

# Genetic variants promoting smooth muscle cell proliferation can result in diffuse and diverse vascular diseases: Evidence for a hyperplastic vasculomyopathy

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**Abstract:** Genetic predisposition to early onset of occlusive vascular diseases, including coronary artery disease, ischemic stroke, and Moyamoya disease, may represent varying presentations of a common underlying dysregulation of vascular smooth muscle cell proliferation. We discuss mutations in two genes, *NF1* and *ACTA2*, which predispose affected individuals to diffuse and diverse vascular diseases. These patients show evidence of diffuse occlusive disease in multiple arterial beds or even develop seemingly diverse arterial pathologies, ranging from occlusions to arterial aneurysms. We also present the current evidence that both *NF1* and *ACTA2* mutations promote increased smooth muscle cell proliferation in vitro and in vivo, which leads us to propose that these diffuse and diverse vascular diseases are the outward signs of a more fundamental disease: a hyperplastic vasculomyopathy. We suggest that the concept of a hyperplastic vasculomyopathy offers a new approach not only to identifying mutated genes that lead to vascular diseases but also to counseling and possibly treating patients harboring such mutations. In other words, this framework may offer the opportunity to therapeutically target the inappropriate smooth muscle cell behavior that predisposes to a variety of vascular diseases throughout the arterial system. *Genet Med* 2010;12(4):196–203.

**Key Words:** smooth muscle cells, proliferation, Moyamoya, neurofibromatosis, *NF1*, neurofibromin, *ACTA2*, smooth muscle  $\alpha$ -actin, aneurysm, arterial occlusion, arterial stenosis, hyperplastic vasculomyopathy

## ATHEROSCLEROSIS, NEOINTIMAL HYPERPLASIA, AND MOYAMOYA DISEASE

Atherosclerosis is a chronic and complex disease process that can ultimately occlude affected arteries and precipitate life-threatening or devastating end-organ damage, such as myocardial infarction or ischemic stroke. Focal lesions occur in medium sized to large arteries and lead to intramural thickening that impinges on the lumen of the vessel. The earliest lesions begin at sites of vascular injury, where endothelial dysfunction and increased permeability of the endothelial barrier allow the abnormal accumulation of plasma-derived lipids and their oxidation products within the matrix of the arterial wall. Intimal cells respond by locally upregulating expression of adhesion molecules to recruit inflammatory cells to the injury site, initiating the migration of monocytes from the peripheral blood into

the intima. The monocytes become activated macrophages and ingest the oxidized lipid, thus acquiring the familiar appearance of “foam cells” readily seen under the pathologist’s microscope.<sup>1</sup> The most commonly described and encountered atherosclerotic plaques contain extensive lipid atheromas. With the obvious role played by lipids in atherogenesis, it is not surprising that hyperlipidemia was one of the earliest and most robust clinical risk factors to be associated with atherosclerosis, and genetic variants leading to hyperlipidemia were some of the earliest heritable risk factors identified for atherosclerotic disease.<sup>2</sup>

However, the recruitment of macrophages and the inflammatory production of cytokines, oxygen free radicals, and other mitotic factors lead to downstream effects whose consequences may be more subtle. Specifically, these signaling events induce the migration of smooth muscle cells (SMCs) from the medial layer of the vessel into the intima, where they proliferate and synthesize matrix molecules to remodel the vascular wall. Although this serves in the acute setting as a protective response to strengthen the weakened vessel, with chronic damage it can maladaptively sclerose the vessel wall and narrow the lumen even further.<sup>1</sup> This process of neointimal formation has been most extensively studied in the context of atherosclerosis. Although most plaques contain extensive lipid deposits, cardiovascular pathologists have distinguished a subset of atherosclerotic lesions that are instead dominated by SMC proliferation and mostly lack lipid.<sup>3</sup> These are often described as fibromuscular lesions to denote the abundance of SMCs and their matrix products.

Genetic advances have identified both single-gene mutations and polymorphisms that predispose individuals to atherosclerosis, which can be loosely grouped based on gene function into the following classes: lipid metabolism; endothelial dysfunction; oxidative stress; inflammation; vascular remodeling; and arterial thrombosis.<sup>2,4,5</sup> It is notable that genetic variants specifically increasing SMC proliferation and migration are not included, although it is likely that all of the above gene classes promote SMC proliferation to some extent. Nonetheless, rare occlusive arterial syndromes that arise in specific and stereotyped arterial beds have been associated with neointimal lesions almost purely consisting of proliferative SMCs. Moyamoya disease is probably the most clinically striking example of such occlusive lesions.

Moyamoya disease is a rare cerebrovascular syndrome often leading to ischemic stroke at a young age. Diagnostic features on angiography include bilateral occlusion or stenosis of the terminal internal carotid artery and the formation of collateral vessel networks at the base of the brain, the so-called “Moyamoya vessels.” The flow of imaging dye through the small collateral vessels forms what looks to be a puff of smoke and lends the disease its Japanese name, as given in 1968 by the neurosurgeon who originally described what appeared to be an exclusively Japanese patient population (Fig. 1).<sup>6</sup> Since then,

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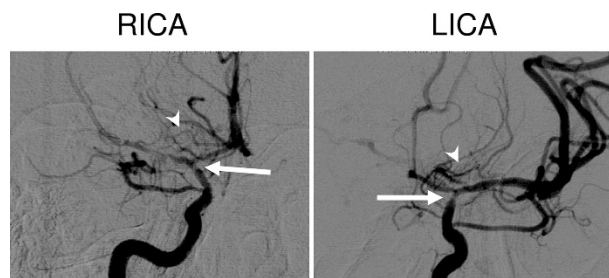
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**Fig. 1.** Angiography of primary Moyamoya disease. These angiograms were performed on a 26-year-old man presenting with the left-sided stroke symptoms. His family history was notable for a paternal grandfather who underwent surgical repair of a thoracic aortic aneurysm in his fifties. Anterior-posterior views of the patient's left internal carotid artery (LICA) and right internal carotid artery (RICA). Arrows indicate bilateral vascular occlusion at the terminal ICA, as denoted by a defect in the flow of imaging dye. Arrowheads indicate the "puff of smoke" appearance of abnormally prominent lenticulostriate vessels (Moyamoya vessels) in the vicinity of the occlusive lesions.

cases of Moyamoya have been identified all over the world, but prevalence is still highest in Japan, suggesting that genetic factors may play a large role in its pathogenesis. Moyamoya is classified into two forms: primary Moyamoya disease of idiopathic etiology and secondary Moyamoya syndrome, which can arise in association with other genetic syndromes or medical conditions, such as neurofibromatosis type 1 (NF1), sickle cell disease, hyperthyroidism, or systemic autoimmune disease.<sup>7,8</sup>

Although the specific genetic underpinnings are still largely unknown, it is possible that candidate genes could be strategically deduced from the clinical characteristics of the Moyamoya syndrome. Fortunately, it was noted 3 decades ago that the pathologic lesions in the occluded internal carotids of patients with Moyamoya disease are dominated by neointimal formation and proliferation of SMCs and bear a remarkable resemblance to the subset of lipid-poor fibromuscular lesions described in atherosclerosis.<sup>9</sup> In this review, we will discuss two genes that, when mutated, have been shown to promote SMC proliferation and to contribute to Moyamoya in affected patients.

