Published in final edited form as:

Curr Atheroscler Rep. 2010 May ; 12(3): 187–193. doi:10.1007/s11883-010-0104-9.

Genomics of Premature Atherosclerotic Vascular Disease

David Seo,

University of Miami Miller School of Medicine, 1501 NW 10th Ave, 809 Biomedical Research Building, Miami, FL 33136, USA

Pascal Goldschmidt-Clermont,

University of Miami Miller School of Medicine, 1600 NW 10th Ave, Rosenstiel Medical Science Building, Miami, FL 33136, USA

Omaida Velazquez, and

University of Miami Miller School of Medicine, 3016 Holtz Center, Miami, FL 33136, USA

Gary Beecham

University of Miami Miller School of Medicine, 1501 NW 10th Ave, 809 Biomedical Research Building, Miami, FL 33136, USA

Abstract

Atherosclerotic vascular disease is a systemic process with a common pathophysiology but with different disease manifestations depending on the vascular site. Over the past two decades, significant efforts have gone toward determining the genomic factors contributing to atherosclerotic vascular disease. Substantial information has been generated regarding the genomics of atherosclerotic coronary heart disease, and recently, several genomic analyses have looked at the cerebrovascular and peripheral vascular beds. This article reviews genomic investigations of atherosclerotic vascular disease in the coronary, cerebrovascular, and peripheral arteries. In this review, we have tried to restrict the discussion to studies of premature atherosclerosis, particularly those using non-biased genomic techniques.

Keywords

Genetics; Genomics; Atherosclerosis; Coronary artery disease; Cerebrovascular disease; Peripheral vascular disease

Introduction

An estimated 80 million Americans have some type of atherosclerotic vascular disease, either with overt manifestations or subclinical involvement. For this discussion, atherosclerotic vascular disease refers to disease in any of the vascular sites, including the

Correspondence to: David Seo.

Disclosure No potential conflicts of interest relevant to this article were reported.

Papers of particular interest, published recently, have been highlighted as:

••Of major importance

coronary, cerebrovascular, and peripheral arteries. The presence of any atherosclerosis significantly increases the risk of adverse cardiovascular events, such as myocardial infarction (MI) and stroke. For example, individuals with peripheral artery disease have a fourfold higher risk of MI and a twofold higher risk for stroke. Given its enormous toll on public health, considerable research efforts have gone toward elucidating atherosclerosis disease pathology. These efforts have led to substantial improvements in the prevention and treatment of atherosclerotic vascular disease. However, causation remains elusive, and over the past two decades, much research has focused on determining the genetic factors that influence atherosclerosis development and progression.

Injury Model of Atherosclerosis

In the current model of atherogenesis, the disease results from the interaction between environmental risk factors, such as hypertension, and an individual's genetic background [1, 2]. Environmental risk factors, such as smoking, elevated cholesterol and hypertension, injure and disrupt the integrity of the arterial endothelial lining, allowing lipids and inflammatory cells to invade the vessel wall. As lipids and inflammatory cells take up residence within arteries, they propagate a cycle of progressive inflammation, vascular remodeling, and end organ damage. This simplified atherosclerosis disease model is known as the "injury model". Genetic factors come into play in the injury model by influencing an individual's susceptibility to injury and augmenting the damaging effects of experienced environmental risk factors. It is likely that multiple genetic risk factors contribute to disease development and occur in the form of gene variants, such as single nucleotide polymorphisms (SNPs). Each SNP represents a variation in DNA that may result in subtle to minor changes in the quantity or function of a translated protein product. However, in an individual experiencing a specific group of environmental risk factors, possessing the right combination of SNPs may dramatically increase disease susceptibility ultimately leading to atherosclerosis [3].

Within the injury model of atherosclerosis, there is growing recognition that stem and/or progenitor cells play a critical and opposing role. (For the purposes of this review, we will use the term "stem cell" to refer to both stem and progenitor cells.) As arteries are injured by environmental stimuli, stem cells from the bone marrow, spleen, peripheral circulation, or resident stem cells within the vascular tissues mobilize to areas of damaged endothelium and enact repair [4, 5]. When stems cells are unable to maintain the balance between injury and repair, either due to a reduction in numbers of stems cells or decline in their reparative capability, atherosclerosis ensues [6–8]. Aging and disease have been shown to drastically alter the number and function of stem cells in human and animal studies [4, 5, 9], but how an individual's genetics influences stem cell injury and repair homeostasis is not clear.

