Marfan syndrome: Getting to the root of the problem
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Relationship between *FBN1* type and severity of cardiovascular involvement in Marfan syndrome

Submitted

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Abstract

**Background:** Patients with Marfan syndrome (MFS) often have aortic disease that limits their survival. This study determined the impact of the FBN1-mutation type upon aortic diameters and dilation rates.

**Methods:** MFS patients were included when carrying a FBN1 mutation classified into dominant negative (DN, incorporation of non-mutated and mutated fibrillin-1 in the extracellular matrix) or haploinsufficiency (HI, only incorporation of non-mutated fibrillin-1, thus decreased amount of fibrillin-1 protein). The study objectives were differences in aortic diameters and aortic dilation rate at the level of the aortic root and arch, and ascending, descending and abdominal aorta by echocardiography, and the combined end-point comprising dissection and death.

**Results:** 290 MFS patients were included, with a HI-FBN1 mutation in 113 (39%), and a DN-FBN1 in 177 (61%) patients. At baseline, HI-FBN1 patients had a larger aortic root diameter than DN-FBN1 patients (HI: 39.3±7.2 mm versus DN: 37.3±6.8 mm, p=0.022), without differences in age nor body surface area. After a mean follow-up duration of 4.9±2.0 years, aortic root and ascending dilation rate were increased in HI-FBN1 patients (HI: 0.57±0.8 versus DN: 0.28±0.5 mm/year, p=0.004, and HI: 0.59±0.9 versus DN: 0.30±0.7 mm/year, p=0.032, respectively). Furthermore, HI-FBN1 patients tended to be at increased risk for the combined end-point of dissection and death compared to DN-FBN1 patients (HR: 3.3, 95%CI: 1.0-11.4, p=0.060).

**Conclusions:** Patients with an HI-FBN1 mutation have a more rapid aortic root and ascending dilation rate compared to patients with a DN-FBN1 mutation.
Chapter 7: FBN1 type relates to cardiovascular severity

Introduction

Marfan syndrome (MFS) is a progressive connective tissue disorder caused by mutations in the FBN1 gene encoding the fibrillin-1 protein.1 MFS patients often have aortic dilation, requiring aortic surgery to prevent aortic dissection and thus to extend their life span.2 However, MFS patients are known to have large variations in phenotypic expression as well as in age of onset of manifestations.3 Previous studies suggest that cardiovascular phenotype depends on FBN1-mutation effect on the fibrillin-1 protein.3-7

Over 2900 different FBN1 mutations have been described in the International Database.8 These mutations can be classified as dominant negative (DN) or haploinsufficiency (HI).4 DN mutations lead to disturbed folding of the protein, owing to interference of mutated with non-mutated fibrillin-1 protein, which result in a disorganized extracellular matrix.9 On the other hand, HI mutations lead to production of only non-mutated fibrillin-1 protein, by FBN1 gene deletions,10 by degradation of mutated mRNA by nonsense mediated decay,11 or due to protein degradation after mRNA synthesis.12

We previously have found that patients with a HI-FBN1 mutation more often have aortic dissections and a shorter survival compared to patients with a DN-FBN1 mutation.13 However, to date a clear correlation between aortic diameter and aortic dilation rate is lacking. To confirm that HI-FBN1 patients have a more severely affected cardiovascular system compared to DN-FBN1 patients, we correlate aortic diameters and aortic dilation rate to genotype classification (HI versus DN) in a well-controlled MFS cohort.

Methods

Patient population

All children and adults diagnosed with MFS, carrying a pathogenic FBN1 mutation, and who were followed up at two specialised MFS centres in Spain between 2004 and 2015 were included. Diagnosis of MFS was determined according to the Ghent criteria during the period of 1996–2010 and according to the revised Ghent criteria thereafter.14,15

Mutation classification

All known pathogenic FBN1 mutations were classified into DN or HI mutations as described previously.4 In short DN mutations lead to stable mutant fibrillin-1 protein with altered or shorter structure, which is incorporated together with normal fibrillin-1 protein (derived from the non-mutated allele). DN is mostly caused by missense and exon-skipping mutations.12,13,16 HI mutations lead to a reduced amount of normal non-mutated fibrillin-1 protein, derived from the non-mutated allele only. HI is caused by whole FBN1 gene deletion,10 degradation of mutated mRNA by nonsense mediated
decay in frameshift and nonsense mutations, or by protein degradation after mRNA synthesis. Mutations, which could not be classified in one of the two groups without skin biopsies were excluded for analysis.

**Study objectives.**

Study objectives were determination of the impact of mutation type on 1) aortic diameters, 2) aortic dilation rate and on 3) cardiovascular events (i.e. aortic dissection and cardiovascular mortality).

