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ORIGINAL INVESTIGATIONS

Comparative Risks of Initial Aortic Events Associated With Genetic Thoracic Aortic Disease

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ABSTRACT

BACKGROUND Pathogenic variants in 11 genes predispose individuals to heritable thoracic aortic disease (HTAD), but limited data are available to stratify the risk for aortic events associated with these genes.

OBJECTIVES This study sought to compare the risk of first aortic event, specifically thoracic aortic aneurysm surgery or an aortic dissection, among 7 HTAD genes and variant types within each gene.

METHODS A retrospective cohort of probands and relatives with rare variants in 7 genes for HTAD (n = 1,028) was assessed for the risk of first aortic events based on the gene altered, pathogenic variant type, sex, proband status, and location of recruitment.

RESULTS Significant differences in aortic event risk were identified among the smooth muscle contraction genes (*ACTA2*, *MYLK*, and *PRKG1*; $P = 0.002$) and among the genes for Loeys-Dietz syndrome, which encode proteins in the transforming growth factor (TGF)-β pathway (*SMAD3*, *TGFB2*, *TGFBR1*, and *TGFBR2*; $P < 0.0001$). Cumulative incidence of type A aortic dissection was higher than elective aneurysm surgery in patients with variants in *ACTA2*, *MYLK*, *PRKG1*, and *SMAD3*; in contrast, patients with *TGFBR2* variants had lower cumulative incidence of type A aortic dissection than elective aneurysm surgery. Cumulative incidence of type B aortic dissection was higher for *ACTA2*, *PRKG1*, and *TGFBR2* than other genes. After adjusting for proband status, sex, and recruitment location, specific variants in *ACTA2* and *TGFBR2* were associated with substantially higher risk of aortic event with childhood onset.

CONCLUSIONS Gene- and variant-specific data on aortic events in individuals with HTAD support personalized aortic surveillance and clinical management. (J Am Coll Cardiol 2022;80:857-869) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ABBREVIATIONS AND ACRONYMS

HTAD = heritable thoracic aortic disease

LDS = Loeys-Dietz syndrome

MAC = Montalcino Aortic Consortium

MFS = Marfan syndrome

PTC-NMD = variant that leads to premature truncation of translation and nonsense-mediated decay of the transcript

PTC-nonNMD = variant that leads to premature truncation of translation but not nonsense-mediated decay of the transcript

SMC = smooth muscle contraction

TGF = transforming growth factor

As thoracic aortic aneurysms progressively enlarge, the risk for life-threatening type A aortic dissections increases. These dissections are preventable if at-risk individuals are identified early and aneurysms surgically repaired in a timely manner.[1,2] Less deadly type B aortic dissections are also part of the thoracic aortic disease spectrum, but typically occur with little or no prior enlargement.[3] Pathogenic variants in both *FBN1* and the genes encoding proteins involved in the canonical transforming growth factor (TGF)-β signaling pathway predispose to highly penetrant thoracic aortic disease in patients with Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS), respectively.[4-8] Importantly, up to 20% of patients with thoracic aortic disease, but without syndromic features, have a family history of the disease.[9] Although both *FBN1* and TGFβ gene pathogenic variants can be responsible for nonsyndromic heritable thoracic aortic disease (HTAD) in families, additional genes have been identified that cause HTAD without MFS or LDS systemic features.[10-13] Eleven genes have a definitive association with HTAD, and based on protein function, these genes are *FBN1, LOX,* and *COL3A1* (extracellular matrix proteins), *TGFBR1, TGFBR2, SMAD3,* and *TGFB2* (proteins involved in TGFβ signaling pathway), and *ACTA2, MYH11, PRKG1,* and *MYLK* (proteins involved in smooth muscle contraction [SMC]).[14,15]

SEE PAGE 870

Clinical management of thoracic aortic disease in patients with MFS has progressively improved over the past 60 years. The initial reports of patients with LDS and pathogenic variants in *TGFBR1* or *TGFBR2* represented the severe end of the phenotypic spectrum and led to more aggressive aortic disease management recommendations than those for patients with MFS.[7,16] The Montalcino Aortic Consortium (MAC) was established to define the natural history associated with the full spectrum of pathogenic variants in novel HTAD genes. The MAC initially assembled data on individuals with *TGFBR1* and *TGFBR2* pathogenic variants[17] followed by data on *SMAD3* variants,[18] and used these data to refine recommendations for management of aortic disease in these patients. The MAC has also examined risk and made management recommendations for individuals with pathogenic variants in *ACTA2* and *MYLK*.[19,20] In this study, we directly compared the risk of first aortic event (ie, elective aortic aneurysm surgery or acute aortic dissection) among 7 HTAD genes, and specific variants within these genes, to more accurately compare and contrast the associated aortic disease risk.

METHODS

STUDY POPULATION. Genotype and clinical data from cohorts of patients and their relatives with rare variants in *ACTA2*, *MYLK*, *PRKG1*, *TGFBR1*, *TGFBR2*, *SMAD3,* and *TGFB2* were combined for these analyses. Patient recruitment, data collection, and sites were previously described.[17,18] This study was reviewed and approved by the Institutional Review Boards of UTHealth and the sites of recruitment. Informed consent and/or authorization to use de-identified data for research were obtained by participating institutions. Detailed methods are available in the Supplemental Appendix.

STATISTICAL METHODS. First aortic event, defined as any elective aortic aneurysm surgery or any aortic dissection, was the primary outcome. Individual first aortic events of elective aortic aneurysm surgery, Stanford type A or type B aortic dissection, and unspecified thoracic aortic dissection were examined as secondary outcomes. Variables were summarized by median, IQR, and minimum-maximum values or frequency. Sex distribution was compared using a z test

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TABLE 1 Characteristics of the Study Population With Rare Variants in the 7 Genes for HTAD

