

Review Article

The complex genetic basis of fibromuscular dysplasia, a systemic arteriopathy associated with multiple forms of cardiovascular disease

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Artery stenosis is a common cause of hypertension and stroke and can be due to atherosclerosis accumulation in the majority of cases and in a small fraction of patients to arterial fibromuscular dysplasia (FMD). Artery stenosis due to atherosclerosis is widely studied with known risk factors (e.g. increasing age, male gender, and dyslipidemia) to influence its etiology, including genetic factors. However, the causes of noninflammatory and nonatherosclerotic stenosis in FMD are less understood. FMD occurs predominantly in early middle-age women, a fraction of the population where cardiovascular risk is different and understudied. FMD arteriopathies are often diagnosed in the context of hypertension and stroke and co-occur mainly with spontaneous coronary artery dissection, an atypical cause of acute myocardial infarction. In this review, we provide a comprehensive overview of the recent advances in the understanding of molecular origins of FMD. Data were obtained from genetic studies using complementary methodological approaches applied to familial, syndromic, and sporadic forms of this intriguing arteriopathy. Rare variation analyses point toward mechanisms related to impaired prostacyclin signaling and defaults in fibrillar collagens. The study of common variation, mainly through a recent genome-wide association study, describes a shared genetic link with blood pressure, in addition to point at potential risk genes involved in actin cytoskeleton and intracellular calcium homeostasis supporting impaired vascular contraction as a key mechanism. We conclude this review with future strategies and approaches needed to fully understand the genetic and molecular mechanisms related to FMD.

Introduction

Fibromuscular dysplasia (FMD) is an idiopathic disease of middle-size arteries, defined by the presence of stenosis due to the thickening of the vascular wall, in the absence of any signs of atheroma deposition, inflammation, or other stenotic disease [1]. FMD has been estimated to affect ~3–4% of the general population [2], but it is often asymptomatic and incidentally detected on angiographical imaging. Diagnosis is usually delayed for several years and mostly obtained following a stroke event or during clinical investigations in hypertensive patients [3,4]. The most frequently affected arterial beds are the renal, carotid, and cervical arteries, although FMD lesions have been described in most muscular arteries such as mesenteric, iliac, splenic, and more rarely in coronary arteries [3,4]. These lesions are commonly found in more than one arterial bed in the same patient, suggesting FMD as a systemic arteriopathy [5]. FMD is also frequently associated to aneurysm, dissection, and arterial tortuosity [3,4], although this type of lesions alone is not considered sufficient for a formal diagnosis in the absence of stenosis [1].

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FMD phenotypic classification is essentially based on imaging. Two main FMD subtypes have been described based on the appearance of FMD lesions from arterial imaging through computed tomography, magnetic resonance, and digital subtraction angiography. Multifocal FMD is characterized by extended arterial lesions with succession of stenosis and dilation with the typical ‘string-of-beads’ phenotype, whereas focal FMD is defined by an isolated stenosis [1]. Multifocal FMD represents approximately 85% of FMD cases and affects predominantly women in a 9:1 ratio, with a mean age at diagnosis between 45 and 55 years [3,4]. Focal FMD is less common, more sex-balanced, and usually presents at a younger age with more severe stenosis and symptoms [6].

There is very little knowledge available about the natural history of FMD lesions. FMD lesions can be detected at any age and are frequently asymptomatic, and thus the context of FMD pathogenesis is generally not known. Given the atypical clinical presentation of FMD and challenging imaging needed for diagnosis, patients are likely to have a delayed diagnosis. Nonetheless, multifocal FMD is rare in pediatric patients, suggesting the lesions mostly arise during early adulthood [7]. A role for female hormones in the development of FMD is suspected but clear evidence for the underlying mechanisms is missing so far [1]. The evolution of FMD lesions is not known and current recommendations include regular follow-up and imaging of affected arterial beds [1]. In this context, investigating the genetic basis of FMD presented as the most promising approach to attempt to unravel the biological mechanisms behind the origin of FMD specific lesions and health complications.

In this review, we will provide a brief recap about the clinical presentation of FMD in the context of common and rare vascular diseases and the current hypotheses of its pathophysiology. In addition to highlighting the challenging features of its genetic model, we will focus on recent advances about our understanding of rare genetic causes of FMD that emerged from the study of familial and syndromic forms. We will then detail the role of common variation in FMD genetic risk that we have recently established. Finally, we will discuss leads provided from these genetic insights to comprehend the links with more common cardiovascular disease and the still challenging aspects of sex-imbalance toward women in FMD. Future directions of research will be suggested to counteract the limitations of the current knowledge about molecular mechanisms of this important and understudied vascular disease.

FMD presents clinically in the context of common cardiovascular diseases

FMD co-occurs with a diverse panel of rare and common cardiovascular diseases. The development of large patient repositories and increasing awareness of FMD allowed to characterize these associations, which were suspected from patient’s series for decades. Hypertension is reported in 72–86% of FMD patients in US and European registries, respectively, and is the most common presentation of FMD [3,4]. Hypertension may be caused by a high degree of stenosis in the renal artery, resulting in a reduced blood flow to the kidney and activation of the renin–angiotensin system, consistent with the renal artery stenosis caused by FMD lesions. Restoration of blood flow using balloon angioplasty usually improves blood pressure in patients [8], but the long delay (9 years on average) between the onset of hypertension and FMD diagnosis may also cause the development of essential hypertension and reduce the efficacy of treatments. Moreover, FMD may be found incidentally during renal artery imaging in hypertensive patients and the causal relationship between FMD lesion and hypertension is not always established. Another frequently co-occurring condition in FMD is headache, often of the migraine type, which is mentioned by 15–60% of FMD patients [3–5]. Similar to FMD, migraine affects most frequently women, although the sex ratio is less biased (2 to 3:1 ratio). Migraine is a multifactorial disease with underlying neurological and vascular pathophysiological mechanisms. Migraine is also a known risk factor for several cardiovascular disorders, especially in women [9]. Unlike hypertension, migraine is not a major presentation of FMD, leading to less than 10% of FMD diagnosis [3–5]. FMD is also frequently associated with arterial dissections in the context of cervical artery dissection (CeAD) and spontaneous coronary artery dissection (SCAD). CeAD is a major cause of ischemic stroke in young to middle-age patients [10]. Contrary to FMD, CeAD affects slightly more men than women and male sex was found as a risk factor for CeAD occurrence in FMD patients [11,12]. Co-occurring cerebrovascular FMD is found in 8% of CeAD patients and is a predictor for CeAD recurrence [13]. SCAD is an increasingly recognized cause of acute coronary syndrome leading to myocardial infarction and may be responsible for up to 35% of acute coronary syndromes in women under 50 years [14]. SCAD is caused by the formation of an intramural hematoma leading to the partial or total obstruction of the true lumen of a coronary artery, sometimes associated with an intimal tear. While SCAD is uncommon in FMD patients, several recent studies identified extra-coronary FMD in 35–70% of SCAD patients, pointing to a potential mechanistic link between these conditions [15–18]. Overall, the diversity of FMD presentation suggests its potential role in the etiology of a large number of cardiovascular diseases. The semi-invasive imaging required for the diagnosis is a major limitation to its

