

## Original article

## Genetic profile in patients with complicated acute aortic syndrome: the GEN-AOR study



Antonio M. Puppo Moreno,<sup>a,b,◇,\*</sup> Nereida Bravo-Gil,<sup>c,d,◇</sup> Cristina Méndez-Vidal,<sup>c,d,◇</sup> Alejandro Adsuar Gómez,<sup>e</sup> F. Tadeo Gómez Ruiz,<sup>f</sup> Carlos Jiménez De Juan,<sup>g</sup> Raquel M. Fernández García,<sup>c,d</sup> Rafael Martín Bermúdez,<sup>a</sup> José María López Sánchez,<sup>a</sup> Sara Martín Sastre,<sup>a</sup> Manuel Fernández Caro,<sup>a</sup> Pastora Gallego,<sup>h</sup> and Salud Borrego<sup>c,d</sup>

<sup>a</sup>Unidad de Cuidados Intensivos, Hospital Universitario Virgen del Rocío, Seville, Spain

<sup>b</sup>Departamento de Medicina, Facultad de Medicina, Universidad de Sevilla, Seville, Spain

<sup>c</sup>Departamento de Medicina Materno-fetal, Genética y Reproducción, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain

<sup>d</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Seville, Spain

<sup>e</sup>Departamento de Cirugía Cardiovascular, Hospital Universitario Virgen del Rocío, Seville, Spain

<sup>f</sup>Departamento de Cirugía Vascular, Hospital Universitario Virgen del Rocío, Seville, Spain

<sup>g</sup>Departamento de Medicina Interna, Hospital Universitario Virgen del Rocío, Seville, Spain

<sup>h</sup>Departamento de Cardiología, Hospital Universitario Virgen del Rocío, Seville, Spain

## Article history:

Received 25 May 2022

Accepted 10 October 2022

Available online 25 October 2022

## Keywords:

Acute aortic syndrome

Thoracic aortic aneurysm and dissection

Genetic testing

Aortic diseases

Next-generation sequencing

Genetic counseling

## ABSTRACT

**Introduction and objectives:** Genetic testing is becoming increasingly important for diagnosis and personalized treatments in aortopathies. Here, we aimed to genetically diagnose a group of acute aortic syndrome (AAS) patients consecutively admitted to an intensive care unit and to explore the clinical usefulness of AAS-associated variants during treatment decision-making and family traceability.

**Methods:** We applied targeted next-generation sequencing, covering 42 aortic diseases genes in AAS patients with no signs consistent with syndromic conditions. Detected variants were segregated by Sanger sequencing in available family members. Demographic features, risk factors and clinical symptoms were statistically analyzed by Fisher or Fisher-Freeman-Halton Exact tests, to assess their relationship with genetic results.

**Results:** Analysis of next-generation sequencing data in 73 AAS patients led to the detection of 34 heterozygous candidate variants in 14 different genes in 32 patients. Family screening was performed in 31 relatives belonging to 9 families. We found 13 relatives harboring the family variant, of which 10 showed a genotype compatible with the occurrence of AAS. Statistical tests revealed that the factors associated with a positive genetic diagnosis were the absence of hypertension, lower age, family history of AAS and absence of pain.

**Conclusions:** Our findings broaden the spectrum of the genetic background for AAS. In addition, both index patients and studied relatives benefited from the results obtained, establishing the most appropriate level of surveillance for each group. Finally, this strategy could be reinforced by the use of statistically significant clinical features as a predictive tool for the hereditary character of AAS.

ClinicalTrials.gov (Identifier: NCT04751058)

© 2022 Published by Elsevier España, S.L.U. on behalf of Sociedad Española de Cardiología.

## Perfil genético asociado a pacientes con síndrome aórtico agudo complicado: el estudio GEN-AOR

## RESUMEN

**Introducción y objetivos:** El papel de la genética en el diagnóstico y la personalización de los tratamientos de las aortopatías, es cada vez mayor. En este estudio se analizó la prevalencia de variantes genéticas en pacientes con síndrome aórtico agudo (SAA) admitidos consecutivamente en una unidad de cuidados intensivos y se evaluó su utilidad clínica.

**Métodos:** Mediante secuenciación masiva, se analizó 42 genes asociados a aortopatías en pacientes con SAA no sindrómico. Las variantes identificadas se segregaron mediante secuenciación Sanger en los familiares disponibles. Además, se estudió la relación entre los resultados genéticos y algunas características clínicas mediante la aplicación de los test exactos de Fisher y de Fisher-Freeman-Halton.

## Palabras clave:

Síndrome aórtico agudo

Diseción y aneurisma aórtico torácico

Estudio genético

Enfermedades aórticas

Secuenciación de nueva generación

Consejo genético

\* Corresponding authors.

E-mail addresses: salud.borrego.sspa@juntadeandalucia.es, antonio.puppo.sspa@juntadeandalucia.es (A.M. Puppo Moreno).

◇ These authors should be considered as joint first authors.

**Resultados:** El análisis de los datos genómicos de 73 pacientes de SAA dio como resultado la identificación de 34 variantes candidatas en 32 individuos, localizadas en 14 genes diferentes. La segregación familiar se realizó en 31 individuos pertenecientes a 9 familias, donde se encontraron 13 portadores de los que 10 mostraron un genotipo compatible con SAA. El estudio estadístico indicó que la ausencia de hipertensión, una menor edad, una historia familiar de SAA y la ausencia de dolor están asociadas con un estudio genético positivo.

**Conclusiones:** Se amplió el espectro mutacional asociado a SAA. Además, tanto los pacientes índice como los familiares estudiados se han visto beneficiados por estos resultados, por lo que se puede establecer el protocolo de seguimiento adecuado para cada uno de ellos. Por último, es importante destacar la posibilidad de utilizar variables clínicas estadísticamente significativas como factores predictores del carácter hereditario del SAA.

ClinicalTrials.gov (Identifier: NCT04751058)

© 2022 Publicado por Elsevier España, S.L.U. en nombre de Sociedad Española de Cardiología.

## INTRODUCTION

Acute aortic syndromes (AAS) encompass 3 life-threatening conditions, including acute aortic dissection, intramural hematoma, and penetrating aortic ulcer.<sup>1</sup> In Spain, AAS affects 20 to 40 cases/million inhabitants/y and is reported more frequently in men (73%).<sup>2</sup> Of these, 70% affect the ascending aorta, 30% the descending portion, and in 78.7%, dissection is the fundamental mechanism.<sup>3</sup> Only an approximate 5% of thoracic aortic aneurysms are symptomatic prior to a life-threatening complication that arises unpredictably and presents as sudden cardiac death.<sup>4</sup> Therefore, timely diagnosis is essential and saves lives.<sup>1</sup> Classically, a number of risk factors have been associated with AAS, including hypertension (76.6%), atherosclerotic disease (27%), use of cocaine (1.8%) or smoking and dyslipidemia.<sup>5</sup> Likewise, AAS may be involved in any condition associated with structural changes of the aortic wall, such as connective tissue disorders, previous aneurysms, the presence of bicuspid aortic valve,<sup>6</sup> or direct aortic injury following surgery or percutaneous intervention.

