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RESEARCH LETTER

Enrichment of Rare Variants in Loeys–Dietz Syndrome Genes in Spontaneous Coronary Artery Dissection but Not in Severe Fibromuscular Dysplasia

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Spontaneous coronary artery dissection (SCAD) is a prime cause of acute myocardial infarction in women < 50 years. Fibromuscular dysplasia (FMD) is present in 17% to 86% of patients with SCAD.[1] In contrast, the prevalence of SCAD in FMD cohorts is <3%.[1] Although the association between these 2 conditions has gained significant interest over the past years, the convergent underlying molecular mechanisms remain elusive.

Familial recurrence studies pointed to a genetic contribution for SCAD and FMD.[2] In case reports and a small-scale exome sequencing-based cohort study, SCAD has occasionally been linked to *FBN1*, *COL3A1*, and *SMAD3* mutations.[2] For FMD, a low yield of molecular testing for syndromic thoracic aortic aneurysm and dissection genes has been reported.[2,3] We aimed to investigate the contribution of rare genetic variants in 25 known thoracic aortic aneurysm and dissection genes to the cause of SCAD and FMD because of the frequent association between the 2 entities. Haloplex-based gene panel sequencing was performed in a large multicenter unrelated patient cohort comprising 179 patients with SCAD±FMD and 102 patients with severe FMD only. The study has full United Kingdom Health Research Authority ethical clearance, and Institutional Review Board approval was obtained from the University of Antwerp and Université Catholique de Louvain. All patients signed informed consent. Variant filtering involved selection of heterozygous nonsynonymous coding or splice site (±2bp and +5bp) variants that are absent in gnomAD v2.1.1, have a minor allele frequency <0.01%, or between 0.01% and 0.1% combined with a CADD score (Combined Annotation Dependent Depletion) >20. After Sanger sequencing validation, selected variants were classified according to the American College of Medical Genetics guidelines. As an independent "control" cohort, gnomAD (genome aggregation database) v2.1.1 (approximately representing the general population) was filtered identically.[4] Two case–control burden analyses were subsequently performed using Fisher exact (expected values <5) or chi-square statistics (expected values ≥5): SCAD±FMD versus gnomAD and FMD only versus gnomAD. To address the multiple testing burden, the significance of the association tests was evaluated using false discovery rate analysis by means of the *q*-value method. A *q* value <0.05 was considered significant. Segregation analysis was carried out for all filtered variants in the associated genes and (likely) pathogenic variants in the other genes if DNA of relatives was available.

Of the patients with SCAD, 38.5% also showed FMD. Connective tissue disease features (including joint hypermobility or dislocations, thin or smooth or hyperelastic skin, varicose veins, pes planus or talipes, hallux valgus, pectus asymmetry or excavatum/carinatum, inguinal hernia, scoliosis, cutis marmorata, joint pain, easy bruising, striae, atrophic scars, tendon rupture, osteoarthritis) were displayed in 26.3% and extracoronary arterial involvement in 26.3%, and 21.8% had a positive

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Table. Overview of Rare Variants in LDS Genes and (Likely) Pathogenic Variants in Other Thoracic Aortic Aneurysm and Dissection Genes

