European Heart Journal (2013) **34**, 3491–3500
doi:10.1093/eurheartj/eht334

Losartan reduces aortic dilatation rate in adults with Marfan syndrome: a randomized controlled trial

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Received 21 June 2013; revised 16 July 2013; accepted 30 July 2013; online publish-ahead-of-print 2 September 2013

See page 3469 for the editorial comment on this article (doi:10.1093/eurheartj/eht349)

Aim Patients with Marfan syndrome have an increased risk of life-threatening aortic complications, mostly preceded by aortic dilatation. Treatment with losartan, an angiotensin-II receptor-1 blocker, may reduce aortic dilatation rate in Marfan patients.

Methods and results In this multicentre, open-label, randomized controlled trial with blinded assessments, we compared losartan treatment with no additional treatment in operated and unoperated adults with Marfan syndrome. The primary endpoint was aortic dilatation rate at any predefined aortic level after 3 years of follow-up, as determined by magnetic resonance imaging. A total of 233 participants (47% female) underwent randomization to either losartan ($n = 116$) or no additional treatment ($n = 117$). Aortic root dilatation rate after 3.1 $\pm$ 0.4 years of follow-up was significantly lower in the losartan group than in controls (0.77 $\pm$ 1.36 vs. 1.35 $\pm$ 1.55 mm, $P = 0.014$). Aortic dilatation rate in the trajectory beyond the aortic root was not significantly reduced by losartan. In patients with prior aortic root replacement, aortic arch dilatation rate was significantly lower in the losartan group when compared with the control group (0.50 $\pm$ 1.26 vs. 1.01 $\pm$ 1.31 mm, $P = 0.033$). No significant differences in separate clinical endpoints or the composite endpoint (aortic dissection, elective aortic surgery, cardiovascular death) between the groups could be demonstrated.

Conclusion In adult Marfan patients, losartan treatment reduces aortic root dilatation rate. After aortic root replacement, losartan treatment reduces dilatation rate of the aortic arch.

Keywords Marfan syndrome • Losartan • Aortic root • Magnetic resonance imaging • Aortic dilatation rate

Introduction

Patients with Marfan syndrome (MFS) have an increased risk of sudden death due to aortic dissection, mostly preceded by aortic dilatation.[1–3] Life expectancy has improved due to surgical techniques for prophylactic aortic root replacement[1] and possibly due to β-blocker therapy.[2–4] However, cardiovascular complications remain a major problem.[5–7]

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Marfan syndrome is usually caused by mutations in the *FBN1* gene, leading to deficiency or malformations of the fibrillin-1 protein.[8] Abnormal or deficient fibrillin-1 probably affects structural integrity of the extracellular matrix and may thereby enhance the release of active transforming growth factor-β (TGF-β).[9,10] These processes are assumed to contribute to the development of aortic medial degeneration and subsequent aortic dilatation and/or dissection.[11,12]

Recently, losartan emerged as a potentially effective novel treatment strategy due to its ability to inhibit TGF-β signalling and thereby preventing progressive aortic root dilatation in an MFS mouse model.[13] The apparent beneficial effect of losartan treatment may also be attributed to other mechanisms. Losartan selectively blocks the angiotensin-II type 1 (AT1) receptor within the renin–angiotensin–aldosterone system[14] and attenuates canonical TGF-β signalling in the aorta. Furthermore, losartan inhibits TGF-β-mediated activation of extracellular signal regulated kinase, by allowing continued signalling thought the AT2 receptor.[15] Indeed, losartan was reported to slow down the aortic root dilatation rate in a small retrospective cohort of paediatric patients with a severe MFS phenotype.[16]

The primary aim of the COMPARE (COzaar in Marfan PAtients Reduces aortic Enlargement) study was to determine whether Cozaar (losartan) reduces the aortic dilatation rate at any predefined aortic level in adults with MFS. Additional aims of the study were to determine whether losartan influences aortic volume and incidence of aortic dissection, elective aortic surgery, or cardiovascular death.[17]

Methods

Study design and participants

The design of the COMPARE study was a randomized, multicentre, open-label trial with blinded assessments of endpoints.[17] Patients were enrolled from January 2008 to December 2009. Patients were identified by all four Dutch university hospitals with a specialized multidisciplinary Marfan screening clinic and by using the national database of adults with congenital heart disease (CONCOR).[18] Eligible patients were adults (≥18 years) who were diagnosed with MFS according to the Ghent criteria of 1996.[19] Patients were ineligible if they (i) had a history of angioedema or other known intolerance for angiotensin-converting enzyme inhibitors (ACEi) and/or angiotensin-II receptor blockers (ARB), (ii) were already using ACEi or ARB, (iii) had renal dysfunction, (iv) had a known intolerance for i.v. contrast agents, (v) had an aortic root diameter >50 mm, (vi) had a history of aortic dissection, (vii) had more than one vascular prosthesis, (viii) were planned for aortic surgery within 6 months of inclusion, or (ix) had the intention to become pregnant in the following 3 years. All previously prescribed medication, including β-blockers and calcium channel blockers, was continued after inclusion. 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).

Medication

Patients in the losartan group started on 50 mg daily, and the dosage was doubled after 14 days. When side effects, such as dizziness, syncope, angioedema, or renal dysfunction, occurred; losartan dosage was either reduced or treatment was terminated. Patients were randomly assigned into a 1:1 ratio to receive losartan daily (losartan group) or no additional treatment (control group). Randomization was performed with a computer generated randomization sequence using randomly permuted blocks of 10. We stratified for the four hospitals.

Assessment and outcomes

Participating patients started losartan treatment after baseline examinations. At baseline and after 3 years of follow-up, we examined patients' medical history and performed magnetic resonance imaging (MRI) of the entire aorta. When MRI was contraindicated, computed tomography (CT) was performed. Annually, patients were evaluated by transthoracic echocardiography (TTE) and interviewed for side-effects, changes in medication use and clinical events. Aortic measurements were evaluated independently by three observers (A.W.d.H., R.F., and A.M.S.) without knowledge of patients' medical therapy.

The primary endpoint of this study was aortic dilatation rate at the six predefined aortic levels, from the aortic root to the bifurcation, measured by means of MRI or CT after 3 years of follow-up. The secondary endpoints were (i) total aortic volume expansion rate and (ii) the incidence of the combined endpoint: cardiovascular mortality/aortic dissection/prophylactic aortic surgery. The decision to perform prophylactic aortic surgery was completely at the discretion of the attending cardiologists, based on European and American guidelines.[20,21] When the surgical threshold was reached, patients underwent either a Bentall or David procedure to replace the dilated aortic root. Anticoagulation therapy was initiated when appropriate.

