Aortic dilatation in Marfan syndrome: role of arterial stiffness and fibrillin-1 variants

Paolo Salvi[a], Andrea Grillo[a,b], Susan Marelli[c], Lan Gao[d], Lucia Salvi[e], Maurizio Viecca[c],
Anna Maria Di Blasio[f], Renzo Carretta[g], Alessandro Pini[c], and Gianfranco Parati[a,b]

Objective: Marfan syndrome (MFS) is an autosomal dominant genetic disorder characterized by aortic root dilation and dissection and an abnormal fibrillin-1 synthesis. In this observational study, we evaluated aortic stiffness in MFS and its association with ascending aorta diameters and fibrillin-1 genotype.

Methods: A total of 116 Marfan adult patients without history of cardiovascular surgery, and 144 age, sex, blood pressure and heart rate matched controls were enrolled. All patients underwent arterial stiffness evaluation through carotid–femoral pulse wave velocity (PWV) and central blood pressure waveform analysis (PulsePen tonometer). Fibrillin-1 mutations were classified based on the effect on the protein, into 'dominant negative' and 'haploinsufficient' mutations.

Results: PWV and central pulse pressure were significantly higher in MFS patients than in controls [respectively 7.31 (6.81–7.44) vs. 6.69 (6.52–6.86) m/s, $P = 0.0008$; 41.3 (39.1–43.5) vs. 34.0 (32.7–35.3) mmHg, $P < 0.0001$], with a higher age-related increase of PWV in MFS (β 0.062 vs. 0.036). Pressure amplification was significantly reduced in MFS [18.2 (15.9–20.5) vs. 33.4 (31.6–35.2)%, $P < 0.0001$]. Central pressure profile was altered even in MFS patients without aortic dilatation. Multiple linear regression models showed that PWV independently predicted aortic diameters at the sinuses of Valsalva (ß = 0.243, $P = 0.002$) and at the sinotubular junction (ß = 0.186, $P = 0.048$). PWV was higher in 'dominant negative' than 'haploinsufficient' fibrillin-1 mutations [7.37 (7.04–7.70) vs. 6.60 (5.97–7.23) m/s, $P = 0.035$], although this difference was not significant after adjustment.

Conclusion: Aortic stiffness is increased in MFS, independently from fibrillin-1 genotype and is associated with diameters of ascending aorta. Alterations in central hemodynamics are present even when aortic diameter is within normal limits. Our findings suggest an accelerated arterial aging in MFS.

Keywords: aortic dilatation, arterial stiffness, fibrillin-1, Marfan syndrome, pulse wave velocity

Abbreviations: ARBs, angiotensin receptor blockers; BP, blood pressure; CPP, central pulse pressure; DT, diastolic time; FBN1, fibrillin-1 protein; HR, heart rate; LVET, left ventricular ejection time; MAP, mean arterial pressure; MFS, Marfan syndrome; PPA, pulse pressure amplification; PWV, carotid–femoral pulse wave velocity; SEVR, subendocardial viability ratio

INTRODUCTION

Marfan syndrome (MFS) is an autosomal dominant genetic disorder, characterized by the synthesis of abnormal fibrillin-1 protein (FBN1). FBN1 plays an important role in connective and elastic tissue morphogenesis and *FBN1* gene is involved in more than 95% of cases of MFS [1]. Prognosis in MFS is mainly determined by the progressive dilatation of the aorta, potentially leading to aortic dissection and death at young age. Early detection of aortic dissection risk could radically change the prognosis of MFS patients. Aortic diameter and dilatation rate, measured with transthoracic echocardiography, are actually considered to be the only clinical predictors of aortic dissection risk [2], but their value is limited, as aortic dissection may also occur unexpectedly in nondilated aortas [3] and after prophylactic aortic root surgery [4]. Moreover, it has not been possible so far to obtain a clear risk profile for vascular complications in MFS by means of genotype characteristics [5,6].

In MFS, genetic defects in structural proteins of the arterial wall, as in the FBN1, lead to changes in the elastic properties of the large arteries. A significant alteration in viscoelastic properties of aorta was shown in murine models of MFS, in which the absence of FBN1 leads to enhanced elastolysis in arterial wall [7]. In humans with MFS, a greater rigidity of the large elastic arteries, and particularly of the

1

aorta [8], was demonstrated with nuclear MRI [9] and with echocardiography [10]. More recent studies, focusing on MRI measurements, showed that aortic stiffness [11,12] is also able to predict the development of aortic luminal growth and dilatation, in all segments of aorta. At present, carotid–femoral pulse wave velocity (PWV) is considered the gold-standard method for assessing arterial stiffness [13]. PWV assessment is a noninvasive, highly reproducible and easy-to-use method with a proven predictive power for cardiovascular morbidity and mortality [14]. In daily clinical practice, PWV estimation with arterial tonometry could offer a reliable and repeatable assessment of aortic visco-elastic properties [15]. However, in previous reports, only small populations of MFS patients have been studied with this method [16], focusing on indirect parameters derived from the analysis of pulse waves morphology, as in the assessment of augmentation index [17] or of wave reflections [18].

This cross-sectional study is aimed to evaluate arterial stiffness in a large cohort of MFS patients, either as PWV or other hemodynamic variables derived from arterial tonometry, and to compare these parameters in MFS with the general population. We then considered the association of the examined parameters with validated risk markers for aortic dissection, such as aortic diameters and Z-scores estimated with transthoracic echocardiography. A further aim of the study was to investigate whether arterial stiffness in MFS may be related to a specific FBN1 genotype, in patients with a positive genetic test for FBN1 mutations.

