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Variants in ADRB1 and CYP2C9: Association with Response to Atenolol and Losartan in Marfan Syndrome

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Abstract

Objective: To test whether variants in *ADRB1* and *CYP2C9* genes identify subgroups of individuals with differential response to treatment for Marfan syndrome through analysis of data from a large randomized trial.

Study design: In a subset of 250 white, non-Hispanic participants with Marfan syndrome in a prior randomized trial of atenolol vs. losartan, the common variants rs1801252 and rs1801253 in *ADRB1* and rs1799853 and rs1057910 in *CYP2C9* were analyzed. The primary outcome was baseline-adjusted annual rate of change in the maximum aortic-root diameter z-score over 3 years, assessed using mixed effects models.

Results: Among 122 atenolol-assigned participants, the 70 with rs1801253 CC genotype had greater rate of improvement in aortic-root z-score compared with 52 participants with CG or GG genotypes (time × genotype interaction p=0.005, mean annual z-score change ± standard error −0.20±0.03 vs −0.09±0.03). Among participants with the CC genotype in both treatment arms, those assigned to atenolol had greater rate of improvement compared with the 71/121 assigned to losartan (interaction $P = .002$, −0.20±0.02 vs. −0.07±0.02; p<0.001). There were no differences in atenolol response by rs1801252 genotype or in losartan response by CYP2C9 metabolizer status.

Conclusions: In this exploratory study, *ADRB1*-rs1801253 was associated with atenolol response in children and young adults with Marfan syndrome. If findings are confirmed in future studies, *ADRB1* genotyping has the potential to guide therapy by identifying those who are likely to have greater therapeutic response to atenolol than losartan.

Keywords

Pharmacogenomics; Pharmacogenetics; Aortic Root Dissection; Angiotensin II receptor blocker; Beta-adrenergic receptor blocker; Personalized Medicine

Marfan syndrome is an autosomal dominant connective tissue disorder affecting approximately 1 in 5000 individuals.(1,2) In most cases, pathogenic variants are identified in *FBN1*, the gene encoding fibrillin 1. Multiple organs are affected; however, progressive aortic-root dilation leading to dissection or rupture is the leading cause of mortality. The angiotensin II type 1-receptor blocker (ARB) losartan is a potential alternative to beta-adrenergic receptor antagonists (beta-blockers) for preventing aortic-root dilation. A randomized trial conducted by the Pediatric Heart Network (PHN) compared atenolol with losartan in children and young adults with Marfan syndrome and found no difference in the rate of aortic-root dilation between the two treatment groups over the 3 year study.(3) However, there was substantial variability in response within treatment groups.

Genetic variation in drug metabolism or response pathways may contribute to inter-individual variability in drug response. For atenolol, polymorphisms in the adrenergic signaling system have been associated with drug response.(4–6) The most widely studied are those in the beta 1 adrenergic receptor gene (*ADRB1*), including two common non-synonymous variants: rs1801252 encoding ADRB1-Ser49Gly and rs1801253 encoding ADRB1-Arg389Gly. Both variants are associated with clinical response to beta-blocker therapy, although there are conflicting data on the effect size and the direction of effect.(7–19) Losartan is metabolized by cytochrome P450 2C9 (encoded by *CYP2C9*), which also has common variants reducing function (rs1799853 encoding *CYP2C9*2* and rs1057910 encoding *CYP2C9*3*). *CYP2C9* variation has been associated with losartan response, as have variants in other genes in the angiotensin pathway, again with heterogeneity of results.(20–26) The impact of genetic variants on response to atenolol or losartan therapy in Marfan syndrome has not been established.

The objective of this study was to determine if variants in *ADRB1* or *CYP2C9* identify subgroups of individuals with superior response to either atenolol or losartan. We studied individuals from the PHN trial who chose to participate in this pharmacogenetic ancillary study. The primary outcome from the PHN trial was assessed, namely the baseline-adjusted rate of change of aortic-root z-score. Pre-specified secondary outcomes were the composite clinical outcome of aortic-root surgery, dissection, and/or death, and for the atenolol cohort, heart rate and atenolol dose. Exploratory analyses of additional variants previously associated with atenolol or losartan response were also performed.

Methods

This pharmacogenetic study was a planned ancillary study of the National Heart, Lung, and Blood Institute-sponsored PHN randomized atenolol vs. losartan in Marfan syndrome trial (NCT00429364). The study design and results for the PHN trial have been previously reported.(3,27) In brief, the PHN trial included participants age 6 months to 25 years with Marfan syndrome diagnosis per Ghent criteria(28) and aortic-root z-score >3.0. Participants in the PHN trial were randomized to treatment with either atenolol, with dose titrated to achieve 20% reduction in heart rate (maximum daily dose 250 mg), or 1.4 mg/kg of losartan (maximum daily dose 100 mg). 17 of 21 sites from the PHN trial invited participants to also enroll in the pharmacogenetic study. Written informed consent and assent for the pharmacogenetic ancillary study were obtained from each participant and/or their parent or guardian, as appropriate, at or after the time of consent for the PHN trial. The PHN trial and the ancillary pharmacogenetic study were approved by the Institutional Review Board or Ethics Committee at each study site.

The primary outcome for the PHN trial and the pharmacogenetic ancillary study was the baseline-adjusted annual rate of change in the aortic-root z-score, based on body surface area, as measured by echocardiography at baseline and 6, 12, 24, and 36-month follow-up visits.(29) The pre-specified composite secondary outcome was freedom from aortic surgery, aortic-root dissection, or death. Because atenolol dose was titrated to achieve 20% heart rate reduction, mean heart rate from 24-hour ambulatory monitoring at baseline and at 36 months and atenolol dose at 36 months were secondary outcomes for the atenolol cohort.