For the first two study objectives, aortic diameters were measured by two experienced observers (R.F., V.G.) using the leading edge-to-leading edge technique in end-diastole at the level of the sinuses of Valsalva, tubular ascending aorta, aortic arch, proximal descending thoracic aorta and abdominal aorta. The progression of aortic dilation was calculated in all patients with at least 1-year of follow-up, as the difference between the first and last echocardiographic follow-up study. For the third study objective, patients were followed up from the first clinical visit until the date of the first cardiovascular event: aortic dissection, cardiovascular death, or the first date of the combined endpoint of dissection and death. Patients were censored at their last clinical visit. Aortic dissection was defined as any dissection (type A and type B) in the aorta confirmed with an imaging modality. Cardiovascular events were updated in April 2015.

**Statistical analysis**

Data are presented as mean value ± standard deviation or as number of patients and percentage. Comparisons between continuous variables were made by Student’s T test. Comparisons between categorical variables were made by Fisher’s exact tests. Aortic dilation rate was evaluated by covariance analysis with baseline aortic diameter as covariate. Cox-regression analysis corrected for age at baseline and gender was used to analyse the mutation-effect on the clinical events. All statistical tests were two-sided and differences were considered statistically significant at p < 0.05. Data analysis was performed using the SPSS statistical package (20.0 for windows; SPSS Inc., Chicago, Illinois, USA).

**Results**

**Baseline Characteristics**

In the two specialised centres 537 patients fulfilling the Ghent criteria were followed up, of whom 351 (65.4%) underwent mutation analysis. In 312 patients (88.9%) a pathogenic FBN1 mutation was found, of whom 22 could not be classified because of uncertainty of classification (Supplemental Table 1). Finally, 290 MFS patients with a classified FBN1 mutation were included in the analysis, with a mean age of 30.2±14.7 years and sex was equally
distributed in the cohort with 48.5% males. A mutation leading to HI was present in 113 patients (39.0%), comprising 54 nonsense mutations, 45 frameshift mutations leading to nonsense mediated decay, 9 missense mutations leading to either unstable fibrilline-1 protein and subsequently protein degradation (n=8) or no conversion of profibrillin-1 to fibrillin-1 and thus no production of fibrillin-1 (n=1), and 5 intronic mutations leading to a splice site mutation and subsequently nonsense mediated decay, confirmed by skin biopsies. A DN mutation was present in 177 patients (61.0%), comprising 136 missense mutations, 21 small in-frame insertions or deletions, 15 in-frame exon skipping mutations, and 5 frameshift mutations in the nonsense mediated decay insensitive area (Supplemental Table 1). The baseline characteristics are shown in Table 1. At baseline, patients with a HI mutation had a significantly larger aortic root diameter than those with a DN mutation (HI: 39.3±7.2 mm versus DN: 37.3±6.8 mm, p=0.012, Figure 1), without differences in age (HI: 28.7±13.8 years versus DN: 31.1±15.2 years, p=0.191) nor body surface area (HI: 1.84±0.38 years versus DN: 1.85±0.31 years, p=0.841). Furthermore, at baseline prevalence of previous aortic surgery (HI: 18.6% versus DN: 16.3%, p=0.635) or aortic dissection (HI: 10.6% versus DN: 6.2%, p=0.186) did not differ between groups. However, age at previous surgery was significantly lower in HI patients (HI: 29.9±10.1 years versus DN: 36.6±12.9 years, p=0.050) than DN patients. No differences existed in cardiovascular treatment at baseline (HI: 53.2% versus DN: 56.1%, p=0.712). Patients with a DN mutation more frequently had ectopia lentis than HI patients (HI: 24.8% versus DN: 52.8%, p<0.001).