What follows is a brief overview of the current state of knowledge regarding the genomics of atherosclerotic vascular disease in the coronary, cerebrovascular, and peripheral arteries. When possible we will focus the discussion to genomic analyses of premature atherosclerotic vascular disease on research that used non-biased methods and technologies such as genome-wide linkage, gene expression, and genome-wide association analyses.

Coronary Heart Disease

Much of what we know about the pathophysiology, epidemiology, and genomics of atherosclerosis come from studies of coronary atherosclerotic heart disease. In general, atherosclerosis is considered a disease of the elderly. Paralleling a lifetime of exposure to risk factors such as hypertension, cholesterol, and smoking, the prevalence of atherosclerosis is high in elderly individuals. Autopsy studies estimate that over 70% of men and 50% of women older than 70 years of age have significant atherosclerosis of the coronary arteries [10]. However, there are large numbers of individuals with premature coronary atherosclerotic heart disease (defined as disease affecting individuals before the ages of 50 to 60 years). In these younger individuals with significantly shorter exposure time to environmental risk factors, genomic factors have a greater influence on disease development and progression. Many genomic studies have examined younger individuals with premature atherosclerosis to increase the ability and likelihood of detecting important genes and gene variants related to atherosclerosis. Such investigations have used functional genomics, linkage, and whole genome association technologies.

The Genetics of Early Onset Coronary Artery Diseases (GeneCard) study looked at families with early-onset coronary atherosclerotic disease, defined as coronary revascularization or MI in men before age 51 years and in women before age 55 years [11]. Using genome-wide linkage analysis of 1,168 individuals from 438 families, the study found that chromosomal loci 1q25 and 3q13 were significantly associated with premature disease. To date, a number of polymorphisms associated with the 3q13 chromosomal locus have been identified in GATA2 (GATA binding protein 2) [12], kalerin [13], and LSAMP (limbic system-associated membrane protein) [14].

A study by Wang et al. [15] utilized genome-wide linkage analysis to identify genomic regions associated with premature MI, with average age of onset being 44 years. Investigators analyzed 1,613 individuals from 428 families and found the 1p34–36 chromosomal locus to be highly associated with early-onset MI. Examination of the genes within this chromosomal region revealed the gene connexin 37 as the putative candidate gene. Other studies have provided further evidence that connexin 37 may be important in atherosclerotic development [16, 17••].

Finally, McPherson et al. [18••] examined premature coronary heart disease by performing genome-wide association analysis on individuals who suffered an MI before age 60. In an analysis of 322 cases and 312 controls, the investigators found a unique locus in chromosomal region 9p21 that was significantly associated with early MI. A number of additional studies have also shown the 9p21 region to be associated with MI and coronary atherosclerosis in other contexts, although not specifically in the context of premature coronary atherosclerotic disease [19••, 20]. There are two potential candidate genes located in the 9p21 region: CDKN2A (cyclin-dependent kinase inhibitor 2A) and CDKN2B (cyclin-dependent kinase 4 inhibitor B).

Cerebrovascular Disease

A number of epidemiologic studies have strongly suggested a role for genomic factors in the development of atherosclerotic cerebrovascular disease. For example, the Danish Twin study found a moderate level of heritability of cerebrovascular disease [21]. Another study of families with a history of atherosclerotic cerebrovascular disease showed that the incidence of strokes was significantly higher in younger individuals [22, 23]. However, our understanding of the genetic factors influencing atherosclerotic disease in the cerebrovascular system remains limited.

One approach for studying the genetics of cerebrovascular disease has been to look at carotid intima media thickness (IMT). Carotid IMT is an intermediate phenotype that is strongly correlated with the development of stroke as well as coronary heart disease [24]. Hernesniemi et al. [25] performed a candidate gene association study for polymorphisms in TLR-4 (toll-like receptor 4) using 2,223 individuals less than 40 years of age. TLR-4 was selected because of its association with carotid IMT in a previous study [26] and its association with inflammatory pathways involved in atherosclerotic vascular disease [27, 28]. The investigators found a polymorphism in TLR-4 that was associated with carotid IMT in this population of young people. On the other hand, in two separate analyses, one involving over 5,000 individuals and the other with over 12,000 individuals, the apoE epsilon variant was significantly associated with carotid IMT as well as with the presence of carotid plaques [29, 30]. There is a preponderance of additional genetic association studies of candidate genes in cerebrovascular disease, but they have been performed in small populations with limited reproducibility [16].

