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ORIGINAL ARTICLE

Postmortem genetic testing should be recommended in sudden cardiac death cases due to thoracic aortic dissection

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Abstract
Background Acute thoracic aortic dissections and ruptures, the main life-threatening complications of the corresponding aneurysms, are an important cause of sudden cardiac death. Despite the usefulness of the molecular diagnosis of these conditions in the clinical setting, the corresponding forensic field remains largely unexplored. The main goal of this study was to explore and validate a new massive parallel sequencing candidate gene assay as a diagnostic tool for acute thoracic aortic dissection autopsy cases.
Materials and methods Massive parallel sequencing of 22 thoracic aortic disease candidate genes performed in 17 cases of thoracic aortic dissection using *AmpliSeq* and *Ion Proton* technologies. Genetic variants were filtered by location, type, and frequency at the *Exome Aggregation Consortium* and an internal database and further classified based on the *American College of Medical Genetics and Genomics (ACMG) recommendations* published in 2015. All prioritized results were confirmed by traditional sequencing.
Results From the total of 10 potentially pathogenic genetic variants identified in 7 out of the 17 initial samples, 2 of them were further classified as pathogenic, 2 as likely pathogenic, 1 as possibly benign, and the remaining 5 as variants of uncertain significance, reaching a molecular autopsy yield of 23%, approximately.
Conclusions This massive parallel sequencing candidate gene approach proved useful for the molecular autopsy of aortic dissection sudden cardiac death cases and should therefore be progressively incorporated into the forensic field, being especially beneficial for the anticipated diagnosis and risk stratification of any other family member at risk of developing the same condition.

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Introduction

Besides the main structural and arrhythmogenic cardiomyopathies, acute thoracic aortic dissection (TAD) or rupture is another important cause of sudden cardiac death (SCD), understood as the natural unexpected decease with cardiac origin of an apparently healthy individual [1, 2]. With an incidence of approximately 3.5/100,000 patients per year [3], the annual risk of this specific type of SCD has been estimated to be greater than 10% [4, 5].

Although TAD may sometimes develop abruptly, it is generally the result of a growing thoracic aortic aneurysm (TAA). The progressive dilation and weakening of the aortic wall could at some point lead to an acute tear in the intima [6]. This typically permits blood to penetrate through the diseased medial layer and dissect along the plane of the aortic wall, forming a true and false lumens with or without communication [6–8]. In a worst case scenario, this lesion would be followed by adventitial disruption and lethal aortic rupture [7].

Approximately, 95% of TAAs are asymptomatic until a life-threatening complication as such occurs as the first manifestation [3, 9, 10], and even acute aortic dissections can be misdiagnosed and difficult to recognize [11]. To further challenge their clinical diagnosis, thoracic aortic aneurysms and dissections (TAADs) have been associated with a wide variety of underlying diseases, typically classified in sporadic (degenerative, inflammatory, autoimmune, infectious, or traumatic, without familial inheritance) or hereditary [12–14]. In contrast to abdominal aortic aneurysms, which are mainly attributed to atherosclerosis, up to 40% of TAADs have been expected to be hereditary with a genetic background as heterogeneous as their broad phenotypic spectrum [14–16]. Those heritable disorders affecting the thoracic aorta can be further classified as syndromic or non-syndromic, depending on the presence or absence of clinical manifestations in other organ systems. Among the 5% corresponding to the syndromic forms of the disease, the most relevant cases involve the main connective tissue syndromes: Marfan, Loeys-Dietz, vascular Ehlers-Danlos, and aneurysm-osteoarthritis [17, 18]. On the other hand, in those non-syndromic, TAAD is the predominant clinical manifestation [14].

No matter the underlying disease, several common non-genetic risk factors for acute TAD exist, such as hypertension, atherosclerosis, male gender, and advanced age [11]. Furthermore, the pathophysiological hallmark of about 80% of TAADs has been described as medial degeneration (formerly "cystic medial necrosis") [19, 20]. It occurs with normal aging of the aorta but can be accelerated by hypertension and brought on by genetic variation that predisposes individuals to the thoracic aortic disease [5, 21, 22]. The last consensus statement from the *Society for Cardiovascular Pathology* and the *Association for European Cardiovascular Pathology* published by Halushka et al. in 2016 described it as a unique but overlapping histopathological condition, involving a collection of different histopathological changes [20]. Those keys for the definition of this condition were (i) elastic fiber fragmentation and/or loss, thinning, and disorganization; (ii) smooth muscle cell disorganization and nuclei loss (at least not clearly identifiable); (iii) laminar medial collapse or compaction of medial elastic fibers; (iv) medial mucoid extracellular matrix accumulation; and (v) medial fibrosis [8, 20].

