

Research Paper

Soluble EMMPRIN levels discriminate aortic ectasia in Marfan syndrome patients

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Abstract

Marfan syndrome (MFS) is a rare genetic disease characterized by a matrix metalloproteases (MMPs) dysregulation that leads to extracellular matrix degradation. Consequently, MFS patients are prone to develop progressive thoracic aortic enlargement and detrimental aneurysm. Since MMPs are activated by the extracellular MMP inducer (EMMPRIN) protein, we determined whether its plasmatic soluble form (sEMMPRIN) may be considered a marker of thoracic aortic ectasia (AE).

Methods: We compared plasma sEMMPRIN levels of 42 adult Caucasian MFS patients not previously subjected to aortic surgery with those of matched healthy controls (HC) by ELISA. In the MFS cohort we prospectively evaluated the relationship between plasma sEMMPRIN levels and the main MFS-related manifestations.

Results: MFS patients had lower plasma sEMMPRIN levels (mean±SD: 2071±637 pg/ml) than HC (2441±642 pg/ml, $p=0.009$). Amongst all considered MFS-related clinical features, we found that only aortic root dilatation associated with circulating sEMMPRIN levels. Specifically, plasma sEMMPRIN levels negatively correlated with aortic Z-score ($r=-0.431$, $p=0.004$), and were significantly lower in patients with AE (Z-score≥2, 1788±510 pg/ml) compared to those without AE (Z-score<2, 2355±634 pg/ml; $p=0.003$). ROC curve analysis revealed that plasma sEMMPRIN levels discriminated patients with AE (AUC [95%CI]: 0.763 [0.610–0.916], $p=0.003$) with 85.7% sensitivity, 76.2% specificity, and 81% accuracy. We defined plasma sEMMPRIN levels ≤2246 pg/ml as the best threshold discriminating the presence of AE in MFS patients with an odds ratio [95%CI] of 19.2 [3.947–93.389] ($p<0.001$).

Conclusions: MFS patients are characterized by lower sEMMPRIN levels than HC. Notably, plasma sEMMPRIN levels are strongly associated with thoracic AE.

Key words: Marfan syndrome; aortic ectasia; EMMPRIN; thoracic aortic aneurysm; Z-score.

Introduction

Marfan syndrome (MFS) is a connective tissue disease with an autosomal dominant inheritance and an estimated incidence of 1:5000 individuals [1]. This condition is caused by mutations in the FBN1 gene encoding for fibrillin-1, an extracellular matrix (ECM)

component that, by forming complex structures called microfibrils, provides elasticity and structural support to tissues [2]. As consequence, this genetic defect leads to pleiotropic manifestations in various body regions such as the skeletal, ocular and cardiovascular

systems [3]. In particular, progressive thoracic aortic aneurysm (TAA) formation and dissection are the leading causes of death in MFS patients [4,5]. For this reason, the efforts of the scientific community are directed towards finding novel therapeutic approaches, as well as early diagnostic tools, to limit MFS aortic dilatation [6].

To date, although the pathways driving aortic disease pathogenesis in MFS have been deeply investigated, a comprehensive molecular mechanism is still missing [7]. Macroscopically, the development of aortic disease in MFS can be explained by simple tissue alterations, due to defective assembly of elastin into elastic fibres. Recent investigations have, however, shown a more complex scenario, with the involvement of transforming growth factor- β (TGF- β) and angiotensin II-dependent pathways [8,9], as well as ECM-dependent alterations [10-12]. The latter involves a group of enzymes called matrix metalloproteases (MMPs) and their inhibitors (tissue inhibitor of metalloproteases, TIMP) that regulate ECM component turnover. In MFS Segura *et al.* established a vicious cycle according to which defects in fibrillin-1 *per se* lead to the up-regulation of MMP synthesis and the accumulation of abnormally aggregated elastin which, in turn, is highly susceptible to be degraded by MMPs [13]. The crucial role of MMPs in MFS was confirmed by treating a mouse model of MFS (Fbn1^{C1039G/+}) with doxycycline which prevents MMP production and secretion, determining a decrease in elastic fibre degeneration, vasomotor function normalization, and inhibition of TGF- β activation [10,14].

The extracellular MMP inducer (EMMPRIN) is a receptor capable of stimulating the production of various MMPs, such as MMP-1, MMP-2, MMP-3, MMP-9, and MMP-11 [15-17]. EMMPRIN is a cell surface transmembrane glycoprotein that, mainly through interacting with cyclophilin A, is involved in several cellular processes including the induction of MMPs, migration, inflammation, and transport of nutrients [18]. These functions provide the molecular basis for the role of EMMPRIN in the pathogenesis of several diseases such as cancer, infectious diseases (e.g. malaria), rheumatoid arthritis, myocardial infarction, multiple sclerosis, and renal fibrosis [17,19-24]. It is important to note that EMMPRIN is present in vascular smooth muscle cells of human aneurysmal aortas [25] and that its expression is induced by angiotensin II and TGF- β administration *in vitro* [25,26].

Emerging evidences indicate the presence of further forms of EMMPRIN in addition to the transmembrane form. Indeed, EMMPRIN has been detected in human serum and plasma where it is

present as a soluble form (sEMMPRIN) derived from the proteolytic cleavage of the transmembrane receptor or from cell secretion *via* microvesicle shedding [27,28]. Currently, it is well known that sEMMPRIN acts in a paracrine fashion to stimulate the production of MMPs [29,30], thereby contributing to numerous diseases. For instance, increased sEMMPRIN levels are present in blood of patients with acute myocardial infarction and stable coronary artery disease, cancer, multiple sclerosis, and systemic sclerosis [31-36].