METHODS

Study cohort
In this study, 116 Marfan patients were recruited in a reference center for MFS (Marfan Clinic, Sacco Hospital, Milan, Italy), from March 2014 to April 2015. Diagnosis of MFS was established according to revised Ghent criteria [1]. Exclusion criteria were: age less than 14 years, history of aortic surgery, aortic dissection or aortic aneurysm distal to aortic root. Patients underwent a clinical and dysmorpho-logical evaluation, transthoracic echocardiography and arterial tonometry on the same day. Anthropometric parameters and clinical history were collected during the clinic visit. BSA was calculated with Du Bois formula. Marfan population was divided in age decades starting from 15 years (15–25, 25–35, etc) and all study parameters were analyzed for each decade. The study protocol was approved by our institutional ethics committee and con-formed to the ethical guidelines of the 1975 Declaration of Helsinki. An informed consent was obtained, depending on age, from patients or their parents or legal representatives, before the enrollment in the study.

Control group
Control group was selected from a large database of indi-viduals undergoing applanation tonometry recordings in the frame of a general population. Detailed characteristics of the control population were previously described in parent studies [19,20]. Patients from these cohorts who were receiving any cardiovascular drug were not included in the current analysis. Thus, data analysis focused only on either normotensive patients or untreated patients with mild-to-moderate hypertension, with preserved functional capacity and without previous clinical cardiovascular events or heart failure. Control patients were selected to match Marfan population for age, mean arterial pressure (MAP), heart rate (HR) and sex, according to age decades. The study protocol was approved by our institutional ethics board, and informed consent was obtained, depending on age, from patients or their parents or legal representatives, before trial enrollment.

Echocardiography
A complete echocardiographic study was performed with a full ultrasound system (Philips EnVisor C-HD; Philips Co, Amsterdam, The Netherland). Aortic root diameters were measured according to current guidelines [2]. Aortic meas-urements were obtained in the parasternal long-axis view. The measurements were taken at the aortic valve 'annulus' at the hinge points of the leaflets, at the aortic root at the largest diameter within the sinuses of Valsalva, at the sinotubular junction at the transition point from sinus to tubular aorta, and at the ascending aorta at the level of the right pulmonary artery. All echocardiographic images were acquired and recorded digitally, and analyzed by a single observer, blinded to the clinical conditions of patients. Aortic Z-score was calculated according to rec-ommendations of Marfan foundation with Devereux's for-mula [21]. Aortic Z-score with correction for body height was used in the regression analysis due to its best clinical performance [22].

Pulse wave velocity and pulse wave analysis
PWV was measured by means of PulsePen device (Dia-Tecne, Milan, Italy), a validated and easy-to-use arterial tonometer. The procedure has been described in detail previously [23]. PulsePen tonometer was also used to mea-sure central blood pressure (BP) and to analyze central BP waveform. Central BP values were obtained by calibrating carotid pulse waveform with brachial MAP and diastolic pressure, acquired by a validated oscillometric device (Omron HEM705IT; Omron Corporation, Kyoto, Japan). Central pulse pressure (CPP) was defined as (central sys-tolic BP – diastolic BP). Pulse pressure amplification (PPA) as percentage of (peripheral pulse pressure – CPP)/CPP. PWV percentile for age was calculated based on a large database concerning a reference general population, according to Arterial Stiffness Collaboration percentiles on European population [24]. Other parameters describing the central pulse waveform, such as diastolic time, left ventricular ejection time (LVET) and subendocardial viabil-ity ratio, were computed from the analysis of pulse wave, as described in detail previously [25].

Genetics
Genetic analysis was performed at the Department of Molecular Genetics of the Istituto Auxologico Italiano, Milan, Italy. Mutation screening, with the consent of the patient or a guardian, was performed on genomic DNA extracted from peripheral-blood cells by using a commercial kit (Puregene Blood Core Kit B; Qiagen,

Minneapolis, Minnesota, USA) following manufacturer's instructions. The entire coding region of the *FBN1* gene was screened by direct sequencing. The PCR fragments were sequenced by using the BigDyeTeminator Kit (Applied Biosystem, Foster City, California, USA) and analyzed on the ABI Prism 3500 automated sequencer (Applied Biosystem). According to the international database UMD-FBN1 [26] and Alamut software (Interactive Biosoftware, Rouen, France), the mutations were classified as: previously described mutation, not previously described mutation, surely disease-causing mutation, probably disease-causing mutation or DNA variation of uncertain significance. Mutations were also categorized according to the exon of place in the *FBN1* gene (1–64), and depending on the type of mutation (missense, nonsense, frameshift and splicing). Moreover, effects of the mutations were predicted by Alamut software, to classify pathogenetic FBN1 mutations as 'haploinsufficient' or 'dominant negative'. This approach was validated in a previous study [27]. Mutations were also listed as familiar or 'de novo'.

Statistical analysis

Qualitative variables are expressed in percentage, continuous variables as mean and confidence interval 95%. Qualitative data were compared with Fisher's exact test, continuous variables with *t* test or analysis of variance with adjustment for covariates statistically correlated with the analyzed variable where appropriate. Continuous variables were tested to detect substantial deviations from normality by computing the Kolmogorov–Smirnov Z, and the assumption of satisfactory normal distribution was met for all of the examined variables. Continuous variables were correlated with simple or multiple linear regression. The degree of correlation is expressed with 'R' or 'R^2'. Stepwise backward multiple linear regression models were constructed by using important covariates from correlation analyses to elucidate independent determinants of aortic diameters. In the regression models, anthropometric variables (age, sex, BSA) and hemodynamic variables MAP, HR were considered as explanatory variables of aortic diameters, together with the hemodynamic measurements. LVET was preferred to HR when measures of PWV were inserted in the models, due to the closer relationship of LVET with PWV reported in previous studies [28]. In the stepwise backward multivariate models, 'P'-to-enter was set at 0.1 and the 'P'-to-stay to 0.05. The differences were defined as significant in the presence of P less than 0.05. Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS for Windows, Release 20.0; SPSS, Chicago, Illinois, USA).

