

Total Serum Transforming Growth Factor- β 1 Is Elevated in the Entire Spectrum of Genetic Aortic Syndromes

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ABSTRACT

Background: Total serum transforming growth factor-beta 1 (tsTGF- β 1) is increased in patients with Marfan syndrome (MFS), but it has not been assessed in thoracic aortic aneurysm and dissection (TAAD), Loeys-Dietz syndrome (LDS), and bicuspid aortic valve disease (BAVD).

Hypothesis: tsTGF- β 1 is increased in genetic aortic syndromes including TAAD, LDS, MFS, and BAVD.

Methods: We measured tsTGF- β 1 and performed sequencing of the genes *FBN1*, *TGFBR1*, and *TGFBR2* in 317 consecutive patients with suspected or known genetic aortic syndrome (167 men, 150 women; mean age 43 ± 14 years). TAAD was diagnosed in 20, LDS in 20, MFS in 128, and BAVD in 30 patients, and genetic aortic syndrome was excluded in 119 patients.

Results: Elevated tsTGF- β 1 levels were associated with causative gene mutations ($P = 0.008$), genetic aortic syndrome ($P = 0.009$), and sporadic occurrence of genetic aortic syndrome ($P = 0.048$), whereas only genetic aortic syndrome qualified as an independent predictor of tsTGF- β 1 ($P = 0.001$). The tsTGF- β 1 levels were elevated in *FBN1* and *NOTCH1* mutations vs patients without mutations (both $P = 0.004$), and in *NOTCH1* mutations vs *ACTA2/MYH11* mutations ($P = 0.015$). Similarly, tsTGF- β 1 levels were elevated in MFS ($P = 0.003$) and in BAVD ($P = 0.006$) vs patients without genetic aortic syndrome. In contrast to specific clinical features of MFS, *FBN1* in-frame mutations ($P = 0.019$) were associated with increased tsTGF- β 1 levels.

Conclusions: tsTGF- β 1 is elevated in the entire spectrum of genetic aortic syndromes. However, gradual differences in the increases of tsTGF- β 1 levels may mirror different degrees of alteration of tsTGF- β 1 signaling in different genetic aortic syndromes.

Introduction

Marfan syndrome (MFS) is an autosomal-dominant inherited disease of connective tissue caused by mutations of the gene fibrillin-1 (*FBN1*), which encodes fibrillin-1 monomers of extracellular microfibrils.¹ The diagnosis of MFS is based on criteria of the revised Ghent nosology.² Intact fibrillin-1

controls the bioavailability of transforming growth factor-beta 1 (TGF- β 1) through binding of the large latent complex in the extracellular matrix. Failure of defective fibrillin-1 to bind latent TGF-beta-binding protein (LTBP)³ results in increased TGF- β 1 activity, which was shown in both tissue and serum of mice with MFS.⁴ Patients with MFS also exhibited increased total TGF-beta1 levels in serum (tsTGF- β 1),⁵⁻⁹ and their successful treatment of aortic aneurysm with beta-adrenergic blockers (BAB), angiotensin receptor blockers (ARB), or angiotensin-converting enzyme inhibitors (ACEi) was shown to result in commensurate decreases of tsTGF- β 1.⁵⁻⁹ These data nourish hope that tsTGF- β 1 may serve as a biomarker of MFS.¹⁰ Interestingly,

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The authors have no funding, financial relationships, or conflicts of interest to disclose.

Additional Supporting Information may be found in the online version of this article.

TGF- β 1 signaling is also involved in other genetic aortic syndromes (GAS), such as Loeys-Dietz syndrome (LDS) caused by mutations of *TGFBR1*, *TGFBR2*, or *TGFB2*,^{11,12} isolated thoracic aortic aneurysm and dissection (TAAD) caused by mutations in *ACTA2*, *SMAD3*, or *MYH11*,¹³ and bicuspid aortic valve disease (BAVD), which in rare cases is caused by mutations in the *NOTCH1* gene.¹⁴ Therefore, we hypothesized that tsTGF- β 1 was elevated in the entire spectrum of GAS comprising TAAD, LDS, MFS, and BAVD as compared to patients without GAS.

