Molecular basis of atheromatosis in children.

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ABSTRACT: Atheromatosis is a lifelong process that begins and develops silently even during fetal development and generally shows clinical manifestations in adult life. The initiation and progressive formation of atheromatic lesions represents a complex interplay of both genetic and environmental factors. Over the last decades there have been numerous studies detecting atherosclerotic lesions in the coronary arteries of children and adolescents and demonstrating the early onset of this disease. Although more research is required in order to unravel the full genetic repertoire of atherogenesis, scientists have already made real progress towards understanding the molecular basis of early atheromatosis and the aetiopathogenesis of this complex disease involves genes related to inflammation, lipid metabolism, MMPs, epigenetic alterations of DNA and gene-environment interactions. Atheromatosis in children and adolescents is really a reversible process and anatomic changes observed in early atheromatosis are modifiable. Thus, primary prevention strategies beginning in childhood have great potential and might result in prevention of adult cardiovascular disease.

Key Words: Early atheromatosis, Atheromatosis in children, Cardiovascular risk factors in children, Atherogenesis.

INTRODUCTION

Atheromatosis is a lifelong, progressive disease of the medium- and large-sized arteries, resulting in the formation of fatty and fibrous lesions in the vessel wall[1]. It's a process that begins and develops silently during the first decades of childhood and adolescence, progresses asymptomatically and generally shows no clinical manifestations until adulthood[2,3]. It is the major cause of cardiovascular diseases leading to complications such as myocardial infarction and stroke[3,4]. Atheromatosis and its complications constitute the main causes of death in western societies[5]. In the United States, cardiovascular disease is responsible for 41.4% of all deaths and constitutes the leading cause of mortality[2].

The initiation and progressive formation of atheromatic lesions represents a complex interplay of both genetic and environmental factors[1,6,7]. In other words atheromatosis and cardiovascular diseases are multifactorial, and their aetiopathogenesis is determined by genetic susceptibility and environmental factors and by gene-gene and gene-environment interactions[8]. Until today scientists have made enormous efforts to study the risk factors and signalling pathways involved in atheromatosis. All these approaches have led to the identification of numerous risk factors that lead to the development of atheromatosis, including cigarette smoking, diabetes and insulin resistance, hypertension, increased levels of circulating lipids, inadequate exercise and obesity[9]. Although several theories have been proposed through the last decades, none of them managed to explain completely the formation and expansion of atheromatic lesions[10]. In spite of this, it is now well accepted that atheromatosis is a chronic inflammatory process resulting from the interactions between plasma lipoproteins, various types of cells (macrophage, T-lymphocytes, endothelial cells and smooth muscle cells) and the extracellular matrix of the arterial wall [10], and that this process is regulated by an intricate network of various chemokines and cytokines produced by the cells involved[11,12].

In the post genomic era, the knowledge of the entire reference genome sequence and some of its frequent variants turned a new page in atherosclerosis

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research concerning the study of genetic alterations. Whole-genome measurement technologies developed both in human and animal genome projects[4], offer the opportunity to uncover entire repertoires of changes in gene expression related to complex diseases such as atheromatosis and reveal their interplay in regulatory networks. We already know that more than 400 genes are involved in this process and that heritability estimates in humans demonstrate that genetic factors explain 50% of its aetiopathogenesis[6]. Atherogenesis-related genes concern endothelium function, thrombogenesis, inflammation, lipid and carbohydrate metabolism[1]. Despite the identification of this complicate network of genes, our knowledge of the roles that these genes play in atherogenesis still remains limited.

ATHEROMATOSIS AND CHILDREN

Atheromatosis in children does not differ from atheromatosis in adults. We just refer to early stage atheromatic lesions, known as ''fatty streaks'', consisting of primarily macrophages and T lymphocytes[10,13]. In a recent review Ross stated that the fatty streak in infants and young children is a pure inflammatory lesion and that this inflammation is transient and does not lead to major endothelial dysfunction[3,14]. Thus, many scientists believe that a considerable number of fatty streaks remain unchanged or disappear during adolescence[14]. Others insist on the fact that endothelium dysfunction in children over time leads to measurable thickening of the intima and media of the vessel wall of large- and medium-sized arteries[3]. This pre-existing thickening, attracts the extracellular deposition of lipids with specific types of proteoglycans, and constitutes the earliest anatomic change of atherosclerosis which will finally result in advanced fibrous atheromatic lesions observed in adults[15].