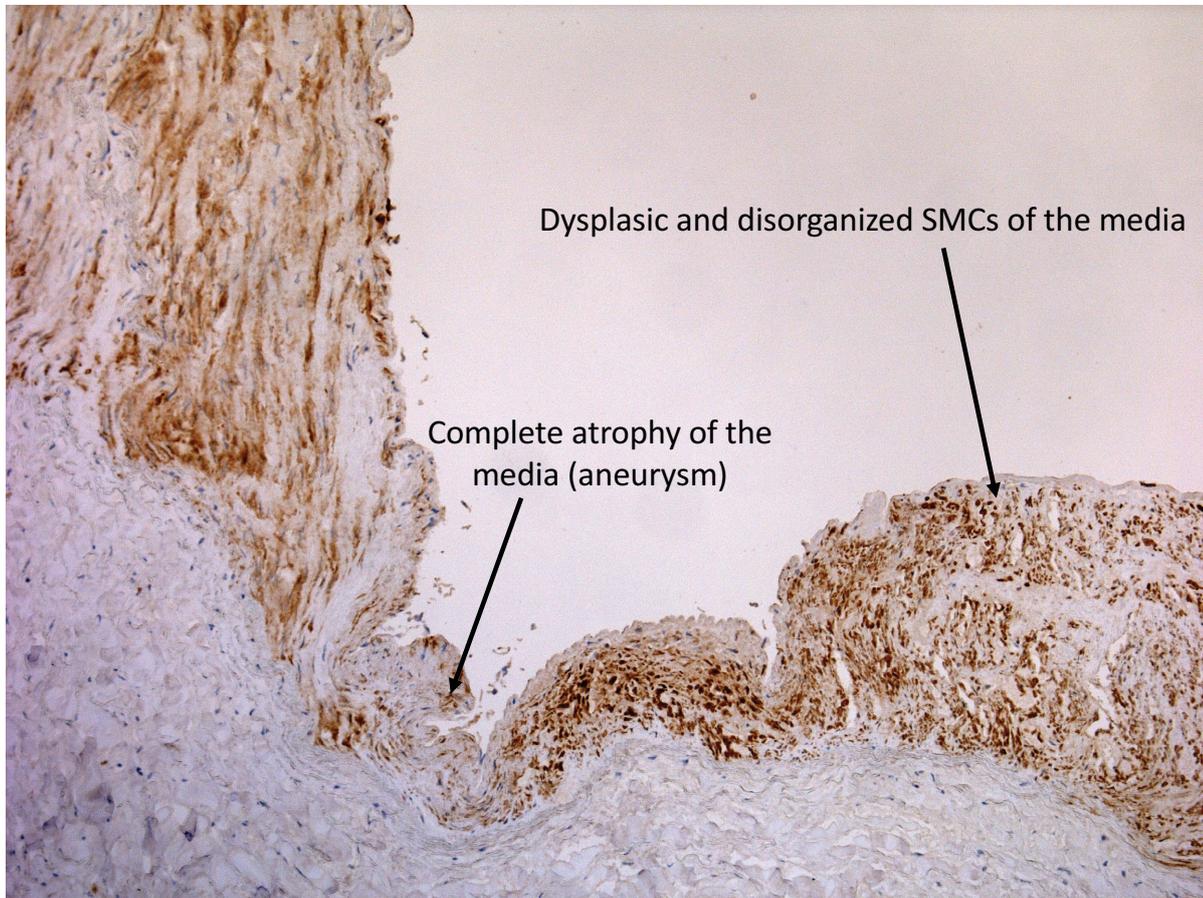


Figure 1. Myosin heavy chain 11 (MYH11) immunostaining of renal artery from 56-year-old woman presenting FMD lesions (Courtesy from Patrick Bruneval, HEGP Pathology Department)

early detection, which slows both diagnosis and adequate treatment, particularly in the case of resistant hypertension. This supports the necessity to search for specific clinical and genetic markers for FMD.

Systemic alterations of arterial physiology in FMD patients

Histological examination of FMD lesions was the main source of indications about the potential mechanisms of FMD for several decades. The most frequently identified phenotype is a global disorganization of the tunica media in affected arteries, termed medial fibroplasia, accounting for the majority of examined cases and corresponding to the multifocal angiographic phenotype [6,19] (Figure 1). Tunica media is composed mostly of smooth muscle cells (SMCs) and is responsible for vessel integrity and the regulation of vascular tone. In normal arteries, SMCs display an elongated shape and are arranged perpendicularly to the blood flow [20]. Conversely, SMCs in dysplastic lesions show more irregular shape and organization, and patches of fibrous tissue are generally visible between SMCs [19]. A rupture of the internal elastic lamina is also a typical marker of FMD lesions, suggesting that structural damage of traumatic origin or degradation of elastin may participate to the pathogenesis of FMD.

Several recent studies attempted to identify biological and imaging markers aiming to simplify FMD diagnosis that heavily relies on semi-invasive and complex imaging, often coupled with prior awareness of its clinical specificities. In a recent study, Olin and colleagues identified 37 proteins and 10 lipids to be detected differentially in the plasma of 90 female FMD patients compared with age and sex matched controls [21]. Global low levels of triglycerides, HDL and LDL cholesterol were observed in plasmas of FMD patients, which was consistent with the absence of dyslipidemia in FMD presentation. Another study by Latosinka and colleagues identified an increase of collagen degradation products in the urine of a cohort of 23 (19 women, 4 men) FMD patients, suggesting that an increased turnover of collagen is associated with FMD pathogenesis [22]. Combined scores using either plasma proteins and lipids or urine biomarkers were generated in these studies and provide promising leads toward improved earlier detection of FMD [21,22].