Other salient features of Moyamoya provide clues to the role of genetics in its pathogenesis. First, multiple case reports of patients with Moyamoya from the literature belie a larger tendency for arterial occlusion—not only within the internal carotid and the anterior cerebral arterial circulation but also within other intracranial and extracranial arteries. Occlusive vascular disease in patients with Moyamoya has been reported to extend to the basilar, posterior cerebral, and superficial temporal arteries of the head, as well as the pulmonary artery, coronary arteries, renal arteries, pancreatic arteries, and digital arteries in the systemic circulation.<sup>10–13</sup> Thus, the insults initiating and driving the pathogenesis of Moyamoya are constitutive to all vessels. This fact suggests that the occlusive disease in the brain causing the hallmark stroke symptoms of Moyamoya may reflect only a portion of a larger, diffuse vasculopathy that can also affect other arterial beds and their associated organs, at least in some patients.

Second, cases of Mendelian inheritance patterns for Moyamoya disease have been reported: one study of 15 Japanese families with multiple affected members revealed an autosomal dominant inheritance pattern with reduced penetrance.<sup>14</sup> Ap-

proximately 7–12% of patients in Japan and 6% in the United States have a positive family history for primary Moyamoya disease.<sup>8</sup> To date, genetic mapping studies have identified several loci associated with primary disease, including regions on chromosomes 3, 6, 8, and 17, which suggests genetic heterogeneity associated with this rare stroke syndrome.<sup>14–18</sup>

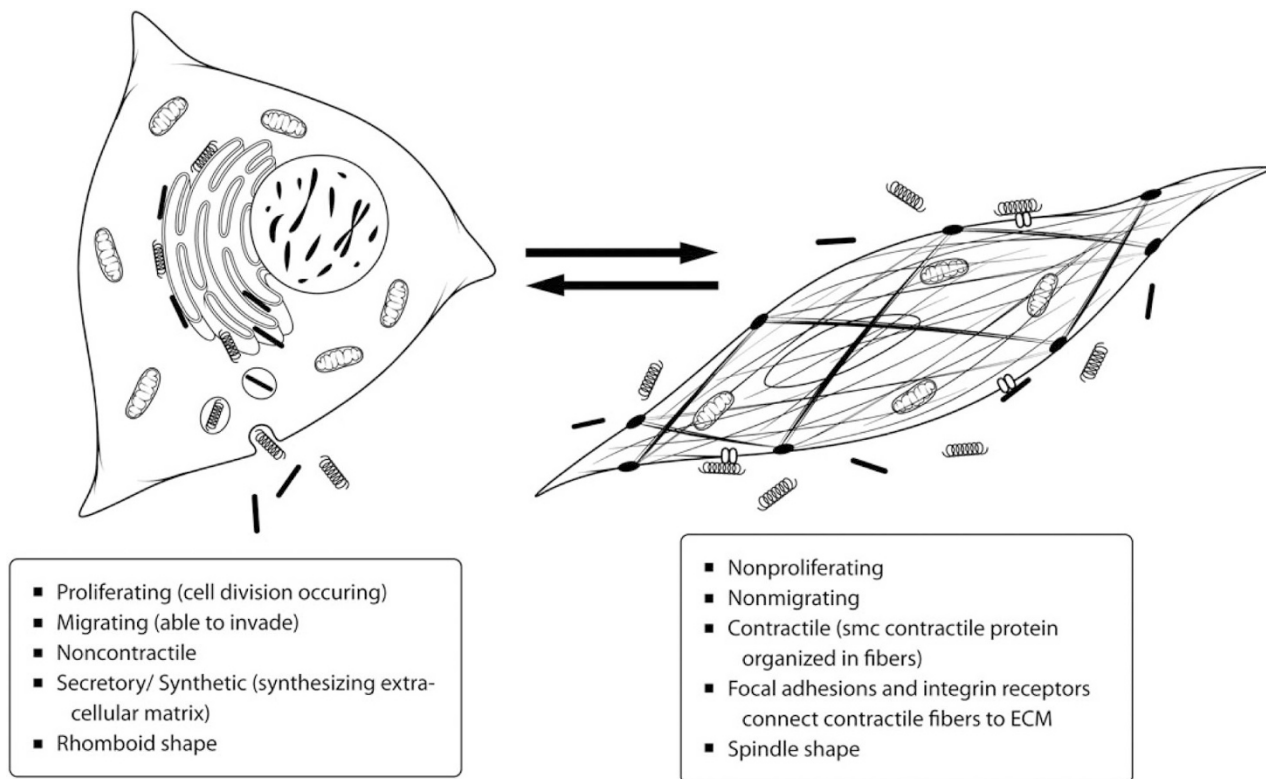
Finally, Moyamoya is a known complication of a number of genetic syndromes, including but not limited to NF1, Turner syndrome, Alagille syndrome, tuberous sclerosis, Noonan syndrome, and Williams syndrome.<sup>19–24</sup> Although it is possible that two rare conditions may occur in a single patient, the greater-than-expected incidence of Moyamoya cooccurring with these syndromes may instead imply that the underlying mutations contribute to a common etiologic process. Again, the clinical evidence supports the hypothesis that a host of genetic alterations can predispose an individual to Moyamoya.

The purpose of this review is to suggest that occlusive vascular disease may be a spectrum of conditions, united by the common process of SMC proliferation, that vary in the degree to which this process dominates the pathogenesis. This spectrum extends from the classic case of vascular damage-induced atherosclerosis, with its association with hyperlipidemia, age, and inflammation, to the more rare cases of intimal lesions characterized almost entirely by SMC hyperplasia, extracellular matrix accumulation, and the remarkable absence of lipid. Moyamoya disease is perhaps the most dramatic example of occlusive disease at this end of the spectrum, where ischemic strokes can occur at extremely young ages in children with no exposure to environmental atheroma-related or age-related risk factors.