Over the last 20 years there are numerous studies detecting atherosclerotic lesions in the coronary arteries of children and adolescents and demonstrating the early onset of this disease[2]. Until recently, our understanding of the childhood antecedents of adult atherosclerosis and cardiovascular disease was limited, having been based on autopsy studies of pathologic findings in teens and young adults who died of accidental causes. However, recent development of new technologies has made it possible to detect early anatomic and physiologic changes in children[3,16]. Observations from the Bogalusa Heart Study have shown that atheromatosis major risk factors of adults are present in childhood and adolescence[17], with documented anatomic changes as early as 5 to 8 years of age[3,18].

Non-invasive functional and structural techniques[19] are now available and provide the opportunity to characterize early arterial disease long before cardiovascular complications present. By using these techniques, it has been possible to quantify the impact of conventional and novel cardiovascular risk factors seen in childhood on the development of preclinical atherosclerotic changes[18], but also to reveal the gene-gene and gene-environment complex interactions that regulate the expression of these genes.

RESEARCH METHOD

We performed a computer-based search using the Pub Med database with the following search terms: genes + early atheromatosis, risk factors + early atheromatosis, fatty streaks, cardiovascular disease + children, inflammation + atheromatosis + children, lipid metabolism + atheromatosis + children. References from the retrieved articles were also screened for additional papers. We reviewed all association studies and publications in English until September 2008.

INFLAMMATION AND ATHEROMATOSIS IN CHILDREN

Atheromatosis is predominantly an inflammatory condition produced by a "response to injury"[20], and atheromatic lesions, from fatty streak to advanced fibrous lesions and the final plaque rupture, possess all features of chronic inflammation[7,10,20,21,22]. In general, an inflammatory response is a normal physiological reaction to daily encountered micro-organisms, tissue damage, and modified self-proteins in an attempt of the body to restore and maintain homeostasis[21]. The immunopathogenesis of atherosclerosis involves a complex interaction between various leukocyte subsets, platelets, endothelial cells, and vascular smooth muscle cells[23]. It is now well accepted that a complex endothelial injury and dysfunction induced by a variety of factors including homocysteine, toxins such as smoking, shear stress, infectious agents, hypertension, hyperglycaemia and oxidized low-density lipoprotein (oxLDL), results in an inflammatory response that is crucial in the initiation and further progression

of atheromatic lesions[7,10,24]. This response to injury, with the following lipid migration and expression of surface adhesion molecules[7], results in a cascade of immunological mediated events characterized by the production of a wide range of chemokines (Ccl2, Ccl9, Ccl11, Ccl19, Ccl21, Cxcl1 και Cxcl2), cytokines (IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, INF-γ and TNF), and growth factors (VEGF)[11,12,21,25]. The role of inflammation in atherogenesis is confirmed by immunohistochemical studies, both in fatty streaks of mice and human, where several cytokines and inflammatory cells (monocyte-derived macrophages and T-lymphocytes) have been found[10,13,20].

Recent research has demonstrated even stronger evidence of the relationship between inflammation and endothelial dysfunction, and numerous systemic markers of inflammation, such as C-reactive protein (CRP) and homocysteine have been associated with the initiation of atheromatosis[3,7,8]. Beyond representing inflammatory markers, these factors have pro-atherogenic functions. Most notably, CRP has been reported to induce expression of leukocyte adhesion molecules, tissue factors, monocyte recruitment to arterial wall with the induction of MCP-1, and increased complement activation[7]. An elevated CRP level even among healthy children has been shown to be associated with a reduction in endothelial function[3]. On the other hand, experimental studies have shown that elevated plasma homocysteine levels may promote the initiation of atheromatosis in different ways[8]. Hyperhomocysteinaemia is believed to promote endothelial damage and dysfunction by inhibiting nitric oxide synthase and subsequently decreasing the bioavailability of nitric oxide[8]. Also, hyperhomocysteinaemia might activate platelets, and thus increase platelet aggregation and adhesion, and can impair DNA methylation affecting both the expression of endothelial and smooth muscle cells. In that way elevated plasma homocysteine levels induce smooth muscle cells proliferation leading to luminal narrowing[8]. Finally, excess homocysteine may initiate atheromatosis through NF-κB induction[8].