Considering the importance of MMPs in MFS pathogenesis, we designed this study to investigate whether sEMMPRIN levels are altered in the plasma of MFS patients presenting different clinical profile, thus offering a rationale for being considered a potential diagnostic tool and/or therapeutic target in MFS-associated phenotypes. To this aim, we firstly examined plasma sEMMPRIN levels in MFS patients and compared the results with healthy subjects. Then, we prospectively evaluated the relationship between plasma sEMMPRIN levels and several clinical markers of MFS extent and TAA progression.

Methods

MFS patients

Adult Caucasian subjects (≥ 18 years) affected by MFS and afferent to the Rare Disease Center-Marfan Clinic (ASST Fatebenefratelli-Sacco, Milan, Italy), in which the presence of *FBN1* mutation was confirmed and without previous aortic surgery, have been screened for inclusion in this study (Figure S1). MFS was diagnosed according to the revised Ghent nosology [37]. Criteria determining the exclusion of MFS patients from the enrolment in this study were the presence of acute or chronic inflammatory state, precocious menopause, cardiac rheumatic disease, asthmatic disease, malignant cancer, systemic or multiple sclerosis, chronic hepatic disease and/or chronic renal failure (creatinine > 1.5 mg/dl), as well as the manifestation of acute myocardial infarction and/or coronary artery disease.

Patient characteristics (e.g. sex, age, height, weight) and clinical data concerning MFS (e.g. systemic score, familial history of the disease, presence of skeletal manifestation, ectopia lentis, dural ectasia, mitral valve prolapse, aortic valve regurgitation, and aortic root diameter at the sinus of Valsalva) were collected and included in our patient registry. Treatments with β -blockers (BB) and/or angiotensin II receptor blockers (ARB) were also recorded and considered for the analyses. Additional/alternative hypotensive drug administration (e.g. calcium channel blockers and/or

angiotensin converting enzyme inhibitors) were also recorded. Importantly, we categorized for sex the cohort of 45 patients eligible for the enrolment in this study. On the basis of the Z-scores of the two subgroups, we excluded 3 outlier patients in order to avoid a gender-related Z-score unbalance (Figure S1). The clinical profile of the eligible vs. enrolled patients has been compared and no statistical difference between the two groups has been found (Table S1). The final study population has therefore included 42 patients of which 29 had at least one MFS affected family member known to carry a mutation in *FBN1* and, thus, were screened only for the *FBN1* mutation. The remaining 13 probands without known familiar MFS cases were evaluated according to phenotype by Next Generation Sequencing (NGS) for both *FBN1* and other genes commonly associated with Heritable Thoracic Aortic Disorders including *FBN1*, *TGFBR1*, *TGFBR2*, *MYH11*, *SMAD3*, *ACTA2*, *COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*, *COL5A2* [38].

This study was authorized by the Ethics Committee of the ASST Fatebenefratelli-Sacco (Prot. N° 39138/2016).

Aortic root echocardiographic evaluation

Patient's aortic root dilatation was evaluated by measuring the vessel diameter at the Valsalva sinus level by using the -EnVisor CHD- Ultrasound System (Philips). The two-dimensional echocardiographic measurements were determined during diastole by the leading edge-to-leading edge technique, as previously described in the literature [39]. At least three replications were performed in blind by the same operator to determine the mean value of the aortic root diameter. The relative intra-observer coefficient of variability between triplicates has been calculated to be 0.84%. Aortic valve regurgitation and mitral valve prolapse were also evaluated by echocardiography.

Patients' parameter calculations

Body surface area (BSA) was calculated with the Du Bois and Du Bois' formula [40]:

$$\text{Height (cm)}^{0.725} * \text{Weight (kg)}^{0.425} * 0.007184$$

As an index to evaluate aortic dilatation at the Valsalva sinus level, the Z-score was calculated by Devereux's formula based on the normalization of the aortic diameter measurement for patients' height, since this index was recently reported as the most appropriate method for aortic root assessment in MFS diagnostic evaluation [41]. The Z-score was calculated considering sex covariate value as 1 for males and 2 for females by using the following the equation:

$$(\text{Sinus of Valsava diameter(cm)} - [1.519 + (\text{age} * 0.010) +$$

$$(\text{height} * 0.010) - (\text{gender} * 0.247)]) / 0.215$$

Aortic ectasia (AE) was diagnosed when Z-score was ≥ 2.0 [37].

MFS patients' sample collection

For each patient enrolled in the study, 8 ml of peripheral whole blood was collected using EDTA Vacutainer tubes (BD). Plasma was obtained after centrifuging the whole blood for 10 minutes at 2500 g, at 4°C.

Healthy controls

Plasma samples collected on EDTA from healthy controls (HC) were purchased by NeoBiotech (Nanterre, France). Then HC were matched for sex and age with MFS counterparts. Age matching was computer-generated selecting minimal age difference between HC and MFS. Moreover, to exclude a possible bias, a statistical analysis to assess possible differences between HC and MFS patient age was also performed. The age of HC (median [IQR]: 35 [29-40]) did not result statistically different from that of MFS patients (35 [29-48]; $p=0.336$).

sEMMPRIN evaluation

Plasma sEMMPRIN levels were quantified by a commercial enzyme-linked immunosorbent assay (Human EMMPRIN/CD147 Quantikine ELISA, R&D Systems) following the manufacturer's instructions. The sEMMPRIN concentration in each sample was determined by interpolation of a linear standard curve obtained by plotting the logarithm of human sEMMPRIN concentrations *versus* the logarithm of the absorbance. Each sample was tested in triplicate.