To make the case for such a spectrum, we will discuss two known single-gene mutations, one syndromic and the other limited to the vascular system, that clearly lead to increased SMC proliferation. Patients harboring these mutations present with occlusive vascular diseases, including Moyamoya, and these occlusions occur diffusely in the vascular system, often affecting multiple arterial beds within the same patient and among family members with the same mutation. More remarkable still is the incidence of arterial aneurysms in these patients, although the mechanisms contributing to the diverse vascular phenotypes of both arterial occlusion and arterial dilation are still unknown. We suggest that the evidence from these relatively rare and severe single-gene disorders indicates a larger disease process—which is characterized by inappropriate and excessive SMC proliferation and which we have termed a hyperplastic vasculomyopathy—that manifests as diffuse and diverse vascular diseases in genetically affected patients and their affected family members. We further expect that genetic variations of any severity that are capable of promoting SMC proliferation will confer a vulnerability to diffuse and diverse vascular disease.

## VASCULAR SMCs AND THEIR DIVERSE ROLES IN THE VASCULAR SYSTEM

Vascular SMCs are unique cells that can alter their phenotypes not only during the development and growth of the arterial tree but also in response to vascular injury and the environmental cues that occur throughout an individual's lifespan. This is in stark contrast to the terminally differentiated cardiomyocytes and skeletal myocytes of the adult, which can respond to injury and increased pre- and afterloads with hypertrophy but not proliferation.<sup>25,26</sup> Under normal physiologic circumstances, quiescent and nonproliferative vascular SMCs contract to regulate vascular tone and distribution of continuous blood flow. These cells acquire their contractile phenotype by expressing smooth muscle-specific myofilaments, such as  $\alpha$ -actin and  $\beta$ -myosin



**Fig. 2.** Phenotypic switching by vascular smooth muscle cells. Mature smooth muscle cells are able to switch between differentiated/contractile and dedifferentiated/synthetic phenotypes. The cell on the right of this figure represents a differentiated smooth muscle cell, whose main feature is the abundance of contractile fibers spanning the cell body. These fibers contract to generate force against the extracellular matrix and regulate tension in the vessel wall. The cell on the left is a dedifferentiated cell, whose main purpose in adults is to respond to vascular injury by proliferating, migrating, and modulating the extracellular matrix to repair the vessel wall. The synthesis and secretion of matrix components is depicted, although smooth muscle cells are also capable of synthesizing and secreting the enzymes responsible for matrix degradation.

heavy chain, and other contractile machinery proteins, such as calponin and smoothelin (Fig. 2). In fact, these molecules are commonly exploited as markers of smooth muscle differentiation in studies of vascular development and function.<sup>27</sup>

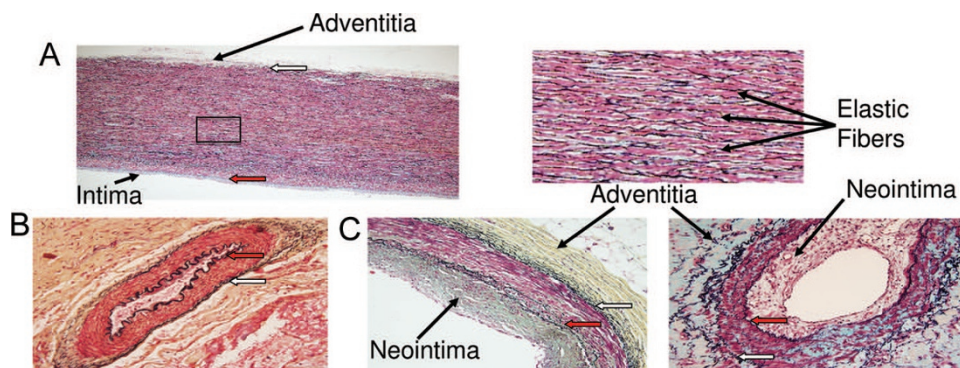
However, the cells also respond to vascular injury or other local stimuli by downregulating contractile gene expression and upregulating the expression of markers denoting a relatively dedifferentiated state, such as S100A4.<sup>28</sup> These injury-responsive cells proliferate rapidly, secrete matrix-digesting enzymes, migrate within focal lesions, and synthesize new extracellular matrix in an effort to repair and remodel the tissue (Fig. 2). Excessive adoption of this synthetic phenotype has been associated with the pathology seen in many sclerotic vascular diseases, the most common by far being atherosclerosis.<sup>29</sup> Although contraction and matrix synthesis are not always mutually exclusive functions of SMCs, for the purpose of this review it is useful to define two polar phenotypes, i.e., the “contractile” and “synthetic” phenotypes, to illustrate the array of potential phenotypes that exist in between them.

The components of the extracellular matrix contribute to the biomechanics of a blood vessel, and thus the SMCs differentially synthesize a variety of matrix proteins according to the needs of the various vessel types comprising a vascular bed. Large elastic arteries—such as the thoracic aorta, carotid, and renal arteries—are characterized by a medial layer arranged into

concentric lamellae that each include elastic fibers with SMCs lined end-to-end between each elastic layer. This architecture allows the stress of pulsatile flow from the heart to be distributed evenly throughout the vessel wall (Fig. 3).<sup>30</sup> The major component of these fibers—elastin—has been shown in vitro to induce the mature contractile phenotype and inhibit the proliferative/synthetic phenotype of SMCs.<sup>31</sup> By contrast, the medial layer of smaller muscular arteries—such as the coronary, cerebral, and mesenteric arteries—are composed almost exclusively of SMCs with only two elastic fibers delineating the boundaries between intima and media (the internal elastic lamina) and between media and adventitia (the external elastic lamina). These arteries are located downstream from elastic arteries and thus bear less force from blood flow. Their SMCs work instead to distribute flow by differential regulation of static vascular tone, so that the presence of the elastic fibers is not as essential to their function.<sup>1</sup>

However, another factor contributing to the unique diversity and complex roles of vascular SMCs are their diverse embryonic origins. For example, they can be derived from cardiac neural crest, the secondary heart field, somites, and mesothelium, among other areas.<sup>32</sup> In addition, adult stem cells from various sources can differentiate into SMCs to populate vessels as they form, remodel with growth, and respond to injury.<sup>33</sup> Although there is some controversy about the origin of different





**Fig. 3.** Artery structure and pathology. Movat-stained sections of human blood vessels are shown, with elastic fibers in black, collagen in yellow, proteoglycans in blue, and smooth muscle cells in red. Red arrows indicate the internal elastic lamina, and white arrows indicate the external elastic lamina. A, Elastic artery (aorta  $\times 40$  magnification, inset is  $\times 100$  magnification). Note the elastic fibers separating each layer of smooth muscle cells. B, Muscular artery (vasa vasorum  $\times 200$  magnification). Note the lack of elastic fibers. C, Intimal hyperplasia and formation of neointima (left, coronary artery  $\times 200$  magnification; right, vasa vasorum  $\times 200$  magnification). Note the proliferation of red-stained smooth muscle cells on the intimal side of the internal elastic lamina.

populations of adult SMCs, there is strong evidence that SMCs in the ascending thoracic aorta and its immediate branches in particular are initially derived from cardiac neural crest cells, whereas SMCs in peripheral blood vessels are derived from other sources.<sup>32,34,35</sup> Although this review will largely focus on the larger influence of single-gene mutations on SMC behavior, the embryonic lineage of the SMC is also likely to influence its preferential response to external signaling factors or to stimuli that direct differentiation, proliferation, and response to stress.