DNA was extracted from either whole blood or a saliva specimen. Given multiple prior associations with atenolol response, two variants in *ADRB1* were the primary candidate pharmacogenetic variants for atenolol: rs1801252 (c.145A>G encoding p.Ser49Gly) and rs1801253 (c.1165C>G encoding p.Arg389Gly). The potential combined effect of both variants was assessed by comparing those with the AA/GG haplotype to those with all other haplotypes. Additional variants associated with beta-blocker response in *AGT* (rs699, rs4762, and rs5051), *LDLR* (rs688), and *PTPRD* (rs12346562) were designated for exploratory analysis.(30–32)

For losartan, the primary candidate variants were in *CYP2C9*. Two variants in this gene, rs1799853 and rs1057910, encoding *CYP2C9*2* and *CYP2C9*3*, respectively, were used to determine the CYP2C9 metabolizer status. Individuals with no *CYP2C9* variants (*CYP2C9*1/*1*) were defined as normal metabolizers, those with one variant (*CYP2C9*1/*2* or **1/*3*) were intermediate metabolizers, and those with two variants (*CYP2C9*2/*2*, **2/*3*, or **3/*3*) were poor metabolizers, per current guidelines for other CYP2C9 substrates.(33,34) Variants in the *ACE*, *AGT*, *AGTR1*, and the TGFβ pathway (*TGFB1, TGFBR1*, and *TGFBR2*) were designated for exploratory analysis in the losartan cohort, as these genes code for proteins in the losartan target pathway.

Variants listed above in *ADRB1*, *AGT*, *LDLR*, *CYP2C9*, and genes in the TGFβ pathway were assessed using target enrichment (Agilent Technologies, Santa Clara, CA) and next generation sequencing on a MiSeq instrument (Illumina, San Diego, CA) performed at the University of Antwerp as part of an ongoing study of Marfan genomics. Variants in *PTPRD*, *ACE*, and *AGTR1* were genotyped for the pharmacogenetic study using MassARRAY (Sequenom Inc., San Diego, CA) in the Vanderbilt Technologies for Advanced Genomics core laboratory. Genotyping results for each variant were confirmed to be in Hardy-Weinberg equilibrium, to approximate known minor allele frequencies, and be concordant across duplicates.

Statistical analyses

The primary analyses were restricted to those who were white and non-Hispanic by self-report, and used an intention-to-treat approach, including all data from each randomized participant. A secondary per-protocol analysis included only echocardiographic data obtained during treatment with study drug (i.e., excluding data from after withdrawal from study drug). Secondary analysis including all individuals (regardless of reported race/ ethnicity) was performed for significant associations. Adjustments were not made to the significance levels of hypothesis tests for the number of genetic variants studied or for secondary trial outcomes; a nominal p-value of 0.05 was considered statistically significant, without correction for multiple comparisons.(35)

To assess whether longitudinal change in aortic-root z-score was associated with the candidate genetic variants or treatment assignment, we employed linear mixed effects regression modeling of the aortic-root z-score with parameterization adjusting for baseline. The study plan included assessment of each variant predictor using an additive model (0 vs. 1 vs. 2 variant alleles with intrinsic ordering of effect), a categorical model (0 vs. 1 vs. 2 variant alleles without intrinsic ordering of effect), and a dichotomous model (0 vs. either 1 or 2 variant alleles). Due to low numbers of individuals with two variant alleles for many genotypes, the dichotomous model was used for the primary analyses.

For the atenolol cohort, the baseline-adjusted annual rates of change in aortic-root z-score were compared in those without vs. with each of the candidate variants using a test of the variant allele-by-time interaction effect. For the losartan cohort, the primary outcome was compared across groups by CYP2C9 metabolizer status (normal vs. intermediate or poor), and exploratory variants in the TGFβ pathway were analyzed by collapsing across all variants (i.e. 0 vs. ≥1 TGFβ pathway variant). Significant variants from the primary analysis within the treatment groups were further evaluated for a differential treatment effect (atenolol vs. losartan) according to genotype by an interaction test (genotype group by treatment by time).

The secondary composite outcome was assessed using a log-rank test, with event rates estimated using the Kaplan-Meier method. Follow-up time was defined as years from randomization to aortic-root surgery date (no deaths or dissections occurred) or censored at last contact date in the trial for event-free subjects. Associations between genotypes and additional secondary outcomes for atenolol (heart rate and atenolol dose) were assessed using Student *t*-tests.

Results

From January 2007 through February 2011, 303 subjects were randomly assigned to atenolol treatment in the PHN trial. Of these, 161 participated in the pharmacogenetic ancillary study; 13 Hispanic, 13 Black, and 9 of other race/ethnicity who were excluded from the primary analysis due to small group size, leading to a primary analysis of 126 white, non-Hispanic individuals (Figure 1). The median age was 11.5 years, and 71 (56.3%) were male (Table I). Demographic and baseline clinical variables were not different in participants in the ancillary study vs. PHN trial participants who did not participate in the ancillary study. A total of 600 echocardiograms were obtained in the atenolol-assigned participants of the pharmacogenetic ancillary study.

