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ARTICLE

Expanding the genetic and phenotypic spectrum of *ACTA2*-related vasculopathies in a Dutch cohort

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ABSTRACT

Purpose: Heterozygous pathogenic/likely pathogenic (P/LP) variants in the *ACTA2* gene confer a high risk for thoracic aortic aneurysms and aortic dissections. This retrospective multicenter study elucidates the clinical outcome of *ACTA2*-related vasculopathies.
Methods: Index patients and relatives with a P/LP variant in *ACTA2* were included. Data were collected through retrospective review of medical records using a standardized questionnaire.
Results: A total of 49 individuals from 28 families participated in our study. In total, 20 different *ACTA2* variants were detected. Aortic events occurred in 65% of the cases (78.6% index patients and 47.6% relatives). Male sex and hypertension emerged as significantly associated with aortic events. Of 20 individuals, 5 had an aortic diameter of <45 mm (1.77 inches) at the time of the type A dissection. Mean age at first aortic event was 49.0 ± 12.4 years. Severe surgical complications for type A and type B dissection occurred in 25% and 16.7% of the cases and in-hospital mortality rates were 9.5% and 0%, respectively.
Conclusion: P/LP *ACTA2* variants are associated with an increased risk for an aortic event and age-related penetrance, which emphasizes the importance of early recognition of the disease. Caregivers should be aware of the risk for aortic dissections, even in individuals with aortic diameters within the normal range.

Introduction

Pathogenic (P) variants in the *ACTA2* gene (OMIM 102620, ORPHA:91387, ORPHA:2573), encoding the smooth muscle cell–specific isoform of alpha-actin, can cause a variety of vascular disorders including thoracic aortic aneurysms and dissections, premature coronary artery disease (CAD), and ischemic stroke.[1,2] In addition, P variants in *ACTA2* are associated with congenital heart diseases (eg, bicuspid aortic valve [BAV] and patent ductus arteriosus), iris flocculi, and livedo reticularis. *ACTA2*-related vasculopathy is inherited as an autosomal dominant condition with reduced and age-related

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penetrance. Little is known about the risk factors for an aortic event and surgical outcome. Male sex, aortic diameter, and specific variants in *ACTA2* are associated with higher risk for aortic events than the general population.[2-4] Although type A dissections occur more frequently in individuals with a P or likely P (LP) *ACTA2* variant, type B dissections are present at younger age. Type B dissections are often complicated by aortic rupture and/or visceral or limb ischemia, for which surgical intervention is needed.[3]

The *ACTA2* gene is located on chromosome 10 at q23.31 and consists of 8 coding exons that encode a 377 amino acid protein. Until October 2021, 39 different variants in the *ACTA2* gene have been included in the Universal Mutation Database (www.umd.be). The vast majority (90%) are missense variants.

In this retrospective multicenter study, we described the clinical presentation of 49 newly identified individuals from 28 families with a P/LP *ACTA2* variant and elucidated the clinical outcome of *ACTA2*-related vasculopathies, including risk factors for an aortic event, detailed surgical outcomes, and survival.

Materials and Methods

Study population

Index patients and relatives with a P/LP variant in the *ACTA2* gene were included in this study at all 7 tertiary referral centers in the Netherlands from 2018 to 2020. Data were collected through retrospective review of available medical records using standardized questionnaire sent to involved clinical geneticists. This questionnaire addressed the genetic test results, the occurrence of thoracic aortic aneurysm and dissection (TAAD) and related treatments, various cardiovascular and ophthalmologic abnormalities, obstetric complications, other relevant medical conditions, and medication use.

The Stanford classification was used to classify thoracic aortic dissections, type A dissection involves the ascending aorta/arch, whereas type B dissection originates in the descending thoracic aorta, distal to the left subclavian artery.[5] Absolute aortic diameters of ≥40 mm (1.57 inches) for the thoracic aorta and ≥30 mm (1.18 inches) for the abdominal aorta were classified as an aneurysm.[6,7] An aortic event was defined as an elective aortic aneurysm repair or an aortic dissection, whether or not preceded by an aneurysm. The *z*-score was calculated using the web-based calculation tool on the Marfan foundation site (https://marfan.org/dx/z-score-adults/), which uses the normograms of Devereux et al[8] and the method of Dubois and Dubois[9] to calculate the body surface area.

Systolic blood pressure of >130 mm Hg and/or diastolic blood pressure of >80 mm Hg and/or the use of antihypertensive agents was defined as hypertension.[10] Severe complications implicated those that are potentially life threatening or are disabling for the individual.