### NF1: A HYPERPLASTIC VASCULOPATHY ASSOCIATED WITH A KNOWN GENETIC SYNDROME

Accumulating evidence from the past few years support the hypothesis that NF1 patients have a predisposition for diffuse and diverse vascular disease resulting from their underlying mutation in the *NF1* gene. NF1 is an autosomal dominant disorder with clinical manifestations that include neurocutaneous signs, such as cafe-au-lait spots, intertriginous freckling, and benign dermal and plexiform neurofibromas, along with a host of more malignant effects on the nervous system, such as glial tumors and learning disabilities. It has recently been recognized that NF1 patients may also develop vascular complications from what appears to be a diffuse vasculopathy, with most patients affected in multiple arteries. Vessels ranging in size from the proximal aorta to small arterioles are prone to stenosis or occlusion or can even develop arterial aneurysms, although they occur less commonly.<sup>19,36</sup> Clinical information concerning NF1 vasculopathy—including prevalence and, by extension, penetrance—is still limited, but it has been known for  $>2$  decades that premature deaths from cerebrovascular disease and myocardial infarctions occur at a higher frequency in NF1 patients than in the general population.<sup>37,38</sup>

The range of vascular diseases and the end-organ effects that may develop as a result of *NF1* mutations are clearly diverse. Cerebrovascular abnormalities in NF1 patients run the gamut from the Moyamoya syndrome occurring most frequently in younger patients to intracranial aneurysms that occur more often in older patients. Coarctation and aneurysms of the aorta, along with aneurysms developing in other arteries, have been

reported.<sup>39,40</sup> Dysplastic renal artery stenosis is the most frequent occlusive vascular complication brought to clinical attention due to the resulting symptoms of secondary hypertension. Although the risk of developing vascular complications is not well established for individual NF1 patients, the picture of an NF1 vasculopathy emerging from NF1 cohorts fits the scenario described in this review for a genetically driven cause of diffuse and diverse vascular diseases. Thus, we suspect the presence of a hyperplastic vasculomyopathy as the more fundamental disease process at work here.

The nature of the genetic insult may help to explain why this might occur. *NF1* encodes the tumor suppressor molecule neurofibromin, a large cytoplasmic protein and member of the GAP family of Ras regulatory proteins, which functions in part as a negative regulator of Ras.<sup>41</sup> The mutations identified in NF1 patients encode dys- or nonfunctional neurofibromin molecules and effectively disinhibit proliferative Ras signaling. Interestingly, lesions in the medium-sized and smaller arteries of NF1 patients display the familiar appearance of hyperplastic SMCs within the intimal layer that crowd the lumen, thereby linking the haploinsufficiency of neurofibromin with pathologic evidence of SMC proliferation.<sup>42,43</sup> Similar lesions can occur in the largest arteries, where intimal proliferation of SMCs has also been associated with aortic coarctation in an NF1 patient.<sup>40</sup>

Mouse models with partial or complete loss of neurofibromin expression in SMCs provide further insight into the pathogenesis of the NF1 vasculopathy. Aortic SMCs explanted from these mice proliferate significantly more rapidly than those from wild-type littermates.<sup>44,45</sup> In vivo, intact carotid arteries from these mice respond to surgical ligation or guidewire injury with marked SMC hyperproliferation in neointimal lesions. Overactivation of the Ras-Erk signaling pathway is suggested by enhanced immunostaining of phosphorylated Erk (its activated form) in the hyperplastic neointimal SMCs. Most definitively—and perhaps most encouragingly—treating the mice with imatinib (Gleevec), a potent inhibitor of the Ras-Erk signaling cascade (and an approved therapeutic agent for neoplasias in humans) was sufficient to prevent the proliferative SMC response to vascular injury.<sup>46</sup> In summary, the *NF1*-deficient mouse models provide evidence of hyperplastic vascular SMCs both in vitro and in response to vascular injury in vivo. Therefore, it seems plausible that the NF1 vasculopathy in

humans could result from a lack of inhibitory cell cycle regulation by neurofibromin in SMCs, and that this may culminate in vascular occlusion from excessive SMC proliferation.

The NF1 vasculopathy can present at a young age as occlusion in any of a diverse set of arterial beds. The most pure, and the most severe, manifestation of this is the Moyamoya syndrome for which NF1 patients are at increased risk.<sup>19</sup> What remains to be seen is the nature of the injury initiating neointimal proliferation and the emergence of Moyamoya. However, a tantalizing clue may lie in the fact that cranial irradiation for the treatment of brain tumors is a recognized risk factor for Moyamoya both in NF1 patients and in individuals without NF1 mutations, although the sensitivity of NF1 patients is thought to be so great that many practitioners now consider cranial irradiation for treatment of tumors to be contraindicated unless all other available therapies have failed.<sup>47</sup>

