

Rare loss-of-function mutations of *PTGIR* are enriched in fibromuscular dysplasia

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Received 22 January 2020; revised 14 May 2020; editorial decision 1 June 2020; accepted 7 June 2020

Time for primary review: 59 days

Aims

Fibromuscular dysplasia (FMD) and spontaneous coronary artery dissection (SCAD) are related, non-atherosclerotic arterial diseases mainly affecting middle-aged women. Little is known about their physiopathological mechanisms. We aimed to identify rare genetic causes to elucidate molecular mechanisms implicated in FMD and SCAD.

Methods and results

We analysed 29 exomes that included familial and sporadic FMD. We identified one rare loss-of-function variant (LoF) (frequency_{gnomAD} = 0.000075) shared by two FMD sisters in the prostaglandin I₂ receptor gene (*PTGIR*), a key player in vascular remodelling. Follow-up was conducted by targeted or Sanger sequencing (1071 FMD and 363 SCAD patients) or lookups in exome (264 FMD) or genome sequences (480 SCAD), all independent and unrelated. It revealed four additional LoF allele carriers, in addition to several rare missense variants, among FMD patients, and two LoF allele carriers among SCAD patients, including one carrying a rare splicing mutation (c.768 + 1C>G). We used burden test to test for enrichment in patients compared to gnomAD controls, which detected a putative enrichment in FMD ($P_{\text{TRAPD}} = 8 \times 10^{-4}$), but not a significant enrichment ($P_{\text{TRAPD}} = 0.12$) in SCAD. The biological effects of variants on human prostacyclin receptor (hIP) signalling and protein expression were characterized using transient overexpression in human cells. We confirmed the LoFs (Q163X and P17RfsX6) and one missense (L67P), identified in one FMD and one SCAD patient, to severely impair hIP function *in vitro*.

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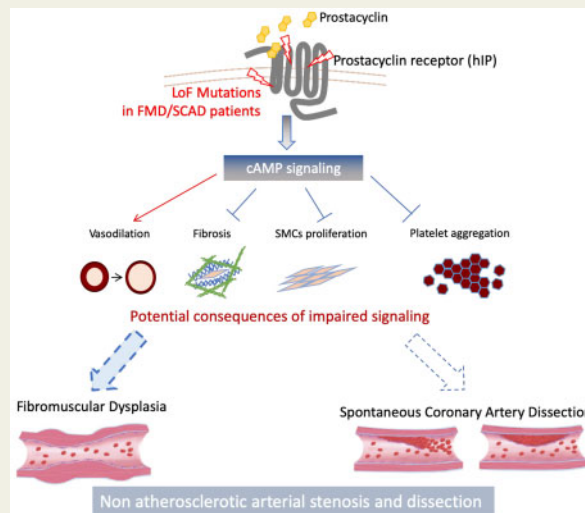
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Conclusions

Our study shows that rare genetic mutations in *PTGIR* are enriched among FMD patients and found in SCAD patients, suggesting a role for prostacyclin signalling in non-atherosclerotic stenosis and dissection.

Graphical Abstract



Keywords

Fibromuscular dysplasia • Spontaneous coronary artery dissection • Rare loss-of-function variants • Prostacyclin signalling

1. Introduction

Fibromuscular dysplasia (FMD) is an atypical and challenging vascular disease. FMD causes non-atheromatous stenosis, dissection, tortuosity, and aneurysms in medium-sized arteries, mainly renal and carotid, but virtually all arteries can be affected.¹ Although frequently asymptomatic, FMD is often associated with debilitating conditions such as hypertension and stroke and presents primarily in middle-aged women (80–90%), without hyperlipidaemia or obesity.²

The pathology and molecular mechanisms of FMD are poorly elucidated, with data coming mainly from observational studies using imaging and histology. Two main types of FMD lesions are defined using angiographic classification.¹ The most common is multifocal FMD, which results in a 'string-of-beads' appearance of the affected artery and represents more than 80% of cases. Focal FMD accounts for the rest of cases and is characterized by isolated stenosis. From the histology, the most commonly described lesions are medial fibroplasia, corresponding to the multifocal angiographic appearance, with an observed excess in fibrous connective tissue in the media of diseased arteries.¹ An overall disorganization of the medial layer is also observed, with clear cellular loss of smooth muscle cells (SMCs).^{3,4} The high proportion of early middle age women among patients suggests a role for female hormones and hormone-associated vascular remodelling in the disease, although clear and direct mechanisms are still missing.⁵ Excessive pulsatility of arteries that results in the accumulation of micro-traumas was also proposed as a potential cause.⁶ Another suggested hypothesis is the occlusion of *vasa vasorum* that would result in intramural ischaemia and myofibroblast transformation of SMCs.⁷

Observational studies report a substantial clinical association between FMD and spontaneous coronary artery dissection (SCAD), an increasingly recognized cause of acute myocardial infarction in young to middle-aged women,^{8,9} with 25–86% of SCAD patients presenting FMD lesions in an additional arterial bed outside of the coronary circulation.⁸ FMD and SCAD share several clinical features; in particular, they both primarily affect middle-aged women and have a notable lack of classical atherosclerotic risk factors. SCAD is characterized by the obstruction of a coronary artery due to the presence of an intramural haematoma and/or a dissected intimal layer. As in the case of FMD, the pathological origin of SCAD lesions is unclear.⁸ An important additional aspect of the overlap between these diseases is that non-coronary arterial dissection (i.e. of the carotid arteries) is a common feature in FMD.

The absence of prospective epidemiological studies and animal models restricts our understanding of the natural history of or FMD and SCAD. A recent systems biology study suggested an association between *CD2AP* encoding CD2-associated protein and its plasma protein levels with FMD.¹⁰ The investigation of the genetic causes offers alternative angles to understand the molecular pathology and mechanisms behind arterial lesions. In a recent study, we identified the first genetic risk locus for FMD, a common variant located in the phosphatase and actin regulator 1 gene (*PHACTR1*).¹¹ We also found that the same risk allele for FMD was also at increased risk of SCAD, independently from the presence of FMD lesions among SCAD patients.¹² This genetic link between FMD and SCAD supported a complex genetic pattern of inheritance for both diseases. Thus, a combination of genetic and environmental factors (local micro-trauma, hormonal fluctuation) could trigger diverse biological mechanisms that result in arterial remodelling and/or coronary artery dissection.

In a complex genetic model, rare pathogenic mutations may also represent causal factors with incomplete penetrance in some patients,¹³ as is the case for several cardiovascular diseases (e.g. hypercholesterolaemia, coronary artery disease). To test this hypothesis, we applied exome sequencing to 29 individuals including FMD siblings and sporadic early-onset cases to search for mutated genes with potential relevance to non-atherosclerotic arterial stenosis. We followed up one candidate gene in 1335 FMD and 843 SCAD patients from four countries. We provide evidence for the prostacyclin receptor to harbour recurrent rare mutations in FMD and SCAD which result in loss of function of receptor signalling *in vitro*.