Methods

Patients

Between March 1, 2010 and February 28, 2013, we obtained blood samples to measure tsTGF- β 1 in 351 consecutive patients age >16 years who we evaluated for suspected or known GAS. We excluded 17 patients in whom we diagnosed genetic syndromes other than TAAD, LDS, MFS, and BAVD, and another 17 patients who did not complete clinical and molecular diagnostic workup. Thus, 317 patients constituted our study group. All patients had complete assessment of manifestations of MFS,¹⁵ and LDS,^{16,17} including sequencing of *FBN1*, *TGFBR1*, and *TGFBR2*. We additionally sequenced *ACTA2*, *COL3A1*, *MYH11*, *NOTCH1*, *SLC2A10*, *SMAD3*, and *TGFB2* genes in 22 patients with clinical features suggestive of mutations of the respective genes. The study was approved by the Hamburg ethical board.

Enzyme-Linked Immunosorbent Assay for TGF- β 1

We collected venous blood samples in a serum separator tube and allowed samples to clot for 30 minutes at room temperature. For complete release of tsTGF- β 1, we incubated the probes overnight at 2°C to 8°C before centrifuging for 20 minutes at 2000g. We removed the serum and stored it at -80°C. We determined tsTGF- β 1 levels by using the Quantikine Human TGF-beta 1 Immunoassay kit according to the manufacturer's protocol (R&D Systems, Minneapolis, MN; catalog number DB100B). We ran all samples in duplicate. The detection limit of the assay was 4.61 pg/mL.

DNA Analysis

We extracted DNA from ethylenediaminetetraacetic acid blood samples, and we amplified and sequenced the entire coding and flanking intronic sequences of *TGFBR1*, *TGFBR2*, and *FBN1* in all 317 patients, and the *ACTA2*, *COL3A1*, *MYH11*, *NOTCH1*, *SLC2A10*, *SMAD3*, and *TGFB2* genes in 22 patients as detailed previously.^{11,18,19} To detect possible (partial) gene deletions or duplications, we used multiplex ligation-dependent probe amplification analysis as described previously.^{11,18,19} All 123 *FBN1* mutations fulfilled criteria for causality (Table 1).²

Final Diagnoses

We diagnosed MFS with a causative *FBN1* mutation, aortic root dilatation ≥ 2 Z-scores, ectopia lentis, a systematic score ≥ 7 points or a family history of MFS using Ghent criteria,²

LDS with a *TGFBR1* or *TGFBR2* mutation and ≥ 1 clinical manifestation of LDS, TAAD according to ACMG,²⁰ and BAVD with a bicuspid aortic valve on echocardiography and exclusion of MFS and LDS. We designated the presence of any of these syndromes as GAS and absence of these syndromes as absence of GAS.

Clinical Variables

We employed standardized history, physical examination, transthoracic echocardiography and magnetic resonance imaging (MRI) to assess independent variables at the time of measuring tsTGF- β 1. We present Ghent systemic score points separately as skeletal scores and as nonskeletal scores, which comprised all nonskeletal features from the list of systemic Ghent features. We used stringent definitions for all clinical variables^{19,21–23} and medications²⁴ as described in the footnotes of Table 2 and Table 3, and as detailed previously.¹⁹ A list of detailed methods has been reviewed and will be made available upon request.

Genetic Variables

To assess *FBN1* mutation characteristics we compared both premature termination codon (PTC) mutations, and splicing mutations vs all other mutations. We classified mutations as follows: elimination or creation of a cysteine, localization in a calcium-binding epidermal growth factor-like domain, in an LTBP-like binding domain, or in the so-called neonatal region spanning exons 24 to 32, where we counted only coding exons. We compared any exon mutation with the respective characteristic vs all other exon mutations, and then we compared only missense mutations with the respective characteristic vs all other missense mutations (Table 1).¹

Data Analysis

We expressed quantitative data as means and qualitative data as numbers. The Kolmogorov-Smirnov test rejected normal distribution of tsTGF- β 1 values in our 317 patients ($P < 0.001$), and therefore we employed the Kruskal-Wallis as a global test to identify differences between more than 2 groups, using and the Mann-Whitney U test for differences between pairs of groups (Table 2 and Table 4, Figure 1). We employed the Spearman correlation coefficient (ρ) to identify an association of independent variables with tsTGF- β 1 levels (Table 3 and Table 1, Figure 1). We performed multivariate testing of variables with univariate P values < 0.05 using multiple linear regression analysis with backward elimination, where variables with $P > 0.1$ were removed from the model. We considered P values < 0.05 as significant, and we used SPSS software (SPSS for Windows, version 17.0; SPSS Inc., Chicago, IL) for statistical analyses.