Although a more specific clinical and pathological picture is likely to be defined in the near future, allowing the recognition of each disease particularities, alternative molecular approaches have already been demonstrated useful in the clinical setting, especially for those patients with non-specific clinical symptoms or a rapid deterioration [7, 11, 20, 23]. The specific underlying genotype could help confirm the diagnosis, perform proper surveillance, determine the best time for surgery, establish an anticipated risk stratification and prognosis, and perform cascade screening for other family members at risk, with the aim of reducing morbidity and mortality [16, 24–27]. In fact, the *American College of Cardiology Foundation* and the *American Heart Association* have already recommended that the underlying genetic variation should dictate the timing of aortic repair, the current standard treatment [16, 28]. In the forensic field, Klintschar et al. discussed the importance of genetic testing for forensic cases of aortic disruption, for the first time [29]. But, despite the positive clinical consequences that the molecular diagnosis has among family members and the high mortality rates obtained when TAAD is left undiagnosed or untreated [11], to date, the corresponding forensic field remains largely unexplored.

Willing to demonstrate the potential benefits of incorporating the molecular diagnosis in the forensic field, the main goal of this study was to explore and validate candidate gene massive parallel sequencing (MPS) as a diagnostic tool for acute TAD autopsy cases. In comparison with traditional sequencing of single genes, MPS has proved to be cost-effective and time-effective for the molecular diagnosis of genetically heterogeneous diseases, in which simultaneous testing of multiple genes is often indicated [17, 30–32]. For this matter, a new MPS candidate gene assay was designed, involving 22 genes already known to be associated with the development of thoracic aortic disease.

Materials and methods

Study samples

Blood or tissue samples (myocardium and spleen) were collected from 17 TAD cases from *Unidad de Valoración del Riesgo de Muerte Súbita Familiar* (*Hospital Universitario La Fe* and *Institutos de Medicina Legal de la Comunidad Valenciana*), 15 of which were obtained from autopsies. The *Comité Ético de Investigación Clínica de Galicia* was the institution in charge of approving the corresponding informed consent, signed by the legal representative. Clinical details of the 17 studied cases are shown in Table 1.

Genomic DNA from the blood samples was extracted using the *phenol-chlorophorm* method, following the manufacturer's instructions. After extraction, the double strand DNA was quantified with QubitTM fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), and DNA purity and integrity were evaluated based on Nanodrop 1000 Spectrophotometer 260/230 and 260/280 ratios (Thermo Fisher Scientific Inc., Waltham, MA, USA) and agarose gels 0.8% (Invitrogen, Thermo Fisher Scientific Inc., Waltham, MA, USA), respectively. Those still doubtful cases were further analyzed with the 2200 Tape Station (Agilent Technologies, Santa Clara, CA, USA).

Candidate gene massive parallel sequencing approach

An *AmpliSeq Custom* assay was designed using the *AmpliSeq Designer v4.2* tool (https://ampliseq.com). The target region, sized 145.52 kb, comprised the coding exons (padding 5 bp) of a total of 22 genes previously associated with TAAD, detailed in Table 2.

Library preparation was performed using the *Ion AmpliSeq Library kit 2.0* (Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturing protocol (*Publication Part Number MAN0006735, Revision B.0*). Briefly, 10 ng of DNA was used for multiplex PCR amplification, followed by digestion of the PCR primers and ligation of a specific barcode sequence adaptor to each sample for identification. All the reactions were performed at half volume of the original fixed in the protocol. Libraries were quantified using the *Ion Library Quantification Kit* (Thermo Fisher Scientific Inc., Waltham,