Recently, investigations of atherosclerotic cerebrovascular disease using non-biased genomic techniques have been reported. Debette et al. [17••] performed a genome-wide association study with 9,401 individuals to find gene variants associated with MRI-proven brain infarctions but no clinical history of stroke. Of the 9,401 participants in the study, there were 1,822 individuals with brain infarcts. The SNP with the highest association of brain infarcts was found in the MACROD2 (MACRO domain containing 2) and FLRT3 (fibronectin leucine = rich transmembrane protein 3) genes. However, the associations of these SNPs with brain infarcts were not replicated in two smaller test cohorts. Interestingly other SNPs within the MACROD2 gene were significantly associated with brain infarcts in one of the test cohorts. The function of MACROD2 is not well known, but may play a role in DNA repair and telomere and chromatin biology [31, 32]. FLRT3 is believed to be involved in cell adhesion, promote fibroblast growth factor growth signaling, and may also be involved in angiogenesis and neurogenesis [33].

In another study, Ikram et al. [34••] performed a genome-wide association study for stroke using 19,602 participants combined from four different registries. Within this group of 19,602 individuals, there were 1,544 strokes. In this study, investigators found two SNPs on chromosome 12p13 that were significantly associated with stroke, and one of these SNPs replicated in two independent validation cohorts. The gene closest to the validated SNP is NINJ2 (ninjurin-2). This gene is involved in cell-cell adhesion [35] and it has been shown in rat stroke models to be involved in nerve regeneration [36, 37].

A final example of genomic analysis relevant to atherosclerotic cerebrovascular disease was a genome-wide linkage analysis performed by Gretarsdottir et al. [38]. The investigators found a strong association between chromosomal locus 5q12 with ischemic stroke. In a follow-up study, the same investigators identified the gene PDE4D (phosphodiesterase 4D) as the likely gene associated with stroke in chromosome 5q12 [39]. The primary function of PDE4D is to degrade secondary messenger cAMP, which is the primary method of signal transduction in smooth muscle cells, T cells, and macrophages. In animal models, increases in cAMP is associated with reduce intimal hyperplasia by downregulation of smooth muscle cell proliferation [40, 41], as well as reduced inflammatory response by T cells and macrophages [22]. Interestingly, in three separate studies, SNPs within PDE4D are strongly associated with early-onset strokes [23, 25, 42].

Peripheral Artery Disease

As is the case with atherosclerotic coronary and cerebrovascular disease, a number of epidemiologic studies support the conclusion that genomic factors contribute to the development of peripheral artery disease (PAD). The National Heart, Lung, and Blood Institute Twin Study assessed the heritability of PAD in 94 monozygotic and 90 dizygotic white male twin pairs [43]. PAD was defined as an ankle-brachial pressure index (ABI) of less than 0.9. Concordance rates for low ABI were 33% for monozygotic pairs and 31% for dizygotic pairs compared with a PAD prevalence of 8.2% in the overall population. Another study examined participants in the Framingham Offspring Study with low ABI [27]. Investigators studied 2,286 individuals and estimated the heritability of PAD as defined by low ABI as 22%. In the Genetic Epidemiology Network of Arteriopathy (GENOA) study, heritability of low ABI was estimated in 1,310 black Americans and 796 white Americans and was found to be 19.5% and 21.2%, respectively [28]. Together, these three studies suggest a moderate heritability for PAD.

The literature regarding genomic analyses of PAD is not as comprehensive as that of coronary atherosclerotic disease, and there are no genomic analyses specifically of premature PAD. There are a small number of case-control studies testing the association between specific candidate gene polymorphisms and PAD, but these studies have not established a consistent genetic marker for PAD [29].