Results

Baseline Characteristics

We measured tsTGF- β 1 levels in 167 men and 150 women at a mean age of 43 ± 14 years (range, 17–75 years). Of these, 158 patients had a causative mutation (49.8%), 198 had GAS (62.5%), 157 had previously undergone cardiovascular

Table 1. Correlation of Mutation Characteristics With tsTGF- β 1 Levels (ng/mL) in 123 Patients With Causative *FBN1* Mutation

Variable	<i>FBN1</i> Characteristic		Spearman Correlation Coefficient (ρ)	<i>P</i>
	Absent	Present		
PTC mutations	42	37	-0.314	0.005
Splicing mutations	109	14	0.026	0.773
Any mutation affecting Cys	70	39	0.064	0.511
Missense mutation affecting Cys	30	39	-0.025	0.839
Any mutation in cbEGF	98	11	0.003	0.976
Missense mutation in cbEGF	58	11	-0.038	0.758
Any mutation in LTBP-bd	106	3	0.002	0.985
Missense mutation in LTBP-bd	67	2	0.121	0.320
Any mutation in exons 24-32	93	16	-0.023	0.815
Missense mutation in exons 24-32	59	10	0.054	0.661

Abbreviations: cbEGF, calcium-binding epidermal growth factor; Cys, cysteine; LTBP-bd, latent transforming growth factor- β -binding protein; PTC, premature termination codon; tsTGF- β 1, total serum transforming growth factor-beta 1.
Criteria of causality of the mutation were amino acid substitutions affecting a cysteine residue in 40, in-frame and out-of-frame deletions/duplications/insertions in 22, other missense mutations in 18, nonsense mutations in 17, splice-site alterations in 14, and mutations in a highly conserved residue in an epidermal growth factor-like domain in 12 patients.

surgery (49.5%) including aortic surgery or intervention in 142 (44.8%), and 57 patients had a history of aortic dissection (18%), with an intimal flap still present on current MRI in 51 patients (16.1%; Table 2).

The tsTGF- β 1 Levels According to Patient Characteristics

The tsTGF- β 1 levels were increased with both presence of a causative mutation ($P=0.008$) and with presence of GAS ($P=0.009$; Table 2). These tsTGF- β 1 levels were elevated in *FBN1* mutations and in *NOTCH1* mutations vs patients without mutations (both $P=0.004$) and in *NOTCH1* mutations versus *ACTA2/MYH11* mutations ($P=0.015$; Figure 1, upper panel). Similarly, tsTGF- β 1 levels were elevated in MFS ($P=0.003$) and in BAVD ($P=0.006$) vs patients without GAS (Figure 1, lower panel). Conversely, previous cardiovascular intervention ($P=0.748$), a history of aortic dissection ($P=0.396$), and a persistent dissecting aortic intimal flap ($P=0.281$) were not correlated with increased tsTGF- β 1 levels (Table 2).

The tsTGF- β 1 Levels in Patients Without GAS

In the 119 patients without GAS, aortic root dilatation was present in 45 (37.8%), previous aortic surgery or intervention in 36 (30.3%), a history with aortic dissection in 18 (15.1%), and a persistent dissecting aortic intimal flap in 17 patients (14.3%). Interestingly, however, none of these features related to increased tsTGF- β 1 serum levels (Table 4).

The tsTGF- β 1 Levels: Univariate Associations

Besides causative gene mutations ($P=0.001$) and GAS ($P<0.001$), only sporadic occurrence of GAS versus familial occurrence of GAS ($P=0.048$) presented as an additional clinical feature with relationship to increased tsTGF- β 1. All other features did not relate to tsTGF- β 1 levels (Table 3).