2. Methods

A more detailed description of methods can be found in the [Supplementary material online, Appendix](#).

2.1 Clinical origin of patients and diagnosis criteria

2.1.1 FMD patients

Familial FMD cases were ascertained as patients with at least one first-degree relative with confirmed FMD and were followed at the Rare Vascular Diseases Reference Center (RVDRC). Sporadic FMD cases were recruited from the RVDRC of the European Hospital Georges Pompidou in Paris, France (HEGP), French ARCADIA (Assessment of Renal and Cervical Artery Dysplasia) registry, Polish ARCADIA-POL registry, US DEFINE-FMD study, University of Michigan Genetic Study of Arterial Dysplasia, Cleveland Clinic FMD Biorepository, as described previously.^{10–12,14} Descriptions of all studies are presented in the [Supplementary material online, Appendix](#). All protocols were validated by ethics committees and involved written informed consent of the included patients, in accordance with the principles of the Declaration of Helsinki. In all studies, an FMD diagnosis was established by clinical experts based on the observation of typical FMD-related lesions of middle-size arteries on imaging (computed tomographic angiography, magnetic resonance angiography, catheter-based angiography, or duplex ultrasound in specialized centres) in absence of features of other causes of arterial stenosis such as atherosclerosis or vasculitis, biochemical evidence of inflammation, or syndromic arteriopathy. FMD experts reviewed imaging to independently confirm the diagnosis. Hypertension was defined as elevated blood pressure ($\geq 140/90$ mmHg) or use of antihypertensive drugs at the time of FMD diagnosis. Except for the exome-sequencing analysis, only unrelated cases were analysed, as confirmed by the examination of medical records and array-based genotyping data, when available.

2.1.2 SCAD patients

SCAD patients were recruited from the French DISCO French register study, the UK SCAD Study, and the Victor Chang Cardiovascular Center (Australia). Description of all studies is presented in the [Supplementary material online, Appendix](#). All protocols were validated by ethics committees and involved written informed consent of the included patients, in accordance with the principles of the Declaration of Helsinki. The diagnosis of SCAD was confirmed by review of the index coronary angiogram by an experienced interventional cardiologist with expertise in the recognition of SCAD, along with contemporaneous medical records. Individuals without a diagnostic angiogram were excluded from all genetic analyses. The majority of patients were of European origin. We obtained individual written informed consent from all participants included. Clinical characteristics of patients are presented in [Table 1](#).

Table 1 General characteristics of study populations

	Targeted Sequencing			PTGIR direct sequencing			Exomes or whole genomes look ups			
	France			New York			Univ. Michigan		UK	
	ARCADIA	ARCADIA-RVDRC	ARCADIA-FMD	Mount Sinai	ARCADIA-FMD	DISCO	Cleveland Clinic	FMD	SCAD	SCAD
N	374	790	150	131	363	264	384	96	96	92%
% Women	84%	82%	98%	81%	90%	97%	96%	92%	92%	92%
% of HTN	78%	84%	57%	88%	NA	64%	NA	NA	NA	NA
Median age at HTN diagnosis (Q1–Q3) (years)	46 (35–53)	41 (30–51)	47 (38–55)	38 (23–46)	NA	51 (43–58)	NA	51 (43–58)	NA	NA
Median age at FMD diagnosis (Q1–Q3) (years)	54 (45–63)	50 (41–61)	54 (46–60)	44 (33–54)	NR	50 (43–57)	NR	50 (43–57)	NR	NR
Median age at inclusion (Q1–Q3) (years)	55 (45–64)	52 (42–62)	57 (50–65)	47 (36–58)	51 (44–59)	55 (47–62)	46 (42–53)	50 (31–57)	46 (42–53)	50 (31–57)
% Multifocal	90%	91%	100%	85%	NR	100%	NR	100%	NR	NR
Median age at event (SCAD) (Q1–Q3) (years)	NR	NR	NR	NR	NR	NR	44 (28–50)	44 (28–50)	44 (28–50)	44 (28–50)
% FMD	NR	NR	NR	NR	NR	35%	NR	NR	NR	NR

FMD, fibromuscular dysplasia; HTN, hypertension; NA, not available; NR, not relevant; Q1, quantile 1; RVDRC, Rare Vascular Diseases Reference Center; SCAD, spontaneous coronary artery dissection.