There have been two genome wide linkage studies of PAD. One was performed as part of the GENOA study [28]. The GENOA investigators performed genome-wide linkage in 1,310 black participants and 796 white participants using ABI as the surrogate marker for PAD. They identified six possible chromosomal regions in the black cohort and two different chromosomal regions in the white cohort, but none reached statistical significance. The second linkage study was performed in Icelandic individuals with PAD [30]. PAD patients either had surgically corrected or angiographically proven peripheral atherosclerotic disease. The investigators found chromosome 1p31 to be significantly associated with PAD. To date, this finding has not been confirmed in a separate population.

Recently, Thorgeirsson et al. [44] performed a recent genome-wide analysis of 10,995 Icelandic smokers to assess gene variants associated with degree of smoking. They identified

a gene variant on chromosome 15q24 in the nicotine acetylcholine receptor gene cluster that was significantly associated with smoking. They also found that that the SNP was significantly associated with the presence of PAD.

There has been one gene expression analysis of human atherosclerotic aortic disease. In this study, investigators collected aortas at the time of harvesting hearts and lungs for transplantation. They generated microarray data from the aortas of cadavers with severe atherosclerosis and those with minimal disease. The average age of the deceased individuals with the severely diseased aortas was 55 years. Using the microarray data, they identified around 200 genes with significant differential expression [45]. These same investigators also performed a parallel study comparing gene expression data from their human aorta analysis with gene expression data generated using the apolipoprotein E knock out mouse model of atherosclerosis [31]. The genes with differential expression from both the human and mouse studies were compared and there was substantial correlation across the two studies. They found that 20% of genes were common to both analyses and the amount of overlap between the two studies was statistically significant. One of the top candidate genes identified in both the human and mouse analyses was osteopontin. Tabibiazar et al. [32] performed a similar gene expression analysis of atherosclerosis comparing human and mouse atherosclerosis. This group also identified osteopontin as one of their top candidate genes [32].

Candidate Genes Point to Inflammation and Stem Cell–Mediated Vascular Repair

In reviewing the genes that have emerged from genomic analyses of the different vascular sites, there are several interesting observations. There is essentially no overlap between the genes with statistically significant association with atherosclerosis in each of the vascular sites (Table 1). Perhaps this is because while the atherosclerotic process is similar across the vascular beds, there are differences in disease timing and progression. This is most likely due to differences in the hemodynamic forces experienced by each site. In addition to the differences in physical stimuli, each vascular site may be comprised of cells from distinct developmental origins [46]. The adventitia may have arisen from a common pool of cells, but the intima and media for each vascular bed may have arisen from distinct and separate cell populations during development. This is particularly important in the context for the role of stem or progenitor cells in arterial homeostasis and vascular repair [4, 47]. For example, Li et al. [48] found significant differences in gene expression between smooth muscle cells from human aorta and coronary arteries. The gene with the greatest differential expression was RGS5 (regulator of G protein signaling 5), which was expressed in smooth muscle cells of aortic tissues but not in the coronary arteries, supporting the notion of regional diversity among cells of the vasculature [48].

Similar findings have been described for endothelial cells, which when exposed to the same hemodynamic forces and experimental conditions, respond differently depending on their vessel type. Investigators exposed endothelial cells from human umbilical veins [49] and human aortas [50, 51] to laminar, static, or distorted flow and found little correlation between the genes identified by microarray analysis in each study. In another investigation,

Deng et al. [52] found that endothelial cells from human saphenous veins and coronary arteries displayed different gene expression changes in response to atherosclerotic stimuli such as oxidized low-density lipoprotein, tumor necrosis factor, and platelet-derived growth factor. But in this study, even when the cell type was identical and remained constant, the gene expression responses to the varying atherosclerotic stimuli were different. This points to the notion that differences in cell origin may influence the genomics of atherosclerosis in different vascular beds.

Although cholesterol management is the mainstay of atherosclerosis prevention and treatment, the majority of the putative candidate genes are not involved in cholesterol metabolism. Rather, a recurring theme among the candidate genes is the involvement in stem cell biology. The gene GATA2 was identified through genomic convergence analysis of the GeneCard linkage study of premature coronary heart disease and gene expression analysis of aortic tissues from younger human organ donors. GATA2 has been shown to be intimately involved in regulation of hematopoietic stem cell homeostasis, with elevated expression of GATA2 leading to hematopoietic stem cell quiescence. Given that endothelial progenitor cells that are likely to play a major role in vascular repair originate from hematopoietic stem cells, GATA2 may play an important role.