RESULTS

The descriptive anthropometric, clinical and echocardiographic characteristics of the study population, according to the presence of aortic dilatation, are shown in Table 1. In our cohort, 71 patients had a significant aortic dilatation defined as aortic Z-score at least 2. Eighty-eight patients (75.8%) were in treatment with angiotensin receptor blockers (ARBs) or ß-blockers for the prevention of aortic dilatation. Eighty-six patients (74.1%) were in therapy with ARBs,

99% of whose were in therapy with Losartan [mean dose: 77.3 ([71.5–83.1) mg; mean dose pro kg of weight: 1.14 (1.05–1.23) mg/kg]. Among ß-blockers (44 patients, 37.9%), atenolol was the most commonly used (34 patients, 29.3%).

Comparison between Marfan and control population

A comparison between MFS patients and controls ($n = 144$) was made for the main hemodynamic variables derived from arterial applanation tonometry (Table 2). MFS showed an increased carotid–femoral PWV compared with controls ($P = 0.0008$). PWV percentile was higher in MFS than controls ($P < 0.0001$). It is worth noting that controls displayed a PWV percentile less than 50th, as they are characterized by BP values lower than the general population (and comparable with MFS cohort). Considering pulse wave analysis variables, MFS had significantly different characteristics of the central pulse wave, with a higher CPP ($P < 0.0001$), a higher systolic mean BP ($P = 0.004$) and a lower diastolic mean BP ($P = 0.02$). A reduced amplification of pulse pressure between central and brachial BP was present in the comparison with controls ($P < 0.0001$). A different trend was observed in PWV correlation with age between MFS ($r = 0.502$, ß $= 0.062$) and controls ($r = 0.456$, ß $= 0.036$), when divided in decades of age (Fig. 1). A sensitivity analysis demonstrated that patients under treatment with ß-blockers ($n = 44$) showed significantly lower values of PPA ($P = 0.001$) and higher values of CPP ($P = 0.041$) than patients receiving a different therapy ($n = 72$, Table in online Supplemental data, http://links.lww.com/HJH/A825), although receiving ß-blockers did not influence the overall results of the study. In fact, even the group not receiving ß-blockers (72 patients) had significantly higher values of PWV ($P = 0.013$) and CPP ($P < 0.001$), and lower values of PPA ($P < 0.001$) when compared with the control group.

Correlation of hemodynamic variables with aortic echocardiographic measurements

In simple linear regression analysis, all of the analyzed hemodynamic variables were significantly related with aortic diameter at the Valsalva sinuses (Table 3). After adjusting for confounders, PWV was the only variable related with aortic diameters at Valsalva sinuses. PWV was also the only variable significantly correlated to the aortic diameter at the sinotubular junction, in simple and adjusted linear regression model (Table 3). No association was found between hemodynamic variables and aortic diameters at aortic annulus and in ascending aorta. PWV percentile was significantly correlated with aortic Z-score, in simple linear regression ($r = 0.192$, $P = 0.039$) and after correction for MAP and LVET ($r = 0.224$, $P = 0.008$).

In stepwise multiple linear regression analysis, PWV was the only hemodynamic variable that significantly predicted aortic diameters, either at Valsalva sinuses or at the sinotubular junction (Table 4). In this analysis, one of the other considered pulse wave analysis variables (CPP, PPA and augmentation index) was also related with aortic diameters or Z-score (Table 4). After correction for confounders, PWV

TABLE 1. Basic characteristics of the Marfan syndrome population

Variables	Total, $n = 116$	Z-score < 2, $n = 45$	Z-score ≥ 2, $n = 71$	P (Z-score < 2 vs. ≥ 2)
Anthropometric characteristics				
Female sex (%)	70 (60%)	29 (64%)	41 (57%)	ns
Age (years)	33.7 (31.2–36.3)	35.3 (30.9–39.7)	32.7 (29.7–35.7)	ns
Height (cm)	178.6 (176.8–180.6)	176.7 (174.3–179.1)	179.8 (176.9–182.7)	ns
BSA (m^2)	1.86 (1.82–1.90)	1.78 (1.29–2.27)	1.90 (1.84–1.96)	0.012
Ghent criteria				
Cardiovascular criterion	98 (85%)	27 (60%)	71 (100%)	<0.0001
Ocular criterion	63 (5%)	28 (62%)	35 (49%)	ns
Family history	82 (71%)	36 (80%)	46 (65%)	ns
FBN1 mutation	93 (80%)	37 (82%)	56 (79%)	ns
Systemic score ≥ 7	104 (90%)	41 (91%)	63 (90%)	ns
Total score	10.1 (9.7–10.5)	10.2 (9.5–10.9)	10.0 (9.4–10.6)	ns
Therapy				
ARBs antagonist	86 (74%)	26 (58%)	60 (85%)	0.001
Beta blockers	44 (38%)	9 (20%)	35 (49%)	0.001
General Marfan features				
Wrist and thumb sign	91 (78%)	35 (78%)	56 (79%)	ns
Severe pectus excavatum	31 (27%)	15 (33%)	16 (23%)	ns
Pectus carinatum	31 (27%)	10 (22%)	21 (30%)	ns
Hindfoot deformity	8 (7%)	5 (11%)	3 (4%)	ns
Pes Planus	76 (66%)	25 (56%)	51 (71%)	ns
Spontaneous pneumothorax	8 (7%)	3 (7%)	5 (7%)	ns
Dural ectasia	69 (60%)	29 (64%)	40 (56%)	ns
Span ratio > 1.05	84 (72%)	33 (73%)	51 (72%)	ns
Scoliosis $> 20°$	103 (89%)	42 (93%)	61 (85%)	ns
Reduced extension of elbows	10 (9%)	5 (11%)	5 (7%)	ns
Facial features	80 (69%)	31 (69%)	49 (69%)	ns
Myopia > 3 diopters	56 (48%)	21 (47%)	35 (49%)	ns
Skin striae	100 (86%)	39 (87%)	61 (86%)	ns
Echocardiographic measurements				
Aortic valve annulus (mm)	22.7 (22.3–23.2)	21.2 (20.6–21.8)	23.7 (23.2–24.2)	<0.0001
Aortic diameter sinuses of Valsalva (mm)	38.2 (37.4–39.0)	34.0 (33.3–34.8)	40.8 (40.0–41.6)	<0.0001
Aortic diameter ST junction (mm)	30.0 (29.1–30.9)	27.3 (26.2–28.2)	31.8 (30.6–33.0)	<0.0001
Aortic diameter ascending aorta (mm)	30.2 (29.3–31.1)	27.6 (26.5–28.7)	31.9 (30.6–33.0)	<0.0001
Aortic Z-score devereux – BSA	2.53 (2.26–2.80)	1.06 (0.84–1.28)	3.47 (3.26–3.68)	<0.0001
Aortic Z-score devereux – height	2.66 (2.33–2.99)	0.80 (0.55–1.05)	3.84 (3.58–4.10)	<0.0001
Mitral valve prolapse	104 (90%)	39 (87%)	65 (92%)	ns
Mitral valve regurgitation	89 (77%)	33 (73%)	56 (79%)	ns
Aortic insufficiency	15 (13%)	8 (18%)	7 (10%)	ns
Ejection fraction (%)	62.3 (61.6–63.0)	62.6 (61.4–63.8)	62.2 (61.5–62.2)	ns