Table 1 Clinical details of the 17 enrolled TAD patients

Sample	Age	Thoracic aorta phenotype	Other manifestations and family history
TAD_1	48	DeBakey type I TAD	Hepatic cavernous hemangioma, hypertension, dyslipidemia, ictus, intracranial aneurysm
TAD_2	33	DeBakey type II TAD	–
TAD_3	27	DeBakey type II TAD, aortic root dilation	BAV, left ventricle hypertrophy. Father abdominal and TAA (root, ascending) and ischemic cardiomyopathy
TAD_4	34	TAD	–
TAD_5	40	Two intervened TAD (alive)	Two brothers with TAD, one surgically treated and another deceased
TAD_6	–	DeBakey type I TAD and re-TAD (alive)	Father SD at 70, aunt SD at <30, uncle angiectasias, cousin fatal TAD at 50, cousin-son TAD at 26, cousin-son TAA
TAD_7	51	DeBakey type I TAD	Pulmonary hypertension, steatohepatitis, colloid goiter
TAD_8	44	TAD	–
TAD_9	35	DeBakey type II TAD, aortic root dilation	Moderate coronary atherosclerosis, myocardial fibrosis
TAD_10	48	DeBakey type II TAD	Hypertrophic cardiomyopathy
TAD_11	58	DeBakey type I TAD, annuloaortic ectasia	Left ventricle hypertrophy
TAD_12	49	DeBakey type II TAD	Left ventricle hypertrophy, severe coronary atherosclerosis
TAD_13	46	DeBakey type I TAD, aortic root dilation	Moderate coronary atherosclerosis
TAD_14	49	DeBakey type II TAD	Left ventricle hypertrophy
TAD_15	34	DeBakey type I TAD, aortic root dilation	BAV
TAD_16	43	DeBakey type I TAD	BAV, left ventricle hypertrophy
TAD_17	46	Standford type A TAD (root, right TSA, left iliac artery)	–

BAV bicuspid aortic valve, *SD* sudden death, *TAA* thoracic aortic aneurysm, *TAD* thoracic aortic dissection, *TSA* supra-aortic trunk

Table 2 Description of the 22 candidate genes considered for MPS and the main diseases affecting the thoracic aorta they have been associated with

Metabolic pathway	Gene	Locus (OMIM)	Differential diagnosis										
			MFS	LDS	EDS	NSAD	Osteogenesis imperfecta	Cutis laxa	JP/ HHTS	ATS	SGS	BAV-TAA syndrome	Noonan/ LEOPARD syndromes
Extracellular matrix	*FBN1*	15q21.1	x			x					x		
	COL1A1	17q21.33			x		x						
	COL1A2	7q21.3			x		x						
	COL3A1	2q32.2			x								
	EFEMP2	11q13.1						x					
	ELN	7q11.23				x		x					
	PLOD1	1p36.22			x								
TGF-β signaling	*TGFBR1*	9q22.33		x		x							
	TGFBR2	3p24.1	x	x		x							
	TGFB2	1q41		x		x							
	TGFB3	14q24.3		x		x							
	SMAD3	15q22.33		x		x							
	SMAD4	18q21.2							x				
	SLC2A10	20q13.12								x			
	SKI	1p36.33-p36.32									x		
Smooth muscle cell contractile apparatus	*ACTA2*	10q23.31				x							
	MYH11	16p13.11				x							
	FLNA	Xq28			x								
	MYLK	3q21.1				x							
	PRKG1	10q11.2-q21.1				x							
Miscellanea	*NOTCH1*	9q34.3										x	
	PTPN11	12q24.13											x

COL1A1 and *COL1A2* cause the classical form of EDS, while *COL3A1* has been associated with the vascular and *PLOD1* with the kyphoscoliotic forms. Alike, *TGFBR1* participates in the development of LDS type 1, *TGFBR2* in type 2, *TGFB2* in type 4, *TGFB3* in type 5, and *SMAD3* in type 3, also known as aneurysm-osteoarthritis syndrome. *TGFBR2* association with MFS type 2 has been controversial. Most MYH11 mutations have been associated with NSAD and concomitant patent ductus arteriosus

ATS arterial tortuosity syndrome, *BAV* bicuspid aortic valve, *JP/HHT* juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome, *LDS* Loeys-Dietz syndrome, *MFS* Marfan syndrome, *NSAD* non-syndromic aortic disease, *SGS* Shprintzen-Goldberg syndrome, *vEDS* vascular Ehlers-Danlos syndrome

MA, USA), and an equimolar pool of 20 samples were prepared at a final concentration of 40 pM. Template preparation was performed on the *Ion Chef* and sequencing on the Ion Proton using the *Ion PI Hi-QTM Chef* and Sequencing Chemistry (Thermo Fisher Scientific Inc., Waltham, MA, USA), respectively.

