Beneficial Outcome of Losartan Therapy Depends on Type of FBN1 Mutation in Marfan Syndrome

Running title: Franken et al.; Losartan is effective in Marfan haploinsufficiency

Romy Franken, MD1,2; Alexander den Hartog, MD1,2; Teodora Radonic, MD, PhD3; Dimitra Micha, PhD4; Alessandra Maugeri, PhD4; Fleur S. van Dijk, MD, PhD4; Hanne E. Meijers-Heijboer, MD, PhD4; Janneke Timmermans, MD5; Arthur J. Scholte, MD, PhD6; Maarten P. van den Berg, MD, PhD7; Maarten Groenink, MD, PhD1,2,8; Barbara J.M. Mulder, MD, PhD1,2; Aeilko H. Zwinderman, PhD9; Vivian de Waard, PhD10*; Gerard Pals, PhD4*

1Depts of Cardiology, 2Radiology, 3Clinical Epidemiology & Biostatistics, 4Medical Biochemistry, Academic Medical Center, Amsterdam, Amsterdam; 5Interuniversity Cardiology Institute of the Netherlands, Utrecht; 6Depts of Pathology, 7Clinical Genetics, VU University Medical Center, Amsterdam; 8Dept of Cardiology, Radboud University Nijmegen Medical Center, Nijmegen; 9Dept of Cardiology, Leiden University Medical Center, Leiden; 10Dept of Cardiology, University Medical Center Groningen, Groningen, the Netherlands

*contributed equally

Correspondence:
G. Pals, PhD
VU University Medical Center
Department of Clinical Genetics
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands
Tel: + 31 20 444 82 78
Fax: + 31 20 444 82 93
E-mail: g.pals@vumc.nl

Abstract:

**Background** - It has been shown that losartan reduces aortic dilatation in patients with Marfan syndrome. However, treatment response is highly variable. This study investigates losartan effectiveness in genetically classified subgroups.

**Methods and Results** - In this predefined sub-study of COMPARE, Marfan patients were randomized to daily receive losartan 100mg or no losartan. Aortic root dimensions were measured by magnetic resonance imaging at baseline and after 3 years. *FBN1* mutations were classified based on fibrillin-1 protein effect into 1) ‘Haploinsufficiency’, decreased amount of normal fibrillin-1, 2) ‘Dominant negative’, normal fibrillin-1 abundance with mutant fibrillin-1 incorporated in the matrix. A pathogenic *FBN1* mutation was found in 117 patients, of whom 79 patients were positive for a dominant negative mutation (67.5%) and 38 for a mutation causing haploinsufficiency (32.5%). Baseline characteristics between treatment groups were similar. Overall, losartan significantly reduced aortic root dilatation rate (no losartan: 1.3±1.5mm/3years, n=59, versus losartan: 0.8±1.4mm/3years, n=58, p=0.009). However, losartan only reduced aortic root dilatation rate in haploinsufficient patients (no losartan: 1.8±1.5mm/3years, n=21, versus losartan 0.5±0.8mm/3years, n=17, p=0.001) and not in dominant negative patients (no losartan: 1.2±1.7mm/3years, n=38, versus losartan 0.8±1.3mm/3years, n=41, p=0.197).

**Conclusions** - Marfan patients with haploinsufficient *FBN1* mutations appear to be more responsive to losartan therapy for inhibition of aortic root dilatation rate compared to dominant negative patients. Additional treatment strategies are needed in Marfan patients with dominant negative *FBN1* mutations.

**Clinical Trial Registration** - http://www.trialregister.nl/trialreg/index.asp; Unique Identifier: NTR1423.

**Key words:** genetic heart disease, angiotension II receptor blocker, aneurysm, aortic valve, magnetic resonance imaging, Marfan syndrome, FBN1 gene, Losartan
Introduction

Marfan syndrome is a progressive disorder caused by mutations in \textit{FBN1} encoding the protein fibrillin-1.\(^1\) Recently, losartan has been found to reduce aortic root dilatation rate in patients with Marfan syndrome.\(^2\) However, there appeared to be large inter-individual variability in response. The aim of this study was therefore to identify patients with a more enhanced effect of losartan therapy.

