE knockout mice showing the phenotype of atherosclerosis with murine CMV and *C. pneumonia* and demonstrated that infection with murine CMV alone, *C. pneumoniae* alone and infection with both pathogens lead to increased lesion sizes of 84, 70, and 45%, respectively. Furthermore, the murine CMV-induced increase in circulating levels of interferon-γ, which may contribute to the increase in lesion size (*22*).

Cardiovascular disease is also a major problem in patients with chronic renal failure leading to increased mortality. A publication by Wolf *et al.* showed that IgA seropositivity for *C. pneumoniae* correlated with elevated values for C-reactive protein, but neither *H. pylori* nor CMV were associated with an increased rate of symptomatic atherosclerosis manifestations such as myocardial infarction or stroke in patients with endstage renal disease (*23*). Other groups also found that C-reactive protein is a risk-factor for subsequent myocardial infarction, but only in older patients infected with *H. Pylori* and not with *C. pneumoniae* (*24*).

There have been published other results indicating that the connection between infection with *C. pneumoniae* and atherosclerosis is not really clear yet. For example, in a study published in 1998, patients with cardiac transplantation showed evidence of past *C. pneumoniae* and CMV infection, but *C. pneumoniae* did not appear to have an independent role in the development of transplant-associated atherosclerosis (*25*). Furthermore, *C. pneumoniae* and CMV are commonly detected in atherosclerotic plaques of the carotid arteries, but their presence is not to be measured by detecting serum antibodies (*26*). Recently, the data of a prospective study investigating the role of systemic and local infection in 109 patients with high-grade internal carotid artery stenosis did not support the hypothesis that systemic *C. pneumoniae*, HSV or CMV-infection in carotid plaques causes plaque destabilization and cerebral thromboembolism. However, plaque infection was only observed in patients with advanced atherosclerosis (*27*). Overexpression of heat shock protein 60, which has been found in atherosclerotic lesions and is involved in the regulation of cell cycle progression and cell proliferation, may be a central intracellular event responsible for the mitogenic effects induced by *C. pneumoniae* (*28*).

Periodontal infections. Since the first observations about 20 years ago (*29*), multiple epidemiologic studies suggest that periodontitis is also an important risk factor for atherosclerosis. Periodontitis induces a peripheral inflammatory and immune response, reflected in elevated concentrations of C-reactive protein and antibodies to periodontal pathogens. Since the major periodontal pathogen, *P. gingivalis*, occur in approximately 40–100% of patients with adult periodontitis, antibody levels to this pathogen are determined most often (*30*, *31*). Antibody levels were found to be significantly associated with coronary heart disease (*32*). Moreover, *P. gingivalis* IgA antibodies could even predict a coronary event (*33*). Studies performed by various groups have shown that *P. gingivalis* is capable of stimulating LDL oxidation, foam cell formation, and rupture of atherosclerotic plaques through inactivation of matrix metalloproteinases (*34–37*). In addition,

among some other pathogens, *P. gingivalis* DNA has even been detected in atherosclerotic plaques (*38*). Choi and colleagues also isolated *P. gingivalis* heat-shock protein specific T-cells in atherosclerotic plaques from subjects with severe atherosclerosis. Bacterial heat shock proteins are believed to be involved in regulating autoimmune mechanisms, and also appear to be associated in the pathogenesis of periodontitis (*39*). As periodontitis and atherosclerosis share common pathogenic factors such as smoking, the nature of their association is difficult to explore. Nevertheless, studies performed so far show clear hints that periodontitis might be an independent risk factor.

In conclusion, the influence of pathogens in the development of atherosclerosis needs further investigation. Interestingly, it was found that present day markers suggested as indicators for heart disease susceptibility such as C-reactive protein and homocysteine are all similarly elevated in tuberculosis, leading to the hypothesis that tuberculosis may also be involved in the development of atherosclerosis (*40*).

DNA damage and DNA repair enzymes in atherosclerosis. Numerous studies have linked generation of reactive oxygen species (ROS) with cellular damage and atherogenesis (*41*,*42*). ROS can provoke cancer by damaging DNA (*43*), leading to DNA strand breaks, and chromosomal aberrations (*44*), and are also involved in oxidation of other macromolecules like lipoproteins, which is considered a fundamental step in the initiation and progression of atherosclerosis. Additionally, oxidative DNA damage is also a prominent feature of atherosclerotic plaques (*45*,*46*). Martinet and co-workers found that several DNA repair enzymes are up-regulated in plaques, either those involved in base excision repair (XRCC1, PARP-1) or in non-specific repair pathways (p53, DNA-PK) (*47*,*48*). Figure 1 summarizes these results. The base excision repair pathway is the major repair pathway for oxidative DNA repair damage (*49*). Overexpression of PARP-1 may contribute to the formation of necrotic cells which occur in atherosclerotic lesions. PARP-1 interacts directly with XRCC1 (*50*) and appears to play an important role in p53 activation and regulation after DNA damage (*51*). XRCC1 gene encodes for a scaffolding protein, which plays an important role in base excision of DNA repair by bringing together DNA polymerase β (*52*) and DNA ligase III (*53*), and also NEIL1 and OGG1 (*54*) at the site of DNA damage. OGG1 plays an essential role in the repair of 8-hydroxyguanine residues in DNA produced by oxidative stress, and possesses glycosylase and apurinic site lyase activity (*55*). DNA-PK serves as an essential upstream activator of p53 (*56*). P53 is activated by two phosphorylation sides, and activation is an important regulatory event in the arterial vessel wall, because p53 deficiency, specifically in macrophages, leads to a significant doubling of atherosclerotic lesion size (*57*). Thus, p53 not only plays a key role in cancer, but also in the development of atherosclerosis.

Homocysteine and Lp(a). Impaired homocysteine metabolism has been implicated as another factor in the development of atherosclerosis. In addition, direct relationships between enhanced homocysteine valus and other risk factors like cigarette smoking, diabetes, obesity, and hypertension have been suggested. Homocysteine is

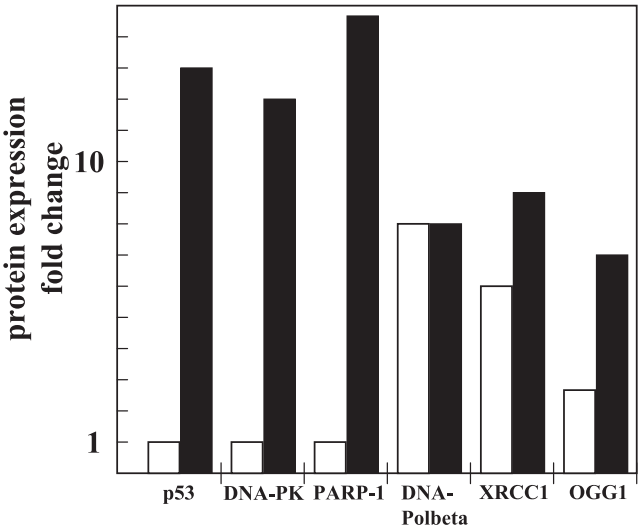

Fig. 1. **Protein expression of several DNA-repair enzymes.** DNA repair enzymes are up-regulated in plaques, either those involved in base excision repair (XRCC1, PARP-1) or in non-specific repair pathways (p53, DNA-PK). P53 and PARP-1 show the highest expression differences in plaques of the carotid artery compared to non-atherosclerotic control vessels. Interestingly, DNA polβ shows no differences in protein expression. β-actin was used as a control. This picture is our densitometric analysis of Western blot data published by Martinet *et al.* (*47*, *48*).

formed during demethylation of methionine. In the British Regional Heart Study, homocysteine levels were significantly higher in patients with stroke (*58*). The exact mechanism by which higher homocysteine levels may lead to CHD or thrombotic risk remains speculative. Toxic effects by the molecule on its own or due to oxidative stress by producing ROS have been postulated.

Studies during the 1970s and 1980s identified elevated plasma Lp(a) concentrations as a risk factor for atherosclerotic disorders including CHD (*59*, *60*). Lp(a) has not only been considered to possess proatherogenic properties by virtue of its similarity to LDL, but also prothrombic properties by virtue of the similarity of apolipoprotein (a) to plasminogen (*61*). It has to be mentioned that to date no clinical trials have shown that lowering Lp(a) levels decreases CHD risk. In contrast, the association of elevated Lp(a) with an increased risk for CHD events is well established. This increased risk may in part due to the activation of monocytes as major cells involved in atherogenesis. High concentrations of plasma Lp(a) were shown to influence the gene expression of human blood lymphocytes. It was shown by Buechler and co-workers that purified Lp(a) led to increased secretion of proinflammatory interleukin-6. Additionally, they found that mRNA abundance of LAL was reduced in monocytes of patients with CHD and selective Lp(a) hyperlipidemia (*62*).

II. Structure and function of LAL

The molecular structure of LAL. LAL [EC 3.1.1.13] shows only small expression values in the tissue. Moreover, the instability and hydrophobicity of this enzyme made biochemical investigations difficult for a long time. In 1991, Anderson and Sando (*63*) succeeded to clone the

cDNA of LAL which finally made it possible to clone it into vector expression systems (*64*). The gene is located on chromosome 10q 23,2 and consists of 10 exons with a size of 39 to 1,487 basepairs and 9 introns. The coding region begins in exon 2 and ends in exon 10. The untranslated 3′-region has a length of 1,255 basepairs, is not interrupted by introns and represents the largest part of exon 10. The LAL is composed of the hydrophobous 27 amino acids long signal sequence which is provided for the transport of the enzyme from the ribosome to the endoplasmatic reticulum. In addition, LAL exhibits an assumed propeptide with 49 amino acids which serves for protein transport and stabilization and is separated in the prelysosomal compartment (*65*). Possibly, this observation explains that after purification of the LAL from human liver tissue two active forms were received with different molecular weights of 41 and 55 kDa, respectively. N-terminal sequencing of the two forms demonstrated that the mature LAL begins with A1 and/or G50 (*66*).

The LAL activity seems to be regulated by the availability of different substrates (Fig. 2). When the uptake of LDL is induced (for example after treatment with either T3 and insulin or cholesterol-rich diet), LAL activity increases 2–3-fold (*67*, *68*). At the same time, inhibition is caused by the formation of the products, which means that in cells showing accumulated amounts of cholesterol the activity of LAL is reduced. The endogene promoter of human LAL in monocytes was characterized by Ries *et al.* (*69*). The promoter region is GC-rich and contains the TATA-box. When the transcription factors Sp1 and AP-2 bind at the 182bp long promoter sequence, an induction of LAL expression is induced during differentiation from monocytes to macrophages (*69*). However, the lysosomal compartment enlarges during differentiation, leading to an induction of all lysosomal enzymes, not only LAL (*70*).

The existence of putative recognition sites for protein kinases, catalyzing reversible phosphorylations, in addition indicates regularization of LAL expression. The sequence of the LAL exhibits four consensus sequences for the protein kinase C, five for the caseine kinase II and one for the tyrosin kinase. To our knowledge, no experimental data concerning phosphorylation of LAL has been published so far.

The formation of disulphide bridges by cysteine residues plays a relevant role in folding and stability and thereby has fundamental meaning for the function of proteins. The sequence of the LAL exhibits six cysteine residues. Based on data by X-ray structure analysis of recombinant HGL expressed in a baculovirus system, a disulphide bridge between C227 and C236 in LAL was determined (*71*) which was previously shown using site-directed mutagenesis by Lohse *et al*. (*72*). The LAL possesses six potential *N*-glycosylation sites and one potential *O*-glycosylation site. LAL does not exhibit O-glycosylation. Using a baculovirus vector system for expression of LAL, it was found that two *N*-glycosylation sites were occupied (N134 and N246) which are essential for stability and activity of the enzyme. First data concerning the *N*-glycosylation site in the propeptide sequence of the LAL suggest that it is also glycosylated (*73*). In contradiction to those findings, Du and co-workers found that five of six N-linked oligosaccharide sites were occupied after expression of human LAL in CHO cells and *Pichia pastoris* (*74*). Probably, the apparent contradiction is due to the different expression systems used.