The frequencies of rs1801252 and rs1801253 variants in the atenolol-assigned cohort are shown in Table 2 (available at www.jpeds.com). To determine whether there were inherent differences in aortic-root size, the maximum aortic-root diameter z-score at baseline was compared across genotypes. No significant differences were found. There were no differences in baseline characteristics by rs1801253 variant (Table 3). The primary outcome, magnitude of the baseline-adjusted annual rate of change in aortic-root z-score, was larger in those with rs1801253 CC genotypes than in those with CG or GG genotypes (interaction p=0.005). The annual rate of change of aortic-root z-score (± standard error) was −0.20 ± 0.03 for those with rs1801253 CC genotype, and −0.09 ± 0.03 for those with CG or GG genotypes. Results were similar when echocardiograms obtained after withdrawal from atenolol study drug were excluded, with additive and categorical genetic models, and using the rs1801253 and rs1801252 variants together as a haplotype (Table 5 and Table 5; available at www.jpeds.com). There was no difference based on the rs1801252 variant (Table 4 and Table 5. When the analysis cohort included all individuals regardless of race/ ethnicity (n=161), the association of rs1801253 and the combined haplotype remained significant in the primary analysis (interaction p=0.04, Table 6[available at www.jpeds.com]).

With respect to the composite clinical outcome, there were a total of four surgeries, no aortic dissections, and no deaths. One of 70 (1.4%) individuals with rs1801253 CC genotype and 3 of 52 individuals with CG or GG genotypes (5.8%) had aortic surgery. Kaplan-Meier estimates demonstrated that three-year freedom from aortic-root surgery was 99% (95% confidence interval 90–100%) for those with rs1801253 CC genotype, and 94% (83–98%) for those with CG or GG genotype (log-rank p=0.19, Figure 2 [available at www.jpeds.com]). There were no differences in the time to composite clinical outcome for the rs1801252 variant. There were no differences in the mean 24-hour heart rates by rs1801253 or rs1801252 variant status at baseline or at 36 months (Table 7 available at www.jpeds.com). There was no difference in atenolol dose at 36 months for either variant (Table 8; available at www.jpeds.com).

To determine whether the rs1801253 variant identifies a subgroup with differential response to atenolol vs. losartan, we examined the dichotomous allele variable as a formal posthoc subgroup factor for the main trial result in a mixed effects model with three-way interaction. There was a significant interaction between the rs1801253 allele and treatment group (p=0.002 for rs1801253 × treatment × time interaction), indicating that the baseline-adjusted annual rate of change in aortic-root z-score by treatment (atenolol or losartan) depends on the presence or absence of rs1801253 variants. Among those with rs1801253 CC genotype, atenolol treatment resulted in an annual rate of change of −0.20 ± 0.02 vs. −0.07 ± 0.02 for losartan (p<0.001, Figure 3).

In the PHN trial, 305 subjects were assigned to losartan treatment, of whom 162 participated in the pharmacogenetic ancillary study. Excluded were 19 Hispanic, 10 Black, and 9 of other race/ethnicity, resulting in 124 were white, non-Hispanic individuals in the primary analysis (Figure 1). The median age was 10.8 years, and 82 (66.1%) were male (Table 1). There were no differences in demographic or clinical variables when compared with losartan-assigned participants in the PHN trial who did not enroll in the ancillary study. A total of 585 echocardiograms were obtained in the losartan-assigned participants of the pharmacogenetic ancillary study.

The frequencies of rs1057910 and rs1799853 variants in the cohort assigned to losartan are shown in Table 9 (available at www.jpeds.com). There were 85 (70.2%) normal metabolizers, 32 (26.4%) intermediate metabolizers, and 4 (3.3%) poor metabolizers. There was no difference in baseline maximum aortic-root diameter z-scores by metabolizer status. There were also no differences in the primary outcome by metabolizer status (Table 10; available at www.jpeds.com), nor in time to the composite outcome.

The frequency of variants in the exploratory candidate genes for atenolol are shown in Table 2. Only *AGT*-rs4762 was associated with the primary outcome (Table 11; available at www.jpeds.com). Individuals with GG genotype had annual aortic-root z-score change of −0.18 ± 0.02 vs. −0.08 ± 0.04 for those with AG or AA genotype (interaction p=0.01). Further analysis revealed no difference in atenolol vs. losartan treatment effect (p=0.23 for rs4762 × treatment × time interaction). The frequencies of variants identified in the exploratory candidate genes for losartan are shown in Table 9. When the presence vs. absence of each of the candidate variants in *ACE, AGT*, and *AGTR1* was tested for association to the primary outcome, no significant result was identified (Table 12; available at www.jpeds.com). Likewise, there was no difference in the primary outcome for individuals with 0 vs. ≥1 variant in the TGFβ pathway (Table 12).

Discussion

There is currently a therapeutic dilemma for the treatment of aortopathy in Marfan syndrome. In the 3-year PHN trial comparing atenolol with losartan for the prevention of aortic-root dilation, no differences in therapeutic response or adverse events were found between treatment groups. In this study, we performed pre-specified exploratory analyses of the response to atenolol based on *ADRB1* variants and response to losartan based on *CYP2C9* variants using data from the PHN randomized trial. We found no differences in losartan response based on CYP2C9 metabolic function. In contrast, atenolol-assigned individuals with the *ADRB1*-rs1801253 CC genotype, (encoding Arg/Arg at position 389) had greater improvement in the aortic-root z-score than those who had CG or GG genotypes (encoding Arg/Gly or Gly/Gly at position 389). This difference motivated us to look for an interaction between rs1801253 genotype and response to atenolol vs. losartan. Although there are no differences between outcomes for atenolol and losartan for those with rs1801253 CG or GG genotypes in our cohort, for those with the CC genotype, we observed greater improvement in aortic-root z-score for atenolol than losartan, indicating better treatment response.

Although prior studies have associated rs1801253 CC with greater heart rate reduction with beta-blockade,(8) we found no significant differences in heart rate by genotype, nor in the final atenolol dose (which had been titrated to achieve 20% reduction in heart rate). In the PHN trial, the rate of change in the aortic-root z-score was not related to atenolol dose, and the relationship between aortic-root z-score and heart rate was not investigated.(3) Considering the greater improvement in aortic-root z-score in those with the CC genotype, it is possible that heart rate alone may not be an accurate indicator of beta-blocker effect when the goal is prevention of progressive aortic dilatation, and atenolol may be exerting an effect that is independent of heart rate. However, due to the limitations of our study, this remains conjecture and requires further investigation.