Statistical analyses

Data with Gaussian distribution were expressed as mean \pm standard deviation (SD), while data with non-parametric distribution were expressed as median and IQR. The Fisher's exact test was performed to compare the distribution of categorical variables. To assess the normal distribution of each covariate the D'Agostino-Pearson omnibus K2 and the Shapiro-Wilk normality tests were performed, as appropriate ($>$ and ≤ 20 samples, respectively). T-student and Mann Whitney U tests were performed to compare plasma sEMMPRIN levels between subgroups of patients, as appropriate. One-way ANOVA was performed to evaluate differences among means, and *post-hoc* multiple comparisons were analysed using Bonferroni's test. Two-way ANOVA was performed to evaluate the influence of two independent categorical variables on one dependent variable and their possible interaction. Pearson and Spearman's rank correlation tests were

used to assess the statistical dependence of two parametric or non-parametric variables, respectively. Receiver Operating Characteristic (ROC) curve analysis was performed to quantify how accurately the sEMMPRIN levels could discriminate patients who developed AE. Specifically, the criterion corresponding with the highest Youden's index was used as a cut-off to categorize the sEMMPRIN levels with the highest sum of sensitivity and specificity [42]. Univariable logistic regression analysis was performed to assess the OR [95%CI] of the association between sEMMPRIN levels and AE development after sEMMPRIN level dichotomization performed on the basis of the cut-off value obtained with the ROC curve analysis. The evaluation of age and sex as possible confounding factors in the association between sEMMPRIN levels and AE development was assessed including these covariates as independent variables in a multivariable logistic regression model. Statistical significance was set at p value <0.05 . All the analyses were performed with GraphPad Prism® software (version 5.0) and with MedCalc.

Results

Patients clinical characteristics

Forty two MFS adult patients fulfilled the inclusion criteria and were enrolled for this study. Patients' clinical profiles are summarized in Table 1, while *FBN1* mutations found in the whole cohort are reported in Table S2. All patients were Caucasians and had not been subjected to any previous aortic surgery. The cohort included 16 males and 26 females. Patients' ages at enrolment ranged from 18 to 61 years, with a median [IQR] of 35 [29-48] (Table 1). Patients' heights ranged from 162 to 198 cm with a mean±SD of 178±10 cm, patients' weights ranged between 42 and 114 kg with a mean of 71.6±16.4 kg, and BSA of the cohort was 1.88±0.22 m² (Table 1). Disease phenotype in our MFS cohort was rather variable: patients' systemic scores ranged from 2 to 16 with a mean of 9.3±3.3. Concerning familial history of the disease, 29 patients had at least one first- and/or second-degree relative with a diagnosis of MFS (69%). Of note, 6 participants had a first-degree ($n=3$) or a second-degree ($n=3$) relative included in the cohort (Table S2). All patients developed skeletal manifestations, 16 out of 42 developed ectopia lentis (38.1%) and 24 out of 33 dural ectasia (72.7%), as reported in Table 1. Cardiovascular manifestations in these patients were prevalent (95.2%), affecting patients at different degrees. Mitral valve prolapse was recorded in 33 out of 40 patients (82.5%) and aortic valve regurgitation in 6 out of 42 patients (14.3%). Patients' Z-scores ranged from -2.26 to +5.48 with a mean of 1.91±1.79 (Table 1). Interestingly, 50%

of patients showed Z-score ≥ 2 at enrolment and, thus, were defined as presenting with AE. As for inclusion criteria, aortic size was similar in males (Z-score: 1.83±1.60) and females (1.95±1.92, $p=0.840$).

Table 1: MFS outpatient clinical setting. Data are expressed as percentage, median [IQR], or mean±SD, as appropriate. *Evaluated only in 33 patients, †evaluated only in 40 patients, ‡calculated with the Devereux's formula with correction for height. BSA: body surface area, BB: β -blockers, ARB: angiotensin receptor blocker.

Patients' profile	n	
Male	16	38.1%
Female	26	61.9%
Age (yr)	42	35 [29-48]
Height (cm)	42	178±10
Weight (kg)	42	71.6±16.4
BSA (m ²)	42	1.88±0.22
Systemic phenotype		
Systemic score	42	9.3±3.3
MFS familial history	29	69%
Skeletal manifestation	42	100%
Ectopia lentis	16	38.1%
Dural ectasia*	24*	72.7%
Mitral valve prolapse†	33†	82.5%
Aortic phenotype		
Aortic valve regurgitation	6	14.3%
Z-score‡	42	1.91±1.79
Aortic ectasia (Z-score ≥ 2)	21	50%
Antihypertensive pharmacological treatment		
BB	1	2.4%
ARB	12	28.6%
BB+ARB	16	38.1%
None	13	30.9%

Concerning hypotensive drug administration at the time of enrolment, 1 out of 42 patients was treated with BB only, 12 were treated with ARB only, 16 with both BB and ARB, while 13 were not subjected to any pharmacological treatment following physician prescription or patients' personal choice (Table 1). It is important to note that none of these patients were treated with calcium channel blockers and/or angiotensin converting enzyme inhibitors.