Data analysis: genetic variant identification and annotation, prioritization protocol, and in silico predictions of causality

The *Torrent Suite Software v5.0.2* (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for the alignment of each sample to the *hg19* human reference genome with the *Torrent Mapping Alignment Program (TMAP) v5.0.7*, while genetic variant calling was performed with the *Torrent Variant Caller* (*TVC*) v5.0.7 and genetic variant annotation with *Annovar* [33].

A pre-established prioritization protocol was applied to the TAD cases, which consisted in six different steps. First of all, seeking genetic variants expected to disrupt protein function, those located in exonic or splicing flanking regions were selected, and the referred as synonymous excluded. A frequency filter towards rare genetic variants with minor allele frequency in the non-Finnish Europeans from the Exome Aggregation Consortium database either unknown or below 1% was then applied [34]. Next, again focusing on rare variants, those reported more than four times in an internal database of around 100 exomes with a highly variable background were excluded. Finally, the genetic variants present in two or less samples from the same MPS run and those that seemed real during the visualization of the raw sequencing results using the *Integrative Genomics Viewer* were prioritized for validation [35, 36].

Available information of each of the prioritized genetic variants was revised in the literature. Their frequency in two extensive reference databases was consulted, the *Exome Variant Server* from the *NHLBI GO Exome Sequencing Project* and the Exome Aggregation Consortium [34, 37, 38]. Prediction of pathogenicity was assessed based on the conservation score and the following four pathogenic prediction tools: *Polyphen-2* [39], *Mutation Taster* [40], *SIFT* [41], and *CADD* [42]. Considering all the available information, candidate genetic variants were further classified according to the *ACMG recommendations* published in 2015 [43].

Traditional sequencing for massive parallel sequencing result confirmation

Traditional sequencing by capillary electrophoresis was chosen as the alternative technology to confirm MPS results. Those exons and the corresponding exon-intron boundaries harboring any of the prioritized genetic variants were sequenced.

Results

Technical performance: coverage and variant calling

The coverage of the submitted region in the assay design was 97.26%, which was achieved based on 789 amplicons distributed in two pools of 401 and 388 primer pairs. The percentage of each gene sequence covered with this assay and the register of the non-covered base pairs are represented in Table 3.

As the majority of the samples were forensic cases with an increased probability of DNA degradation, before starting any prioritization protocol, we ensured that the corresponding coverage exceeded the minimum read depth of 30×, the recommended standard in most publications. Only one sample, *TAD_13*, did not reach the required threshold, probably as a consequence of a low-quality DNA, and was therefore excluded from the subsequent analysis. The average percentage of gene sequence with a depth of coverage greater than 30× in the remaining 15 samples is also shown in Table 3.

Prioritized genetic variants

Table 4 contains a summary of the results obtained after each step of the prioritization protocol, shown as medians calculated after the exclusion of *TAD_13*. Briefly, from a median of 32.5 candidate genetic variants, 32 were located in exons or flanking splicing regions, 14 were not synonymous, and just 2.5 had a frequency below 1% and could be therefore considered not polymorphisms, but rare genetic variants. From those, a median of 1 was present less than four times in the internal exome database and also in a maximum of two samples from the same MPS run. After the evaluation of this latter with the *Integrative Genomic Viewer* [35, 36], Table 5 shows each of the genetic variants that overcame the whole prioritization protocol, met ACMG recommendations [43], and were further confirmed by traditional sequencing.

Table 3 Assay design expected versus observed coverage per gene

Gene	Target (bp)	Assay design		Mean % of the gene covered >30×
		Missed (bp)	Covered (%)	
ACTA2	1214	0	100	83.90
COL1A1	4905	291	94.07	95.31
COL1A2	4621	0	100	99.64
COL3A1	4911	13	99.74	96.85
EFEMP2	1432	51	96.44	98.83
ELN	2734	0	100	97.34
FBN1	9266	0	100	100
FLNA	8414	498	94.08	95.66
MYH11	6391	119	98.14	97.67
MYLK	6055	99	98.36	95.49
NOTCH1	8088	658	91.78	89.62
PLOD1	2374	0	100	94.80
PRKG1	2517	0	100	90.04
PTPN11	1936	24	98.76	98.44
SKI	2257	77	96.59	92.12
SLC2A10	1676	38	97.73	98.75
SMAD3	1452	43	97.04	86.78
SMAD4	1769	0	100	99.90
TGFB2	1409	100	92.90	93.28
TGFB3	1309	26	98.01	87.41
TGFBR1	1602	107	93.32	92.73
TGFBR2	1859	0	100	99.89