Currently, over 2000 mutations have been described in the International Database.\(^3,4\) \textit{FBN1} mutations can be classified as ‘dominant negative’ or ‘haploinsufficient’ depending on the effect of the \textit{FBN1} mutation on the fibrillin-1 protein.\(^5\) Dominant negative mutations lead to disturbed function, or folding of the protein, causing disturbed interactions with fibrillin-1 and other proteins. Hence a disorganized extracellular matrix.\(^6\) Both the strength of the fibrillin-1 matrix may be changed, as well as the release of binding proteins, such as transforming growth factor-\(\beta\) (TGF-\(\beta\)), which is (indirectly) captured in the fibrillin-1 network. Indeed, plasma TGF-\(\beta\) levels are elevated in Marfan patients, and are correlated to aortic dissection and aortic root dilatation.\(^7,8\)

On the other hand, a lower production of normal fibrillin-1 protein by haploinsufficient mutations, will also lead to reduced binding of TGF-\(\beta\),\(^9,10\) either due to whole gene deletions of \textit{FBN1},\(^11\) or degradation of the mutant protein,\(^12\) or nonsense mediated decay by degradation of fibrillin-1 encoding messenger RNA (mRNA).\(^13\) The latter type of mutations leads to a homogeneous phenotype, since there is no mutant protein.\(^5\) The reduced level of normal fibrillin-1 protein presumably results in a thinner fibrillin-1 matrix in the vasculature and thus in reduced aortic wall strength.

The objective of this study is to investigate the losartan effect on aortic root dilatation.
rate between Marfan patients with a FBN1 mutation leading to haploinsufficiency and Marfan patients with a FBN1 mutation leading to a dominant negative fibrillin-1 effect.

Methods

Patient population

In order to investigate the losartan effect on aortic root dilatation rate between haploinsufficient and dominant negative FBN1 mutations, we included all patients with a pathogenic FBN1 mutation and a native aortic root at time of inclusion and exclusion scan of the COMPARE study. In short, the COMPARE trial investigated the effects of losartan (100 mg) on aortic dimensions in a multi-centre randomized, controlled clinical trial on top of prescribed cardiovascular medication.2 Inclusion criteria were diagnosis of Marfan syndrome14 and age ≥ 18 years.15 Exclusion criteria were angiotensin converting enzyme inhibitor usage and previous replacement of more than one part of the aorta.15 The trial complied with the Declaration of Helsinki and was conducted with approval of the Medical Ethical Committees of all participating hospitals. Written informed consent was obtained from all participants. This trial is registered at the Netherlands Trial Register (number NTR1423).

Baseline examination

As part of the COMPARE trial, during the baseline visit, medical specialists extensively examined all patients on clinical features of Marfan syndrome in cardiovascular, ocular and skeletal systems according to the Ghent criteria of 1996.14 Punch skin biopsies were taken from the upper thigh of 55 patients at the onset of the trial after local anaesthesia with ethyl chloride spray. Aortic root diameter was measured at end diastole by ECG triggered Magnetic Resonance Imaging (MRI) in short axis views at the level of the sinus of Valsalva during end-expiration. After a mean follow-up of three years, patients underwent a second MRI and subsequently aortic
root dilatation rate was calculated. Clinical events, including aortic root surgery, aortic dissection type A and B, mitral valve surgery and cardiovascular death were also evaluated at the end of the study. Sanger sequencing of the 65 coding FBN1 exons in genomic DNA (gDNA) from blood cells was used to detect mutations. Large deletions were tested with multiplex ligation-dependent probe amplification (MRC-Holland, MLPA kit P065 and P066).\(^{11}\)

**Mutation classification**

Mutations were classified as ‘dominant negative’ in the case of: 1) missense mutations leading to stable mutant fibrillin-1 protein with altered structure; 2) mutations leading to exon skipping or deletion resulting in in-frame events and consequently a shorter stable protein;\(^ {16}\) 3) Premature Termination Codon (PTC) or frameshift mutations leading to a shorter fibrillin-1 protein without causing nonsense mediated decay (NMD).