ARBs, angiotensin receptor bockers; FBN1, fibrillin-1 protein.

TABLE 2. Pulse wave analysis parameters of Marfan syndrome patients compared with controls

Variables	Marfan syndrome, $N = 116$	Controls, $N = 144$	P
Age (years)	33.7 (31.1–36.2)	34.3 (32.1–36.4)	ns
Female sex (%)	70 (60.3%)	101 (70.1%)	ns
Carotid–femoral distance (mm)	627.5 (619.3–635.6)	597.5 (590.6–604.3)	<0.0001
Mean BP (mmHg)	77.1 (75.3–78.8)	77.6 (76.7–78.4)	ns
SBP (mmHg)	109 (106.3–111.6)	107.3 (105.9–108.6)	ns
DBP (mmHg)	61.2 (59.5–62.8)	62.7 (61.7–63.6)	ns
Pulse pressure (mmHg)	47.8 (45.7–49.8)	44.6 (43.0–46.1)	0.013
Central SBP (mmHg)	102.5 (99.9–105.0)	96.7 (95.6–97.7)	0.0001
Central pulse pressure (mmHg)	41.3 (39.0–43.5)	34.0 (32.5–35.4)	<0.0001
Heart rate (min^{-1})	63.5 (61.4–65.5)	65.1 (63.6–66.5)	ns
PP amplification	18.2 (15.8–20.5)	33.4 (31.5–35.2)	<0.0001
LVET (ms)	314.2 (309.5–318.8)	308.4 (305.2–311.5)	0.04
DT (ms)	660.9 (634.7–687.0)	632.6 (612.5–652.6)	ns
Ti (ms)	143.2 (134.8–151.5)	161.4 (155.1–167.6)	ns
Augmentation index	1.6 (−1.5–4.71)	1.2 (−1.7–42)	ns
Mean SBP (mmHg)	92.2 (89.9–94.4)	88.9 (87.9–89.8)	0.004
Mean DBP (mmHg)	69.9 (68.2–71.5)	72.0 (71.1–72.8)	0.02
SEVR	1.66 (1.60–1.71)	1.58 (1.53–1.62)	0.04
End-systolic BP (mmHg)	79.6 (77.5–81.6)	81.5 (80.4–82.5)	ns
Carotid–femoral PWV (m/s)	7.31 (6.99–7.62)	6.69 (6.51–6.86)	0.0008
PWV (for age percentile)	57.5 (52.2–62.7)	31.8 (28.8–34.7)	<0.0001

Values are mean and confidence interval 95%. BP, blood pressure; DT, diastolic time; LVET, left ventricular ejection time; PP, pulse pressure; PWV, pulse wave velocity; SEVR, subendocardial viability ratio; Ti, time to inflection point.
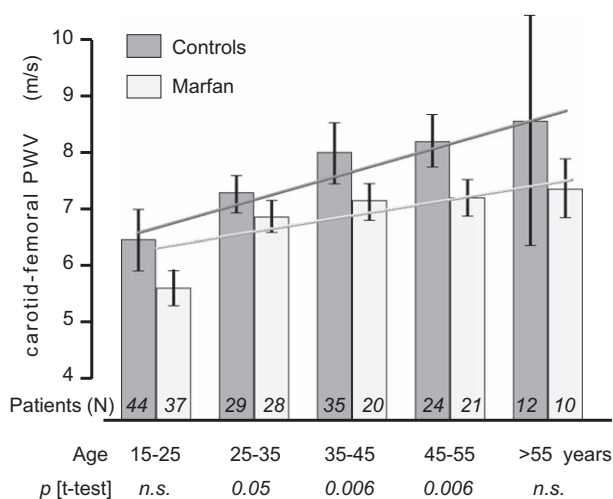
FIGURE 1 Carotid–femoral pulse wave velocity in Marfan syndrome patients and controls, divided by decades of age. Means and confidence intervals 95%.