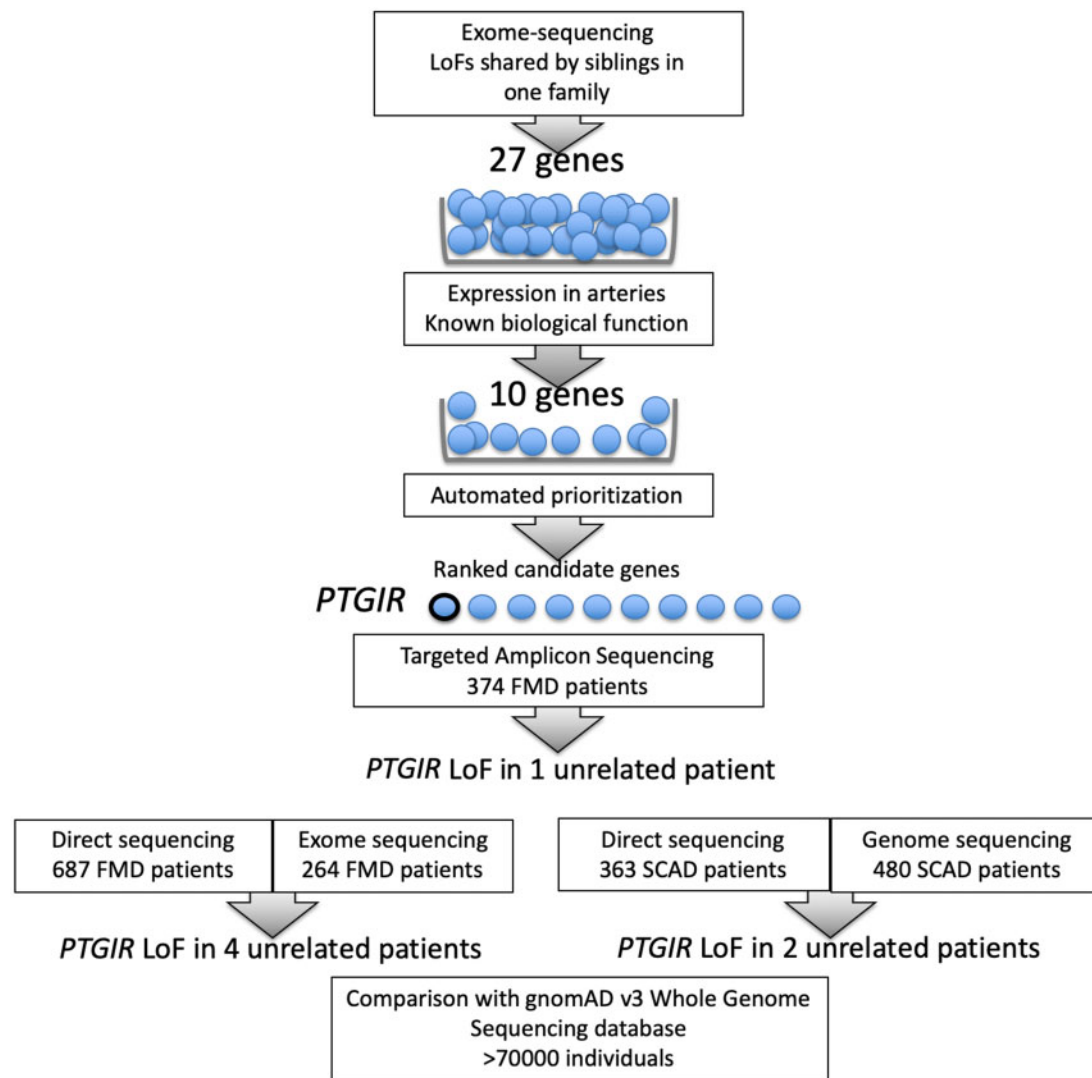


Figure 1 Flowchart illustrating the genetic approach undertaken in this study. FMD, fibromuscular dysplasia; LoF, loss-of-function; SCAD, spontaneous coronary artery dissection.

2.1.3 Immunohistochemistry

For immunohistochemistry, paraffin blocks of arterial tissues (three renal arteries) were obtained from surgical pathology archives of the European Hospital Georges Pompidou and belonged to patients who gave written informed consent to donate remaining tissue for research purposes (protocol 'Collection d'échantillons biologiques en pathologie cardiovasculaire, métabolique et rénale de l'Hôpital Européen Georges-Pompidou', ethically validated by the Comité de Protection des Personnes Île-de-France II on 4 January 2017, no. CPP 2016-13-09). We used two normal renal arteries and one FMD artery where incident diagnosis was made from the histology.

2.2 Study patients

Figure 1 summarizes the exome filtering and the follow-up strategies applied in this study.

2.2.1 Exome-sequencing patients

We studied 21 FMD familial cases formed by 4 FMD sib pairs, 2 sib trios, 1 cousins pair, and 1 family with 5 affected sibs (Supplementary material

online, Figure S1).¹⁵ We also studied four sporadic FMD cases and had access to DNAs from unaffected parents of two of these cases, who were analysed as family trios and had an early-onset FMD (12 and 16 years old at diagnosis). In total, we analysed 29 exome sequences. Exome and targeted resequencing were performed by Integrage[®] Genomics (Evry, France), as previously described.¹⁵

We also interrogated whole-exome sequences for mutations in *PTGIR* from 264 FMD patients (University Michigan), and whole-genome sequences of 384 British (Leicester University) and 96 Australian (Victor Chang Cardiovascular Center) SCAD patients. Sanger sequencing was used to validate genotypes presenting low coverage (<10 reads) in the exome-sequencing data.

2.2.2 Direct resequencing patients

Direct sequencing of *PTGIR* was performed in a total of 1071 unrelated FMD patients. Seven hundred and ninety were French patients from ARCADIA or the RVDRC, including the 374 analysed by target resequencing, 150 were US patients from the DEFINE-FMD study and 131

were Polish from the ARCADIA-POL registry. We also screened 363 SCAD patients from the DISCO registry.¹² Sanger sequencing was performed on a 3730xl DNA Analyzer system (Applied Biosystems).

2.2.3 GnomAD database

GnomAD v3 aggregates the whole-genome sequencing of 71 702 samples from unrelated individuals sequenced as part of various disease specific and population genetic studies.¹⁶ Ancestry was assigned using principal component analysis on the first 10 components using a set of high-quality variants, and 32 299 samples were assigned as non-Finnish Europeans.

2.3 Molecular characterization of mutants

Wild-type or mutant human prostacyclin receptor (hIP) protein were overexpressed in human embryonic kidney cells (HEK293, ATCC CRL-1573) for 48 h. Cyclic adenosine monophosphate (cAMP) quantification was performed using cAMP-Glo assay (Promega, WI, USA), according to the manufacturer's description. Western blot and immunofluorescence assays were performed as previously described.¹¹ Mouse anti-hIP (sc-365268, Santa Cruz Biotechnologies, TX, USA) was used to detect prostacyclin receptor.

2.4 Statistical analyses

Associations between *PTGIR* [loss-of-function (LoF) variants], FMD, and SCAD were tested using a gene-based burden test implemented in TRAPD (Testing Rare vAriants using Public Data) package. We took advantage of the availability of latest version of gnomAD¹⁶ (v3) to evaluate the enrichment of rare *PTGIR* alleles in patients.¹⁷ GnomAD v3 uses only whole-genome sequencing from >70 000 individuals (>30 000 individuals of European ancestry), which provides a homogeneous and large dataset with high read depth (>99.9% of samples with >15× coverage at *PTGIR* locus) allowing robust variant calling and estimation of the frequencies of these rare alleles. It was estimated that whole-genome sequencing provides >95% sensitivity in SNP detection above a read depth of 15×, which is less than the minimum required in gnomAD v3.¹⁸ We used two-sided Fisher's exact test to estimate the enrichment *P*-values, as recommended by the authors.¹⁷

Unless otherwise noted, *P*-values were evaluated from a Student's *t*-test in functional characterization experiments.

3. Results

3.1 Gene prioritization from exome-sequencing data

We re-analysed our previous exome sequences that included four affected sib pairs, two affected sib trios, and one cousin pair¹⁵ in combination with a new sample of one sib quintet, two sporadic cases analysed with both parents (two trios) and two unrelated sporadic cases (Figure 1 and Supplementary material online, Figure S1). Relevant variants were defined as non-synonymous predicted deleterious or LoF unobserved or with low frequency in gnomAD [minor allele frequency (MAF) < 0.001]. The inter-family analysis and trio filtrations were inconclusive with no gene containing relevant variants shared in at least two families, or recessive transmitted from parents to cases or *de novo* in the trios.

We then restricted the analyses to genes with LoF variants following the potential transmission pattern of each family, including dominant, recessive, and compound heterozygotes. A shortlist of 27 genes was

identified to which we applied three prioritization tools.^{19–21} Using DAVID and STRING algorithms for functional annotations, we found that 10 genes had potential roles in blood vessel biology and signalling. We then provided a training list of genes involved directly or as regulators/effectors in stenosis and aneurysm-related phenotypes in human diseases and mouse knockout models to the ENDEAVOUR tool. Using basic machine learning techniques to model the arterial dysfunctions observed in FMD, ENDEAVOUR ranked the prostaglandin I₂ receptor gene (*PTGIR*) as the best candidate. *PTGIR* contained one rare LoF (rs199560500/Q163X, MAF = 0.00075 in gnomAD) that was shared by two affected sisters from Family 2. *PTGIR* encodes prostaglandin I₂ (prostacyclin) receptor (hIP), a G protein-coupled receptor well known for its cardioprotective, anti-atherosclerotic, and anti-thrombotic functions.²² For follow-up, we selected a shortlist of candidate genes based on prioritization tools, literature link with arterial stenosis, smooth muscle contraction, or extracellular matrix organization, in addition to *PTGIR* (Supplementary material online, Table S1).