Cardiovascular imaging

All MRI scans were performed at two centres (AMC Amsterdam and LUMC Leiden). Aortic diameters were measured at six landmark levels on the MRI and CT scans; the aortic root, the ascending and descending thoracic aorta at the level of the pulmonary bifurcation, the aortic arch, the descending thoracic aorta at the level of the diaphragm and the abdominal aorta just proximal to the aortic bifurcation. When aortic aneurysms were detected between these landmark levels, separate aneurysm measurements were performed. Aortic volume was measured from the aortic annulus to the aortic bifurcation. Additionally, the aortic root was measured by TTE. (See Appendix A for a detailed description of MRI, CT, and TTE acquisitions.)

Statistical methods

Sample size calculation (330 patients) was based on the primary endpoint. We assumed that the mean aortic root dilatation rate in MFS patients would be 0.9 mm/year,[22] and that losartan would reduce this to 0.5 ± 1.5 mm/year (two-sided $\alpha = 0.05$; $\beta = 0.2$).[17] The effect of losartan on aortic dilatation rate was evaluated by covariance analysis with baseline aortic dimension as covariate. As our primary outcome parameter consisted of the changes of aortic diameters at six levels that were possibly correlated, we performed multiple testing correction by using a permutation approach of 1000 permutations that took the correlations between the diameters at the six levels into account. From the permutation distribution we derived that when using a significance level of 0.0159, the family wise error was maintained at 0.05. The *P*-values of the aorta diameter changes at the six levels were obtained from the permutation Null-distribution. All analyses were performed on the basis of intention-to-treat. Additionally, per protocol and sensitivity analyses were performed. Per protocol analyses were performed to evaluate change in diameter between the two groups of MFS patients who continued their losartan treatment throughout the entire study and in whom losartan treatment was not started during the study, respectively. For the sensitivity analyses, patients who experienced a clinical endpoint were also included. Data shown are mean ± SD. The combined secondary endpoint (aortic dissection, elective aortic

surgery, or cardiovascular death) was evaluated by means of the χ^2 test. The proportions of patients with a stable aortic root diameter during 3 years of follow-up (dilatation rate $\leq$0 mm/3 year) were compared using Fisher's exact test. Covariance analysis was also used to evaluate the losartan effect on aortic dilatation rate in subgroups of patients: males vs. females, with or without a known *FBN1* mutation or β-blocker therapy, mean arterial pressure $\leq$ or $>$90 mmHg, baseline aortic root diameter $\leq$ or $>$45 mm and age $\leq$ or $>$40 years. The mean differences in aortic root dilatation rate between losartan-treated patients and control patients were plotted in a forest plot[23] and tested for significance using the interaction test between treatment-indicator (losartan or no losartan) and subgroup. Data analysis was performed using the SPSS statistical package (19.0 for windows; SPSS, Inc., Chicago, IL, USA).

Results

Patients

From January 2008 until December 2009, 233 patients (38 $\pm$ 13 years, 47% females) were enrolled; 116 were randomly assigned to treatment with losartan and 117 to no additional treatment (*Figure 1*). Patient characteristics at baseline are shown in *Table 1*. Follow-up was 3.1 $\pm$ 0.4 years, similar in both arms. A losartan dosage of 100 mg daily was achieved in 63 patients (54%). In 34 patients (29%), losartan dosage was 50 mg daily; in 2 patients (2%), losartan dosage was reduced to 25 mg and in 17 patients (15%) losartan treatment was ceased due to side-effects, including dizziness caused by low blood pressure ($n = 14$), renal dysfunction ($n = 1$), extreme fatigue ($n = 1$), or angioedema ($n = 1$). In one patient randomized to the control group, losartan was initiated after 2 years (*Figure 1*). Other cardiovascular medicinal treatment regimens did not change during the study between baseline and follow-up. Five patients underwent a contrast-enhanced ECG-triggered CT scan instead of MRI.

Primary endpoint

Aortic root dilatation rate could be evaluated in 145 patients with a native aortic root at the time of exclusion (*Figure 1*). Baseline characteristics were comparable between patients with and without a native aortic root, with exception of the distal aortic dimensions (aortic volume; 222 $\pm$ 56 mL vs. 271 $\pm$ 70 mL, respectively, $P <$ 0.001). There were no statistical significant differences between the losartan treated and control group in these 145 MFS patients with a native aortic root (aortic root diameter; 43.8 $\pm$ 5.0 vs. 43.2 $\pm$ 4.4 mm, $P = 0.436$). The aortic root dilatation rate was significantly lower in the losartan group than in the control group, 0.77 $\pm$ 1.36 vs. 1.35 $\pm$ 1.55 mm/3 years, respectively, $P = 0.014$ (*Table 2*, *Figures 2* and *3*). Aortic root dilatation rate in patients on only losartan therapy was 0.91 $\pm$ 1.25 mm/3 years ($n = 17$) and in patients without losartan or any other form of cardiovascular medical therapy was 1.34 $\pm$ 1.12 mm/3 years ($n = 21$, $P = 0.268$). The per protocol and sensitivity analyses rendered similar results. Losartan was also significantly associated with reduced aortic root dilatation rate as measured by TTE in the intention-to-treat analysis, respectively, 1.34 $\pm$ 1.51 vs. 1.93 $\pm$ 1.39 mm/3 years, $P = 0.021$ (*Table 2*).

As expected, losartan significantly reduced mean arterial blood pressure by 6 $\pm$ 11 mmHg compared with baseline ($P < 0.001$) and differed significantly from blood pressure changes in the control group after 3 years (3 $\pm$ 9 mmHg, $P = 0.032$). No correlation was found between mean arterial blood pressure or systolic blood pressure with aortic root dimension ($P = 0.855$ and $P =$ 0.819, respectively) or aortic root dilatation rate ($P = 0.716$ and $P = 0.967$, respectively). Furthermore, regression analysis showed that change in the mean arterial blood pressure or change in systolic blood pressure was not correlated with aortic root dilatation rate in patients treated with losartan or controls (respectively, $r = 0.058$; $P = 0.630$ and $r = 0.001$; $P = 0.993$, *Figure 4*). The percentage of participants with a stable aortic root (defined as a dilatation rate $\leq$0 mm/3 years) was 50% in the losartan group and 31% in the control group ($P = 0.022$), with a number-needed-to-treat of 5.3 patients.

Aortic dilatation rate beyond the aortic root was evaluated in 218 patients (*Figure 1*). Aortic dilatation rate in the trajectory beyond the aortic root was not significantly reduced by losartan (*Table 2*).

Secondary outcomes

Aortic volume increase was assessed in 168 MFS patients with a native aortic root or aortic root replacement prior to study inclusion (excluded due to technical issues: 33, clinical endpoints: 20, refusal: 12, see *Figure 1*). In the intention-to-treat-analysis, the total aortic volume increase was similar in both groups (*Table 2*). The per protocol and sensitivity analyses rendered similar results.

A total of 19 patients underwent prophylactic aortic surgery due to progressive aortic dilatation. No difference in separate clinical endpoints or the composite endpoint was found between the groups (prophylactic aortic root surgery: 10 vs. 8, distal aortic surgical intervention: 0 vs. 1, type B aortic dissection: 0 vs. 2, respectively, for the losartan and control groups). No cardiovascular deaths occurred during the study.