Differences in plasma sEMMPRIN levels between MFS patients and healthy controls

Circulating sEMMPRIN levels in 42 MFS patients and 42 matched HC have been measured. Plasma sEMMPRIN levels were significantly lower in MFS patients (2071±637 pg/ml) than in HC (2441±642, $p=0.009$; Figure 1A). To exclude a possible bias determined by familiar clustering in this cohort, a sensitivity analysis on the 36 unrelated probands was performed. Interestingly, plasma sEMMPRIN levels in these MFS patients remained statistically lower in respect to matched HC (2099±648 vs. 2445±608, $p=0.022$).

To exclude possible confounding effects of age or pharmacological treatment in our MFS cohort, we firstly correlated sEMMPRIN plasmatic concentration

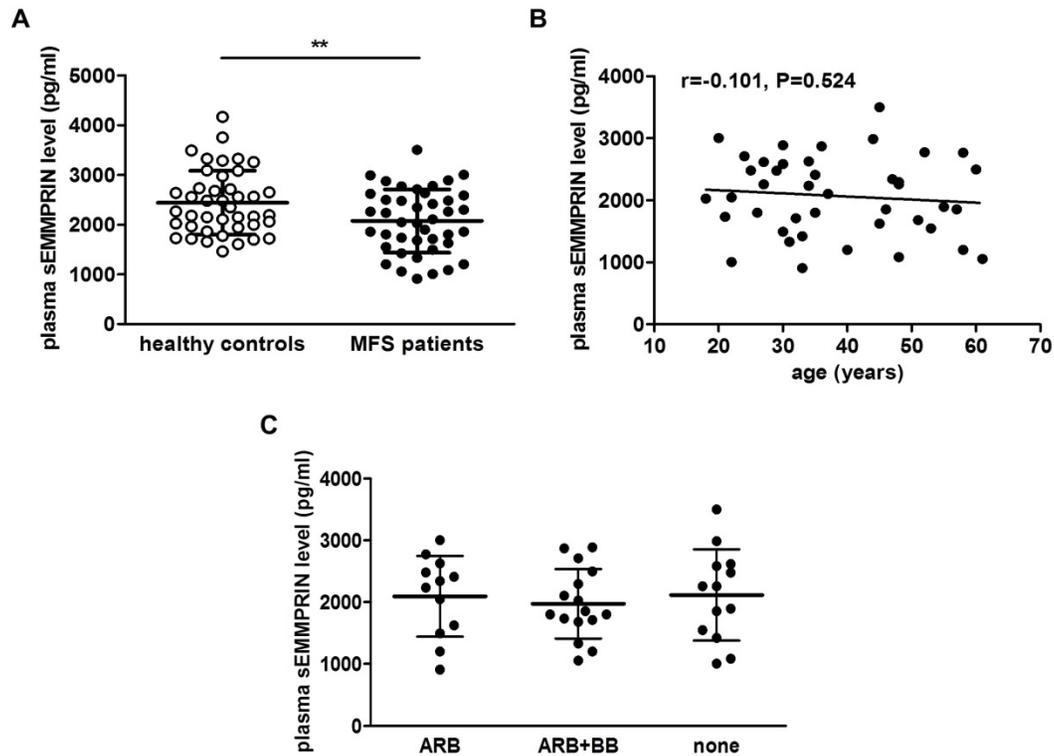


Figure 1: Plasma sEMMPRIN levels in healthy control and MFS patient cohorts. Differences in plasma sEMMPRIN levels of MFS patient and healthy control cohorts considered overall (A). Correlation between plasma sEMMPRIN levels and age in MFS patients (B). Plasma sEMMPRIN levels in patients treated with different hypotensive drugs (C). ARB: angiotensin II receptor blocker, BB: β -blockers. ** $p < 0.01$.

with age and then we categorized patients for their pharmacological treatment. As shown in Figure 1B and C, neither patients' age (Spearman $r = -0.101$, $p = 0.524$) nor the different antihypertensive treatment (ANOVA $p = 0.813$) influenced plasma sEMMPRIN levels.

We then checked whether sex might impact circulating sEMMPRIN. Interestingly, we found that plasma sEMMPRIN levels were significantly higher in males than in females both in HC ($p = 0.031$, Figure 2A) and MFS patients ($p = 0.022$, Figure 2B). Nevertheless, the breakdown of individual changes between matched HC and MFS patients did not reveal significant sex-related differences (males vs. females, 336 ± 612 vs. 446 ± 957 pg/ml; $p = 0.692$, Figure 2C).

To further assess the influence of being affected by MFS and of sex on circulating sEMMPRIN levels we performed a two-way ANOVA that confirmed the significant influence of the MFS disease ($p = 0.009$) and of sex ($p = 0.003$) on sEMMPRIN level expression, but failed to prove an interaction between the two variables ($p = 0.784$).

sEMMPRIN levels and MFS systemic features

We then investigated whether plasma sEMMPRIN levels correlate with patients' clinical phenotype. In particular, we focused our attention on the most important MFS-related systemic and aortic

manifestations. As for the systemic manifestations, circulating sEMMPRIN levels did not correlate with systemic score (Pearson $r = 0.157$, $p = 0.319$; Figure 3A). Moreover, plasma sEMMPRIN levels were similar between patients with and without a MFS familial history (2073 ± 680 vs. 2067 ± 554 pg/ml, $p = 0.976$; Figure S2). Similarly, sEMMPRIN levels did not differ in patients presenting or not ectopia lentis (2030 ± 774 vs. 2097 ± 552 pg/ml, $p = 0.746$; Figure 3B), dural ectasia (2020 ± 624 vs. 2327 ± 712 pg/ml, $p = 0.234$; Figure 3C), and mitral valve prolapse (2088 ± 656 vs. 2093 ± 584 pg/ml, $p = 0.985$; Figure 3D).

