

Rationale and design of a randomized clinical trial of β -blocker therapy (atenolol) versus angiotensin II receptor blocker therapy (losartan) in individuals with Marfan syndrome

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Background Cardiovascular disease, including aortic root dilation, dissection, and rupture, is the leading cause of mortality in patients with Marfan syndrome (MFS). The maximal aortic root diameter at the sinuses of Valsalva is considered the best predictor of adverse cardiovascular outcome. Although advances in therapy have improved life expectancy, affected individuals continue to suffer cardiovascular morbidity and mortality. Recent studies in an *FBN1*-targeted mouse model of MFS with aortic disease similar to that seen in humans showed that treatment with losartan normalized aortic root growth and aortic wall architecture.

Methods The Pediatric Heart Network designed a randomized clinical trial to compare aortic root growth and other short-term cardiovascular outcomes in subjects with MFS receiving atenolol or losartan. Individuals 6 months to 25 years of age with a body surface area-adjusted aortic root z score >3.0 will be eligible for inclusion. The primary aim is to compare the effect of atenolol therapy with that of losartan therapy on the rate of aortic root growth over 3 years. Secondary end points include progression of aortic regurgitation; incidence of aortic dissection, aortic root surgery, and death; progression of mitral regurgitation; left ventricular size and function; echocardiographically derived measures of central aortic stiffness; skeletal and somatic growth; and incidence of adverse drug reactions.

Conclusion This randomized trial should make a substantial contribution to the management of individuals with MFS and expand our understanding of the mechanisms responsible for the aortic manifestations of this disorder. (Am Heart J 2007;154:624-31.)

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The Marfan syndrome

Marfan syndrome (MFS) is a systemic disorder of connective tissue with autosomal dominant inheritance and a prevalence of approximately 1 per 5000 population.¹ The cardinal features of this disorder involve the ocular, musculoskeletal, and cardiovascular systems. Cardiovascular disease, including aortic root dilation, aortic dissection, and myxomatous mitral valve changes, is the leading cause of mortality in MFS. Although early diagnosis and refined medical and surgical management have increased median life expectancy from approximately 40 to 70 years, individuals with MFS continue to suffer important morbidity.²

Up to 90% of individuals with classic MFS will have a cardiovascular "event" during their lifetime, including surgical repair of the aortic root, fatal or nonfatal aortic dissection, or mitral valve surgery.^{3,4} In addition, individuals with MFS may have lens dislocation; skeletal

involvement including anterior chest deformity, scoliosis, and joint hypermobility; lung disease most commonly manifested by spontaneous pneumothorax; decreased skeletal muscle mass and fat stores; and dural ectasia.¹

Etiology of MFS

Marfan syndrome is caused by mutations in *FBNI*, the gene encoding fibrillin 1.⁵ More than 600 *FBNI* mutations have been reported.⁶ Because fibrillin 1 is an important component of the extracellular matrix microfibril,^{7,8} this protein was initially thought to play mainly a structural role in connective tissue. Structural abnormalities leading to weakness in connective tissue seemed to explain some clinical findings such as lens dislocation, joint hypermobility, lung bullae, and aortic dissection but not other features such as bone overgrowth, myxomatous valve changes, and craniofacial abnormalities. Therefore, a more plausible explanation for the changes seen in MFS invokes some combination of altered cellular migration, proliferation, and programmed cell death.

Transforming growth factor β (TGF- β) recently emerged as a potential mediator of these morphogenetic perturbations. The TGF- β s are pluripotential cytokines that regulate cell performance and tissue morphogenesis and homeostasis. They are synthesized and secreted as an inactive precursor (the large latent complex) that binds to the extracellular matrix and requires regulated activation to release free TGF- β for biologic activity.^{9,10} The latent TGF- β -binding protein component of the large latent complex has been localized to extracellular microfibrils and specifically binds to fibrillin 1.^{11,12} The current hypothesis is that abnormal fibrillin causes failure of latent complex sequestration and consequent excessive TGF- β activation,¹³ resulting in the MFS phenotype.