Another type of biomarkers was also suggested from less invasive imaging techniques, such as high-resolution echo-tracking. This method was applied to examine nonclinically affected radial and carotid arteries in 70 renal FMD patients (90% women) and revealed that patients had thicker carotid and radial arteries [23]. Additionally, the presence of echo-acoustic inhomogeneities (additional interfaces or ruptures in the of the blood–intima acoustic interface) was more frequently seen in FMD cases than in controls [23]. Through a more physiological exploration, recent imaging-based examination of systemic arterial involvement in FMD patients, who were matched to hypertensive patients and healthy control, showed an impaired endothelium-independent dilation and reduced brachial artery diameter in patients with FMD [24]. Altogether, these studies suggest that a systemic alteration of arterial physiology may occur in FMD, including outside of the mainly affected arterial beds that are the renal and cervical arteries. However, these clinical approaches did not provide a major insight into the pathophysiological mechanisms of FMD nor demonstrated potential inherited and environmental risk factors. In the absence of longitudinal studies assessing the development and evolution of FMD lesions, genetics appeared as an alternative to provide clues into the mechanisms underlying FMD.

Defining the genetic model for FMD and candidate gene studies

Familial occurrence of FMD has been recurrently reported since the 70s, and represents 3–7% of all FMD cases in modern registries [3–5]. However, most known families are generally small (e.g. sibpairs, mother and daughter pairs), which has highly limited the application of powerful genetic linkage studies to identify potential genetic causes. This may be explained by the incomplete penetrance of symptoms in FMD and the lack of justification to conduct semi-invasive diagnosis procedures in seemingly healthy relatives [1]. To circumvent this limitation, Perdu and colleagues used high resolution multidimensional echo-tracking of the carotid artery to detect subclinical arterial lesions in FMD patients and relatives, unaffected controls and sporadic FMD patients [25]. They found that FMD patients, both from familial and sporadic cases, had a significantly higher ‘arterial score’ than controls. Interestingly, unaffected relatives also obtained higher arterial scores than controls, although lower than FMD cases, suggesting that an underlying arterial phenotype could be used as a proxy for FMD detection and diagnosis, and ultimately help identifying FMD in relatives using non-invasive procedures. This method has, however, a limited discrimination power to distinguish between intima-media thickening due to atherosclerosis plaque deposition and media thickening specific to FMD lesions [25]. Efforts making use of more modern and optimized imaging techniques are needed to consider large-scale screening applications.

In the absence of linkage studies, several candidate gene studies were applied to relatively small samples of FMD patients. The search for mutations in candidate genes was motivated by the occurrence of vascular abnormalities mimicking FMD stenosis, aneurysm, or vascular tortuosity in Mendelian connective tissue disorders such as Marfan, Loeys-Dietz, vascular Ehlers-Danlos, or type I neurofibromatosis syndromes [26–28]. It is, however, unclear whether these lesions arise from the same pathophysiological mechanisms [28,29]. Unfortunately, genetic screening studies targeting these genes, mainly, *FBNI*, *ACTA2*, *SMAD3*, *TGFBR1/2*, and *COL3A1* only identified mutations of uncertain significance in few FMD patients [30,31]. Interestingly, elevated plasma levels of TGF- β 1 and TGF- β 2 were detected in a cohort of 47 (43 women) FMD patients, suggesting common features with the spectrum of genetic syndromes that involve altered TGF- β (e.g. Loeys-Dietz syndrome) [31]. However, a recent study found no enrichment for mutations in genes in the TGF- β pathway in FMD patients [32].

The constitution of the US and the European registries, in addition to few nation-wide cohorts of FMD patients, were instrumental to conceive powerful genetic studies for FMD. On the one hand, family-based studies associated with screening in larger cohorts of patients enabled the identification of rare genetic variants that were demonstrated to be enriched among FMD patients, while other efforts came from the study of syndromes involving FMD as a primary manifestation. On the other hand, the very low yield of mutations screen in sporadic FMD cases from registries and the recent re-evaluation of FMD prevalence motivated the use of population-based approaches such as genome-wide association studies (GWAS), leading to the identification of several susceptibility loci. Altogether, these efforts detailed below showed that FMD follows a complex genetic pattern of inheritance, with rare and common variants potentially involved in its genetic architecture.

Insights from exome sequencing studies

The first exome sequencing study on FMD analyzed sequences from 16 familial FMD cases (7 families, involving sib-pairs and sib-trios, 14 women, 2 men) recruited from the Rare Vascular Diseases Referral Centre of the European

Hospital Georges Pompidou (HEGP) [33]. This study identified no gene with protein-changing variants shared between three or more families, which ruled out the existence of a major and obvious causal gene in the context of these small familial settings. Potential mutations in known causal genes for connective tissue diseases in these families were also ruled out [33]. Nonetheless, the follow-up of genes with at least four identified variants using genotypes of rare variants from 249 sporadic cases and 689 controls showed a nominal gene-based association for several genes including the myosin light chain kinase (*MYLK*), previously reported to harbor mutations in familial forms of thoracic aorta aneurysm [34], obscurin (*OBSCN*), dynein cytoplasmic heavy chain 1 (*DYNC2H1*) and E3 ubiquitin-protein ligase RNF213 (*RNF213*), associated with MoyaMoya disease, a progressive cerebral angiopathy involving intracranial carotid artery stenosis [35]. Further replication in larger series of patients is required to confirm the real implication of these genes in FMD genetic risk.

An interesting genetic and molecular mechanism for FMD emerged in a follow-up study from the same recruitment center, where a sample of 29 exomes partially overlapping with the previous study was investigated. Here, exome sequences were combined from FMD familial cases formed by siblings, cousins' pair, and one family with five affected sibs, in addition to sporadic cases, including two trios [33,36]. The analyses were focused on rare loss-of-function (LoFs) alleles identified and applied an automated literature-based prioritization algorithm to narrow down a list of 10 candidate genes. Aiming to identify more LoFs carriers among patients, candidate genes were sequenced in 374 unrelated FMD patients, mostly multifocal FMD, which led to the identification of an additional LoF in one of the prioritized genes, prostaglandin I₂ receptor (*PTGIR*). *PTGIR*, which encodes the receptor for prostacyclin, a potent vasodilator hormone and a highly relevant candidate gene [37], was Sanger sequenced in ~1000 FMD patients from three recruiting centers from Europe and the U.S.A., in addition to look up for LoFs in exome sequences from 264 additional FMD patients from the U.S.A. Altogether, six unrelated patients were found to carry LoF alleles of *PTGIR*. Although rare genetic events, LoFs in *PTGIR* were described in unselected individuals in the gnomAD database. Nonetheless, *PTGIR* showed a significant enrichment for LoFs in FMD patients compared to gnomAD controls [36]. Of note, *PTGIR* also harbored four rare missense mutations in other FMD patients that were functionally characterized in human embryonic kidney cells. Among these, at least one missense variant, p.Leu67Pro, showed a significant reduction in sensitivity to Iloprost, an analog of prostacyclin. Patients carrying *PTGIR* mutations were all women and did not present any specific clinical features related to FMD presentation suggesting potentially more subtle clinical specificities of impaired signaling of this receptor to be at play.