AAS may be part of well-characterized genetic syndromes, including Marfan syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome. In addition, AAS may present as an isolated disease confined to the thoracic aorta (nonsyndromic AAS), and can be sporadic (80% of nonsyndromic AAS<sup>4,7</sup>) or hereditary, depending on the presence of other affected family members. AAS is a genetically heterogeneous disease with overlapping clinical presentations; hence, patients' genetic data has become crucial during diagnosis, for the identification of other at-risk relatives<sup>8</sup> and personalized patient management, including timing of prophylactic surgical repair.<sup>9</sup>

Since the introduction of next-generation sequencing (NGS), notable achievements have been made in the discovery of genetic variants and key regulatory molecules associated with AAS.<sup>7,10–12</sup> To date, variants in about 40 genes have been associated with AAS, accounting for 20% to 30% of all cases. Autosomal dominant transmission has been proven in the vast majority of patients with the disease, although autosomal recessive and X-linked inheritance have also been observed.<sup>13</sup>

Aortopathies are more likely to be associated with a genetic cause when the affected individual is younger than 50 years old and/or has a family history of aortic aneurysm or dissection or sudden unexplained death.<sup>14</sup> In addition, the presence of some systemic manifestations may be suggestive of syndromic heritable thoracic aortic disease.

In this study, we analyzed the prevalence of genetic variants in a group of nonsyndromic AAS patients admitted to the intensive care unit to explore the clinical usefulness of AAS-associated variants in treatment decision-making and family traceability of this clinically silent and highly morbid disease.

## METHODS

### Patients cohort and surveillance

This is a prospective observational study of a group of patients consecutively admitted to intensive care unit, University Hospital Virgen del Rocío Seville, with a diagnosis of AAS from October 2017 to January 2020. The study was authorized by the Andalusian Biomedical Research Ethics Platform (PEIBA) (GEN-AOR Internal Code 2436-N-20, Clinical trials [NCT04751058]). Informed consent for the inclusion of the genetic study and for research purposes was collected from all patients or legal guardians when appropriate.

The clinical manifestations considered to be suggestive signs of AAS during initial patient evaluation were the following: signs of sudden, severe chest pain with typical irradiation, hypertension, pulse deficit, difference in blood pressure readings between arms, semiology of aortic insufficiency, signs of ventricular failure, nonspecific electrocardiogram (normal or with left ventricular hypertrophy), normal or with mediastinal widening radiograph and other physical findings that could alert the clinician to the presence of an underlying Marfan syndrome using the Ghent scale. Confirmation of AAS was performed using multislice computed tomography (CT) angiography (angio-CT) (1 mm slices), with and without iodinated contrast, for subsequent reconstruction. After confirmation and image analysis, patients were treated according to the recommendations of the European Society of Cardiology Guidelines<sup>15</sup> for the affected segment and angiographic conditions.

After clinical evaluation and imaging tests, patients meeting the following criteria were enrolled in the genetic study: a) AAS confirmed by angio-CT; b) aged > 16 years and c) absence of signs consistent with syndromic conditions. On the other hand, lifeless entry patients were excluded. Once the patient cohort was selected, the electronic health record was used to collect information on their personal and family history, clinical presentation, and disease course. In this light, the family history was only considered positive if other relatives with aortopathies and/or sudden death were found.

A family segregation analysis was recommended for all cases in which a genetic variant was detected, including likely pathogenic and variants of uncertain significance. After genetic counseling, family members that agreed to undergo clinical examination after a positive or uncertain result in the genetic test were evaluated by echocardiography. Angio-CT was applied during the clinical assessment only after an altered echocardiogram. All positive relatives were referred to the departments of comprehensive care for familial heart diseases and rare diseases of our center.

### DNA isolation and quality analysis

Automatic genomic deoxyribonucleic acid (DNA) extraction was performed using a Chemagic 360 instrument (Perkin Elmer,

United States). DNA integrity and purity were checked by gel electrophoresis and using both fluorometric and spectrophotometric methods. A detailed description of the complete process can be found in the methods of the supplementary data.

### Aortic diseases gene panel design

A capture panel (Roche, USA) including 42 genes (table 1 of the supplementary data) related to different aortic diseases was used. The selection of captured genes was based on current guidelines, public databases, and expert consensus. Further details are shown in the methods of the supplementary data.

### Library preparation and sequence data generation

One microgram of genomic DNA was used for each library preparation using the SeqCap EZ Library SR version 5.1 (Roche, USA) according to the manufacturer's protocol with minor modifications (methods of the supplementary data). Sequencing was performed in the Illumina NextSeq500 platform (Illumina, USA).

### Bioinformatic analysis

Data analysis was performed using a previously validated pipeline<sup>16</sup> with some modifications (methods of the supplementary data).

### Variant prioritization and pathogenicity assessment

Variants were prioritized based on established clinical and genetic criteria (methods of the supplementary data). Finally, only variants classified as pathogenic, likely pathogenic or variants of unknown significance were reported.

### Statistical analysis

Continuous variables are expressed as mean  $\pm$  standard deviation, while categorical variables are expressed as absolute numbers and percentages. All cases were classified in 2 groups: a) individuals with clearly pathogenic or likely pathogenic variants, and b) individuals with no variants or with clinically uncertain variants. Differences between both groups for categorical variables were estimated by Fisher's exact test, except for contingency tables larger than 2x2, for which the Fisher-Freeman-Halton Exact test was used. The obtained results were considered statistically significant for 2-sided *P*-values < .05. All statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., USA).

To evaluate the diagnostic accuracy of significant clinical variables and their combination to be used as heritability-prediction tools, the following attributes were calculated<sup>17,18</sup>: true positive rate (or sensitivity), true negative rate (or specificity), false positive rate, false negative rate, and the odds ratio (OR), representing the predicted probability that a case with a given clinical variable is genetically positive against the probability that a case without that clinical variable has a positive genetic test. An OR greater than 1 implies a fold-increased odd that a positive genetic case manifests a given clinical variable vs it does not. Of note, cases classified as uncertain were not included in these calculations.

## RESULTS

### Demographic and clinical characteristics of acute aortic syndrome cases

From October 2017 to January 2020, 83 AAS patients admitted to the intensive care unit met the inclusion criteria for the genetic study. However, 10 of them died in the first hours without the possibility of obtaining DNA samples, leaving a total of 73 patients for the study. Table 1 shows the general clinical characteristics of this group of patients, while detailed information for each of the cases is shown in table 2 of the supplementary data. The median age was 57 years with an approximate male/female ratio of 3:1. Based on Stanford classification, 63% of patients were Stanford A and 37%, Stanford B cases. Dissection (74%) was the most frequent clinical form. Hypertension and smoking were the most common risk factors, being even more represented in patients with aortic dissection type B (70.3% and 81.4%, respectively). In 57 patients, the first symptom was pain, accompanied by a hypertensive crisis in 27 patients. Sixty patients (82.2%) underwent surgical treatment, 46.2% on an emergency and/or urgent basis. Of note, 47.9% of the patients had an aortic diameter less than 5 cm.