In the fibrillin 1-deficient mouse model,¹³⁻¹⁶ excessive TGF- β signaling has been associated with progressive aortic root dilation, myxomatous mitral valve changes, and failure of lung alveolar septation. Moreover, the aortic, valve, and lung phenotypes can be attenuated or prevented in these mice by systemic administration of an antibody that specifically antagonizes the activity of TGF- β in vivo.^{13,14,16} These data support the paradigm that perturbation of matrix sequestration of TGF- β can contribute to the pathogenesis of MFS.

Current medical approach to aortic root dilation in MFS

The aortic root diameter at the sinuses of Valsalva is considered the best predictor of adverse cardiovascular outcome.⁴ The optimal medical therapy for aortic root dilation has been a matter of vigorous debate.¹⁷⁻²² Because several, although not all, studies have shown that therapy with β -adrenergic blockers (BBs) reduces the rate of aortic growth,¹⁷⁻²⁰ many clinicians consider

BB to be the standard of care. The presumed mechanisms—decreasing proximal aortic shear stress and heart rate—are plausible based on the pathophysiologic analysis; however, treatment with BBs does not prevent attainment of important clinical end points including aortic regurgitation, surgery, dissection, and death. In cases where BB therapy is contraindicated or not tolerated, or by clinician preference, calcium-channel blockers or angiotensin-converting enzyme (ACE) inhibitors are used to reduce the ejection impulse.²² There are no reported randomized trials of these drugs, but ACE inhibitors have the theoretical advantage of inhibiting vascular smooth muscle cell apoptosis based on observations in cultured Marfan aortic media cells.^{21,23}

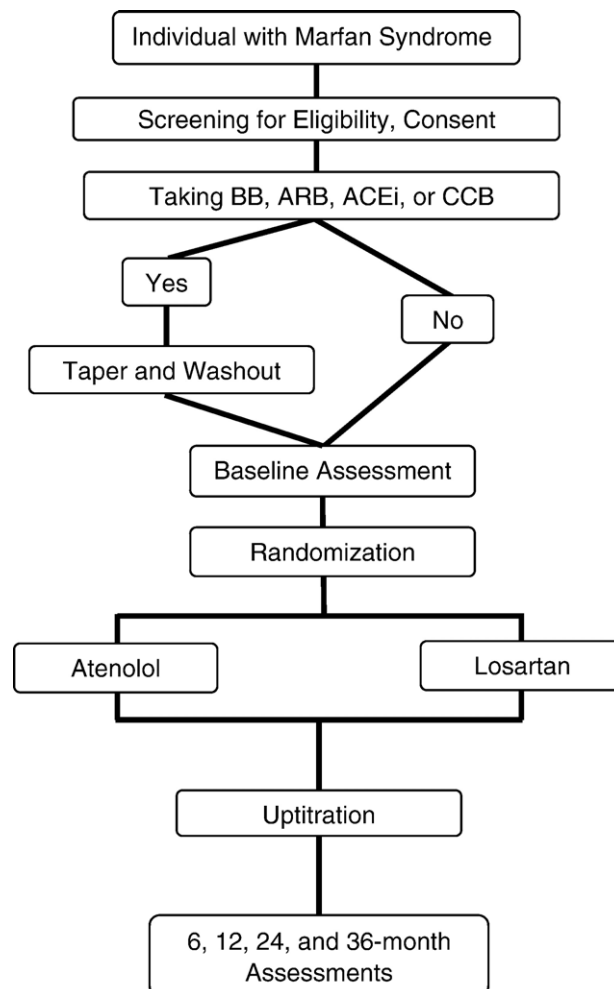
Pharmacologic trials in mouse models of MFS

Numerous studies describe the ability of the angiotensin receptor blocker (ARB), losartan, to achieve clinically relevant inhibition of TGF- β signaling in vivo (reviewed in Habashi et al¹⁶). In several disease states, including chronic renal disease and cardiomyopathy, antifibrotic effects of losartan, independent of hemodynamic effects, have been directly linked to TGF- β inhibition.