Variant analysis

All index patients underwent sequencing of a TAAD gene panel, including at least the definitive and strong genes for TAAD.[11] The pathogenicity of variants in the *ACTA2* gene (NG_011541.1, NM_1613.3) was assessed using (Alamut Visual v2.11) software, which integrates data from large-scale population genetic studies, evolutionary conservation of nucleotides and amino acids, and in silico missense and messenger RNA (mRNA) splicing prediction tools. The Mendelian Clinically Applicable Pathogenicity Score was added to improve classification of rare missense variants.[12] A threshold of 0.025 correctly dismisses 60% of variants of uncertain significance at 95% sensitivity.

ACTA2 variants with a minor allele frequency of >1% in the Genome Aggregation Database v2.1.1[13] and synonymous and intronic variants without predicted effect on mRNA splicing were excluded from further analysis. If possible, cosegregation of the variant with the disease phenotype in the family was analyzed. RNA sequencing on RNA isolated from fibroblasts obtained from skin biopsies was performed for the splice variant p.[Ala206Gly,-Glu207_Ile269del] in the last exon of *ACTA2*. Variants were interpreted according to the 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines[14] and classified into 5 categories ie, P, LP, uncertain significance, likely benign, and benign. Only variants classified as P or LP were included in this study.

Of note, de novo *ACTA2* variants disrupting the Arg179 residue are associated with a specific phenotype referred to as multisystemic smooth muscle dysfunction syndrome (OMIM 613834, ORPHA:404463) and were excluded from this study.

Statistical analysis

Continuous variables were summarized by mean and SD. Categorical variables are shown in frequencies and percentages. Comparisons were made using the Student *t* test and χ^2 test with Yates' correction, respectively. A *P* value of <.05 was considered statistically significant. A binary logistic regression analysis was used to identify risk factors associated with aortic event. Variables with a *P* value around or <.05 in the univariate analysis were included in the multiple logistic regression model. Odds ratios, CIs, and *P* values were used for interpretation.

Results

Characteristics of the study population

A total of 49 individuals from 28 families were included. The number of men and women were almost equal (Table 1). Median age at genetic diagnosis was 50.0 (interquartile range = 25.0) years.

Table 1 Clinical characteristics

Variable	All ($N = 49$)	With Aortic Event ($n = 32$)	Without Aortic Event ($n = 16$)	Unknown Aortic Event ($n = 1$)	P value
Median age at diagnose, y (IQR)	50.0 (25.0)	50.5 (20.3)	45.00 (33.0)		.754
Sex					.051
Male	23	19	4		
Female	26	13	12	1	
Blood pressure					.005
Hypertension	22	17	5		
No hypertension	19	8	11		
Unknown	8	7		1	
BMI, mean (SD)	27.7 (4.0)	28.2 (7.3)	27.2 (3.7)		.354
Smoking history					.507
Smoking	22	14	8		
No smoking	14	9	5		
Unknown	13	9	3	1	

BMI, body mass index; *IQR*, interquartile range.

Molecular studies

A total of 20 different P/LP *ACTA2* variants were detected in this cohort, including 18 missense, 1 splice site, and 1 frameshift variant (Figure 1, Table 2). Functional assay for the splice variant p.[Ala206Gly,Glu207_Ile269del] showed no aberrant splicing and an equal presence of both alleles. The frameshift variant p.(Ser340Cysfs*26) is a deletion of 2 nucleotides, leading to a frameshift and a premature protein termination in the last exon. In total, 8 variants were novel. A total of 4 variants were found in more than 1 family; p.(Arg39Cys) was found in 3 families and p.(Arg149Cys), p.(His175Asp), and p.(Arg208His) were each found in 2 families. In 17 families, 63 relatives were screened for the familial P/LP *ACTA2* variants, including 30 heterozygotes and 33 nonheterozygotes of the P/LP variants in *ACTA2*. Medical records of 21 heterozygotes from 11 families were available: 16 first-degree and 5 second-degree relatives. Relatives from 6 of the 17 families were not included because they live abroad, their test result was unknown at the time of inclusion, or none of the relatives were heterozygotes of the P/LP variants in *ACTA2*.

No proven de novo variants were found, but not all parents were available for genetic testing.