### Identification of genetic variants

Our NGS application allowed us to cover 97.1% of target bases, resulting in a mean coverage of 357.9x and a percentage of reads mapped on target of 83.4%. All coding regions corresponding to genes associated with aortic diseases (table 1 of the supplementary data), were covered > 20x. NGS data analysis led to the detection of 34 heterozygous candidate genetic variants in a total of 32 patients (table 2 and figure 1), including 24 missense, 4 splicing, 3 frameshift, 2 nonframeshift deletions, and 1 copy number variation (figure 2A).

These variants were found in 14 different genes, with *MYH11* being the most prevalent mutated gene followed by *MYLK* and *FBN1* (figure 2B). Although these genes are involved in a wide spectrum of molecular pathways (table 1 of the supplementary data), most of them (22 cases, 69%) were associated with aortic dissection in our cohort (figure 3A). In all cases, our results were compatible with an autosomal dominant inheritance pattern except for family 10, for which compound heterozygosity of 2 changes in *GAA* was considered the most likely disease cause.

Approximately, 40.7% of patients (n = 13) harbored genetic variants in genes previously associated with familial, nonsyndromic forms, 21.8% (n = 7) with syndromic conditions, and 37.5% (n = 12) carried variants in genes associated with both syndromic and nonsyndromic forms (figure 3B). Interestingly, 14 *a priori* nonsyndromic cases harbored pathogenic or likely pathogenic variants in genes previously associated with a potential syndromic aortic disease. Consequently, patients 10, 53, 64, and 69 were diagnosed with Pompe disease, arterial tortuosity syndrome, neurofibromatosis, and Marfan syndrome, respectively, and patients 28 and 65 were diagnosed with Ehler-Danlos syndrome. This allowed us to reclassify 18.7% of cases to a syndromic aortopathy, which has important implications for patient and family management.

### Assessment of the clinical significance of identified variants

Ten of the 34 identified variants had previously been reported as pathogenic or likely pathogenic in public databases and/or the literature, whereas 8 were previously described as variants of unknown significance, and 16 were novel without any clinical

**Table 1**  
Characteristics of the study group

	Number/mean $\pm$ SD	Percentage/intervals	P
<i>Patients description</i>			
Patients in total	73	100	-
Age at the AAS presentation <sup>a</sup>	57 $\pm$ 13.16	16-81	-
< 55 y	33	45.2	< .001 <sup>c</sup>
Male	54	74	.241
Female	19	26	
<i>Geography, race</i>			
Andalusia	70	95.8	.557
Other	3	4.2	
Caucasian race	73	100	-
<i>Type of clinical presentation</i>			
Acute (< 15 d)	54	74	1.000 <sup>b</sup>
Subacute (> 15 d)	7	9.6	
Chronic (> 1 mo)	12	16.4	
Stanford A	46	63	.278
Stanford B	27	37	
Diameter of the aorta, cm <sup>a</sup>	4.57 $\pm$ 1.5	2.5-12.5	-
<i>Type</i>			
Dissection	52	74	.562 <sup>b</sup>
Penetrating ulcer	5	6.8	
Intramural hematoma	7	9.6	
Rupture aneurysm	2	2.7	
Aneurysm	7	9.6	
<i>Risk factors</i>			
Smoking	44	60.3	.424
Hypertension	42	57.3	.007 <sup>c</sup>
Cardiac surgery	3	4.1	.557
DM	5	6.8	.610
COPD	8	11	.432
Family history	15	20.5	.003 <sup>c</sup>
Hyperlipidemia	19	26	.241
Drug abuse	15	20.5	.267
Bicuspid valve	2	2.7	1.000
<i>Clinical symptoms</i>			
Pain	57	78.1	.009 <sup>c</sup>
Hypertensive crisis	28	38.4	1.000
Pulse deficit	23	31.5	1.000
Shock	13	17.8	.326
Acute renal failure	21	28.8	.393
Cardiac tamponade	6	8.2	.663
Visceral ischemia	12	16.4	1.000
Syncope	14	19.4	.744
<i>Treatment</i>			
Medical treatment	13	17.8	.742
Surgical treatment	60	82.2	
Open surgery	40	66.6	.371
Endovascular	20	27.4	
<i>Treatment criteria</i>			
Emergent	28	38.4	.833 <sup>b</sup>
Urgent	13	17.8	
Delayed	19	26	
ICU stay <sup>a</sup>	11 $\pm$ 11.2	1-51	-
Hospital stay <sup>a</sup>	21 $\pm$ 17.5	1-70	-
AAS-related deaths	14	19.2	.747

AAS, acute aortic syndrome; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; SD, standard deviation.

The data are expressed as the mean  $\pm$  standard deviation and intervals for continuous variables<sup>(a)</sup> and absolute numbers and percentages for categorical variables. P-values show the significance obtained by applying the statistical test (Fisher exact test or Fisher-Freeman-Halton Exact Test<sup>(b)</sup>, as appropriate) to relate the genetic results with categorical variables, with those marked with a letter c being statistically significant<sup>(c)</sup>.