The genes CDKN2A and CDKN2B were identified in genome-wide association study of early-onset MI. These two genes are tumor suppressor genes that act as key regulators of cell cycle signaling and have been found to be particularly important in stem cell biology [53, 54]. As stem cells age, expression levels of these genes increase, leading to cellular apoptosis and senescence. The gene BMI, a *Polycomb* group repressor, is essential for the self-renewal in adult stem cells [53, 55]. The BMI protein has been shown to control the expression activity of the CDKN2A and CDKN2AB genes.

In the gene expression analysis of aortas from human organ donors, the great majority of genes identified belonged to the gene ontology categories of immune response, inflammation, chemotaxis, and cell signaling. These included genes such as CXCR4, ALOX5, and AIF1, which are all genes heavily involved in inflammation. In the context of stem cell biology, CXCR4, also plays a crucial role in stem cell recruitment and mobilization [56–58].

In the study by Karra et al. [31], when the microarray results between the human and mouse aorta atherosclerosis studies were compared, a top candidate gene from both analyses was osteopontin. Osteopontin is well known for its role in bone metabolism [36, 37] but is also highly expressed in multiple cancer types, likely participates in cancer metastasis, and is also highly expressed in atherosclerotic plaques [59, 60]. In the context of stem cell biology, osteopontin plays a critical role in regulating the location and proliferation of hematopoietic stem cells [61, 62]. In mice that lack osteopontin, they have a marked increase in the cycling of the hematopoietic stem cell population [63, 64].

Conclusions

The past decades of genomic research in atherosclerotic vascular disease show us that there is a heritable genomic component, especially in the content of premature disease. Investigations to determine the genomic component contributing to atherosclerosis across different vascular sites have yielded a number of plausible candidate genes. Not surprisingly, the candidate genes tend to be different for each vascular bed. However, from the compendium of genomic results generated to date, there is an emerging theme common across each vascular site, which is the likely role of stem cell-mediated vascular repair in atherosclerosis. Whether the pathways found are relevant to immune response, inflammation, or cell signaling, many of the putative candidate genes play a significant role in stem cell biology. It may very well be that the interplay between vascular injury, stem cell-mediated repair, individual genetics, and environment risk factors represents the next wave of investigation in the vascular biology field.

References

1. Nabel EG. Cardiovascular disease. *N Engl J Med*. 2003; 349:60–72. [PubMed: 12840094]
2. Milewicz DM, Seidman CE. Genetics of cardiovascular disease. *Circulation*. 2000; 102:IV103–111. [PubMed: 11080139]
3. Williams R, Goldschmidt-Clermont P. The genetics of cardiovascular disease: from genotype to phenotype. *Dialogues Cardiovasc Med*. 2004; 9:3–223.
4. Rauscher F, Goldschmidt-Clermont P, Davis B, et al. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation*. 2003; 108:457–463. [PubMed: 12860902]
5. Hill JM, Zalos G, Halcox JP, et al. circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med*. 2003; 348:593–600. [PubMed: 12584367]
6. Dimmeler S, Zeiher A. Vascular repair by circulating endothelial progenitor cells: the missing link in atherosclerosis? *J Mol Med*. 2004; 82:671–677. [PubMed: 15322703]
7. Shaw C, Gatz C, Fisk D, et al. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS Biol*. 2007; 5:e201. [PubMed: 17676974]
8. Zhu S, Liu X, Li Y, et al. Aging in the atherosclerosis milieu may accelerate the consumption of bone marrow endothelial progenitor cells. *Arterioscler Thromb Vasc Biol*. 2007; 27:113–119. [PubMed: 17095715]
9. Heeschen C, Lehmann R, Honold J, et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. *Circulation*. 2004; 109:1615–1622. [PubMed: 15037527]
10. Wenger NK, Helmy T, Patel AD, Lerakis S. Evidence-based management of coronary artery disease in the elderly—current perspectives. *MedGenMed: Medscape General Medicine*. 2005; 7:75.
11. Hauser ER, Crossman DC, Granger CB, et al. A genomewide scan for early-onset coronary artery disease in 438 families: the GENECARD Study. *Am J Hum Genet*. 2004; 75:436–447. [PubMed: 15272420]
12. Connelly JJ, Wang T, Cox JE, et al. GATA2 is associated with familial early-onset coronary artery disease. *PLoS Genet*. 2006; 2:e139. [PubMed: 16934006]
13. Wang L, Hauser ER, Shah SH, et al. Peakwide mapping on chromosome 3q13 identifies the kalirin gene as a novel candidate gene for coronary artery disease. *Am J Hum Genet*. 2007; 80:650–663. [PubMed: 17357071]
14. Wang L, Hauser ER, Shah SH, et al. Polymorphisms of the tumor suppressor gene LSAMP are associated with left main coronary artery disease. *Ann Human Genet*. 2008; 72:443–453. [PubMed: 18318786]