One of the key regulators of inflammation is the transcription nuclear factor-κB (NF-κB), which is actually a family of transcription factors consisting of 5 members: p65 (RelA), c-Rel, RelB, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100)[21]. NF-κB is believed to regulate more than 160 genes, some of them involved in the early stages of atheromatosis[21]. Those genes are related to inflammatory and immunologic pathways, apoptosis and cell proliferation and thus for a long time NF-κB has been regarded as a pro-atherogenic factor mainly because of the regulation of various pro-inflammatory genes[11]. In details, NF-κB regulates the transcription of several enzymes important in the early modification of LDL and the formation of inflammatory lipid mediators[21]. Also NF-κB controls the transcription of monocyte chemoattractant protein-1 (MCP-1), a significant chemokine for the attraction of monocytes in early lesion development and of several adhesion molecules, including P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), all implicated in the initiation of atheromatosis[21]. The crucial role of this factor in atherogenesis was finally established by experiments showing that NF-κB was highly expressed in endothelial cells of regions prone to developing atherosclerotic lesions compared to the cells of regions that were less prone[21].

During the last decade, studies in animal models have established cytokines and chemokines as pivotal players in the regulation of endothelial and smooth muscle cell (SMC) function[13]. Thus, it is now accepted that apart from their potent effects on immune system and inflammatory cells, these molecules also promote endothelial cell dysfunction and alter SMC phenotype and function, which can contribute to or retard vascular pathologies[13]. Cytokines and chemokines can also significantly enhance the function of endothelial cell adhesion molecules at all stages of transendothelial cell migration[13]. Also someone should emphasize on the fact that many cytokines, such as tumor necrosis factor-α (TNF-α), can possibly induce a number of other cytokines and chemokines[13]. According to in vitro studies, pro-inflammatory factors such as interferon-gamma (IFN-γ), TNF-α and interleukin-1 beta (IL-1β), can be atherogenic by impairing reverse cholesterol transport system (RCT), a pathway through which accumulated cholesterol is transported from the vessel wall to the liver for excretion, thus preventing atherosclerosis[26]. IL-1 has been studied in atherogenesis using either IL-1β-deficient mice or

mice with lack of the IL-1 receptor. Absence of IL-1β in apoE$^{-/-}$ mice reduced atheromatosis by almost 30% and this effect was accompanied by a significant decrease in the expression of one of the major adhesion molecules in the early stages of atheromatosis, VCAM-1, and chemokine MCP-1[21]. The expression of VCAM-1, which binds predominantly monocytes and T lymphocytes, is also induced by many pro-inflammatory cytokines such as TNFα and IL-6[27]. Endothelial cell dysfunction is also promoted by INF-γ and experiments have already shown that blockade of this pro-inflammatory cytokine inhibits atherogenesis[13]. In contrast there are some cytokines which retard lesion formation[13]. The absence of the anti-inflammatory cytokine IL-10 has also been evaluated in mice and this interleukin shows inhibitory activity for SMC recruitment, and its action may counteract the pro-atherogenic activity of other cytokines observed in fatty streaks[13]. Except for IL-10, IL-18 also appears to negatively regulate SMC proliferation in early atheromatic lesions[13]. Finally IL-10 and TGF-β can notably inhibit NF-κB's pro-inflammatory signalling pathway, and thus prevent atherogenesis[13]. It is clear that cytokine stimulation of endothelial cells can both positively and negatively modulate expression of endothelial gene products and regulate the process of atheromatosis.

Previous studies have already shown that elevated myeloperoxidase (MPO) levels were associated with the presence of coronary heart disease[28]. MPO is considered to be pro-atherogenic due to the inflammatory effects that possesses. Mutations on the MPO gene, such as the -463G/A polymorphism, can have a functional significance and lead to higher reactive oxygen species (ROS), which finally results in the formation of early lesions[28].

Fetuin-A (AHSG), is a liver-secreted, plasma glycoprotein whose biological function remained obscure until recently. We already know that AHSG inhibits ectopic calcification and can act as a natural inhibitor of the insulin receptor tyrosine kinase in liver and skeletal muscle[29]. It was recently reported that AHSG suppresses atheroprotective adiponectin production and generates an atherogenic profile of lipoproteins which results in the induction of several inflammatory cytokines[29]. All these alterations affect significant pathways of atherogenesis and lead to the early ''fatty streaks'' formation.