**Table 2**  
Likely causative mutations and variants of unknown significance identified in the study cohort

ID	Gen	Mutation		Presence in GnomAD	Ref.	Reported clinical significance	In silico prediction tools					ACMG	Class
		cDNA	Protein				Sift	PPhen	CADD	HSF	MutTast		
3	MYH11	c.3766_3768del	p.Lys1263del	13 het	1	LP <sup>a,b</sup>	-	-	-	-	DC	VUS	LP
8	MYLK	c.3929T > G	p.Leu1310Arg	None	Novel	NA	D	PD	P	-	DC	VUS	LP
10	GAA	c.-32-13T > G	NA	856 het/1 hom	2	pa,b	-	-	B	-	DC	P	P
		c.2237G > C	p.Trp746Ser	20 het	3	pa,b	D	PD	P	-	DC	P	P
12	MYLK	c.4749C > A	p.His1583Gln	None	Novel	NA	D	PsD	P	-	DC	VUS	LP
15	FBN1	c.7334T > C	p.Leu2445Pro	None	Novel	NA	D	PD	P	-	DC	LP	LP
18	FBN1	c.7412C > G	p.Pro2471Arg	7 het	4	VUS <sup>a,b,c</sup>	T	PD	P	-	DC	VUS	VUS
19	MYH11 <sup>e</sup>	Duplication 16p13.11	NA	NA	5	CIP <sup>b,d</sup>	-	-	-	-	-	VUS	VUS
22	MYLK	c.5329C > T	p.Pro1777Ser	4 het	rs <sup>1</sup>	VUS <sup>a</sup>	D	PD	P	-	DC	VUS	VUS
27	ELN	c.1150+1G > A	NA	17 het	7	P <sup>a</sup>	-	-	P	WSB	DC	P	P
28	COL1A1	c.2995C > T	p.Pro999Ser	None	Novel	NA	T	PD	P	-	DC	LP	LP
29	NOTCH1	c.4372G > A	p.Ala1458Thr	23 het	rs <sup>2</sup>	VUS <sup>a</sup>	T	B	B	-	PM	B	VUS
31	MYLK	c.2149G > A	p.Asp717Asn	25 het	8	VUS <sup>a,h</sup>	D	PD	P	-	DC	VUS	VUS
35	ACTA2	c.897T > G	p.Asn299Lys	None	Novel	NA	D	PsD	P	-	DC	VUS	LP
38	MYH11	c.2156G > A	p.Arg719Gln	None	9	pa,b	D	PD	P	-	DC	LP	P
39	MYLK	c.1315G > A	p.Gly439Arg	7 het	rs <sup>3</sup>	VUS <sup>a</sup>	D	PsD	P	-	DC	VUS	LP
40	TGFBR2	c.1061C > T	p.Ala354Val	7 het	rs <sup>4</sup>	NA <sup>h</sup>	T	B	P	-	DC	VUS	VUS
44	ACTA2	c.412A > G	p.Ile138Val	None	Novel	NA	T	PD	P	-	DC	VUS	LP
48	NOTCH1	c.2263_2265del	p.Asn755del	60 het	rs <sup>5</sup>	VUS <sup>a,c</sup>	-	-	-	-	DC	B	VUS
49 <sup>f</sup>	GAA	c.2237G > C	p.Trp746Ser	20 het	10	pa,b,c	D	PD	P	-	DC	P	P
52	ELN	c.1921C > A	p.Leu641Ile	None	Novel	NA	T	PD	B	-	PM	LB	VUS
53	SLC2A10	c.1334del	p.Gly445Glu <sup>fs*40</sup>	24 het	11	pa,b,c	-	-	-	-	DC	P	P
60	ELN	c.1288G > A	p.Gly430Arg	6 het	rs <sup>6</sup>	NA	T	PD	B	-	PM	LB	VUS
61	MYH11	c.4599+2dup	NA	None	rs <sup>7</sup>	P <sup>a</sup>	-	-	-	WSB	DC	VUS	P
62	FBN1	c.2671A > G	p.Ile891Val	None	Novel	NA	T	B	B	-	DC	VUS	VUS
63	FBN1	c.8517del	p.Glu2841Asn <sup>fs*4</sup>	None	Novel	NA	-	-	-	-	DC	P	LP
64 <sup>g</sup>	NF1	c.2816del	p.Asn939Ile <sup>fs*14</sup>	None	12	P <sup>b</sup>	-	-	-	-	DC	P	LP
65	COL5A2	c.2806G > A	p.Gly936Arg	None	rs <sup>8</sup>	NA	D	PD	P	-	DC	VUS	LP
67	FBN1	c.5917+1G > T	NA	None	rs <sup>9</sup>	pa,c	-	-	P	WSB	DC	P	P
68	MYH11	c.3397G > C	p.Ala1133Pro	None	Novel	NA	D	PD	P	-	DC	VUS	LP
69	FBN1	c.1130G > T	p.Cys377Phe	None	Novel	NA <sup>h</sup>	D	PD	P	-	DC	LP	LP
71	MYH11	c.214G > A	p.Gly72Arg	None	Novel	NA	T	B	P	-	DC	LB	VUS
72	TGFBR1	c.934G > T	p.Gly312Cys	None	Novel	NA <sup>h</sup>	D	PD	P	-	DC	LP	LP
		c.1067G > A	p.Arg356Gln	5 het	rs <sup>10</sup>	VUS <sup>a,c</sup>	T	B	P	-	DC	LP	LP

ACMG, variant classification based on American College of Medical Genetics and Genomics criteria; B, benign; CADD, combined annotation dependent depletion (Variants were considered pathogenic with a score  $\geq 15$ ); CIP, conflicting interpretation of pathogenicity; D, damaging; DC, disease-causing. Het, heterozygotes; Hom, homozygotes; HSF, human splicing finder; ID, identification number; LB, likely benign; LP, likely pathogenic; MutTast, mutation taster; NA, not available; Our Class, variant classification based on all the collected information; P, pathogenic; PD, probably damaging; PM, polymorphism; Pphen, Polyphen-2; PsD, possibly damaging; Ref, references; rs1, rs748200926; rs2, rs200495793; rs3, rs190877071; rs4, rs376752333; rs5, rs587778559; rs6, rs274470120; rs7, rs794728677; rs8, rs1471655044; rs9, rs363808; rs10, rs727504292; T, tolerated; VUS, variant of unknown significance; WSB, wild-type splicing site broken.

All detected variants were found in heterozygous state. The reported clinical significance was checked both in the databases ClinVar (<sup>a</sup>), LOVD (<sup>b</sup>) and HGMD (<sup>c</sup>), and the literature (<sup>d</sup>).

<sup>e</sup> Copy number variation affecting a genomic region including MYH11 and additional loci.

<sup>f</sup> Unsolved case due to only 1 heterozygous variant was identified in an autosomal recessive gene.

<sup>g</sup> Variant allele fraction of 27%, compatible with somatic mosaicism, which is a common mechanism in NF1 and leads to variable symptoms.

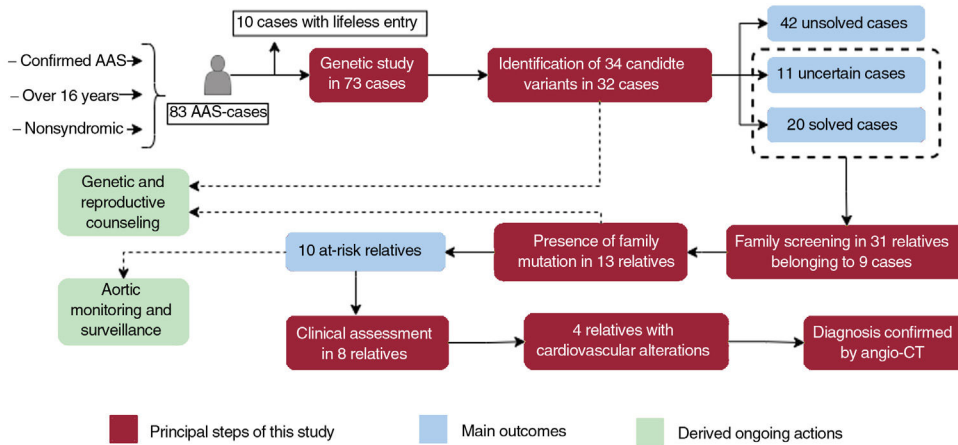
<sup>h</sup> Another change in the same genomic position was reported as pathogenic or likely pathogenic.

association so far (table 2 and figure 4). A re-evaluation of variants was performed according to the previous clinical associations, the allele count in gnomAD, the *in silico* pathogenicity predictions and the American College of Medical Genetics and Genomics criteria, resulting in the reclassification of 19 variants (figure 4). This resulted in the identification of 11 variants of unknown significance in 11 cases (15.07%) and 23 pathogenic or likely pathogenic variants in 21 patients, although only 20 cases were considered solved (27.39%), since a unique heterozygous variant in the autosomal recessive gene GAA was found in patient 49. If a clinical association is confirmed for these variants, the diagnostic rate of this study could reach up to 42.46%.

### Family screening and risk stratification

Family members belonging to 9 families with detected candidate variants were willing to be studied (table 3 and figure 1). We confirmed the presence of the detected variants in 13 out of 31 relatives analyzed by Sanger sequencing. Three (1:4, 1:5 and 1:17) of the 13 relatives were carriers of genetic variants associated with an autosomal recessive trait and hence their genotypes were not compatible with a AAS phenotype. The remaining 10 individuals harbored genetic variants compatible with an autosomal dominant inheritance and therefore their genotypes were consistent with the occurrence of AAS.