Losartan treatment and aortic root replacement

A history of aortic root replacement prior to inclusion was present in 63 patients (27 in the losartan group). At baseline, patients with aortic root replacement demonstrated greater aortic dimensions in the remaining aortic trajectory when compared with the total patient cohort. Furthermore, patients randomized to losartan demonstrated smaller dimensions of the aortic arch and the descending thoracic aorta at the level of the diaphragm when compared with the control group at baseline (respectively, 24 $\pm$ 3 vs. 26 $\pm$ 4 mm, $P =$ 0.029 and 21 $\pm$ 2 vs. 23 $\pm$ 4 mm, $P = 0.009$).

Patients with prior aortic root replacement demonstrated greater distal aortic dilatation rates when compared with unoperated patients (*Tables 3* and *4*). After aortic root replacement, aortic arch dilatation rate was significantly lower in the losartan group than in the control group (0.50 $\pm$ 1.26 vs. 1.01 $\pm$ 1.31 mm/3 years, respectively, $P = 0.033$). Aortic dilatation rate in the descending aorta at the level of the pulmonary artery and diaphragm was comparable between the groups (*Table 3*). No significant difference in aortic volume increase between groups could be demonstrated (*Table 3*). However, operated patients in the control group showed a significantly larger increase in aortic volume during the follow-up than unoperated patients (20 $\pm$ 18 vs. 8 $\pm$ 13 mL/3 years, respectively, $P = 0.004$). Losartan-treated patients in the operated and

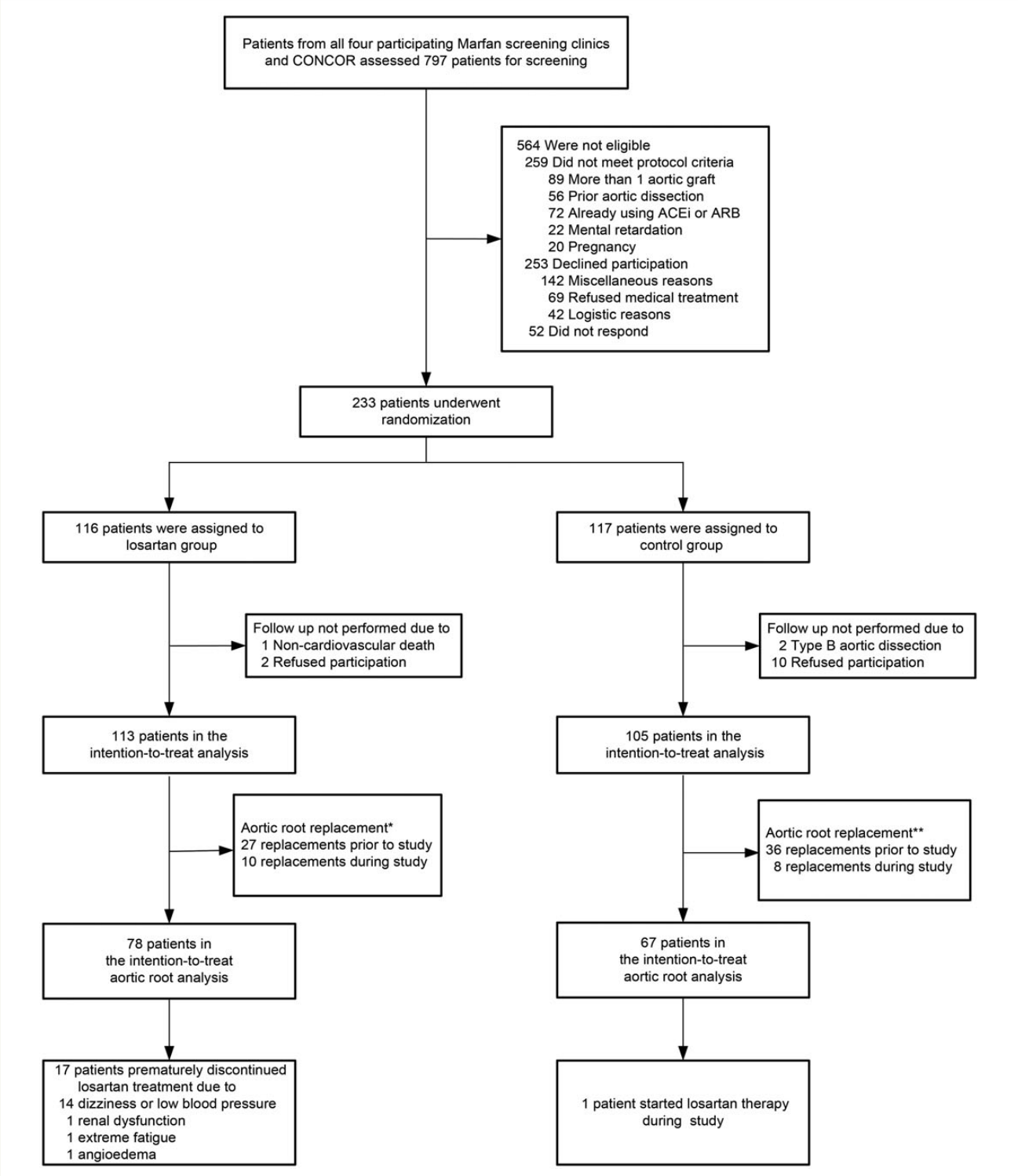

Figure 1 Randomization and follow-up for aortic diameter analysis. Patients were excluded from aortic diameter analysis due to refusal of participation in follow-up, non-cardiovascular death in the losartan group or type B aortic dissection in the control group. ACEi denotes angiotensin-converting enzyme inhibitors and ARB denotes angiotensin-II receptor blockers. *After 3 years, a total of 37 patients had an aortic root graft (27 prior to the study and 10 during the study) in the losartan group. Of these 37 patients, 2 are not included in the box 'aortic root replacement' due to non-cardiovascular death and refusal to participate in follow-up. **After 3 years, a total of 44 patients had an aortic root graft (36 prior to the study and 8 during the study) in the control group. Six of the 44 patients are not included in the box 'aortic root replacement' due to refusal to participate in follow-up.