Children's obesity is reaching alarming proportions and is considered to be a major health problem. World Health Organization estimates that there are 3 millions obese children in Europe and this huge number is raised by 85.000 more children every year[30]. Obesity represents a chronic inflammatory status and obese children of all ages have evidence of a low-grade chronic inflammatory state[2,31]. Adipocytes release either cytokines or an array of adipokines such as leptin, which regulate immunologic and systemic activities[32]. There have been numerous studies associating child obesity with various changes in lipid profile and inflammatory markers, and thus scientists believe not only that this status might accelerate atheromatosis and cardiovascular disease later on, but may also be associated with the initiation of atheromatosis in early life[3,18,31,33]. In details, the involvement of cytokines in obesity as well as of the adipokine leptin is supported by studies showing that weight reduction normalizes mediators of inflammation. In fact, absolute numbers of CD4+ cells and CD4/CD8 ratio increase, while leptin values fluctuate within normal ranges, after a hypocaloric diet for a six month period[32]. The correlation of circulating CRP, inflammatory cytokines and complications of obesity has been studied in obese children with mixed results. There have been large population studies showing that body mass index (BMI) is the best predictor of elevated CRP in children[3]. Elevated levels of serum TNF-α have been found in some studies of obese children although there are also studies showing no correlation between obesity and this inflammatory marker. Finally, IL-6 and adiponectin were positively and negatively correlated with BMI respectively[31].

An important goal of future studies will be a more-detailed investigation of all genes and inflammatory-related pathways regulated by different cytokines in atherogenesis. The relatively recent appreciation that inflammatory response plays a key role in atherogenesis implies that inhibiting inflammation may provide new anti-atherosclerosis therapy.

LIPID METABOLISM

Intracellular processes that regulate lipid metabolism

are crucial for our health status both in childhood and adulthood, but our understanding of lipid homeostasis is limited because of the complexity that characterizes their metabolic pathways involving cholesterol uptake, biosynthesis, transport, metabolism, and secretion[26]. The role of cholesterol in atheromatosis is well established and has been elegantly reviewed[34] and hypercholesterolemia is an independent risk factor in early atheromatosis well described and documented even 20 years ago[3,35]. Excess on cholesterol blood levels results in atherogenesis through early endothelial activation or dysfunction accompanied by the expression of adhesion molecules and chemokines[10]. In details, it has been demonstrated that oxidized low-density lipoprotein (LDL), remnant lipoprotein (β-VLDL) and lipoprotein-a (LPa) play a critical role in the pro-inflammatory reaction and the formation of early stage lesions and are considered to be atherogenic when elevated in the plasma[10]. Elevated blood levels of these lipoproteins up-regulate vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), P-selectin and E-selectin, molecules considered to be responsible for the adherence of macrophages and T-lymphocytes to endothelium[10]. After the deposition in the intima, these lipoproteins are subjected to chemical modification leading to a series of biological reactions, as mentioned above[10]. A recent study showed that lowering plasma cholesterol eliminates the formation of atheromatic lesions observed in mice with sustained high levels of plasma cholesterol[4] and that the mRNA levels of thirty-seven atherosclerosis genes responded strongly to this cholesterol lowering[4]. Beyond that, there is recent evidence that lipids and lipoproteins may act as nuclear factors affecting basic biological phenomena such as transcription, genome stability, and cell differentiation. In particular, experimental studies provide new insights into the mechanisms of interaction between DNA and lipid molecules such as short-chain fatty acids and cholesterol and it has been shown that hyperlipidaemic lipoprotein profiles are associated with aberrant DNA methylation patterns at early stages of atheromatosis in mice and in cultured human macrophages, suggesting that a rearrangement of DNA methylation patterns is among early molecular changes associated with atherogenesis[36].

Familial hypercholesterolemia (FH) is the most common lipid disorder in childhood affecting 1 out of 500 individuals and the frequency of homozygotes is about 1:1,000,000[37]. FH is a dominantly inherited autosomal disorder caused by mutations of the low-density lipoprotein receptor (LDLR) gene on chromosome 19[37,38]. LDLR is a cell- surface glycoprotein responsible for the uptake and catabolism of plasma LDL. The defective protein fails to catabolize LDL particles causing higher concentrations of total cholesterol (TC) and LDL cholesterol (LDL-C) in the plasma[39]. More than 600 different mutations have been described in the literature so far[37]. Some of the LDLR variants which were already reported to play a significant role in early atheromatosis are C201-D206, C297F, G528D, Q81X, C358R, D97Y, G571E and a recent study discovered three new missense mutations G266C, T368M, and D451Y on exons 6, 8 and 10 of the LDLR gene respectively[39].