The tsTGF- β 1 Levels: Multivariate Testing

Because sporadic occurrence of GAS was exclusively assessed in 198 patients with GAS, we analyzed those 3 variables with univariate association with tsTGF- β 1 ($P<0.05$) in 2 separate models. In the first model we included the 2 variables of GAS and causative gene mutation and identified GAS (regression coefficient $b=12.497$ with 95% confidence interval [CI]: 5.288 to 19.706; $P=0.001$) as the exclusive predictor of tsTGF- β 1. In the second model we included the 2 variables causative gene mutation and sporadic occurrence of GAS, where a causative gene mutation was excluded ($P=0.654$) with identification of a weak association with sporadic occurrence of GAS ($b=8.495$; 95% CI: -1.102 to 18.092; $P=0.082$).

The tsTGF- β 1 Levels' Association With *FBN1* Mutation Characteristics

In-frame mutations related to higher tsTGF- β 1 levels than PTC mutations in the subgroup 123 patients with causative *FBN1* mutation ($P=0.005$), whereas all other *FBN1* mutation characteristics were unrelated to tsTGF- β 1 levels (Table 1).

Discussion

The major result of this study was corroboration of the hypothesis that GAS was associated with elevated tsTGF- β 1 levels. These tsTGF- β 1 levels were elevated in *FBN1* mutations and in *NOTCH1* mutations vs patients without mutations, and in *NOTCH1* mutations vs *ACTA2/MYH11* mutations. Similarly, tsTGF- β 1 levels were elevated in MFS and in BAVD vs patients without GAS. Besides GAS, only sporadic occurrence of GAS related marginally to increased tsTGF- β 1 levels. Other clinical features including aortic

Table 2. Total TGF-β1 Serum Levels According to Patient Characteristics

Patient Characteristics	No. of Patients ^a	Total TGF-β1 Serum Level, ng/mL		<i>p</i> ^b
		Mean ± SD	Min – Max	
Causative gene mutation				
No causative mutation	159	34.7 ± 29.6	0.01–182	0.008
<i>ACTA2</i> , <i>MYH11</i> ^c	8 (4/4)	39.5 ± 41.1	14.5–139.1	
<i>TGFBR1</i> , <i>TGFBR2</i> , <i>TGFB2</i> ^d	20 (11/9)	41.4 ± 26.7	6.4–102.8	
<i>FBN1</i>	123 (54/69)	45.2 ± 35.1	1.2–249.6	
<i>NOTCH1</i>	7 (0/7)	59 ± 9.8	39.9–69.6	
Final diagnosis				
No genetic aortic syndrome	119	32 ± 26.8	1.5–135.1	0.009
Thoracic aortic aneurysm or dissection	20 (14/6)	39.1 ± 31.6	1.5–139.1	
Loeys-Dietz syndrome	20 (11/9)	41.4 ± 26.7	6.4–102.8	
Marfan syndrome	128 (55/73)	44.4 ± 34.9	1.2–249.6	
Bicuspid aortic valve disease	30 (6/24)	50.8 ± 37.4	0.02–182	
Previous cardiovascular intervention ^e				
None	160 (16.1%)	39.3 ± 32.5	1.2–182	0.748
Aortic intervention	142 (44.8%)	39.7 ± 31.4	1.7–249.6	
Isolated heart valve intervention	15 (4.7%)	46.3 ± 36.1	0.2–110.4	
Aortic dissection				
None	260 (82%)	40.3 ± 30.8	0.2–182	
History of aortic dissection	57 (18%)	38 ± 37.8	2.4–249.6	0.396 ^f
Persistent dissecting aortic intimal flap ^g	51 (16%)	37.6 ± 39.1	2.4–249.6	0.281 ^f
Total	317 (90/108)	39.8 ± 32.1	0.02–249.6	

Abbreviations: Max, maximum; Min, minimum; SD, standard deviation; TGF-β1, transforming growth factor-beta 1.