Negative correlation between plasma sEMMPRIN levels and MFS patients' aortic features

As for MFS aortic phenotype, plasma sEMMPRIN levels were similar in patients who developed or not aortic valve regurgitation (2164 ± 689 vs. 2056 ± 637 pg/ml, $p = 0.706$; Figure 4A).

We next found that patients' Z-scores significantly negatively correlated with plasma sEMMPRIN levels (Pearson $r = -0.431$, $p = 0.004$), as shown in Figure 4B. Specifically, lower levels of plasma sEMMPRIN were found in patients characterized by higher Z-scores, thus presenting a more severe aortic dilatation. Interestingly, after performing a categorization for sex, the correlation

between Z-score and plasma sEMMPRIN levels was confirmed both in males (Pearson $r=-0.551$, $p=0.027$) and in females (Pearson $r=-0.408$, $p=0.039$; Figure S3). Then, we adopted the Z-scores to categorize patients showing AE, defined as patients with a Z-score level \geq

2, and those without AE (Z-score level < 2). Consistently with previous findings, patients with AE showed significantly lower plasma sEMMPRIN levels (1788 ± 510 pg/ml) versus those without AE (2355 ± 634 pg/ml, $p=0.003$; Figure 4C).

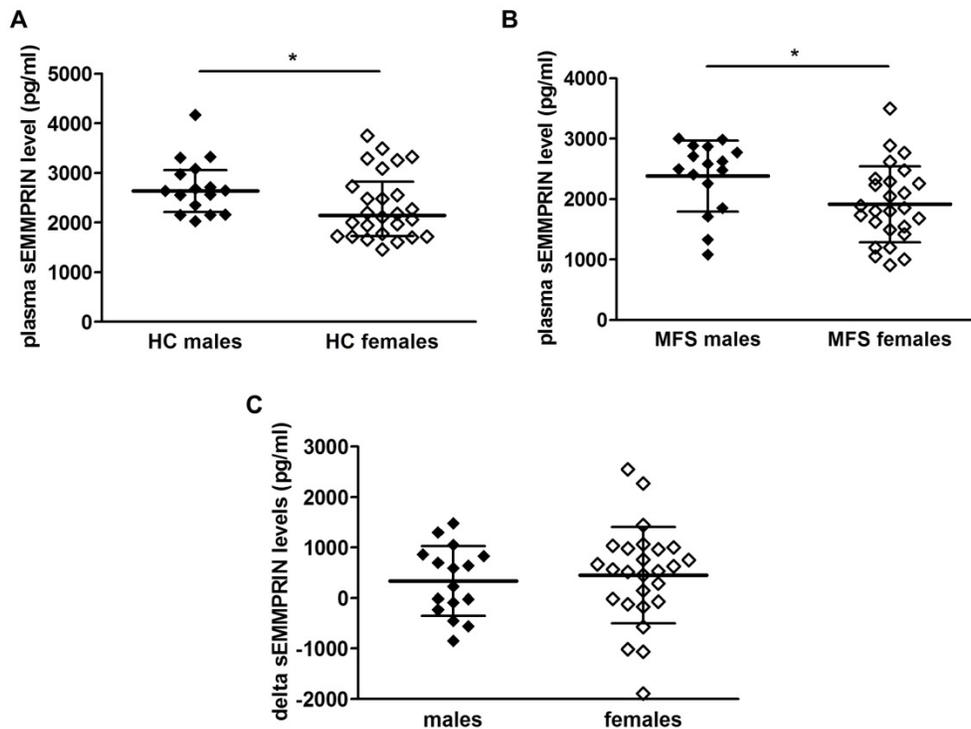


Figure 2: Circulating sEMMPRIN levels in male and female HC and MFS patients. Differences in plasma sEMMPRIN levels in HC (A) and MFS patients (B) categorized for sex. Delta sEMMPRIN levels between matched HC and MFS patients categorized for sex (C). HC: healthy controls; MFS: Marfan syndrome. * $p<0.05$.