Function of the LAL. Lipoproteins and in particular LDL, circulating in the blood, are endocytosed by lipoprotein receptors into the cells and transported to the lysosomes, where LDL receptors and particles of lipoproteins dissociate. The latters are hydrolyzed by lysosomal enzymes. In the lysosomes, LAL plays a crucial role by catalyzing the hydrolysis of triglycerides and cholesteryl esters. LAL was also found to be co-localized in the membrane fractions of early and late endosome markers (*75*). Fatty acids, mono glycerides and cholesterol are set free and transported to the cytoplasm. The free fatty acids are used for energy production (β-oxidation) or are re-esterified. Free cholesterol contributes to cholesterol equilibrium

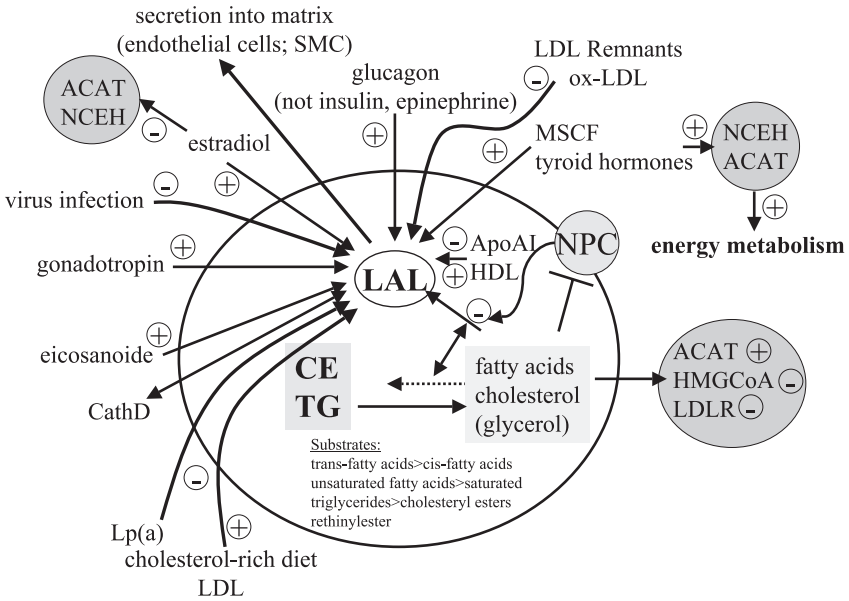

Fig. 2. **Impacts on expression and activity of LAL enzyme and interaction with endo- and exogenous parameters.** An up-regulation of expression or activity of LAL is indicated by ⊕ and down-regulation by ⊖. In addition, the substrates of LAL and which kind of substrate is preferred if more than one is available are shown. Abbreviations used are: HMGCoA: hydroxymethylglutaryl-CoA; LDLR: LDL-receptor; SMC: smooth muscle cells; CE: cholesteryl ester; TG: triglyceride; NPC: Niemann Pick-protein C; Apo A-I: apolipoprotein A-I; MSCF: mouse stem cell factor; NCEH: neutral cholesteryl ester hydrolase; ACAT: acyl-coenzyme A–cholesteryl-acyl-transferase; CathD: cathepsin D; Lp(a): lipoprotein (a).

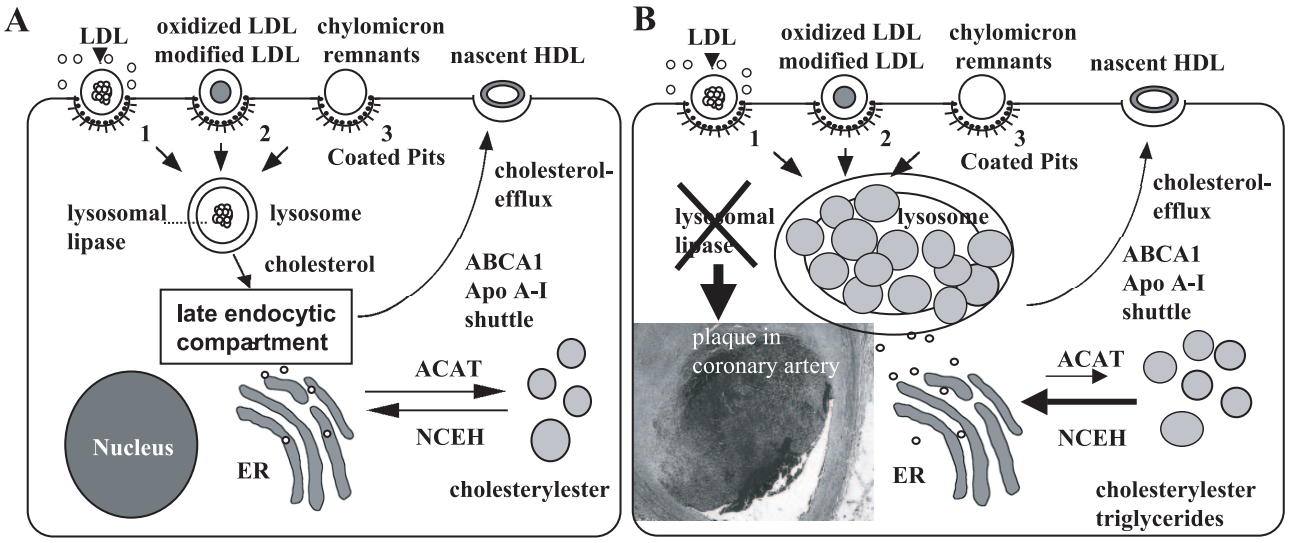

Fig. 3. **Lipid metabolism in presence of active LAL (A) and in a LAL-deficient cell (B).** LDL and other lipoproteins enter the cell via endocytosis and are hydrolyzed in the lysosomes by LAL releasing cholesterol, free fatty acids and glycerol. LAL-deficiency or reduced activity leads to an accumulation of cholesteryl esters and triglycerides in the lysosomal compartment, which may promote formation of atherosclerotic plaques. The ABCA1 transporter converts pools of late endocytic lipids associated with endocytosed apo A-I which is released from the cell as nascent HDL (*105*). Please note that cholesterol is liberated from LDL cholesteryl ester in the hydrolytic compartment containing LAL and then moves to late endosome/lysosome before reaching the ER (*75*). Abbreviations used are: LDL: low-density lipoprotein; HDL: high-density lipoprotein; ER: endoplasmatic reticulum; ACAT: Acyl-coenzyme A–cholesteryl-acyl-transferase; NCEH: neutral cholesteryl ester hydrolase.

and is used for membrane stabilization or is transported out of the cell (efflux), see Fig. 3. It should be taken into account that altering cellular cholesterol levels induces mechanism to maintain cholesterol equilibrium. For example, there is induction of a number of genes via the transcription factor SREBP if cellular cholesterol levels are low. These genes include HMG-CoA reductase for increased production of cholesterol and the LDL-Receptor for increased endocytosis of cholesterol-rich proteins (*76*). If the LAL is not active and/or is missing, triglycerides and cholesteryl esters accumulate in the cell, resulting in foam cell formation and as a consequence in atherosclerotic plaques (Fig. 3).

Wolman disease (WD) and cholesterol ester storage disease (CESD). The clinical relevance of the LAL is described by two diseases caused by deficiencies of the enzyme, namely Wolman disease (WD) and cholesteryl ester storage disease (CESD) (*77*).

WD is a pediatric disease with accumulation of triglycerides and cholesteryl esters inside the cells. This is caused for most different tissues by complete loss of the catalytic activity of the LAL. WD children suffer from strong vomiting, diarrhoea, and hepatosplenomegaly and normally die within the first year after birth. Meanwhile, more than 90 cases of WD have been described caused by missense and nonsense, insertion and deletion mutants in LAL (*78*, *79*).

Patients with CESD exhibit a remainder activity of 10–30% of the LAL. CESD is characterized by an accumulation of triglycerides in the cells and shows a more benign process. After autopsy of adult patients diagnosed with CESD, an early outcome of atherosclerosis was found. To date, CESD has been examined with approximately 70 cases on the molecular level. As is the case for the Wolman patients, different mutations in the gene of the

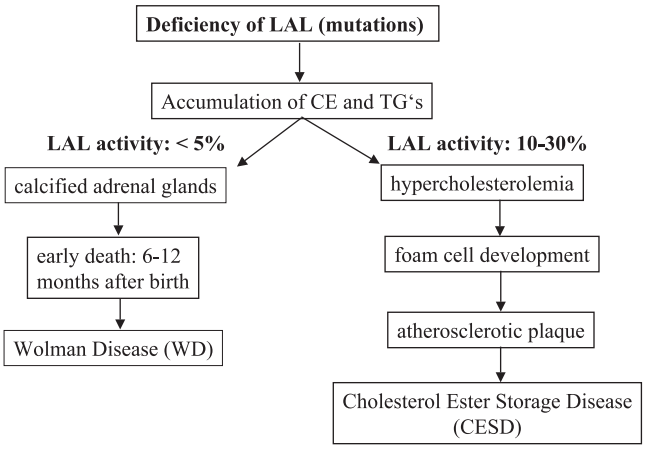

Fig. 4. **Differences of the two diseases, Wolman disease and cholesteryl ester storage disease, caused by deficiencies of LAL.** For both diseases, there is an accumulation of cholesteryl esters and triglycerides in the cells, by which cholesteryl ester storage disease is the benign form, which can be treated with statins and a low-fat diet. The reason is the more active LAL in these patients. Wolman patients, dying within the first 12 months of their lives, show only residual LAL activity.

LAL were detected (*80–82*). An overview of the differences between WD and CESD is described in (*83*) and summarized in Fig. 4.

At present, state of the art therapy for patients causing CESD is treatment with HMGCoA reductase inhibitors to minimize endogenous synthesis of cholesterol, as well as treatment with cholestyramin, an anion exchanger binding bile acid. Treatment has to be combined with a low fat diet. To our knowledge from literature search, the only functioning therapy for Wolman patients

so far is bone marrow transplantation. One patient reached the age of four years after bone marrow transplantation (*84*).

Animal models of LAL. We established a transgenic mouse strain overexpressing LAL which is described briefly in section III of this review. For the detailed description and data read the up-coming publication by Mayet *et al.* (*85*).

Some animals of a strain of Donryu rats showed typical symptoms of the WD (*86*). A substantial accumulation of cholesteryl esters, free cholesterol and triglycerides was detected in the liver of these animals. Cholesteryl esters and free cholesterol were also found in the spleen, but no triglycerides had accumulated. The activity of LAL was detected neither in the liver nor in the spleen of the animals. Heterozygous animals exhibited a middle activity of LAL and showed no lipid accumulation in liver and spleen. The cause of the deficiency of LAL in these rats is a 4.5-kb deletion in the genomic DNA.

Some years ago, Du *et al*. established the homozygous LAL knock out mice (*87*). LAL-deficient mice showed a normal phenotype at birth, developed normally and were fertile. However, a substantial accumulation of triglycerides and cholesteryl esters developed in liver, suprarenal bodies and small intestine of the animals. Additionally, the animals developed an insulin resistance after injection of glucose and showed an increased plasma level of free fatty acids. This model phenotypically resembles CESD, but resembles biochemically and histopatically WD. A few years later, the same group succeeded in substituting LAL transiently in these mice by means of an adenovirus overexpressing LAL (*88*). A substantial decomposition in hepatocytes and macrophages was shown after treatment. Thus, a therapy of human CESD or WD appears at least possible by substitution with recombinant enzyme.

III. Stable overexpression of LAL in a transgenic mouse model

To fully understand the molecular backgrounds of the regulation and function of specific genes and their failure in pathologic processes, it is absolutely necessary to carry out experiments in the whole organism. The transgenic technology offers the possibility for such an approach and has brought new aspects to scientists in the fields of basic research, medicine and pharmacology (*89*, *90*). The advantage of transgenic animal models is that the induced phenotype is transmitted from generation to generation, which makes it possible to analyze individuals not only in the stage of different ages but also in different generations.

In liver homogenates of female transgenic mice overexpressing LAL a threefold, in male animals a sevenfold increase in LAL activity was detected. Probably, the different values of LAL activity are caused by sex specific hormonal differences. Mice fed with western type diet showed significant elevations of plasma VLDL-cholesterol and hepatocellular lipids compared to controls.