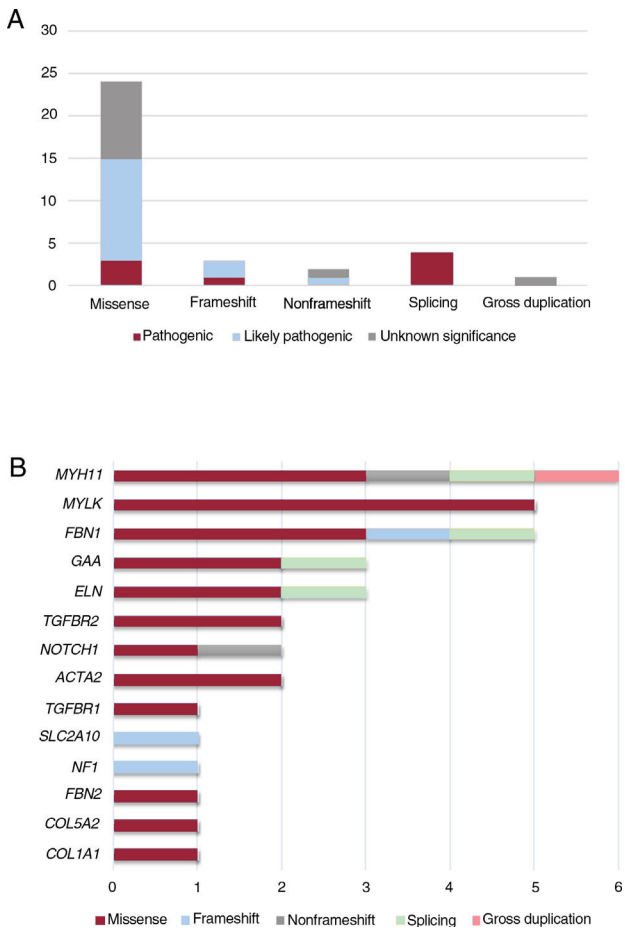
October 2017 January 2020



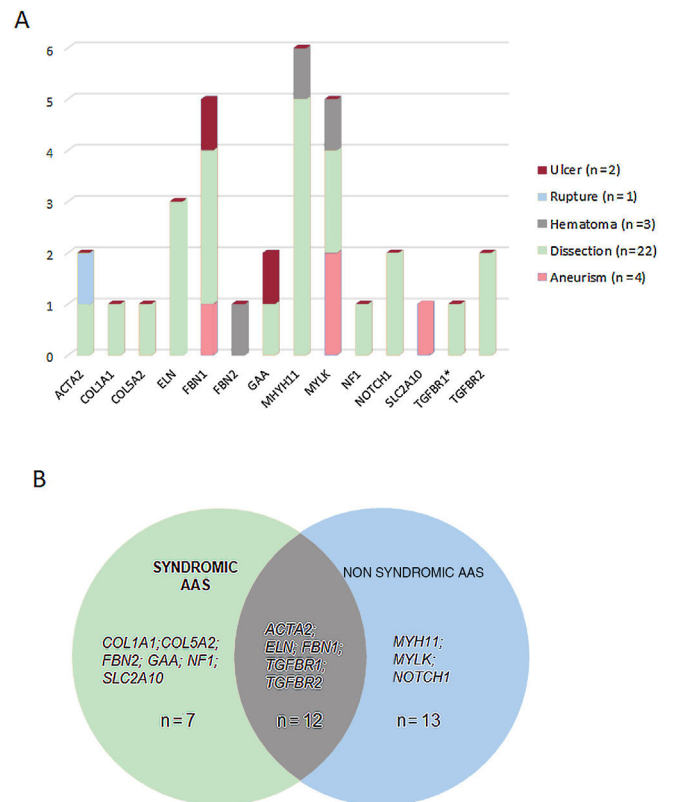
**Figure 1.** Central illustration. Flowchart of patients, family screening, and genetic testing outcomes. Orange boxes reflect the principal steps performed in our workflow, while the main outcomes are shown in red boxes. The ongoing actions derived as a consequence of this study are indicated in green boxes. AAS, acute aortic syndrome; Angio-CT, multislice computed tomography angiography.

A clinical reassessment was offered to the 10 relatives with a positive result, but only 8 have attended to date. Among them, 4 patients already showed cardiovascular alterations: *a*) individual I:16 (16 years) showed a resting cardiac electrical activity with QTc

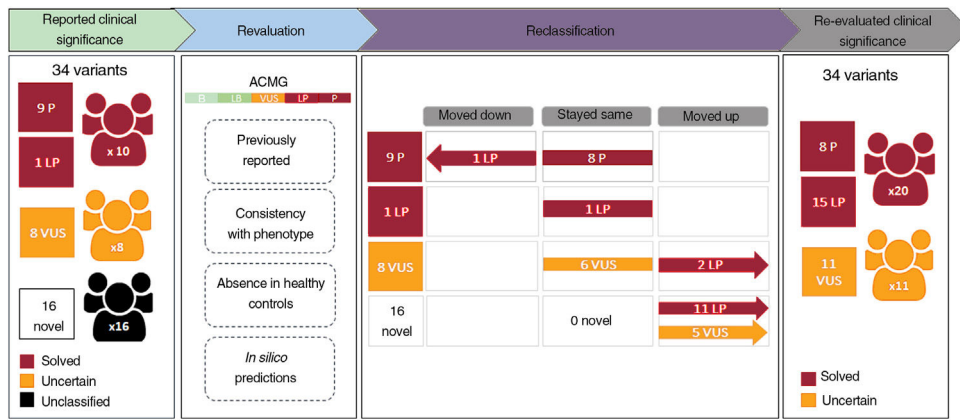
in upper limits (464 ms); *b*) individual I:20 (67 years) showed mild dilatation of the root (37 mm) and ascending aorta (40 mm), and a grade II aortic insufficiency; *c*) individual I:21 (63 years) showed mild dilatation of the root (43 mm) and ascending aorta (38 mm), and a mild diffuse calcified atheromatosis; and *d*) individual I:31 (15 years) showed a mild dilatation of pulmonary artery. Remarkably, with the exception of individual I:7, all relatives with positive genetic results and no current signs of aortopathies were younger than the index patient of their family. Indeed, 11 out



**Figure 2.** Classification of the 34 identified variants associated with aortic disease in our cohort. A: type of variants according to consequence type. B: distribution of variant types, including missense, frameshift, nonframeshift, splicing and gross duplication in genes associated with aortic disease in our cohort.



**Figure 3.** Genotype-phenotype correlations. A: representation of the type of aortic disease associated with the genes identified in this study. B: number of cases harboring variants in genes associated with syndromic, nonsyndromic or to both forms of aortic disease. The asterisk denotes a variant in the *TGFB1* gene present in 1 of the cases harboring a concomitant variant in *TGFB2*.