Mutations were classified as ‘haploinsufficient’ in case of: 1) deletion of the whole FBN1 gene; 2) deletion of at least the first (exon 1) or the last exon (exon 65) of the FBN1 gene, which prevents transcription and/or translation of the gene; 3) PTC or frameshift mutations leading to a null-allele as a consequence of NMD\(^ {13}\); 4) missense mutations leading to degradation of the mutant protein\(^ {12}\); and 5) mutations leading to a very short truncated protein (translation of less than the first 10 exons of the FBN1 gene), which will not participate in fibril formation.

Effects of the mutations were predicted by Alamut\(^ {®}\) software (Interactive Biosoftware, Rouen, France). In order to confirm the mutation classification, fibrillin-1 messenger RNA (mRNA) production was measured in fibroblast cultures from available skin biopsies. In 55 patients the effect of the mutation was tested on mRNA expression. mRNA expression in cultured fibroblasts was studied by mRNA isolation, complementary DNA (cDNA) synthesis and QPCR on a Lightcycler LC480 (Roche), using UPL probe #78 (Roche) with
primers: agcggggattctcacttgat (forward) and cctcacactcgtccacgtc (reverse), with the GAPD UPL reference gene kit (Roche).

**Statistical analysis**

Data are presented as mean value ± standard deviation or as number of patients (percent). Comparisons between continuous variables were made by Mann-Whitney U test. Comparisons between categorical variables were made by Fisher’s exact tests. A two way analysis of variance was used after ranking of the aortic root dilatation rates, to calculate the difference in effect size of losartan between both groups. The spearman’s rank correlation coefficient was used to analyse the correlation between mean arterial blood pressure and change in aortic root dilatation rate. 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 (19.0 for windows; SPSS Inc., Chicago, Illinois, USA).

**Results**

**Classification into dominant negative and haploinsufficient FBN1 mutations**

All patients in our cohort were diagnosed with MFS based on the Ghent criteria of 1996 and 2010, and did not have distinguishing features of other connective tissue disorders. In our COMPARE cohort, we included 233 patients, and in 186 patients a pathogenic FBN1 mutation was found (Fig. 1) In the remainder 47 patients, we did not perform genetic analysis because of refusal of the patient (n=1), we did not found a mutation even after sequencing and MLPA analysis (n=37), and in 9 patients we found a pathogenic mutation in another connective tissue disorder gene: TGFBR2 (n=2), TGFBR1 (n=1), MYH11 (n=2), TGFBR2 (n=3), and in the MYLK1 gene (n=1). Of the 186 patients with a pathogenic FBN1 mutation, 69 patients were excluded for our current analysis, because they already had an aortic root replacement at the start of the study
(n=53), or during the study (n=16). (Fig. 1) Thus, we included 117 patients with a pathogenic

\(FBN1\) mutation and a native aortic root at the time of the exclusion scan (mean age: 35.3 years
(range 18-71 years). Classification of mutations revealed that 79 patients were positive for a

dominant negative mutation (67.5%) and 38 patients were positive for a mutation causing

haploinsufficiency (32.5%).

In order to validate the predicted mutation effect by Alamut, we compared gDNA with

complementary DNA (cDNA), synthesized from mRNA of cultured fibroblasts from skin

biopsies. In total we tested the effect of the mutation on cDNA sequence analyse of 55 Marfan

patients. Examples are shown in Fig. 2.