To investigate whether this was an effect of alteration of lipid uptake into the cells due to the stable overexpression of the LAL in the transgenic mice, kinetic studies were performed. Firstly, male transgenic mice were treated with ^{125}Iod-labeled chylomicrons by intravenous injection. Changes in the lipoprotein metabolism and/or content could be determined neither in the serum nor in the examined tissues (liver, kidney, intestine, heart) compared to wild-type mice. These experiments were repeated with labelled ^{14}C-cholesterylester and ^{3}H-cholesteryloleylether lipoproteins (LDL). Only the ester binding is hydrolyzed by LAL, while the ether binding is not. Thus, the molecules containing the ether binding remain within the cells and could be used as an internal standard to calculate the rates of hydrolysis in comparison to the concentrations of ^{14}C-cholesterylester. In these experiments, no significant differences were found in the plasma between wild-type and LAL transgenic mice (Fig. 5). Nevertheless, it was shown that the rate of intracellular hydrolysis of the lipids in transgenic mice was significantly elevated compared to wild type mice (data not shown).

We hypothesize that the higher amounts of cytoplasmatic lipids are due to intracellular mechanisms, which are up-regulated through the high rates of hydrolysis. The uptake from the blood is not altered by the high activity of the LAL. Additionally, the mechanisms to route out the intracellular amount of lipids are up-regulated, leading to elevated VLDL plasma levels.

Thus, we suggest that since the LAL does not play a direct role in the homeostasis of the plasmalipids, its effect on it and on atherogenesis remains limited. But further experiments are necessary to finally clear the effect of LAL when overexpressed.

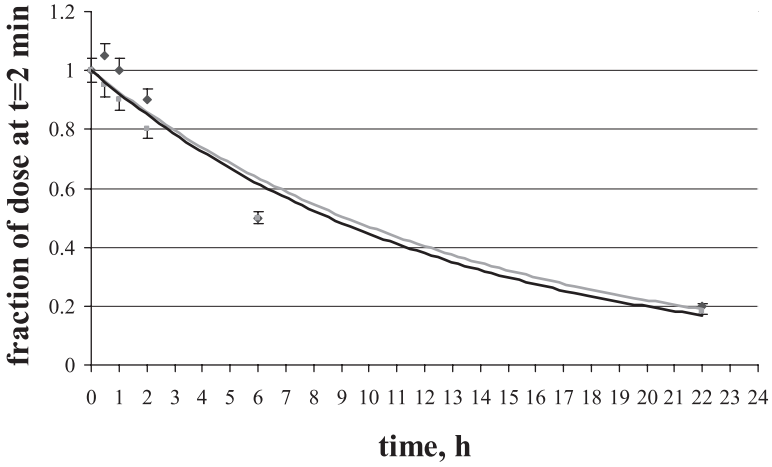

Fig. 5. **Kinetic study of plasma lipids in transgenic mice stably overexpressing LAL shows no differences compared to wild-type mice.** The figure shows the content of ^{14}C-cholesterylester in the plasma of transgenic and wild-type mice, respectively. The grey curve shows the content for wild-type mice, while the black curve for LAL-transgenic mice. The experiment was performed for a period of 22 hours, and curves are calculated from the amount of radioactive substrate at 2 min.

IV. Other transgenic mice models for atherosclerosis

The first famous study in the field of atherosclerosis using transgenic mice was published in *Science* magazine in the year 1993. The study revealed that transgenic mice overexpressing mouse apolipoprotein (apo) A-II had elevated HDL cholesterol concentrations, but nevertheless had exhibited increased atherosclerotic lesion development as compared to normal mice. This was by means of larger HDL molecules in transgenic mice and an increased ratio of apo A-II to apo A-I in HDL particles. Thus, both the composition and the amount of HDL appear to be important determinants of atherosclerosis (*91*).

In order to assess the role of human apolipoprotein A-I and HDL in atherosclerosis susceptibility, transgenic mice overexpressing the human apo-AI gene were crossed with apo E–deficient mice. Apo E–deficient mice are highly susceptible to atherosclerosis. It was shown that human apo A-I gene expression as well as overexpression using an adenovirus vector in mice increased HDL and suppresses atherosclerosis in the apo E–deficient mouse (*92*).

The two studies clearly demonstrated that transgenic mice overexpressing either apo A-I or apo A-II both exhibit elevated levels of HDL cholesterol, but whereas the apo A-I transgenic mice are protected against atherosclerosis, the apo A-II transgenic mice show increased lesion development. The reason for this observation is based on two effects. Firstly, HDL taken from apo A-II transgenic mice but not from either the apo A-I transgenics or non-transgenic littermate controls by itself stimulates lipid hydroperoxide formation in artery wall cells, indicating that the apo A-II transgenic HDL is in fact proinflammatory. Secondly, the loss of function of apo A-II transgenic HDL as an antioxidant is associated with a decreased content of paraoxonase 1, an enzyme that may protect against oxidation (*93*). The results from paraoxonase 1 *in vitro* studies and experiments using knock out and transgenic mice suggest that this protective effect may be attributed to the ability of paraoxonase 1 to attenuate the oxidative modification of lipoprotein particles. This member of the paraoxonase gene family is down-regulated by the influence of oxidative stress, whereas paraoxonase 2 is up-regulated in response to oxidative stress [for details see review by Ng *et al.* (*94*)]. In addition to the two effects described, expression of apo A-II in apo E–deficient mice induced a dose-dependent increase in VLDL of up to 24-fold in these kind of animals (*95*). In conclusion, higher expression levels of apo A-II might contribute to an increased susceptibility to atherosclerosis.

Higher plasma levels of triglycerides were also measured when apo C-I was overexpressed in apo E knockout mice. Additionally, these mice showed a near doubling of cholesterol, leading to increased atherosclerosis. HDL from apo C-I transgenic mice have a marked inhibitory effect on hepatic lipase activity, but Lipoprotein Lipase is only minimally affected. Probably, inhibition of hepatic lipase is an important mechanism of the decrease in lipoprotein clearance mediated by apo C-I (*96*).

Toll-like receptors (TLR) are expressed in atherosclerotic plaques and are associated with inflammatory activation of endothelial cells and macrophages. Lipopolysaccharide interacts with ligand-binding protein and CD14, a LPS receptor, to present LPS to TLR4 which then activates the expression of inflammatory genes (*97*). Thus, lipopolysaccharides released during acute infection might link bacterial infection, immune response and inflammation through TLR activation in plaque cells, endothelial cells and macrophages. Some of these potential TLR-activating bacterial factors may be of oral origin. The potential of periodontal and some other oral pathogens to activate TLRs is suggested by findings from cell culture experiments and animal models. It is known that P. gingivalis, one of the major pathogens involved in periodontitis (see Section I), is capable of stimulating LDL oxidation (*37*). Miller and colleagues clearly demonstrated that an early form of ox-LDL, minimally modified LDL (mmLDL), activates TLR4-dependent and -independent signaling pathways in J774 and primary peritoneal macrophages, resulting in secretion of proinflammatory cytokines (*98*). Moreover, in a femoral artery cuff model in atherosclerotic apo E3 (Leiden) transgenic mice, TLR4 activation by lipopolysaccharides stimulated plaque formation and subsequent outward arterial remodeling. Carotid artery ligation in wild-type mice resulted in outward remodeling in the contralateral artery, which was associated with an increase in TLR4 expression and heat-shock protein mRNA levels. In contrast, outward remodeling was not observed in TLR-deficient mice (*99*). Thus, elevation in TLR4 expression leads to outward remodeling, which was found to be associated with aneurysm formation (*100*). Future research using animal models will provide more insights into TLR implication in oral-pathogen–mediated atherosclerotic processes.

Lipoprotein lipase is a key enzyme in the hydrolysis of triglyceride-rich lipoproteins. It was shown that LDL-receptor knockout mice overexpressing lipoprotein lipase were resistant to diet-induced atherosclerosis due to the suppression of remnant lipoproteins (*101*). Levels of cholesterol, apo A-I, and apo A-IV were increased in HDL in apo E–knockout mice overexpressing the human lipoprotein lipase and had lower triglyceride content than controls (apo E knockout mice). Therefore, the apo E-knockout mice overexpressing lipoprotein lipase developed 2-fold smaller fatty streak lesions in the aortic sinus. In conclusion, overproduction of lipoprotein lipase is protective against atherosclerosis even in the absence of apo E (*102*).

Macrophage-specific overexpression of cholesteryl ester hydrolysis in hormone-sensitive lipase (HSL) transgenic female mice paradoxically increases cholesterol esterification and cholesteryl ester accumulation in macrophages, and thus increases susceptibility to diet-induced atherosclerosis compared to non-transgenic control C57BL/6 mice (*103*). It was suggested that whereas increased cholesterol uptake could contribute to transgenic foam cell formation, there are no differences in cholesterol synthesis and the expression of cholesterol efflux mediators like ABCA1, ABCG1, apo E, PPARγ, and LXRα compared to wild-type macrophages. Increased atherosclerosis in HSL transgenic mice appears to be due to coupling of the efficient re-esterification of excess free cholesterol to its limited removal mediated by the cholesterol acceptors in these mice. Thus, the overexpression of cholesterol acceptors in HSL-apo A-IV double-transgenic mice increases plasma HDL levels and decreases diet-induced atherosclerosis

compared to HSL transgenic mice with aortic lesions reduced to sizes compared to those in non-transgenic littermates (*104*).

Tangier disease (TD) is a genetic disorder caused by mutations in the ABCA1 gene. The function of ABCA1 in normal cells is to mediate phospholipids efflux to lipid-poor apo-AI which generates the HDL particle (*105*). Cholesterol efflux to the nascent HDL particle *via* ABCA1 will depend upon the cholesterol status of the cell. Individuals with TD are unable to eliminate cholesterol from cells, leading to its build-up in the tonsils and other organs (*106*). To investigate the role of ABCA1 in atherogenesis, diet-induced atherosclerosis in transgenic mice overexpressing ABCA1 was analyzed. Beneficial changes in the lipid profile led to significantly lower aortic atherosclerosis in those mice, giving evidence of an anti-atherogenetic role for the ABCA1 transporter. Thus, overexpression of ABCA1 leading to increased HDL levels is clearly atheroprotective (*107*).

A subset of patients with high plasma HDL concentrations shows enhanced rather than reduced atherosclerosis. Analysis of transgenic mice overexpressing human lecithin cholesteryl acyl-transferase (LCAT) showed both elevated HDL and increased diet-induced atherosclerosis (*108*). Another study by Mehlum and co-workers found that LCAT activity was 35-fold higher in serum of the homozygous transgenic mice than in murine controls and decreased 11–20% in the transgenic mice when fed with atherogenic diet. They found that the development of atherosclerosis was similar in transgenic and control mice. Thus, the higher LCAT activity had no significant influence on the development of diet-induced atherosclerosis (*109*). However, it is not clear so far why there are these differences in the two studies discussed.

Plasma phospholipids transfer protein (PLTP) is thought to be involved in the remodelling of HDL, which are atheroprotective. It is also involved in the metabolism of VLDL. From experiments with PLTP overexpressing mice it is concluded that PLTP increases susceptibility to atherosclerosis by lowering HDL concentrations (*110*). However, mice do not express cholesteryl ester transfer protein (CETP), which is involved in the same metabolic pathways as PLTP. Therefore, Lie and co-workers (*111*) studied atherosclerosis in heterozygous LDL-receptor–deficient mice expressing both human CETP and human PLTP. Expression of PLTP in this animal model resulted in increased atherosclerosis in spite of reduced apo B–containing lipoproteins, by reduction of HDL and HDL-associated antioxidant enzyme activities.

As already mentioned, studies that have investigated the effect of antioxidants on atherosclerosis showed inconsistent results, *e.g.* atherosclerosis was either retarded or not changed by dietary antioxidants. Overexpression of Cu/Zn-superoxide dismutase (SOD; removing superoxide radicals) and /or catalase (removing hydrogen peroxide) in apo E–lacking mice showed that endogenously produced hydrogen peroxide but not superoxide radicals contribute to the formation of oxidized lipids and the development of atherosclerosis in apo E knockout mice (*112*).

The initiation step of atherosclerosis is accompanied by the accumulation of modified lipoproteins in the vessel wall. Group IIa secretory phospholipase A2 (sPLA2 IIa) may be a key player in the enzymatic modification of LDL. After 10 weeks of high-fat diet, mice overexpressing sPLA2 AIIa in macrophages showed 2.3-fold larger lesions compared with control mice. However, smooth muscle cells or fibroblasts in the lesions were not affected. Nevertheless, data evaluated so far clearly indicate that macrophage sPLA2 IIa is a proatherogenic factor and suggest that the enzyme regulates collagen production in the plaque and as a consequence fibrotic cap development (*113*).