3.2 Direct sequencing identifies recurrent LoF variants in *PTGIR* among FMD patients

We analysed 20 genes using targeted amplicon sequencing in 374 patients (Table 1). A full list of identified LoF variants is shown in Supplementary material online, Table S1. We found truncating variants in five genes (*P2RX6*, *P2RY4*, *P2RY11*, *P2RX4*, and *PTGIR*). The variants identified in *P2RX6*, *P2RY4*, *P2RY11*, and *P2RX4* were either as common in FMD patients as in gnomAD or direct sequencing did not confirm their presence. However, we found an additional patient carrying Q163X, the same LoF variant identified by exome sequencing in one FMD sib pair, which supported further the investigation of *PTGIR* coding sequences in more patients.

Overall, we analysed 1071 unrelated FMD patients by direct sequencing (Supplementary material online, Figure S2). First, we confirmed Q163X carriers among the 374 patients and screened both *PTGIR* coding exons in this sample, especially exon 2, which is incompletely covered by targeted amplicon sequencing. Then, we Sanger sequenced 632 additional FMD patients from France (*N* = 416), Poland (*N* = 131), and USA (*N* = 150). We identified Q163X in one additional FMD patient and one rare frameshift variant in two FMD patients (rs754755149/P17RfsX6, MAF = 9×10^{-5} in gnomAD) (Table 2). We also looked up exome-sequencing results in 264 FMD patients from the University of Michigan/Cleveland Clinic biorepository and found one additional carrier of Q163X at the heterozygote state. As the region coverage by exome sequencing at this locus was low in this cohort (3× depth), we confirmed the presence of the variant using Sanger sequencing (Supplementary material online, Figure S2).

Using TRAPD burden test, we found that the FMD cohort of patients (*N* = 1335) was significantly enriched for LoF alleles in *PTGIR* compared to a control population from gnomAD v3 (*N* = 71 702, *P* = 4×10^{-4} , TRAPD burden test, Supplementary material online, Table S2). All LoF carriers are of European ancestry. Similar enrichment was observed when we restricted the comparison to non-Finnish European controls (*N* = 32 399, *P* = 8×10^{-4} , Supplementary material online, Table S2).

3.3 Functional characterization of rare LoF and missense variants in *PTGIR*

In addition to the two *bonafide* LoFs, we identified four rare missense variants in FMD patients (MAF_{gnomAD} < 0.001, Table 2) for which functional characterization is not reported. rs201261904 affects the

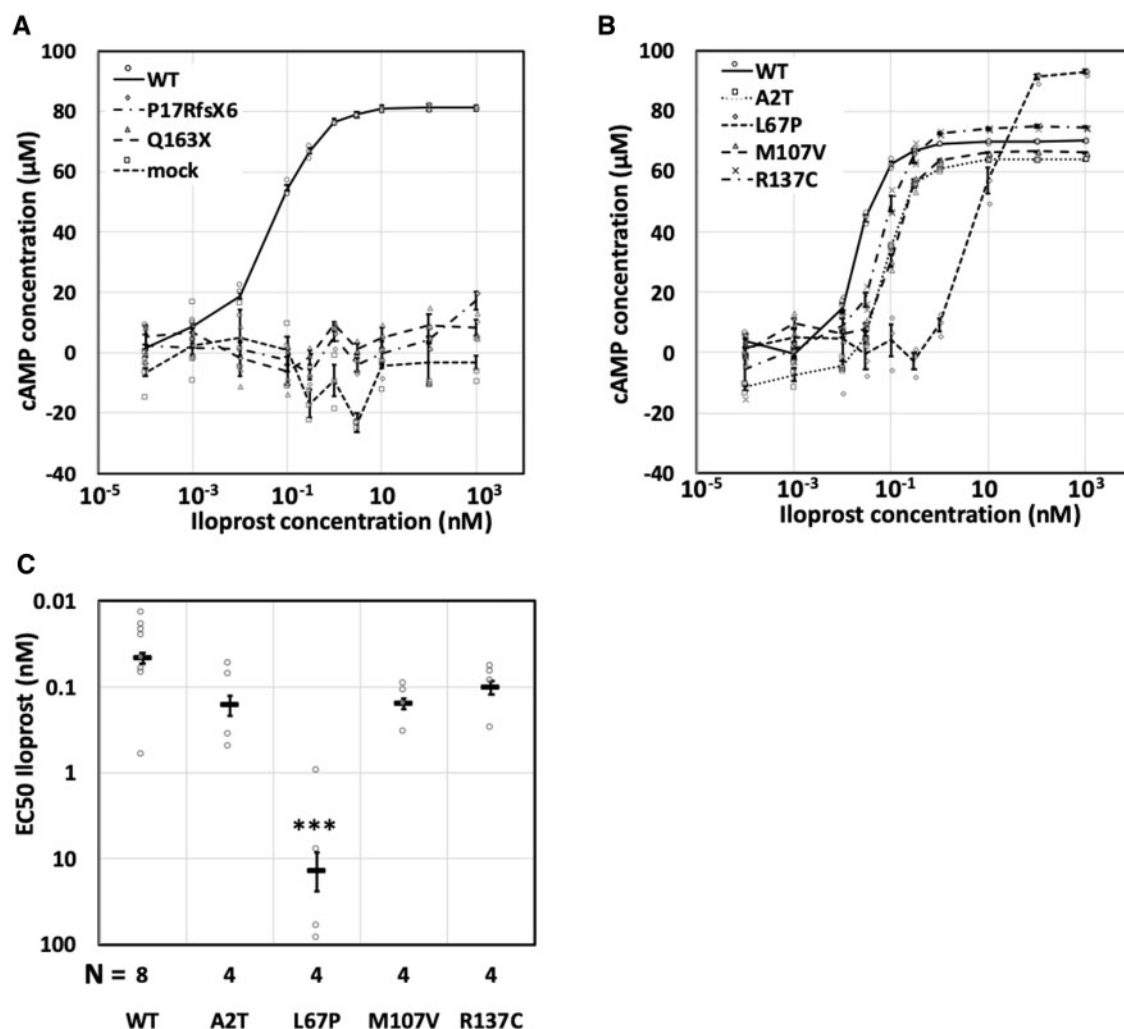


Figure 2 Analysis of cAMP synthesis in response to iloprost in HEK293 cells overexpressing WT or mutant prostacyclin receptors. (A and B) Concentration of cAMP in HEK293 cells overexpressing WT prostacyclin receptor (hIP), mutant hIP (A: P17RfsX6 or Q163X, B: A2T, L67P, M107V, or R137C) or transfected with mock plasmid (pcDNA-FLAG-HA). Error bars represent the standard deviation of three technical replicates. (C) Measurement of 50% response concentration to iloprost (EC₅₀) in HEK293 cells overexpressing WT or mutant hIP. *N* represents the number of independent experiments. Error bars represent the standard error of the mean. Student's *t*-test *P*-value (bilateral test with homoscedastic variance) ****P* < 10⁻³. cAMP, cyclic adenosine monophosphate; WT, wild-type.