	Total (N = 1,028)	*ACTA2* (n = 306)	*MYLK* (n = 55)	*PRKG1* (n = 37)	*SMAD3* (n = 211)	*TGFB2* (n = 42)	*TGFBR1* (n = 141)	*TGFBR2* (n = 236)
No. of unique variants	218	43	7	1	52	12	41	62
No. of probands	376 (37)	100 (33)	7 (13)	4 (11)	60 (28)	14 (33)	72 (51)	119 (50)
Type of variant								
Missense substitution	795 (78)	306 (100)	12 (22)	37 (100)	99 (47)	14 (33)	144 (100)	186 (79)
PTC-NMD	128 (12)	0 (0)	40 (73)	0 (0)	71 (34)	17 (40)	0 (0)	0 (0)
PTC-nonNMD	89 (9)	0 (0)	0 (0)	0 (0)	28 (13)	11 (26)	0 (0)	50 (21)
IF small deletion	7 (1)	0 (0)	0 (0)	0 (0)	7 (3)	0 (0)	0 (0)	0 (0)
CNV deletion	9 (1)	0 (0)	3 (5)	0 (0)	6 (3)	0 (0)	0 (0)	0 (0)
Median age, y	33 (19-49)	34 (20-49)	46 (29-64)	32 (21-46)	42 (28-53)	36 (26-49)	27 (16-44)	25 (15-38)
Sex								
Female	502 (49)	143 (47)	27 (49)	22 (60)	90 (43)	17 (40)	81 (57)	122 (52)
Male	526 (51)	163 (53)	28 (51)	15 (40)	121 (57)	25 (60)	60 (43)	114 (48)
Location of recruitment								
North America	618 (60)	267 (87)	35 (64)	37 (100)	103 (49)	31 (74)	55 (39)	90 (38)
Europe	351 (34)	31 (10)	20 (36)	0 (0)	108 (51)	11 (26)	62 (44)	119 (50)
Australia	35 (3)	5 (2)	0 (0)	0 (0)	0 (0)	0 (0)	16 (11)	14 (6)
Japan	24 (2)	3 (1)	0 (0)	0 (0)	0 (0)	0 (0)	8 (6)	13 (6)

Values are n, n (%), or median (IQR).

CNV = copy number variant (exon or whole gene); IF = in-frame; PTC-NMD = truncating variants that result in a premature termination codon and nonsense mediated decay; PTC-nonNMD = truncating variants that result in a premature termination codon and escape nonsense mediated decay.

for one sample proportion. Cumulative probability of the primary outcome was calculated using the Kaplan-Meier method and compared between groups by univariable Cox regression with adjustment for family clustering. Cumulative incidence of individual first aortic events were calculated using a competing risks analysis and compared between groups using the method of Fine and Gray with adjustment for family clustering. Variables with a P value <0.20 by the univariable analysis were included in the multivariable model. Multivariable analyses of any first aortic event associated with variant subtypes were performed using Cox regression, stratified by gene and adjusted for proband status, sex, and geographic location of recruitment, and intrafamilial correlation using the clustered robust method. Statistical analysis was performed using the Stata statistical package version 16.1.

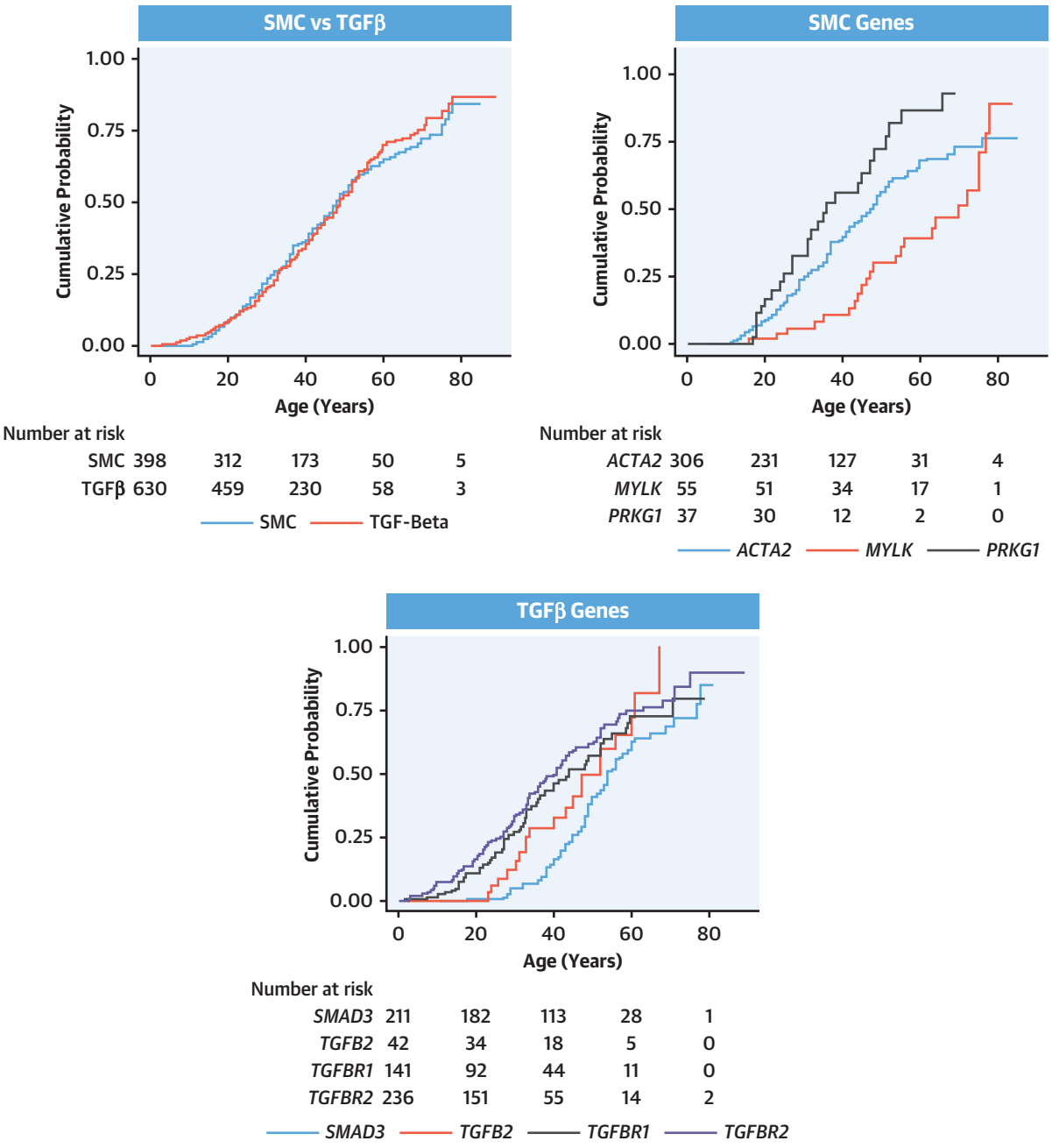
RESULTS

Data from 1,028 individuals from 376 families with 218 unique variants in 7 HTAD genes were used for this analysis. Patients with *ACTA2* variants represented 30% of the cohort, followed by *TGFBR2* (23%), *SMAD3* (20%), *TGFBR1* (14%), *MYLK* (5%), *PRKG1* (4%), and *TGFB2* (4%) (**Table 1,** Supplemental Table 1). Of the 1,028, 456 (44%) had their first aortic event, defined as presentation with an acute thoracic aortic dissection (214 type A, 66 type B, and 25 unspecified thoracic aortic dissection) or elective surgical repair of an aortic aneurysm (n = 151), at median age of 36 years (IQR: 24-48 years). Only 2 of the first elective aortic aneurysm surgeries involved the abdominal aorta. Median age at last follow-up, without an aortic event, was 29 years (IQR: 16-50 years). Eighteen percent (n = 187) were deceased at median age of 45 years (IQR: 29-56 years). Fifty-one percent of cases were male. By location of recruitment, 60% of cases were recruited in the United States and Canada, 35% in Europe, and 5% in Australia and Japan.