Prior mouse data supported the potential for ARB therapy to be superior to beta-blockade, (36) but both the PHN trial and an additional randomized trial by Forteza et al with 140 pediatric and adult Marfan patients found no difference with respect to aortic-root dilation over three years of follow up.(37) Long-term follow up (>5 years) of 128 of the 140 participants in the Forteza et al trial also indicated no difference in aortopathy between treatment groups, and the authors suggest that losartan might be a useful alternative to beta-blockers for Marfan patients.(38) The addition of ARBs to current therapy (often including beta-blockers) has also been investigated, with initial results indicating benefit of adding losartan.(39,40) However, more recent studies have not shown a difference between losartan with beta-blockade vs. beta-blockade alone.(41–43) Given the apparent equipoise between losartan and atenolol with respect to therapeutic outcomes and adverse events, identification of a biomarker to guide the clinical management may improve outcomes for these patients. In our study, the difference between atenolol and losartan response among those with *ADRB1*-rs1801253 CC genotype was statistically significant and larger than the treatment effect observed for those with *ADRB1*-rs1801253 CG or GG genotypes. These findings suggest that this subgroup of Marfan patients may receive more benefit from treatment with atenolol than with losartan. This raises the possibility that clinical testing for *ADRB1* variants, available through commercial laboratories, could assist in determining optimal medical therapy.

The functional impact of the rs1801253 variant on ADRB1 function has been assessed; the C>G missense variant leads to a single amino acid substitution (Arg389Gly) that reduces receptor G-protein coupling.(44) Individuals with the CC genotype, encoding two copies of the more functional Arg389 protein, are expected to have a more robust response to beta-blockade. A blunted response is expected in those whose genetic variation has already reduced beta-adrenoreceptor activity. This has been observed, as the rs1801253 CC genotype is associated with increased response to beta-blockers in healthy individuals(13,14) and those with essential hypertension and heart failure.(7–9,11,12,15–18) However, negative studies have also been published, particularly in heart failure.(18,45–49) There are also reports of increased response to rate control in atrial fibrillation patients with the CG or GG genotype, rather than CC genotype.(18,19) These variable findings highlight the need to assess the impact of pharmacogenetic variants in the specific patient population of interest.

Our findings, indicating that a genetic biomarker identifies a subset of patients for whom atenolol may be more effective than losartan, exemplify the clinical potential of pharmacogenomics. This study also illustrates many of the challenges in building evidence for precision medicine approaches in children. We are limited in the conclusions we can draw from our data due to small sample size, despite recruiting participants from 17 clinical sites. Marfan syndrome is a rare disease, precluding recruitment of large cohorts. Ideally, our findings would be replicated in an independent data set prior to clinical implementation. Assembly of a replication cohort of appropriate size will require coordination across ongoing or future Marfan studies and/or multiple biobanks to identify atenolol-exposed individuals with documentation of comparable endpoints. Also, the rarity of definitive clinical endpoints such as death and aortic dissection necessitate use of a surrogate outcome. Thus our data provide evidence of a difference in efficacy of these two drugs based on the rs1801253 genotype but do not prove a survival difference between groups nor that clinically-guided therapy will provide clinical benefit.

This study has several additional limitations. As a substudy of the PHN trial, this study was subject to the same cohort selection as the trial (e.g. requirement for an aortic-root z-score > 3 at the time of study enrollment; study drug dosing with titration of atenolol but not losartan to achieve heart rate reduction). Approximately half of those who participated in the PHN trial chose to enroll in this ancillary study; we found no differences in demographic and clinical characteristics between those who did vs. did not participate in the ancillary study, but there may be unmeasured selection bias. Despite beginning with a large trial cohort, our sample size for analysis of each variant is small, particularly for variants with low frequency, limiting power and precluding analysis of heterozygotes vs. homozygotes. Our analyses do not include correction for multiple comparisons. Due to this small sample size, we focused on variants with the most robust associations to the drugs of interest. Additional variants may play an important role in response to atenolol or losartan in patients with Marfan syndrome. For the *ADRB1*-rs1801253 variant, we did perform stratified analyses that confirmed the association among white, non-Hispanic participants. However, we do not have adequate sample size to analyze other subsets of individuals by race or ancestry, where distinct genetic variants may be important predictors of response to atenolol and/or losartan.

The rs1801253 variant may identify a subgroup of patients in whom atenolol therapy is superior to losartan. If differences in drug response by genotype are replicated and demonstrated to be clinically meaningful, *ADRB1* testing may identify those who are likely to have greater therapeutic response to atenolol than to losartan.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and Acronyms:

ARB	angiotensin II type 1-receptor blocker
PHN	Pediatric Heart Network
ADRB1	beta 1 adrenergic receptor gene
CYP2C9	cytochrome P450 2C9 gene