Table 1 Baseline demographic and clinical characteristics of the patients[a]

Variables	Control, $n = 117$	Losartan, $n = 116$
General features		
Gender (female)	62 (53.0)	47 (40.5)
Body surface area (m^2)	2.0 ± 0.2	2.0 ± 0.2
Age (years)	38.3 ± 13.4	36.8 ± 12.3
≤40 years	69 (59.0)	70 (60.3)
>40 years	48 (41.0)	46 (39.7)
Cardiovascular medication usage		
β-blocker	82 (70.1)	87 (75.0)
Ca^{2+} channel blocker	3 (2.6)	2 (1.7)
Blood pressure		
Systolic (mmHg)	125 ± 13	124 ± 14
Diastolic (mmHg)	74 ± 10	74 ± 11
Mean arterial pressure		
≤90 mmHg	65 (55.6)	63 (54.3)
>90 mmHg	52 (44.4)	53 (45.7)
Aortic root		
≤45 mm	50 (61.7)	44 (49.4)
>45 mm	31 (38.3)	45 (50.6)
FBN1 mutation[b]	97 (88.2)	86 (74.8)
Aortic root surgery	36 (30.8)	27 (23.3)
Distal aorta surgery	5 (4.3)	2 (1.7)
Mitral valve prolapse	65 (55.6)	63 (54.3)
Mitral valve surgery	5 (4.3)	4 (3.4)
Aortic dimensions by MRI (mm)		
Aortic root	43.7 ± 4.8	44.8 ± 5.6
Z-score aortic root	3.8 ± 1.6	3.9 ± 1.5
Ascending aorta	28.1 ± 3.9	28.0 ± 3.6
Aortic arch	24.4 ± 3.3	23.6 ± 2.8
Descending aorta		
Pulmonary artery	23.9 ± 3.6	23.7 ± 3.7
Diaphragm	21.2 ± 3.5	20.3 ± 2.5
Abdominal	16.2 ± 3.4	16.4 ± 3.9
Aortic volume (mL)	244 ± 70	226 ± 55
Aortic dimensions by TTE		
Aortic root (mm)	42.7 ± 4.4	43.3 ± 5.0

TTE, transthoracic echocardiography; MRI, magnetic resonance imaging.
[a]Plus–minus values are means ± SD.
[b]FBN1 analyses were not performed in one patient in the losartan group and in three patients in the control group. In five patients from the losartan group mutations were found in the TGFB2 and MYH11 ($n = 1$) gene. In four patients from the control group mutations were found in *TGFB2* ($n = 1$), *MYLK* ($n = 1$), *MYH11* ($n = 1$), and *TGFBR1* ($n = 1$) gene.

Table 2 Primary outcomes in the intention-to-treat population during the study period[a]

Outcome	Control, $n = 105$	Losartan, $n = 113$	*P*-value[†]
Aortic dilatation rate by MRI			
Aortic root[b]	1.35 ± 1.55	0.77 ± 1.36	0.014
Ascending aorta	0.85 ± 1.23	0.78 ± 1.32	0.726
Aortic arch	0.61 ± 1.35	0.52 ± 1.37	0.598
Descending aorta			
Pulmonary artery	0.72 ± 1.40	0.54 ± 1.40	0.366
Diaphragm	0.43 ± 1.13	0.31 ± 1.13	0.472
Abdominal	0.37 ± 1.12	0.51 ± 2.18	0.594
Aortic volume	12 ± 16	12 ± 14	0.812
Aortic dilatation rate by TTE			
Aortic root	1.93 ± 1.39	1.34 ± 1.51	0.021

TTE, transthoracic echocardiography; MRI, magnetic resonance imaging.
[a]Data are change in millimetre per 3 years, with the exception of aortic volume (millilitre per 3 years) (Plus–minus values are means ± SD).
[b]Aortic root assessed in 145 patients (67 in the control group, 78 in the losartan group).
[†]*P*-value after multiple testing correction.

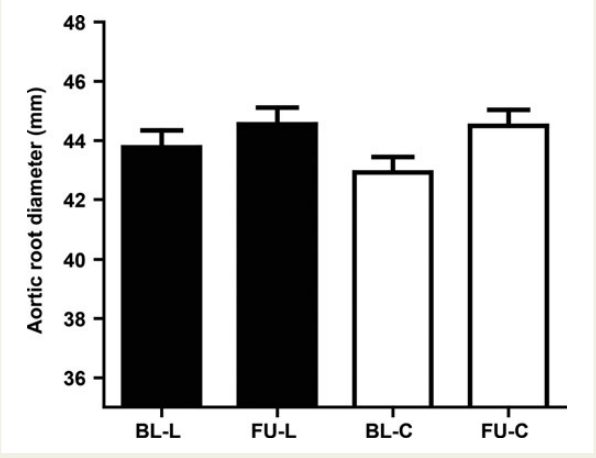

Figure 2 Aortic root dimensions at baseline and after 3 years of follow-up of both groups. BL-L denotes baseline aortic root diameter of patients treated with losartan, FU-L denotes follow-up aortic root diameter of patients treated with losartan, BL-C denotes baseline aortic root diameter of patients without losartan therapy, FU-C denotes follow-up aortic root diameter of patients without losartan therapy. Data shown are mean ± standard error of the mean.

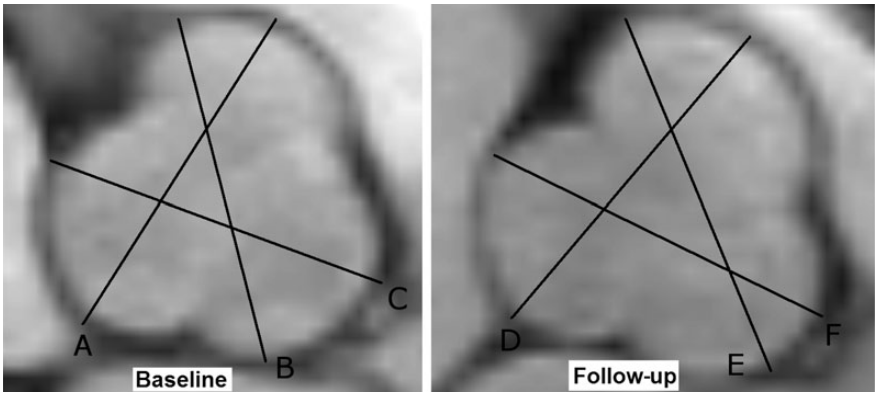

Figure 3 Magnetic resonance images showing the aortic root in short axis of a COMPARE patient with Marfan syndrome at baseline and after 3 years of follow-up. Greatest aortic root diameter of three measured distances was used; (*A* and *D*) non coronary cusp to right coronary cusp increased from 41 to 43 mm; (*B* and *E*) right coronary cusp to left coronary cusp increased from 42 to 44 mm; (*C* and *F*) non-coronary cusp to left coronary cusp increased from 41 to 42 mm.

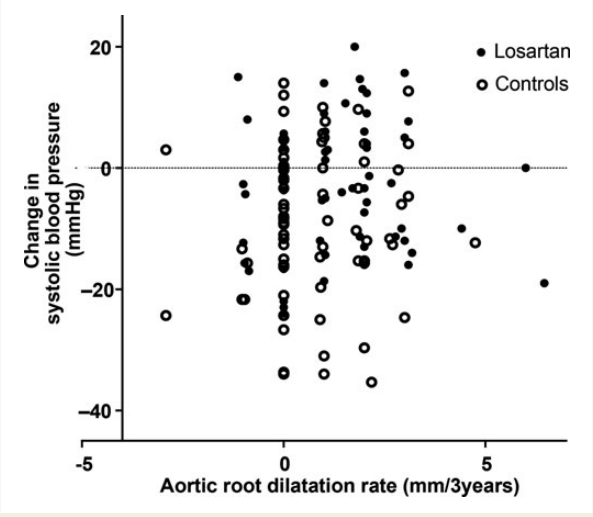

Figure 4 No correlation between systolic blood pressure change and aortic root dilatation rate in both groups. Correlation between change in systolic blood pressure and aortic root dilatation rate in patients treated with losartan ($r = 0.058$, $P = 0.630$) and in controls ($r = 0.001$, $P = 0.993$).

unoperated subgroups did not show this disparity (15 ± 10 vs. 11 ± 15 mL/3 years, respectively, $P = 0.488$).