Table 2 Description of rare LoFs and missense variants identified in *PTGIR* in FMD patients

rs	cDNA	Protein	Consequence	FR	POL	NY	MI	gnomAD (alleles/10 000)		CADD score	SIFT score	Polyphen score
								NFE	all			
rs754755149	c.48del	P17RfsX6	Frameshift	2	0	0	0	1.2	0.9	NA	NA	NA
rs199560500	c.487C>T	Q163X	Stop gained	3	0	0	1	1.5	0.8	48	NA	NA
rs201261904	c.4G>A	A2T	Missense	1	0	0	0	0.5	1.0	16.9	0.1	0.1
rs1397542892	c.200T>C	L67P	Missense	0	0	1	0	0.2	0.1	31	0	1
rs775137134	c.319A>G	M107V	Missense	0	1	0	0	0.0	0.1	23.9	0	0.3
rs199969416	c.409C>T	R137C	Missense	1	0	0	0	0.0	0.0	24.9	0.1	0.9

The number of alleles is indicated (no homozygous patients identified).

FMD, fibromuscular dysplasia; LoF, loss-of-function variant; NA, not available, NFE, Non-Finnish Europeans.

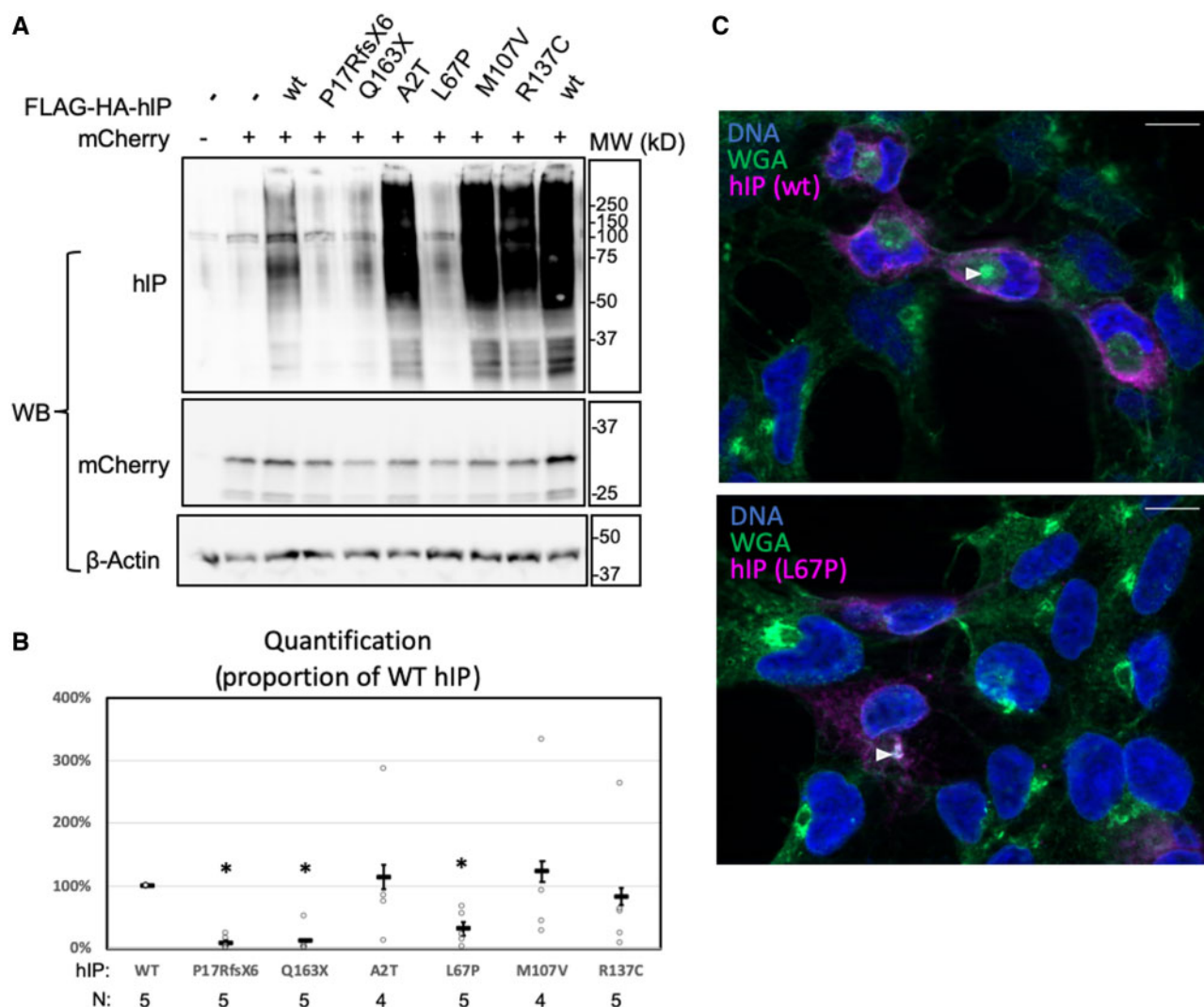


Figure 3 Evaluation of the expression and localization of the prostacyclin receptor mutated proteins. (A) WT and mutant protein expression. SDS-PAGE/western blot assay on whole cells extracts of HEK293 cells overexpressing WT or mutant prostacyclin receptor (hIP) included a FLAG-HA N-terminal tag and the transfection control (mCherry). Proteins (hIP, mCherry, and β -actin) were detected using specific primary antibodies. We found that hIP is detected as a mix of bands and higher molecular weight smear due to its extensive post-translational modification. Full size blots are presented in [Supplementary material online, Figure S7](#). (B) Protein quantification. We performed five independent experiments (hIP-A2T and M107V were assayed in four experiments), and the average is shown with standard error of the mean as error bars. We indicate Student's *t*-test *P*-value (paired test) when $P < 0.05$ as *. (C) Immunofluorescence visualization of HEK293 cells overexpressing wild-type or L67P hIP. Protein localization was assayed using hIP specific antibody (purple signal). Fixed cells were incubated with Alexa488-conjugated WGA (green signal), and with 4',6-diamidino-2-phenylindole (DAPI) (blue signal). Images were taken with a 100 \times objective on a Zeiss ApoTome system. hIP, human prostacyclin receptor; WGA; Wheat Germ Agglutinin; WT, wild-type.