Genetic basis of FMD presenting in rare syndromes

Two recent studies identified likely causal genes in syndromic arterial diseases involving severe arterial dysplasia suggesting common mechanistic grounds with FMD. Grange syndrome was described in 1998 as an autosomal recessive syndromic disease involving early-onset progressive occlusion or stenosis of middle-size arteries, notably renal artery lesions causing hypertension and cerebral artery lesions resulting in transient ischemic stroke events [38]. Based on affected arterial beds and imaging, these lesions were classified as FMD-like, with occurrences of both multifocal and focal types of lesions [39]. Exome-sequencing analysis of three affected siblings (two women and one man) identified compound heterozygous protein-truncating variants in the gene coding for YY1-associated protein 1 (*YY1AP1*). Sequencing of *YY1AP1* in three unrelated patients suspected to suffer from Grange syndrome (one man and two women with FMD-like arteriopathies) showed the presence of homozygous LoFs in all three cases, with heterozygous mutations in parents confirming the recessive mode of inheritance [39]. At least four studies identified compound heterozygous mutations of *YY1AP1* in Grange syndrome patients, further confirming *YY1AP1* as the only known causal gene for Grange syndrome [40–43]. Most identified mutations are protein-truncating variants with only one example of homozygous missense variants [40]. Given the severity of vascular alterations in Grange syndrome patients, a lookup in exome sequences of 286 sporadic multifocal FMD patients (96% women) for heterozygous mutations in *YY1AP1* identified one heterozygous protein-truncating mutation whereas no mutation was identified in 282 controls [39]. Further analysis of *YY1AP1* in larger samples is however required to determine if there is a significant burden of *YY1AP1* mutations in FMD. YY1AP1 was initially identified as a coactivator of ying yang 1 (YY1), a multifunctional transcription factor with ubiquitous expression [44]. Functional analysis using short hairpin RNAs in immortalized human SMCs showed that *YY1AP1* was required for the induction of SMC markers elicited by TGF- β treatment (e.g. smooth muscle actin, Figure 1). YY1AP1 deficiency also led to decreased cell proliferation associated with increased expression of cyclin-dependent kinase p21 and G2 cell cycle arrest [39]. The authors hypothesize that the loss of SMC-differentiation participates in vascular remodeling, although it remains unclear how defects in *YY1AP1* may result in the observed vascular phenotypes. One major limitation to functional studies on this gene is the absence of a *YY1AP1* ortholog in rodents, preventing the use of mice or rat animal models. Introduction of

the identified pathogenic mutations in controlled human cell lines (e.g. induced pluripotent stem cells) could help further investigations on the mechanisms of Grange syndrome and FMD-like arteriopathy.

Recently, Richer and colleagues described a recurrent *COL5A1* missense mutation (p.Gly514Ser) in four unrelated patients (three women, one man) presenting with arterial aneurysms, dissections, tortuosity and multifocal FMD affecting multiple arteries [45]. While *COL5A1* mutations account for approximately 80% of classical Ehlers-Danlos syndrome (cEDS) cases [46], only one of the patients fulfilled the criteria for cEDS, which is characterized by hyper-extensible skin, hypermobile joints and poor wound healing. Medial fibroplasia could be confirmed histologically in two patients, with disorganization of SMCs, patches of fibrous tissue and ruptures in the elastin layer in affected arteries. Further examination of *COL5A1* sequence in 264 multifocal FMD patients identified 6 additional *COL5A1* rare variants predicted as deleterious, while no predicted deleterious variants were identified in 284 controls. Altogether, these results support *COL5A1* variants as potential cause of a systemic arterial disease involving arterial dissection, tortuosity and FMD, outside of the typical cEDS clinical pattern. Further research on FMD and dissection would highly benefit from an accurate molecular classification of these variants, together with the identification of potential gene modifiers explaining the variable penetrance of connective tissue and arterial manifestations in the affected patients.

Genetic susceptibility to FMD is mostly determined by common genetic variation

The first susceptibility locus for FMD was identified in 2016 under the hypothesis that common genetic variants involved in common cardio-metabolic traits may play a role in FMD genetic risk. In a two-stage case-control study, we analyzed the association of ~26,000 common variants in 249 FMD patients and 689 controls and replicated in a total of 1154 cases (85% women) and 3895 controls from four case-control studies [47]. The top associated variant was rs9349379 and is located in an intronic region to *PHACTR1* on chromosome 6. This variant is an expression quantitative trait locus (eQTL) for *PHACTR1* in artery tissue and is located ~50 kb upstream of the promoter reported to be active in artery tissue [48]. Significant correlations of rs9349379 genotype with *PHACTR1* expression were also reported in primary human macrophages, primary human fibroblasts and induced pluripotent stem cells (iPSCs)-derived endothelial cells (ECs) [47,49,50]. rs9349379 is located in an arterial specific regulatory element, and its genotype interferes with the binding of myocyte-specific enhancer factor 2 (MEF2) transcription factors, an essential group of regulators for vascular homeostasis through specific functions in ECs [51], SMCs [52], and macrophages [53]. It was proposed that rs9349379 may regulate the expression of *EDN1*, the gene coding for endothelin-1, located 600 kb of the variant, in stem cell-derived ECs [54]. In addition, rs9349379-G allele was associated with higher plasmatic concentration of endothelin-1 and a correlation of rs9349379 with plasmatic endothelin-1 was found in healthy subjects [54], as well as in patients with angina pectoris [55] and SCAD patients [56], although this result could not be replicated in FMD patients and healthy controls [21]. The correlation of rs9349379 with *EDN1* expression could not be observed in iPSC-derived ECs in another study [50]. The link of rs9349379 to *EDN1* expression is unclear, as recent single-cell approaches examining chromatin accessibility in multiple human tissues revealed that while the region encompassing rs9349379 was highly accessible in vascular SMCs and macrophages, they were not accessible in ECs, the main site for *EDN1* transcription [57]. *PHACTR1*, on the other hand, is involved in the regulation of actin stress fibers assembly [58], which play an important role in the motility and contractility of SMCs [59]. Recently, a role for *PHACTR1* in efferocytosis, i.e. clearance of apoptotic cells by macrophages, was demonstrated in human and mouse cells proposing that reduced macrophage expression of *PHACTR1* may participate to CAD risk by promoting necrosis in atheromatous plaques [60]. The specific mechanisms through which rs9349379 may affect the susceptibility to FMD and arterial dissection remain however elusive.