TABLE 4. Independent predictors of aortic diameters

Aortic diameter	R^2	Predictors	B	ß	P
Annulus	0.266	BSA	3.972	0.356	<0.001
		Gender	−1.153	−0.226	0.021
Valsalva sinuses	0.400	Gender	−3.012	−0.322	<0.001
		BSA	7.691	0.374	<0.001
		PWV	0.653	0.243	0.002
		MAP	−0.082	−0.176	0.025
ST junction	0.321	BSA	7.976	0.351	<0.001
		LVET	0.046	0.229	0.006
		Age	0.074	0.202	0.035
		PWV	0.550	0.186	0.048
Ascending	0.312	BSA	8.337	0.378	<0.001
		Age	0.115	0.310	<0.001
		LVET	0.034	0.174	0.035
Aortic Z-score	0.037	PWV %	0.012	0.192	0.039

Stepwise backward multiple linear regression analysis in Marfan syndrome patients ($n = 116$). Gender: male = 1, female = 2. B, unstandardized regression coefficient; ß, standardized regression coefficient; LVET, left ventricular ejection time; MAP, mean arterial pressure; PWV, carotid-femoral pulse wave velocity; PWV%, carotid–femoral pulse wave velocity for age percentile.

was significantly higher in patients with aortic dilatation, defined as aortic Z-score at least 2 ($P = 0.018$), than patients without a significant dilatation, whereas CPP and PPA were not significantly different between these two groups (Table 5).

We then separately analyzed the subgroup of MFS patients with aortic diameter within limits of normality (Z-score < 2, $n = 45$), by comparing them with a matched control population ($n = 90$, Table 5). Although PWV was not significantly different from controls in the group of MFS patients with aortic diameter within normal limits, CPP was significantly higher ($P = 0.004$) and PPA lower ($P < 0.001$) than matched controls.

Correlation of hemodynamic variables with fibrillin-1 protein genotype

Genetic data were available for 103 patients (88.8%). Either the remaining patients refused to give consent to genetic analysis or to data publication (10 patients), or genetic data analysis was not completed (three patients). A pathogenetic FBN1 mutation was identified in 93 patients (80.1%). Patients with 'missense' or 'splicing' mutations displayed higher PWV values than patients with 'frameshift' mutations [7.37 (6.95–7.78) m/s; 7.29 (6.73–7.85) m/s; 6.01 (4.76–7.27) m/s; $P < 0.05$, Fig. 2]. Among patients with FBN1 mutation, in 89 patients it was possible to define the categorization of FBN1 mutation between 'haploinsufficient' ($n = 21$) and a

'dominant negative' ($n = 68$). Patients with 'dominant negative' mutations had higher PWV values than 'haploinsufficient' patients [7.37 (7.04–7.70) vs. 6.60 (5.97–7.23) m/s, $P = 0.035$]. These differences did not remain significant after adjustment for anthropometric variables, MAP and LVET. No association was found between hemodynamic parameters and clustered exons of FBN1 mutations [exons 24–32 (10 patients) vs. other exons].

DISCUSSION

Our study provides clear evidence that in MFS patients, aortic stiffness is significantly increased when compared with matched controls, by using for the first time a recommended noninvasive methodology (arterial applanation tonometry) in a large population. PWV showed a higher increase rate in the MFS than in the control population, suggesting an accelerated arterial aging, and emerged as an independent predictor of aortic diameter at the sinuses of Valsalva and at the sinotubular junction. Central pressure parameters (CPP and PPA) were significantly different from controls even in patients with aortic diameter within normal limits (aortic Z-score < 2). PWV was higher in MFS patients with 'dominant negative' than in patients with 'haploinsufficient' mutations in fibrillin-1 gene, although this difference did not remain significant after adjustment for confounders.

TABLE 3. Relationships between hemodynamic variables and aortic diameters

	Aortic diameter Valsalva sinuses				Aortic diameter ST junction			
$N = 116$	Adjusted data for age, sex, BSA, MAP, HR				Adjusted data for age, sex, BSA, MAP, HR			
Variables	Univariate R	P	Partial R	P	Univariate R	P	Partial R	P
Carotid-femoral PWV	0.174	0.031	0.244	0.005	0.288	0.002	0.222	0.010
Central pulse pressure	0.212	0.011	−0.011	0.453	0.084	0.187	−0.058	0.272
Augmentation Index	−0.201	0.015	−0.009	0.462	0.079	0.201	0.084	0.192
Pulse Pressure Amplification	−0.187	0.022	0.026	0.394	−0.018	0.425	0.081	0.200

Bivariate correlations between hemodynamic variables and aortic diameters at Valsalva sinuses and at sinotubular junction in Marfan syndrome patients. HR, heart rate; MAP, mean arterial pressure; PWV, pulse wave velocity.

Variables	MFS Z-score < 2, n = 45	Controls (matched for group Z-score < 2), n = 90	MFS Z-score ≥ 2, n = 71	P (Z-score < 2 vs. controls)	P* (Z-score < 2 vs. ≥2)
Age (years)	35.3 (30.9–39.7)	35.3 (32.2–38.3)	32.7 (29.7–35.7)	ns	ns
Female sex (%)	29 (64%)	60 (66%)	41 (57%)	ns	ns
Mean arterial pressure (mmHg)	77.8 (75.1–80.4)	77.8 (76.6–78.9)	76.7 (74.2–79.2)	ns	ns
Heart rate (min^{-1})	63.4 (60.4–66.1)	63.6 (61.9–65.3)	63.5 (60.6–66.4)	ns	ns
Hemodynamic variables					
Central pulse pressure (mmHg)	40.3 (36.5–44.1)	34.1 (32.5–35.7)	41.9 (39.1–44.7)	0.004	ns*
Pulse pressure amplification	19.7 (15.8–23.6)	32.7 (30.6–34.8)	17.3 (14.3–20.4)	<0.001	ns*
Carotid-femoral PWV (m/s)	7.05 (6.57–7.52)	6.69 (6.46–6.91)	7.48 (7.06–7.90)	ns	0.018*

Values are mean and confidence interval 95%. MFS, Marfan syndrome; PWV, pulse wave velocity.
*P corrected for age, sex, BSA, mean arterial pressure and left ventricular ejection time.