N-terminal cytoplasmic extension of hIP (A2T), whereas the three other variants are located in the transmembrane helices (L67P, M107V, R137C, [Supplementary material online, Figure S3a](#)). Among these, rs1397542892 (L67P) affects a strictly conserved residue among vertebrate orthologs of PTGIR and is predicted to be deleterious (SIFT score of 0, Polyphen score of 1, [Table 2, Supplementary material online, Figure S3b](#)).

As mentioned, PTGIR encodes for the prostacyclin receptor. Therefore, to assess the potential impact of PTGIR rare variants on cellular functions, we overexpressed wild-type and mutant hIP in the human embryonic kidney cell line HEK293 and measured production of cAMP

in response to iloprost, a synthetic analogue of prostacyclin. We measured a 50% maximal effective concentration of iloprost ($EC_{50_{ilop}}$) of 0.05 nM for wild-type hIP ([Figure 2](#)). As expected, transfection with plasmids encoding any of the two nonsense mutants Q163X and P17RfsX6 resulted in a total loss of cell sensitivity to iloprost ([Figure 2A](#)). All four missense mutant proteins were at least partially functional in overexpressing cells ([Figure 2B](#)). However, L67P hIP had a 100-fold decrease in iloprost sensitivity ($EC_{50_{ilop}} = 14.1$ nM, $P = 1.1 \times 10^{-4}$, [Figure 2C](#)) whereas other mutants were not significantly different from wild-type hIP. Western blot using anti-hIP antibody could detect all tested missense mutants, and the band pattern was similar between wild-type and mutant

Table 3 Clinical characteristics of FMD patients carrying non-functional *PTGIR* alleles

	Patient 1a ^a	Patient 1b ^a	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
rs ID			rs199560500			rs754755149		rs1397542892
cDNA position			c.487C->T			c.48del		c.200T->C
Protein position			Q163X			P17RfsX6		L67P
Sex (M/F)	F	F	F	F	F	F	F	F
Age at inclusion (years)	61	57	53	46	57	26	62	65
Age at diagnosis (years)	61	53	52	46	56	26	61	62
Familial (Y/N)	Y	Y	N	N	ND	N	N	N
FMD subtype	Multifocal	ND	Multifocal	Multifocal	Multifocal	Focal	Multifocal	Multifocal
Multisite (Y/N/ND)	ND	ND	N	N	Y	ND	N	Y
Number of vascular beds	ND	ND	1	1	3	ND	1	3
Arterial beds involved	Renal (both)	Vertebral	Carotid (both)	Renal (unilateral)	Carotid, iliac, renal	Renal (unilateral)	Renal (both)	Internal carotid, vertebral, renal
Hypertension (Y/N)	Y	Y	N	Y	N	Y	Y	Y
Age at onset (years)	51	47		46		24	61	62
Family history for HTN (Y/N)	Y	Y	N	Y	Y	ND	Y	Y
Dissection? (Y/N)	N	N	N	N	N	N	N	N
Known aneurysms? (Y/N)	N	Y	N	N	N	N	N	N
Required intervention (dilation)? (Y/N)	N	N	N	N	N	Y (+restenosis)	ND	Y (no restenosis)
Smoking status	Smoker	Smoker	Former smoker	Smoker	Smoker	Smoker	Non-smoker	Non-smoker

Patients are organized in three groups, according to the rare variant they carry.

FMD, fibromuscular dysplasia; HTN, hypertension; N, no; ND, not determined, Y, yes.

^aSisters from Family 2.

proteins (Figure 3A and Supplementary material online, Figure S4a), showing that no gross changes of post-translational modifications were caused by the mutations. L67P mutant expression was strongly affected, sometimes undetectable (Figure 3A), with an average signal reduction by 70–90% ($N = 4$, $P = 0.03$), whereas other mutants did not cause a significant reduction of hIP expression. Using immunofluorescence in HEK293 cells, we observed that wild-type hIP was mostly visible in the cytoplasm and at the plasma membrane (Figure 3B and Supplementary material online, Figure S4b). Conversely, we observed a clear colocalization of L67P hIP protein with intracellular organelles bound by Wheat Germ Agglutinin, a widely used lectin that binds to sialic acid and N-acetylglucosaminyl modified proteins, thus suggesting L67P mutation partly impairs hIP maturation (Figure 3B and Supplementary material online, Figure S5b). Finally, co-expression of wild-type hIP (fused with mCherry) with wild type or mutant versions of hIP did not affect cellular response to iloprost (Supplementary material online, Figure S5a), which excludes the existence of a full dominant-negative effect of hIP mutants. However, co-expressing hIP-mCherry protein with mutant hIP, we found that mature forms of hIP-mCherry were almost undetectable in the presence of Q163X and L67P hIP, whereas the other mutants had no effect on hIP (Supplementary material online, Figure S5b). This suggests a potential dominant effect of these two mutants, providing a potential mechanism for the pathogenesis of heterozygous *PTGIR* mutations.

3.4 Clinical features of FMD patients carrying *PTGIR* non-functional alleles

The patients harbouring *PTGIR* non-functional alleles were all women, with ages at FMD diagnosis ranging from 26 to 61 years (Table 3). Six of the eight patients had multifocal FMD, and one patient had only a focal

lesion. Lesions were first identified in the renal artery for four patients and in the carotid/vertebral arteries for the other four patients. Five patients were screened for multivessel FMD, and two of them had FMD lesions in other vascular beds. Six patients had hypertension, with onset 1–10 years prior to FMD diagnosis, all of them having renal FMD. Family history of hypertension was reported for six patients, but no other history of unexplained vascular issue was reported. No dissection was detected in any of the eight patients, and only Patient 1b had a known aneurysm. Patient 5, carrying the rs754755149 frameshift variant, was a 26-year-old woman with focal FMD affecting one renal artery. She underwent angioplasty, which was initially successful, but restenosis was seen during follow-up. All other patients had multifocal lesions. Patient 7, carrying L67P missense, underwent angioplasty of both renal arteries, restoring normal blood flow with a moderate benefit on the management of hypertension. Upon follow-up for >5 years, no restenosis was detected. This patient has no family history of aneurysm, dissection, sudden death, or any early cardiovascular disease. She has one hypertensive sister aged 54 whose clinical exploration and DNA are not available. The other patients had no angioplasty.

3.5 Histological localization of the prostacyclin receptor

Immunohistochemistry staining of three renal arteries from pathology archives (two normal, one FMD) with hIP antibody showed protein presence in all arterial layers, with a stronger expression in SMCs of the medial layer, both in normal and FMD tissues (Supplementary material online, Figure S6). Of note, none of the patients carrying *PTGIR* mutations had vascular surgery, which limited access to their arteries to evaluate the mutant proteins expressions.