CUMULATIVE PROBABILITY OF COMPOSITE FIRST AORTIC EVENT ASSOCIATED WITH HTAD GENES. Kaplan-Meier estimates of cumulative risk of composite first aortic event by grouping genes based on the function of the corresponding protein found that the cumulative risk of first aortic event did not differ between patients with variants in the TGFβ genes (ie, LDS genes, *TGFBR2*, *TGFBR1*, *SMAD3,* and *TGFB2*) and those with variants in the SMC genes (*ACTA2*, *MYLK,* and *PRKG1*) (**Figure 1,** Supplemental Table 2). Among patients with variants in the SMC genes, cumulative risks of aortic event were significantly different ($P = 0.002$) and highest among patients with *PRKG1*, followed by those with *ACTA2* and *MYLK* variants. Similarly, cumulative risks of aortic event differed significantly among patients with variants in the TGFβ genes ($P < 0.0001$), with the highest and lowest risk observed for *TGFBR2* and *SMAD3*, respectively.

Overall, cumulative risk of composite aortic event at age 65 years was 70% (95% CI: 66.0-74.5). Risk of

FIGURE 1 **Cumulative Risks for Aortic Event Based on Genes and Variants**



Kaplan-Meier graphs of the cumulative risks of composite first aortic event (elective aortic aneurysm surgery, type A and type B aortic dissection) based on groups of genes for heritable thoracic aortic disease, the individual genes, and variant types within each gene. **Upper panels** show the graphs of genes grouped together based on the function of the corresponding protein (smooth muscle contraction [SMC] and transforming growth factor [TGF] β) and the individual genes within these groups. The middle and bottom panels show graphs of variant types within the individual genes (Supplemental Table 2). PTC-NMD = variant that leads to premature truncation of translation and nonsense-mediated decay of the transcript; PTC-nonNMD = variant that leads to premature truncation of translation but not nonsense-mediated decay of the transcript; TGF = transforming growth factor.

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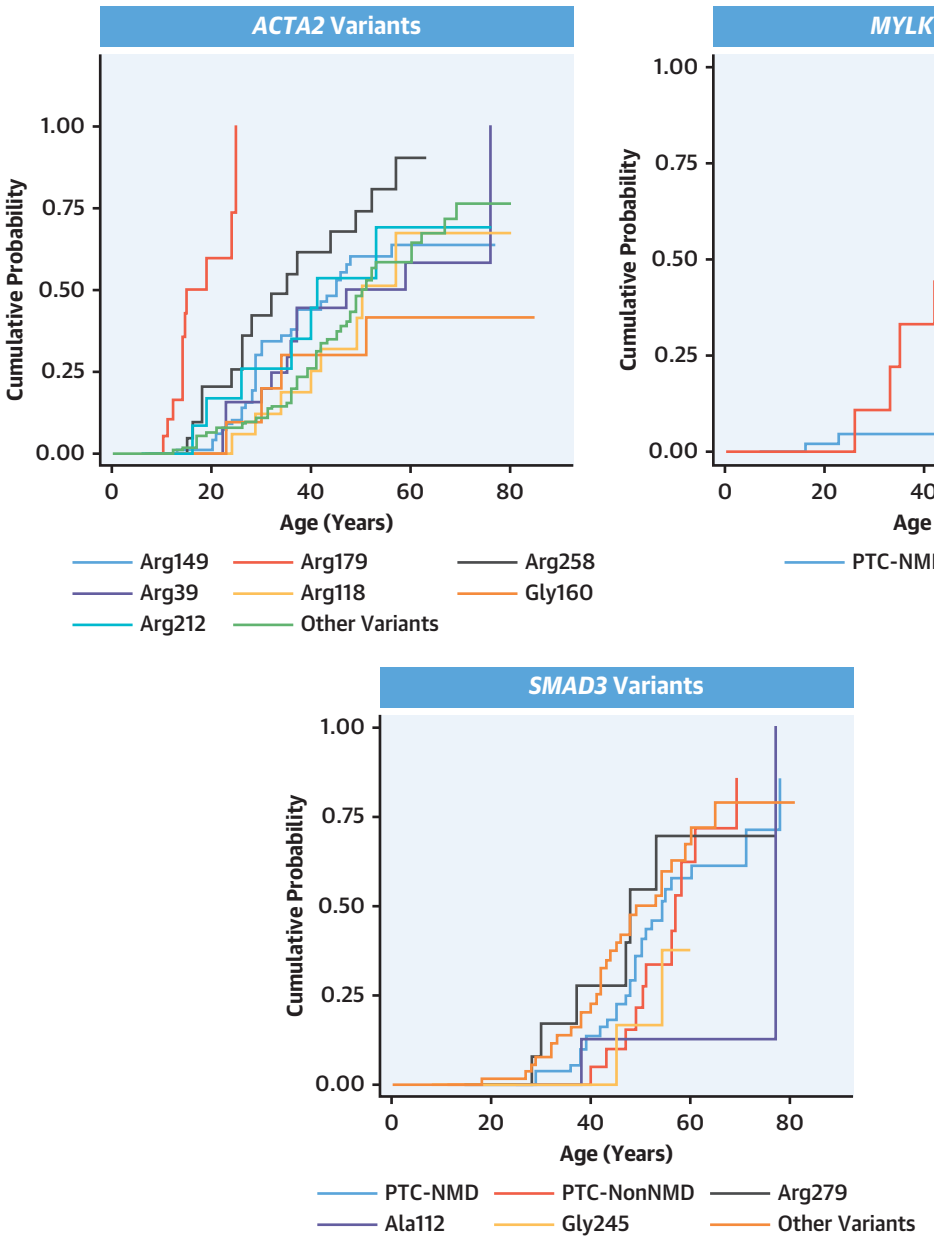
FIGURE 1 Continued