Differently from the previous studies reporting an increase in aortic stiffness in MFS, which applied MRI [9] or echocardiographic methods [10,29], we performed for the first time a detailed analysis of aortic pressure wave variables in a large group of MFS patients with arterial applanation tonometry, a noninvasive and easy-to-use methodology. Not only PWV was higher in MFS than in the control population for all age groups, but also the average age-normalized PWV percentile was higher. We should underline that the control population displayed a reduced PWV percentile due to the occurrence of BP values lower than the median values found in a general population, and comparable with BP values of MFS patients.

Analysis of central pressure wave also showed substantial differences in MFS. The central-to-periphery pressure amplification phenomenon was significantly reduced and central BP values were significantly higher than in a matched control population. The reduced amplification of pulse pressure is present in MFS despite the increased height and the consequent increased length of the aorta, demonstrated by the increased carotid-to-femoral distance in the comparison with controls, which theoretically would reduce pressure amplification, due to dampened wave reflections [30]. Significantly, even in patients without a significant aortic dilatation (Z-score < 2), CPP and PPA were significantly different from the control population, suggesting that these variables derived from the central BP profile are able to identify the initial hemodynamic abnormalities present in MFS, before an aortic involvement becomes evident. Although a close relationship between CPP and aortic diameters was reported in a previous study [14], our article is the first showing that the alterations in central pressure are present even when aortic diameters do not exceed the limits of normality. This finding suggests that the central, more than the peripheral, BP values may constitute a hemodynamic determinant of aortic dilatation in MFS.

It is interesting to note that the increased rate of PWV with age was higher in MFS than in controls. It appears that in MFS, aortic stiffness is just above normal levels in younger age, and becomes increasingly different from the reference population as affected patients become older. A role of FBN1 in the aging process of arteries and in arterial stiffening was already demonstrated in the general population [31], confirming that FBN1 contributes to determining the elastic properties of arteries. The FBN1 network is connected to the elastin and collagen matrix to limit excessive stretch during the cardiac cycle. Data from murine models of MFS (mgR/mgR mouse) [7] suggest that fragmentation of the medial elastic network occurs later in life when lamellar structure is already established. It is possible that MFS mutations in FBN1 contribute to alter arterial wall during lifetime, and produce a time-dependent stiffening, leading to large arterial dilatations and dissections. Our results are compatible with a more pronounced and progressive arterial aging in MFS, confirming previous findings from animal studies in the mgR/mgR mouse [32].

In this context, the explanation of the difference in PWV between MFS patients with haploinsufficient and dominant negative FBN1 mutations is not straightforward. Although the presence of different baseline cofactors between the groups may have influenced the data, the effect of FBN1 mutations could contribute to explain the observed difference. Haploinsufficient patients produce a reduced amount of functionally normal FBN1, whereas dominant negative patients have a dysfunctional FBN1 protein, produced by a broad spectrum of genetic alterations. It is possible that a thinner, but with normal viscoelastic properties, arterial wall is present with haploinsufficient mutations, leading
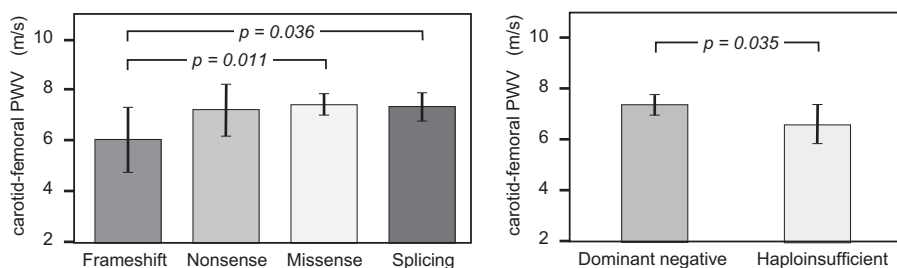

FIGURE 2 Carotid–femoral pulse wave velocity according to fibrillin-1 mutation type in Marfan syndrome patients. Mean and confidence interval 95%.

to a significantly lower PWV. Recent prospective studies [33] suggest a significant impact of pathogenic FBN1 mutations on cardiovascular phenotype severity, with haploinsufficient mutations being associated with an increased risk of aortic dissection and death. Although the observed differences in hemodynamic parameters between different FBN1 variants were NS in the model adjusted for covariates, we believe that the relationship between PWV and FBN1 genotype needs to be further elucidated in future studies and could open new perspectives in the understanding of the pathophysiology of vascular damage in MFS. It should be acknowledged that our study was not specifically designed to find a difference between these two groups, and a larger sample size is probably needed for this purpose.

In our study, aortic stiffness in MFS appears to be not only increased, but also related to aortic diameters at the Valsalva sinuses and at the sinotubular junction, which are considered at present the most reliable markers of aortic dissection risk in MFS [2]. Previous studies have tried to relate aortic elastic properties with aortic diameters and dissection risk. In MRI studies, local aortic distensibility seems to independently predict progressive aortic dilatation [11], and regional PWV estimation has a good specificity in predicting absence of regional luminal aortic growth [12]. Also wave reflections, measured as heart-rate adjusted augmentation index, were found to independently impact aortic disease progression [17]. Our study, by considering a larger sample than previous reports, confirms that the evaluation of aortic viscoelastic properties could have a role in the cardiovascular stratification of MFS patients. Our study also clearly supports the suggestion that applanation tonometry, a reliable and affordable methodology, validated as the gold-standard method for arterial stiffness measurement [13], is a suitable choice to assess aortic properties in MFS.