15. Wang Q, Rao S, Shen G, et al. Premature myocardial infarction novel susceptibility locus on chromosome 1P34–36 identified by genomewide linkage analysis. *Am J Hum Genet.* 2004; 74:262–271. [PubMed: 14732905]
16. Bird T, Jarvik G, Wood N. Genetic association studies. *Neurology.* 2001; 57:1153–1154. [PubMed: 11591829]
- 17••. Debette S, Bis JC, Fornage M, et al. Genome-wide association studies of MRI-defined brain infarcts: meta-analysis from the CHARGE Consortium. *Stroke.* 2010; 41:210–217. [PubMed: 20044523]
- 18••. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A. A common allele on chromosome 9 associated with coronary heart disease. *Science.* 2007; 316:1488–1491. [PubMed: 17478681]
- 19••. Helgadottir A, Thorleifsson G, Manolescu A. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science.* 2007; 316:1491–1493. [PubMed: 17478679]
20. Lemmens R, Abboud S, Robberecht W, et al. Variant on 9p21 strongly associates with coronary heart disease, but lacks association with common stroke. *Eur J Hum Genet.* 2009; 17:1287–1293. [PubMed: 19319159]
21. Bak S, Gaist D, Sindrup S, et al. Genetic liability in stroke: a long-term follow-up study of Danish twins. *Stroke.* 2002
22. Tilley S, Coffman T. ... of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest.* 2001; 108:15–23. [PubMed: 11435451]
23. van Rijn MJE, Slooter AJ, Schut AF, et al. Familial aggregation, the PDE4D gene, and ischemic stroke in a genetically isolated population. *Neurology.* 2005; 65:1203–1209. [PubMed: 16162858]
24. O'Leary DH, Polak JF, Kronmal RA, et al. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med.* 1999; 340:14–22. [PubMed: 9878640]
25. Hernesniemi JA, Raitakari OT, Kähönen J, et al. Toll-like receptor 4 gene (Asp299Gly) polymorphism associates with carotid artery elasticity. The cardiovascular risk in young Finns study. *Atherosclerosis.* 2008; 198:152–159. [PubMed: 17996871]
26. Kiechl S, Lorenz E, Reindl M. Toll-like receptor 4 polymorphisms and atherogenesis. *N Engl J Med.* 2002; 347:185–192. [PubMed: 12124407]
27. Murabito J, Guo C, Fox C, D' ... R. Heritability of the anklebrachial index: the Framingham Offspring study. *Am J Epidemiol.* 2006; 164:963–968. [PubMed: 16928729]
28. Kullo IJ, Turner ST, Kardia SL. A genome-wide linkage scan for ankle-brachial index in African American and non-Hispanic white subjects participating in the GENOA study. *Atherosclerosis.* 2006; 187:433–438. [PubMed: 16280126]
29. Knowles JW, Assimes TL, Li J, et al. Genetic susceptibility to peripheral arterial disease: a dark corner in vascular biology. *Arterioscler Thromb Vasc Biol.* 2007; 27:2068–2078. [PubMed: 17656669]
30. Gudmundsson G, Matthiasson S, Arason H. ... of a gene for peripheral arterial occlusive disease to chromosome 1p31. *Am J Hum Genet.* 2002; 70:586–592. [PubMed: 11833003]
31. Karra R, Vemullapalli S, Dong C, et al. Molecular evidence for arterial repair in atherosclerosis. *Proc Natl Acad Sci USA.* 2005; 102:16789–16794. [PubMed: 16275914]
32. Tabibiazar R, Wagner RA, Ashley EA, et al. Signature patterns of gene expression in mouse atherosclerosis and their correlation to human coronary disease. *Physiol Genom.* 2005; 22:213–226.
33. Karaulanov E, Böttcher R, Stannek P, et al. Unc5B interacts with FLRT3 and Rnd1 to modulate cell adhesion in *PLoS One.* 2009; 4:e5742. [PubMed: 19492039]
- 34••. Ikram MA, Seshadri S, Bis JC, et al. Genomewide association studies of stroke. *N Engl J Med.* 2009; 360:1718–1728. [PubMed: 19369658]
35. Zhang S, Dailey GM, Kwan E, et al. An MMP liberates the Ninjurin A ectodomain to signal a loss of cell adhesion. *Genes Dev.* 2006; 20:1899–1910. [PubMed: 16815999]
36. Asou Y, Rittling S, Yoshitake H, et al. Osteopontin facilitates angiogenesis, accumulation of osteoclasts, and resorption in ectopic bone 1. *Endocrinology.* 2001; 142:1325–1332. [PubMed: 11181551]