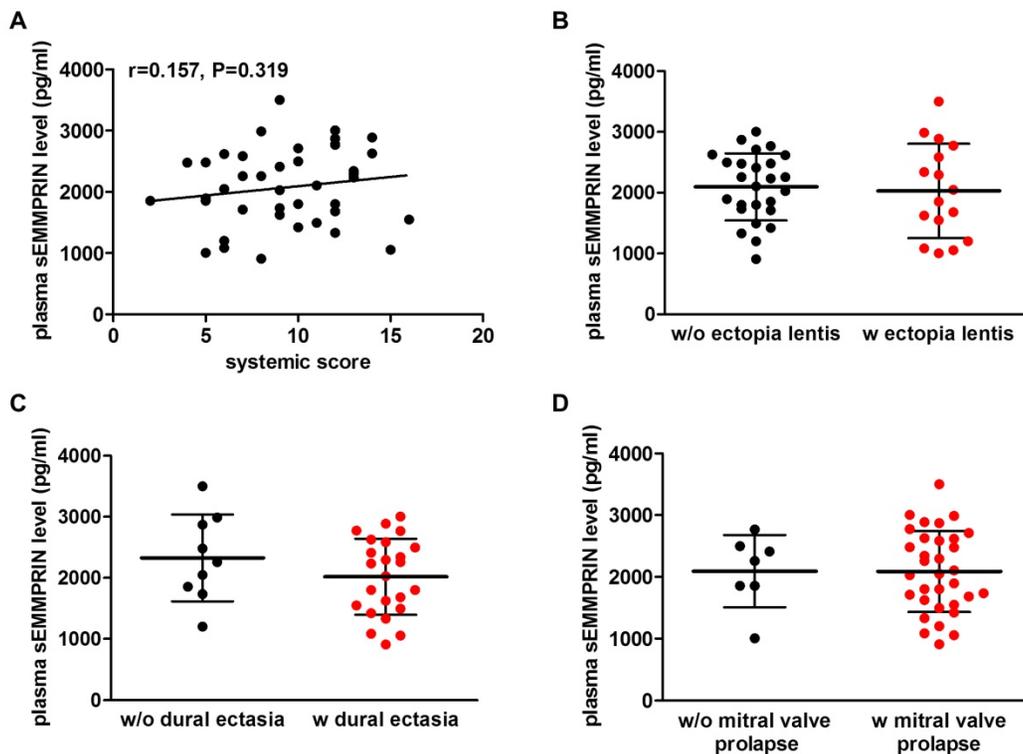


Figure 3: Plasma sEMMPRIN levels in MFS patients characterized by different systemic phenotype. Correlation between plasma sEMMPRIN levels and patients' systemic score (A). Differences in plasma sEMMPRIN levels in patients developing or not ectopia lentis (B), dural ectasia (C) or mitral valve prolapse (D). w/o: without, w: with.

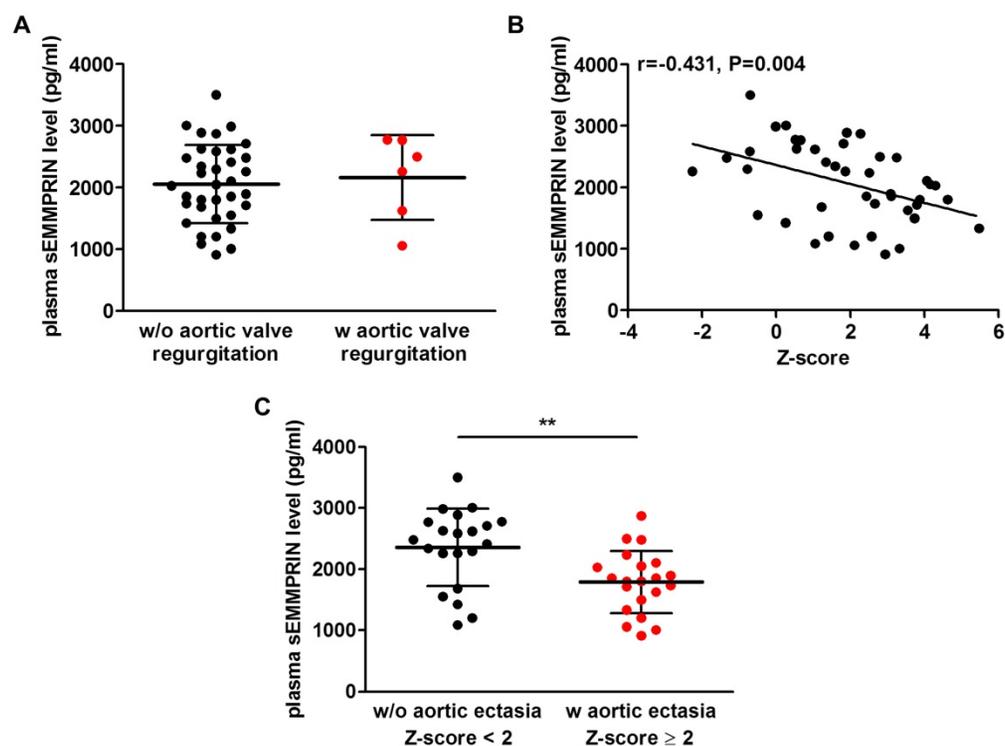


Figure 4: Plasma sEMMPRIN levels in MFS patients characterized by different aortic phenotype. Difference in plasma sEMMPRIN levels in patients with or without aortic valve regurgitation (A). Correlation between plasma sEMMPRIN levels and patients' Z-score (B) and difference in plasma sEMMPRIN levels in patients with or without aortic ectasia (C). w/o: without, w: with. $**p < 0.01$.

To further investigate the sEMMPRIN value as discriminator, we performed a comparison with other circulating molecules previously found to be related to aortic dilatation (e.g. MMP-2, MMP-9, TIMP-1, and TGF- β 1) [43-46] by correlating their levels detected in patients' circulation with the Z-scores. Interestingly, none of the evaluated plasma biomarkers in our MFS cohort significantly correlated with patients' Z-scores (Figure S4). Moreover, to evaluate whether sEMMPRIN yielded independent information in respect to other proposed biomarkers, we correlated plasma sEMMPRIN levels with MMP-2, MMP-9, TIMP-1, and TGF- β 1 levels. Interestingly, we did not find any significant correlation between plasma sEMMPRIN and the evaluated molecules (Figure S5).