Among the measured arterial stiffness-related hemodynamic parameters, the only one that was independently associated with aortic diameters and with aortic dilatation, after adjustment for multiple confounders, was PWV. Arterial tonometry-derived PWV percentile, the age-normalized value of PWV, being in direct relation with aortic Z-score could thus be a useful clinical parameter for cardiovascular evaluation in MFS. However, considering the cross-sectional design of our study, the predictive ability of PWV for the risk of aortic dilatation or dissection cannot be definitely determined. Further longitudinal studies are therefore needed to validate arterial tonometry and in particular, PWV for the evaluation of aortic dissection risk in MFS.

Limitations of our study should be acknowledged. First, a large majority of MFS patients were using a therapy for aortic dilatation prevention (ARBs or ß-blockers). Given that antihypertensive drugs like ARBs or ß blockers have been shown to *reduce* PWV [34], the increase in PWV due to MFS could hypothetically be even more pronounced, with greater differences as compared with control patients and a tighter relationship with aortic dilatation, in the absence of antihypertensive therapies. It is also possible that therapy with ARBs may have influenced the observed difference in PWV between dominant negative and haploinsufficient FBN1 mutations, on the grounds of the demonstration by

recent studies that losartan is more beneficial in MFS with haploinsufficient mutation [27]. Thus, a more effective reduction in PWV may have been produced by ARBs in this group of patients. The possible effects of treatment with ß-blockers should also be acknowledged, given the relevant effect of this drug class on pressure amplification [35], although in our study this type of therapy should not be considered as the most relevant factor influencing central pressure in MFS. In the subgroup analysis of patients not taking ß-blockers, the differences with controls in CPP, PPA and PWV remained significant, suggesting that the alteration of these parameters in MFS is not determined by ß-blocker therapy, but rather by the syndrome itself.

Another limitation in our study is the survival selection bias. It is possible that MFS patients included in our cohort are the patients with milder and slower progressive cardiovascular manifestation, as patients undergoing aortic surgery in earlier life were systematically excluded from this study. It is not possible on the basis of our data to understand if patients undergoing aortic surgery in earlier life could have different aortic viscoelastic properties from other MFS patients. Only studies considering patients at younger ages (including children and/or adolescents) and long follow-up could answer this question.

The current study shows that aortic stiffness is increased in patients affected by MFS and that aortic PWV is independently associated with diameters of aorta at the sinuses of Valsalva and at the sinotubular junction, which are markers of aortic dissection risk. For the first time, a recommended and noninvasive methodology for the evaluation of arterial stiffness (arterial applanation tonometry) was used in a large cohort of MFS patients. The discovery of steeper increase of PWV with age in MFS than in the general population suggests an accelerated arterial aging in this syndrome. PWV was increased in MFS independently from fibrillin-1 genotype, a result that, in the light of recent studies suggesting a significant impact of genotype on cardiovascular phenotype severity [33], could help to understand the pathophysiology of vascular damage in this syndrome. Further longitudinal studies are needed to evaluate the diagnostic and prognostic ability of arterial stiffness parameters derived from arterial tonometry for the assessment of aortic dissection risk in MFS, beyond echocardiographic risk markers.

REFERENCES
1. Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, Devereux RB, *et al*. The revised Ghent nosology for the Marfan syndrome. *J Med Genet* 2010; 47:476–485.
2. Baumgartner H, Bonhoeffer P, De Groot NM, de Haan F, Deanfield JE, Galie N, *et al*. ESC Guidelines for the management of grown-up congenital heart disease (new version 2010). *Eur Heart J* 2010; 31:2915–2957.
3. Groenink M, Lohuis TA, Tijssen JG, Naeff MS, Hennekam RC, van der Wall EE, *et al*. Survival and complication free survival in Marfan's syndrome: implications of current guidelines. *Heart* 1999; 82:499–504.

4. Finkbohner R, Johnston D, Crawford ES, Coselli J, Milewicz DM. Marfan syndrome. Long-term survival and complications after aortic aneurysm repair. *Circulation* 1995; 91:728–733.

5. Faivre L, Collod-Beroud G, Loeys BL, Child A, Binquet C, Gautier E, *et al*. Effect of mutation type and location on clinical outcome in 1013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. *Am J Hum Genet* 2007; 81:454–466.

6. Dietz HC. Potential phenotype-genotype correlation in marfan syndrome: when less is more? *Circ Cardiovasc Genet* 2015; 8:256–260.

7. Marque V, Kieffer P, Gayraud B, Lartaud-Idjouadiene I, Ramirez F, Atkinson J. Aortic wall mechanics and composition in a transgenic mouse model of Marfan syndrome. *Arterioscl Throm Vas* 2001; 21:1184–1189.

8. Jondeau G, Boutouyrie P, Lacolley P, Laloux B, Dubourg O, Bourdarias JP, *et al*. Central pulse pressure is a major determinant of ascending aorta dilation in Marfan syndrome. *Circulation* 1999; 99:2677–2681.

9. Groenink M, de Roos A, Mulder BJ, Verbeeten B Jr, Timmermans J, Zwinderman AH, *et al*. Biophysical properties of the normal-sized aorta in patients with Marfan syndrome: evaluation with MR flow mapping. *Radiology* 2001; 219:535–540.