Aortic events

In total, 32 of 49 (65.0%) individuals had an aortic event, including elective aortic aneurysm repair in 4 and aortic dissection in 29 (20 type A, 12 type B, and 2 abdominal) (Table 3). Three individuals underwent elective aortic aneurysm repair because of ascending aortic aneurysms of diameters of 50, 50, and 60 mm (1.97, 1.97, and 2.36 inches, respectively) and 1 for an abdominal aortic aneurysm of 51 mm (2.01 inches). A total of 5 individuals had both type A and type B dissection, with several years between the events and 1 had an elective aortic aneurysm repair followed by a type A dissection years later. Mean age at first aortic event was 49.0 (±12.4) years. The youngest individual was a 24-year old male with a type B dissection extending from the

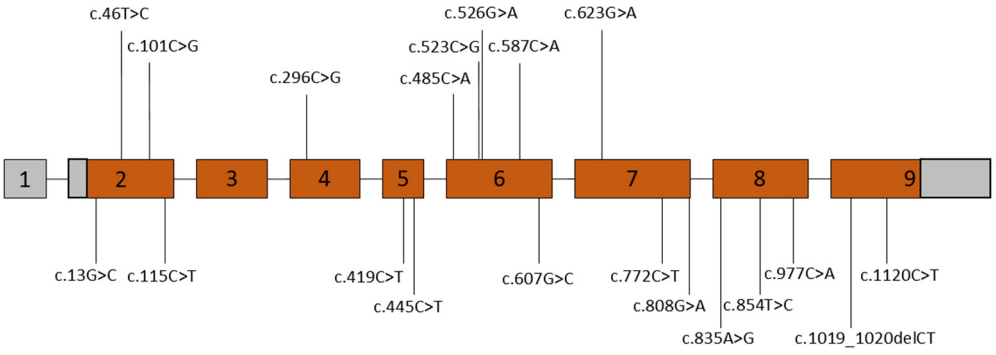

Figure 1 Schematic representation of the *ACTA2* gene. Schematic overview of the *ACTA2* gene, with the location of pathogenic and likely pathogenic variants in *ACTA2* found in our cohort. The variants displayed above the exons are novel and the variants displayed below the exons are known. Orange boxes represent coding exons, gray boxes represent untranslated region (UTR), and connecting lines represent introns (unscaled).

Table 2 Overview of pathogenic and likely pathogenic *ACTA2* variants identified in this study

Nucleotide Change	Protein Change	Coding Effect	MAF gnomAD	M-CAP Score	Evidence[14,a,b,c,d]	Classification	Known/Novel
c.13G>C	p.(Glu5Gln)	Missense	1/31390	0.416	PS4, PM2(s), PP2, PP1	Likely pathogenic	Described previously[15]
c.46T>C	p.(Ser16Pro)	Missense	Absent	0.765	PS4, PM2(s), PP1, PP2, PP3	Likely pathogenic	Novel
c.101C>G	p.(Pro34Arg)	Missense	Absent	0.692	PS4, PM2(s), PP1, PP2, PP3	Likely pathogenic	Novel
c.115C>T	p.(Arg39Cys)	Missense	1/31374	0.347	PS4, PM2(s), PM5, PP2, PP3, PP5	Pathogenic	Known[16]
c.296C>G	p.(Ala99Gly)	Missense	5/251008	0.389	PS4, PM2(s), PP1, PP2, PP3	Likely pathogenic	Novel
c.419C>T	p.(Ala140Val)	Missense	Absent	0.592	PS1, PS4, PM2(s), PP2, PP3, PP5	Pathogenic	Known[17]
c.445C>T	p.(Arg149Cys)	Missense	1/278732	0.509	PS1, PS4, PM2(s), PM5, PM6, PP2, PP3, PP4, PP5	Pathogenic	Known[2]
c.485C>A	p.(Thr162Asn)	Missense	Absent	0.550	PS4, PM2(s), PP1, PP2, PP3, PP4	Likely pathogenic	Novel
c.523C>G	p.(His175Asp)	Missense	Absent	0.525	PS4, PM2(s), PP1, PP2, PP3	Likely pathogenic	Novel
c.526G>A	p.(AIa176Thr)	Missense	1/251062	0.288	PS4, PM2(s), PP1, PP2, PP3	Likely pathogenic	Novel
c.587C>A	p.(Thr196Asn)	Missense	Absent	0.194	PS4, PM2(s), PP1, PP2, PP3	Likely pathogenic	Novel
c.607G>C	p.(Val203Leu)	Missense	1/250930	0.082	PS4, PM2(s), PP1, PP2	Likely pathogenic	Described previously[15]
c.623G>A	p.(Arg208His)	Missense	3/250572	0.559	PS4, PM2(s), PP1, PP2, PP3	Likely pathogenic	Novel
c.772C>T	p.(Arg258Cys)	Missense	1/31384	0.516	PS1, PS4, PM2, PM5, PP2, PP3, PP5	Pathogenic	Known[1]
c.808G>A	p.[Ala206Gly,Glu207_Ile269del]	Splice	1/250820	0.696	PS3, PS4, PM2(s), PP1, PP4, PP5	Pathogenic	Known[3]
c.835A>G	p.(Thr279Ala)	Missense	Absent	0.100	PS1, PS4, PM2(s), PP2	Pathogenic	Described previously[15]
c.854T>C	p.(Met285Thr)	Missense	1/251106	0.367	PS1, PS4, PM2(s), PP2, PP3	Pathogenic	Described previously[15]
c.977C>A	p.(Thr326Asn)	Missense	15/250886	0.141	PS4, PM2(s), PP1, PP2, PP5	Likely pathogenic	Known[1]
c.1019_1020delCT	p.(Ser340Cysfs*26)	Frameshift	Absent	N/A	PS1, PS4, PM2(s), PP5	Pathogenic	Known[18]
c.1120C>T	p.(Arg374Cys)	Missense	1/251242	0.275	PS1, PS4, PM2(s), PP2, PP3	Pathogenic	Described previously[15]