^aIn parentheses are the number of mutations that occurred in families/number of sporadic mutations. ^bKruskal-Wallis test. ^cSeven *ACTA2* mutations, and 1 *MYH11* mutation. ^dFour *TGFBR1* missense mutations, 15 *TGFBR2* missense mutations, and 1 *TGFB2* mutation. ^eCardiovascular interventions comprised the presence of isolated aortic root replacement including conduit operations and David Procedure in 110 patients, isolated aortic stent-graft implantation in 6, isolated aortic valve surgery in 8, isolated mitral valve surgery in 7, combined aortic root replacement and mitral valve surgery in 10, combined aortic root replacement and stent-graft placement in 10, combined surgical replacement of thoracic descending aorta in 2, and combined replacement of the aortic root and of thoracic descending aorta in 4 patients. ^fThe Mann-Whitney *U* test identified no differences of tsTGF-β1 levels according to presence or absence of both history of aortic dissection and presence of a persistent dissecting aortic intimal flap. ^gDemonstration of a residual intimal flap in patients with a history of aortic dissection on magnetic resonance imaging at the time of tsTGF-β1 measurement.

diameters, Z-scores, clinical features of MFS, or aortic dissection were unrelated to tsTGF-β1 levels.

GAS exhibited increased tsTGF-β1 levels as compared to patients without GAS. Thus, increased tsTGF-β1 may generally indicate presence of GAS rather than a single disease entity such as MFS. Gillis et al reviewed the complex metabolism of GAS where they argued that TGF-β1 dysregulation was the common pathogenetic mechanism of all types of GAS.¹⁴ Therefore, our findings support the view of GAS as a TGF-β1-related disease.

However, our results exhibited gradual differences of tsTGF-β1 elevations across different GAS, where the highest levels were present in BAVD and the lowest levels in TAAD. These differences were significant between tsTGF-β1 in

NOTCH1 mutations vs *ACTA2/MYH11* mutations. This finding fits into the current understanding of different mechanisms of aneurysm formation in different gens.¹⁴ *NOTCH1* mutations cause BAVD, and relate to both alteration of Notch signaling in genetic aortic aneurysms²⁵ and to increased TGF-β1 signaling.²⁶ Conversely, *ACTA2/MYH11* mutations affect the contractile apparatus of the vascular smooth muscle cells rather than TGF-β1 signaling. Therefore, lower TGF-β1 levels may be expected from the underlying major pathomechanism despite some evidence for upregulation of TGF-β1 signaling in *ACTA2* and *MYH11* mutations with TAAD.²⁷ Finally, our finding of increased tsTGF-β1 in LDS is novel and supports LDS as a disease with upregulation of TGF-β1 signaling.¹⁴

Table 3. Correlation of Clinical Features With tsTGF- β 1 Levels (ng/mL) in 317 Patients

Variable	Spearman Correlation Coefficient (ρ)	P
Age, y	-0.080	0.153
Male	-0.001	0.992
Body weight (kg)	0.005	0.929
Body height (m)	0.029	0.608
Body surface area (m ²) ^a	0.013	0.825
Total cholesterol (mg/dL)	-0.045	0.444
High-density lipoprotein cholesterol (mg/dL)	0.040	0.500
Low-density lipoprotein cholesterol (mg/dL)	-0.022	0.711
Current smoking	0.018	0.743
β -Adrenergic blocker	-0.034	0.547
Angiotensin receptor blocker	0.072	0.203
ACEi	0.026	0.650
Any medication (BAB, ARB, ACEi) ^b	0.067	0.235
Systolic blood pressure (mm Hg)	0.059	0.330
Diastolic blood pressure (mm Hg)	0.077	0.202
Aortic root diameter (cm)	0.030	0.688
Aortic root Z-score ^c	0.084	0.260
LV ejection fraction (%) ^d	0.048	0.564
Indexed end-systolic LV diameter (mm/m ²) ^d	0.016	0.861
Indexed end-diastolic LV diameter (mm/m ²) ^d	0.067	0.372
Indexed left-atrial diameter (mm/m ²) ^d	0.021	0.797
Aorta ascendens diameter (cm) ^e	-0.102	0.086
Aorta descendens diameter (cm) ^e	-0.089	0.145
NT-proBNP (pg/mL) ^f	-0.028	0.622
Sporadic occurrence of syndrome ^g	0.141	0.048
Aortic root dilatation ^h	0.059	0.296
Ectopia lentis	-0.012	0.837
Dural ectasia (Habermann)	0.080	0.159
Mitral valve prolapse	0.054	0.335
History of recurrent hernia	0.110	0.051
Skeletal score (points) ⁱ	0.029	0.612
Nonskeletal score (points) ⁱ	0.063	0.264
Systemic total score points ^j	0.046	0.418