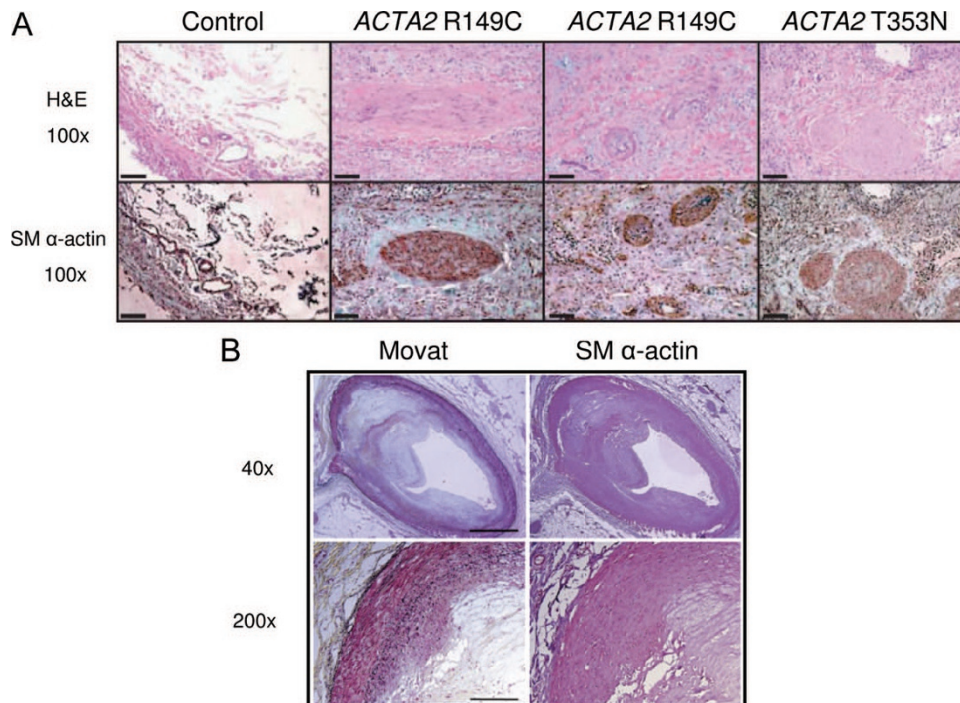
### ACTA2 VASCULOPATHY: A SINGLE-GENE MUTATION LEADING BOTH TO ARTERIAL OCCLUSION AND TO ARTERIAL ANEURYSM

Mutations in the *ACTA2* gene are responsible for 10–15% of all familial thoracic aortic aneurysms and dissections and are currently the most commonly identified cause of an inherited predisposition for thoracic aortic disease.<sup>48</sup> However, it was noted by our group while studying one large family to map the *ACTA2* locus that family members affected by aortic aneurysms and dissections were also marked with an unusual skin finding. These individuals displayed a severe form of livedo reticularis,

a purplish, lacy, net-like rash caused by occlusion of the dermal capillaries. Although it may be precipitated by cold temperatures in normal people, the rash was always present in affected family members, regardless of temperature. In fact, livedo reticularis was completely predictive of all family members harboring an *ACTA2* mutation, both in members with aortic aneurysms and in those with no evidence of aortic disease.<sup>48</sup> This observation provided the first clue that *ACTA2* mutations could also lead to occlusive arterial lesions and may lead to a more diverse range of vascular diseases than was originally obvious.

Further linkage analysis and association studies from 20 families with *ACTA2* mutations began to uncover a concurrent predisposition among mutation carriers for occlusive vascular diseases, including greater-than-expected incidences of early-onset ischemic stroke and coronary artery disease. Surprisingly, our cohort even included an unusual number of cases of primary Moyamoya disease, thereby establishing the first causal link between a genetic mutation and idiopathic Moyamoya disease.<sup>49</sup> These occlusive lesions occur in young and middle-aged adults despite minimal risk factors for vascular disease—namely the risk factors of severe vascular damage associated with atherosclerosis—such as hyperlipidemia, smoking, or diabetes.

Vascular pathology observed in carriers of *ACTA2* mutations include aortic medial findings typically associated with thoracic aortic aneurysms: specifically, a medial degeneration characterized by focal loss of SMCs and elastic fiber disruption and the accumulation of proteoglycans. However, the atypical appearances of focal SMC hyperplasia and disarray were also obvious



**Fig. 4.** Occlusive *ACTA2* arterial pathology. A, The vaso vasorum of patients with *ACTA2* mutations are larger due to SMC hyperplasia, as demonstrated by smooth muscle  $\alpha$ -actin immunostaining, which was absent in the vaso vasorum of an age-matched control (scale bar represents 100  $\mu$ m). Reprinted with permission from *Nat Genet.* 2007;39:1488–1493.<sup>48</sup> B, Coronary artery from a 28-year-old, stained with Movat's stain and smooth muscle  $\alpha$ -actin antibody (upper panels, scale bar represents 1.0 mm; lower panels, scale bar represents 200  $\mu$ m). The artery showed 70% narrowing due to a fibrocellular atherosclerotic plaque. Immunostaining revealed that the fibrocellular accumulation resulted from smooth muscle cells containing smooth muscle  $\alpha$ -actin. Reprinted with permission from *Am J Hum Genet.* 2009;84:617–627.<sup>49</sup>

in other areas of pathologic specimens. Surprisingly, when compared with control aortas, we were able to measure in mutation carriers a significant increase in vessel diameter and thickening in the media of the vasa vasorum, the muscular arteries which course through the adventitial layer of the aorta and supply nutrients to the outermost lamellae of the aortic media. Immunostaining for SMC  $\alpha$ -actin confirmed that the thickening resulted from higher numbers of SMCs in the medial layer of these muscular arteries (Fig. 4).<sup>48</sup>

Pathology slides at autopsy from a 28-year-old mutation carrier who died from an acute aortic dissection also demonstrated clinically significant stenosis (>70%) in a coronary artery due to intimal fibrocellular accumulation (Fig. 4). The presumably atherosclerotic lesion was more cellular and contained far fewer lipids than more typical atherosclerotic plaques. This case represents an extremely compelling example of both diverse and diffuse disease arising within a single patient carrying an *ACTA2* mutation. Unfortunately, arterial lesions from stroke and patients with Moyamoya disease with *ACTA2* mutations were not available for study, but we would expect similar fibrocellular lesions in the vessels of the head and neck, especially in our cases of Moyamoya disease.

Therefore, the clinical characteristics and pathology of the *ACTA2* vasculopathy overlap remarkably with those of the NF1 vasculopathy. In both disorders, a single-gene mutation predisposes to diverse and diffuse vascular diseases dominated by occlusive lesions, although more than occasionally presenting as aneurysms. To explore the possibility that the vasculopathies resemble each other on the cellular level, we determined that SMCs and skin myofibroblasts explanted from patients heterozygous for *ACTA2* mutations also proliferate more rapidly than age- and sex-matched control cells.<sup>49</sup> Also, similar to neurofibromin-deficient mice, *Acta2* null mice display normal cardiovascular system development.<sup>50</sup> It has already been shown that mesangial myofibroblasts explanted from the kidneys of these mice proliferate more rapidly than wild-type cells,<sup>51</sup> whereas experiments performed in our laboratory demonstrate that aortic SMCs explanted from *Acta2* null mice also proliferate more rapidly than those from wild-type littermates (unpublished data). Whether these *Acta2* null mice are similarly sensitive to vascular injury is not yet known. Nonetheless, current data indicate that both *NF1* and *ACTA2* mutations lead to increased proliferation of SMCs both in vitro and in vivo, which corresponds to the diffuse vascular disease found in patients harboring these mutant genes.