NCBI reference sequence: NG_011541.1, NM_001613.3.

ClinGen, Clinical Genome Resource; *gnomAD*, Genome Aggregation Database; *MAF*, minor allele frequency; *M-CAP*, Mendelian Clinically Applicable Pathogenicity; *N/A*, not applicable.

[a]PS4 is given after calculating BayesianOR according to the recommendation of Kyung Cho et al (Cho YK, Won D, Keum C, Lee BH, Seo GH, Lee BC. A novel PS4 criterion approach based on symptoms of rare diseases and in-house frequency data in a Bayesian framework. https://doi.org/10.1101/2020.07.22.215426). Odds ratios are > 5.0 and CI does not include 1.0.

[b]PM2 is used as a supportive argument according to the latest recommendations, ClinGen Sequence Variant Interpretation Recommendation for PM2 version 1.0

[c]Specification of PP2: the missense constraint score (*z*-score ≥ 3.09) from gnomAD was used.[19,20] *z*-score for *ACTA2* was 3.2 (https://gnomad.broadinstitute.org); the missense variants were spread over all exons.

[d]Specification for PP4: presence of iris flocculi, which is highly specific for ACTA2 related vasculopathies.

Table 3 Surgical data

Variable	Elective Aortic Aneurysm Repair ($n = 4$)	Type A Dissection ($n = 20$)	Type B Dissection ($n = 12$)	Dissection Abdominal Aorta ($n = 2$)
Operation performed (% of total)	4 (100)	17 (85.0)	6 (50.0)	1 (50.0)
Mean diameter, mm (SD)		47.2 (15.1)[a]	33 (7.1)[b]	70[c]
Type of intervention (% of operations)				
SCAR	1 (25)			
SCAR and hemiarch replacement	1 (25)	9 (52.9)		
SCAR and aortic arch replacement	1 (25)	1 (5.9)		
AVR plus ascending aorta and hemiarch replacement		5 (29.4)		
AVR plus ascending aorta and arch replacement		2 (11.8)		
Descending aortic replacement			1 (16.7)	
TEVAR procedure			5 (83.3)	
EVAR procedure	1 (25)			
Aortic bifurcation prosthesis				1 (100%)
Number of patients with severe complications (%)	1 (25)	4 (25)	1 (16.7)	
Severe complications				
Death		2		
Sternum infection		1		
Perioperative rupture of descending aorta			1	
Myocardial infarction		1		
Cardiac tamponade	1			

AVR, aortic valve replacement; *EVAR,* endovascular aortic repair; *TEVAR,* thoracic endovascular aortic repair; *SCAR,* supracoronary ascending aorta replacement.

[a]1.86 inches

[b]1.30 inches

[c]2.76 inches

aortic arch to the renal arteries. Of the 18 individuals without an aortic event, 9 had an aortic aneurysm, including 3 at the level of the aortic root, 5 on the ascending aorta, and 1 was abdominal.

The mean age at the time of diagnosis of the aortic aneurysm was 50.9 (± 10.4) years. The mean diameter of the thoracic aortic aneurysms was 46.0 (± 4.3) mm (1.81 [± 0.17] inches) and the diameter of the abdominal aortic aneurysm was 35 mm (1.38 inches). In total, 8 individuals were asymptomatic, which is defined as the absence of an aortic event and/or an aortic aneurysm. The mean diameter in asymptomatic individuals was 30.6 (20-38) mm (1.20 [0.79-1.5] inches) and the mean age was 37.0 (± 17.0) years. One individual was a post mortem diagnosed heterozygote; no information about aortic diameters was present.