Table 3. Continued

Variable	Spearman Correlation Coefficient (ρ)	P
Previous cardiovascular intervention	0.040	0.473
History of aortic dissection	-0.048	0.397
Persistent dissecting aortic intimal flap	-0.061	0.282
Causative gene mutation	0.180	0.001
Genetic aortic syndrome	0.198	<0.001

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BAB, β -adrenergic receptor blocker, LV, left ventricle; NT-proBNP, N-terminal pro-brain natriuretic peptide serum levels; tsTGF- β 1, total serum transforming growth factor-beta 1. ^aDu Bois and Du Bois.²¹ ^bA total of 154 patients were on any medication at the time of TGF- β 1 measurement including isolated or combined intake of BAB in 105, ARB in 58, and ACEi in 40 patients as described previously.²⁴ ^cAssessment according to Devereux.²² ^dTwo-dimensional echocardiography according to Simpson's rule and current guidelines.²³ ^eMeasurements were obtained on magnetic resonance imaging at the time of tsTGF- β 1 measurements. ^fElectrochemiluminescence sandwich immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) on an Elecsys System 2010. ^gThis variable was considered only in 198 patients with thoracic aortic aneurysm and dissection, Loeys-Dietz syndrome, Marfan syndrome, and bicuspid aortic valve disease, where we defined sporadic occurrence in all patients with exclusion of diagnostic criteria of his or her genetic aortic syndrome in his or her parent, child, or sibling. ^hAortic root Z-score ≥ 2 or previous aortic root replacement. ⁱAccording to Ghent criteria as detailed previously.²

Table 4. Total TGF β 1 Serum Levels According to Aortic Features in 119 Patients Without Genetic Aortic Syndrome

Aortic Feature	Absence/ Presence	Total TGF- β 1, ng/mL		P ^a
		Absence	Presence	
Aortic root dilatation	74/45	30.6 \pm 28.6	34.4 \pm 23.9	0.144
Previous aortic surgery or intervention	83/36	31.1 \pm 28.9	34.2 \pm 21.3	0.120
History of aortic dissection	101/18	32.4 \pm 27.8	30.1 \pm 21.2	0.959
Persistent dissecting aortic intimal flap	102/17	32.2 \pm 27.7	30.9 \pm 21.6	0.832
Any aortic feature ^b	70/49	31.4 \pm 29.1	32.9 \pm 23.5	0.301

Abbreviations: TGF- β 1, transforming growth factor-beta 1. ^aMann-Whitney U test. ^bAny aortic feature comprised aortic dissection, aortic surgery or aortic intervention, or presence of aortic root dilatation, or combinations.

The tsTGF- β 1 levels were increased in sporadic GAS, which may reflect a generally more severe disease in de novo mutations. Interestingly, we did not identify any other clinical features that related to increased tsTGF- β 1. This argues for GAS as a systemic disease with multifocal rather than organ-specific dysregulation of TGF- β 1.