To test the hypothesis that angiotensin II type 1 blockade decreases aortic damage, Habashi et al¹⁶ randomized cohorts of 2-month-old mice with a fibrillin 1 mutation found in a patient with classic MFS to receive placebo, propranolol, or losartan. Echocardiography was used to monitor the aortic root. After 6 months, the rate of aortic growth in losartan-treated animals was indistinguishable from that seen in wild-type controls ($P = .55$). Aortic growth in propranolol-treated mice was significantly less than that in the placebo group ($P < .001$) but greater than that in losartan-treated mice ($P < .02$). Aortic wall architecture showed progressive deterioration in untreated and propranolol-treated mice, but the aortic wall architecture in losartan-treated mice could not be distinguished from that in wild-type littermates. Losartan also improved noncardiovascular manifestations of MFS, including distal airspace disease. Furthermore, improvements in the losartan-treated mice correlated with reduced TGF- β signaling. Because blood pressure and heart rate were decreased similarly in BB- and losartan-treated mice, the protection afforded by ARBs goes beyond alteration of hemodynamics to modification of the underlying disease, presumably through antagonism of TGF- β .

Rationale for this trial

Despite the major advances in the medical and surgical management of MFS, morbidity persists. Existing medical therapies do not target the pathogenic basis for MFS; these therapies simply aim to reduce hemodynamic stress on

Figure 1

Flow diagram for the trial. BB; β -Blocker; ARB, angiotensin II receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; CCB, calcium-channel blocker.

predisposed tissue. Angiotensin receptor blocker therapy has the theoretical advantage of modifying the abnormal tissue directly by antagonism of TGF- β . The compelling results of losartan therapy in mice prompted a desire to translate these results systematically to humans. Neither the safety nor the efficacy of administration of losartan in humans with MFS can be evaluated in the absence of a randomized clinical trial. This multicenter, randomized clinical trial will compare outcomes in individuals with MFS randomized to either atenolol or losartan.

Study design and methods

Study overview

This trial is designed to test the hypothesis that ARB therapy with known TGF- β antagonism will reduce the

rates of aortic root diameter growth and progression of aortic regurgitation compared to BB therapy. We will enroll 604 children and young adults who will be randomly assigned to receive BB (atenolol) or ARB (losartan) for 36 months. This study was designed by the National Heart, Lung, and Blood Institute (NHLBI)-funded Pediatric Heart Network (PHN)²⁴ and will be conducted at 14 clinical centers. A flow chart of the study design is shown in Figure 1.

Patient selection

The inclusion criteria was as follows:

1. diagnosis of MFS according to Ghent criteria²⁵;
2. age of 6 months to 25 years;
3. body surface area (BSA)-adjusted aortic root z score >3.0 (sinuses of Valsalva); and

4. informed consent and assent of participant, parent(s), or legal guardian as applicable.

The exclusion criteria was as follows:

1. prior aortic surgery;
2. aortic root dimension at the sinuses of Valsalva >5 cm;
3. planned aortic surgery within 6 months of enrollment;
4. aortic dissection;
5. clinical or molecular diagnosis of other connective tissue disorders that have overlap with MFS (Shprintzen-Goldberg syndrome²⁶ or Loeys-Dietz syndrome¹⁴);
6. therapeutic (eg, for systemic hypertension, arrhythmia, ventricular dysfunction, or valve regurgitation) rather than prophylactic use of ACE inhibitor, BB, or calcium-channel blocker;
7. history of angioedema while taking an ACE inhibitor or BB;
8. intolerance to ARB that resulted in termination of therapy;
9. intolerance to BB that resulted in termination of therapy;
10. renal dysfunction (creatinine level more than upper limit of age-related normal values);
11. asthma;
12. diabetes mellitus;
13. pregnancy or planned pregnancy within 36 months of enrollment; or
14. inability to complete study procedures including history of poor acoustic windows (inability to obtain accurate measurement of aortic root).