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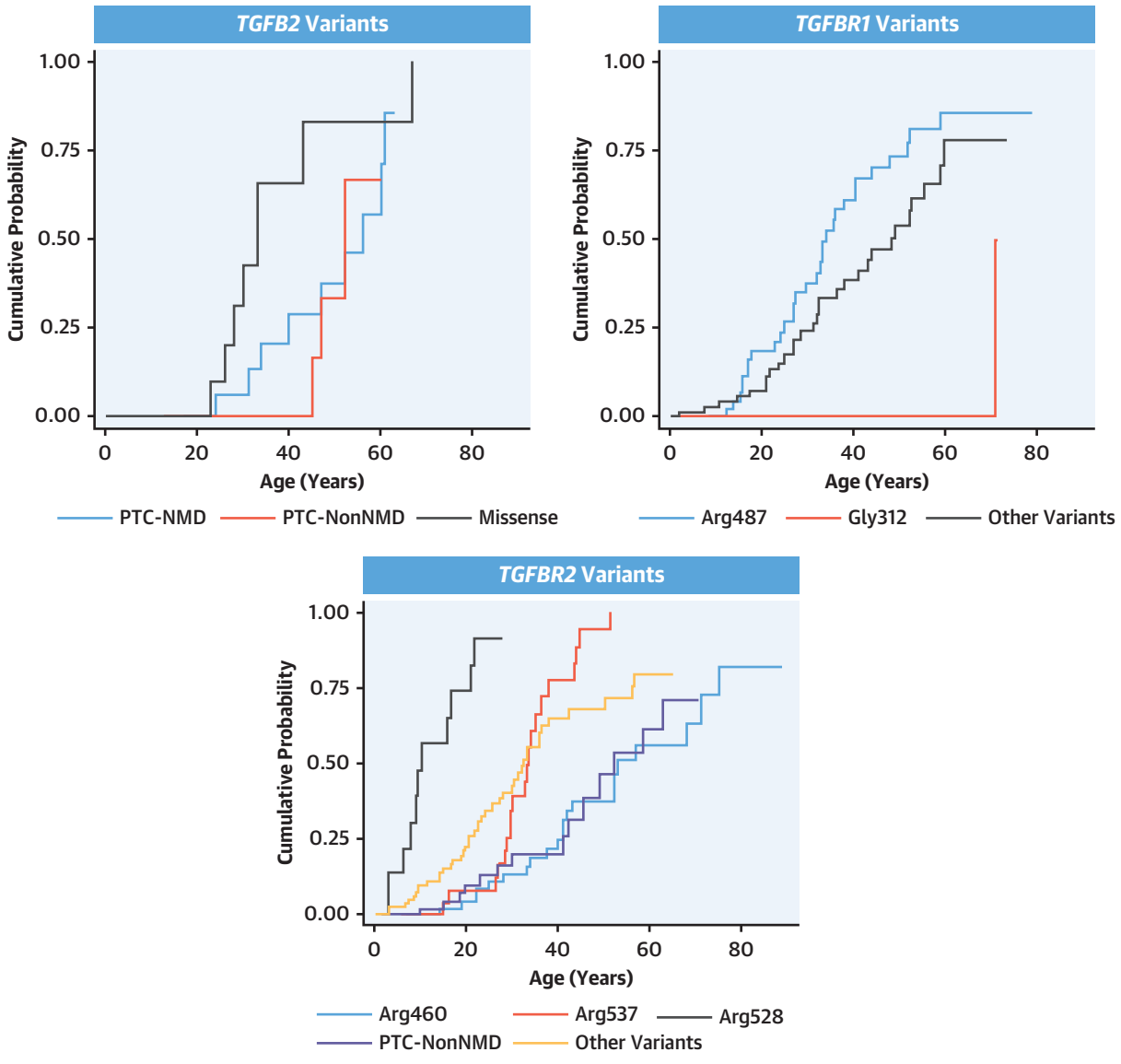
composite aortic event at age 25 years (Table 2) was 15% to 27% for *ACTA2*, *PRKG1*, *TGFBR1,* and *TGFBR2*, but much lower, 1% to 6%, for *MYLK*, *SMAD3,* and *TGFB2*. At age 65 years, cumulative risk was higher than average for *PRKG1*, *TGFB2*, *TGFBR1,* and *TGFBR2*, and lower on average for *MYLK* and *SMAD3*.

CUMULATIVE INCIDENCE OF INDIVIDUAL FIRST AORTIC EVENTS ASSOCIATED WITH HTAD GENES. The cumulative incidence of individual first aortic events (elective aortic aneurysm surgery, acute type A or type B aortic dissection) was compared using a competing risks analysis of all probands and relatives, and relatives only (Figure 2, Supplemental Table 3) (thoracic aortic dissections of unspecified location were excluded). There are markedly different patterns of aortic disease presentation among the HTAD genes, with the cumulative incidence of type A aortic dissection higher than elective aortic aneurysm surgery among patients with variants in *ACTA2*, *MYLK*,

FIGURE 1 Continued



PRKG1, and *SMAD3*. In contrast, a lower incidence of type A aortic dissection than elective aortic aneurysm surgery was observed in patients with *TGFBR2* and *TGFB2* variants, whereas the cumulative incidence of these events did not differ in patients with *TGFBR1* variants. When relatives only were analyzed, these patterns persisted for *ACTA2*, *PRKG1*, *MYLK*, *TGFB2,* and *TGFBR1*; however, cumulative incidences of type A dissection and elective aneurysm surgery were similar in relatives with *SMAD3* and *TGFBR2* variants.

The cumulative incidences of individual first aortic events at age 25 and 65 years and *P* values of comparison tests with adjustment for family clustering are shown in **Table 2**.[21] Compared with *ACTA2*, cumulative incidence of any first aortic event at age 65 years was lower for *MYLK* ($P = 0.019$) and *SMAD3* ($P = 0.001$), but not significantly different for *PRKG1*, *TGFB2*, *TGFBR1,* and *TGFBR2*. Similarly, cumulative incidence of type A aortic dissection at age 65 years was significantly higher for *ACTA2* compared with *TGFBR2* ($P = 0.049$) but was not statistically different compared with the other genes. There was a significant incidence of type B aortic dissection at ages 25 and 65 years in patients with *ACTA2*, *PRKG1,* and *TGFBR2* variants, and relatively few type B

dissections as the presenting event in patients with variants in other HTAD genes.

ADJUSTED AND UNADJUSTED RISK OF COMPOSITE FIRST AORTIC EVENT BY VARIANT TYPE. The risk of first aortic event associated with different variant types, within the individual genes, was examined by analysis of subgroups of 10 or more individuals (related and unrelated) carrying variants predicted to result in a similar effect on the protein, specifically haploinsufficiency (entire gene deletion, exon deletions and truncating variants resulting in premature truncation and nonsense-mediated decay [PTC-NMD]), truncated proteins (PTC-nonNMD), missense substitutions, and recurrent missense variants disrupting the same amino acid. A list of variants and variant types, as well as median age at first aortic event or last follow-up without an event, are shown in Supplemental Table 1. We have previously reported differences in event rates with age for specific variants or variant types in *ACTA2*, *MYLK*, and *SMAD3*.[14–16] Here, we show different event rates with age for specific variant types in *TGFBR2*, including Arg528 substitutions associated with highly penetrant events in childhood and Arg537 substitutions associated with complete penetrance and intermediate age of onset compared with Arg528 and other variant types in *TGFBR2* (**Figure 1**). In addition, Arg487 substitutions in *TGFBR1* conferred a higher risk for aortic events.