<table>
<thead>
<tr>
<th>Value</th>
<th>HI mutation (n=113)</th>
<th>DN mutation (n=177)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.7 (±13.8)</td>
<td>31.1 (±15.2)</td>
<td>0.191</td>
</tr>
<tr>
<td>sex (male)</td>
<td>50.4%</td>
<td>47.2%</td>
<td>0.631</td>
</tr>
<tr>
<td>body surface area (mm²)</td>
<td>1.84 (±0.38)</td>
<td>1.85 (±0.31)</td>
<td>0.841</td>
</tr>
<tr>
<td>ectopia lentis</td>
<td>24.8%</td>
<td>52.8%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>previous aortic complication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>previous surgery</td>
<td>19.5%</td>
<td>16.9%</td>
<td></td>
</tr>
<tr>
<td>age at previous surgery (years)</td>
<td>29.9 (10.1)</td>
<td>36.6 (±12.9)</td>
<td>0.050</td>
</tr>
<tr>
<td>previous dissection</td>
<td>10.6%</td>
<td>6.2%</td>
<td>0.186</td>
</tr>
<tr>
<td>age at previous dissection (years)</td>
<td>34.9 (±8.3)</td>
<td>39.7 (±12.8)</td>
<td>0.307</td>
</tr>
<tr>
<td>cardiovascular treatment</td>
<td>53.2%</td>
<td>56.1%</td>
<td>0.712</td>
</tr>
<tr>
<td>losartan</td>
<td>18.3%</td>
<td>20.2%</td>
<td>0.759</td>
</tr>
<tr>
<td>betablockers</td>
<td>30.3%</td>
<td>27.2%</td>
<td>0.590</td>
</tr>
<tr>
<td>losartan and betablockers</td>
<td>1.8%</td>
<td>2.9%</td>
<td>0.710</td>
</tr>
<tr>
<td>aortic dimensions (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sinus of valsalva</td>
<td>39.3 (±7.2)</td>
<td>37.3 (±6.8)</td>
<td>0.022</td>
</tr>
<tr>
<td>tubular ascending aorta</td>
<td>31.1 (±7.2)</td>
<td>30.9 (±7.4)</td>
<td>0.853</td>
</tr>
<tr>
<td>arch</td>
<td>23.4 (±6.2)</td>
<td>24.3 (±6.9)</td>
<td>0.436</td>
</tr>
<tr>
<td>proximal descending thoracic aorta</td>
<td>18.4 (±7.3)</td>
<td>18.7 (±7.3)</td>
<td>0.834</td>
</tr>
<tr>
<td>abdominal aorta</td>
<td>19.0 (±6.2)</td>
<td>17.2 (±4.0)</td>
<td>0.127</td>
</tr>
</tbody>
</table>
Aortic root dilation rate between genotype subgroups

The clinical follow-up data is shown in Table 2. Corrected for baseline aortic diameter, aortic dilation rate was significantly increased in HI patients at the aortic root (HI: 0.57±0.8 versus DN: 0.28±0.5 mm/year, p=0.004) and at the tubular ascending aorta (HI: 0.59±0.9 versus DN: 0.30±0.7 mm/year, p=0.032). For the arch and the proximal descending thoracic and abdominal aorta no differences were demonstrated between DN and HI patients (Figure 2).

Table 2. Aortic dilation rate between genotype subgroups

<table>
<thead>
<tr>
<th>Value</th>
<th>HI mutation (n=98)</th>
<th>DN mutation (n=150)</th>
<th>P</th>
<th>Corr P</th>
</tr>
</thead>
<tbody>
<tr>
<td>follow-up (years)</td>
<td>5.0 (±2.3)</td>
<td>4.9 (±1.9)</td>
<td>0.960</td>
<td></td>
</tr>
<tr>
<td>aortic dilation rate (mm/year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sinus of valsalva</td>
<td>0.57 (±0.8)</td>
<td>0.28 (±0.5)</td>
<td>0.008</td>
<td>0.004</td>
</tr>
<tr>
<td>tubular ascending aorta</td>
<td>0.59 (±0.9)</td>
<td>0.30 (±0.7)</td>
<td>0.044</td>
<td>0.032</td>
</tr>
<tr>
<td>arch</td>
<td>0.66 (±0.9)</td>
<td>0.59 (±0.8)</td>
<td>0.732</td>
<td>0.799</td>
</tr>
<tr>
<td>proximal descending thoracic aorta</td>
<td>0.61 (±0.7)</td>
<td>0.42 (±1.0)</td>
<td>0.408</td>
<td>0.403</td>
</tr>
<tr>
<td>abdominal aorta</td>
<td>0.50 (±0.4)</td>
<td>0.49 (±0.8)</td>
<td>0.948</td>
<td>0.912</td>
</tr>
</tbody>
</table>

Corr P, is the p-value corrected for baseline aortic dimension

Aortic complications between genotype subgroups

During follow-up 5 patients died (mean age: 42.2±13.2 years), 11 patients had an aortic dissection (mean age 41.4±10.3 years) and 55 patients needed aortic surgery (mean age 36.7±11.4 years).