37. Standal T, Borset M, Sundan A. Role of osteopontin in adhesion, migration, cell survival and bone remodeling. *Exp Oncol*. 2004; 26:179–184. [PubMed: 15494684]
38. Gretarsdottir S, Sveinbjörnsdottir S, Jonsson H, et al. Localization of a susceptibility gene for common forms of stroke to 5q12. *Am J Hum Genet*. 2002; 70:593–603. [PubMed: 11833004]
39. Gretarsdottir S, Thorleifsson G, Reynisdottir S. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nat Genet*. 2003; 35:131–138. [PubMed: 14517540]
40. Indolfi C, Avvedimento EV, Di Lorenzo E, et al. Activation of cAMP-PKA signaling in vivo inhibits smooth muscle cell proliferation induced by vascular injury. *Nat Med*. 1997; 3:775–779. [PubMed: 9212106]
41. Indolfi C, Di Lorenzo E, Rapacciuolo A, et al. 8-chloro-cAMP inhibits smooth muscle cell proliferation in vitro and neointima formation induced by balloon injury in vivo. *J Am Coll Cardiol*. 2000; 36:288–293. [PubMed: 10898448]
42. Song Q, Cole JW, O'Connell JR, et al. Phosphodiesterase 4D polymorphisms and the risk of cerebral infarction in a biracial population: the Stroke Prevention in Young Women Study. *Hum Mol Genet*. 2006; 15:2468–2478. [PubMed: 16835261]
43. Carmelli D, Fabsitz R, Swan G, et al. Contribution of genetic and environmental influences to ankle-brachial blood pressure index in the NHLBI Twin Study. National Heart, Lung, and Blood Institute. *Am J Epidemiol*. 2000; 151:452–458. [PubMed: 10707913]
44. Thorgeirsson T, Geller F, Sulem P, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature*. 2008; 452:638–642. [PubMed: 18385739]
45. Seo D, Wang T, Dressman H, et al. Gene expression phenotypes of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2004; 24:1922–1927. [PubMed: 15297278]
46. Schwartz SM, Virmani R, Rosenfeld ME. The good smooth muscle cells in atherosclerosis. *Curr Atheroscler Rep*. 2000; 2:422–429. [PubMed: 11122774]
47. Zenovich AG, Panoskaltsis-Mortari GJ, Caron R, et al. Sex-based differences in vascular repair with bone marrow cell therapy: relevance of regulatory and Th2-type cytokines. *Transpl Proc*. 2008; 40:641–643.
48. Li J, Adams L, Wang X, et al. Regulator of G protein signaling 5 marks peripheral arterial smooth muscle cells and is downregulated in atherosclerotic plaque. *J Vasc Surg*. 2004; 40:519–528. [PubMed: 15337883]
49. García-Cardena G, Comander J. Biomechanical activation of vascular endothelium as a determinant of its functional phenotype. *Proc Natl Acad Sci U S A*. 2001; 98:4478–4485. [PubMed: 11296290]
50. Chen BP, Li YS, Chen KD, et al. DNA microarray analysis of gene expression in endothelial cells in response to 24-h shear stress. *Physiol Genom*. 2001; 7:55–63.
51. Brooks AR, Lelkes PI, Rubanyi GM. Gene expression profiling of human aortic endothelial cells exposed to disturbed flow and steady laminar flow. *Physiol Genom*. 2002; 9:27–41.
52. Deng DX-F, Tsalenko A, Vailaya A, et al. Differences in vascular bed disease susceptibility reflect differences in gene expression response to atherogenic stimuli. *Circ Res*. 2006; 98:200–208. [PubMed: 16373601]
53. Shenghui H, Nakada D, Morrison SJ. Mechanisms of stem cell self-renewal. *Annu Rev Cell Develop*. 2009; 25:377–406.
54. Janzen V, Forkert R, Fleming HE, et al. *Stem*-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature*. 2006; 443:421–426. [PubMed: 16957735]
55. Liu J, Cao L, Chen J, et al. Bmi1 regulates mitochondrial function and the DNA damage response pathway. *Nature*. 2009; 459:387–392. [PubMed: 19404261]
56. Zernecke A, Schober A, Bot I, et al. SDF-1 α /CXCR4 Axis is instrumental in neointimal hyperplasia and recruitment of smooth muscle. *Circ Res*. 2005; 96:784–791. [PubMed: 15761195]
57. Hristov M, Zernecke A, Liehn EA, Weber C. Regulation of endothelial progenitor cell homing after arterial injury. *Thromb Haemost*. 2007; 98:274–277. [PubMed: 17721606]
58. Sainz J, Sata M. CXCR4, a key modulator of vascular progenitor cells. *Arterioscler Thromb Vasc Biol*. 2007; 27:263–265. [PubMed: 17229969]