Untranslated regions were considered for design, but not for coverage estimations

bp base pairs

Table 4 Description of the prioritization protocol applied to the candidate gene massive parallel sequencing results

Prioritization step	Median of the remaining genetic variants
Total number	32.5
Exonic and located in splicing flanking regions	32
Non-synonymous, frame shift, or stop codon	14
Minor allele frequency ≤1%	2.5
Presence in internal database ≤4 times	1
Presence in ≤2 samples from the same MPS run	1

MPS massive parallel sequencing

Table 5 Summary of the candidate gene massive parallel sequencing results

Sample	Gene	Ref seq	Nucleotide change	AA change	Zigosity	*ACMG* classification	References
TAD_1	*ELN*	NM_000501	c.767C>T	p.(Ala256Val)	het	VUS	
	SMAD3	NM_005902.3	c.1140G>A	p.(Trp380*)	het	Pathogenic	
TAD_2	*PLOD1*	NM_000302.3	c.1495C>T	p.(Arg499Trp)	het	VUS	
	FBN1	NM_000138.4	c.2243G>A	p.(Cys748Tyr)	het	Pathogenic	[44]
TAD_4	*FBN1*	NM_000138.4	c.165-4T>A		het	VUS	
TAD_6	*FBN1*	NM_000138.4	c.7412C>G	p.(Pro2471Arg)	het	Likely pathogenic	[45]
TAD_10	*COL1A2*	NM_000089.3	c.304C>T	p.(Pro102Ser)	het	Likely benign	
TAD_16	*MYLK*	NM_053025.3	c.454C>T	p.(Arg152Cys)	het	VUS	
TAD_17	*MYH11*	NM_002474.2	c.3766_3768del	p.(Lys1256del)	het	Likely pathogenic	[46, 47]
			c.3611C>T	p.(Ala1204Val)	het	VUS	

ACMG American College of Medical Genetics and Genomics, *het* heterozygosis, *VUS* variant of unknown significance

Molecular autopsy yield

Unexpectedly, we were able to confirm the presence of a total of 10 potentially pathogenic mutations in 7 out of the 17 initial samples, representing approximately a 41% of the total samples analyzed. The classification of these genetic variants according to ACMG recommendations [43] (Table 5) proved two of them as pathogenic and two other as likely pathogenic. Of the six remaining variants, one was classified as possibly benign and the other five as variants of uncertain significance (VUS), partly due to lack of information. Therefore, the molecular autopsy yield achieved with this approach has been of 4 out of 17 cases in which an established molecular diagnosis was reached, which represents a 23%, approximately.

Discussion

In the present study, we have transferred to the forensic field a molecular diagnostic tool based on MPS of candidate genes, an approach that Proost et al., among others, had already shown in 2016 to be clinically useful [30]. In this case, 22 different genes were analyzed in a total of 17 TAD SCD cases, resolving about 23% of all of them. Despite the general complications associated with forensic samples, this latter yield was close to the obtained by Proost et al. in 2016. In fact, only *TAD_13* had low-quality DNA, demonstrating the usefulness of the PCR-based targeted enrichment methods for MPS [32]. In addition, the only 10 ng of starting DNA is another important advantage, especially when compared with the higher amounts needed for the hybridization-based target enrichment of postmortem samples.

Although the TAAD outcomes are often fatal [11], the diagnosis of aortic dissection remains clinically challenging. It is difficult to accurately distinguish it from conditions such as acute coronary artery syndrome, pericarditis, pulmonary thromboembolism, or cholecystitis/pancreatitis [11]. Even during a macroscopic physical examination, a false lumen completely occupied by thrombus could disguise the aortic wall tear [11]. All these existing confounding factors support the need for alternative diagnostic approaches to be widely implemented.

In the clinical setting, *European Society of Cardiology* current recommendations already suggest to include the molecular diagnosis of TAAD as part of routine clinical care for any affected patient and relatives [7]. Hiratzka et al. had already conditioned TAAD clinical management based on the genetic background in 2010 [28]. No matter the underlying disease causing TAAD, it is essential to accurately recognize which patients would benefit the most from a genetic diagnosis [16]. Bowdin et al. proposed in 2016 different situations in which genetic testing should be recommended: (i) a medical history and/or examination consistent with a syndromic form of the disease; (ii) a family history of TAAD, SCD, or family members with syndromic features; (iii) an early age development (under 65 years), especially in the absence of cardiovascular risk factors such as hypertension, smoking, or hyperlipidemia; (iv) the involvement of the aortic root and ascending aorta, aortic arch, or descending aorta; or (v) a histopathological examination compatible with medial degeneration [16].