Gene Variant	MAF gnomAD	ACMG Classification	M/F	CTD	HT	SCAD	FMD	Extracoronary Arterial Involvement	Family History
SMAD2 c.619C>G (p.Pro207Ala)	0	VUS (PP2; PM2)	F	N	Y	Cx/RCA	…	…	…
SMAD2 c.1082A>C (p.Asn361Thr)	0	LP (PP2-3; PM1-2)	M	Y	Y	LAD	…	Tortuosity carotid/ iliac artery	…
SMAD2 c.1108C>T (p.Pro370Ser)	0	LP (PP2-3; PM1-2)	F	N	N	RCA	R/CC/other	Arterial dissection	…
SMAD3 c.871+1G>A	0	LP (PP3; PM1-2,4)	F	Y	N	LAD	…	Infrarenal aneurysm	Sister: incidentally identified small brain aneurysm (–)
									Brother: splenic aneurysm requiring surgery (+)
									Mother: abdominal aortic rupture (?)
TGFB2 c.440C>T (p.Pro147Leu)	4.1E-4	VUS (PM1)	F	N	Y	LAD	R	…	…
TGFB2 c.440C>T (p.Pro147Leu)	4.1E-4	VUS (PM1)	F	N	N	LAD	…	…	…
TGFB2 c.631C>T (p.Arg211Cys)	3.2E-5	VUS (PP3; PM1)	F	N	N	LAD	R/CC	Vertebral artery dissection	…
TGFB2 c.740G>T (p.Gly247Val)	0	VUS (PP3; PM1-2)	F	N	N	LAD	…	…	…
TGFB3 c.454C>T (p.Arg152Trp)	1.4E-5	VUS (PP3; PM1)	F	Y	N	LAD	R	Common iliac artery aneurysm	…
TGFB3 c.463C>T (p.Arg155Trp)	0	VUS (PP3; PM1-2)	F	Y	N	OM2	…	Tortuosity vertebral arteries	…
TGFB3 c.813G>C (p.Lys271Asn)	7.4E-5	VUS (PM1)	F	?	Y	Cx	?	…	Brother: thoracic aortic aneurysm (?)
TGFB3 c.1202T>C (p.Leu401Pro)	0	LP (PP3,5; PM1-2)	F	N	N	…	NA	…	Father: abdominal and thoracic aortic aneurysm (+)
TGFBR1 c.739G>A (p.Glu247Lys)	0	VUS (PP3; PM1-2)	M	N	N	LAD	…	…	…
TGFBR1 c.1499G>C (p.Gly500Ala)	4.0E-6	VUS (-)	F	N	Y	LAD	R/CC	Arterial dissection	…
TGFBR2 c.1082A>G (p.Tyr361Cys)	0	VUS (PP3; PM1-2)	F	N	Y	NA	…	CC aneurysm and arterial dissection	…
TGFBR2 c.1718C>T (p.Ser573Leu)	1.6E-5	VUS (PP3)	F	N	N	RDP	R	…	…
TGFBR2 c.1396+5G>T	0	LP (PP3; PM2,4,6)	F	Y	N	Cx	…	…	(de novo)
LOX c.893T>G (p.Met298Arg)	0	P (PP3; PM1-2; PS1,3)	F	Y	Y	Cx	R	…	Mother: brain aneurysm (?)
FLNA c.3806G>A (p.Gly1269Asp)	0	LP (PP2-3; PM2,4)	F	N	Y	LAD	…	Mild aortic dilation	Mother: thoracic aortic aneurysm and rupture (?)
									Daughter: BAV with mild ascending aortic aneurysm (?)
COL3A1 c.737delG (p.Gly246Aspfs*17)	0	P (PP3; PM2; PVS1)	F	N	?	…	R	Arterial aneurysm	…
COL3A1 c.2177G>T (p.Gly726Val)	0	LP (PP2-3; PM1-2,5)	F	N	Y	LAD/Cx	…	…	…

Extracoronary arterial involvement includes tortuosity, aneurysm, or dissection of noncoronary arteries. Family history is deemed positive on documentation of at least 1 first- or second-degree relative with SCAD, FMD, or arterial aneurysm/dissection. ? indicates unknown variant status; (–), variant is absent; (+), variant is present; ACMG, American College of Medical Genetics; BAV, bicuspid aortic valve; CC, cervico-cephalic; CTD, connective tissue disease findings; Cx, circumflex coronary artery; F, female; FMD, fibromuscular dysplasia; gnomAD, genome aggregation database; HT, hypertension; LAD, left anterior descending coronary artery; LDS, Loeys–Dietz syndrome; LP, likely pathogenic; M, male; MAF, minor allele frequency; N, no; NA, yes, but the location is unknown; OM2, obtuse marginal 2; P, pathogenic; R, renal; RCA, right coronary artery; RDP, right descending posterior coronary artery; SCAD, spontaneous coronary artery dissection; TAAD, thoracic aortic aneurysm and dissection; VUS, variant of uncertain significance; and Y, yes.

family history. In the FMD-only cohort, these frequencies were 0%, 84.3%, and 2.9%, respectively. Multifocal FMD was observed in 84.3% of cases, and in 46.1% of patients, FMD was observed in multiple vascular beds.