Following the identification of a common variant associated to FMD, the first GWAS meta-analysis of FMD was performed, based on data from six case-control studies from Europe and the U.S.A. [61]. Through the analysis of ~5.5 million common genetic variants (minor allele frequency > 1%) in 1556 FMD cases and 7100 controls, and analyses were focused on multifocal FMD, the most common imaging phenotype [61]. A global SNP-based heritability was reported to be ~0.43, confirming the substantial contribution of polygenic heritability in FMD genetic risk. Four independent genetic risk loci were identified, including *PHACTR1*. The other three loci are located on chromosome 12 close to LIM domain and actin-binding protein 1 (*LIMA1*), low-density lipoprotein receptor-related protein 1 (*LRP1*), and plasma membrane calcium-transporting ATPase1 (*ATP2B1*) genes. *LRP1* and *ATP2B1* were identified as bona-fide target genes based on Bayesian colocalization analyses using arterial correlation with gene expression data, whereas several candidate genes were identified at the *LIMA1* locus including *LIMA1* itself, cyclic

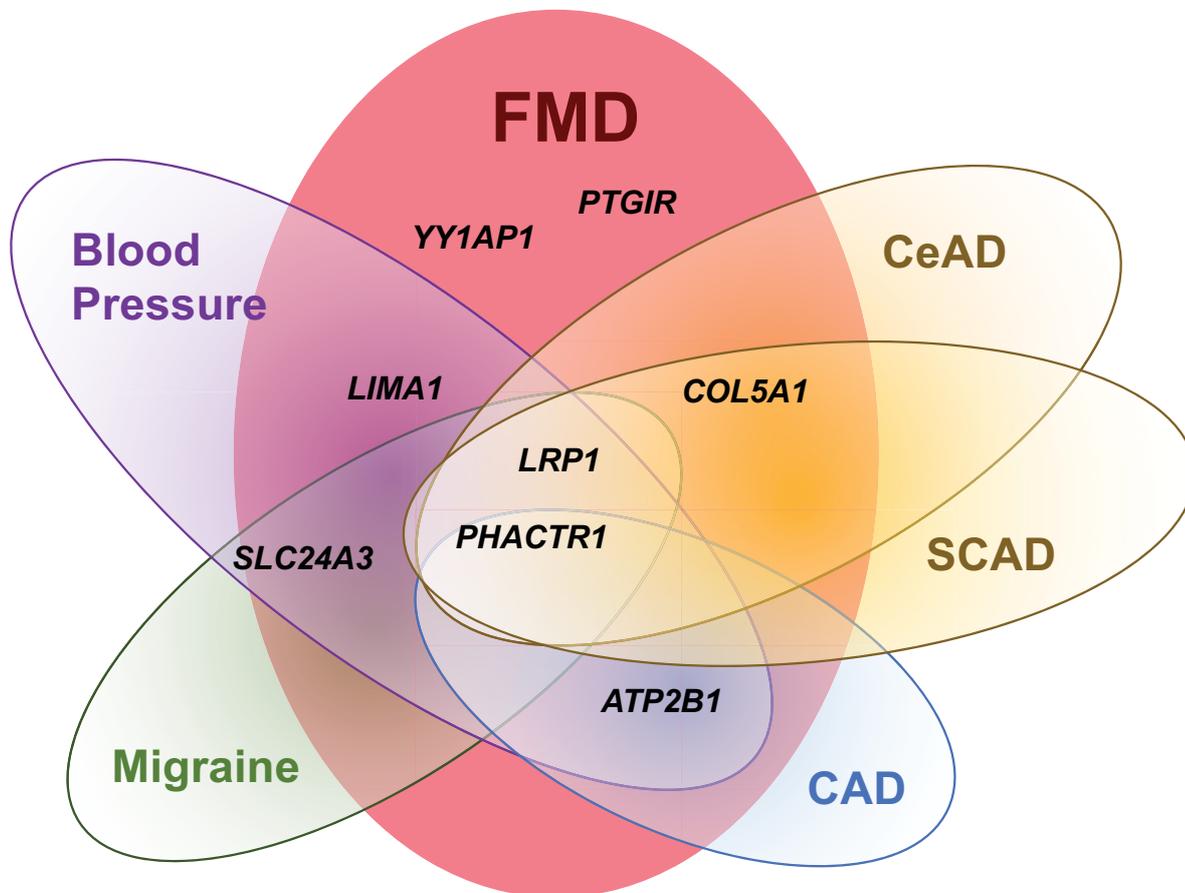


Figure 2. Venn diagram summarizing the genetic overlap between FMD and associated cardiovascular diseases

AMP-dependent transcription factor ATF1 (*ATF1*) and SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1 (*SMARCD1*) (Table 2). Integration of genetic and arterial expression datasets through transcriptome-wide association analysis led to the identification of another gene associated to multifocal FMD, sodium/potassium/calcium exchanger 3 (*SLC24A3*), located on chromosome 20 [61].

In summary, FMD genetic risk is substantially determined by common genetic variations, although only a small fraction (five risk loci) were described so far. Current functional annotation support four genes to be confidently predicted as target genes of at those loci, namely *PHACTR1*, *LRP1*, *ATP2B1* and *SLC24A3*. These findings provided useful unbiased information about the potential mechanisms involved in FMD pathogenesis and allowed the exploration of genome-wide links with clinically overlapping vascular diseases.