The potential clinical consequences of a molecular TAAD diagnosis based on strong genotype-phenotype correlations, such as a personalized surgical timing or anticipated prognosis, are more obvious. We defend this also concerns other family members at risk, because early diagnosis of the latent stage of the heritable disease would allow preventive management [2]. They could undergo cascade screening to anticipate the development of this potentially fatal condition, no matter if the proband was originally a clinical or forensic case.

In fact, nowadays, the molecular autopsy has already been implemented for the diagnosis of other cardiovascular diseases causing SCD, such as the hyperthrophic or dilated cardiomyopathies. The candidate gene MPS strategy is particularly useful for those Mendelian cases expected to be monogenic, caused by necessary and sufficient high impact

mutations. By way of example, Meder et al. defined a candidate gene MPS approach for the hypertrophic and dilated cardiomyopathies in 2011; Hertz et al. did likewise with the main channelopathies (long and short QT and Brugada syndromes) in 2014, and finally, Pua et al. embraced all the main inherited cardiac conditions in the assay they published in 2016 [48–50]. Based on the relatively large number of genes that had already been associated with TAAD, we developed a new candidate gene MPS approach involving the 22 most frequently associated, shown in Table 2. In contrast with the previously mentioned cardiovascular diseases, that have been extensively explored, this has been one of the first candidate gene MPS approaches to TAAD, despite also being a potentially lethal condition. Once again, candidate gene MPS has been proved a useful approach for the molecular diagnosis and molecular autopsy of genetically heterogenic conditions, allowing simultaneous testing of an increasing number of TAAD candidate genes. We believe that many more will be discovered in the coming years, resulting in a constant update of any MPS design [16].

However, any MPS approach entails challenges regarding genetic variant interpretation. When a large number of genes are tested at the same time, there is a higher chance of finding VUS [16]. They therefore need to be accompanied by a corresponding prioritization protocol, adapted to the disease characteristics. The one here described is just an example. It is essential to remember that every prioritization step implies a certain bias, and we could be missing important genetic variants as well as detecting others of unknown significance. For this reason, MPS results should be re-evaluated as the scientific community progresses in the field. In the case of a deceased individual, if there were to be sufficient banked DNA, an updated design could be offered in the same way as for living individuals [16]. The interpretation of MPS results is even more difficult when dealing with a frequently asymptomatic disease with an incomplete penetrance and variable expression, which means that not all carriers of the causal mutation develop the clinical manifestations and that a wide range of clinical manifestations exists.

Once a putative causal mutation has been identified, the next step would be to support causality through in silico predictions of pathogenicity and conservation, cascade screening, and functional studies. In this case, we were able to perform the first and would offer the segregation analysis to every willing family member. This latter provides one of the strongest evidences of causation, but it is not always feasible.

Based on all the aforementioned advantages in both the clinical and forensic fields, we propose TAAD molecular autopsy to be offered at clinical centers and not only restricted to research laboratories. In the forensic field, the identification of a potentially causal genetic variant often leads to appropriate genetic counseling in relatives that could benefit the most from this diagnostic and prognostic tool.

Conclusions

The MPS candidate gene approach here proposed, involving 22 already known TAAD genes, has proved useful for the molecular autopsy of acute TAD SCD cases, with a diagnostic yield of about 23%. Considering that the survival rate is directly related to a prompt diagnosis and individualized treatment, this alternative diagnostic tool should be progressively incorporated in the forensic field and appropriately correlated with clinical and histopathological findings. It would help to anticipate prognosis and establish accurate risk stratification of any family member at risk of developing the same disease.

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Compliance with ethical standards All samples were processed and preserved by *Biobanco La Fe* (PT13/0010/0026), integrated in the *Plataforma Nacional de Biobancos*, with the approval of the corresponding *Scientific* and *Ethics Committees*. Furthermore, the corresponding informed consent, signed by either the individual or legal representative, was approved by *Comité Ético de Investigación Clínica de Galicia*. All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

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