Plasma sEMMPRIN levels discriminate the presence of aortic ectasia

We subsequently performed a ROC curve analysis to assess whether plasma sEMMPRIN levels could reveal the presence of AE in MFS patients. Interestingly, the area under the curve (AUC) obtained by the ROC analysis was as high as 0.763 (95% CI: 0.610-0.916), denoting the ability of plasma sEMMPRIN levels to discriminate patients with or without AE ($p = 0.003$, Figure 5A). We also calculated that the optimal cut-off value for plasma sEMMPRIN was 2246 pg/ml. This threshold, obtained considering the highest sum of sensitivity and specificity,

provided 85.7% sensitivity (correctly classifying 18 out of the 21 patients with AE) and 76.2% specificity (correctly classifying 16 out of the 21 patients without AE). Furthermore and notably, such a cut-off for plasma sEMMPRIN level showed a positive predictive value of 78.3%, a negative predictive value of 84.2% and an accuracy of 81.0% in predicting AE in MFS patients. Then, we estimated the Odd ratio (OR) by a logistic regression model to evaluate the association between circulating sEMMPRIN levels and the development of AE. Consistently with our previous results, plasma sEMMPRIN levels ≤ 2246 pg/ml were associated with a considerably higher frequency of AE development (OR [95%CI]: 19.2 [3.947-93.389], $p < 0.001$; Figure 5B). Moreover, to exclude sex and age as possible confounding factors, we performed a multivariable logistic regression analysis, confirming the association between plasma sEMMPRIN levels and the development of AE independently from these two variables (adjusted OR [95%CI]: 36.05 [3.815-340.621], $p = 0.002$; Figure 5C).

Finally, we performed a sensitivity analysis on the 36 unrelated probands to exclude a possible bias determined by familiar clustering. Among these patients the 17 with AE had plasma sEMMPRIN levels lower than the 19 patients without AE (1805 ± 535 vs. 2362 ± 637 pg/ml, $p = 0.008$). Furthermore, also in this subgroup of patients, plasma sEMMPRIN levels resulted significantly associated with the

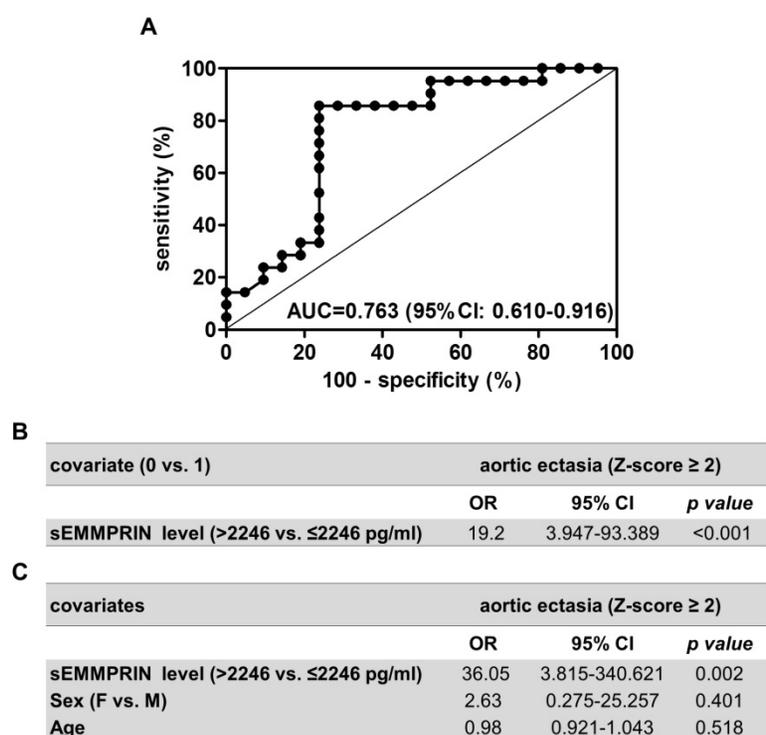


Figure 5: Performance of the plasma sEMMPRIN level as discriminator for AE. ROC curve analysis of plasma sEMMPRIN levels found in MFS patients who developed (n=21) or not (n=21) aortic ectasia (A). Logistic regression analyses performed to assess the association between plasma sEMMPRIN levels and AE development without (B) and with (C) the inclusion of sex and age covariates in the model. AUC: area under the curve, CI: confidence intervals, OR: Odd ratio, F: female, M: male.

presence of AE both by ROC curve analysis (AUC=0.757 [95% CI: 0.590-0.924], $p=0.009$) and by OR (17.5 [3.311-92.477], $p<0.001$).

Discussion

ECM remodeling is crucial in determining MFS-TAA development and dissection. In this context, several determinants of ECM remodeling have been investigated as potential circulating biomarkers [43-48]. Circulating levels of fibrillin-1 fragments have been found to be associated with the development of TAA in MFS patients [49]. Moreover, TGF- β levels were positively correlated with aortic root dimensions and aortic root growth [44], even if previous evidence failed to correlate TGF- β levels with Z-score or aortic diameter at the Valsalva sinus level [50,51]. Finally, homocysteine plasma levels, as well as the prevalence of homozygous genotypes of folic acid metabolism enzymes, correlated with severe cardiovascular manifestations and aortic dissection in MFS patients [52,53]. Nevertheless, at present an established clinical-grade circulating biomarker is still lacking.

Of interest, MMPs are key players in exacerbating ECM degradation triggered by functional fibrillin-1 deficiency [13]. Notably, MMP-2 and MMP-9 were up-regulated in TAA tissue and

aortic valves of MFS patients [13]. A more recent study showed higher levels of MMP-12, MMP-14, and TIMP-2 in MFS-TAA when compared to other TAA, indicating that TAA in MFS possess a very distinctive tissue expression profile of MMPs and TIMPs, suggestive of a specific modulator [43].