Comparison of variant types by univariable Cox regression identified variant type(s) with significantly different risks than the reference (**Table 3**). Multivariable analysis confirmed that some variant types were associated with significantly different aortic event risks after adjusting for sex, proband status, and location of recruitment (**Table 3**). For *ACTA2*, risk of Arg179 missense substitutions was significantly higher (HR: 5.89; 95% CI: 3.02–11.48), whereas the Arg118 (HR: 0.52; 95% CI: 0.34–0.79) and other variants (HR: 0.43; 95% CI: 0.26–0.72) were significantly lower compared with the reference variant type, Arg149 missense substitutions. Similarly, risk of aortic event was significantly higher for missense variants in *TGFB2* (2.23; 95% CI: 1.11–4.50) and lower for PTC-nonNMD variants (HR: 0.32; 95% CI: 0.12–0.86) compared with the reference, PTC-NMD variants. Among the *SMAD3* variant types, only Arg279 was significantly different from the reference, PTC-NMD variants, with HR: 1.76 (95% CI: 1.01–3.06). Adjusted risk of aortic event of *TGFBR1* Arg487 substitutions was significantly higher than all other variants in *TGFBR1*. Last, adjusted risks of aortic event associated with *TGFBR2* Arg528 and Arg537 substitutions (HR: 11.76; 95% CI: 6.17–22.40 and HR: 1.95; 95% CI: 1.03–3.69, respectively) were significantly higher compared with the reference, Arg460 substitutions, but all other *TGFBR2* variants, including PTC-nonNMD variants, were not significantly different from the Arg460 group. Other variants in *TGFBR2* and *TGFBR1* (**Figure 1,** Supplemental Table 1) may be associated with childhood events and higher than average risks but could not be analyzed separately because of the small numbers of cases.

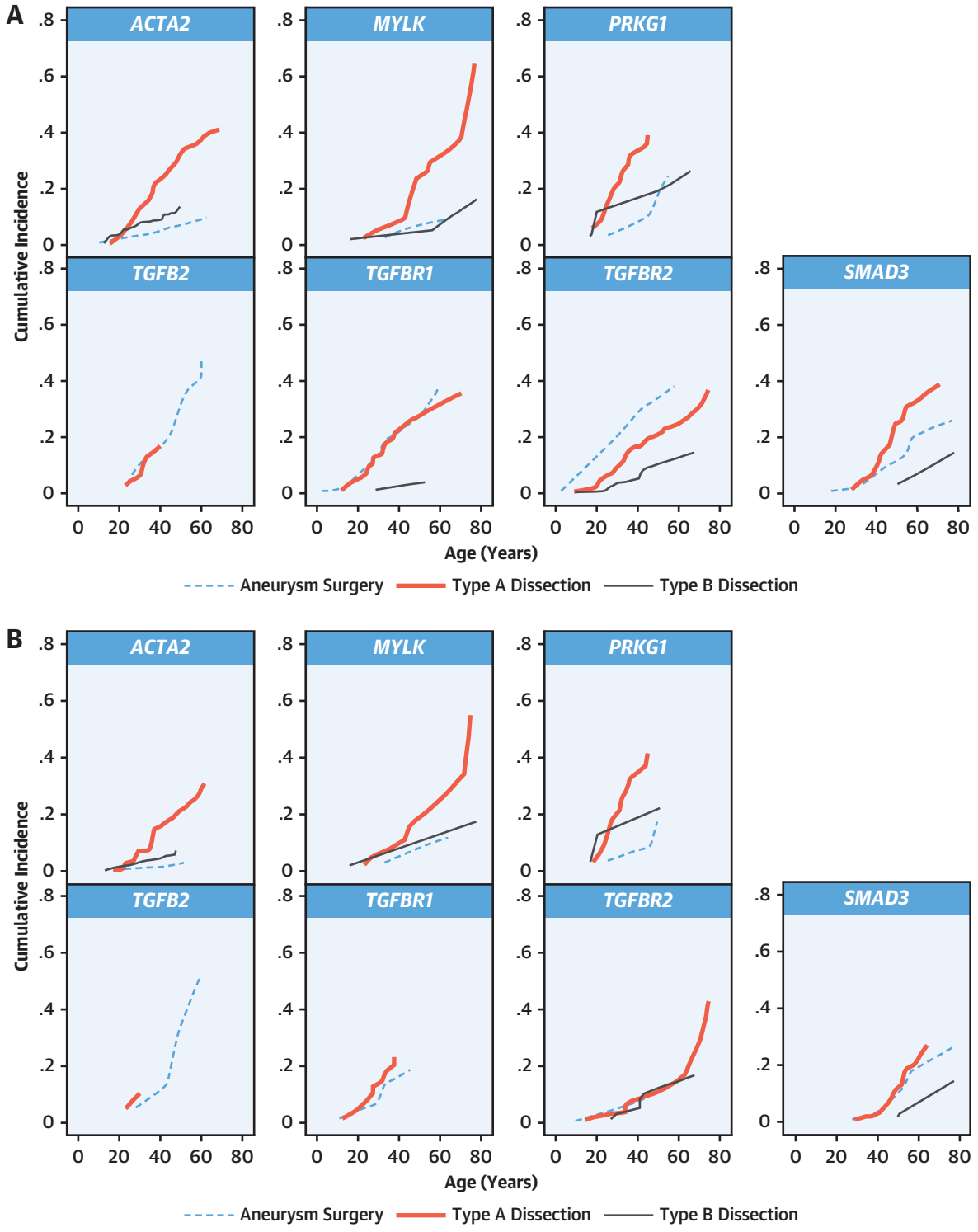
ADJUSTED RISK OF AORTIC EVENT BY SEX AND LOCATION OF RECRUITMENT. Analysis of sex and

TABLE 2 Cumulative Incidence of First Aortic Event (Composite and Individual Events) for the 7 HTAD Genes