The decision to restrict the study subjects to a younger population derives from the likelihood that older individuals with MFS who have not yet required surgery are biased toward milder variants of the disorder and are less likely to demonstrate a treatment effect within the 3-year time frame of this study. A similar rationale led to the requirement for a BSA-adjusted aortic root z score >3.0 at the time of enrollment.

Randomization and stratification

Eligible subjects will be randomly assigned in a 1:1 ratio to receive atenolol or losartan using randomly permuted blocks within strata defined by attainment of maximum height (defined here as 16 years of age for males and 15 years for females²⁷) and BSA-adjusted aortic root z score at baseline (<4.5/ \geq 4.5 SD). Dynamic allocation within center will be used to ensure equal numbers of subjects in each treatment arm at each center.

Study treatments

All subjects on prophylactic therapy with BB, ARB, ACE inhibitor, or calcium-channel blocker before enrollment

will be weaned off medication over a 14-day period. After this, a drug washout period of 14 to 21 days will occur before baseline assessment and randomization.

After the baseline clinical evaluation, subjects will enter the up-titration period, during which they will receive atenolol or losartan. Study drugs will be administered in either liquid or pill form depending on the subject's ability to swallow pills. The goal of the up-titration period is to reach the effective dose (defined below) that will be continued throughout the maintenance phase. Each up-titration cycle will last for 21 to 28 days.

The average starting dose of atenolol will be 0.5 mg/kg, and the dose will be increased in each subsequent cycle by approximately 1 mg/kg to a maximum daily dose of 4 mg/kg, not to exceed 250 mg. The mean heart rate measured using a 24-hour ambulatory electrocardiogram (24-hour ECG) will guide up-titration. The goal of treatment with atenolol will be a \geq 20% decrease in the mean heart rate, which reflects adequate β blockade.^{28,29}

The average starting dose of losartan will be 0.4 mg/kg and the daily dose will be increased as tolerated by approximately 0.4 mg/kg in each subsequent cycle to a maximum daily dose of between 1.0 and 1.4 mg/kg, not to exceed 100 mg.

Masking of treatment group assignment

The primary end point and many of the secondary end points will be measured in the echocardiography core laboratory by personnel masked to treatment group assignment. However, given the difference in heart rate response between the 2 therapies and the differences in up-titration (to heart rate response for atenolol but not for losartan), study personnel supervising up-titration will be aware of the subject's treatment assignment. No one else including subjects, their families, and primary care providers will be informed of the subject's treatment assignment.

Study measurements and subject follow-up

The following will be obtained at baseline and at 6, 12, 24, and 36 months after randomization:

- review of medical history;
- height, weight, ratio of upper to lower segment, and blood pressure;
- electrocardiographic images;
- 24-hour ECG;
- questionnaire regarding adverse drug reactions.

After the maintenance dose is established, subjects will be contacted quarterly to assess for adverse effects. Date of aortic dissection, aortic surgery, and death will be recorded, if applicable.

Trial outcomes

The primary outcome is the rate of change in the BSA-adjusted aortic root (sinuses of Valsalva) z score. Aortic