As mentioned in Section I, efforts to elucidate the role of Lp(a) in atherogenesis had been hampered by the lack of an animal model with high plasma Lp(a) levels. Recently, Schneider and co-workers were able to produce two lines of transgenic mice expressing apo A in the liver and crossed them with mice expressing human apo B-100, generating two lines of Lp(a) mice. They found an increase in oxidized lipids specific to Lp(a) in high-level apo A–expressing mice, suggesting a mechanism by which increased circulating levels of Lp(a) could contribute to atherosclerosis (*114*).

Ox-LDL has been implicated in the pathogenesis of atherosclerosis. Increased expression of lectin-like oxidized LDL receptor-1 (LOX-1), the receptor for Ox-LDL in endothelial cells has been found in atherosclerotic plaques. Analysis of mice overexpressing LOX-1 in C57BL/6 and apo E knockout backgrounds demonstrated that LOX-1 overexpression promotes inflammatory intramyocardial vasculopathy in a hyperlipidemic mouse model, and this effect is probably mediated through the endothelial dysfunction induced by overexpression of LOX-1 (*115*).

As a conclusion, there are many proteins which seem to have huge influences in the development or protection of atherosclerosis. Nevertheless, there are conflicting results remaining from transgenic mice and other animal models.

SNPs in genes expressed in transgenic mice. Most forms of atherosclerosis are the product of many genes of different pathways with small effects which are modified by the environment or the effects of other genes, rather than of a single highly penetrant gene. For an overview of candidate genes for genetic susceptibility see Lusis *et al.* (*116*). Several approaches using those candidate genes have been performed in order to identify genetic differences among individuals and common genetic variations, so-called "single-nucleotide polymorphisms" (SNPs), which are thought to underlie the different susceptibility to atherosclerosis.

The human genome contains approximately ten million SNPs with an estimated two common missense variants per gene (*117*). SNPs that might be relevant for studies of human diseases are non-synonymous SNPs leading to an amino acid exchange after translation, or lie within putative regulatory regions of genes affecting the expression, tissue specificity or function of relevant proteins, or are localized in splice-sites, leading to a gain or loss of active splice-variants of proteins in the cell. In atherosclerosis, SNP's in genes involved in lipid and lipoprotein metabolism, inflammation, oxidative stress, lipid oxidation, endothelial cell function, and those involved in maintaining the integrity of extracellular matrix are primary candidates for susceptibility.

Ath1 is a quantitative trait locus on mouse chromosome 1 that renders C57BL/6 mice susceptible and C3H/He mice resistant to diet-induced atherosclerosis. Tnfsf4 is one gene encompassed by this region, which encodes OX40 ligand, the ligand for OX40 on activated CD4+ cells. Ligand of

OX40 seems to be involved in allergic processes and play a critical role in autoimmunity, perhaps leading to systemic anti-tumor immunity (*118*, *119*). It was found that mice with targeted mutations in Tnfsf4 had significantly smaller atherosclerotic lesions than did control mice. In addition, mice overexpressing Tnfsf4 had significantly larger atherosclerotic lesions. That is interesting, because in two independent human populations the less common allele of SNP rs3850641, which is localized in an intron region of TNFSF4, was significantly more frequent in individuals with myocardial infarction than in controls (*120*).

There are many other studies showing SNPs having either a protective or promoting effect on atherosclerosis in human beings (*121*, *122*). However, this is not the topic of this review. For further information, readers should read the references mentioned.

A summary of proteins leading to an increase of atherosclerotic processes if overexpressed in transgenic mice is given in Fig. 6.

V. Adenoviral expression of LAL

Recombinant adenoviruses are commonly used vectors for experimental gene transfer. Uptake of adenovirus into cells takes place by high-affinity binding receptor-mediated endocytosis. Adenoviruses can infect a variety of both dividing and non-dividing cells and can also be grown to high titer [10^{10-12} plaque-forming units (pfu)/ml], making these one of the best tools for gene transfer. Disadvantages include only a transient expression of the transducted gene and immune reactions of the host. However, second- and third-generation vectors have been constructed by deleting virus genes responsible for the immune response (*123*, *124*). Strategies for increasing the effectiveness of adenoviral gene transfer have been studied widely. For example, such a strategy has been made in the development of tissue-specific adenoviral vectors for arterial gene transfer (*125*). Further progress has been made also in improving adenovirus endocytosis efficiency, influencing factors that affect delivery of transgenes into the cell, and ensuring the expression of the transgene (*126*).

To produce the adenoviral vector overexpressing LAL, the cDNA of human LAL was cloned into the transfer vector pAcCMVpLpA (*127*). The vector was co-transfected with adenovirus type 5-plasmid pJM17 (*128*) using the helper cell line 293 (human embryonic kidney cells) (*129*). Adenoviruses were plaque-purified using the same cell line and titers determined were between 5×10^{10} and 1×10^{12} plaque forming units (pfu/ml).

Cultivated skin fibroblasts (MRC5-cells, ATCC No. CCL 171) were transfected with an adenovirus in different solutions expressing human LAL (AdCMVhLAL). High expression of LAL was detectable up to 12 days after transfection. In addition, a significant mobilization of accumulated cholesteryl esters was detected in transduced fibroblasts of Wolman patients. These data clearly demonstrate that the deficient activity of LAL enzyme is substitutable in cultivated fibroblasts (*130*).

In studies performed by Du and co-workers, recombinant human LAL was given intravenously or intraparitoneally in an effort to decrease atherosclerotic lesions in mouse models (*131*). In the LAL-deficient mice, recombinant LAL showed direct effects on mannose receptor positive cells, but had relatively minor, if any, effects on lysosomal storage within hepatocytes (*132*). Based on this study, the LDL-receptor deficient mouse on a high-fat/high-cholesterol diet was used to test the effects of LAL administration. Interestingly, a 50% reduction in lesion size was found in the treated mice compared to untreated mice. To evaluate targeting of LAL to the lesions, a LAL:Apo E double knockout mice was used. Compared to apo E knockout mice, the atherosclerosis development is accelerated in the double knockout mice. By immunohistochemical staining, LAL was found to be localized to the macrophages of the lesions and to sub-endothelial regions in advanced lesions. When considering the role of a particular protein in atherosclerosis, studies using both transgenic and knockout animals should be used.

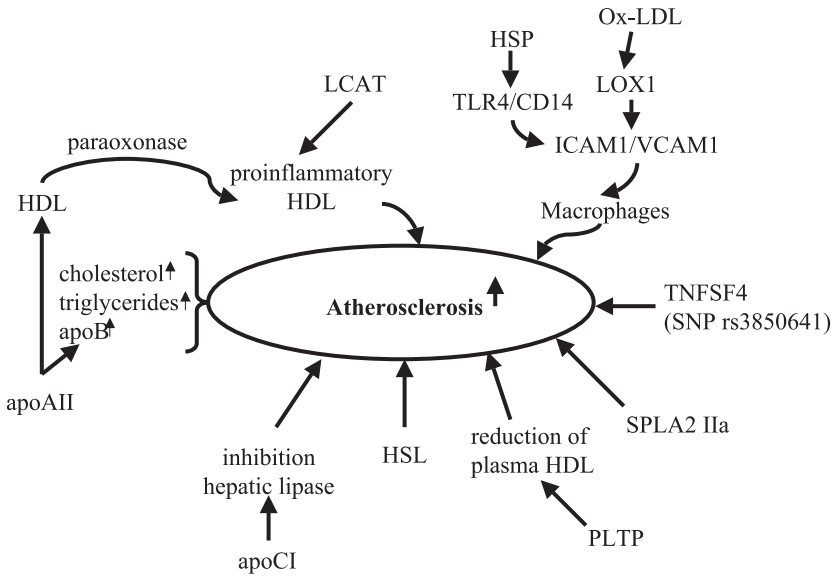

Fig. 6. **Proteins causing atherosclerosis if overexpressed stably in transgenic mice.** Abbreviations used are: LCAT: lecithin cholesterol acyl-transferase; HSP: heat shock protein; LDL: low-density lipoprotein; LOX1: oxidized LDL receptor 1; ICAM1: intracellular adhesion molecule 1; VCAM1: vascular cell adhesion molecule 1; TLR4/CD14: complex of Toll-like receptor/CD14; TNFSF4: encodes OX40 ligand; SPLA2 IIa: group IIa secretory phospholipase A2; PLTP: plasma phospholipids transfer protein; HSL: hormone sensitive lipase.

VI. Adenoviral expression of other proteins involved in atherosclerosis

For CHD, hypercholesterolemia and dyslipoproteinemia are the major risk factors. A therapeutical strategy consists in increasing the serum HDL cholesterol concentration in order to improve the 'reverse cholesterol transport'. Studies in transgenic mice (see Section III and IV) and rabbits for human apo A-I showed that overexpression of these proteins increases serum HDL cholesterol concentration and reduces diet-induced atherogenesis. Adenovirus-mediated transfer of human apo A-I gene in mice also increases circulating apo A-I. Apo A-I is one potential target for gene therapy of patients with atherosclerosis associated with a low HDL cholesterol level.

The experimental proof that overexpression of apo A-I in mice really inhibits atherosclerosis was brought by Zhang and co-workers (*133*). In this study, mice were injected with apo A-I adenovirus (10^{11} particles per animal) 3 days before ^{3}H-labeled J774-macrophages loaded with cholesterol by incubation with acetylated LDL were injected. Apo A-I overexpression led to significantly higher ^{3}H-cholesterol in plama, liver, and feces. The authors concluded that injection of ^{3}H-cholesterol–labeled macrophage foam cells is a method of measuring reverse cholesterol transport specifically from macrophages to feces *in vivo*, and apo A-I promotes macrophage-specific reverse cholesterol transport.

The scavenger receptor class B, type I (SR-BI) is an HDL receptor that mediates selective cholesterol uptake from HDL to cells (*134*). In rodents, SR-BI has a critical influence on plasma HDL-cholesterol concentration and structure, the delivery of cholesterol to steroidogenic tissues, female fertility, and biliary cholesterol concentration. SR-BI can also serve as a receptor for non-HDL lipoproteins and appears to play an important role in reverse cholesterol transport. Different studies using adenovirus-mediated expression of SR-BI indicate a protective effect of SR-BI against atherosclerosis. Thus, it may also become an attractive target for therapeutic intervention (*135*).

Endothelial cells produce superoxide anions (O_2^-), which are removed in the cells by copper/zinc superoxide dismutase (Cu/ZnSOD). Adenoviral infection with an adenoviral vector containing cDNA for human Cu/ZnSOD increased content and activity of the enzyme in bovine aortic endothelial cells, leading to reduced cellular superoxide radical release. When cells infected with the adenovirus were incubated with LDL, formation of malondialdehyde and oxidation of LDL were decreased (*136*). It seems that SOD overexpression leads to protection against atherosclerosis and may also be a good gene therapy target. In another study, intravenous administration of an adenovirus overexpressing extracellular SOD in LDL receptor knockout mice induced a 3.5- to 7-fold increase in plasma total SOD-activity. Results showed a tendency for a reduction in the overall lesion area after SOD gene transfer as compared with LacZ transduced control mice. However, the difference did not reach statistical significance. It was concluded that short-term overexpression of extracellular SOD *in vivo* does not affect atherogenesis in LDL-receptor knockout mice (*137*). In contrast to this study, Fennell and co-workers found that adenovirus-mediated overexpression of extracellular SOD, but not MnSOD *in vivo* resulted in improved endothelial function in a rat model of hypertension (*138*). In conclusion, further studies are necessary to prove whether SOD is really a protective protein against atherosclerosis and if yes as a co-factor or by localization.

Catalase, another anti-oxidant, attenuates ROS production and cell apoptosis when overexpressed in human arterial endothelial cells using an adenoviral vector. The protective effect is mediated through the down-regulation of JNK and the up-regulation of ERK1/2 phosphorylation as well as AP-1inactivation (*139*).

Apo E is a multifunctional protein synthesized by the liver and by tissue macrophages. Plasma apo E (derived primarily from the liver) regulates plasma lipoprotein metabolism, but macrophage-derived apoE was shown to slow the progression of atherosclerosis independent of plasma lipid levels. The hypothesis was tested in a study, whether hepatic expression of human apo E could inhibit atherogenesis even in a model in which apo E expression had little effect on plasma lipoproteins. Using a second generation recombinant adenovirus, hepatic expression of apo E in LDL-Receptor knockout mice fed with Western type diet was associated with significantly reduced atherosclerosis without reducing plasma cholesterol levels. This finding indicated that liver-derived plasma apo E can influence early atherogenesis through mechanisms other than modulation of lipoprotein metabolism and that liver-directed gene transfer and overexpression of apo E may also be a therapeutic approach to atherosclerosis.