Excluding the index patients, 16 of 21 (76.2%) relatives with a P/LP variant in *ACTA2* had a phenotype, including 10 (47.6%) with an aortic event at a mean age of 53.3 (±12.8) years, 3 with an aortic aneurysm, 2 with iris flocculi, and 1 with premature CAD. The 5 relatives without signs of the disease had a mean age of 53.4 (±18.4) years.

The mean diameter of the ascending aorta at the time of an aortic dissection was 47.2 (30-79) mm (1.86 [1.18- 3.11] inches) for a type A dissection based on available data from 9 individuals. Five of them, in 4 females and 1 male, had a diameter of ≤45 mm (1.77 inches) at the time of dissection, namely 30 mm (1.18 inches) (z-score = –1.3), 30 mm (1.18 inches) (z-score = –0.9), 40 mm (1.57 inches) (z-score = 3.0), 41 mm (1.61 inches) (z-score = 2.9), and 45 mm (1.77 inches) (z-score = 3.5). The diameter of the aorta at the time of a type B dissection was known for 2 patients: 28 (1.10 inches) (z-score = –2.47) mm and 38 mm (1.50 inches) (z-score = 0.4). The diameter of the abdominal aorta at the time of dissection in 1 patient was 70 mm (2.76 inches) (z-score = 12.5). In the 4 individuals with an elective aortic aneurysm repair, the diameter at the time of surgery was 50 mm (1.97 inches), 50 mm (1.97 inches), and 60 mm (2.36 inches) for the ascending aorta and 51 mm (2.01 inches) for the abdominal aorta. In most individuals (17/28) with a thoracic aortic aneurysm, the maximum diameter was found at the level of the ascending aorta.

Assessment of the cardiovascular risk factors revealed that the prevalence of aortic events was higher in men than in women ($P = .051$) and significantly higher in individuals with hypertension than in individuals without hypertension before dissection ($P = .005$) (Table 1). Hypertension (odds ratio = 9.768, CI = 1.701-56.076, $P = .011$) and male sex (odds ratio = 8.526, CI = 1.412-51.493, $P = .020$) were identified as independent risk factors for the occurrence of an aortic event in the multivariable analysis. No correlation was found between sex and hypertension ($P = .366$). For 8 individuals, blood pressure measurements were not available. For 2 individuals, no medical records were available; they were diagnosed as heterozygotes post mortem. Other cardiovascular risk factors like body mass index and smoking did not significantly increase the risk for an aortic event (Table 1).

Survival

In total, 8 individuals died at a mean age of 51.5 years (±10.6 years), including 6 deaths caused by an aortic event and in 2 the cause of death was unknown. Three individuals died before surgical intervention for a type A dissection could be performed, including 1 post mortem diagnosed heterozygote. One individual underwent a supracoronary ascending aorta replacement (SCAR) plus hemiarch and died 1 month later because of a new acute type B dissection. Two individuals died as a result of complications of the surgery. One individual first had an SCAR with replacement of the aortic arch because of a type A dissection extending till below the renal arteries and died in hospital after a second surgery for the remaining dissection. The other received aortic valve replacement with aortic ascendens and arch replacement because of an acute type A dissection. After the operation, a myocardial infarction occurred caused by an occlusion of the left anterior descending artery. Because of hemodynamic instability, extracorporeal membrane oxygenation was needed. The individual died of cardiogenic shock after explant of the extracorporeal membrane oxygenation.

Surgical data

In total, 17 individuals (85.0%) with a type A dissection underwent surgery (Table 3). Severe complications occurred in 4 (25%) of the type A dissection surgeries, including 2 deaths as a result of multiple complications as described before. One individual suffered from a sternum infection and 1 had a myocardial infarction.

In total, 6 individuals (50.0%) with a type B dissection underwent surgery, including 5 with a thoracic endovascular aortic repair (TEVAR) procedure. One individual had 7 years of medical treatment for a type B dissection, and after an elective ascending aortic and aortic arch replacement due to an aneurysm, the descending aorta was replaced by a prosthesis a few months later (Table 3). A total of 4 individuals had a TEVAR immediately after the type B dissection was diagnosed, in 3 because of an acute ischemic leg and in 1 because of a small rupture in the aortic arch. In 1 individual, a TEVAR was performed 9 days after the type B dissection was diagnosed; information about the reason for surgical intervention could not be obtained.