## DISCUSSION

We propose that occlusive vascular diseases may exist on a spectrum anchored at one end by atherosclerosis, where a long, slow cascade of events initiated by environmentally derived vascular injury, endothelial dysfunction, and lipid deposition drives vascular remodeling over time. At the other end lies hyperplastic vasculomyopathy as we have described it, similarly driven by vascular injury but instead characterized by a more dynamic process where an underlying genetic predisposition inappropriately favors synthetic rather than contractile SMCs and promotes their increased migration, proliferation, and matrix production. For mutations both in *NF1* and in *ACTA2*, it seems that the genetic insults are not sufficient to cause disease. Rather, these changes may confer a pathologic sensitivity to more subtle injuries. Neither in humans nor in mice do these genetic insults interrupt the development of or overall distribution of blood through the vascular system. Instead, the vasculopathy seems to arise from an exaggerated response to local-

ized postdevelopmental insults to the arteries, as suggested by the NF1 mouse model.<sup>42,45</sup> Similarly, we predict that the largely clinical diagnosis of a patient with an inherited predisposition for hyperplastic vasculomyopathy would involve evidence of occlusive vascular disease occurring at a young age in the absence of obviously injurious vascular risk factors. We would predict neointimal SMC proliferation with minimal lipid deposition or inflammation on pathologic examination and recommend autopsy studies for more definitive diagnosis, especially in the case of an unexplained death within a suspicious familial pattern of diseases.

Vascular occlusions would occur diffusely in the vascular tree, as the genetic alterations conferring the gain of proliferative function would make all arterial SMCs vulnerable to hyperplasia. Although the locations of occlusions within a particular vascular bed would not be random—and would likely be stereotyped to particular vascular geometries, as occurs in atherosclerosis<sup>52</sup>—we predict that virtually any vascular bed could be a possible location for occlusion in these patients. Such is the case in *ACTA2* mutation carriers who simultaneously possess the occlusive livedo rash and the greater-than-average risk for early stroke or myocardial infarction. Such is also the case in the mounting number of patients with Moyamoya who possess arterial stenoses elsewhere in the head or elsewhere in the body. Because it is the most dramatic presentation of an occlusive vascular phenotype originating from SMC hyperplasia, in fact, we predict that primary Moyamoya disease in a young patient with no vascular risk factors may actually be a clinical marker for an underlying genetic predisposition to hyperproliferative arterial SMCs. Although we have discussed here the specific vasculopathies associated with *NF1* and *ACTA2* mutations, we hypothesize that investigations of mutations in many other potential genes will uncover similar relationships between SMC proliferation and the presentation of diffuse vascular disease.

The presence of a hyperplastic vasculomyopathy would also entail the presentations of diverse vascular diseases among affected patients and their family members. This became apparent as we more extensively evaluated our patients with *ACTA2* mutations. We found that individuals with early-onset ischemic stroke shared the same mutation with relatives showing early-onset myocardial infarction and even with relatives presenting with thoracic aortic aneurysms. Previously, it may have been difficult to identify the common thread unifying the various diseases, as patients are often referred to different specialists—such as neurologists or cardiologists—based on the differing end-organ effects of disease arising in the vessels. By establishing the underlying pathogenic framework for hyperplastic vasculopathies, we hope to alter the clinical assessment of early-onset vascular disease, so that more patients can be identified and possibly treated for their more fundamental problems in the vasculature, i.e., problems regulating the proliferation of vascular SMCs.

By this framework of a hyperplastic vasculomyopathy, it is logical that the proliferative tendency of SMCs in *ACTA2* and *NF1* mutation carriers would predispose to occlusive diseases in some arteries, such as the luminal narrowing seen in patients with lipid-poor coronary artery disease or Moyamoya. However, an unanswered question is why these same mutations can also lead to dilation and aneurysm formation in other arteries, as occurs in the intracranial aneurysms in some NF1 patients and the thoracic aortic aneurysms that occur quite frequently in *ACTA2* mutation carriers. It seems that various types of arteries are differentially sensitive to the effect of the underlying mutation, contributing to the diversity of pathology. For example, the differential presence of elastin in elastic arteries, when



compared with muscular arteries, may influence the presentation of genetic vasculomyopathy by influencing the sensitivity of the SMC to the proliferative mutation. As discussed above, elastin is a known potent inhibitor of SMC proliferation, a fact emphasized by the absence of elastin in studies of mice whose genetically engineered elastin deficiencies enable a severe aortic stenosis through increased proliferation of SMCs.<sup>53</sup>

We have previously hypothesized that, for *ACTA2* mutations, an underlying loss of SMC contractility is the fundamental defect leading to aortic aneurysms.<sup>48</sup> *ACTA2* mutations are predicted to disrupt proper structure and/or function of the smooth muscle-specific actin thin filaments that are necessary for contractile interaction with smooth muscle myosin thick filaments.<sup>54</sup> Measurements from *Acta2* knockout mice confirm decreased aortic contractility.<sup>50</sup> At the same time, mutations in other actin isoforms disrupt proper muscle function and result in diseases of contractility, including the *ACTA1* mutations that cause skeletal myopathies and the *ACTC* mutations that have been associated with both dilated and hypertrophic cardiomyopathy.<sup>54</sup>

The aorta bears the major force of pulsatile blood flow from the heart. A loss of function effect on contractility would cause a decreased ability to limit pulse pressure during systole and ensure continuous blood flow to downstream arterial beds during diastole. We hypothesize that the contractile overload sensed by the aortic SMCs may chronically activate vascular “repair” pathways in an attempt to fortify the wall and compensate for the contractile defect. Unfortunately, the chronic activation of matrix degradation and resynthesis may lead to the observed degenerative pathologies and over time to aortic aneurysm formation. Furthermore, pathologic specimens from *ACTA2* mutant patients show dedifferentiated, noncontractile SMCs in the ascending aorta and reveal the loss of lamellar architecture and the disruption of elastic fibers as SMCs proliferate in focal lesions. It is clear that this histologic disarray would also contribute to decreased contractility and to a focal weakening in the aortic wall by disrupting the continuous elastic reservoir necessary for distributing wall stress, creating a local area highly vulnerable to dissection or rupture.

Thus, we are proposing that *ACTA2* mutations result in a gain-of-proliferative-function in SMCs and may lead to occlusive disease in smaller arteries, whereas the relative loss-of-contractile-function may lead to aneurysms in the aorta. At the same time, it is important to note that published data have suggested that hyperproliferative SMCs may contribute to aortic aneurysm formation. A mouse model genetically engineered with a deficiency of low-density lipoprotein receptor-related protein 1 has revealed activation of proliferative PDGF signaling that leads both to SMC hyperplasia and to aortic aneurysms. Importantly, the antiproliferative effects of imatinib (Gleevec) were sufficient to prevent this aneurysm formation.<sup>56</sup> Although the investigators did not determine whether imatinib contributed to the adoption of a contractile phenotype in the mutant SMCs, these results suggest that blocking SMC proliferation may also prevent aneurysm formation in the larger arteries.