Table 4 Clinical characteristics of SCAD patients carrying non-functional *PTGIR* alleles

	Patient 8	Patient 9	Patient 10
rs	rs199560500	rs1397542892	rs1302581755
cDNA	c.487C->T	c.200T->C	c.768 + 1C>G
Protein	Q163X	L67P	ND
Sex (M/F)	F	F	F
Age at 1st event (years)	38	66	58
Clinical presentation	Non-STEMI	STEMI	Non-STEMI
SCAD subtype	1	2	2
P-SCAD (Y/N)	N	N	N
Recurrent SCAD (Y/N)	N	N	N
Other ischaemic events	N	Y	N
Migraine (self-reported)	N	Y	N
Hypertension (Y/N)	Y	N	N
Dyslipidaemia (Y/N)	N	N	N
Smoking status	ND	Non-smoker	Smoker
FMD in other arterial beds (Y/N)	N	N	N

SCAD subtype is based on Saw classification.

FMD, fibromuscular dysplasia; N, no; ND, not determined; SCAD, spontaneous coronary artery dissection; STEMI: ST-elevation myocardial infarction; Y, yes.

3.6 Rare LoF mutations also identified in SCAD patients

Given the established clinical and genetic overlap between FMD and SCAD, we also screened 363 French SCAD patients from the DISCO registry for rare variants in *PTGIR* by direct sequencing. We found one patient with a splicing variant not reported in gnomAD (rs1302581755). The variant is a G to C substitution in position +1 of the 3' end of exon 2, disrupting the donor splicing site, resulting in a predicted LoF (Supplementary material online, Table S3). We also found one more patient with L67P mutation. A look up for variants in genome sequences of 384 British and 96 Australian SCAD patients identified one additional patient carrying the Q163X, rs199560500 variant allele (Supplementary material online, Table S3). We also identified additional rare missense variations of *PTGIR* in SCAD patients, for which functional effects are not known (Supplementary material online, Table S3). Although *PTGIR* LoF alleles were relatively more frequent in SCAD patients than in gnomAD control populations, we did not find a significant enrichment for LoFs in *PTGIR* among SCAD patients compared to gnomAD controls using TRAPD burden test ($P = 0.12$).

3.7 Clinical features of SCAD patients carrying *PTGIR* LoF alleles

The SCAD patients were all women, aged 38–66 years at first SCAD event (Table 4). SCAD patient 1, bearing rs199560500 variant, presented with non-ST elevation myocardial infarction, hypertension on treatment with a history of gestational hypertension. Upon coronary angiography, a very extensive dissection of the left mainstem coronary could be identified, with no signs of atherosclerosis. The two other patients were older at the time of their SCAD event (58 and 66 years), and SCAD lesions presented as a diffuse stenosis leading to a partial or complete occlusion of the coronary artery, with a visible haematoma in the arterial wall. Upon full screening of other arterial beds, these three SCAD patients did not exhibit detectable FMD lesions. Clinical record for Patient 8 indicates the father died at age 58 from myocardial infarction and had a history of obstructive pulmonary disease and the patient's mother died

from multiple sclerosis. No familial history of systemic or inflammatory disease was reported for Patients 9 and 10.

4. Discussion

Here we describe rare LoF and missense mutations in the prostaglandin I_2 receptor gene (*PTGIR*) in FMD and SCAD patients (Central Illustration). We found that these mutations severely impair prostacyclin receptor signalling *in vitro* and are prevalent at a rate significantly higher in a cohort of ~1300 FMD patients, compared to large unselected publicly available cohorts. We also describe some of these rare mutations in SCAD patients, without an overt significant enrichment. Our study describes an unprecedented genetic impairment in the prostacyclin signalling in non-atheromatous arterial stenosis and dissection.

4.1 Further support for a shared genetic basis between FMD and SCAD

Understanding the accurate genetic model has been a challenging endeavour for FMD and SCAD. We have recently established their complex genetic mode of inheritance through the association between the common variant rs9349379 in *PHACTR1* and an increased risk for both diseases.^{11,12} After a first negative exome-sequencing study in FMD,¹⁵ we increased the sample numbers and applied a prioritization strategy that allows the identification of *PTGIR* as a novel gene mutated in FMD. A recent exome study conducted on SCAD families described rare mutations in *TLN1*.²³ The identification of *PTGIR* as an additional gene involved in FMD and potentially SCAD further supports their complex genetic basis involving both common and rare mutations. We suspect incomplete penetrance for the mutations described in *PTGIR*. Several LoF variants are reported in gnomAD, which include population-based and diseased cohorts, but in lower frequency compared to FMD patients. On the other hand, the high estimated prevalence of asymptomatic FMD in unselected populations (1–6%)¹ is compatible with a substantial number of undiagnosed FMD patients carrying *PTGIR* mutations among these large public cohorts. Here we estimate in our cohorts that at least 0.5%

of FMD patients carry a non-functional *PTGIR* allele. Of note, this rate of *PTGIR* mutation carriers is comparable to the percentage reported for *PSCK9* mutation carriers (0.6%) among cohorts of hypercholesterolaemia.²⁴

4.2 Rare non-functional LoFs in *PTGIR* among FMD and SCAD patients

We provide evidence for complete loss of protein function *in vitro* caused by the two LoFs and showed that missense mutations are functional, except L67P that strongly impairs the receptor signalling and protein function. Previous studies have implicated *PTGIR* missense variants, in particular rs4987262 (R212C), in atherosclerosis and thrombosis.²⁵ It should be noted that this variant is relatively frequent (1% in Non-Finnish European populations in gnomAD) and was identified in 22 FMD patients and 12 SCAD patients in our cohorts, including one SCAD patient with homozygous mutation (Supplementary material online, Table S3). Apart from L67P mutation, all missense mutations studied here show a two- to five-fold decrease in sensitivity to iloprost, similar to previous observations for R212C mutation.²⁶ We note that several rare missense variants identified in SCAD patients only were not functionally characterized. It is therefore possible that we underestimate the pathogenicity of *PTGIR* missense mutations.