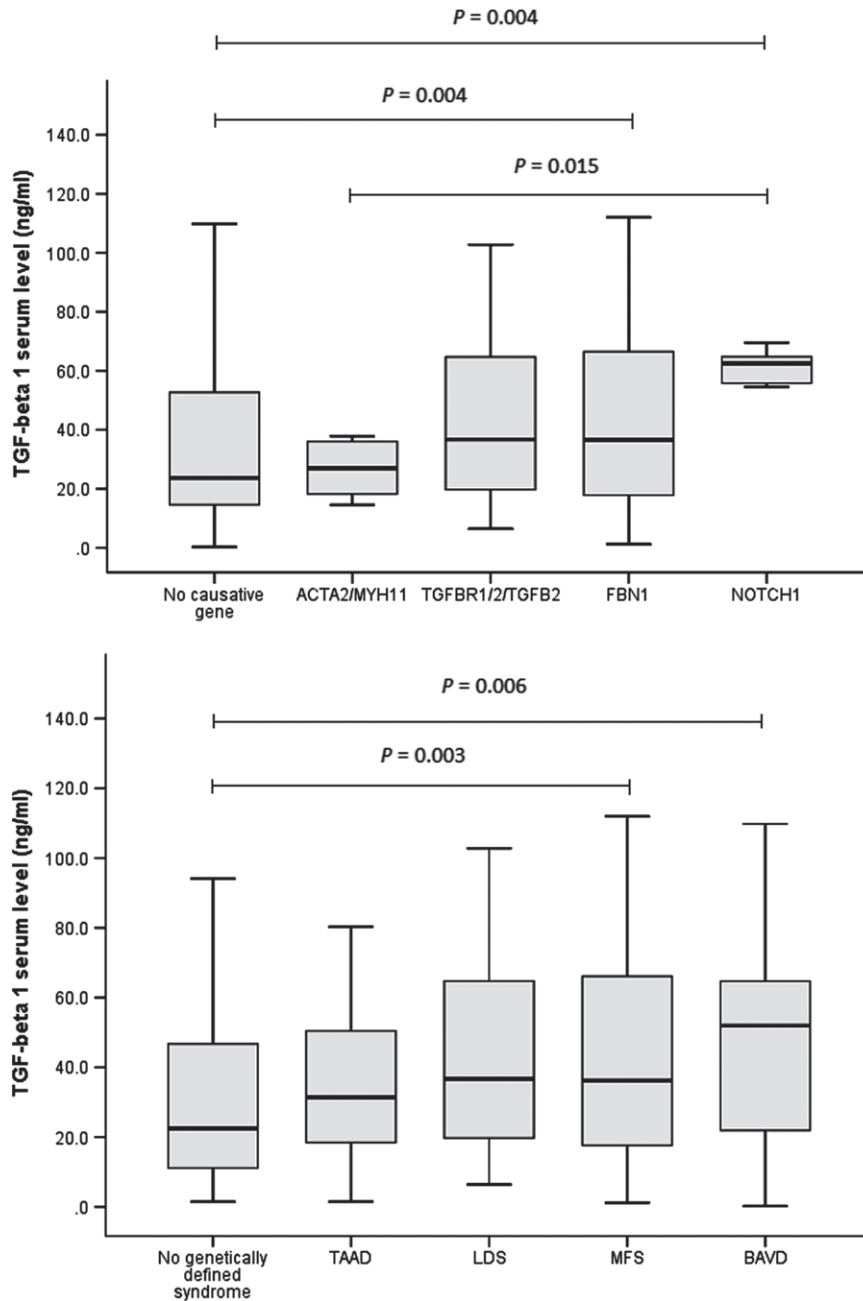


Figure 1. The box and whiskers plots of total serum transforming growth factor- β 1 (tsTGF- β 1) identify higher levels in *FBN1* mutations ($P = 0.004$) and in *NOTCH1* mutations ($P = 0.004$) than in patients with no mutations, and higher levels in *NOTCH1* mutations than in *ACTA2/MYH11* mutations ($P = 0.015$; upper panel). Similarly, tsTGF- β 1 levels are higher in Marfan syndrome (MFS) ($P = 0.003$) and in bicuspid aortic valve disease (BAVD) than in patients with no genetic aortic syndrome ($P = 0.006$; lower panel). Abbreviations: LDS, Loeys-Dietz syndrome; TAAD, thoracic aortic aneurysm and dissection.