Lipoprotein(a) or LP(a), is a member of the plasma lipoproteins with general properties of LDL and high LP(a) plasma levels have been associated with an increased incidence of cardiovascular disease, due to both atherogenic and thrombogenic potentials of this molecule[40]. In particular, studies in animal models showed that LP(a) may act as a pro-inflammatory agent that accelerates early lesion formation[10] and thus represents an independent predictor of the development of premature atherosclerosis in humans[41]. The LP(a) particle consists of two disulfide-linked proteins, apolipoprotein B (APO-B) and APO(a). The APO(a) is a highly glycosylated protein and there have been reported more than 20 genetic polymorphisms in this molecule related to early atheromatic lesions[43]. APO-B measured in children and adolescents, reflects a lipoprotein profile predisposing to the development of subclinical atherosclerosis and is considered to be a marker which might have value in paediatric lipid risk assessment[42]. Also, familial deficiency of APO-B 100 is an autosomal dominant disorder related to hyperlipidaemia and premature atheromatosis in children[42]. Among the genetic alterations associated with lipid metabolism, the APO-E genotype has gained particular prominence within the problem of early atheromatosis. This is a polymorphic gene with 3 co-dominant alleles (ε 2, ε 3, ε 4) that give rise to 6 genotypes: ε 2ε 2, ε 2ε 3, ε 3ε 3, ε 3ε 4, ε 4ε 4 and ε 2ε 4. Several population studies have shown that the ε 4 al-

lele, and in particular the ε 3ε 4 genotype, is strongly associated with early atheromatosis and fatty streak formation[2,42].

A critical part of RCT, which was previously mentioned, is cholesterol efflux, in which accumulated cholesterol is removed from macrophages of the vessel wall with the ATP-binding membrane cassette transporter A1 (ABCA1) or other mechanisms (SR-B1, caveolins, sterol 27-hydroxylase) and collected by HDL and apoA-I[26]. This cholesterol-HDL molecule is then delivered to the liver for excretion. ABCA1 is an integral membrane protein which facilitates cellular cholesterol efflux and has generated considerable interest as a potential anti-atherogenic agent. In patients with mutated ABCA1 genes, RCT and cholesterol efflux are impaired and atheromatosis is increased. In studies with transgenic mice, disruption of ABCA1 genes can induce atheromatosis[26]. Other studies with animal models showed that mice, strongly expressing ABCA1, exhibit an anti-atherogenic lipid profile, with elevated levels of HDL-C and apoA-I, and significantly less aortic atherosclerosis[26]. Mutation of this gene in humans is the genetic cause of Tangier disease (TD), an autosomal recessive disorder of lipid metabolism associated with cardiovascular disease[19,26]. In TD, fibroblasts, HDL-mediated cholesterol efflux and intracellular lipid trafficking and turnover are abnormal, so these patients have low levels of HDL and high levels of triglycerides[26].

Cholesterol ester transfer protein (CETP) is a molecule that facilitates the transport of cholesteryl ester from HDL to APO B-containing lipoproteins. These cholesteryl esters are then delivered back to the liver via LDLR, and finally eliminated through the gastrointestinal tract[26]. Recent studies in transgenic mice have already shown that the introduction of the human CETP gene with an atherogenic diet results in decreased HDL levels, increased VLDL, LDL and APO B levels and finally initiates the formation of spontaneous atheromatic lesions, supporting the hypothesis that CETP is pro-atherogenic[26]. In humans, a decrease of CETP activity may increase HDL and decrease VLDL and LDL and CETP inhibition has attracted scientists' attention[26]. JTT-705 is a direct inhibitor of CETP that raises HDL in a dose-dependent manner and also a CETP vaccine is manufactured, which induces auto-antibodies that specifically bind and inhibit endogenous CETP, with the intention of increasing HDL and reducing the development of atheromatosis[26].

Thus, it is clear that identifying the genetic determinants of dyslipidemia and all the pathways involved in lipid metabolism is a crucial step towards understanding the molecular basis of early atheromatosis and developing new therapeutic approaches.