References

1. Judge DP, Dietz HC. Marfan's syndrome. Lancet. 2005 12 3;366:1965–76. [PubMed: 16325700]
2. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991 7 25;352:337–9. [PubMed: 1852208]
3. Lacro RV, Dietz HC, Sleeper LA, Yetman AT, Bradley TJ, Colan SD, et al. Atenolol versus losartan in children and young adults with Marfan's syndrome. N Engl J Med. 2014 11 27;371:2061–71. [PubMed: 25405392]
4. Johnson JA, Terra SG. Beta-adrenergic receptor polymorphisms: cardiovascular disease associations and pharmacogenetics. Pharm Res. 2002 12;19:1779–87. [PubMed: 12523655]
5. Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. Annu Rev Pharmacol Toxicol. 2003;43:381–411. [PubMed: 12540746]
6. Mottet F, Vardeny O, de Denus S. Pharmacogenomics of heart failure: a systematic review. Pharmacogenomics. 2016;17:1817–58. [PubMed: 27813451]
7. Johnson JA, Zineh I, Puckett BJ, McGorray SP, Yarandi HN, Pauly DF. Beta 1-adrenergic receptor polymorphisms and antihypertensive response to metoprolol. Clin Pharmacol Ther. 2003 7;74:44–52. [PubMed: 12844134]
8. Liu J, Liu Z-Q, Yu B-N, Xu F-H, Mo W, Zhou G, et al. beta1-Adrenergic receptor polymorphisms influence the response to metoprolol monotherapy in patients with essential hypertension. Clin Pharmacol Ther. 2006 7;80:23–32. [PubMed: 16815314]
9. Terra SG, Pauly DF, Lee CR, Patterson JH, Adams KF, Schofield RS, et al. beta-Adrenergic receptor polymorphisms and responses during titration of metoprolol controlled release/extended release in heart failure. Clin Pharmacol Ther. 2005 3;77:127–37. [PubMed: 15735607]
10. Kurnik D, Muszkat M, Li C, Sofowora GG, Friedman EA, Scheinin M, et al. Genetic variations in the α(2A)-adrenoreceptor are associated with blood pressure response to the agonist dexmedetomidine. Circ Cardiovasc Genet. 2011 4;4:179–87. [PubMed: 21325151]
11. Lobmeyer MT, Gong Y, Terra SG, Beitelshees AL, Langaee TY, Pauly DF, et al. Synergistic polymorphisms of beta1 and alpha2C-adrenergic receptors and the influence on left ventricular ejection fraction response to beta-blocker therapy in heart failure. Pharmacogenet Genomics. 2007 4;17:277–82. [PubMed: 17496726]
12. O'Connor CM, Fiuzat M, Carson PE, Anand IS, Plehn JF, Gottlieb SS, et al. Combinatorial pharmacogenetic interactions of bucindolol and β1, α2C adrenergic receptor polymorphisms. PLoS ONE. 2012;7:e44324. [PubMed: 23071495]
13. Huntgeburth M, La Rosée K, ten Freyhaus H, Böhm M, Schnabel P, Hellmich M, et al. The Arg389Gly β1-adrenoceptor gene polymorphism influences the acute effects of β-adrenoceptor blockade on contractility in the human heart. Clin Res Cardiol. 2011 8;100:641–7. [PubMed: 21311897]
14. Sofowora GG, Dishy V, Muszkat M, Xie HG, Kim RB, Harris PA, et al. A common beta1-adrenergic receptor polymorphism (Arg389Gly) affects blood pressure response to beta-blockade. Clin Pharmacol Ther. 2003 4;73:366–71. [PubMed: 12709726]
15. Liggett SB, Mialet-Perez J, Thaneemit-Chen S, Weber SA, Greene SM, Hodne D, et al. A polymorphism within a conserved beta(1)-adrenergic receptor motif alters cardiac function and

beta-blocker response in human heart failure. Proc Natl Acad Sci USA. 2006 7 25;103:11288–93. [PubMed: 16844790]