Franken et al reported increased tsTGF- β 1 in 28 MFS patients with previous aortic root replacement vs 71 MFS patients without such surgery and a correlation of tsTGF- β 1 with aortic root diameters.⁹ However, in a larger study on tsTGF- β 1 in MFS patients, the GenTAC Consortium did not find correlations between tsTGF- β 1 levels and aortic root diameters or aortic root Z-scores.⁶ Our study extends these GenTAC Consortium findings in MFS to the entire spectrum of GAS, where tsTGF- β 1 levels were unrelated to aortic root replacement, aortic dissection, or aortic root dilatation.

Medications such as BAB, ACEi, or ARB were found to impact tsTGF- β 1 levels in treatment studies of MFS.^{6,9,28} However, our study was not designed to assess medication effects; patients were on various drugs, at different dosages and combinations and types of a BAB, ARB, and ACEi over different time periods, and included treatment of various diseases rather than MFS alone. Therefore, our finding that medications did not relate significantly to tsTGF- β 1 levels does not exclude that a BAB, ARB, or ACEi modulates tsTGF- β 1 signaling in GAS.

Although the mechanisms by which *FBN1* mutations cause increased TGF- β 1 signaling are currently unclear, it has been surmised that domain-specific functions of *FBN1* are needed for its modulation of TGF- β 1 signaling.¹⁴ Our findings support this view, because the relationship of *FBN1* in-frame mutations with higher tsTGF- β 1 levels than in frameshift and PTC mutations suggests that haploinsufficiency of *FBN1* has less impact on TGF- β 1 signaling than expression of mutant *FBN1* proteins. In line with this, specific missense mutations in exons 41 and 42 that encode the TGF- β -binding protein-like domain 5 associate with higher TGF- β levels in acromiolic and geleophysic dysplasias compared with controls.²⁹

Study Limits

Some potential limits of the study require elucidation. The total of 351 persons who we evaluated for GAS were likely to represent the prevalence of GAS in the general Hamburg metropolitan population.³⁰ Because we only excluded a small fraction of patients (34 patients, 10.8%) from this study, our patients were likely to represent GAS in the general Hamburg population without major bias.

We identified significantly higher tsTGF- β 1 levels in patients with GAS as in patients without GAS. Patients without GAS were not healthy, but 49 patients exhibited aortic features including a history of aortic dissection, aortic surgery or intervention, or aortic root dilatation (41.2%; Table 4). However, patients without GAS exhibited mean tsTGF- β 1 levels of 32 ng/mL, which was lower than the normal mean tsTGF- β 1 levels of 39.6 ng/mL provided by the manufacturer of our TGF- β 1 assay.³¹ Thus, the presence of aortic disease alone without a genetic cause does not seem to increase tsTGF- β 1 levels as compared to a normal healthy population.

Similarly, we excluded mutations in *TGFBR1*, *TGFBR2*, *ACTA2*, *NOTCH1*, and *TGFBR2* only in those 22 of 119 patients without GAS who exhibited specific clinical features of syndromes associated with the respective genes. Therefore, we may have missed GAS in some patients despite extensive clinical and molecular diagnostic workup. This may explain higher maximum tsTGF- β 1 levels of 135.1 ng/mL in our patients without GAS as compared to maximum levels of 63.4 ng/mL reported in healthy volunteers.³¹

Moreover, tsTGF- β 1 measurements can be distorted by platelet degranulation. However, we followed the manufacturer's recommendations for elimination of platelets. Comparison with tsTGF- β 1 values from other studies of MFS was not possible, because the methods differed across studies (see Supporting Information, Table, in the online version of this article).

Conclusion

TsTGF- β 1 is elevated in the entire spectrum of GAS. Therefore, dysregulation of TGF- β 1 signaling may be a common pathogenetic mechanism of GAS. However, gradual differences of tsTGF- β 1 with higher levels in *NOTCH1* mutations and BAVD, and lower levels in *ACTA2/MYH11* mutations and TAAD, may mirror different degrees of tsTGF- β 1 signaling alterations in GAS. Because

no single clinical feature related to increased tsTGF- β 1 levels, GAS appears as a systemic disease with multifocal rather than organ-specific dysregulation of TGF- β 1. Finally, tsTGF- β 1 may emerge as a diagnostic marker of GAS. However, larger studies with completely healthy controls are needed to establish diagnostic thresholds of tsTGF- β 1 levels.

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