Subgroup analysis

No subgroups (*FBN1* mutation, mean arterial pressure, aortic root diameter, concomitant β-blocker usage, gender, and age) could be identified in whom losartan therapy was more beneficial in reducing aortic root dilatation rate (*Figure 5*). No interaction between treatment-indicator and subgroups could be demonstrated ($P > 0.467$).

Table 3 Aortic dilatation rate by magnetic resonance imaging in patients with aortic root replacement at baseline[a]

Outcome	Control, *n* = 36	Losartan, *n* = 27	*P*-value
Aortic arch	1.01 ± 1.31	0.50 ± 1.26	0.033
Descending aorta			
Pulmonary artery	1.00 ± 1.25	0.50 ± 1.79	0.249
Diaphragm	0.48 ± 1.37	0.41 ± 1.04	0.376
Abdominal	0.16 ± 1.37	0.71 ± 3.02	0.348
Aortic volume	20 ± 18	15 ± 10	0.438

MRI, magnetic resonance imaging.
[a]Data are change in millimetre per 3 years, with the exception of aortic volume (millilitre per 3 years) (Plus–minus values are means ± SD).

Table 4 Aortic dilatation rate by magnetic resonance imaging in patients with a native aortic root at baseline[a]

Outcome	Control, *n* = 73	Losartan, *n* = 82	*P*-value
Aortic arch	0.44 ± 1.35	0.52 ± 1.41	0.809
Descending aorta			
Pulmonary artery	0.60 ± 1.45	0.55 ± 1.27	0.833
Diaphragm	0.40 ± 1.02	0.28 ± 1.15	0.441
Abdominal	0.46 ± 1.00	0.46 ± 1.92	0.348
Aortic volume	8 ± 13	11 ± 15	0.292

MRI, magnetic resonance imaging.
[a]Data are change in millimetre per 3 years, with the exception of aortic volume (millilitre per 3 years) (Plus–minus values are means ± SD).

Discussion

This is the first prospective, randomized, controlled trial indicating a beneficial effect of losartan treatment on aortic root dilatation rate in adults with MFS. The reduction of mean aortic root dilatation rate in the losartan group was present, irrespective of age, sex, blood pressure, aortic root size, presence of a *FBN1* mutation and concomitant β-blocker use (*Figure 5*). As subgroup analyses were performed on relatively small groups of patients, these results should be interpreted with some prudence. Subgroup analysis on aortic root dilatation rate between patients on only losartan and those without medical therapy did not show a significant difference, due to limited sample size. The effects of losartan monotherapy on aortic root dilatation rate will have to be awaited from other studies.

Although we could not demonstrate a significant association of losartan treatment with reduced aortic dilatation rate beyond the aortic root or with clinical events, losartan was significantly associated with reduced dilatation rate of the aortic arch in the subgroup of patients with a history of aortic root surgery. However, this result should be interpreted with some caution as baseline aortic dimensions of patients with prior aortic root replacement were not completely comparable between the groups.

We found large variability in individual aortic root dilatation rates in the losartan group and losartan treatment did not normalize the dilatation rate to that of the healthy population (aortic root dilatation rate of 0.8–0.9 mm for each advancing decade of life).[24] The large interindividual differences in response to losartan treatment may be partly explained by genetic factors, such as different types of *FBN1* mutations[25] and genetic modifiers, especially those involved in other inflammatory pathways[26] and partly by interindividual variation in forces acting on the aortic tissue. However, subgroup analyses could not identify any patient group with larger or smaller reduction in aortic root dilatation rate, most likely due to lack of power.

Furthermore, aortic root diameters measured by MRI were larger than measured by TTE (mean difference 0.9 ± 1.6 mm); however, aortic root dilatation rate reduction measured by MRI was comparable with TTE findings (0.58 and 0.59 mm). This phenomenon has been observed and explained previously.[27]

The incidence of clinical events was low in our study. Therefore, the clinical relevance of losartan treatment on aortic surgery and aortic dissection could not be determined by this trial and requires a prospective study with longer follow-up and a much larger sample size. The low incidence of aortic dissections and the absence of death in our study may have been caused by the low threshold for prophylactic aortic root surgery in MFS at 45–50 mm according to current guidelines.[20,21]

In the current era of aggressive surgical prophylactic treatment, ascending aortic dissection has become a rare event in patients with known MFS. As a corollary, the fate of the aortic trajectory beyond the aortic root has become a major clinical issue. We