59. Proudfoot D, Skepper JN, Shanahan CM, Weissberg PL. Calcification of human vascular cells in vitro is correlated with high levels of matrix Gla protein and low levels of osteopontin expression. *Arterioscler Thromb Vasc Biol.* 1998; 18:379–388. [PubMed: 9514406]
60. Giachelli C, Liaw L, Murry C, et al. Osteopontin expression in cardiovascular diseases. *Ann NY Acad Sci.* 1995; 760:109–126. [PubMed: 7785890]
61. Burdo T, Wood M, Fox H. Osteopontin prevents monocyte recirculation and apoptosis. *J Leukocyte Biol.* 2007; 81:1504–1511. [PubMed: 17369493]
62. Haylock DN, Nilsson SK. Stem cell regulation by the hematopoietic stem cell niche. *Cell Cycle.* 2005; 4:1353–1355. [PubMed: 16123595]
63. Nilsson SK, Johnston HM, Whitty GA, et al. Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. *Blood.* 2005; 106:1232–1239. [PubMed: 15845900]
64. Stier S, Ko Y, Forkert R, et al. Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. *J Exp Med.* 2005; 201:1781–1791. [PubMed: 15928197]

Table 1

Chromosomal loci and genes identified by genomic techniques in the different vascular beds

Loci/gene	Associated with	Genomic technique
Cardiovascular		
9p21 (CDKN2A/B)	Early-onset myocardial infarction	Genome-wide association
LSAMP	Myocardial infarction/atherosclerosis	Genomic convergence, microarray, and linkage
kalirin	Myocardial infarction/atherosclerosis	Genomic convergence, microarray, and linkage
GATA2	Myocardial infarction/atherosclerosis	Genomic convergence, microarray, and linkage
1q25 and 3q13	Early-onset myocardial infarction	Linkage study
1p34–36	Early-onset myocardial infarction	Linkage study
Cerebrovascular		
TLR-4	Carotid IMT at <40 y	Candidate gene association
apoE epsilon variant	Carotid IMT	Candidate gene association
5q12 (PDE4D)	Stroke	Genome-wide association
20p12 (MACROD2 and FLRT-3)	Silent stroke	Genome-wide association
12p13 (NINJ2)	Stroke	Genome-wide association
Peripheral		
1p31	Peripheral artery disease	Linkage study
Numerous genes, including osteopontin	Peripheral artery disease	Genomic convergence, human and mouse microarray analysis