The therapeutic implications of a hyperplastic vasculomyopathy are striking, as we have proposed that genetic variants driving SMC proliferation are a common mechanism predisposing affected patients to both occlusive vascular diseases and aneurysms. Thus, we are suggesting a new class of candidate genes to be associated with vascular disease. Furthermore, we suggest that strategies to treat inherited predispositions to occlusive disease, and possibly to prevent aneurysm formation, could similarly rely on treating the underlying predisposition to SMC hyperplasia. In fact, it has already been shown in separate

experiments with genetically vulnerable animal models that both arterial narrowing, by neointimal proliferation, and arterial dilation, by medial degeneration, can be prevented by treatment with imatinib (Gleevec).<sup>46,55</sup> These are exciting results, as this agent is already approved for chronic, preventive use in humans as an antineoplastic agent with an extremely favorable side-effect profile. It is tempting to imagine a similar situation where patients with an identified hyperplastic vasculomyopathy are also chronically treated with imatinib to prevent the progression of vascular disease.

Using the framework of a hyperplastic vasculomyopathy, we hope that practitioners can begin to recognize clustering of diffuse and diverse vascular diseases within the families of their patients and suspect an underlying genetic predisposition, especially when a sentinel case of Moyamoya is involved. We suggest that a family history can be a very powerful tool, not only for genetic counselors, genetic researchers, and specialists in medical genetics, but also for the general physician, in that the mechanisms for disease in any one patient come more clearly into view as patterns emerge among their affected relatives. This was certainly the case in reviewing the vascular disease patterns emerging among those carrying *NF1* and *ACTA2* mutations, and we have every expectation that it will continue to be the case as more genes associated with hyperplastic vasculomyopathy are identified. Furthermore, it seems within reach that the same practitioners could offer screening, particularly in the case of mutations identified in single genes. Management of probands and potentially affected family members may also benefit from periodic imaging of the aorta, intracranial circulation, and coronary arteries, which may identify early lesions in the most critical locations. However, we present the common pathogenic mechanism of SMC hyperproliferation as a tantalizing therapeutic target, which may hold the potential for preventive therapies in the near future, to more effectively treat a wide array of devastating vascular diseases that arise with the genetic predisposition to hyperplastic vasculomyopathy.

## REFERENCES

1. Fauci AS, Kasper DL, Longo DL, et al., editors. Harrison's principles of internal medicine, 17th ed. New York: McGraw-Hill, 2008.
2. Topol EJ, Smith J, Plow EF, Wang QK. Genetic susceptibility to myocardial infarction and coronary artery disease. *Hum Mol Genet* 2006;15: R117–R123.
3. Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb Vasc Biol* 1995;15:1512–1531.
4. Roy H, Bhardwaj S, Yla-Herttuala S. Molecular genetics of atherosclerosis. *Hum Genet* 2009;125:467–491.
5. Wang Q. Molecular genetics of coronary artery disease. *Curr Opin Cardiol* 2005;20:182–188.
6. Kudo T. Spontaneous occlusion of the circle of Willis. A disease apparently confined to Japanese. *Neurology* 1968;18:485–496.
7. Fukui M. Guidelines for the diagnosis and treatment of spontaneous occlusion of the circle of Willis ('moyamoya' disease). Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease) of the Ministry of Health and Welfare, Japan. *Clin Neurol Neurosurg* 1997; 99(suppl 2):S238–S240.
8. Roach ES, Golomb MR, Adams R, et al. Management of stroke in infants and children—a scientific statement from a special writing group of the American Heart Association Stroke Council and the Council on Cardiovascular Disease in the young. *Stroke* 2008;39:2644–2691.
9. Haltia M, Iivanainen M, Majuri H, Puranen M. Spontaneous occlusion of the circle of Willis (Moyamoya Syndrome). *Clin Neuropathol* 1982;1:11–22.
10. Masuda J, Ogata J, Yutani C. Smooth-muscle cell-proliferation and localization of macrophages and T-cells in the occlusive intracranial major arteries in Moyamoya disease. *Stroke* 1993;24:1960–1967.
11. Aoyagi M, Fukai N, Yamamoto M, Nakagawa K, Matsushima Y, Yamamoto K. Early development of intimal thickening in superficial temporal arteries