It was already described that cholesteryl ester-loaded foam cells are a hallmark of atherosclerosis. Stimulation of the hydrolysis of cytosolic cholesteryl esters may be used as a therapeutic modality of atherosclerosis. For this purpose, hormone-sensitive Lipase (HSL) was overexpressed by adenovirus-mediated gene delivery in THP-1 macrophage-like cells. Overexpression of HSL increased neutral cholesterylester hydrolase (NCEH) activity and eliminated cholesteryl esters completely in the cells which were preloaded with cholesteryl esters by incubation with acetylated low density lipoprotein (*140*). Data show that cholesterol efflux was stimulated in the absence or presence of HDL which might be at least partially explained by the expression of ABCA1. Importantly, these effects were achieved without the addition of ACAT inhibitor, cAMP, or even HDL. Moreover, it is important to note that elimination of cholesteryl esters did occur not only by increasing efflux but also by decreasing influx of cholesterol.

Mice with combined leptin and LDL receptor deficiency are obese and show severe dyslipidemia and insulin resistance. Double mutant mice have higher levels of HDL and non-HDL cholesterol, and triglycerides. They also show higher oxidative stress, determined by higher titers of autoantibodies against malondialdehyde-modified LDL. Higher macrophage homing and accumulation of oxidized apolipoprotein B-100–containing lipoproteins are associated with larger plaque volumes in the aortic root. In addition, the activities of the HDL-associated antioxidant enzymes paraoxonase 1 and LCAT are lower in double-mutant mice. Adenovirus-mediated LCAT gene transfer in this mouse strain increased plasma LCAT activity and reduced the titer of the antibodies and plaque volume in the aortic root (*141*). It was concluded from this study that transient LCAT overexpression is associated with a

reduction of oxidative stress and atherosclerosis which is in contradiction to the results found by overexpression experiments in LCAT transgenic mice (see Section IV), making it necessary to further evaluate the data concerning overexpression of LCAT and its influence on atherosclerosis.

Atherosclerosis results not only from accumulation of macrophages but also of extracellular matrix in the arterial wall. Decorin, a small matrix proteoglycan, regulating cell proliferation, migration and growth factors' activity, was overexpressed in apo E–deficient mice. Systemic overexpression of decorin using an adenoviral vector reduced inflammation, triglycerides and fibrosis in atherosclerotic plaques, the latter by forming complexes with transforming growth factor-β (TGFβ-1) (*142*).

Matrix metalloproteinases (MMPs) are often in suspicion of having an effect on atherosclerosis (*143*). These proteins cause matrix degradation and may be involved in the rupture of atherosclerotic plaques by degrading fibrous caps, resulting in the intravascular thrombus formation. For example, overexpression of MMP-9 by injection of adenovirus in porcine coronary arteries led to intravascular thrombus formation at the MMP-transfected site but not at the LacZ-transfected site. Co-transfection of tissue inhibitor of MMP (TIMP-1) with MMP-9 prevented the thrombus formation. Western Blot analysis revealed the reduced expression of intact tissue factor pathway inhibitor-1 and the increased tissue factor (TF) expression at the MMP-9–transfected cells (*144*). Thus, overexpression of MMP-9 promotes intravascular thrombus formation due in part to the activation of TF-mediated coagulation cascade. In addition, Rouis and co-workers investigated the impact of adenovirus-mediated elevation in the circulating levels of human TIMP-1 in atherosclerosis-susceptible apo E–knockout mice. They found that overexpression of TIMP-1 in fact reduces atherosclerotic lesions in the mouse model (*145*).

Atherosclerosis susceptibility is decreased in mice with PLTP deficiency. To study the effect of PLTP on the development of atherosclerosis, PLTP was overexpressed in apo E–knockout mice using an adenovirus-associated (AAV)-mediated system. It was found that increased PLTP activity results in (a) a decrease in HDL cholesterol, HDL phospholipids, and apo A-I levels, (b) a decrease in vitamin E contents, (c) an increase in lipoprotein oxidizability, (d) an increase in autoantibodies against oxidized apo B–containing particles, and (e) an increase in atherosclerosis lesions in proximal aorta (*146*). These results confirm data obtained by studying transgenic mice overexpressing PLTP (see Section IV).

The presence of the tumor-suppressor gene p53 in advanced atherosclerotic plaques and the sensitivity to p53-induced cell death of smooth muscle cells isolated from these plaques gave rise to speculation about the role of p53 in atherogenesis (see also Section I). To give insights, atherosclerotic plaques in apo E knockout mice were incubated transluminally with recombinant adenovirus carrying either a p53 or LacZ transgene as a control. P53 transfection led to an increase in cap cell apoptosis 1 day after transfer. Overexpression of p53 resulted in a marked decrease in the cellular and extracellular content of the cap which is a characteristic feature of plaque vulnerability to rupture (*147*). It is well established that plaques with a high-lipid content are more vulnerable to rupture. However, coronary arteries may respond to plaque growth by either outward expansion of the vessel wall (positive remodeling), or vessel shrinkage (negative remodeling). There is the apparent paradox that positive remodelling may be advantageous (providing benefit in terms of avoiding luminal stenosis), but also harmful in that marked compensatory remodelling, which may make the plaque more vulnerable. In contrast, lesions with negative remodelling may be associated with higher grade stenosis, but may appear more stable. It was reported that plaques with positive remodelling have a higher lipid content and macrophage count, both markers of plaque vulnerability. This might stimulate MMP production, leading to cell matrix breakdown (*148*). Thus, in our opinion there is no doubt that plaque vulnerability is a dangerous event (Fig. 7).

The death receptor Fas and Fas ligand (FasL), like p53 playing a crucial role in apoptosis, are present in human advanced atherosclerotic plaques. In a study by Zadelaar and co-workers, carotid atherogenesis was initiated in apo E–deficient mice. The resulting plaques were incubated with recombinant adenovirus overexpressing FasL or lacZ as a control. Interestingly, three days after transfer, FasL expression led to a 38% reduction in the number of cap cells. However, two weeks after transfer, non-thrombic rupture, intra-plaque haemorrhage, buried caps and iron

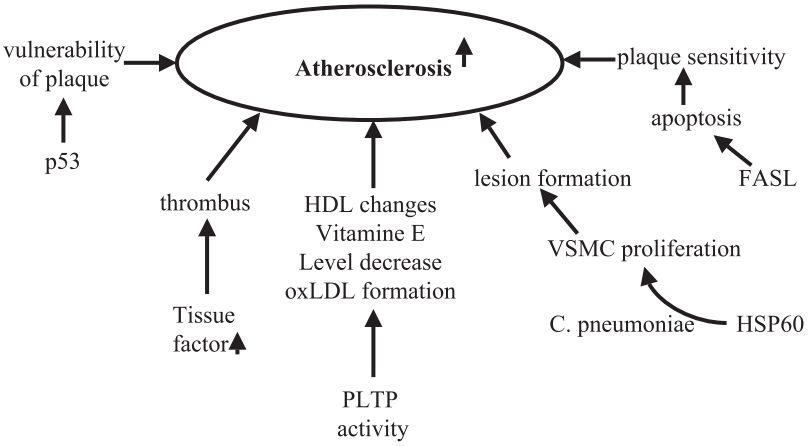

Fig. 7. **Proteins causing atherosclerosis if overexpressed transiently in mice by adenoviral gene transfer.** Abbreviations used are: PLTP: plasma phospholipids transfer protein; VSMC: vascular smooth muscle cells; FASL: Fas ligand; HSP: heat shock protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

deposits were observed in 6 out of 17 Ad-FasL–treated carotid arteries *versus* 0 out of 17 controls, indicating enhanced plaque vulnerability (*149*).

A summary of proteins leading to an increase of atherosclerotic processes if overexpressed after adenoviral transfer in mice is given in Fig. 7.

VII. Conclusion and future perspectives

Diet, hormone status, and in addition many other factors have influence on the activity and expression of the LAL. Changes of the activity and expression rate of this enzyme can change the expression of many other enzymes and the meaning of other pathways of the lipid metabolism. The influences and reciprocal effects on the LAL known so far are summarized in Fig. 2.

Without going into details, it should be mentioned that NPC (see Fig. 2) belongs to the gene products of NPC1 or NPC2, respectively. Mutations in either the NPC1 or NPC2 gene cause Nieman-Pick disease type C which is inherited in an autosomal recessive kind of way and is characterized by accumulation of lipids in the spleen, liver, lungs, bone marrow, and brain. The NPC1 gene produces a protein that is located in membranes of the cell and is involved in the movement of cholesterol and lipids within cells. The NPC2 gene produces a protein that binds and transports cholesterol, although its exact function is not fully understood. Extremely low levels or deficiency of these proteins lead to abnormal accumulation of lipids and cholesterol within the cells which is similar to the effect of non-functioning LAL. Additional information about Nieman-Pick disease is summarized by Ory (*150*).

Despite the central role of the LAL, a stable overexpression does not have huge effects on the lipid metabolism at least in the mouse model. Further crossing experiments of the LAL transgenic mouse-strain with apo E–knockout mouse and other atherosclerosis-sensitive mouse strains need to be performed. Hereby it should be examined whether there are differences in development and expiration of atherosclerosis in LAL overexpressing mice. Although it was recently shown in the mouse model that by administration of LAL atherosclerotic plaques were reduced quantitatively and qualitatively (*131*), the results won so far lead to the assumption that despite the activity and expression of the LAL still many other factors on the molecular level for the development of atherosclerosis play an important role. As shown in this review, many proteins especially if overexpressed and other factors seem to have huge influences on the development of atherosclerosis. Thus, the molecular causes of atherosclerosis should not be examined by investigations of only one enzyme after diagnosis, but rather by means of many proteins and perhaps SNP's in certain genes in combination mentioned in this review to determine a spectrum as broad as possible of potential targets for therapy.

The authors want to thank PD Dr. Martin Merkel and Dr. Stefanie Mayet for producing the transgenic LAL mouse strain and for their help in carrying out the experiments. Thank you to Mrs. Julia Rethmeier for the experiments with the adenovirus. Oliver Zschenker thanks especially his wife, Yvonne, who made all this possible.