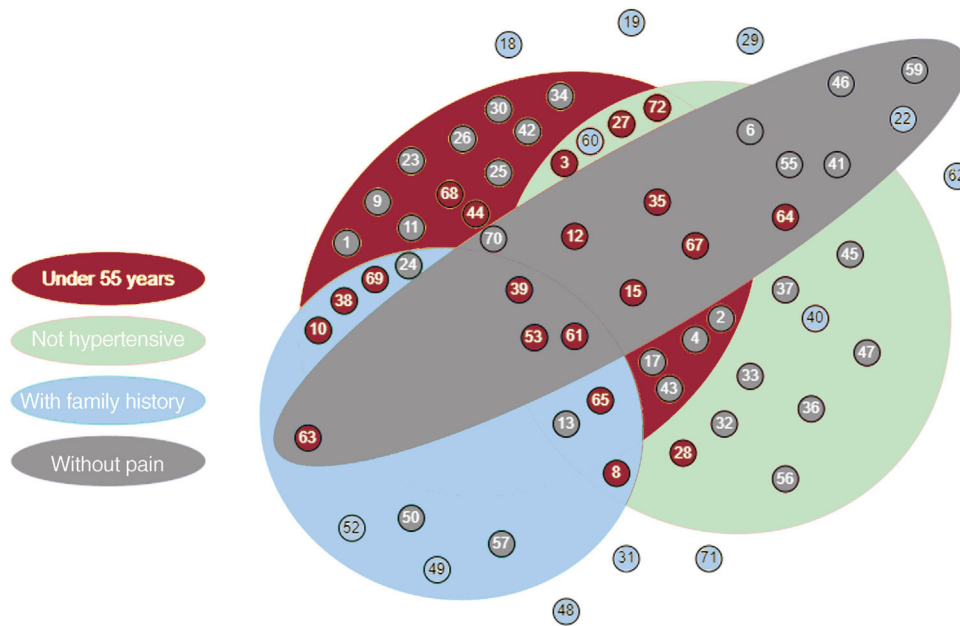


**Figure 4.** Workflow of variant re-evaluation, classification, and clinical interpretation. ACMG, variant classification based on American College of Medical Genetics and Genomics criteria; B, benign; LB, likely benign; LP, likely pathogenic; P, pathogenic; VUS, variant of unknown significance.

**Table 3**  
Family segregation of the genetic variants identified in the 9 patients with available relatives

Family ID	Gene	Genetic variant	Total relatives	Individual ID	Relationship to index	Presence of mutation	Current signs of AAS	Recent clinical assessment
8	MYLK	c.3929T > G	3	I:1	Daughter	No	No	NA
				I:2	Sister	No	No	NA
				I:3	Sister	No	No	NA
10	GAA	c.-32-13T > G	2	I:4	Daughter	Yes (c.-32-13T > G) No (c.2237G > C)	No	NA*
		c.2237G > C		I:5	Daughter	No (c.-32-13T > G) Yes (c.2237G > C)	No	NA*
12	MYLK	c.4749C > A	4	I:6	Brother	No	No	NA
				I:7	Sister	Yes	No	Yes
				I:8	Son	No	No	NA
				I:9	Son	No	No	NA
18	FBN1	c.7412C > G	4	I:10	Son	No	No	NA
				I:11	Daughter	Yes	No	Yes
				I:12	Daughter	No	No	NA
				I:13	Daughter	No	No	NA
38	MYH11	c.2156G > A	3	I:14	Son	No	No	NA
				I:15	Son	No	No	NA
				I:16	Niece	Yes	Yes	Yes
49	GAA	c.2237G > C	2	I:17	Daughter	Yes	No	NA*
				I:18	Son	No	No	NA
61	MYH11	c.4599+2dup	10	I:19	Sister	Yes	No	No
				I:20	Mother	Yes	Yes	Yes
				I:21	Maternal aunt	No	No	NA
				I:22	Maternal uncle	Yes	Yes	Yes
				I:23	Cousin	Yes	No	Yes
				I:24	Cousin	No	No	NA
				I:25	Cousin	Yes	No	Yes
				I:26	Niece	Yes	No	No
				I:27	Nephew	No	No	NA
I:28	Nephew	No	No	NA				
63	FBN1	c.8517del	2	I:29	Sister	No	No	NA
				I:30	Sister	No	No	NA
69	FBN1	c.1130G > T	1	I:31	Daughter	Yes	Yes	Yes

AAS, acute aortic syndrome; ID, identification number; NA, not applicable. All individuals harboring the mutations are heterozygous. Individuals with current signs of AAS showed cardiovascular alterations according to the latest imaging tests. A recent clinical assessment was offered to patients in whom a causative genotype was detected, which was not applicable for wild-type patients and carriers of autosomal recessive variants (\*).



**Figure 5.** Association between hereditary acute aortic syndrome cases and significant clinical features. Each circle corresponds to 1 of the families under study, labeled with its identification number. The color of the circles indicates whether pathogenic or likely pathogenic variants (red), variants of unknown significance (blue), or no candidate variants (grey), were found.

of 13 relatives with a finding of familial variants are currently of reproductive age (< 50 years).

**Correlation of genetic results with clinical variables**

Statistical tests showed significant differences between patients with a clear genetic diagnosis and those with no causative or uncertain variants, for the following clinical variables: hypertension, patient age, family history, and pain (table 1 and figure 5). The absence of hypertension, an age < 55 years old, having a family history of AAS and without pain as a first symptom, significantly correlated with the detection of pathogenic or likely pathogenic genetic variants and, therefore, with hereditary cases.

All solved cases were included in at least 1 of these 4 statistically significant groups whereas the combination of the 4 significant variables encompassed only 3 of these cases (figure 5). Indeed, table 4 shows that, while sensitivity was highest for age and hypertension (a true positive rate of 70% and 80%, respectively), family history and pain were the most specific (a true negative rate of 90% and 85%, respectively). The combination of these clinical variables allowed us to obtain a balance between sensitivity and specificity, as well as to improve their predictive ability according to the calculation of OR. If we analyze the highest values of this attribute, the combination of age and pain (OR = 21.54) and the combination of hypertension and family history (OR = 13.33) would be the sets with a greatest predictive strength, although

**Table 4**  
Attributes calculated to measure the diagnostic accuracy of statistically significant clinical variables

			TP	TN	FP	FN	TPR (Sens.)	TNR (Spec.)	FPR	FNR	OR
HT			14	26	15	6	0.7	0.63	0.37	0.3	4.04
Age			16	25	16	4	0.8	0.61	0.39	0.2	6.25
FH			9	37	4	11	0.45	0.90	0.10	0.55	7.57
Pain			9	35	6	11	0.45	0.85	0.15	0.55	4.77
HT	Age		11	36	5	9	0.55	0.88	0.12	0.45	8.8
HT	FH		5	40	1	15	0.25	0.98	0.02	0.75	13.33
HT	Pain		8	38	3	12	0.4	0.93	0.07	0.6	8.44
Age	FH		7	39	2	13	0.35	0.95	0.05	0.65	10.50
Age	Pain		7	40	1	13	0.35	0.98	0.02	0.65	21.54
FH	Pain		4	41	0	16	0.2	1	0	0.8	NA
HT	Age	FH	4	40	1	16	0.2	0.98	0.02	0.8	10
HT	FH	Pain	3	41	0	17	0.15	1	0	0.85	NA
HT	Age	Pain	7	41	0	13	0.35	1	0	0.65	NA
Age	FH	Pain	3	41	0	17	0.15	1	0	0.85	NA
HT	Age	FH	3	41	0	17	0.15	1	0	0.85	NA
Age+Pain		HT+FH	9	39	2	11	0.45	0.95	0.05	0.55	15.96

FH, family history; FN, false negatives; FNR, false negative rate; FP, false positives; FPR, false positive rate; HT, hypertension; OR, odds ratio; TN, true negatives; TNR, true negative rate or specificity (Spec.); TP, true positives; TPR, true positive rate or sensitivity (Sens.).  
The effective sample size was 61 since cases with variants of unknown significance were not included in calculations.



their sensitivities were decreased (35% and 25%, respectively). However, the joint use of these 2 combinations allowed us to maintain a high predictive value (OR = 15.96) without excessively reducing sensitivity (45%) and ensuring specificity (95%).