16. Chen L, Meyers D, Javorsky G, Burstow D, Lolekha P, Lucas M, et al. Arg389Gly-beta1-adrenergic receptors determine improvement in left ventricular systolic function in nonischemic cardiomyopathy patients with heart failure after chronic treatment with carvedilol. Pharmacogenet Genomics. 2007 11;17:941–9. [PubMed: 18075464]
17. Mialet Perez J, Rathz DA, Petrashevskaya NN, Hahn HS, Wagoner LE, Schwartz A, et al. Beta 1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. Nat Med. 2003 10;9:1300–5. [PubMed: 14502278]
18. Rau T, Düngen H-D, Edelmann F, Waagstein F, Lainščak M, Dimković S, et al. Impact of the β1-adrenoceptor Arg389Gly polymorphism on heart-rate responses to bisoprolol and carvedilol in heart-failure patients. Clin Pharmacol Ther. 2012 7;92:21–8. [PubMed: 22617224]
19. Parvez B, Chopra N, Rowan S, Vaglio JC, Muhammad R, Roden DM, et al. A common β1-adrenergic receptor polymorphism predicts favorable response to rate-control therapy in atrial fibrillation. J Am Coll Cardiol. 2012 1 3;59:49–56. [PubMed: 22192668]
20. Bae J-W, Choi C-I, Lee H-I, Lee Y-J, Jang C-G, Lee S-Y. Effects of CYP2C9*1/*3 and *1/*13 on the pharmacokinetics of losartan and its active metabolite E-3174. Int J Clin Pharmacol Ther. 2012 9;50:683–9. [PubMed: 22735459]
21. Babaoglu MO, Yasar U, Sandberg M, Eliasson E, Dahl M-L, Kayaalp SO, et al. CYP2C9 genetic variants and losartan oxidation in a Turkish population. Eur J Clin Pharmacol. 2004 7;60:337–42. [PubMed: 15197523]
22. Allabi AC, Gala J-L, Horsmans Y, Babaoglu MO, Bozkurt A, Heusterspreute M, et al. Functional impact of CYP2C95, CYP2C96, CYP2C98, and CYP2C911 in vivo among black Africans. Clin Pharmacol Ther. 2004 8;76:113–8. [PubMed: 15289788]
23. Miller JA, Thai K, Scholey JW. Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II. Kidney Int. 1999 12;56:2173–80. [PubMed: 10594793]
24. Sookoian S, Castaño G, García SI, Viudez P, González C, Pirola CJ. A1166C angiotensin II type 1 receptor gene polymorphism may predict hemodynamic response to losartan in patients with cirrhosis and portal hypertension. Am J Gastroenterol. 2005 3;100:636–42. [PubMed: 15743363]
25. Trevelyan J, Needham EWA, Morris A, Mattu RK. Comparison of the effect of enalapril and losartan in conjunction with surgical coronary revascularisation versus revascularisation alone on systemic endothelial function. Heart. 2005 8;91:1053–7. [PubMed: 16020596]
26. Felehgari V, Rahimi Z, Mozafari H, Vaisi-Raygani A. ACE gene polymorphism and serum ACE activity in Iranians type II diabetic patients with macroalbuminuria. Mol Cell Biochem. 2011 1;346:23–30. [PubMed: 20830509]
27. Lacro RV, Dietz HC, Wruck LM, Bradley TJ, Colan SD, Devereux RB, et al. Rationale and design of a randomized clinical trial of beta-blocker therapy (atenolol) versus angiotensin II receptor blocker therapy (losartan) in individuals with Marfan syndrome. Am Heart J. 2007 10;154:624–31. [PubMed: 17892982]
28. De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet. 1996 4 24;62:417–26. [PubMed: 8723076]
29. Selamet Tierney ES, Levine JC, Chen S, Bradley TJ, Pearson GD, Colan SD, et al. Echocardiographic methods, quality review, and measurement accuracy in a randomized multicenter clinical trial of Marfan syndrome. J Am Soc Echocardiogr. 2013 6;26:657–66. [PubMed: 23582510]
30. Kurland L, Liljedahl U, Karlsson J, Kahan T, Malmqvist K, Melhus H, et al. Angiotensinogen gene polymorphisms: relationship to blood pressure response to antihypertensive treatment. Results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. Am J Hypertens. 2004 1;17:8–13. [PubMed: 14700505]
31. Liljedahl U, Lind L, Kurland L, Berglund L, Kahan T, Syvänen A-C. Single nucleotide polymorphisms in the apolipoprotein B and low density lipoprotein receptor genes affect response to antihypertensive treatment. BMC Cardiovasc Disord. 2004 9 28;4:16. [PubMed: 15453913]

32. Gong Y, McDonough CW, Beitelshees AL, El Rouby N, Hiltunen TP, O'Connell JR, et al. PTPRD gene associated with blood pressure response to atenolol and resistant hypertension. J Hypertens. 2015 11;33:2278–85. [PubMed: 26425837]
33. Johnson JA, Gong L, Whirl-Carrillo M, Gage BF, Scott SA, Stein CM, et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. Clin Pharmacol Ther. 2011 10;90:625–9. [PubMed: 21900891]
34. Caudle KE, Rettie AE, Whirl-Carrillo M, Smith LH, Mintzer S, Lee MTM, et al. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and HLA-B genotypes and phenytoin dosing. Clin Pharmacol Ther. 2014 11;96:542–8. [PubMed: 25099164]
35. Althouse AD. Adjust for Multiple Comparisons? It's Not That Simple. Ann Thorac Surg. 2016 May;101:1644–5.
36. Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. N Engl J Med. 2008 6 26;358:2787–95. [PubMed: 18579813]
37. Forteza A, Evangelista A, Sánchez V, Teixidó-Turà G, Sanz P, Gutiérrez L, et al. Efficacy of losartan vs. atenolol for the prevention of aortic dilation in Marfan syndrome: a randomized clinical trial. Eur Heart J. 2016 3 21;37:978–85. [PubMed: 26518245]
38. Teixido-Tura G, Forteza A, Rodríguez-Palomares J, González Mirelis J, Gutiérrez L, Sánchez V, et al. Losartan Versus Atenolol for Prevention of Aortic Dilation in Patients With Marfan Syndrome. J Am Coll Cardiol. 2018 10 2;72:1613–8. [PubMed: 30261963]
39. Groenink M, den Hartog AW, Franken R, Radonic T, de Waard V, Timmermans J, et al. Losartan reduces aortic dilatation rate in adults with Marfan syndrome: a randomized controlled trial. Eur Heart J. 2013 12;34:3491–500. [PubMed: 23999449]
40. Chiu H-H, Wu M-H, Wang J-K, Lu C-W, Chiu S-N, Chen C-A, et al. Losartan added to β-blockade therapy for aortic root dilation in Marfan syndrome: a randomized, open-label pilot study. Mayo Clin Proc. 2013 3;88:271–6. [PubMed: 23321647]
41. Milleron O, Arnoult F, Ropers J, Aegerter P, Detaint D, Delorme G, et al. Marfan Sartan: a randomized, double-blind, placebo-controlled trial. Eur Heart J. 2015 8 21;36:2160–6. [PubMed: 25935877]
42. Muiño-Mosquera L, De Nobele S, Devos D, Campens L, De Paepe A, De Backer J. Efficacy of losartan as add-on therapy to prevent aortic growth and ventricular dysfunction in patients with Marfan syndrome: a randomized, double-blind clinical trial. Acta Cardiol. 2017 12;72:616–24. [PubMed: 28657492]
43. Sandor GGS, Alghamdi MH, Raffin LA, Potts MT, Williams LD, Potts JE, et al. A randomized, double blind pilot study to assess the effects of losartan vs. atenolol on the biophysical properties of the aorta in patients with Marfan and Loeys-Dietz syndromes. Int J Cardiol. 2015 1 20;179:470–5. [PubMed: 25465809]
44. Mason DA, Moore JD, Green SA, Liggett SB. A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. J Biol Chem. 1999 4 30;274:12670–4. [PubMed: 10212248]
45. Metra M, Covolo L, Pezzali N, Zacà V, Bugatti S, Lombardi C, et al. Role of beta-adrenergic receptor gene polymorphisms in the long-term effects of beta-blockade with carvedilol in patients with chronic heart failure. Cardiovasc Drugs Ther. 2010 2;24:49–60. [PubMed: 20352314]
46. Filigheddu F, Argiolas G, Degortes S, Zaninello R, Frau F, Pitzoi S, et al. Haplotypes of the adrenergic system predict the blood pressure response to beta-blockers in women with essential hypertension. Pharmacogenomics. 2010 3;11:319–25. [PubMed: 20235788]
47. Baudhuin LM, Miller WL, Train L, Bryant S, Hartman KA, Phelps M, et al. Relation of ADRB1, CYP2D6, and UGT1A1 polymorphisms with dose of, and response to, carvedilol or metoprolol therapy in patients with chronic heart failure. Am J Cardiol. 2010 8 1;106:402–8. [PubMed: 20643254]
48. White HL, de Boer RA, Maqbool A, Greenwood D, van Veldhuisen DJ, Cuthbert R, et al. An evaluation of the beta-1 adrenergic receptor Arg389Gly polymorphism in individuals with heart failure: a MERIT-HF sub-study. Eur J Heart Fail. 2003 8;5:463–8. [PubMed: 12921807]