Severe complications occurred in one (16.7%) of the surgeries for type B dissection (Table 3). This individual had a perioperative rupture of the descending aorta during the TEVAR procedure with paraplegia afterward. Hemodynamic instability occurred immediately after the procedure due to a rupture, requiring a lateral thoracotomy.

Two individuals had surgeries for a consecutive type A and B dissection and type B and A dissection, respectively. One individual received aortic bifurcation prosthesis because of an abdominal aortic dissection. In total, 5 individuals were treated conservatively for a type B dissection and 1 for an abdominal aortic dissection.

A total of 4 individuals underwent elective aortic aneurysm repair. The SCAR plus hemiarch replacement was complicated by cardiac tamponade. The individual who underwent the endovascular aortic repair procedure underwent a secondary intervention after 9 years because of an endoleak. The individual with an SCAR, underwent an aortic valve replacement plus ascending aorta and arch replacement procedure because of a type A aortic dissection a few years later.

Congenital heart malformations

In total, 45 individuals were examined by a cardiologist: 6 (13%) had a congenital heart malformation, including persistent ductus arteriosus (PDA) in 3, BAV in 2, and atrial septum defect type II in 1. These 6 individuals all had different P/LP variants in *ACTA2* (Table 4). Four individuals were not examined by a cardiologist; 2 of them were obligate heterozygotes and 2 individuals were diagnosed as heterozygotes post mortem.

Table 4 Other health conditions in heterozygotes of pathogenic or likely pathogenic variant in *ACTA2*

Variable	*ACTA2* Variant	Frequency (% of Total Patients Screened)
Congenital heart malformation		6 (13.3)
PDA	p.(Arg39Cys)	3 (6.7)
	p.(His175Asp)	
	p.(Ser16Pro)	
BAV	p.(Glu5Gln)	2 (4.4)
	p.(Met285Thr)	
ASD type II	p.(Ser340Cysfs*26)	1 (2.2)
Eye anomalies		10 (40)
Iris flocculi	p.(Arg149Cys)	7 (28)
	p.[Ala206Gly,Glu207_Ile269del]	
	p.(Thr162Asn)	
Tortuosity retinal vessels	p.(Arg39Cys)	1 (4)
Keratoconus	p.[Ala206Gly,Glu207_Ile269del]	1 (4)
Retinal tear	p.(AIa176Thr)	1 (4)

ASD, atrium septum defect; *BAV*, bicuspid aortic valve; *PDA*, persistent ductus arteriosus.

Ocular abnormalities

Ophthalmologic examination was performed in 25 individuals and showed abnormalities in 10 (40%), of which 7 individuals had iris flocculi. All heterozygotes of the p.(Arg149Cys) variant in *ACTA2* with ophthalmologic investigation ($n = 5$) had iris flocculi (Table 4). Other ocular abnormalities, including tortuosity of the retinal vessels, keratoconus, and retinal tear, were described once.

Other clinical manifestations

Occlusive vascular diseases were reported in 9 individuals, including CAD in 6, livedo reticularis in 2, and an ischemic cerebrovascular accident (CVA) in 1. Two individuals suffered from venous thrombosis.

In total, 11 individuals underwent imaging of the coronary arteries, including computed tomography angiography in 8, coronary angiogram in 2, and myocardial perfusion imaging in 1. A myocardial infarction was diagnosed in 3, dissection of the left anterior descending artery in 1, CAD in 1, and stenosis of the right coronary artery in 1. All 3 myocardial infarctions were caused by atheromatosis. Two individuals underwent coronary artery bypass graft and 2 underwent percutaneous coronary intervention. Of the 6 individuals with CAD, 2 were younger than 45 years.

Imaging of the cerebral arteries by computed tomography angiography or magnetic resonance angiography was performed in 16 individuals without neurologic symptoms and revealed abnormalities in 1 (6%), namely a dilated and tortuous course of the distal vertebral artery.

Other vascular abnormalities included dilatation and elongation of the iliac arteries and tortuosity of the renal artery, each reported once. However, only 14 individuals underwent imaging of cerebral, thoracic, and abdominal vascular tree.

An umbilical hernia was reported in 4 unrelated individuals, including 1 with an umbilical and inguinal hernia. Two related individuals had an inguinal hernia only. In 1 individual, the correction of an umbilical hernia with a mesh implantation was complicated by a perforation of the sigmoid, requiring reoperation.

The obstetric history was available for 22 women with a total of 52 pregnancies. One individual underwent a cesarean section at 38 weeks of pregnancy because of resistant hypertension. Five days postpartum, a type A dissection occurred. The diameter of the ascending aorta was <40 mm (1.57 inches) and the sinotubular junction was 25 mm (0.98 inches) at the time of the valve sparing aortic replacement.