Table 1. Outcome variables

Primary outcome
Rate of change in aortic root (sinuses of Valsalva) BSA-adjusted z score
Secondary outcomes
Rate of change in aortic root (sinuses of Valsalva) absolute dimension
Rate of change in ascending aorta absolute dimension and BSA-adjusted z score
Rate of change in aortic annulus absolute dimension and BSA-adjusted z score
Rate of change of aortic regurgitation, measured as change in vena contracta area indexed for BSA
Aortic dissection, aortic root surgery, or death at 36 months after randomization
Time to first occurrence of aortic dissection, aortic root surgery, or death up to 36 months after randomization
Rate of change of mitral regurgitation, measured as change in vena contracta area indexed for BSA
Rate of change in z scores for left ventricular mass, volume, mass-volume ratio, and ejection fraction by 2-dimensional echocardiography
Rate of change in z scores for left ventricular end-diastolic and end-systolic dimensions, diastolic septal and posterior wall thickness, left ventricular mass and shortening fraction by M-mode
Rate of change of aortic root and ascending aortic elastic modulus and stiffness index
Rate of change in z scores for weight, height, and BMI corrected for age in subjects as determined by availability of z scores
Rate of change in weight and BMI with covariate adjustment for age in all subjects
Incidence of adverse drug reactions reported during routine surveillance

BMI, Body mass index.

root size and growth rate are considered the best predictors of the risk of aortic dissection and remain the most commonly used measures to determine the timing of surgery in both adults and children.^{4,30-32} Echocardiograms will be performed by echocardiographers trained for this protocol and interpreted centrally to minimize bias and interobserver error. Secondary outcomes are listed in Table 1.

Statistical considerations

Longitudinal data from 2 participating centers were used to estimate rate of change and covariance structure of the primary outcome. Data for potentially eligible patients were also collected from participating clinical centers to characterize the expected study population. Estimates from these analyses were used to calculate target sample size.

The potential decrease in z score change rate is greater in those who have not attained maximum height (children, defined here as those <16 years of age²⁷) than in those who have (adults); therefore, the minimum clinically significant difference (MCS D) between the treatment groups was assessed separately for adults and children.

The MCS D for children was chosen with the goal of reaching adulthood (16 years) with minimal aortic root dilation, defined as BSA-adjusted aortic root z score of 2 SDs. For adults, the MCS D was defined as an effect

that would delay surgery by 10 years, assuming that surgery is performed when aortic root dimension exceeds 5 cm. Preliminary results and other data² led to estimated MCS Ds of 0.25 and 0.08 SD per year for children and adults, respectively. In the preliminary analysis data set, 67% of the subjects were children, so the overall MCS D was calculated as the weighted average, 0.194 SD per year.

After 20% inflation to account for subject dropout, 3 interim analyses, and potential crossover, a total of 604 subjects will be required to detect the MCS D with 85% power at significance level .05. Because the power to detect the overall MCS D is much greater in children than in adults, the power of the primary analysis will be compromised if a large proportion of adults are enrolled in the trial. Therefore, adult enrollment will be capped at 33%.

Primary analyses will be performed on an intention-to-treat basis. The primary outcome of rate of change in BSA-adjusted aortic root z score will be modeled using the parametric curves longitudinal model³³ with treatment efficacy assessed by a likelihood ratio test of whether the treatment group by time interaction effect is zero. If significant dropout occurs, the reasons for dropout will be evaluated. If the data appear to be missing at random, all available data will be included in analysis, and multiple imputation methods will be used to impute values for missing data.

Secondary analyses will compare treatment groups: (1) with covariate-adjusted analysis; (2) according to treatment actually received; and (3) after exclusion of any randomized subjects found subsequently to have been trial ineligible at the time of enrollment.

Four interaction analyses are planned to estimate the effect of the following characteristics on treatment effect:

- attainment of maximum height at baseline: subjects with no change in height after the baseline visit versus subjects with increased height after the baseline visit;
- age at baseline as a continuous variable;
- baseline BSA-adjusted aortic root z score (<4.5 vs ≥4.5);
- prior use of BB (yes vs no).

To monitor the trial for large treatment differences, 3 formal interim analyses are planned, timed to occur when one third, one half, and three quarters of postbaseline measurements are expected to be available. An O'Brien-Fleming stopping boundary, with a Lan-DeMets adjustment, will be used for this purpose.^{34,35} A data and safety monitoring board and an independent medical monitor have been established by the NHLBI to monitor this trial for safety.