Genetic data supports etiological links between FMD and common vascular diseases

FMD loci overlap with genetic risk loci for several vascular diseases

A striking common feature of the genetic determinants of FMD identified so far is the systematic overlap with other cardiovascular or neurovascular diseases, both for rare (Table 1) and common genetic variants (Table 2 and Figure 2). For instance, all FMD risk loci identified through GWAS were known risk loci for blood pressure [61]. This overlap at top risk loci for FMD was also observed at the genomic level through genetic correlation assessed using the linkage disequilibrium score regression method [61]. Globally, genetic loci for FMD are positively correlated with both systolic and diastolic blood pressure [61]. As hypertension is one of the most common presentations for FMD, this global correlation at the genomic level supports shared etiological factors between FMD and high blood pressure. At least for the currently validated risk loci, genetic associations with FMD do not seem to be modified when the analyses were stratified by hypertension status, or after adjusting the genetic associations for systolic blood pressure. Further

Table 1 Rare mutations identified in FMD patients

Gene	Alleles	Protein change	Disease	Reference
PTGIR	c.487C>T (Het.)	p.Glu163*	FMD/SCAD	[36]
	c.48del (Het.)	p.Pro17Argfs*6	FMD	
	c.768 + 1C>G (Het.)	?	SCAD	
	c.200T>C (Het.)	p.Leu67Pro	FMD/SCAD	
YY1AP1	c.634G>A (Het./ Hom.)	p.Arg212Cys.	Coronary heart disease	[79]
	c.724C>T / c.2390 T>A	p.Gln242* / p.Leu797*	Grange syndrome	[39]
	c.1903_1906delTCTG (Hom.)	p.Glu636Profs*13		
	c.2401G>T (Hom.)	p.Glu801*		
	c.664C>T (Hom.)	p.Gln222*		
	c.997+23T>G / c.826-1G>A	p.Ala333Glyfs*10 / p.Lys276Profs*32		[41]
	c.1169del (Hom.)	p.Lys390Argfs*12		[42]
	c.1079C>T (Hom.)	p.Pro360Leu		[40]
	c.1616del / chr1_155652668_155659515_del	p.Lys539Argfs*12 / ?		[43]
	COL5A1	c.1540G>A (Het.)	p.Gly514Ser	Dysplasia-associated arterial disease
c.341C>A		p.Ala114Asp	FMD	[45]
c.367C>G		p.Gln123Glu		
c.1304C>T		p.Pro435Ala		
c.1831C>T		p.Arg611Trp		
c.3491C>T		p.Pro1164Leu		
c.4198C>T		p.Pro1400Ser		
c.1535T>C		p.Leu512Pro	SCAD	[90]
c.1291G>A		p.Gly431Arg		
c.2419A>G		p.Lys807Glu		
c.193C>T		p.Arg65Trp	CeAD	[89]
c.514G>T		p.Val172Phe		
c.5270C>T		p.Thr1757Met		
c.487G>A		p.Gly163Ser	Aortic dissection	[91]
c.1372C>T		p.Pro458Ser		
c.2768C>T		p.Pro923Leu		
c.2842C>T		p.Arg948Trp		
c.3292G>A		p.Ala1098Thr		
c.3398G>A	p.Arg1133Gln			
c.3431G>C	p.Gly1144Ala			
c.3445G>A	p.Val1149Met			
c.3752C>T	p.Pro1251Leu			
c.4241G>C	p.Gly1414Ala			
c.5263G>A	p.Ala1755Thr			
	Several variants, LoFs, structural variants, missense variants...		cEDS	[46]

For each gene, mutations involved in other vascular diseases and connective tissue disorders were mentioned. Het.: heterozygous. Hom.: homozygous.

analyses using Mendelian Randomization are needed to confirm the exact direction of causality between FMD and hypertension, which is currently limited by the small number of available risk loci [61].

Of note, two of FMD loci are highly pleiotropic and were reported in multiple cardiovascular and neurovascular diseases (Figure 2). *PHACTR1* locus is associated with several cardiovascular or neurovascular diseases including coronary artery disease (CAD) [62], CeAD [11], blood pressure [63], and migraine [64]. This locus is also reported to associated with SCAD, independently from FMD through the analysis of stratified SCAD cohorts [56]. FMD risk allele, rs9349379-A, is the major allele in European ancestry populations (~60%) and also the risk allele for CeAD, SCAD and hypertension. However, it is reported to be at opposite direction for CAD, where the minor allele is at risk [65]. Similarly, the lead SNP at *LRP1* locus, rs11172113, is also associated with migraine [66], SCAD [67], abdominal aortic aneurysm [68], MoyaMoya disease [69] and is suggestively associated with CeAD and CAD [70]. In the same

Table 2 Lead common variants reported in genetic risk loci for FMD

Variant ID	Chr	Position (GRCh37)	FMD risk allele	Reported target gene(s)	Association with other cardiovascular diseases					
					SCAD	CeAD	CAD	BP	Migraine	Other
rs9349379	6	12903957	A	<i>PHACTR1</i> (up)	Y	Y	Y	Y	Y	Y
rs11172113	12	57527283	T	<i>LRP1</i> (up)	Y	y	y	y	Y	Y
rs7301566	12	50581647	T	<i>LIMA1</i> (up), <i>ATF1</i> (down) <i>SMARCD1</i> (down) <i>COX14</i> (down)				Y		
rs2681492	12	90013089	T	<i>ATP2B1</i> (down)			Y	Y		
rs2424245	20	19649716	T	<i>SLC24A3</i> (down)				Y	Y	

Reported target genes are genes where a correlation between the variant and the expression of the gene was reported in arterial tissues, with the indication of the direction of the correlation (up: FMD risk allele correlated with higher gene expression, and down: for correlation with lower expression). Associations of FMD lead SNPs with other vascular traits/diseases is reported. Y: genome-wide significant association ($P < 5 \times 10^{-8}$). y: suggestive association ($P < 1 \times 10^{-5}$). Abbreviations: BP: systolic, diastolic blood pressure or pulse pressure; CAD, coronary artery disease; CeAD, cervical artery dissection; SCAD, spontaneous coronary artery disease. Other vascular disorders: coronary artery calcification (rs9349379), abdominal aortic aneurysm and Moyamoya disease (rs11172113).

line, lead variants at *ATP2B1* and *SLC24A3* loci were previously associated with CAD [65] and migraine [66], respectively. At the genome level, a significant positive genetic correlation was observed between FMD and migraine [61]. A high correlation was also observed between FMD and CeAD, although this was not statistically significant due to a lack of power of CeAD GWAS data set [11]. Interestingly, a negative genetic correlation was observed between FMD and CAD, which seems to be independent from the shared genetic basis between CAD and blood pressure. These genetic findings are supported by the clinical overlaps observed between FMD, and these cardiovascular disease [3,13]. However, the exact mechanisms at play are still to be identified and whether the genetic correlations observed globally are related to specific biological mechanisms is a highly relevant information that is still missing to inform the specific genomic regions, and ultimately genes, involved in the shared or opposite risk with FMD.