Discussion

We provide detailed phenotypic and genotypic data of 49 individuals from 28 families harboring 20 different heterozygous P/LP *ACTA2* variants. We have identified 8 novel *ACTA2* variants. There is no mutational hotspot because the P/LP variants found in our cohort were distributed all over the gene, in concordance with previous studies. Most P/LP variants in *ACTA2* found in our cohort were missense variants. Only 1 frameshift in the last exon of *ACTA2* and 1 splice site variant were identified.

Guo et al[2] suggested a dominant negative disease mechanism in *ACTA2*-related vasculopathies. In smooth muscle cells of individuals with a P/LP *ACTA2* variant, reduced *ACTA2*-containing fibers were shown, suggesting disruption of actin fiber assembly or stability. Renard et al[18] described 2 nonsense variants in *ACTA2*, which suggested that premature truncating variants in *ACTA2* can cause TAAD as well. Although no functional assays were performed, both variants are predicted to escape from nonsense-mediated mRNA decay because they are located at the end of *ACTA2* and may still exert a dominant negative effect. The functional assay for the splice variant performed in our study supported the suggestion of a dominant negative effect. No functional assay has been performed for the frameshift variant, but this variant is also located in the last exon of *ACTA2*, therefore, a dominant negative effect seems plausible. The other novel variants identified in this study are all missense variants.

An aortic event occurred in 65.0% the total study cohort. After the exclusion of index patients, 47.6% of family members had an aortic event at a mean age of 53.3 years (±12.8 years), which probably better represents the penetrance for aortic events of *ACTA2* variants. Nevertheless, our family-based approach, starting from symptomatic individuals, will potentially result in an overestimation of the penetrance of disease-causing variants. An overall penetrance of 76% for aortic events at the age of 85 years has been described previously in a study with a similar approach in identifying heterozygotes of a P/LP *ACTA2* variant.[3] When including all phenotypical features of *ACTA2* related vasculopathy, the penetrance of *ACTA2* variants in relatives was 76.2%. The penetrance of *ACTA2* variants in asymptomatic individuals without a family history of aortic disease, such as secondary or incidental findings from genetic testing, might be significantly lower.[21]

Assessment of known cardiovascular risk factors revealed that the presence of hypertension significantly increased the risk for an aortic event ($P = .011$). Hypertension has not been described before as a specific risk factor for aortic events in heterozygotes of a P/LP variant in *ACTA2*. In individuals without a genetic predisposition, hypertension is a well-known risk factor for thoracic aortic aneurysms and aortic dissections.[22,23] Therefore, it is important to treat all hypertensive *ACTA2* individuals with medication that reduces hemodynamic stress and provide lifestyle advices. In a recent consensus statement for the management of individuals with P *ACTA2* variants, it was even proposed that antihypertensive medication should be considered in all *ACTA2* individuals, ie, also in normotensive individuals.[24]

Male sex also seems to be a predisposing factor for the occurrence of an aortic event in heterozygotes of a P/LP variant in *ACTA2*, as was reported previously by Regalado

et al.[3] This was also seen in individuals with Marfan syndrome caused by P variants in the *FBN1* gene.[25,26] The underlying mechanism for this gender difference is still unclear and warrants further study.

The vast majority of the individuals with a type A dissection underwent surgical intervention and half of the type B dissections were treated surgically. The type of surgeries performed in our cohort did not differ from the standard procedure for aortic dissections. Mortality rate in individuals without a known genetic predisposition for TAAD and with Marfan syndrome treated surgically for an acute type A dissection was 16.7% to 18% and treated with endovascular management for an acute type B dissection was 7.4% to 12.3%.[27-30] For heterozygotes of a P/LP variant in *ACTA2* with an acute dissection, Regalado et al[3] found an in-hospital mortality rate of 14%, but no details about the complications after surgical intervention were available. In our cohort, the in-hospital mortality rate after surgery for a type A dissection was 12.5% and for patients with endovascular treatment for a type B dissection was 0%. Therefore, it seems that individuals with a P/LP variant in *ACTA2* do not have a higher in-hospital mortality rate after aortic dissection intervention than the individuals without a genetic predisposition for TAAD. The general consensus is to avoid a TEVAR procedure in individuals with genetic aortopathies, except in case of a complicated type B dissection. Our data can support the general consensus to perform a TEVAR procedure for complicated type B dissections in individuals with a P/LP variant in *ACTA2* as in individuals with other genetic aortopathies.[24,31]