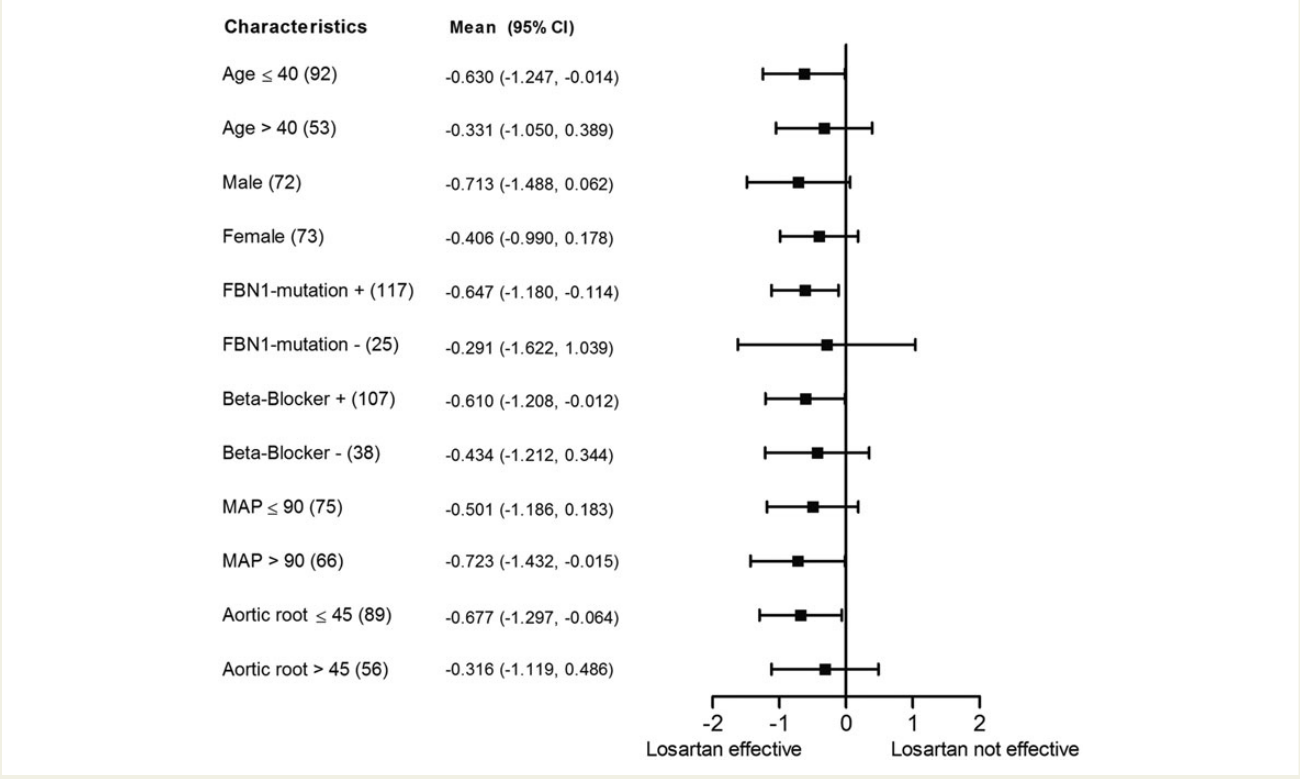

Figure 5 Effect of losartan treatment on aortic root dilatation rate in subgroups of Marfan patients. Among subgroups of patients, the mean differences in aortic root dilatation rate between losartan treated patients and control patients are indicated by solid squares. Horizontal lines represent 95% confidence intervals (95% CI). (*n*) denotes number of patients in subgroup-analysis, MAP (mean arterial pressure, mmHg), aortic root is presented in mm, age is presented in years.

could not demonstrate a reduction of aortic dilatation rate beyond the aortic root associated with losartan treatment in the entire cohort, most likely due to lack of power. Another possible explanation may relate to the different developmental origin of aortic root (neural crest cells) when compared with the remaining aorta. Neural crest cells showed to have a different response to TGF-β signalling.[28,29]

By blocking the AT1-receptor, losartan reduces arterial blood pressure and wall stress by vaso-active mechanisms.[14] In our study, the mean arterial blood pressure was significantly reduced in the losartan treatment group when compared with the controls. However, no correlation was found between change in the mean arterial pressure or systolic blood pressure with aortic root dilatation rate. A possible explanation for the beneficial effect of losartan might be due to the ability of losartan to inhibit TGF-β signalling. Transforming growth factor-β antagonists, other than losartan, that have no effect on blood pressure, provide overt vascular protection in mouse models.[15] However, wall stress appears to be an essential precondition for the development of aortic media degeneration, as shown by the completely normal architecture of the aortic wall in newborn MFS mice. Therefore, the beneficial effects of losartan may be caused by both signalling and blood pressure-lowering effects.

Targeted treatment dosage of 100 mg losartan daily was reached in only 54% of the patients, mainly due to side-effects, such as hypotension by concomitant β-blocker use. However, in the intention-to-treat analysis, which includes the lower losartan treatment dosage as well, a beneficial effect of losartan on aortic root dilatation rate could be demonstrated. A possible explanation could be that a low dosage of losartan daily might already be enough to inhibit a TGF-β signalling cascade.

We observed that the trajectory beyond the aortic root dilates more progressively in patients with a history of aortic root replacement, as previously reported.[6,30,31] The aortic dilatation rate of the aortic trajectory beyond the aortic root may be enhanced by haemodynamic factors, altered wall mechanics, loss of the Windkessel effect with higher pulsatile forces acting onto the descending aorta, or clamping of the aorta during the operation.[6]

In summary, losartan treatment reduces aortic root dilatation rate in adults with MFS with a number-needed-to-treat of 5.3 patients when comparing the percentage of patients with stable aortic root between both groups. Following prophylactic aortic root replacement, losartan treatment also has a beneficial effect on dilatation rate in the aortic arch.

Study limitations

We were unable to enrol the original defined total sample size of 330 patients, mainly due to our strict inclusion and exclusion criteria. Second, inclusion was limited to the patients known at the designated four Dutch university hospitals with a specialized multidisciplinary Marfan screening clinic and by using the national database of adults with congenital heart disease (CONCOR).

Aortic root dilatation rate was overestimated in our original sample size analysis (dilatation rate: 1.35 mm/3 years as opposed to the expected 2.7 mm/3 years). Although this could be interpreted as a result of a less severely affected study cohort, similar aortic root dilatation rates have been reported previously.[32]

Another limitation of our study design is that aortic root dilatation rate could only be assessed in 145 MFS patients with a native aortic root. The decision to include MFS patients with prior aortic root replacement in our study was based on the hypothesis that losartan might also have a beneficial effect on the aortic trajectory beyond the aortic root. Furthermore, this is clinically highly relevant as a large proportion of the current MFS population already underwent aortic root replacement or most likely will undergo in the near future.

Furthermore, the open label character of this study is a limitation. We were persuaded by the clinical urgency to rapidly assess the effect of losartan after the positive results in mice. A double-blinded study would have delayed this process. More and more patients were already being treated with losartan before any evidence of a beneficial effect in humans (see *Figure 1*). Therefore, we decided to perform an open label study in collaboration with the Dutch Marfan Organisation. Nevertheless, the endpoints were evaluated without knowledge of patients' medical therapy.

Appendix A

Image acquisition

Magnetic resonance imaging acquisition was performed by either an Avanto (Siemens, Erlangen, Germany) or a Philips (Intera, release 11 and 12; Philips Medical Systems, Best, the Netherlands) 1.5 Tesla MRI scanner using a phased array cardiac receiver coil.

Contrast-enhanced magnetic resonance angiography (MRA) of the total aorta was performed by first pass imaging of 0.2 mL/kg body weight contrast bolus of gadovist (Bayer Schering AG, Berlin, Germany) with a molarity of 1 mmol/L. Contrast agent was injected intravenously in the brachial vein at an infusion rate of 2 mL/s, and subsequently flushed by 20 mL saline at 2 mL/s, using contrast power injectors (Mallinckrodt, Inc., St Louis, MO, USA or Medrad Spectris Solaris EP MR Injection System, Warrendale, USA). Contrast enhanced MRA image acquisition was triggered by scout imaging of the contrast bolus and aimed to visualize the total aorta during first pass of the contrast bolus in the aorta. Imaging occurred during breath-holding at end-inspiration. The contrast enhanced MRA of the full aorta was acquired by means of a standard, commercially available non-ECG gated 3D, T1-weighed, spoiled gradient-echo sequence (either 3DFLASH on the Siemens system or 3DFFE on the Philips system). This resulted in a 3D presentation of the entire aorta with a near-isotropic resolution of 1.4 × 1.3 × 1.4 mm/voxel.