MMPs

It is widely accepted that oxLDL promotes atherogenic events which are in part mediated by matrix metalloproteinases (MMPs), inflammatory cytokines, and prostaglandins, produced and secreted from all kinds of cells involved in atheromatic process[43]. Among these mediators, MMPs are zinc-dependent endoproteinases that participate in the remodelling of the extracellular matrix (ECM)[44]. The isozymes of MMPs identified to date have been classified into 5 groups including collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, and -11), membrane-type MMPs (MMP-14, -15, -16, -17, -24, and -25), and matrilysins (MMP-7 and -26). MMPs are believed to play an important role in every stage of atheromatosis and numerous studies have already shown that MMP-1, -2, -3, -7, -8, -9, -12, -13, and -14 are highly expressed at atheromatic lesions[43,45]. Among them, MMP-9, MMP-3 and MMP-12 seem to act as atherogenic agents[43,45,46]. MMP-9 is involved in the development and progression of atheromatosis[43] and studies in MMP-9-deficient SMC and MMP-9 knockout mice showed that this MMP-9 deficiency resulted in decreased migratory activity, less intima hyperplasia and finally less atheromatosis[44]. The function of MMP-12 in the initiation of atheromatosis was studied in human MMP-12 transgenic (Hmmp-12 Tg) rabbits[46]. Fatty streaks in hMMP-12 Tg rabbits fed a 1% cholesterol diet for 6 weeks (cholesterol-induced model of atherosclerosis) were more pronounced and were associated with more significant degradation of the internal elastic layer compared with wild-type (WT) animals. Also the numbers of infiltrating macrophages and smooth muscle cells in the lesions were increased in hMMP-12 Tg compared with WT animals[46].

Thus, the elucidation of the role of other MMPs variants will permit a better understanding of the events observed in the early stage of atheromatosis and will potentially result in new therapeutic approaches.

OTHER RISK FACTORS OF ATHEROMATOSIS IN CHILDREN

Apart from all the genetic components of the early stage of atheromatosis which were previously described, scientists have identified even more agents believed to interfere in atherogenesis:

Group IIA phospholipase A_2 (secretory PLA_2 or sP-LA_2) has been found in human atherosclerotic lesions and is implicated in chronic inflammatory conditions such as atheromatosis[24]. $sPLA_2$ IIA is considered to be a pro-atherogenic factor and has been suggested to regulate collagen deposition in the plaque and fibrotic cap development[24].

Sphingomyelinase (SMase) is an enzyme that hydrolyzes sphingomyelin (SM) to release ceramide, a substance which is elevated in atherosclerotic plaques as well as in LDL isolated from these lesions. Ceramide is believed to play a crucial role in the early accumulation of LDL within the arterial wall, which is a critical step in the initiation of atheromatosis[24].

AMPK is a protein kinase, whose activation represses the proliferation of many cells involved in atherogenesis and also regulates the expression of genes related to lipid metabolism [45]. AMPK's signaling pathway involves a significant number of tumor suppressing genes (LKB1, p53, TSC1 and TSC2) and enhances various growth factors[45]. Thus, this molecule might be used as a therapeutic agent in atherogenesis through inhibiting the proliferation of the cells participating in early formation of atheromatic lesions.

Considering atheromatosis to be a metabolic complication has directed scientists' attention towards peroxisome proliferator-activated receptors (PPARs). PPARs, as ligand-activated transcription factors belonging to the nuclear hormone receptor family, can regulate multiple target genes. Extensive data establish expression of all three PPAR isotypes (PPARα, -γ, and -δ/β) throughout the vasculature and inflammatory cells[27] and the potential importance of these factors in atheromatosis is evident by their transcriptional regulation of pathways involved in atherogenic dyslipidemia, extracellular matrix remodeling, cholesterol efflux, thrombogenicity, and inflammation[27]. As such, PPARs may well be involved in all stages of atheromatosis, from the earliest fatty streaks to the late stage complicated lesions[27]. In details, PPARα is believed to play an important role in atherogenesis by reducing leukocyte adhesion to activated endothelial cells and inhibiting subsequent transendothelial leukocyte migration[27]. Also this receptor exerts anti-inflammatory effects in the vascular wall through a decrease in the expression of inflammatory cytokines such as IFN-γ, TNFα, and IL-2. PPARα inhibits the expression of NF-κB[21] and improves lipid profile by reducing triglycerides levels and increasing HDL levels[27]. Finally, PPARγ in SMC suppresses the expression of genes responsible for the adhesion of monocytes to the endothelium (VCAM-1, ICAM-1) and their transendothelial migration, which are both crucial early processes for the subsequent development of atheromatic lesions[47].