Trial organization and timeline

The PHN Marfan Study Subcommittee and the PHN Steering Committee, together with the NHLBI, will be

responsible for all aspects of this study. The protocol has been approved by an independent protocol review committee and a data and safety monitoring board and by the institutional review board at each clinical center and at the data coordinating center. This study will be conducted under an investigational new drug application with the Federal Drug Administration. The trial is registered at ClinicalTrials.gov (NCT00429364). Infants, children, and young adults will be recruited for this trial from among patients at the clinical centers over a period of approximately 36 months, with data accrual to continue for an additional 3 years. Enrollment began in February 2007; as of June 15, 2007, 66 subjects had been enrolled. All centers will follow the same study procedures.

Discussion

Choice of primary outcome

The major clinical cardiovascular end points for individuals with MFS are aortic root surgery, aortic dissection, and death. It is fortunate that aortic dissection and death are rare in children and young adults with MFS, but this also means that a trial designed to assess differences in these events would require an impractical number of patients and years. The decision to intervene surgically is a function of aortic root size or growth rate and is relatively standardized. Therefore, a primary end point related to change in aortic root size was considered to be clinically relevant as well as feasible. In addition, aortic root size and growth rate were favorably affected by losartan treatment in the mouse model.

Importance of knowledge to be gained

The results of this trial will make an important contribution to the management of individuals with MFS by determining whether the rates of aortic growth and progression of aortic regurgitation are lower in those subjects receiving ARB therapy when compared to those receiving BB therapy and by determining the effect of these 2 drugs on the secondary end points. Without this trial, we will not be able to assess the efficacy or the safety of losartan administration in humans with MFS across a broad range of genotypes and severities.

Limitations

Although the study will be a prospective, randomized trial, it will not be possible to mask all of the subjects or their care providers to the study drug assignment. Valid treatment comparisons can be made without masking, as long as care is taken to avoid treatment-related biases in outcome assessment. This will be achieved because the physicians at the echocardiography core laboratory evaluating the primary end point and many secondary end points will be masked to treatment assignment. In addition, every effort will be made to prevent the study subjects and their families from learning their treatment assignment.

The lack of a placebo arm is another potential limitation. During protocol development, several designs and drugs were considered, including designs with a placebo arm. After extensive discussion, and because BB therapy is considered by many to be the standard of care for patients with MFS, most of the trial subcommittee members concluded that a placebo arm would not be acceptable to many patients, families, study investigators, and primary cardiologists. Without a placebo arm, our study will not be able to evaluate the efficacy of each of the therapies independently, only the efficacy of one therapy relative to the other.

The study may not detect an effect that is smaller than that for which the study is powered and may be underpowered for subgroup analyses and some secondary end points. In particular, the study will be underpowered to determine whether atenolol or losartan is superior in preventing or delaying aortic dissection, surgery, and death because these events are expected to be rare in our study population. Thus, the primary end point is a surrogate rather than a true clinical end point, but it is a predictor of more serious MFS outcomes. Finally, the study results may not be generalizable to individuals with MFS who have BSA-adjusted aortic root z scores ≤ 3.0 or to those individuals with variants of MFS who do not meet the Ghent diagnostic criteria for MFS.

Conclusions

The appeal of a trial of losartan therapy in patients with MFS reflects its rational derivation from disease pathogenesis, its novel mechanism of action, and its performance in validated mouse models of MFS. One of the primary goals of the PHN is to promote evidence-based clinical care. Given the widespread publicity and excitement regarding the performance of losartan in animal models and the lack of practical barriers for its widespread clinical application, we sought to take advantage of a unique but time-limited opportunity to assess the utility of this therapy with a randomized study design while clinical equipoise is still maintained. This trial should make a substantial contribution to the management of individuals with MFS and will expand our understanding of the mechanisms responsible for the aortic manifestations of this disorder.

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Appendix A

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