In patients without aortic root replacement, aortic root size was assessed by cine imaging sequences (Steady State Free Precession, SSFP) perpendicular to the long axis of the aortic root as shown by coronal and sagittal scouts (either TrueFisp on the Siemens system or Balanced TFE on the Philips system) during end-expiration. Typical SSFP characteristics were: slice thickness 6 mm, flip angle 60–80°, field of view 300–400 mm, matrix size 256 × 192, 25–50 frames per cardiac cycle. These acquisitions resulted in a CINE short-axis representation of the aortic root at the level of the sinus of Valsalva with an in-plane spatial resolution of 1.2–1.8 × 1.4–1.8 mm/pixel and a temporal resolution of ∼20–30 ms.

Transthoracic echocardiography was performed with a Vivid 7 (GE, Vingmed Ultrasound, Horton, Norway) ultrasound system by experienced ultrasound technicians. Aortic root diameters were

measured in end-diastole at the level of the sinus of Valsalva, by using the leading edge to leading edge technique in parasternal long axis, consistent with the current American Society of Echocardiography guidelines.[33]

Computed tomography was performed by the use of a Philips 64 slice CT scanner, using generally available iodine-based contrast agents in a small number of patients.

Image processing

Aortic root diameter was assessed by greatest end-diastolic diameter of three cusp-cusp dimensions from the outer to inner wall on the SSFP images. All measurements beyond the aortic root were performed on multiplanar MRA reconstructions from inner to inner edge.

The Vessel analysis software (3 mensio vascular, 3mensio Medical Imaging BV, Bilthoven, the Netherlands) was used to calculated aortic volumes. Intra- and inter-observer variability of aortic volume assessment showed excellent reproducibility.[34] Images were loaded in the software with window and level settings acquired from the DICOM data. The total aortic volume was determined by the following technique; a central lumen line was created by manually placing a seeding point through the lumen of the aorta in the axial, the sagittal, and the coronal plane. A complete set of multi-planar reformats was reconstructed by the computer perpendicular to this central lumen line, resulting in a stretched vessel view of the aorta, from the aortic valve to the aortic bifurcation. The aortic lumen was manually separated from the surrounding tissue by placing a cut-off line between the enhanced aortic lumen voxels and the surrounding voxels in four cross-sections. The volume of the contrast-enhanced aortic lumen was reconstructed from the individually segmented axial slices, starting at the level of the aortic root and ending at the level of the aortic bifurcation.

Acknowledgments

The authors would also like to thank 3Mensio Medical Imaging, suppliers of the semi-automated vessel analysis software.

Funding

This study was funded by a grant from the Dutch Heart Association (2008B115) and supported by the Interuniversity Cardiology Institute of the Netherlands (ICIN).

Conflict of interest: None declared.

References

1. Silverman DI, Burton KJ, Gray J, Bosner MS, Kouchoukos NT, Roman MJ, Boxer M, Devereux RB, Tsipouras P. Life expectancy in the Marfan syndrome. *Am J Cardiol* 1995;**75**:157–160.
2. Engelfriet P, Mulder B. Is there benefit of beta-blocking agents in the treatment of patients with the Marfan syndrome? *Int J Cardiol* 2007;**114**:300–302.
3. Hartog AW, Franken R, Zwinderman AH, Groenink M, Mulder BJ. Current and future pharmacological treatment strategies with regard to aortic disease in Marfan syndrome. *Expert Opin Pharmacother* 2012;**13**:647–662.
4. Shores J, Berger KR, Murphy EA, Pyeritz RE. Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. *N Engl J Med* 1994;**330**:1335–1341.
5. Cameron DE, Alejo DE, Patel ND, Nwakanma LU, Weiss ES, Vricella LA, Dietz HC, Spevak PJ, Williams JA, Bethea BT, Fitton TP, Gott VL. Aortic root replacement in 372 Marfan patients: evolution of operative repair over 30 years. *Ann Thorac Surg* 2009;**87**:1344–1349.
6. Engelfriet PM, Boersma E, Tijssen JG, Bouma BJ, Mulder BJ. Beyond the root: dilatation of the distal aorta in Marfan's syndrome. *Heart* 2006;**92**:1238–1243.
7. Groenink M, Lohuis TA, Tijssen JG, Naeff MS, Hennekam RC, Van Der Wall EE, Mulder BJ. Survival and complication free survival in Marfan's syndrome: implications of current guidelines. *Heart* 1999;**82**:499–504.
8. Robinson PN, Arteaga-Solis E, Baldock C, Collod-Beroud G, Booms P, De Paepe A, Dietz HC, Guo G, Handford PA, Judge DP, Kielty CM, Loeys B, Milewicz DM, Ney A, Ramirez F, Reinhardt DP, Tiedemann K, Whiteman P, Godfrey M. The molecular genetics of Marfan syndrome and related disorders. *J Med Genet* 2006;**43**:769–787.
9. Ramirez F, Dietz HC. Marfan syndrome: from molecular pathogenesis to clinical treatment. *Curr Opin Genet Dev* 2007;**17**:252–258.
10. Franken R, den Hartog AW, de Waard V, Engele L, Radonic T, Lutter R, Timmermans J, Scholte AJ, van den Berg MP, Zwinderman AH, Groenink M, Mulder BJ. Circulating transforming growth factor-beta as a prognostic biomarker in Marfan syndrome. *Int J Cardiol*; doi: 10.1016/j.ijcard.2013.03.033. Published online ahead of print 10 April 2013.
11. Carlson RG, Lillehei CW, Edwards JE. Cystic medial necrosis of the ascending aorta in relation to age and hypertension. *Am J Cardiol* 1970;**25**:411–415.
12. Nagashima H, Sakomura Y, Aoka Y, Uto K, Kameyama K, Ogawa M, Aomi S, Koyanagi H, Ishizuka N, Naruse M, Kawana M, Kasanuki H. Angiotensin II type 2 receptor mediates vascular smooth muscle cell apoptosis in cystic medial degeneration associated with Marfan's syndrome. *Circulation* 2001;**104**(12 Suppl. 1): I282–I287.
13. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi C, Podowski M, Neptune ER, Halushka MK, Bedja D, Gabrielson K, Rifkin DB, Carta L, Ramirez F, Huso DL, Dietz HC. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science* 2006;**312**: 117–121.
14. Burnier M. Angiotensin II type 1 receptor blockers. *Circulation* 2001;**103**:904–912.
15. Habashi JP, Doyle JJ, Holm TM, Aziz H, Schoenhoff F, Bedja D, Chen Y, Modiri AN, Judge DP, Dietz HC. Angiotensin II type 2 receptor signaling attenuates aortic aneurysm in mice through ERK antagonism. *Science* 2011;**332**:361–365.
16. Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC III. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. *N Engl J Med* 2008;**358**: 2787–2795.
17. Radonic T, de Witte P, Baars MJ, Zwinderman AH, Mulder BJ, Groenink M. Losartan therapy in adults with Marfan syndrome: study protocol of the multi-center randomized controlled COMPARE trial. *Trials* 2010;**11**:3.
18. van der Velde ET, Vriend JW, Mannens MM, Uiterwaal CS, Brand R, Mulder BJ. CONCOR, an initiative towards a national registry and DNA-bank of patients with congenital heart disease in the Netherlands: rationale, design, and first results. *Eur J Epidemiol* 2005;**20**:549–557.
19. De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996;**62**:417–426.
20. Baumgartner H, Bonhoeffer P, De Groot NM, de HF, Deanfield JE, Galie N, Gatzoulis MA, Gohlke-Baerwolf C, Kaemmerer H, Kilner P, Meijboom F, Mulder BJ, Oechslin E, Oliver JM, Serraf A, Szatmari A, Thaulow E, Vouhe PR, Walma E. ESC Guidelines for the management of grown-up congenital heart disease (new version 2010). *Eur Heart J* 2010;**31**:2915–2957.
21. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE Jr, Eagle KA, Hermann LK, Isselbacher EM, Kazerooni EA, Kouchoukos NT, Lytle BW, Milewicz DM, Reich DL, Sen S, Shinn JA, Svensson LG, Williams DM. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. *Circulation* 2010;**121**:e266–e369.
22. Roman MJ, Rosen SE, Kramer-Fox R, Devereux RB. Prognostic significance of the pattern of aortic root dilation in the Marfan syndrome. *J Am Coll Cardiol* 1993;**22**: 1470–1476.
23. Lewis S, Clarke M. Forest plots: trying to see the wood and the trees. *BMJ* 2001;**322**: 1479–1480.
24. Vasan RS, Larson MG, Levy D. Determinants of echocardiographic aortic root size. The Framingham Heart Study. *Circulation* 1995;**91**:734–740.
25. Judge DP, Biery NJ, Keene DR, Geubtner J, Myers L, Huso DL, Sakai LY, Dietz HC. Evidence for a critical contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. *J Clin Invest* 2004;**114**:172–181.
26. Radonic T, de Witte P, Groenink M, de Waard V, Lutter R, van Eijk M, Jansen M, Timmermans J, Kempers M, Scholte AJ, Hilhorst-Hofstee Y, van den Berg MP, van Tintelen JP, Pals G, Baars MJ, Mulder BJ, Zwinderman AH. Inflammation aggravates disease severity in Marfan syndrome patients. *PLoS One* 2012;**7**:e32963.