Several MMPs are regulated by EMMPRIN, a transmembrane glycoprotein receptor also found in a soluble plasmatic form [28]. While the receptor directly acts as a MMP inducer [15-17], the soluble protein appears to work in a paracrine fashion and it was recently proposed as a circulating biomarker in several diseases [31-36]. Although it is reported that sEMMPRIN levels are elevated in the blood of patients with acute myocardial infarction, coronary artery disease, cancer, and multiple and systemic sclerosis [31-36], in our study we detected lower circulating sEMMPRIN levels in MFS patients compared to HC. Moreover, in our MFS cohort we found that sEMMPRIN levels negatively correlated with patients' aortic dilatation extent at the Valsalva sinus level.

It can be speculated that these findings may reflect an elevated presence of the full-length and active form of EMMPRIN in the cell membrane. Indeed, it has been recently reported that active transmembrane EMMPRIN is abundant in the vascular smooth muscle cells of TAA tissue [25]. Thus, it is possible that, in the MFS pathological context, the cleavage of the full-length form of EMMPRIN, as well as its secretion from cells through vesicles, are blocked which leads to higher EMMPRIN expression in the cell membrane, consequently inducing MMP expression and activation in the tissue. Future studies are, however, warranted to define the precise mechanisms regulating sEMMPRIN in MFS-TAA.

In this work, we also found that sEMMPRIN levels are influenced neither by age nor by treatment with the ARB Losartan and/or the BB Atenolol. Together these results allow us to exclude age and antihypertensive treatment as confounding factors for the subsequent analyses. Interestingly, we found that circulating sEMMPRIN levels were lower in females than in males both in HC and MFS patients. Mechanistically, several studies indicate MMPs and other metalloproteinases as candidates responsible for sEMMPRIN cleavage and/or secretion in different pathological contexts [28]. Notably, it has been reported that MMP expression is sex-related both in pathological and healthy conditions [54-57]. Such a

sex-dependent association between MMP expression and sEMMPRIN levels needs further investigations in the MFS-TAA setting. Nevertheless, we believe our data clearly show that sex-related differences in sEMMPRIN levels are independent from the presence of the disease. Moreover, we can also exclude that these differences in sEMMPRIN levels are due to variability in thoracic aortic phenotype severity, since male and female patients have been selected at enrolment with a similar Z-score.

We evaluated whether the circulating sEMMPRIN levels could predict some MFS pathological features. Interestingly, beside the pathological systemic and cardiovascular features considered, plasma sEMMPRIN levels were strongly and selectively associated with the severity of aortic dilatation. In fact, our results indicate that sEMMPRIN levels negatively correlated with the extent of the aortic dilatation according to the Z-score. Specifically, patients with higher Z-scores –and, thus, more pronounced aortic enlargement– had lower circulating sEMMPRIN levels.

Furthermore, we also uncovered that patients showing AE (Z-score ≥ 2) have lower sEMMPRIN levels than those with a milder aortic enlargement (Z-score < 2) and, even more important, circulating sEMMPRIN levels ≤ 2246 pg/ml can discriminate patients with AE with a sensitivity as high as 85.7%. To the best of our knowledge, this is the first study to introduce a potential role for circulating sEMMPRIN in predicting AE amongst MFS patients which opens new perspectives in this pathological context for mechanistic studies on the involvement of the underlying pathway in aortic tissue remodeling. Importantly, the high sensitivity reached with this test along with the 81.0% accuracy indicate that sEMMPRIN levels may have implications in the management of MFS patients, since the identification of patients at higher risk of AE onset is of primary importance to prevent TAA development and dissection, the leading cause of death in MFS [4]. To date, the most widespread tools for measuring patient aortic diameter are imaging techniques such as transthoracic echocardiography, computed tomography and magnetic resonance imaging which allow an effective monitoring of aortic diameter progression [58]. The added value, on top of current clinical practice, of new biochemical information in this context may help to further discriminate subset of patients at higher risk [59]. Although still preliminary, our data suggest sEMMPRIN as a novel and promising circulating molecule among others already introduced in the literature as related to AE.

Study limitations and future perspectives

Our current findings are not conclusive to define sEMMPRIN levels as biomarker for aortic ectasia in MFS patients, due to the relatively small sample size of this proof-of-concept study. Nevertheless, it is important to highlight that the identification of sEMMPRIN as a novel potential specific discriminator for thoracic AE in MFS patients may represent the basis for future larger multicentric confirmative longitudinal studies.

Conclusions

In conclusion, our results suggest that plasma sEMMPRIN could be a useful and valuable adjuvant tool complementing imaging techniques for detecting AE development in MFS patients. Moreover, beside its potential diagnostic value, our data provide a rationale for further studying EMMPRIN glycoprotein in MFS pathological context with the aim to mechanistically investigate associated downstream pathways related to ECM remodeling, as well as to identify possible therapeutic targets to prevent or limit TAA progression.

Abbreviations

AE: aortic ectasia; ARB: angiotensin II receptor blockers; BB: beta-blockers; BSA: body surface area; ECM: extracellular matrix; EMMPRIN: extracellular matrix metalloproteases inducer; HC: healthy controls; MFS: Marfan syndrome; MMP: matrix metalloproteases; NGS: next generation sequencing; sEMMPRIN: soluble extracellular matrix metalloproteases inducer; TAA: thoracic aortic aneurysm; TGF- β 1: transforming growth factor beta 1; TIMP: tissue inhibitor of metalloproteases.

Supplementary Material

Supplementary figures and tables.

<http://www.thno.org/v09p2224s1.pdf>

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Competing Interests

The authors have declared that no competing interest exists.

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