REFERENCES

1. Eckel, R.H. and Krauss, R.M. (1998) American Heart Association call to action: obesity as a major risk factor for coronary heart disease: AHA Nutrition Committee. *Circulation* **97**, 2099–2100
2. Grundy, S.M. (2002) Obesity, metabolic syndrome, and coronary atherosclerosis. *Circulation* **105**, 2696–2698
3. Fridman, J.M. and Halaas, J.L. (1998) Leptin and the regulation of body-weight in normals. *Nature* **395**, 763–770
4. Cooke, J.P. and Oka, R.K. (2002) Does leptin cause vascular disease? *Circulation* **106**, 1904–1905
5. Dichtl, W., Nilsson, L., Goncalves, I., Ares, M.P., Banfi, C., Calara, F., Hamsten, A., Eriksson, P., and Nilsson, J. (1999) Very low-density lipoprotein activated nuclear factor-kB in endothelial cells. *Circ. Res.* **84**, 1085–1094
6. Blake, G.J. and Ridker, P.M. (2000) Are statins inflammatory? *Curr. Control Trials Cardiovasc. Med.* **1**, 161–165
7. Corti, R., Farkouh, M.E., and Badimon, J.J. (2002) The vulnerable plaque and acute coronary syndromes. *Am. J. Med.* **113**, 668–680
8. Dichtl, W., Dulak, J., Frick, M., Alber, H.F., Schwarzacher, S.P., Ares, M.P., Nilsson, J., Pachinger, O., and Weidinger, F. (2003) HMG-CoA reductase inhibitors regulate inflammatory transcription factors in human endothelial and vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **23**, 58–63
9. Liao, J.K. (2005) Clinical implications for statin pleiotropy. *Curr. Opin. Lipidol.* **16**, 624–629
10. Steenland, K. (1992) Passive smoking and the risk of heart disease. *JAMA* **267**, 94–99
11. Ahijevych, K. and Wewers, M.E. (2003) Passive smoking and vascular disease. *J. Cardiovasc. Nurs.* **18**, 69–74
12. Han, Y., Runge, M.S., and Brasier, A.R. (1999) Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. *Circ. Res.* **84**, 695–703
13. Dagenais, N.J. and Jamali, F. (2005) Protective effects of angiotensin II interruption: evidence for antiinflammatory actions. *Pharmacotherapy* **25**, 1213–1229
14. Deedwania, P.C. (2003) Diabetes and vascular disease: common links in the emerging epidemic of coronary artery disease. *Am. J. Cardiol.* **91**, 68–71
15. Eckel, R.H., Wassef, M., Chait, A., Sobel, B., Barrett, E., King, G., Lopes-Virella, M., Reusch, J., Ruderman, N., Steiner, G., and Vlassara, H. (2002) Prevention Conference VI: Diabetes and Cardiovascular Disease: Writing Group II: pathogenesis of atherosclerosis in diabetes. *Circulation* **105**, 138–143
16. Ford, E.S. (1999) Body mass index, diabetes, and C-reactive protein among U.S. adults. *Diabetes Care.* **22**, 1971–1977
17. Guerci, B., Bohme, P., Kearney-Schwartz, A., Zannad, F., and Drouin, P. (2001) Endothelial dysfunction and type 2 diabetes. Part 2: altered endothelial function and the effects of treatments in type 2 diabetes mellitus. *Diabetes Metab.* **27**, 436–447
18. Lusis, A.J. (2000) Atherosclerosis. *Nature* **407**, 233–241
19. Ismail, A., Khosravi, H., and Olson, H. (1999) The role of infection in atherosclerosis and coronary artery disease: a new therapeutic target. *Heart Dis.* **1**, 233–240
20. Shi, Y. and Tokunaga, O. (2002) Chlamydia pneumonia and multiple infections in the aorta contribute to atherosclerosis. *Pathol. Int.* **52**, 755–763
21. van der Veen, A.J., Hommels, M.J., Kroon, A.A., Kessels, A., Flobbe, K., van Engelshoven, J., Bruggeman, C.A. and de Leeuw, P.W. (2002) *Chlamydia pneumoniae* seropositivity and systemic and renovascular atherosclerotic disease. *Arch. Intern. Med.* **162**, 786–790
22. Burnett, M.S., Gaydos, C.A., Madico, G.E., Glad, S.M., Paigen, B., Quinn, T.C., and Epstein, S.E. (2001)

Atherosclerosis in apoE knockout mice infected with multiple pathogens. *J. Infect. Dis.* **183**, 226–231
23. Wolf, S.C., Brehm, B.R., Mayer, O., Jurgens, S., Schultze, G., and Risler, T. (2004) Infectious risk factors for atherosclerotic vascular disease in hemodialysis patients—*Chlamydia pneumoniae* but not *Helicobacter pylori* or cytomegalovirus is associated with increased C-reactive protein. *Ren. Fail.* **26**, 279–287
24. Witherell, H.L., Smith, K.L., Friedman, G.D., Ley, C., Thom, D.H., Orentreich, N., Vogelman, J.H., and Parsonnett, J. (2003) C-reactive protein, *Helicobacter pylori*, *Chlamydia pneumoniae*, cytomegalovirus and risk for myocardial infarction. *Ann. Epidemiol.* **13**, 170–177
25. Fang, J.C., Kinlay, S., Kundsin, R., and Ganz, P. (1998) *Chlamydia pneumoniae* infection is frequent but not associated with coronary arteriosclerosis in cardiac transplant recipients. *Am. J. Cardiol.* **82**, 1479–1483
26. Chiu, B., Viira, E., Tucker, W., and Fong, I.W. (1997) *Chlamydia pneumoniae*, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. *Circulation* **96**, 2144–2148
27. Muller, B.T., Huber, R., Henrich, B., Adams, O., Berns, G., Siebler, M., Jander, S., Muller, W., Loncar, R., Godehardt, E., and Sandmann, W. (2005) *Chlamydia pneumoniae*, herpes simplex virus and cytomegaovirus in symptomatic and asymptomatic high-grade internal carotid artery stenosis. Does infection influence plaque stability? *VASA* **34**, 163–169
28. Hirono, S., Dibrov, E., Hurtado, C., Kostenuk, A., Ducas, R., and Pierce, G.N. (2003) stimulates proliferation of vascular smooth muscle cells through induction of endogenous heat shock protein 60. *Circ. Res.* **93**, 710–716
29. Mattila, K.J., Nieminen, M.S., Valtonen, V.V., Rasi, V.P., Kesaniemi, Y.A., Syrjala, S.L., Jungell, P.S., Isoluoma, M., Hietaniemi, K., and Jokinen, M.J. (1989) Association between dental health and acute myocardial infarction. *BMJ.* **298**, 1579–1580
30. Slots, J. and Ting, M. (1999) *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: occurence and treatment. *Periodontol 2000* **20**, 82–121
31. Kinane, D.F., Mooney, J., and Ebersole, J.L. (1999) Humoral immune response to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontal disease. *Periodontol 2000* **20**, 289–340
32. Pussinen, P.J., Jousilahti, P., Alfthan, G., Palosuo, T., Asikainen, S., and Salomaa, V. (2003) Antibodies to periodontal pathogens are associated with coronary heart disease. *Arterioscler. Thromb. Vasc. Biol.* **23**, 1309–1311
33. Pussinen, P.J. and Mattila, K. (2004) Periodontal infections and atherosclerosis: mere associations? *Curr. Opin. Lipidol.* **15**, 583–588
34. Buhlin, K., Gustafsson, A., Pockley, A.G., Frostegard, J. and B., K. (2003) Risk factors for cardiovascular disease in patients with periodontitis. *Eur. Heart J.* **24**, 2099–2107
35. Katz, J., Flugelman, M.Y., Goldberg, A., and Heft, M. (2002) Association between periodontal pockets and elevated cholesterol and low density lipoprotein cholesterol levels. *J. Periodontol.* **73**, 494–500
36. Qi, M., Miyakawa, H., and Kuramitsu, H.K. (2003) *Porphyromonas gingivalis* induces murine macrophage foam cell formation. *Microb. Pathog.* **35**, 259–267
37. Schenkein, H.A., Berry, C.R., Purkall, D., Burmeister, J.A., Brooks, C.N., and Tew, J.G. (2001) Phosphorylcholine-dependent cross-reactivity between dental plaque bacteria and oxidized low-density lipoproteins. *Infect. Immun.* **69**, 6612–6617
38. Haraszthy, V.I., Zambon, J.J., Trevisan, M., Zeid, M., and Genco, R.J. (2000) Identification of periodontal pathogens in atheromatous plaques. *J. Periodontol.* **71**, 1554–1560
39. Choi, J.I., Chung, S.W., Kang, H.S., Rhim, B.Y., Kim, S.J., and Kim, S.J. (2002) Establishment of *Porphyromonas gingivalis* heat-shock-protein-specific T-cell lines from atherosclerosis patients. *J. Dent. Res.* **81**, 344–348
40. Broxmeyer, L. (2004) Heart disease: the greatest 'risk' factor of them all. *Med. Hypotheses* **62**, 773–779
41. Kunsch, C. and Medford, R.M. (1999) Oxidative stress as a regularator of gene expression in the vasculature. *Circ. Res.* **85**, 753–766
42. Irani, K. (2000) Oxidant signaling in vascular cell growth, death, and survival: a review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. *Circ. Res.* **87**, 179–183
43. Wang, D., Kreutzer, D.A., and Essigmann, J.M. (1998) Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions. *Mutat. Res.* **400**, 99–115
44. Beckman, K.B. and Ames, B.N. (1997) Oxidative decay of DNA. *J. Biol. Chem.* **272**, 19633–19636
45. Ballinger, S.W., Patterson, C., Yan, C.-N. *et al.* (2000) Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. *Circ. Res.* **86**, 960–966
46. Botto, N., Rizza, A., Colombo, M.G. *et al.* (2001) Evidence for DNA damage in patients with coronary artery disease. *Mutat. Res.* **493**, 23–30
47. Martinet, W., Knaapen, M.W.M., De Meyer, G.R.Y., Herman, A.G., and Knockx, M.M. (2001) Oxidative DNA-damage and repair in experimental atherosclerosis are reserved by dietary lipid lowering. *Circ. Res.* **88**, 733–739
48. Martinet, W., Knaapen, M.W.M., De Meyer, G.R.Y., Herman, A.G., and Knockx, M.M. (2002) Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. *Circulation* **106**, 927–932
49. Lindahl, T. and Wood, R.D. (1999) Quality control by DNA-repair. *Science* **286**, 1897–1905
50. Masson, M., Niedergang, C., Schreiber, V., Muller, S., Ménissier-de Murcia, J. and de Murcia, G. (1998) XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. *Mol. Cell. Biol.* **18**, 3563–3571
51. Pieper, A.A., Verma, A., Zhang, J., and Snyder, S.H. (1999) Poly(ADP-ribose) polymerase, nitric oxide and cell death. *Trends Pharmacol. Sci.* **20**, 171–181
52. Kubota, Y., Nash, R.A., Klungland, A., Schar, P., Barnes, D.E., and Lindahl, T. (1996) Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase β and the XRCC1 protein. *EMBO J.* **15**, 6662–6670
53. Nash, R.A., Caldecott, K.W., Barnes, D.E., and Lindahl, T. (1997) XRCC1 protein interacts with one of two distinct forms of DNA ligase III. *Biochemistry* **36**, 5207–5211
54. Campalans, A., Marsin, S., Nakabeppu, Y., O'Connor, T.R., Boiteux, S., and Radicella, J.P. (2005) XRCC1 interacts with multiple DNA glycosylases: A model for its recruitment to base excision repair. *DNA Repair* **4**, 826–835
55. Nishimura, K., Tsumagari, H., Morioka, A., Yamauchi, Y., Miyashita, K., Lu, S., Jisaka, M., Nagaya, T., and Yokota, K. (2002) Regulation of apoptosis through arachidonate cascade in mammalian cells. *Appl. Biochem. Biotechnol.* 102–103, 239–250
56. Woo, R.A., McLure, K.G., Lees-Miller, S.P. and al., e. (1998) DNA-dependent protein kinase acts upstream of p53 in response to DNA damage. *Nature* **394**, 700–704
57. van Vlijmen, B.M., Gerritsen, G., Franken, A.L. *et al.* (2001) Macrophage p53 deficiency leads to enhanced atherosclerosis in APOE*3-Leiden transgenic mice. *Circ. Res.* **88**, 780–786
58. Perry, I.J., Refsum, H., Morris, R.W., Ebrahim, S.B., Ueland, P.M., and Shaper, A.G. (1995) Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* **346**, 1395–1398