Gene	No. Events	Cumulative Incidence at 25 y, % (95% CI)	Cumulative Incidence at 65 y, % (95% CI)	*P* Value[a]
Composite aortic event				
ACTA2	141	15 (11.4–20.4)	69 (61.3–76.1)	reference
MYLK	23	4 (1.0–14.4)	47 (31.8–65.4)	0.019
PRKG1	26	27 (14.8–45.0)	86 (69.7–96.3)	0.051
SMAD3	78	1 (0.1–3.7)	66 (55.8–75.6)	0.001
TGFB2	20	6 (1.5–21.5)	81 (58.9–95.9)	0.959
TGFBR1	60	19 (12.8–27.8)	73 (61.0–83.2)	0.53
TGFBR2	108	25 (19.1–31.4)	76 (67.2–84.7)	0.1
Elective aneurysm surgery				
ACTA2	18	3 (1.4–5.7)	8 (4.9–12.9)	reference
MYLK	3	0 (0–0)	9 (2.3–22.4)	0.741
PRKG1	6	3 (0.2–14.0)	25 (10.3–42.2)	0.018
SMAD3	24	1 (0.0–2.7)	20 (13.1–28.3)	0.035
TGFB2	11	3 (0.2–13.0)	51 (27.0–71.4)	<0.001
TGFBR1	31	10 (5.1–16.0)	40 (28.0–51.2)	<0.001
TGFBR2	58	16 (11.5–21.7)	38 (29.7–46.8)	<0.001
Type A aortic dissection				
ACTA2	77	6 (3.8–9.9)	39 (31.3–45.9)	reference
MYLK	17	2 (0.2–9.1)	33 (18.1–48.4)	0.846
PRKG1	12	12 (3.8–25.3)	39 (22.0–55.4)	0.458
SMAD3	42	0 (0–0)	36 (26.3–44.9)	0.098
TGFB2	5	3 (0.2–13.0)	17 (6.0–32.0)	0.141
TGFBR1	26	9 (4.8–15.9)	29 (19.3–38.7)	0.531
TGFBR2	35	7 (3.6–11.0)	26 (18.3–34.9)	0.049
Type B aortic dissection				
ACTA2	30	5 (2.8–8.1)	14 (9.3–18.5)	reference
MYLK	3	2 (0.2–8.6)	5 (0.8–15.4)	0.096
PRKG1	7	11 (3.6–24.2)	19 (7.6–34.8)	0.009
SMAD3	7	0 (0–0)	7 (2.6–13.0)	0.01
TGFB2	1	0 (0–0)	0 (0–0)	0.105
TGFBR1	3	0 (0–0)	4 (1.1–10.8)	0.017
TGFBR2	15	2 (0.5–4.6)	12 (6.7–18.8)	0.35
Unspecified thoracic aortic dissection				
ACTA2	16	1 (0.2–2.7)	8 (4.7–13.3)	reference
PRKG1	1	0 (0.0–0.0)	4 (0.3–15.7)	0.455
SMAD3	5	0 (0.0–0.0)	4 (1.2–8.3)	0.109
TGFB2	3	0 (0.0–0.0)	13 (3.2–30.7)	0.586

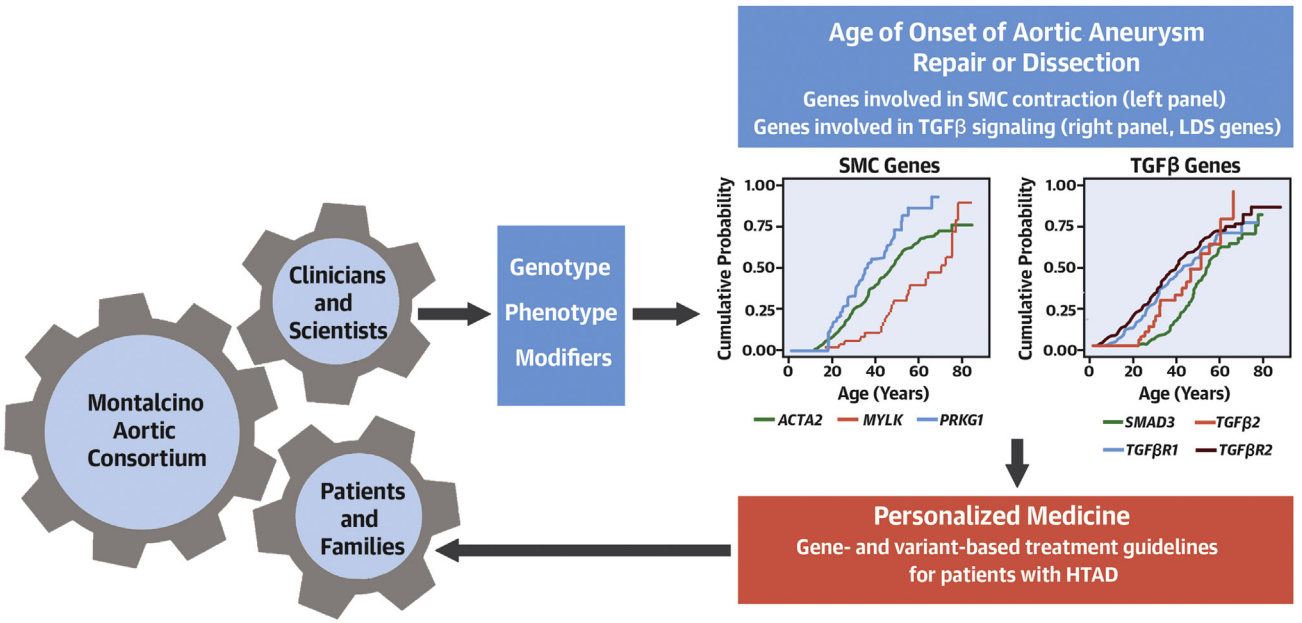
[a]*P* values from Cox or competing risk regression with adjustment for family clustering are shown for each gene compared with the reference gene group (*ACTA2*).

FIGURE 2 **Cumulative Incidence and Type of Aortic Event Based on Gene**



Cumulative incidence of individual first aortic events using a competing risks analysis in patients with rare variants in the 7 genes for heritable thoracic aortic disease. Graphs of the data from both probands and relatives (**A**; n = 1,028) and data from relatives only (**B**; n = 652) (Supplemental Table 3).

CENTRAL ILLUSTRATION **Gene-Specific Thoracic Aortic Risk Models From the Montalcino Aortic Consortium**



Data were collected from probands and family members with rare variants in seven genes predisposing to heritable thoracic aortic disease. Using these data, risk and type of first aortic event were stratified based on both the altered gene and recurrent variants within the genes.

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The international Montalcino Aortic Consortium (MAC) collects patient data to generate gene- and pathogenic variant-specific risk models to improve outcomes for individuals with heritable thoracic aortic disease (HTAD).

location of recruitment showed these factors influenced risk of aortic event with variable effects depending on the gene. Adjusted risk of aortic event was significantly higher in male than female patients, as previously reported for *ACTA2* and *TGFBR1*, but also for *TGFB2* (HR: 3.49; 95% CI: 1.41-8.64) and *TGFBR2* (HR: 1.53; 95% CI: 1.04-2.23). Location of recruitment also appeared to significantly influence risk of aortic event, particularly in patients with *MYLK* or *SMAD3* variants who had a higher and lower risk of aortic events, respectively, when recruited in Europe compared with North America.