## DISCUSSION

Aortic disease is a major diagnostic challenge due to its silent nature; however, its complications constitute an important source of extreme severity and mortality. Because more than 20% of patients report a positive family history, AAS genetics has been extensively investigated as a potential avenue for both diagnosis and risk stratification.

In this study, we used NGS-based multigene testing as a screening method to identify disease-related genetic variants in the context of the molecular diagnosis of aortic diseases. The identification of marked differences in the diagnostic yield (3% to 37%) is common in this group of diseases,<sup>10,11,19</sup> which has been attributed to differences in patient inclusion criteria, variant classification rules, and the selection of genes to be included in the study. In line with previously reported data,<sup>10,11,19</sup> we achieved a diagnostic rate of 27.39%, which may increase to 42.46% if variants classified as variant of unknown significance are eventually upgraded to likely pathogenic.

Bioinformatic analysis enabled us to detect 34 heterozygous candidate genetic variants in 14 AAS-associated genes, with *MYH11* being the most prevalent mutated gene followed by *MYLK* and *FBN1*, which is consistent with previously reported prevalence data.<sup>19,20</sup> In contrast, genetic variants in the *ACTA2* gene are underrepresented (6.2%) in our population in comparison with previous reports (12%–21%),<sup>21</sup> which could indicate a less relevant role of this gene in our population, or a bias due to diverse inclusion criteria.

Establishing genotype-phenotype correlations is especially hampered in these disorders due to the wide spectrum of physical manifestation, suggesting the involvement of multifactorial genetic or environmental risk factors.<sup>10,22</sup> Our results reinforce the notion that a hypothesis-free approach regardless of clinical assumptions involving genes carefully selected by experts considerably improves the genetic management of these patient.<sup>23</sup> Moreover, most life-threatening autosomal dominant diseases notoriously hamper diagnosis prior to complications.<sup>4</sup> Thus, a common limitation is sample size and the availability of affected family members to undergo conclusive segregation studies. Indeed, in this study, familial segregation was only performed in 9 out of 13 families, highlighting the importance of conducting periodic reassessments to refine the categorization of variants. This arduous task is especially relevant when concomitant genetic defects are detected.<sup>24</sup> Of note, 2 likely pathogenic variants in *TGFBR1* and *TGFBR2*, respectively, were found in patient 72; however, additional studies are needed to discern the degree of involvement of each of them in the appearance or severity of the disease.

In addition, beyond clarifying genotype-phenotype correlations and achieving a genetic diagnosis of index patients, genetic testing in AAS has a crucial role in determining familial risk.<sup>25</sup> Asymptomatic older and younger at-risk relatives of an affected individual should be promptly evaluated to clarify their genetic status and to establish the appropriate level of surveillance. Genetic screening for familial aortopathy in 31 family members identified a genotype compatible with the development of AAS in 10 individuals who are currently being monitored and treated upon symptom appearance (I:16, I:20, I:21 and I:31). Furthermore, 3 additional individuals (I:4, I:5 and I:17) carried potential AAS-causing variants in the context of an autosomal recessive disorder. Therefore, a total of 13 individuals may also benefit from reproductive genetic counseling and preimplantation genetic

tests, which is especially relevant considering that most of the studied family members are of reproductive age. Furthermore, the identification of at-risk relatives prevented the monitoring of 15 individuals lacking the disease-causing variant, reducing associated costs, and providing a sense of relief for these patients. Nevertheless, the absence of a genetic variant with unknown clinical significance in an individual (ie family 18) is not enough to be exempt that individual from surveillance.<sup>20</sup>

Importantly, the establishment of genotype-phenotype correlations not only allows us to anticipate the symptoms in patients harboring causative genetic variants, but also to predict the hereditary character of AAS even when the underlying genetic defect has not yet been identified. In this study, 4 clinical characteristics were significantly associated with the presence of pathogenic variants. Whereas clinical variables including normal blood pressure, early presentation of the disease and a positive family history have been previously associated with hereditary AAS,<sup>24,26</sup> this study also points to the absence of pain as an indicator of a heritable thoracic aortic disease. Indeed, we suggest that the combination of the clinical variables age and pain, together with the combination of hypertension and family history, may be applied as a useful predictive tool for heritability. Remarkably, other clinical variables commonly associated with genetic results, such as the type of aortic dissection,<sup>27</sup> were not associated in this study, suggesting differences among populations or a bias related to our limited sample size.

## CONCLUSIONS

In summary, the implementation of this NGS strategy guided the clinical management of our patients with aortic diseases by preventing emergency events for them and their relatives and providing appropriate genetic and reproductive counseling. As genetic testing becomes more widespread and accurate, a comprehensive library of pathogenic genetic variants could be developed, improving risk stratification, the possibility of genetically informed personalized surgical interventions, discrimination between AAS from other serious conditions, and management of clinically challenging AAS cases.

## FUNDING

This work was supported by *Instituto de Salud Carlos III* (ISCIII), Spanish Ministry of Economy and Competitiveness, Spain and co-funded by the European Union (ERDF, “A way to make Europe”) [P118-00612; P119/01550; P121-00244; IMP/00009], Regional Ministry of Health and Families of the Autonomous Government of Andalusia [PEER-0501-2019; PEER-0470-2019], Regional Ministry of Economic Transformation, Industry, Knowledge and Universities of Andalusia [P20\_00887] and the Foundation Isabel Gemio/Foundation Cajasol [FGEMIO-2019-01]. NBG is supported by a fellowship RH-0118-2020, which has been funded by the Regional Ministry of Health and Families of the Autonomous Government of Andalusia.

## AUTHORS' CONTRIBUTIONS

A.M. Puppo Moreno, and S. Borrego conceived the study. A.M. Puppo Moreno, S. Borrego, N. Bravo-Gil, and C. Méndez-Vidal designed the study and drafted the manuscript. A.M. Puppo Moreno, A. Adsuar Gómez, F. Tadeo Gómez Ruiz, C. Jiménez de Juan, R. Martín Bermúdez, J.M. López Sánchez, S. Martín Sastre, and M. Fernández Caro collected clinical data and performed clinical management. A.M. Puppo Moreno, P. Gallego and S. Borrego

interpreted clinical data. N. Bravo-Gil, C. Méndez-Vidal and R.M. Fernández García analyzed and interpreted genomic data. S. Borrego, N. Bravo-Gil, and C. Méndez-Vidal collected genomic data. A. Adsuar Gómez, F. Tadeo Gómez Ruiz, C. Jiménez de Juan, R.M. Fernández García, R. Martín Bermúdez, J.M. López Sánchez, S. Martín Sastre, M. Fernández Caro, and P. Gallego revised the manuscript critically for important intellectual content. All authors approved the final version to be published. A.M. Puppo Moreno, N. Bravo-Gil and C. Méndez-Vidal should be considered as joint first authors.