59. Craig, W.Y., Neveux, L.M., Palomaki, G.E., Cleveland, M.M., and Haddow, J.E. (1998) Lipoprotein (a) as a risk factor for ischemic heart disease: metaanalysis of prospective studies. *Clin. Chem.* **44**, 2301–2306
60. Marcovina, S.M. and Koschinsky, M.L. (2002) A critical evaluation of the role of Lp(a) in cardiovascular disease: can Lp(a) be useful in risk assessment? *Sem. Vasc. Med.* **2**, 335–344
61. Marcovina, S.M. and Koschinsky, M.L. (2003) Evaluation of lipoprotein (a) as a prothrombotic factor: progress from bench to bedside. *Curr. Opin. Lipidol.* **14**, 361–366
62. Buechler, C., Ullrich, H., Aslanidis, C., Bared, S.M., Lingenhel, A., Ritter, M., and Schmitz, G. (2003) Lipoprotein (a) downregulates lysosomal acid lipase and induces interleukin-6 in human blood monocytes. *Biochim. Biophys. Acta* **1642**, 25–31
63. Anderson, R.A. and Sando, G.N. (1991) Cloning and expression of cDNA encoding human lysosomal acid lipase/cholesteryl ester hydrolase. *J. Biol. Chem.* **266**, 22479–22484
64. Tilkorn, A.-C., Merkel, M., Greten, H., and Ameis, D. (1999) High-level baculoviral expression of lysosomal lipase. *Methods Mol. Biol.* **109**, 177–185
65. Zschenker, O., Oezden, D., and Ameis, D. (2004) Lysosomal acid lipase as a preproprotein. *J. Biochem.* **136**, 65–72
66. Ameis, D., Merkel, M., Eckerskorn, C., and Greten, H. (1994) Purification, characterization and molecular cloning of human hepatic lysosomal acid lipase. *Eur. J. Biochem.* **219**, 905–914
67. Brecher, P., Pyun, H.Y., and Chobanian, A.V. (1977) Effect of atherosclerosis on lysosomal cholesterol esterase activity in rabbit aorta. *J. Lipid Res.* **18**, 154–163
68. Haley, N.J., Fowler, S. and de Duve, C. (1980) Lysosomal acid cholesterol esterase activity in normal and lipid-laden aortic cells. *J. Lipid Res.* **21**, 961–969
69. Ries, S., Buechler, C., Langmann, T., Fehringer, P., Aslanidis, C., and Schmitz, G. (1998) Transcriptional regulation of lysosomal acid lipase in differentiating monocytes is mediated by transcription factors Sp1 and AP-2. *J. Lipid Res.* **39**, 2125–2134
70. Gabig, T.G. and Babior, B.M. (1981) The killing of pathogens by phagocytes. *Annu. Rev. Med.* **32**, 313–326
71. Canaan, S., Roussell, A., Verger, R., and Cambillau, C. (1999) Gastric lipase: crystal structure and activity. *Biochim. Biophys. Acta* **1441**, 197–204
72. Lohse, P., Lohse, P., Chahrokh-Zadeh, S., and Seidel, D. (1997) Human lysosomal acid lipase/cholesteryl ester hydrolase and human gastric lipase: site-directed mutagenesis of Cys227 and Cys 236 results in substrate-dependent reduction of enzymatic activity. *J. Lipid Res.* **38**, 1896–1905
73. Zschenker, O., Baehr, C., Hess, U.-F., and Ameis, D. (2005) Systemic mutagenesis of potential glycosylation sites of lysosomal acid lipase. *J. Biochem.* **137**, 387–394
74. Du, H., Levine, M., Ganesa, C., Witte, D.P., Cole, E.S., and Grabowski, G.A. (2005) The role of mannosylated enzyme and the mannose receptor in enzyme replacement therapy. *Am. J. Hum. Genet.* **77**, 1061–1074
75. Sugii, S., Reid, P.C., Ohgami, N., Du, H., and Chang, T.Y. (2003) Distinct endosomal compartments in early trafficking of low density lipoprotein-derived cholesterol. *J. Biol. Chem.* **278**, 27180–27189
76. Wang, X., Sato, R., Brown, M.S., Hua, X., and Goldstein, J.L. (1994) SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* **77**, 53–62
77. Aslanidis, C., Ries, S., Fehringer, P., Buechler, C., Klima, H., and Schmitz, G. (1996) Genetic and biochemical evidence that CESD and Wolman disease are distinguished by residual lysosomal acid lipase. *Genomics* **33**, 85–93
78. Pagani, F., Pariyarath, R., Garcia, R., Stuani, C., Burlina, A.B., Ruotolo, G., Rabusin, M., and Baralle, F.E. (1998) New lysosomal acid lipase gene mutations explain the phenotype of Wolman disease and cholesteryl ester storage disease. *J. Lipid Res.* **39**, 1382–1388
79. Zschenker, O., Jung, N., Rethmeier, J., Trautwein, S., Hertel, S., Zeigler, M., and Ameis, D. (2001) Characterization of lysosomal acid lipase mutations in the signal peptide and mature polypeptide region causing Wolman disease. *J. Lipid Res.* **42**, 1033–1040
80. Anderson, R.A., Bryson, G.M., and Parks, J.S. (1999) Lysosomal acid lipase mutations that determine phenotype in Wolman and cholesterol ester storage disease. *Mol. Gen. Metab.* **68**, 333–345
81. Lohse, P., Maas, S., Sewell, A.C., van Diggelen, O.P., and Seidel, D. (1999) Molecular defects underlying Wolman disease appear to be more heterogenous than those resulting in cholesterol ester storage disease. *J. Lipid Res.* **40**, 221–228
82. Muntoni, S., Wiebusch, H., Funke, H., Ros, E., Seedorf, U., and Assmann, G. (1995) Homozygosity for a splice junction mutation in exon 8 of the gene encoding lysosomal acid lipase in a Spanish kindred with cholesterol ester storage disease (CESD). *Hum. Genet.* **95**, 491–494
83. Assmann, G. and Seedorf, U. (2002) Acid lipase deficiency: Wolman's disease and cholesterol ester storage disease in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valee, L., eds.) pp. 3551–3573, McGraw Hill, New York
84. Krivit, W., Peters, C., Dusenbery, K., Ben-Yoseph, Y., Ramsay, N.K., Wagner, J.E., and Anderson, R. (2000) Wolman disease successfully treated by bone marrow transplantation. *Bone Marrow Transplant.* **26**, 567–570
85. Mayet, S., Merkel, M., Breslow, J.L., Beisiegel, U., Ameis, D., and Heeren, J. (submitted) A new transgenic mouse strain overexpressing lysosomal acid lipase. *J. Lipid Res.*
86. Kuriyama, M., Yoshida, H., Suzuki, M., Fujiyama, J., and Igata, A. (1990) Lysosomal acid lipase deficiency in rats: lipid analysis and lipase activities in liver and spleen. *J. Lipid Res.* **31**, 1605–1612
87. Du, H., Duanmu, M., Witte, D., and Grabowski, G.A. (1998) Targeted disruption of the mouse lysosomal acid lipase gene: long-term survival with massive cholesteryl ester and triglyceride storage. *Hum. Mol. Genet.* **7**, 1347–1354
88. Du, H., Heur, M., Witte, D.P., Ameis, D., and Grabowski, G.A. (2002) Lysosomal acid lipase deficiency: correction of lipid storage by adenovirus-mediated gene transfer in mice. *Hum. Gene Ther.* **13**, 1361–1372
89. Ge, R. (1999) Genetically manipulated animals and their use in experimental research. *Ann. Acad. Med. Singapore* **28**, 560–564
90. Craigen, W. (2001) Mouse models of human genetic disorders in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valee, L., eds.) pp. 379–416, McGraw Hill, New York
91. Warden, C.H., Hedrick, C.C., Qiao, J.H., Castellani, L.W., and Lusis, A.J. (1993) Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II. *Science* **261**, 469–472
92. Plump, A.S., Scott, C.J., and Breslow, J.L. (1994) Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse. *Proc. Natl. Acad. Sci. USA* **91**, 9607–9611
93. Castellani, L.W., Navab, M., Van Lenten, B.J., Hedrick, C.C., Hama, S.Y., Goto, A.M., Fogelman, A.M., and Lusis, A.J. (1997) Overexpression of apolioprotein AII in transgenic mice converts high density lipoproteins to proinflammatory particles. *J. Clin. Invest.* **100**, 464–474
94. Ng, C.J., Shih, D.M., Hama, S.Y., Villa, N., Navab, M., and Reddy, S.T. (2005) The paraoxonase gene family and atherosclerosis. *Free Radic. Biol. Med.* **38**, 153–163
95. Escola-Gil, J.C., Julve, J., Marzal-Casacuberta, A., Ordonez-Llanos, J., Gonzalez-Sastre, F., and Blanco-Vaca, F. (2000) Expression of human apolipoprotein A-II in

apolipoprotein E-deficient mice induces features of familial combined hyperlipidemia. *J. Lipid Res.* **41**, 1328–1338
96. Conde-Knape, K., Bensadoun, A., Sobel, J.H., Cohn, J.S., and Shachter, N.S. (2002) Overexpression of apoC-I in apoE-null mice: severe hypertriglyceridemia due to inhibition of hepatic lipase. *J. Lipid Res.* **43**, 2136–2145
97. Aderem, A. and Ulevitch, R.J. (2000) Toll-like receptors in the induction of the innate immune response. *Nature* **406**, 782–787
98. Miller, Y.I., Viriyakosol, S., Worrall, D.S., Boullier, A., Butler, S., and Witztum, J.L. (2005) Toll-like receptor 4-dependent and -independent cytokine secretion induced by minimally oxidized low-density lipoprotein in macrophages. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1085–1087
99. Hollestelle, S.C., De Vries, M.R., Van Keulen, J.K., Schoneveld, A.H., Vink, A., Strijder, C.F., Van Middelaar, B.J., Pasterkamp, G., Quax, P.H., and De Kleijn, D.P. (2004) Toll-like receptor 4 is involved in outward arterial remodeling. *Circulation* **109**, 393–398
100. Grange, J.J., Davis, V., and Baxter, B.T. (1997) Pathogenesis of abdominal aortic aneurysm: an update and look toward the future. *Cardiovasc. Surg.* **5**, 256–265
101. Shimada, M., Ishibashi, S., Inaba, T., Yagyu, H., Harada, K., Osuga, J., Ohashi, K., Yazaki, Y., and Yamada, N. (1996) Suppression of diet-induced atherosclerosis in low-density lipoprotein receptor knockout mice overexpressing lipoprotein lipase. *Proc. Natl. Acad. Sci. USA* **93**, 7242–7246
102. Yagyu, H., Ishibashi, S., Chen, Z., Osuga, J., Okazaki, M., Perrey, S., Kitamine, T., Shimada, M., Ohashi, K., Harada, K., Shionoiri, F., Yahagi, N., Gotoda, T., Yazaki, Y., and Yamada, N. (1999) Overexpressed lipoprotein lipase protects against atherosclerosis in apolipoprotein E knockout mice. *J. Lipid Res.* **40**, 1677–1685
103. Escary, J.L., Choy, H.A., Reue, K., Wang, X.P., Castellani, L.W., Glass, C.K., Lusis, A.J., and Schotz, M.C. (1999) Paradoxical effect on atherosclerosis of hormone-sensitive lipase overexpression in macrophages. *J. Lipid Res.* **40**, 397–404
104. Choy, H.A., Wang, X.P., and Schotz, M.C. (2003) Reduced atherosclerosis in hormone-sensitive lipase transgenic mice overexpressing cholesterol acceptors. *Biochim. Biophys. Acta* **1634**, 76–85
105. Neufeld, E.B., Stonik, J.A., Demosky, S.J.J., Knapper, C.L., Combs, C.A., Cooney, A., Comly, M., Dwyer, N., Blanchette-Mackie, J., Remaley, A.T., Santamarina-Fojo, S., and Brewer, H.B.J. (2004) The ABCA1 transporter modulates late endocytic trafficking: insights from the correction of the genetic defect in Tangier disease. *J. Biol. Chem.* **279**, 15571–15578
106. Young, S.G. and Fielding, C.J. (1999) The ABCs of cholesterol efflux. *Nat. Genet.* **22**, 316–318
107. Joyce, C.W., Amar, M.J., Lambert, G., Vaisman, B.L., Paigen, B., Najib-Fruchart, J., Hoyt, R.F.J., Neufeld, E.D., Remaley, A.T., Fredrickson, D.S., Brewer, H.B.J., and Santamarina-Fojo, S. (2002) The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apoE-knockout mice. *Proc. Natl. Acad. Sci. USA* **99**, 407–412
108. Berard, A.M., Foger, M., Remaley, A., Shamburek, R., Vaisman, B.L., Talley, G., Paigen, B., Hoyt, R.F.J., Marcovina, S.M., Brewer, H.B.J., and Santamarina-Fojo, S. (1997) High plasma HDL concentrations associated with enhanced atherosclerosis in transgenic mice overexpressing lecithin-choleseryl acyltransferase. *Nat. Med.* **3**, 744–749
109. Mehlum, A., Gjernes, F., Solberg, L.A., Hagve, T.A., and Prydz, H. (2000) Overexpression of human lecithin:cholesterol acyltransferase in mice offers no protection against diet-induced atherosclerosis. *APMIS* **108**, 336–342
110. van Haperen, R., van Tol, A., van Gent, T., Scheek, L., Visser, P., van der Kamp, A., Grosveld, F. and de Crom, R. (2002) Increased risk of atherosclerosis by elevated plasma levels of phospholipid transfer protein. *J. Biol. Chem.* **277**, 48938–48943
111. Lie, J., de Crom, R., van Gent, T., van Haperen, R., Scheek, L., Sadeghi-Niaraki, F. and van Tol, A. (2004) Elevation of plasma phospholipid transfer protein increases the risk of atherosclerosis despite lower apolipoprotein B-containing lipoproteins. *J. Lipid Res.* **45**, 805–811
112. Yang, H., Roberts, L.J., Shi, M.J., Zhou, L.C., Ballard, B.R., Richardson, A., and Guo, Z.M. (2004) Retardation of atherosclerosis by overexpression of catalase or both Cu/Zn-superoxide dismutase and catalase in mice lacking apolipoprotein E. *Circ. Res.* **95**, 1075–1081
113. Ghesquiere, S.A., Gijbels, M.J., Anthonsen, M., van Gorp, P.J., van der Made, I., Johansen, B., Hofker, M.H. and de Winther, M.P. (2004) Macrophage-specific overexpression of group IIa sPLA2 increases atherosclerosis and enhances collagen deposition. *J. Lipid Res.* **46**, 201–210
114. Schneider, M., Witztum, J.L., Young, S.G., Ludwig, E.H., Miller, E.R., Tsimikas, S., Curtiss, L.K., Marcovina, S.M., Taylor, J.M., Lawn, R.M., Innerarity, T.L., and Pitas, R.E. (2005) High-level lipoprotein [a] expression in transgenic mice: evidence for oxidized phospholipids in lipoprotein [a] but not in low density lipoproteins. *J. Lipid Res.* **46**, 769–778
115. Inoue, K., Arai, Y., Kurihara, H., Kita, T., and Sawamura, T. (2005) Overexpression of lectin-like oxidized low-density lipoprotein receptor-1 induces intramyocardial vasculopathy in apolipoprotein E-null mice. *Circ. Res.* **97**, 176–184
116. Lusis, A.J., Fogelman, A.M., and Fonarow, G.C. (2004) Genetic bases of atherosclerosis: Part I New genes and pathways. *Circulation* **110**, 1868–1873
117. Cargill, M., Altshuler, J., Ireland, J., Sklar, P., Ardlie, K., Patil, N., Shaw, N., Lane, C.R., Lim, E.P., Kalyanaraman, N., Nemesh, J., Ziaugra, L., Friedland, L., Rolfe, A., Warrington, J., Lipshutz, R., Daley, G.Q., and Lander, E.S. (1999) Characterization of single-nucleotide-polymorphisms in coding regions of human genes. *Nat. Genet.* **22**, 231–238
118. Andarini, S., Kikuchi, T., Nukiwa, M., Pradono, P., Suzuki, T., Ohkouchi, S., Inoue, A., Maemondo, M., Ishii, N., Saijo, Y., Sugamura, K., and Nukiwa, T. (2004) Adenovirus vector-mediated in vivo gene transfer of OX40 ligand to tumor cells enhances antitumor immunity of tumor-bearing hosts. *Cancer Res.* **64**, 3281–3287
119. Arestides, R.S., He, H., Westlake, R.M., Chen, A.I., Sharpe, A.H., Perkins, D.L., and Finn, P.W. (2002) Costimulatory molecule OX40L is critical for both Th1 and Th2 responses in allergic inflammation. *Eur. J. Immunol.* **32**, 2874–2880
120. Wang, X., Ria, M., Kelmenson, P.M., Eriksson, P., Higgins, D.C., Samnegard, A., Petros, C., Rollins, J., Bennet, A.M., Wiman, B., de Faire, U., Wennberg, C., Olsson, P.G., Ishii, N., Sugamura, K., Hamsten, A., Forsman-Semb, K., Lagercrantz, J., and Paigen, B. (2005) Positional identification of TNFSF4, encoding OX40 ligand, as a gene that influences atherosclerosis susceptibility. *Nat. Genet.* **37**, 365–372
121. Endler, G., Exner, M., Schillinger, M., Marculescu, R., Sunder-Plassmann, R., Raith, M., Jordanova, N., Wojta, J., Mannhalter, C., Wagner, O.F., and Huber, K. (2004) A microsattelite polymorphism in the heme oxygenase-1 gene promoter is associated with increased bilirubin and HDL levels but not with coronary artery disease. *Thromb. Haemost.* **91**, 155–161
122. Jormsjo, S., Whatling, C., Walter, D.H., Zeiher, A.M., Hamsten, A., and Eriksson, P. (2001) Allele-specific regulation of matrix metalloproteinase-7 promoter activity is associated with coronary artery luminal dimensions among hypercholesterolemic patients. *Arterioscler. Thromb. Vasc. Biol.* **21**, 1834–1839
123. Moorhead, J.W., Clayton, G.H., Smith, R.L., and Schaack, J. (1999) A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduced mouse ears. *J. Virol.* **73**, 1046–1053