DISCUSSION

These data from the Montalcino Aortic Consortium illustrate the intergenic and intragenic variability of both risk for and type of first aortic events associated with 7 genes for HTAD, information critical for the clinical management of these patients (Central Illustration). For example, the results delineate individuals with early-onset and aggressive thoracic aortic disease and, conversely, individuals at risk for late-onset, low-penetrant aortic disease. Initial descriptions of LDS were of children and young adults with *TGFBR1* and *TGFBR2* pathogenic variants at the severe end of the phenotypic spectrum, with marked syndromic features and aggressive aortic disease associated with a high risk of dissection.[16] The MAC sought to define the broader spectrum of disease associated with all HTAD genes. This study shows that pathogenic variants in SMC and TGFβ genes confer a similar risk for first aortic event. Surprisingly, SMC genes are associated with a significantly higher risk for aortic dissections than TGFβ genes, with the *PRKG1* group associated with the highest risk for any aortic dissection or aortic event. *ACTA2*, *MYLK*, and *PRKG1* variants have a higher risk of presenting with type A aortic dissections than elective aneurysm surgery, regardless of proband status. At the same time, *TGFBR1* and *TGFBR2* groups have the highest burden of childhood-onset aortic events.

TABLE 3 Cox Regression Analysis of First Aortic Event by Variant Type and Stratified by HTAD Gene

		Univariable Analysis		Multivariable Analysis[a]	
	n	Unadjusted HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
ACTA2					
Variant type[a]					
Arg149	71	reference		reference	
Arg179	35	15.72 (8.26–29.93)	<0.001	5.89 (3.02–11.48)	<0.001
Arg258	23	1.85 (0.91–3.75)	0.09	1.20 (0.58–2.47)	0.63
Arg39	30	0.85 (0.49–1.48)	0.56	0.94 (0.56–1.58)	0.83
Arg118	17	0.67 (0.42–1.09)	0.11	0.52 (0.34–0.79)	0.002
Gly160	11	0.48 (0.12–1.87)	0.29	0.43 (0.13–1.50)	0.19
Arg212	17	1.00 (0.53–1.89)	0.99	0.88 (0.38–2.02)	0.76
Other variants	102	0.79 (0.47–1.33)	0.37	0.43 (0.26–0.72)	0.001
Proband	100	4.90 (3.43–6.98)	<0.001	5.18 (3.36–7.98)	<0.001
Male	163	1.47 (1.07–2.01)	0.02	1.64 (1.12–2.40)	0.01
MYLK					
Variant type					
PTC-NMD	43	reference		reference	
Missense	12	2.93 (1.57–5.46)	0.001	2.33 (1.03–5.28)	0.04
Proband	7	2.23 (0.57–8.81)	0.25		
Male	28	1.16 (0.58–2.31)	0.67		
Location of recruitment[b]					
North America	35	reference		reference	
Europe	20	2.51 (1.13–5.56)	0.02	2.01 (1.01–3.99)	0.05
PRKG1					
Proband	4	0.76 (0.41–1.43)	0.40		
Male	15	1.24 (0.46–3.33)	0.67		
SMAD3					
Variant type					
PTC-NMD	77	reference		reference	
PTC-nonNMD	28	0.95 (0.55–1.63)	0.84	1.51 (0.74–1.78)	0.53
Arg279	15	1.47 (0.86–2.51)	0.16	1.76 (1.01–3.06)	0.04
Ala112	16	0.40 (0.09–1.82)	0.24	0.50 (1.06–2.38)	0.38
Gly245	12	0.51 (0.41–0.64)	<0.001	1.00 (0.62–1.60)	0.99
Other variants	63	1.41 (0.90–2.23)	0.14	1.52 (0.94–2.47)	0.09
Proband	60	3.43 (2.28–5.14)	<0.001	4.05 (2.64–6.22)	<0.001
Male	121	1.28 (0.84–1.94)	0.25		
Location of recruitment					
North America	103	reference		reference	
Europe	108	0.60 (0.38–0.92)	0.02	0.52 (0.32–0.85)	0.01

Continued on the next page

There were few childhood-onset events associated with *ACTA2* (mostly due to the Arg179 variant) and only rare childhood aortic events associated with *TGFB2*, *SMAD3*, *PRKG1,* and *MYLK*.

The higher risk for dissections associated with SMC genes could be because of type A dissections occurring without significant enlargement of the aorta. The *PRKG1* pathogenic variant is associated with dissections at significantly younger ages than aneurysm repair as well as type A dissections occurring with minimal to no aortic enlargement.[11] Similarly, type A dissections in *MYLK* mutation carriers can also occur with little to no enlargement of the aorta.[20] In contrast, most *ACTA2* mutation carriers have dilation of the aorta before type A dissections, raising the alternative hypothesis that a lack of syndromic features is delaying the diagnosis of HTAD and leading to dissections.[19,22] Further supporting this alternative hypothesis is the fact that the *SMAD3* group had a higher incidence of presentation with type A dissections and lower elective aneurysm surgery than the other LDS genes, and the individuals with *SMAD3* variants have fewer LDS features and instead have atypical complications not observed with other LDS genes, including early-onset osteoarthritis and age-related Charcot-Marie-Tooth-like neuropathy.[23,24] Also, *TGFB2* pathogenic variants are associated with significant MFS features,[6] and the *TGFB2* cases less commonly presented with type A dissections. These data emphasize that in the absence of syndromic features, a family history of thoracic aortic disease is the only clinical finding that identifies individuals who have a high risk for aortic dissections due to HTAD.

There was a significantly higher incidence of presenting with type B aortic dissections in patients with *ACTA2*, *PRKG1,* and *TGFBR2* variants when compared with the other genes, and when compared with type B dissections incidence in patients with MFS.[25] Importantly, type B aortic dissections in *PRKG1* and *ACTA2* cases occurred at younger ages than type A dissections. Type B dissections are associated with lower acute mortality than type A dissections, but they do cause increased long-term morbidity and mortality.[26] Because the proximal descending aortic diameter does not predict type B dissections, clinical markers are limited for predicting these dissections.[27] Based on these data, treatment with β-adrenergic blocking agents should be considered in children >10 years of age with *ACTA2, MYLK,* and *PRKG1* pathogenic variants, even in the absence of enlargement of the ascending aorta or hypertension, to reduce the risk of both type A and B aortic dissections.[1,28]

We found significant differences in the cumulative risk of aortic events among the TGFβ genes, thus questioning the classification of these genes as a single syndrome. Systemic complications also differ among these genes, for example, craniosynostosis is associated with *TGFBR1* and *TGFBR2* variants and peripheral neuropathy and severe osteoarthritis with *SMAD3* variants.[5,23] Other observations further complicate the classification of disorders associated with variants in TGFβ genes: these genes trigger aortic disease in the absence of systemic features[29]; individuals with TGFβ gene variants can meet diagnostic criteria for MFS,[4,29-31] and pathogenic variants in another TGFβ gene, *TGFB3,* are associated with a

low penetrant risk for aortic events and unique systemic manifestations.[32] Thus, the systematic designation of LDS to all patients with pathogenic variants in genes that encode proteins involved in canonical TGFβ signaling fails to communicate important gene-specific vascular and systemic complications. The systemic features of LDS have been associated with increased aortic risk in patients with pathogenic variants in *TGFBR1* or *TGFBR2* and therefore may inform vascular risk.[16,17] These observations emphasize that the diagnosis of individuals with HTAD should be based both on the disease-causing gene and the clinical phenotype of the patient, a dyadic classification recently recommended for all Mendelian disorders.[33] Thus, a patient with a pathogenic *TGFBR2* variant could be diagnosed with *TGFBR2*-related LDS, *TGFBR2*-related MFS, or *TGFBR2*-related HTAD based on the presence or absence of other systemic features of LDS and MFS.