## CONFLICTS OF INTEREST

All authors report no disclosures.

### WHAT IS KNOWN ABOUT THE TOPIC?

- Genetic testing has become a crucial element during the diagnosis, management, surveillance, risk stratification and familial evaluation of AAS patients.
- To date, variants in about 40 genes have been associated with AAS.
- Specific genotype-phenotype correlations continue to emerge, allowing for more precise and individualized management and treatment of patients with AAS.

### WHAT DOES THIS STUDY ADD?

- The identification of novel variants associated with AAS, expanding our knowledge of the genetic background of this disorder.
- The description of how the genetic findings can contribute to strategies to prevent and treat AAS.
- The possibility to predict the heritable character of AAS through the assessment and combination of clinical features such as hypertension, age, pain, and family history.

## APPENDIX. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version, at <https://doi.org/10.1016/j.rec.2022.10.005>

## REFERENCES

1. Sorber R, Hicks CW. Diagnosis and Management of Acute Aortic Syndromes: Dissection, Penetrating Aortic Ulcer, and Intramural Hematoma. *Curr Cardiol Rep.* 2022;24:209–216.
2. Evangelista A, Rabasa JM, Mosquera VX, et al. Diagnosis, management and mortality in acute aortic syndrome: results of the Spanish Registry of Acute Aortic Syndrome (RESA-II). *Eur Heart J Acute Cardiovasc Care.* 2018;7:602–608.
3. Evangelista Masip A, Lopez-Sainz A, Barros Membrilla AJ, et al. Spanish Registry of Acute Aortic Syndrome (RESA). Changes in therapeutic management and lower mortality in acute aortic syndrome. *Rev Esp Cardiol.* 2022;75:816–824.
4. Kuzmik GA, Sang AX, Elefteriades JA. Natural history of thoracic aortic aneurysms. *J Vasc Surg.* 2012;56:565–571.
5. Evangelista A, Isselbacher EM, Bossone E, et al. Insights From the International Registry of Acute Aortic Dissection: A 20-Year Experience of Collaborative Clinical Research. *Circulation.* 2018;137:1846–1860.
6. Baxter BT. Heritable diseases of the blood vessels. *Cardiovasc Pathol.* 2005;14:185–188.
7. Luyckx I, Callejon IV, Bucciolini L, Loeys B. Update on the molecular landscape of thoracic aortic aneurysmal disease. *Curr Opin Cardiol.* 2022;37:201–211.
8. Robertson EN, van der Linde D, Sherrah AG, et al. Familial non-syndromal thoracic aortic aneurysms and dissections - Incidence and family screening outcomes. *Int J Cardiol.* 2016;220:43–51.
9. Harris SL, Lindsay ME. Role of Clinical Genetic Testing in the Management of Aortopathies. *Curr Cardiol Rep.* 2021;23:10.
10. Poninska JK, Bilinska ZT, Franaszczyk M, et al. Next-generation sequencing for diagnosis of thoracic aortic aneurysms and dissections: diagnostic yield, novel mutations, and genotype phenotype correlations. *J Transl Med.* 2016;14:115.
11. Renner S, Schuler H, Alawi M, et al. Next-generation sequencing of 32 genes associated with hereditary aortopathies and related disorders of connective tissue in a cohort of 199 patients. *Genet Med.* 2019;21:1832–1841.
12. Proost D, Vandeweyer G, Meester JA, et al. Performant Mutation Identification Using Targeted Next-Generation Sequencing of 14 Thoracic Aortic Aneurysm Genes. *Hum Mutat.* 2015;36:808–814.
13. Albornoz G, Coady MA, Roberts M, et al. Familial thoracic aortic aneurysms and dissections—incidence, modes of inheritance, and phenotypic patterns. *Ann Thorac Surg.* 2006;82:1400–1405.
14. Demo E, Rigelsky C, Rideout AL, et al. Genetics and Precision Medicine: Heritable Thoracic Aortic Disease. *Med Clin North Am.* 2019;103:1005–1019.
15. Erbel R, Aboyans V, Boileau C, et al. Corrigendum to: 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases. *Eur Heart J.* 2015;36:2779.
16. Bravo-Gil N, Gonzalez-Del Pozo M, Martin-Sanchez M, et al. Unravelling the genetic basis of simplex Retinitis Pigmentosa cases. *Sci Rep.* 2017;7:41937.
17. Persoskie A, Ferrer RA. A Most Odd Ratio: Interpreting and Describing Odds Ratios. *Am J Prev Med.* 2017;52:224–228.
18. Ranganathan P, Aggarwal R. Common pitfalls in statistical analysis: Understanding the properties of diagnostic tests - Part 1. *Perspect Clin Res.* 2018;9:40–43.
19. Zheng J, Guo J, Huang L, et al. Genetic diagnosis of acute aortic dissection in South China Han population using next-generation sequencing. *Int J Legal Med.* 2018;132:1273–1280.
20. Milewicz DM, Guo D, Hostetler E, et al. Update on the genetic risk for thoracic aortic aneurysms and acute aortic dissections: implications for clinical care. *J Cardiovasc Surg (Torino).* 2021;62:203–210.
21. Morisaki H, Akutsu K, Ogino H, et al. Mutation of ACTA2 gene as an important cause of familial and nonfamilial nonsyndromic thoracic aortic aneurysm and/or dissection (TAAD). *Hum Mutat.* 2009;30:1406–1411.
22. Landis BJ, Schubert JA, Lai D, et al. Exome Sequencing Identifies Candidate Genetic Modifiers of Syndromic and Familial Thoracic Aortic Aneurysm Severity. *J Cardiovasc Transl Res.* 2017;10:423–432.
23. Renard M, Francis C, Ghosh R, et al. Clinical Validity of Genes for Heritable Thoracic Aortic Aneurysm and Dissection. *J Am Coll Cardiol.* 2018;72:605–615.
24. Fang M, Yu C, Chen S, et al. Identification of Novel Clinically Relevant Variants in 70 Southern Chinese patients with Thoracic Aortic Aneurysm and Dissection by Next-generation Sequencing. *Sci Rep.* 2017;7:10035.
25. Milewicz DM, Regalado ES. Use of genetics for personalized management of heritable thoracic aortic disease: how do we get there? *J Thorac Cardiovasc Surg.* 2015;149:S3–S5.
26. Weerakkody R, Ross D, Parry DA, et al. Targeted genetic analysis in a large cohort of familial and sporadic cases of aneurysm or dissection of the thoracic aorta. *Genet Med.* 2018;20:1414–1422.
27. Hicks KL, Byers PH, Quiroga E, Pepin MG, Shalhub S. Testing patterns for genetically triggered aortic and arterial aneurysms and dissections at an academic center. *J Vasc Surg.* 2018;68:701–711.