124. Kochanek, S., Clemens, P.R., Mitani, K., Chen, H.H., Chau, S., and Caskey, C.T. (1996) A new adenoviral vector: replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and beta-galactosidase. *Proc. Natl. Acad. Sci. USA* **93**, 5731–5736
125. Turunen, M.P., Puhakka, H.L., Koponen, J.K., Hiltunen, M.O., Rutanen, J., Leppanen, P., Turunen, A.M., Narvanen, A., Newby, A.C., Baker, A.H., and Yla-Herttuala, S. (2002) Peptide-retargeted adenovirus encoding a tissue inhibitor of metalloproteinase-1 decreases restenosis after intravascular gene transfer. *Mol. Ther.* **6**, 306–312
126. Russell, W.C. (2000) Update on adenovirus and its vectors. *J. Gen. Virol.* **81**, 2573–2604
127. Gomez-Foix, A.M., Coats, W.S., Baque, S., Alam, T., Gerard, R.D., and Newgard, C.B. (1992) Adenovirus-mediated transfer of the muscle glycogen phosphorylase gene into hepatocytes confers altered regulation of glycogen metabolism. *J. Biol. Chem.* **267**, 25129–25134
128. McGrory, W.J., Bautista, D.S., and Graham, F.L. (1988) A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. *Virology* **163**, 614–617
129. Graham, F.L., Smiley, J., Russell, W.C., and Nairn, R. (1977) Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen Virol.* **36**, 59–74
130. Tietge, U.J., Sun, G., Czarnecki, S., Yu, Q., Lohse, P., Du, H., Grabowski, G.A., Glick, J.M., and Rader, D.J. (2001) Phenotypic correction of lipid storage and growth arrest in wolman disease fibroblasts by gene transfer of lysosomal acid liapse. *Hum. Gene Ther.* **12**, 279–289
131. Du, H., Schiavi, S., Wan, N., Levine, M., Witte, D.P., and Grabowski, G.A. (2004) Reduction of atherosclerotic plaques by lysosomal acid lipase supplementation. *Arterioscler. Thromb. Vasc. Biol.* **24**, 147–154
132. Du, H., Schiavi, S., Levine, M. and al., e. (2001) Enzyme therapy for lysosomal acid lipase deficiency in the mouse. *Hum. Mol. Genet.* **10**, 1639–1648
133. Zhang, Y., Zanotti, I., Reilly, M.P., Glick, J.M., Rothblat, G.H., and Rader, D.J. (2003) Overexpression f apolipoprotein A-I promotes reverse transport of cholesterol from macrophages to feces in vivo. *Circulation* **108**, 661–663
134. Kozarsky, K.F., Donahee, M.H., Rigotti, A., Iqbal, S.N., Edelman, E.R., and Krieger, M. (1997) Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. *Nature* **387**, 414–417
135. Krieger, M. and Kozarsky, K. (1999) Influence on the HDL receptor SR-BI on atherosclerosis. *Curr. Opin. Lipidol.* **10**, 491–497
136. Fang, X., Weintraub, N.L., Rios, C.D., Chappell, D.A., Zwacka, R.M., Engelhardt, J.F., Oberley, L.W., Yan, T., Heistad, D.D., and Spector, A.A. (1998) Overexpression of human superoxide dismutase inhibits oxidation of low-density lipoprotein by endothelial cells. *Circ. Res.* **82**, 1289–1297
137. Laukkanen, M.O., Leppanen, P., Turunen, P., Porkkala-Sarataho, E., Salonen, J.T., and Yla-Herttuala, S. (2001) Gene transfer of extracellular superoxide dismutase to atherosclerotic mice. *Antioxid. Redox. Signal.* **3**, 397–402
138. Fennell, J.P., Brosnan, M.J., Frater, A.J., Hamilton, C.A., Alexander, M.Y., Nicklin, S.A., Heistad, D.D., Baker, A.H., and Dominiczak, A.F. (2002) Adenovirus-mediated overexpression of extracellular superoxide dismutase improves endothelial dysfunction in a rat model of hypertension. *Gene Ther.* **9**, 110–117
139. Lin, S.J., Shyue, S.K., Liu, P.L., Chen, Y.H., Ku, H.H., Chen, J.W., Tam, K.B., and Chen, Y.L. (2004) Adenovirus-mediated overexpression of catalase attenuates oxLDL-induced apoptosis in human aortic endothelial cells via AP-1 and C-Jun N-terminal kinase/extracellular signal-regulated kinase mitogen-activated protein kinase pathways. *J. Mol. Cell Cardiol.* **36**, 129–139
140. Okazaki, H., Osuga, J., Tsukamoto, K., Isoo, N., Kitamine, T., Tamura, Y., Tomita, S., Sekiya, M., Yahagi, N., Iizuka, Y., Ohashi, K., Harada, K., Gotoda, T., Shimano, H., Kimura, S., Nagai, R., Yamada, N., and Ishibashi, S. (2002) Elimination of cholesterol ester from macrophage foam cells by adenovirus-mediated gene transfer of hormone-sensitive lipase. *J. Biol. Chem.* **277**, 31893–31899
141. Mertens, A., Verhamme, P., Bielicki, J.K., Phillips, M.C., Quarck, R., Verreth, W., Stengel, D., Ninio, E., Navab, M., Mackness, B., Mackness, M., and Holvoet, P. (2003) Increased low-density lipoprotein oxidation and impaired high-density lipoprotein antioxidant defense are associated with increased macrophage homing and atherosclerosis in dyslipidemic obese mice: LCAT gene transfer decreases atherosclerosis. *Circulation* **107**, 1640–1646
142. Al Haj Zen, A., Caligiuri, G., Sainz, J., Lemitre, M., Demerens, C., and Lafont, A. (2005) Decorin overexpression reduces atherosclerosis development in apolipoprotein E-deficient mice. *Atherosclerosis* Epub ahead of print
143. Xu, Q. (2002) Role of heat shock proteins in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **22**, 1547–1559
144. Morishige, K., Shimokawa, H., Matsumoto, Y., Eto, Y., Uwatoku, T., Abe, K., Sueishi, K., and Takeshita, A. (2003) Overexpression of matrix metalloproteinase-9 promotes intravascular thrombus formation in porcine coronary arteries in vivo. *Cardiovasc. Res.* **57**, 572–585
145. Rouis, M., Adamy, C., Duverger, N., Lesnik, P., Horellou, P., Moreau, M., Emmanuel, F., Caillaud, J.M., Laplaud, P.M., Dachet, C., and Chapman, M.J. (1999) Adenovirus-mediated overexpression of tissue inhibitor of metalloproteinase-1 reduces atherosclerotic lesions in apolipoprotein E-deficient mice. *Circulation* **100**, 533–540
146. Yang, X.P., Yan, D., Qiao, C., Liu, R.J., Chen, J.G., Li, J., Schneider, M., Lagrost, L., Xiao, X., and Jiang, X.C. (2003) Increased atherosclerotic lesions in apoE mice with plasma phospholipid transfer protein overexpression. *Arterioscler. Thromb. Vasc. Biol.* **23**, 1601–1607
147. von der Thusen, J.H., van Vlijmen, B.M., Hoeben, R.C., Kockx, M.M., Havekes, L.M., van Berkel, T.J., and Biessen, E.A. (2002) Induction of atherosclerotic plaque rupture in apolipoprotein E–/– mice after adenovirus-mediated transfer of p53. *Circulation* **105**, 2064–2070
148. Varnava, A.M., Mills, P.G., and Davies, M.J. (2002) Relationship between coronary artery remodeling and plaque vulnerability. *Circulation* **105**, 939–943
149. Zadelaar, A.S., Thusen, J.H., M Boesten, L.S., Hoeben, R.C., Kockx, M.M., Versnel, M.A., van Berkel, T.J., Havekes, L.M., L Biessen, E.A. and van Vlijmen, B.M. (2005) Increased vulnerability of pre-existing atherosclerosis in apo E-deficient mice following adenovirus-mediated Fas ligand gene transfer. *Atherosclerosis* Epub ahead of print
150. Ory, D.S. (2004) The nieman-pick disease genes; regulators of cellular cholesterol homeostasis. *Trends Cardiovasc. Med.* **14**, 66–72