27. Meijboom LJ, Groenink M, Van Der Wall EE, Romkes H, Stoker J, Mulder BJ. Aortic root asymmetry in marfan patients; evaluation by magnetic resonance imaging and comparison with standard echocardiography. *Int J Card Imaging* 2000;**16**:161–168.
28. ten Dijke P, Arthur HM. Extracellular control of TGFbeta signalling in vascular development and disease. *Nat Rev Mol Cell Biol* 2007;**8**:857–869.
29. Topouzis S, Majesky MW. Smooth muscle lineage diversity in the chick embryo. Two types of aortic smooth muscle cell differ in growth and receptor-mediated transcriptional responses to transforming growth factor-beta. *Dev Biol* 1996; **178**:430–445.
30. Mulder BJ. The distal aorta in the Marfan syndrome. *Neth Heart J* 2008;**16**:382–386.
31. Nollen GJ, Meijboom LJ, Groenink M, Timmermans J, Barentsz JO, Merchant N, Webb GD, Lamb HJ, Tijssen JG, Van Der Wall EE, Mulder BJ. Comparison of aortic elasticity in patients with the Marfan syndrome with and without aortic root replacement. *Am J Cardiol* 2003;**91**:637–640.
32. Meijboom LJ, Timmermans J, Zwinderman AH, Engelfriet PM, Mulder BJ. Aortic root growth in men and women with the Marfan's syndrome. *Am J Cardiol* 2005;**96**: 1441–1444.
33. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 2005;**18**:1440–1463.
34. den Hartog AW, Franken R, de Witte P, Radonic T, Marquering HA, van der Steen WE, Timmermans J, Scholte AJ, van den Berg MP, Zwinderman AH, Mulder BJ, Groenink M. Aortic disease in patients with Marfan syndrome: aortic volume assessment for surveillance. *Radiology*; doi: 10.1148/radiol.13122310. Published online ahead of print 25 June 2013.

CARDIOVASCULAR FLASHLIGHT

doi:10.1093/eurheartj/eht228
Online publish-ahead-of-print 2 July 2013

Renal sympathetic denervation in resistant hypertension late after surgical repair for aortic coarctation

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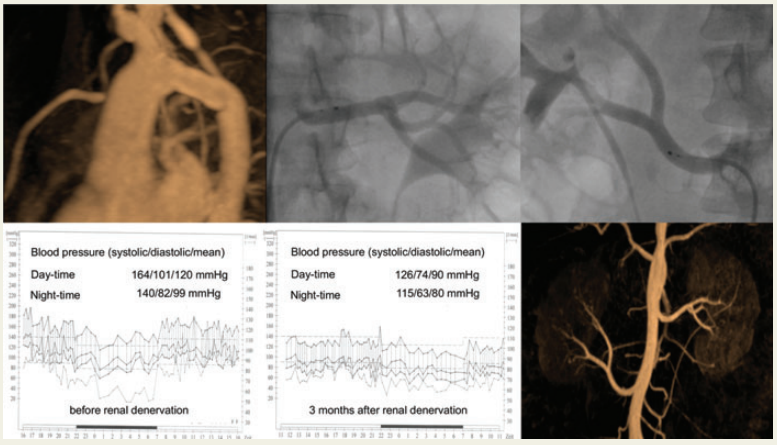

Here, we report the case of a 32-year-old male with previous surgery for aortic coarctation, who was referred for diagnostics and management of resistant arterial hypertension. The patient had undergone subclavian flap repair at the age of 10 followed by second open-heart surgery including partial replacement of the descending aorta and conduit insertion to the left subclavian artery 6 years later. Additionally, coiling of an intracranial aneurysm was performed at the age of 30. Ambulatory blood pressure measurements revealed inadequate blood pressure control (*Panel*, lower left) despite treatment with five different antihypertensive drugs including diuretics. Magnetic resonance imaging confirmed an anatomically satisfying repair with no evidence for recurrent coarctation (*Panel*, upper left). Haemodynamic assessment demonstrated a gradient across the site of previous repair of 5 mmHg. We, therefore, proceeded with renal sympathetic denervation using the Simplicity^{TM} catheter (Medtronic, MN, USA). Six ablations were performed in the right and seven in the left renal artery (*Panel*, upper mid and right). During the follow-up, antihypertensive medication remained unchanged. At 3-month follow-up, ambulatory blood pressure measurements showed marked improvements in daytime and nighttime blood pressure control (*Panel*, lower left and mid). Also, magnetic resonance angiography excluded any stenosis of the renal arteries (*Panel*, lower right).

The observed positive effects of renal denervation in this specific type of secondary hypertension bare the hope that this innovative technique might extend the currently very limited armory against hypertension in young adults with previous repair of aortic coarctation.