**Clinical features between dominant negative and haploinsufficient patients**

Medical specialists completely mapped our Marfan patients, resulting in a sample rate of clinical

features of 100% (Table 1). Clinical features of the cardiovascular system which are scored in

MFS comprise aortic root dilatation with a Z-score > 2 and mitral valve prolapse. There were no
differences between patients with dominant negative or haploinsufficient mutations for each

variable of the cardiovascular system. However, when we divided aortic root diameter by age,

patients with a haploinsufficient \(FBN1\) mutation had a trend towards an increased aortic root
diameter compared to dominant negative patients (1.49 ± 0.5 versus 1.35 ± 0.5, respectively,
p=0.093).

Interestingly, patients with haploinsufficient \(FBN1\) mutations had more frequently

presence of pectus carinatum (50% versus 29%, p=0.035), dural ectasia (61% versus 32%,
p=0.003), and skin striae (84% versus 65%, p=0.017) compared to patients with dominant

negative \(FBN1\) mutations (Table 1). Baseline characteristics were similar between the losartan

and no losartan treatment group of haploinsufficient patients, as well as dominant negative
patients (Table 2).

**Losartan effect on haploinsufficient and dominant negative patients**

Losartan is commonly used as a blood pressure regulator. Overall in the Marfan patients, losartan significantly reduced mean arterial pressure (-6 ± 10 mmHg versus -0.8 ± 8, p=0.002). However, this blood pressure lowering effect of losartan was only found in the patients with a dominant negative FBN1 mutation (-7 ± 9 mmHg versus 0.7 ± 7, p<0.001) and not in patients with a haploinsufficient FBN1 mutation (-4 ± 10 mmHg versus -3 ± 9, p=0.864). No correlation was found between mean arterial blood pressure with aortic root dilatation rate or between haploinsufficient and dominant negative patients (Fig. 3).

We recently showed that losartan significantly reduces aortic root dilatation rate in Marfan patients. Here, in our selected cohort of patients with a pathogenic FBN1 mutation, patients treated with losartan also showed significant reduction in aortic root dilatation rate compared to patients without losartan therapy (no losartan: 1.3 ± 1.5 mm/3years, n=59 versus losartan: 0.8 ± 1.4 mm/3years, n=58, p=0.009).

Noteworthy, losartan therapy was highly beneficial for patients with a haploinsufficient mutation, since these patients showed a prominent and significant reduction in aortic root dilatation rate (no losartan: 1.8 ± 1.5 mm/3/year, n=21 versus losartan: 0.5 ± 0.8 mm/3/year, p=0.001, n=17)(Fig 4). In contrast, in patients with a dominant negative mutation the effect of losartan was not significant (no losartan: 1.2 ± 1.7 mm/3/year, n=38, versus losartan: 0.8 ± 1.3 mm/3/year, n=41, p=0.197)(Fig. 4). In absolute terms, the percentage of haploinsufficient patients with a stable aortic root (defined as a dilatation rate ≤0 mm/3years) was 58.8% in the losartan group and 19.0% in the control group (p=0.014). The percentage of dominant negative patients with a stable aortic root was 51.2% in the losartan group and 60.5% in the control group.
(p=0.498). When a two way analysis of variance was used to calculate the difference in effect size of losartan between both groups, no statistical significance in this relatively small cohort (p=0.147) was shown.

Discussion

This is the first study to demonstrate the value of classification of FBN1 mutations based on their effect on fibrillin-1 protein level. Our study shows that classification into haploinsufficient (32.5%) and dominant negative (67.5%) patients is feasible and that both groups have similar cardiovascular pathology. Interestingly, losartan therapy significantly reduced the aortic root dilatation rate in the haploinsufficient patients, whereas only a modest insignificant reduction was found in dominant negative patients.