4.3 The prostacyclin signalling and physiopathology of arterial stenosis and dissection

The loss or decrease in prostacyclin signalling has a wide range of biological consequences, all compatible with arterial remodelling and dissection observed in FMD and SCAD patients. Prostacyclin is a well-established vasodilator, with anti-thrombotic and anti-atherosclerotic properties. Prostacyclin is also a potent repressor of the proliferation and migration of SMCs from the vascular wall and promotes their contractile phenotype,²² which is compatible with the lesions observed in FMD. A high level of fibrotic tissue characterizes FMD lesions. Interestingly, prostacyclin signalling is known to repress fibrosis in several tissues.²² The cAMP signalling downstream hIP is a well-known regulator of fibroblast function and a repressor of fibrosis through the direct inhibition of extracellular matrix synthesis, myofibroblast differentiation, and fibroblast proliferation.²⁷ Prostacyclin effects are often seen as opposite to the effects of Thromboxane A₂, a closely related prostaglandin, which favours thrombosis and vasoconstriction, and their balance is a key to several pathologies.²² Aspirin, widely prescribed for its anti-thrombotic properties, inhibits the production of both prostacyclin and thromboxane and may compensate the effect of unbalanced prostacyclin/thromboxane signalling in FMD patients. However, anti-thrombotic medication may represent an additional risk in patients developing aneurysms in middle size arteries, and the screening for *PTGIR* mutations may help identify patients who could benefit of such medication. On the other hand, iloprost is used to treat pulmonary arterial hypertension, scleroderma, Raynaud's phenomenon, and other diseases with prominent vasoconstriction, and may have unpredictable effects in patients bearing hIP mutations.²² Whether FMD and SCAD patients with *PTGIR* mutations may exhibit increased platelet aggregation and an increased risk of developing pulmonary arterial hypertension or thrombosis would be an interesting investigation to conduct in the future. Given the high relevance of the prostacyclin to thromboxane balance to vascular function, the genetic investigation of more genes linked to these pathways could provide additional clues about the role of this mechanism in FMD and SCAD.

4.4 Study limitations

The prevalence of the identified *PTGIR* LoFs is low and affects a small fraction of FMD and SCAD patients. On the other hand, the study samples are relatively limited, compared to other complex cardiovascular diseases and may result in inaccurate estimation in mutation rate among patients. We lack large pedigrees with clinical and genetic information in affected and unaffected members to assess the extent of the penetrance of *PTGIR* mutations and to fully establish these as causal factors. We do not provide significant support for enrichment in SCAD, compared to FMD, potentially due to differences in samples sizes. We identified heterozygous LoFs, with biological consequences that are hard to predict considering the multiple biological functions of hIP, and its possible interactions/compensations with related prostaglandin receptors in a more complex physiological system. Biological and vascular samples from patients bearing *PTGIR* rare alleles were not available to identify direct biological effects of these mutations.

5. Conclusions

We identified genetic defaults in *PTGIR* that impair its cellular function and are likely to be rare genetic causes for FMD and SCAD. According to our genetic screen of FMD and SCAD cohorts, we estimate *PTGIR* mutations to be present in ~0.5% of FMD patients and ~0.3% of SCAD patients. Larger studies are needed to refine these estimations, which should include a greater number of patients. This finding, and the availability of multiple drugs targeting this pathway, may help clinicians to design specific therapeutic approaches for FMD and SCAD patients. Further genetic analyses involving functionally related genes are required to fully evaluate the influence of this pathway on the pathogenesis of non-inflammatory stenosis and dissection observed in FMD and SCAD arteries.

Data availability

Exome-sequencing and whole-genome sequencing data underlying this article cannot be shared publicly due to the protection of the privacy of individuals who participated in the study. The data will be shared on reasonable request to the corresponding author with agreement of the respective cohort managers.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Authors' contributions

A.G and N.B.-N. designed the study, analysed the data, drafted the work, and wrote the manuscript. J.A. participated to study conception and design, collected genetic and medical data. A.G., D.D., and P.B. performed experiments. T.B. performed statistical analyses. A.L., V.D.'E., A.F.D.N., D.K.-D., J.W.O., E.W.-C., A.P., A.J., A.A.B., T.R.W., S.E.H., N.J.S., D.A., N.F.-M., S.H., Y.W., M.-L.Y., K.H., N.C., P.M., A.C., B.F., P.-F.P., E.M., A.A., L.A., M.A., H.L.G., S.K.G., J.C.K., and X.J. participated to study design of the different cohorts, collected genetic and medical data. A.J., D.A., N.C., P.M., E.M., L.A., M.A., H.L.G., S.K.G., J.C.K., and X.J. revised the manuscript for important intellectual content. All authors gave final approval of the

version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements

We thank Drs Robert Graham and Eleni Giannoulou from Victor Chang Cardiac Research Institute, Sydney, New South Wales, Australia for providing exome-sequencing data for SCAD patients. We thank recruiting clinicians in all centres and the patients who participated in this study. We acknowledge the Fibromuscular Dysplasia Society of America for enabling study enrolments at meetings into the University of Michigan Genetic Study of Arterial Dysplasia. We thank AstraZeneca's Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D for funding the sequencing of and providing the bioinformatics support related to subjects recruited at Leicester University. We acknowledge the leadership of the ESC-ACCA SCAD Study Group and thank our patients and volunteers for their support.

Conflict of interest: M.A. reports grants from French Ministry of Health, during the conduct of the study; grants and non-financial support from Recor, grants from Idorsia, Novartis, Quantum Genomics, and French Federation of Cardiology; personal fees from CVRx and Novartis, for work unrelated to this work. S.K.G. is a non-compensated member of the Medical Advisory Board of the Fibromuscular Dysplasia Society of America, a non-profit organization. D.A. has received research funding from Abbott vascular to support a clinical research fellow, funding from Astra Zeneca Inc. for unrelated research and has conducted unrelated consultancy for GE Inc. All other authors declared no conflict of interest.

Funding

The French studies are supported by a European Research Council grant [ERC-Stg-ROSALIND-716628 to N.B.N.]. The ARCADIA study was sponsored by the Assistance Publique-Hôpitaux de Paris and funded by a grant from the French Ministry of Health [Programme Hospitalier de Recherche Clinique 2009, AOM 08192] and the Fondation de Recherche sur l'Hypertension Artérielle. The French SCAD study is supported by the French Society of Cardiology foundation 'Cœur et recherche', the French Coronary Atheroma and Interventional Cardiology Group (GACI). The DEFINE-FMD is supported by the US National Institutes of Health [R01HL130423, R01HL135093, T32HL007824]. The University of Michigan study is supported by NHLBI [R01HL139672], Doris Duke Charitable Foundation, University of Michigan Frankel Cardiovascular Center, the University of Michigan Taubman Institute, sequencing services by the Northwest Genomics Center at the University of Washington, Department of Genome Sciences, under U.S. Federal Government contract number HHSN268201j0037C from the NHLBI. The Cleveland Clinic FMD Biorepository was supported in part by the NIH, National Center for Research Resources, CTSA 1UL1RR024989, Cleveland, Ohio. GeneBank was supported in part by grants from NHLBI and Office of Dietary Supplements [P01 HL076491, P01 HL147823, R01HL128300, and R01HL103866 to S.H.]. The UK SCAD study was supported by BeatSCAD, the British Heart Foundation [PG/13/96/30608], and the Leicester NIHR Biomedical Research Centre.

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Translational perspective

This study adds evidence to the possibility of fibromuscular dysplasia (FMD) and spontaneous coronary artery dissection (SCAD) share a common genetic basis. We show that rare loss of function variants in the gene encoding the prostaglandin I_2 receptor (*PTGIR*) are enriched in FMD patients and present in SCAD patients. This pathway is a target of widely used drugs such as aspirin or iloprost. If this mechanism is confirmed by further