Patients with an HI mutation tended to at 3.3-fold increased risk for the combined clinical endpoint (HI: 6.2% versus DN: 2.3%, HR: 3.3, 95%CI: 1.0-11.4, p=0.060), corrected for age at baseline and gender. For the individual endpoints, HI patients were not at significant increased risk for cardiovascular mortality (HI: 2.7% versus DN: 1.1%, HR: 2.8, 95%CI: 0.5-17.1, p=0.265), aortic dissection (HI: 6.2% versus DN: 2.3%, HR: 3.3, 95%CI: 1.0-11.4, p=0.060), nor for aortic surgery corrected for sex and age at baseline between the two groups (HI: 18.6% versus DN: 17.4%, HR: 1.1, 95%CI: 0.6-1.9, p=0.787). Figure 2 demonstrates the barcharts of the aortic dilation rates between HI and DN patients.
Discussion

This is the first study to demonstrate that patients with a HI-FBN1 mutation had a larger aortic root diameter, and a more rapid dilation rate of the aortic root and ascending aorta compared to patients with a DN-FBN1 mutation.

Although genotype-phenotype correlations in MFS are difficult due to the clinical variability within and between families, a couple of genotype-phenotype correlations are established. In a large cohort of 1013 probands, Faivre et al, have demonstrated that patients with a cysteine missense mutation (DN) are at increased risk of ectopia lentis, compared to patients with a premature termination codon (HI). In addition, a study of Aoyama et al. already demonstrated in 1995 that mutations leading to a very low deposition of the fibrillin-1 protein (HI) were associated with shortened event-free survival and more severe cardiovascular complications. Moreover, among Ghent-positive MFS patients, a higher frequency of HI-FBN1 mutations was found in patients with cardiovascular events (79%) versus patients without an event (48%, p=0.0039) even at a younger age, when compared to patients with a DN-FBN1 mutation. Furthermore, in a review summarizing 1511 patients, it was shown that HI-FBN1 patients have increased skeletal and cardiovascular involvement and DN-FBN1 patients more often have ectopia lentis. Finally, we recently confirmed that patients with a HI-FBN1 mutation are at increased risk for hard clinical endpoints, including aortic dissection and mortality, compared to patients with a DN-FBN1 mutation. These studies revealed intrinsic differences between MFS populations with a HI- or DN-FBN1 mutation. In the present study, we retrospectively confirmed in a large cohort that patients with a HI-FBN1 mutation have increased dilation rate of the aortic root and ascending aorta, the most affected parts of the MFS aorta. Furthermore, we validated that HI-FBN1 patients tended to have an increased risk for the combined clinical end-point (i.e. aortic dissection and mortality) compared to DN-FBN1 patients.

Currently, it is unknown why patients with an HI-FBN1 mutation are at increased cardiovascular risk compared to DN-FBN1 patients. Interestingly, Aubart et al., have
demonstrated a 4-fold variation in \textit{FBN1} mRNA synthesis level between controls without MFS with a very stable 50/50 allelic contribution\textsuperscript{7} and a similar \textit{FBN1}-mRNA variation was found in MFS patients with a HI-\textit{FBN1} mutation; however the level of \textit{FBN1} mRNA was half of those in the controls and >90% was transcribed from the non-mutated allele, confirming the concept of HI. Finally, the authors demonstrated that a low level of \textit{FBN1} mRNA is an important determinant for severity of phenotype and tends to increase the risk of aortic dilation.\textsuperscript{7} It seems to be that a low amount of fibrillin-1 protein leads to a more severe phenotype.

This HI-\textit{FBN1} in MFS is in contrast with that in other disorders, such as \textit{COL3A1}, where a null-allele (HI) leads to a milder and variable phenotype of Ehlers-Danlos syndrome, another aortic aneurysm disorder.\textsuperscript{18} The reason for concerted mRNA expression of the mutated and non-mutated allele in MFS is unknown. However, this may lead to interesting novel therapeutic options for HI-\textit{FBN1} patients, for example by stimulate the non-mutated allele by \textit{FBN1} expression regulators located outside the \textit{FBN1} locus.\textsuperscript{7} In addition, in a sub-study of the COMPARE study, HI-\textit{FBN1} patients responded to the addition of losartan on top of beta-blockers with a significant reduction of aortic dilation rate.\textsuperscript{4}

The present retrospective study has several limitations. First, in only 64% of the patients, mutation analysis was performed; others refused mutation analysis or analysis was not considered necessary for diagnosis and counselling. Second, our classification is based on software programming, knowledge of previous mutations and literature. We did not have skin biopsies to test protein stability and effect on mRNA. Third, only few patients have died or have had an aortic dissection during follow-up, thus the clinical endpoint is based on a small numbers of patients.

In conclusion, patients with a HI mutation had a more severely affected aortic phenotype, with larger aortic root diameters, a more rapid dilation rate and tended to have an increased risk of death and dissections compared to patients with a DN mutation.

\textbf{References}