In the single-gene burden analyses, only *SMAD2* reached significant enrichment for rare variants in patients with SCAD±FMD compared with the general population after false discovery rate correction (3/358 [0.8%] versus 172/241 932 [0.1%], *P*=1E-3, *q*=1.7E-2). However, we did observe a higher frequency of variants in all 6 Loeys–Dietz syndrome (LDS) genes (ie, *TGFBR1/2*, *SMAD2/3*, *TGFB2/3*; Table). A combined LDS gene burden analysis confirmed a highly significant *P* value surviving false discovery rate correction (16/358 [4.5%] versus 3485/235 972 [1.5%]; *P*=3E-6, q=1E-4). The patient cohort with variants in the LDS genes was not statistically significantly different from the whole SCAD±FMD cohort with respect to sex, age at SCAD event, family history, SCAD location, FMD co-occurrence, connective tissue disease findings, history of hypertension, and concurrence with extracoronary tortuosity or aneurysm/dissection. None of the variant carriers was clinically diagnosed with LDS or presented with the typical LDS triad, suggesting that these variants can cause a milder phenotype. Segregation analysis in the limited number of relatives from which DNA was available hints to variable expressivity (Table). Although no significance was reached for the other analyzed thoracic aortic aneurysm and dissection genes, we also identified likely pathogenic variants in *COL3A1*, *FLNA*, and *LOX* in patients with SCAD±FMD with a positive family history of arteriopathy (Table). Whereas pathogenic *COL3A1* variants were described in patients with SCAD previously,[2] *FLNA* and *LOX* variants have not. Altogether, thoracic aortic aneurysm and dissection panel analysis of the SCAD±FMD cohort reached a rare variant uptake of 10.6%.

Despite previous identification of elevated TGFB1 and TGFB2 plasma levels in patients with mostly severe FMD,[2] neither the single-gene analyses nor combined LDS gene analysis (1/204 [0.5%] versus 3485/235 972 [1.5%]; *P*=3.8E-1) yielded significant *P* values in our FMD-only series. However, we did identify 1 multifocal FMD patient with a *TGFB3* variant (p.Leu401Pro) that was previously reported in LDS,[5] and for the first time identified a patient with FMD (multifocal) with a pathogenic *COL3A1* variant (p.Gly246Aspfs*17).

In conclusion, we demonstrated that rare variants in the known LDS genes impinge on SCAD risk, implying a pathophysiological role for dysregulated transforming growth factor β signaling and, hence, opening new avenues for SCAD research and future prevention and therapy. Although validation in other SCAD cohorts is warranted, our results advocate for routine molecular diagnostic screening of LDS genes in patients with SCAD, even in those without connective tissue disease manifestations. We showed that pathogenic *FLNA* and *LOX* variants are occasionally found in SCAD±FMD cases and revealed the presence of pathogenic *COL3A1* variants in both patients with SCAD±FMD and patients with FMD only.

Gene panel sequencing was done for *ACTA2*, *BGN*, *COL3A1*, *ELN*, *EMILIN1*, *FBN1/2*, *FLNA*, *FOXE3*, *LOX*, *MAT2A*, *MFAP5*, *MYH11*, *MYLK*, *NOTCH1*, *PRKG1*, *SKI*, *SMAD2/3/4/6*, *TGFB2/3*, and *TGFBR1/2*. For all samples, at least 99% of the target region was covered at ≥30×. The following transcripts were used to annotate all variants in the associated genes or (likely) pathogenic variants in any of the other genes: ENST00000402690, ENST00000327367, ENST00000366929, ENST00000238682, ENST00000374994, ENST00000359013, ENST00000304636, ENST00000369850, and ENST00000231004.

ARTICLE INFORMATION

Data sharing: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX

Collaborators of the European/International Fibromuscular Dysplasia Registry and Initiative (FEIRI)

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