DNA METHYLATION AND ATHEROMATOSIS IN CHILDREN

Gene expression can be regulated at a genetic level by structural changes in DNA sequence and at an epigenetic level by mechanisms that are mutation independent. This epigenetic regulation of gene expression involves modifications of chromatin such as DNA methylation and several types of histone post-translational modifications such as acetylation, methylation, phosphorylation, and ubiquitination[48]. DNA methylation is the post-replication addition of methyl groups to the 5' position of cytosine rings within the context of CpG dinucleotides[9]. P. E. Newman was the first scientist that made the hypothesis that aberrant DNA methylation patterns play a key role in atherogenesis[49]. Since late 1990s the study of DNA methylation progressed and researchers started to reveal the relationship between this epigenetic modification and various genes involved in the process of atheromatosis. These studies show that the initial stages of atheromatosis are associated with a rearrangement of genomic DNA methylation patterns including both hyper- and hypomethylation, of atheromatosis-protective and atheromatosis-susceptible genes respectively[48]. For example DNA hypermethylation at the human estrogen recep-

tor gene (ERα) is associated with early atheromatosis and also genes related to lipid metabolism are likely candidate factors behind such changes[48]. Despite remarkable progress, the study of DNA methylation in atheromatosis is still at the beginning. With the advent of new technologies, it is possible that in the future we will be able to analyze and understand epigenotype-phenotype relationships, which may unravel an epigenetic mechanism underlying atherogenesis.

GENE-ENVIRONMENT INTERACTIONS

So far, we have mentioned all those genes and their variants believed to be involved in atherogenesis and the formation of fatty streaks in children. Beside this intricate network of genes and their interactions, gene-environment interactions might also play a significant role in early atheromatosis, given the fact that gene expression is partially regulated by environmental factors.

In general, evidence based on human epidemiological studies and dietary interventions in animal models, suggest that maternal nutritional imbalance and metabolic disturbances during gestation and fetus development may have a persistent effect on the health of the offspring and may even be transmitted to the next generation[50,51]. In the last decade a series of studies have demonstrated that common disorders take root in early nutrition and thus, Hales and Barker first introduced the term ''fetal programming''[52]. The fact that early atheromatic lesions are already formed during fetal development was first documented 25 years ago[53] and it is widely recognized that the uterus environment determines significantly adult disease[54]. Recent research, links dysmetabolic conditions during pregnancy with increased atheromatosis in childhood and scientists focus on the impact of specific maternal risk factors, such as obesity, metabolic syndrome, and diabetes, on cardiovascular risk in the offspring[54]. As previously mentioned inflammation plays a crucial role in atherogenesis, and two key factors that influence inflammation, maternal hypercholesterolemia and maternal immune mechanisms, have been shown to affect the developmental programming of early atheromatosis. Maternal hypercholesterolemia during pregnancy is associated with increased fatty streak formation in human fetal arteries and accelerated postnatal atherogenesis in normocholesterolemic children[53,54]. Gene expression between offspring of normo- and hypercholesterolemic mothers differs, and these differences persist long after birth, supporting the assumption that fetal lesion formation is associated with genetic programming[53]. On the other hand CRP level during gestation is believed to be an important predictor of increased atherogenesis in offspring of hypercholesterolemic mothers[55]. Finally, another study relates early atheromatosis in 9 year-old children, as measured by intima media thickening, with a lower energy intake of mothers during pregnancy, reinforcing the idea that maternal nutritional condition might influence susceptibility to childhood atheromatosis[51].

Not only the in utero nutritional status but also postnatal diet is believed to program early atheromatosis in children. This assumption was based on postnatal nutritional manipulations in animal models and human epidemiological data that relate markers of early nutrition, such as the size of birth or in infancy, to atherogenesis[56]. For example in one epidemiological study, scientists associated prolonged breast-feeding with a greater incidence of atherosclerosis in adult life[56]. Also there are numerous studies concerning the identification and characterization of genes regulated by the level of dietary fat, and researchers demonstrated that the expression of Lfm-1 and Lfm-2 genes, which are associated with atheromatosis, changed in relation to different levels of fat intake[57]. Thus, readjusting our early nutritional policies might result in a better nutritional programming and prevention of atheromatosis and cardiovascular disease.