A key finding of this study is that the age of onset of aortic events can be variable among patients who harbor the same or different pathogenic variants in the same gene. Our data support regular, comprehensive aortic surveillance, beginning in childhood, for individuals with *ACTA2* Arg179, *TGFBR2* Arg537 and Arg528, as well as *TGFBR1* Arg487 variants. The significant burden of aortic events in childhood for individuals with *TGFBR1* and *TGFBR2* variants indicates that aortic surveillance should begin in the first decade of life. Although there were no events in childhood in the *TGFB2* and *SMAD3* group, an inherited *SMAD3* mutation associated with adult-onset aortic disease was identified in a neonate with massive aortic enlargement, most likely because of an unidentified second genetic hit.[34] Thus, imaging of the aorta should be pursued at the time of genetic diagnosis no matter the age. Follow-up aortic surveillance should be based on the initial scan, the aortic event risk associated with the HTAD gene or the specific variant in the gene, the presence of extra-aortic LDS features, and the family history.

Data from 1,575 patients with *FBN1* pathogenic variants (mean age 34.1 ± 17.8 years) similarly found that specific variant types, as well as location of the variant in the gene, predicted a differential risk of aortic events. There were variable effects of gender based on genotype, that is, male sex was associated with higher aortic event risk for all variant types examined, with the exception of cysteine altering variants. Based on 455 aortic events reported in this population with *FBN1* variants, the risk of any first aortic event was 8% (95% CI: 6.2%-9.4%) and 64% (95% CI: 59.3%-69.1%) at ages 25 and 65 years, respectively, which is intermediate when compared with the gene-specific risks reported in this study (**Table 2**) (G. Jondeau, written communication, 2021).[35]

TABLE 3 Continued

		Univariable Analysis		Multivariable Analysis[a]	
	n	Unadjusted HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
TGFB2					
Variant type					
PTC-NMD	17	reference		reference	
PTC-nonNMD	11	0.66 (0.24–1.78)	0.41	0.32 (0.12–0.86)	0.02
Missense	14	2.62 (0.96–6.53)	0.06	2.23 (1.11–4.50)	0.02
Proband	14	2.30 (0.51–10.34)	0.28		
Male	25	2.27 (1.39–3.71)	0.001	3.49 (1.41–8.64)	0.01
Location of recruitment					
North America	31	reference		reference	
Europe	11	0.42 (0.14–1.30)	0.13	0.34 (0.10–1.17)	0.09
TGFBR1					
Variant type					
Arg487	46	reference		reference	
Gly312	14	0.06 (0.03–0.11)	<0.001	0.09 (0.04–0.20)	<0.001
Other variants	81	0.60 (0.32–1.14)	0.12	0.58 (0.31–1.06)	0.08
Proband	72	2.52 (1.27–5.01)	0.01	2.01 (1.12–3.62)	0.02
Male	60	2.79 (1.74–4.49)	<0.001	2.77 (1.53–5.00)	0.001
Location of recruitment					
North America	55	reference		reference	
Europe	62	0.66 (0.27–1.59)	0.35	0.62 (0.27–1.40)	0.25
Australia	16	1.40 (0.66–2.96)	0.38	1.31 (0.50–3.40)	0.58
Japan	8	2.12 (0.97–4.64)	0.06	1.69 (0.81–3.56)	0.16
TGFBR2					
Variant type					
Arg460	57	reference		reference	
Arg537	29	3.62 (2.11–6.22)	<0.001	1.95 (1.03–3.69)	0.04
Arg528	15	22.67 (9.99–51.42)	<0.001	11.76 (6.17–22.40)	<0.001
PTC-nonNMD	50	1.11 (0.38–3.22)	0.84	0.96 (0.46–2.03)	0.92
Other variants	85	2.82 (1.53–5.20)	0.001	1.67 (0.91–3.08)	0.10
Proband	119	6.69 (3.65–12.26)	<0.001	5.05 (2.85–8.95)	<0.001
Male	114	1.38 (1.00–1.92)	0.05	1.53 (1.04–2.23)	0.03
Location of recruitment					
North America	90	reference		reference	
Europe	119	0.73 (0.34–1.55)	0.41	0.63 (0.36–1.10)	0.11
Australia	14	3.13 (1.32–7.40)	0.01	2.21 (0.91–5.38)	0.08
Japan	13	3.24 (1.43–7.33)	0.01	1.53 (0.74–3.18)	0.25

[a]Categorization of variant type is shown in Supplemental Table 1. [b]Location of recruitment was included in the model when the patients were recruited from different sites.

STUDY LIMITATIONS. A limitation of this study is the fact that probands were ascertained after presenting with either syndromic features or an aortic event and tend to be more severely affected, particularly in cases with de novo mutations. Thus, analysis of relatives may provide a closer approximation of the disease risk in familial cases. Furthermore, studies

are needed to examine the role of additional risk factors in predicting aortic events, such as family history of aortic dissections, degree of vascular tortuosity, systemic features of LDS, medications, comorbid conditions, and imaging biomarkers (ie, aortic size, stiffness, and compliance).[17,36]

CONCLUSIONS

Our findings demonstrate the value of the multisite Montalcino Aortic Consortium to characterize the risk of aortic disease associated with rare variants in HTAD genes with adjustments for geographic differences in recruitment and clinical management. These data emphasize the utility of genetic testing, not only to identify family members at risk for aortic disease, but also in stratifying aortic disease risk and tailoring aortic surveillance and surgical management. The accumulating data on gene- and variant-specific aortic outcomes for HTAD genes may be used to develop algorithms for incorporating molecular diagnosis, along with clinical features and family history, into medical decision-making to improve outcomes and minimize costs for the care of patients with HTAD.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The risk of thoracic aortic aneurysm and dissection in patients with pathogenic variants in 7 genes associated with heritable thoracic aortic disease, along with recurrent variants in each gene, can be used to stratify risk and predict type of first aortic event.

TRANSLATIONAL OUTLOOK: Further research is needed to clarify algorithms for surveillance and management of patients with pathogenic variants in these genes.

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APPENDIX For supplemental material and tables, please see the online version of this paper.