49. de Groote P, Helbecque N, Lamblin N, Hermant X, Mc Fadden E, Foucher-Hossein C, et al. Association between beta-1 and beta-2 adrenergic receptor gene polymorphisms and the response to beta-blockade in patients with stable congestive heart failure. Pharmacogenet Genomics. 2005 3;15:137–42. [PubMed: 15861037]

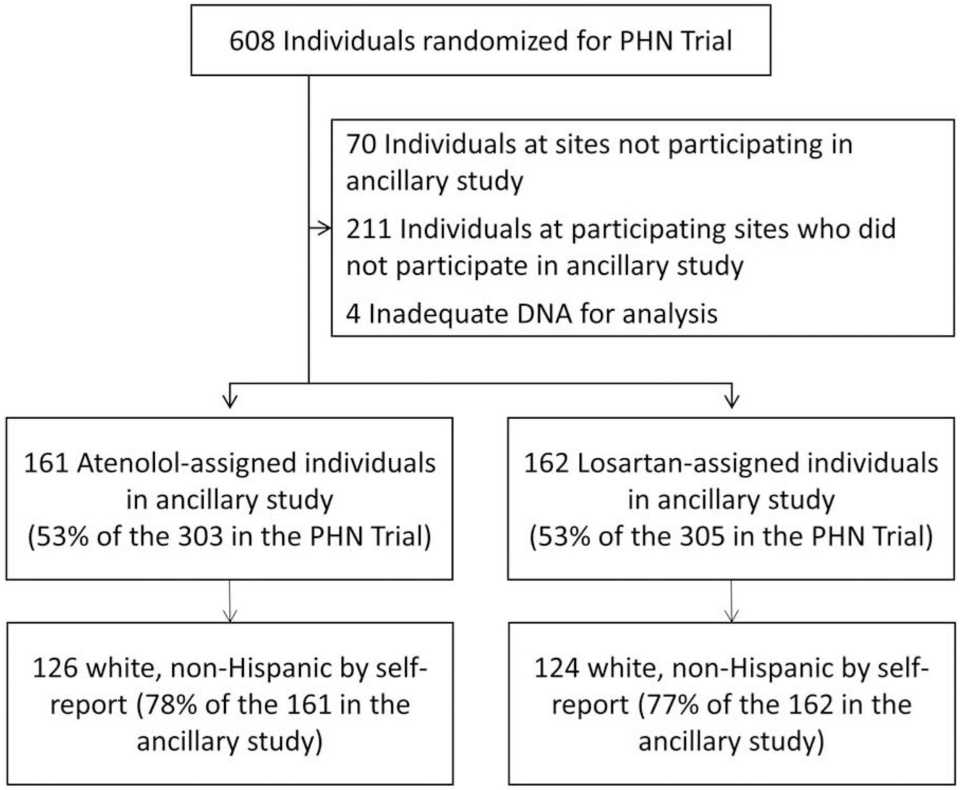

Figure 1. Study cohorts.

This ancillary study included 161 (126 white, non-Hispanic) of the 303 participants of the main randomized trial who were assigned to atenolol, and 162 (124 white, non-Hispanic) of the 305 participants assigned to losartan. For atenolol the 161 enrolled included 13 Hispanic, 13 Black, and 9 of other race/ethnicity who were excluded from the primary analysis due to small group size; Similarly, the 162 enrolled in the Losartan group had 19 Hispanic, 10 Black, and 9 of other race/ethnicity who were excluded from the primary analysis.