10. Vitarelli A, Conde Y, Cimino E, D'Angeli I, D'Orazio S, Stellato S, *et al*. Aortic wall mechanics in the Marfan syndrome assessed by transesophageal tissue Doppler echocardiography. *Am J Cardiol* 2006; 97:571–577.

11. Nollen GJ, Groenink M, Tijssen JG, Van Der Wall EE, Mulder BJ. Aortic stiffness and diameter predict progressive aortic dilatation in patients with Marfan syndrome. *Eur Heart J* 2004; 25:1146–1152.

12. Kroner ES, Scholte AJ, de Koning PJ, van den Boogaard PJ, Kroft LJ, van der Geest RJ, *et al*. MRI-assessed regional pulse wave velocity for predicting absence of regional aorta luminal growth in marfan syndrome. *Int J Cardiol* 2013; 167:2977–2982.

13. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, *et al*. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; 27:2588–2605.

14. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, *et al*. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; 37: 1236–1241.

15. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, *et al*. Recommendations for improving and standardizing vascular research on arterial stiffness a scientific statement from the American Heart Association. *Hypertension* 2015; 66:698–722.

16. Payne RA, Hilling-Smith RC, Webb DJ, Maxwell SR, Denvir MA. Augmentation index assessed by applanation tonometry is elevated in Marfan Syndrome. *J Cardiothorac Surg* 2007; 2:43.

17. Mortensen K, Aydin MA, Rybczynski M, Baulmann J, Schahidi NA, Kean G, *et al*. Augmentation index relates to progression of aortic disease in adults with Marfan syndrome. *Am J Hypertens* 2009; 22:971–979.

18. Segers P, De Backer J, Devos D, Rabben SI, Gillebert TC, Van Bortel LM, *et al*. Aortic reflection coefficients and their association with global indexes of wave reflection in healthy controls and patients with Marfan's syndrome. *Am J Physiol Heart Circ Physiol* 2006; 290: H2385–H2392.

19. Salvi P, Meriem C, Temmar M, Marino F, Sari-Ahmed M, Labat C, *et al*. Association of current weight and birth weight with blood pressure levels in Saharan and European teenager populations. *Am J Hypertens* 2010; 23:379–386.

20. Alecu C, Labat C, Kearney-Schwartz A, Fay R, Salvi P, Joly L, *et al*. Reference values of aortic pulse wave velocity in the elderly. *J Hypertens* 2008; 26:2207–2212.

21. Devereux RB, de Simone G, Arnett DK, Best LG, Boerwinkle E, Howard BV, *et al*. Normal limits in relation to age, body size and gender of two-dimensional echocardiographic aortic root dimensions in persons ≥15 years of age. *Am J Cardiol* 2012; 110:1189–1194.

22. van Kimmenade RR, Kempers M, de Boer MJ, Loeys BL, Timmermans J. A clinical appraisal of different *Z*-score equations for aortic root assessment in the diagnostic evaluation of Marfan syndrome. *Genet Med* 2013; 15:528–532.

23. Salvi P, Lio G, Labat C, Ricci E, Pannier B, Benetos A. Validation of a new noninvasive portable tonometer for determining arterial pressure wave and pulse wave velocity: the PulsePen device. *J Hypertens* 2004; 22:2285–2293.

24. Arterial Stiffness Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J* 2010; 31: 2338–2350.

25. Salvi P, Revera M, Faini A, Giuliano A, Gregorini F, Agostoni P, *et al*. Changes in subendocardial viability ratio with acute high-altitude exposure and protective role of acetazolamide. *Hypertension* 2013; 61:793–799.

26. Collod-Beroud G, Le Bourdelles S, Ades L, Ala-Kokko L, Booms P, Boxer M, *et al*. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. *Hum Mutat* 2003; 22:199–208.

27. Franken R, den Hartog AW, Radonic T, Micha D, Maugeri A, van Dijk FS, *et al*. Beneficial outcome of losartan therapy depends on type of FBN1 mutation in Marfan syndrome. *Circ Cardiovasc Genet* 2015; 8:383–388.

28. Salvi P, Palombo C, Salvi GM, Labat C, Parati G, Benetos A. Left ventricular ejection time, not heart rate, is an independent correlate of aortic pulse wave velocity. *J Appl Physiol* 2013; 115:1610–1617.

29. Hirata K, Triposkiadis F, Sparks E, Bowen J, Wooley CF, Boudoulas H. The Marfan syndrome: abnormal aortic elastic properties. *J Am Coll Cardiol* 1991; 18:57–63.

30. London GM, Guerin AP, Pannier B, Marchais SJ, Stimpel M. Influence of sex on arterial hemodynamics and blood pressure. Role of body height. *Hypertension* 1995; 26:514–519.

31. Powell JT, Turner RJ, Sian M, Debasso R, Lanne T. Influence of fibrillin-1 genotype on the aortic stiffness in men. *J Appl Physiol* 2005; 99:1036–1040.

32. Eberth JF, Taucer AI, Wilson E, Humphrey JD. Mechanics of carotid arteries in a mouse model of Marfan Syndrome. *Ann Biomed Eng* 2009; 37:1093–1104.

33. Franken R, Groenink M, de Waard V, Feenstra HM, Scholte AJ, van den Berg MP, *et al*. Genotype impacts survival in Marfan syndrome. *Eur Heart J* 2016; 37:3285–3290.

34. Ong KT, Delerme S, Pannier B, Safar ME, Benetos A, Laurent S, *et al*. Aortic stiffness is reduced beyond blood pressure lowering by short-term and long-term antihypertensive treatment: a meta-analysis of individual data in 294 patients. *J Hypertens* 2011; 29: 1034–1042.

35. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, *et al*. Differential impact of blood pressure–lowering drugs on central aortic pressure and clinical outcomes. *Circulation* 2006; 113:1213–1225.