FUTURE PERSPECTIVES

Atheromatosis in children and adolescents is really a reversible process and anatomic changes observed in early atheromatosis are modifiable[3,18]. Thus, primary prevention strategies beginning in childhood have great potential and might result in prevention of adult cardiovascular disease. Several guidelines have been already published[16,18] and some of the recommended measures are low-fat diets and regular high-energy exercise to prevent children's obesity, screening of blood pressure, lipid profile and glucose levels and finally pharmacologic intervention in case our targets cannot be achieved in any other way[3,14,16]. The identification of atheromatosis susceptible genes with the use

of candidate gene approach and genome-wide linkage studies[1], have been very useful in shedding light on this complex disease. Advances in statistical analysis and bioinformatics as well as the development of new methods such as array analysis, suppression subtractive hybridization (SSH) and cDNA representational analysis, provide scientists the ability to study the expression level of thousands of genes and uncover the intricate gene-network of atheromatosis[58,59,60]. After all, discovering these genes and unravelling their gene-gene and gene-environment interactions is a crucial step in order to facilitate drug target development. Gene therapy for atheromatosis and subsequent cardiovascular disease is in progress although, neither interference of target gene expression nor the transfer of therapeutic genes is clinically used[61]. The relative accessibility of blood vessel and myocardium makes them ideal targets for gene therapy and we have already obtained encouraging results with genes encoding growth factors (VEGF), transcriptional factors (NF-κB) and inherited genetic defects in lipid metabolism such as familial hypercholesterolemia[61].

In the future, it may be possible that administering a single genetic test will be adequate to reveal patients likely to develop atheromatosis and cardiovascular disease. Until then, more research is required in order to unravel the full genetic repertoire of this complex process.

Μοριακή βάση της παιδικής αθηρωμάτωσης.

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ΠΕΡΙΛΗΨΗ: Η αθηρωμάτωση αποτελεί μία χρόνια εξελισσόμενη πάθηση των μέσου και μεγάλου μεγέθους αγγείων, η οποία ξεκινά σιωπηρά κατά την περίοδο της εμβρυικής ανάπτυξης, εξελίσσεται κατά την παιδική και εφηβική ηλικία και εκδηλώνεται κλινικά κατά την ενήλικο ζωή. Αιτιοπαθογενετικά αποτελεί μία πολύπλοκη διαδικασία, στην γένεση και την εξέλιξη της οποίας συμμετέχουν τόσο γενετικοί όσο και περιβαλλοντικοί παράγοντες. Μέχρι σήμερα έχουν πραγματοποιηθεί πολυάριθμες μελέτες με σκοπό την ανίχνευση αθηρωματικών ελκών στις στεφανιαίες αρτηρίες των παιδιών και εφήβων, αποδεικνύοντας την πρώιμη έναρξη της νόσου. Αν και η έρευνα για την ανακάλυψη του συνόλου των γονιδίων που συμμετέχουν στην διαδικασία της αθηρωγένεσης δεν έχει ολοκληρωθεί, εντούτοις έχουν ήδη γίνει τα πρώτα βήματα για την κατανόηση της μοριακής βάσης της παιδικής αθηρωμάτωσης. Στην αιτιοπαθογένεια της πολύπλοκης αυτής διαδικασίας εμπλέκεται ένας μεγάλος αριθμός γονιδίων που σχετίζονται με την φλεγμονή, τον μεταβολισμό των λιπιδίων, τις μεταλλοπρωτεάσες αλλά και πλήθος επιγενετικών τροποποιήσεων και αλληλεπιδράσεων μεταξύ γονιδίων και περιβάλλοντος. Η πρώιμη αθηρωμάτωση αποτελεί μία αναστρέψιμη διαδικασία και για τον λόγο αυτό με την σωστή οργάνωση στρατηγικών πρόληψης από την παιδική ηλικία, μπορεί να επιτευχθεί η πρόληψη των καρδιαγγειακών παθήσεων κατά την ενήλικο ζωή.

Λέξεις Κλειδιά: Αθηρωγένεση, Παιδική αθηρωμάτωση, Πρώιμη αθηρωμάτωση.

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