Potential mechanisms linking FMD to common cardiovascular diseases

Among the statistically validated loci and suspected causal genes, the regulation of arterial musculature contraction and intracellular calcium homeostasis emerged as two particularly promising pathophysiological mechanisms (Figure 3). For instance, two genes are directly involved in intracellular calcium homeostasis and as known actors of the regulation of vascular contraction. *ATP2B1* encodes an ATP-dependent calcium channel exporting calcium to the extracellular space and is a regulator of smooth muscle contraction [71]. Smooth-muscle specific deletion of *Atp2b1* in mice leads to increased blood pressure due to enhanced vasoconstriction [72]. Similarly, *SLC24A3* encodes a sodium/calcium/potassium exchanger exporting one calcium and one potassium ions toward the extracellular space while importing four sodium ions. Studies in rodents and in vascular human SMCs showed a role for *SLC24A3* in vascular contraction through the regulation of calcium homeostasis [73,74]. Vascular contraction and calcium release in response to contractile signals were also partly impaired in mice in which *LRP1* was specifically deleted in SMCs [75]. Interestingly, an alteration of endothelium-independent vascular dilation was observed in FMD patients, in nonaffected arterial beds, suggesting that a systemic alteration of vascular tone regulation may participate to FMD pathogenesis [24].

The genetic involvement of *PTGIR* in FMD may provide leads to understand the link between altered smooth-muscle contraction and the development of fibrodysplasia lesions (Figure 3). Prostacyclin, one of the most potent vasodilator hormones, is an endothelium-derived prostaglandin that exerts multiple roles in the vascular wall physiology and platelet aggregation [76]. Deletion of the prostacyclin receptor gene in mice enhances injury-induced vascular proliferation and platelet activation [77], while *Ptgir* knockout in a pro-atherogenic background (*ApoE*^{-/-}) accelerates the development of atherosclerosis [78]. Consistent with this finding, *PTGIR* variants, in particular rs4987262 (p.Arg212Cys), have been associated with increased atherosclerosis and thrombosis in patients with high

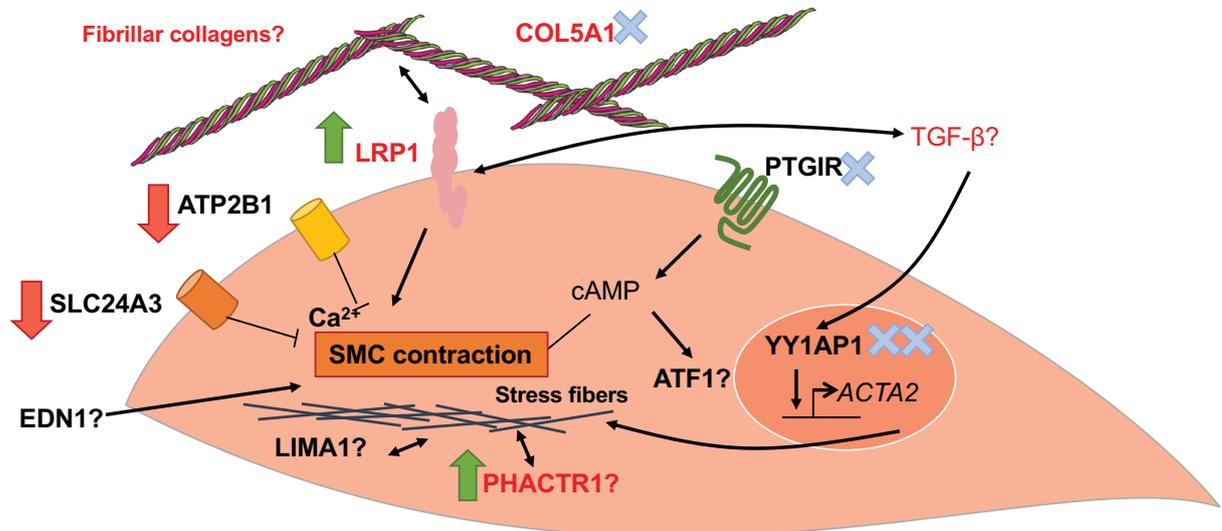


Figure 3. Molecular functions where FMD associated genes are suspected to act in SMCs

Arrows indicate the risk of FMD is correlated with up-regulation (green) or down-regulation (red) of gene expression. Simple cross symbolizes heterozygous and double cross homozygous missense or loss of function mutations which were reported in the genes. Interrogation marks indicate suspected biological mechanisms. Mechanisms or genes in red are also associated with arterial dissection.

cardiovascular risk [79,80]. Vascular effects of prostacyclin are known to balance the effects of thromboxane A_2 , another prostaglandin with strong vasoconstrictor and pro-thrombotic effects [77,78]. Interestingly, depending on local available concentration, and relative expression of prostacyclin and thromboxane A_2 receptors, prostacyclin may activate the thromboxane A_2 receptor in arterial SMCs, resulting in vasoconstriction and increased SMC proliferation [76,81,82]. Prostacyclin receptors interact directly with thromboxane A_2 receptor, modifying its subcellular localization and downstream signaling [82,83]. The current hypothesis is that *PTGIR* associated mutations could exert dominant effects both through prostacyclin and thromboxane signaling [84].

Another pathway that may play an important role in FMD pathogenesis is the extracellular matrix homeostasis (Figure 3). *LRP1* is an endocytic receptor able to interact with a wide variety of ligands including lipoprotein particles, growth factors (e.g. $TGF-\beta$ and $PDGF-BB$), in addition to multiple extracellular proteases and extracellular matrix components. *LRP1* is also a major regulator of extracellular matrix composition [85], which is profoundly modified and disorganized in FMD lesions (Figure 1). Using animal models, several functions of *LRP1* were identified in the vasculature. Smooth muscle specific inactivation (*smLRP1*) in a pro-atherogenic background (*Ldlr*^{-/-}) resulted in increased susceptibility to atherosclerosis, disruption of the elastic layer and aneurysm formation, which were at least in part mediated by ectopic activation of the $PDGF$ pathway [86]. Similar to *PHACTR1*, *LRP1* is also involved in efferocytosis by macrophages in atheromatous plaques, and lower expression of *LRP1* may contribute to enhanced atherosclerosis, necrotic core formation and plaque rupture [87]. A proteomic analysis of the vascular wall in *smLRP1* mice study found an accumulation of *HtrA1* serine protease and connective tissue growth factor, consistent with the observed degradation of the elastic layer, increased thickness of the aorta and global disorganization of the vascular wall [88]. Mutations of *COL5A1* leading to the deposition of aberrant collagen V fibers were also identified in a dysplasia-associated arterial disease, supporting a role for altered extracellular matrix in FMD. It is interesting to note that *COL5A1* mutations were correlated to a high probability of dissection in a FMD patients [45]. Rare variants of *COL5A1* and other fibrillar collagens were recently also reported in CeAD [89], SCAD [90], and aortic dissection [91], in the absence of FMD in those patients. Several genes involved in deposition of the extracellular matrix were recently associated to SCAD, but were not involved in the genetic risk for FMD for now. The strongest genetic signal in SCAD GWAS was located on chromosome 1, near a disintegrin and metalloproteinase with thrombospondin-like protein 4 (*ADAMTSL4*) gene, which encodes a protein potentially involved in fibrillin deposition [67,92,93]. Another SCAD genetic locus overlaps fibrillin-1 gene (*FBN1*), the causal gene for Marfan syndrome, also associated with thoracic artery aneurysm and dissection [26]. Mutations in fibrillar collagen were strongly enriched in SCAD but remain rare in FMD cohorts. Thus, while the implication of extracellular matrix homeostasis in arterial dissection is established,