Losartan, an angiotensin II receptor type 1 (AT1) inhibitor, is known to reduce TGF-β signalling in a well-known mouse model with Marfan syndrome. Therefore, it was hypothesized that losartan has a beneficial effect in Marfan patients by lowering blood pressure and by reducing TGF-β signalling. However, we demonstrated that mean arterial blood pressure did not correlate with aortic root dilatation rate in dominant negative, as well as haploinsufficient patients treated with losartan. Therefore, the effect of losartan on the aortic root dilatation rate is not merely blood pressure related. There is a multitude of signaling cascades downstream of AT1 activation. It seems that one of the other pathways has a detrimental effect on the vessel wall upon chronic AT1 activation.17–20

A possible explanation for the more enhanced beneficial effect of losartan in haploinsufficient patients may be that their aortic wall suffers from hyperextension because of a thinner fibrillin-1 network. The fibrillin-1 network is connected to the elastin and collagen network to limit excessive stretch, which may be hampered in these patients.21 Hyperextension
of the aorta or ‘aortic stretch’ may damage the aortic wall directly, causing AT1 activation for a rapid damage response by producing TGF-β.\textsuperscript{22} Furthermore, local Angiotensin II has been associated with aneurysm formation.\textsuperscript{19} Altogether, haploinsufficient patients may have more locally produced Angiotensin II and thus a more beneficial effect of losartan on local aneurysm forming.

Traditionally, most \textit{FBN1} mutations were thought to cause a dominant negative effect on the fibrillin-1 protein, resulting in Marfan syndrome.\textsuperscript{6,10} However, multiple studies, including this study, have revealed that \textit{FBN1} haploinsufficiency leads to the full spectrum of Marfan syndrome and is present in a substantial part (32.5%) of the adult Marfan population.\textsuperscript{11,13} Haploinsufficient patients have a homogeneous phenotype, due to similar mutation effect on fibrillin-1: reduced but functionally normal fibrillin-1 protein. In contrast, patients with dominant negative mutations display a broad spectrum dysfunctional fibrillin-1 proteins. This probably explains the highly variable response to losartan in this patient group. In addition, we demonstrated a different effect of losartan therapy, based on this phenotypic classification, underlining the relevance of categorization Marfan patients genetically.

In conclusion, our results show that the beneficial effect of losartan therapy on aortic root dilatation rate is more pronounced in patients with a mutation causing \textit{FBN1} haploinsufficiency. We recommend to analyze and categorize the \textit{FBN1} mutations in all Marfan patients by genetic specialists in order to predict individual losartan effectiveness. Moreover, enhanced effort should be made to further characterize the Marfan patients with dominant negative \textit{FBN1} mutations,\textsuperscript{23} to find novel therapeutics reducing aortic dilatation rate for this patient population.

**Funding Sources:** Supported by Dutch Heart Association (2008B115) and by Interuniversity Cardiology Institute of the Netherlands (ICIN). The work described in this study was carried out
in the context of the Parelsnoer Institute (PSI). PSI is part of and funded by the Dutch Federation of University Medical Centers.