- in patients with moyamoya disease. *Stroke* 1996;27:1750–1754.
12. Ikeda E. Systemic vascular changes in spontaneous occlusion of the circle of Willis. *Stroke* 1991;22:1358–1362.
  13. Goldberg HJ. Moyamoya associated with peripheral vascular occlusive disease. *Arch Dis Childhood* 1974;49:964–966.
  14. Mineharu Y, Liu W, Inoue K, et al. Autosomal dominant moyamoya disease maps to chromosome 17q25.3. *Neurology* 2008;70:2357–2363.
  15. Inoue TK, Ikezaki K, Sasazuki T, Matsushima T, Fukui M. Linkage analysis of moyamoya disease on chromosome 6. *J Child Neurol* 2000;15:179–182.
  16. Nanba R, Tada M, Kuroda S, Houkin K, Iwasaki Y. Sequence analysis and bioinformatics analysis of chromosome 17q25 in familial moyamoya disease. *Childs Nerv Syst* 2005;21:62–68.
  17. Ikeda H, Sasaki T, Yoshimoto T, Fukui M, Arinami T. Mapping of a familial moyamoya disease gene to chromosome 3p24.2-p26. *Am J Hum Genet* 1999;64:533–537.
  18. Sakurai K, Horiuchi Y, Ikeda H, et al. A novel susceptibility locus for moyamoya disease on chromosome 8q23. *J Hum Genet* 2004;49:278–281.
  19. Friedman JM, Arbiser J, Epstein JA, et al. Cardiovascular disease in neurofibromatosis 1: report of the NF1 Cardiovascular Task Force. *Genet Med* 2002;4:105–111.
  20. Spengos K, Kosmaidou-Aravidou Z, Tsvigoulis G, Vassilopoulou S, Grigori-Kostaraki P, Zis V. Moyamoya syndrome in a Caucasian woman with Turner's syndrome. *Eur J Neurol* 2006;13:e7–e8.
  21. Gaba RC, Shah RP, Muskovitz AA, Guzman G, Michals EA. Synchronous moyamoya syndrome and ruptured cerebral aneurysm in Alagille syndrome. *J Clin Neurosci* 2008;15:1395–1398.
  22. Imaizumi M, Nukada T, Yoneda S, Takano T, Hasegawa K, Abe H. Tuberosclerosis with moyamoya disease. Case report. *Med J Osaka Univ* 1978;28:345–353.
  23. Schuster JM, Roberts TS. Symptomatic moyamoya disease and aortic coarctation in a patient with Noonan's syndrome: strategies for management. *Pediatr Neurosurg* 1999;30:206–210.
  24. Kawai M, Nishikawa T, Tanaka M, et al. An autopsied case of Williams syndrome complicated by moyamoya disease. *Acta Paediatr Jpn* 1993;35:63–67.
  25. Ahuja P, Sdek P, MacLellan WR. Cardiac myocyte cell cycle control in development, disease, and regeneration. *Physiol Rev* 2007;87:521–544.
  26. Strohmman RC, Paterson B, Fluck R, Przybyla A. Cell fusion and terminal differentiation of myogenic cells in culture. *J Anim Sci* 1974;38:1103–1110.
  27. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* 2004;84:767–801.
  28. Brisset AC, Hao H, Camenzind E, et al. Intimal smooth muscle cells of porcine and human coronary artery express S100A4, a marker of the rhomboid phenotype in vitro. *Circ Res* 2007;100:1055–1062.
  29. Owens GK. Regulation of differentiation of vascular smooth muscle cells. *Physiol Rev* 1995;75:487–517.
  30. Wagenseil JE, Mecham RP. Vascular extracellular matrix and arterial mechanics. *Physiol Rev* 2009;89:957–989.
  31. Karnik SK, Brooke BS, Bayes-Genis A, et al. A critical role for elastin signaling in vascular morphogenesis and disease. *Development* 2003;130:411–423.
  32. Majesky MW. Developmental basis of vascular smooth muscle diversity. *Arterioscler Thromb Vasc Biol* 2007;27:1248–1258.
  33. Hirschi KK, Majesky MW. Smooth muscle stem cells. *Anat Rec A Discov Mol Cell Evol Biol* 2004;276:22–33.
  34. Jiang X, Rowitch DH, Soriano P, McMahon AP, Sucov HM. Fate of the mammalian cardiac neural crest. *Development* 2000;127:1607–1616.
  35. Stoller JZ, Epstein JA. Cardiac neural crest. *Semin Cell Dev Biol* 2005;16:704–715.
  36. Rosser TL, Vezina G, Packer RJ. Cerebrovascular abnormalities in a population of children with neurofibromatosis type 1. *Neurology* 2005;64:553–555.
  37. Sorensen SA, Mulvihill JJ, Nielsen A. Long-term follow-up of von Recklinghausen neurofibromatosis. Survival and malignant neoplasms. *N Engl J Med* 1986;314:1010–1015.
  38. Rasmussen SA, Yang Q, Friedman JM. Mortality in neurofibromatosis 1: an analysis using U.S. death certificates. *Am J Hum Genet* 2001;68:1110–1118.
  39. Chew DK, Muto PM, Gordon JK, Straceski AJ, Donaldson MC. Spontaneous aortic dissection and rupture in a patient with neurofibromatosis. *J Vasc Surg* 2001;34:364–366.
  40. Oderich GS, Sullivan TM, Bower TC, et al. Vascular abnormalities in patients with neurofibromatosis syndrome type I: clinical spectrum, management, and results. *J Vasc Surg* 2007;46:475–484.
  41. Dasgupta B, Gutmann DH. Neurofibromatosis 1: closing the GAP between mice and men. *Curr Opin Genet Dev* 2003;13:20–27.
  42. Hamilton SJ, Friedman JM. Insights into the pathogenesis of neurofibromatosis 1 vasculopathy. *Clin Genet* 2000;58:341–344.
  43. Stewart DR, Cogan JD, Kramer MR, et al. Is pulmonary arterial hypertension in neurofibromatosis type 1 secondary to a plexogenic arteriopathy? *Chest* 2007;132:798–808.
  44. Li F, Munchhof AM, White HA, et al. Neurofibromin is a novel regulator of RAS-induced signals in primary vascular smooth muscle cells. *Hum Mol Genet* 2006;15:1921–1930.
  45. Xu J, Ismat FA, Wang T, Yang J, Epstein JA. NF1 regulates a Ras-dependent vascular smooth muscle proliferative injury response. *Circulation* 2007;116:2148–2156.
  46. Lasater EA, Bessler WK, Mead LE, et al. Nf1 +/- mice have increased neointima formation via hyperactivation of a Gleevec sensitive molecular pathway. *Hum Mol Genet* 2008;17:2336–2344.
  47. Ullrich NJ, Robertson R, Kinnamon DD, et al. Moyamoya following cranial irradiation for primary brain tumors in children. *Neurology* 2007;68:932–938.
  48. Guo DC, Pannu H, Papke CL, et al. Mutations in smooth muscle alpha-actin (*ACTA2*) lead to thoracic aortic aneurysms and dissections. *Nat Genet* 2007;39:1488–1493.
  49. Guo DC, Papke CL, Tran-Fadulu V, et al. Mutations in smooth muscle alpha-actin (*ACTA2*) cause coronary artery disease, stroke, and moyamoya disease, along with thoracic aortic disease. *Am J Hum Genet* 2009;84:617–627.
  50. Schildmeyer LA, Braun R, Taffet G, et al. Impaired vascular contractility and blood pressure homeostasis in the smooth muscle alpha-actin null mouse. *FASEB J* 2000;14:2213–2220.
  51. Takeji M, Moriyama T, Oseto S, et al. Smooth muscle alpha-actin deficiency in myofibroblasts leads to enhanced renal tissue fibrosis. *J Biol Chem* 2006;281:40193–40200.
  52. Cunningham KS, Gotlib AI. The role of shear stress in the pathogenesis of atherosclerosis. *Lab Invest* 2005;85:9–23.
  53. Li DY, Brooke B, Davis EC, Mecham RP, et al. Elastin is an essential determinant of arterial morphogenesis. *Nature* 1998;393:276–280.
  54. Milewicz DM, Guo D, Tran-Fadulu V, et al. Genetic basis of thoracic aortic aneurysms and dissections: focus on smooth muscle cell contractile dysfunction. *Annu Rev Genomics Hum Genet* 2008;9:283–302.
  55. Boucher P, Gotthardt M, Li WP, Anderson RG, Herz J. LRP: role in vascular wall integrity and protection from atherosclerosis. *Science* 2003;300:329–332.