further investigation will be required to establish the causal link to FMD lesions. Of note, dissection is rare in FMD affected arterial beds [5]. On the other hand, genes involved in the regulation of vascular contraction were found primarily as associated to FMD. More extensive investigation about the mechanisms through which alterations in vascular contraction may lead to the development of FMD is needed to better understand the nature and extent of shared mechanisms between both arteriopathies.

Hypotheses about the role of female sex in FMD pathogenesis

The major sex-bias towards women in FMD prevalence suggests a role for female hormones and or sex chromosomes in FMD pathogenesis. However, no direct genetic or clinical observational evidence is available to date to incriminate any of these factors in FMD. The limited available data for male patients in the current cohorts prevent certainly robust comparative genetic association results to inform for potential differences by sex [61]. Of note, the current GWAS meta-analysis reported no significant genetic association signal on the X chromosome. Increasing the statistical power of the current genetic data available may provide more precise estimations about the role of sexual chromosomes on FMD risk.

An interesting observation related to gene expression and regulation deserves highlight for one of the target genes identified from the FMD GWAS. A lower expression of *SLC24A3* was observed in artery tissues from healthy women compared with men [61]. The rodent ortholog of this gene, *Slc24a3*, was previously demonstrated to be regulated along estrous cycle in the uterus of mice and rats, and to be a transcriptional target of estradiol and progesterone [94,95]. Investigating the expression of *Slc24a3* during the estrous cycle in arteries could certainly delineate further this potential mechanism.

Finally, if the absence of genetic factors from sexual chromosomes is confirmed, this may support female hormones to likely act as environmental modifiers of the risk of FMD. Female hormones, and in particular estrogens, typically act as vasodilators [96] while genetic signals and observation in patients suggest an enhanced vascular contraction or impaired relaxation in FMD patients. Estrogen is a potent activator of prostacyclin synthesis in the endothelium [97] and exerts at least part of its athero-protective effects through prostacyclin pathway [98]. Thus, alterations of prostacyclin signaling in SMCs could potentially have greater consequences for women compared with men. Further functional analyses of FMD-associated *PTGIR* variants could thus provide insights on sex-related molecular mechanisms and their involvement in FMD pathogenesis.

Future directions

Recent efforts have provided a wealth of knowledge about the genetic basis of FMD, an intriguing and understudied arteriopathy affecting mostly women with atypical clinical presentation of cardiovascular disease. Important advances helped generating new leads about the pathophysiology of FMD and related vascular disease form these studies. However, many important questions remain unanswered today. First, none of the identified genetic signals provided a clear mechanism explaining the overwhelming sex imbalance in FMD. More powered genetic analysis and greater inclusion of men in FMD cohorts may help circumvent this limitation. Identification of realistic animal models for FMD could also help tackle this issue. Second, the difficulty to establish FMD diagnosis remains a strong limiting factor for future genetic studies involving substantially larger cohorts of patients in the aim to improve our knowledge about the genetic architecture of FMD. Despite a relatively high estimated prevalence in the general population, its detection remains mostly incidental, which introduces important inclusion biases during the recruitment of patients for genetic studies (e.g under-recruitment of men), which is needed to conduct sex stratified analyses that are still missing. The study of focal FMD, where more men are reported among patients, is also impeded by this limitation. Third, the identification of noninvasive and accessible biomarkers for diagnosis procedures represents a highly promising strategy to improve the currently lacking knowledge about fundamental mechanisms of FMD origin [21–23]. Finally, the striking genetic overlap between FMD and other vascular diseases suggests the existence of common pathophysiological mechanisms, despite very different clinical presentations. Precise definition of shared and distinct genetic determinants should help identify these mechanisms, while combination of genetic analyses through the integration of genetic data from all the diseases may improve the precision and power for future genetic studies [99].

Data Availability

The article does not include original data but reviews existing and published work.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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CRedit Author Contribution

Adrien Georges: Conceptualization, Investigation, Visualization, Writing—original draft, Writing—review & editing. **Nabila Bouatia-Naji:** Supervision, Funding acquisition, Validation, Visualization, Project administration, Writing—review & editing.

Abbreviations

ACTA2, actin alpha 2, smooth muscle; *ATP2B1*, ATPase plasma membrane Ca^{2+} transporting 1 gene; *ATF1*, activating transcription factor 1 gene; CAD, coronary artery disease; cAMP, cyclic adenosine monophosphate; CeAD, cervical artery dissection; cEDS, classical Ehlers-Danlos syndrome; *COL5A1*, collagen type V alpha 1 chain gene; EC, endothelial cell; *EDN1*, endothelin-1 gene; eQTL, expression quantitative trait locus; *FBN1*, fibrillin-1 gene; FMD, fibromuscular dysplasia; GWAS, genome-wide association study; iPSC, induced pluripotent stem cell; *LIMA1*, LIM domain and actin binding 1 gene; LoF, loss-of-function; *LRP1*, low-density lipoprotein receptor related protein 1 gene; MEF2, myocyte-specific enhancer factor 2; MYH11, myosin heavy chain 11, smooth muscle-specific; PDGF, platelet-derived growth factor; *PHACTR1*, phosphatase and actin regulator 1 gene; *PTGIR*, prostaglandin I₂ receptor gene; SCAD, spontaneous coronary artery disease; *SLC24A3*, solute carrier family 24 member 3 gene; SMC, smooth muscle cell; TGF- β , transforming growth factor beta; *YY1AP1*, YY1 associated protein 1 gene.

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