According to current literature for individuals with P/LP *ACTA2* variants, elective aortic root replacement should be considered in asymptomatic patients with a maximal aortic diameter between 45 and 50 mm (1.77 and 1.97 inches, respectively).[22,32] The American Heart Association (AHA) recommends elective surgery at a maximal ascending aortic diameter of 45 mm (1.77 inches).[33] Although the diameter of the ascending aorta is a well-established risk factor for dissection, an interesting finding in our study is that more than half (55%) of the individuals had a diameter of ≤45 mm (1.77 inches) at the time of dissection. In literature, among 47 individuals with a P/LP variant in *ACTA2* whose aortic diameters were available, 9 (19%) individuals had a diameter of ≤45 mm (1.77 inches) at the time of aortic dissection.[2-4,34] Two type A dissections at a diameter of <45 mm (1.77 inches) occurred in the peri- or postpartum period.[35] We conclude that aortic diameter and *z*-scores solely are not reliable to estimate the risk for an aortic event and that hypertension and male sex should also be taken into account.

PDA is described previously in individuals with an *ACTA2*-related vasculopathies, especially in individuals with the typical p.(Arg179His) variant causing multisystemic smooth muscle dysfunction syndrome.[36,37] Beside the typical p.(Arg179His) variant in *ACTA2*, PDA is also described previously in 2 sisters with the *ACTA2* variant p.(Asn117Lys).[38] However, we showed that it is can also be found in patients with other *ACTA2* variants.

In our cohort, 4% (2/49) of the individuals with different *ACTA2* variants had a BAV, which is in line with the study of Guo et al.[2]

Less than half of the individuals in this cohort had ocular abnormalities, mainly iris flocculi. As reported previously, most individuals with iris flocculi were heterozygote of the p.(Arg149Cys) *ACTA2* variant.[2,39] The other 2 variants in our study associated with iris flocculi were novel, namely p.[Ala206Gly,Glu207_Ile269del] and p.(Thr162Asn).

Tortuosity of the retinal vessels was observed in 1 patient and has also been reported previously, therefore, there might be a correlation with *ACTA2*-related vasculopathy.[36] Keratoconus and retinal tear were identified once and have not been described thus far. It is uncertain whether these features are truly related to the *ACTA2* variants. Previous studies have also reported aniridia and retinal detachment.[36] Premature CAD, defined as age of onset less than 55 and 60 years in men and women, respectively, has been described in 20% of the individuals with a P/LP variant in *ACTA2*.[1] The frequency of premature CAD in our cohort was lower (8%), which might be because of the low imaging rate of the coronary arteries. Both individuals with livedo reticularis carried the p.(Arg149Cys) *ACTA2* variant and this is the only variant described so far in individuals with livedo reticularis.

Only 1 individual had a dilated and tortuous course of the distal vertebral artery (2%), which is similar to the 2% of cerebral aneurysms reported by Guo et al.[1] Other cerebral arteriopathy that have been described included abnormal straightening of the cerebral arteries, stenosis of the intra- and supraclinoid carotid artery, and the midbasilar artery.[40,41] These abnormalities were not seen in our cohort.

In 2018, a group of experts from the European reference network for rare vascular diseases had written a consensus statement for surveillance of individuals with a P/LP *ACTA2* variant.[24] This consensus statement recommends vascular surveillance of the asymptomatic adult heterozygotes of a P/LP *ACTA2* variant using a 2-dimention–transthoracic echocardiography yearly and complete vascular imaging of thorax and abdomen every 2 to 5 years. Besides vascular surveillance, prophylactic treatment with beta-blocker from the age of 4 years is recommended.[24] Despite the small cohort, our data give no reason to question the surveillance recommendations for asymptomatic individuals with a P/LP *ACTA2* variant.

Conclusion

Heterozygous P *ACTA2* variants show reduced penetrance for aortic disease, with hypertension and male sex as predisposing factors. Caregivers should be aware of aortic dissection, even in individuals with aortic diameters within the normal range. We conclude that mere aortic diameter and *z*-scores are not sufficient to predict the risk for an aortic dissection. Therefore, it is important to take into account other risk factors in individuals with an *ACTA2* related vasculopathy like hypertension and male sex. We recommend aggressive treatment of hypertension. There seems to

be no increased risk for complications with acute or prophylactic interventions.

Data Availability

Data are available upon request.

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Ethics Declaration

This study was approved by the Medical Ethics Committees of the Erasmus MC, University Medical Centre Rotterdam (MEC 2017-1162) and all affiliated centers. All patients provided written informed consent before their inclusion in this study.

Conflict of Interest

The authors declare no conflicts of interest.

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