**Conflict of Interest Disclosures:** None.

**References:**


Table 1: Characteristics of Dominant Negative and Haploinsufficient FBN1 patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Dominant Negative (n=79)</th>
<th>Haploinsufficiency (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basic features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>43 (54.4)</td>
<td>19 (50.0)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.7 ± 12.9</td>
<td>32.5 ± 12.1</td>
</tr>
<tr>
<td>Body Surface Area (m²)</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>59 (74.7)</td>
<td>27 (71.1)</td>
</tr>
<tr>
<td><strong>Cardiovascular features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic root diameter (mm)</td>
<td>43.7 ± 4.6</td>
<td>43.2 ± 5.2</td>
</tr>
<tr>
<td>Aortic root diameter / age (mm/year)</td>
<td>1.35 ± 0.5</td>
<td>1.49 ± 0.5</td>
</tr>
<tr>
<td>Distal aortic surgery</td>
<td>2 (2.5)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Mitral valve prolapse</td>
<td>41 (51.9)</td>
<td>24 (63.2)</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>89.4 ± 9.2</td>
<td>91.5 ± 10.1</td>
</tr>
<tr>
<td><strong>Ocular features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ectopia Lentis</td>
<td>44 (55.7)</td>
<td>15 (39.5)</td>
</tr>
<tr>
<td>Myopia &gt; 3 diopters</td>
<td>15 (19.0)</td>
<td>8 (21.1)</td>
</tr>
<tr>
<td>Flat cornea</td>
<td>4 (5.1)</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>Increased axial length</td>
<td>6 (7.6)</td>
<td>7 (18.4)</td>
</tr>
<tr>
<td>Hypoplastic iris</td>
<td>6 (7.6)</td>
<td>4 (10.5)</td>
</tr>
<tr>
<td><strong>Skeletal features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wrist and thumb sign</td>
<td>31 (39.2)</td>
<td>21 (55.3)</td>
</tr>
<tr>
<td>Severe Pectus Excavatum</td>
<td>12 (15.2)</td>
<td>4 (10.5)</td>
</tr>
<tr>
<td>Pectus Carinatum*</td>
<td>23 (29.1)</td>
<td>19 (50.0)</td>
</tr>
<tr>
<td>Hindfoot deformity</td>
<td>21 (26.6)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>Pes Planus</td>
<td>32 (40.5)</td>
<td>16 (42.1)</td>
</tr>
<tr>
<td>Spontaneous pneumothorax</td>
<td>9 (11.4)</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>Dural Ectasia†</td>
<td>25 (31.6)</td>
<td>23 (60.5)</td>
</tr>
<tr>
<td>Joint hypermobility</td>
<td>16 (20.3)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>Highly arched palate</td>
<td>50 (63.3)</td>
<td>28 (73.7)</td>
</tr>
<tr>
<td>Span ratio &gt; 1.05</td>
<td>15 (19.0)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>Scoliosis &gt; 20°</td>
<td>17 (21.5)</td>
<td>13 (34.2)</td>
</tr>
<tr>
<td>Reduced extension of elbows</td>
<td>10 (12.7)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>Facial features</td>
<td>22 (27.8)</td>
<td>15 (39.5)</td>
</tr>
<tr>
<td>Skin striae*</td>
<td>51 (64.6)</td>
<td>32 (84.2)</td>
</tr>
<tr>
<td>Skeletal score</td>
<td>7.6 ± 3.3</td>
<td>7.5 ± 3.5</td>
</tr>
</tbody>
</table>

* p<0.05, † p<0.01
Table 2A: Characteristics between losartan and no losartan Dominant negative *FBN1* patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>No Losartan (n=38)</th>
<th>Losartan (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>21 (55.3)</td>
<td>22 (53.7)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.0 ± 13.2</td>
<td>36.4 ± 12.8</td>
</tr>
<tr>
<td>Body Surface Area (m²)</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Aortic root diameter (mm)</td>
<td>43.6 ± 4.3</td>
<td>43.9 ± 4.8</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>89.0 ± 8.6</td>
<td>89.8 ± 9.7</td>
</tr>
<tr>
<td>Ectopia Lentis</td>
<td>19 (50.0)</td>
<td>25 (61.0)</td>
</tr>
<tr>
<td>Systemic score</td>
<td>7.5 ± 3.4</td>
<td>7.7 ± 3.1</td>
</tr>
</tbody>
</table>

Table 2B: Characteristics between losartan and no losartan Haploinsufficient *FBN1* patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>No Losartan (n=21)</th>
<th>Losartan (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>10 (47.6)</td>
<td>9 (52.9)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.8 ± 13.5</td>
<td>30.9 ± 10.4</td>
</tr>
<tr>
<td>Body Surface Area (m²)</td>
<td>2.0 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Aortic root diameter (mm)</td>
<td>42.8 ± 4.8</td>
<td>43.6 ± 5.7</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>90.6 ± 8.8</td>
<td>92.7 ± 11.6</td>
</tr>
<tr>
<td>Ectopia Lentis</td>
<td>6 (28.6)</td>
<td>9 (52.9)</td>
</tr>
<tr>
<td>Systemic score</td>
<td>7.1 ± 3.3</td>
<td>8.0 ± 3.8</td>
</tr>
</tbody>
</table>
Figure Legends:

**Figure 1:** Flow chart of study population. In the COMPARE trial 233 patients were included. For this additive analysis we excluded 47 patients not having a pathogenic *FBN1* mutation, and 69 patients due to replacement of the aortic root before or during study. The black boxes include patients with a haploinsufficient mutation; the grey boxes comprise patients with a dominant negative mutation. AoRR: aortic root replacement

**Figure 2:** Examples of testing the effect of mutations on mRNA. Mutations were detected in genomic DNA (gDNA: top panels) and compared with complementary (cDNA) that was prepared from messenger RNA (mRNA) of patient fibroblasts. Three examples are shown: (A) The mutation c.6412T>A is detected in genomic DNA (top panel). It changes a codon for Phenylalanin (AAA) into a stop (TAA), which may lead to a truncated protein, or to degradation of the mutant mRNA by nonsense mediated decay (NMD). In the cDNA sequence (bottom panel) the mutation is not present, which proves absence of mutant mRNA, which is evidence of haploinsufficiency. (B) The single base insertion c.2851insG is detected in genomic DNA (top panel). This leads to a shift in the DNA reading frame and subsequently a stop codon. The double sequence peaks after the mutation are caused by overlap of normal and mutant sequence. In cDNA (bottom panel) only the normal sequence is seen, showing evidence of NMD and haploinsufficiency. (C) The intronic mutation c.6164-1G>A destroys the canonical splice site and may lead to exon skipping or intron retention with NMD. The cDNA sequence shows 2 sequences, with evidence of skipping of exon 50. This is confirmed by a double band of the PCR product on an agarose gel. This is an inframe deletion in the mRNA and will result in a shorter
protein with a dominant negative effect. (D) The PCR products of the cDNA samples that were used for sequence analysis show a double band for the exon skipping.

**Figure 3:** Aortic root dilatation rate is not associated with change in blood pressure. Reduction of aortic root dilatation rate was independent of change in mean arterial blood pressure in haploinsufficient patients (black), as well as in dominant negative patients (grey).

**Figure 4:** Losartan effect between haploinsufficient and dominant negative patients. We classified the 117 Marfan patients with a pathogenic *FBN1* mutation and a native aortic root in four groups: patients with a haploinsufficient mutation without (black) and with losartan therapy (black with blocks), and patients with a dominant negative mutation without (grey) and with losartan therapy (grey with blocks). The median is marked with a black or a white line, and the boxes delineate all data between 10-90%. Remarkably, losartan therapy only significantly reduced aortic root dilatation rate in patients with a haploinsufficient mutation.
233 Marfan patients

47 exclusion because of
- 37 no mutation in FBN1
- 1 analysis not performed
- 9 mutation in other gene

186 FBN1 mutations

69 exclusion because of
- 53 AoRR before start of study
- 16 AoRR during study

117 FBN1 mutations

42 cysteine missense
23 other missense
11 frameshift
15 intron mutation
18 nonsense
6 inframe deletion
2 deletion whole gene
Change in mean arterial blood pressure (mmHg)

Aortic root dilatation rate (mm/3 years)

- HI, $r^2=0.01$, p=ns
- DN, $r^2=0.02$, p=ns
Beneficial Outcome of Losartan Therapy Depends on Type of FBN1 Mutation in Marfan Syndrome

Circ Cardiovasc Genet. published online January 22, 2015;
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/early/2015/01/22/CIRCGENETICS.114.000950

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Genetics can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org//subscriptions/