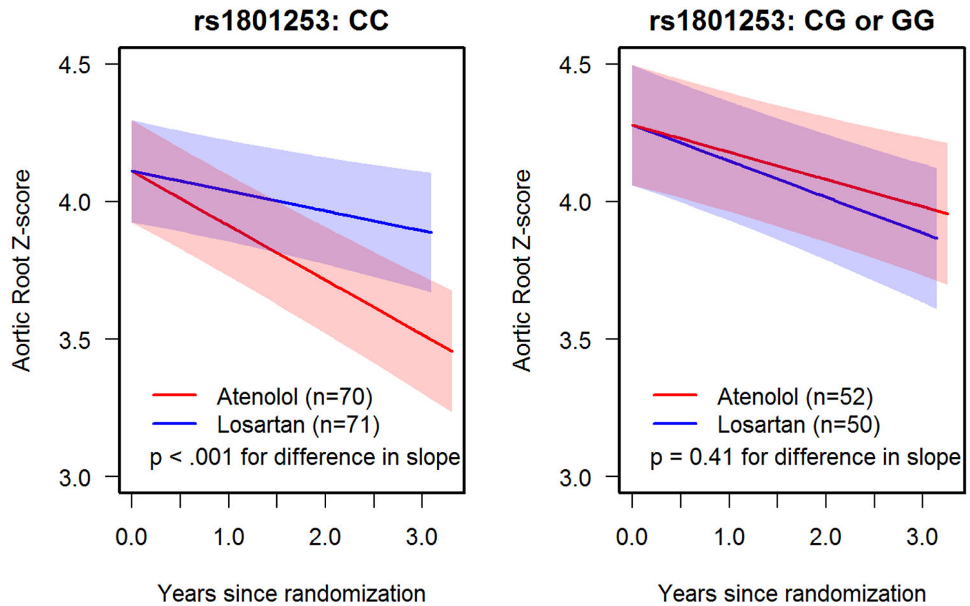

Figure 3. Predicted baseline-adjusted change in maximum aortic-root z-score over time for atenolol and losartan assignment by *ADRB1*-rs1801253 genotype.

The model-predicted aortic-root z-score is plotted on the y-axis and time since randomization on the x-axis. The atenolol group is shown in red and the losartan group in blue. Shading indicates 95% pointwise confidence bands. The genotype × treatment × time interaction p=0.002. The left panel shows individuals with CC genotype for *ADRB1*-rs1801253 (slope=−0.20±0.02 for atenolol and −0.07±0.02 for losartan, p<0.001 for difference in slope). The right panel shows individuals with the CG or GG genotype for *ADRB1*-rs1801253 (no significant difference in slope).

Table 1.

Demographics and clinical characteristics at study baseline by assigned treatment

	Atenolol (n=126)	Losartan (n=124)
Age at randomization, years		
Mean ± SD	12.0 ± 6.5	11.6 ± 6.2
Median (IQR)	11.5 (6.6, 16.4)	10.8 (6.3, 16.0)
Male	71 (56.3%)	82 (66.1%)
Presence of causal *FBN1* variant *	41 (32.5%)	36 (29.0%)
Family history of Marfan syndrome	71 (56.3%)	74 (59.7%)
Maximum aortic-root diameter		
Mean ± SD	3.4 ± 0.7	3.4 ± 0.7
Median (IQR)	3.5 (2.8, 4.0)	3.5 (2.9, 3.9)
Maximum aortic-root diameter z-score		
Mean ± SD	4.2 ± 1.0	4.3 ± 1.2
Median (IQR)	4.0 (3.5, 4.7)	4.0 (3.4, 5.0)
Max aortic-root diameter z-score ≥ 4.5	38 (30.2%)	44 (35.5%)
Prior cardiac surgery	2 (1.6%)	4 (3.2%)
Other cardiovascular disorder	20 (15.9%)	16 (12.9%)
Prior beta-blocker	76 (60.3%)	75 (60.5%)
Endocrine disorder	5 (4.0%)	0 (0%)
Neurodevelopment disorder	25 (19.8%)	28 (22.6%)
Psychiatric disorder	7 (5.6%)	9 (7.3%)

SD – Standard Deviation; IQR – Interquartile Range; p-values comparing atenolol to losartan for each variable were >0.05.

* no presence of causal *FBN1* variant includes absent and unknown status.

Table 3.

Demographics and clinical characteristics at study baseline by assigned treatment and *ADRB1*-rs1801253 genotype

	Atenolol (n=122)*		**Losartan (n=121)†**	
	CG or GG Genotype (n=52)	**CC Genotype (n=70)**	**CG or GG Genotype (n=50)**	**CC Genotype (n=71)**
Age at randomization, years				
Mean ± SD	13.2 ± 6.4	11.3 ± 6.6	11.1 ± 6.3	11.8 ± 6.2
Median (IQR)	11.9 (8.1, 18.7)	11.0 (5.3, 14.9)	9.5 (5.9, 16.1)	12.1 (6.9, 15.4)
Male	28 (53.8%)	41 (58.6%)	33 (66.0%)	48 (67.6%)
Presence of causal *FBN1* variant‡	14 (26.9%)	25 (35.7%)	10 (20.0%)	25 (35.2%)
Family history of Marfan syndrome	28 (53.8%)	42 (60.0%)	29 (58.0%)	44 (62.0%)
Maximum aortic-root diameter				
Mean ± SD	3.5 ± 0.7	3.3 ± 0.7	3.4 ± 0.7	3.4 ± 0.7
Median (IQR)	3.6 (3.0, 4.0)	3.3 (2.8, 3.9)	3.5 (2.8, 3.9)	3.3 (2.9, 4.0)
Maximum aortic-root diameter z-score				
Mean ± SD	4.3 ± 1.1	4.1 ± 1.0	4.5 ± 1.2	4.1 ± 1.1
Median (IQR)	4.1 (3.6, 4.7)	4.0 (3.5, 4.7)	4.0 (3.5, 5.4)	3.9 (3.3, 4.8)
Max aortic-root diameter z-score ≥ 4.5	18 (34.6%)	19 (27.1%)	21 (42.0%)	20 (28.2%)

SD – Standard Deviation; IQR – Interquartile Range.

[*] 4 / 126 individuals in the atenolol treatment group failed genotyping for rs1801253.

[†] 3/124 individuals in the losartan treatment group failed genotyping for rs1801253.

[‡] no presence of causal *FBN1* variant includes absent and unknown status.