Genetics

Renin-Angiotensin-Aldosterone Genotype Influences Ventricular Remodeling in Infants With Single Ventricle

Seema Mital, MD; Wendy K. Chung, MD, PhD; Steven D. Colan, MD; Lynn A. Sleeper, ScD; Cedric Manlhiot, BSc; Cammon B. Arrington, MD; James F. Cnota, MD; Eric M. Graham, MD; Michael E. Mitchell, MD; Elizabeth Goldmuntz, MD; Jennifer S. Li, MD; Jami C. Levine, MD; Teresa M. Lee, MD; Renee Margossian, MD; Daphne T. Hsu, MD; on behalf of the Pediatric Heart Network Investigators

Background—We investigated the effect of polymorphisms in the renin-angiotensin-aldosterone system (RAAS) genes on ventricular remodeling, growth, renal function, and response to enalapril in infants with single ventricle.

Methods and Results—Single ventricle infants enrolled in a randomized trial of enalapril were genotyped for polymorphisms in 5 genes: angiotensinogen, angiotensin-converting enzyme, angiotensin II type 1 receptor, aldosterone synthase, and chymase. Alleles associated with renin-angiotensin-aldosterone system upregulation were classified as risk alleles. Ventricular mass, volume, somatic growth, renal function using estimated glomerular filtration rate, and response to enalapril were compared between patients with ≥2 homozygous risk genotypes (high risk), and those with <2 homozygous risk genotypes (low risk) at 2 time points: before the superior cavopulmonary connection (pre-SCPC) and at age 14 months. Of 230 trial subjects, 154 were genotyped: Thirty-eight were high risk, and 116 were low risk. Ventricular mass and volume were elevated in both groups pre-SCPC. Ventricular mass and volume decreased and estimated glomerular filtration rate increased after SCPC in the low-risk (P<0.05), but not the high-risk group. These responses were independent of enalapril treatment. Weight and height z-scores were lower at baseline, and height remained lower in the high-risk group at 14 months, especially in those receiving enalapril (P<0.05).

Conclusions—Renin-angiotensin-aldosterone system–upregulation genotypes were associated with failure of reverse remodeling after SCPC surgery, less improvement in renal function, and impaired somatic growth, the latter especially in patients receiving enalapril. Renin-angiotensin-aldosterone system genotype may identify a high-risk subgroup of single ventricle patients who fail to fully benefit from volume-unloading surgery. Follow-up is warranted to assess long-term impact.

Clinical Trial Registration—http://www.clinicaltrials.gov. Unique identifier: NCT00113087.

Key Words: angiotensin-converting enzyme inhibitors ■ congenital heart disease ■ genetic association analysis ■ heart surgery ■ hypertrophy

Infants with single-ventricle lesions account for a significant proportion of the health burden related to congenital heart defects.1 In infancy, these lesions are characterized by a single functioning ventricle that supports both the systemic and pulmonary circulations, resulting in significant ventricular volume overload. Two subsequent palliative surgeries are performed, one at 4 to 6 months of age, the superior cavopulmonary connection (SCPC), and another between 18 to 36 months of age, the Fontan procedure. The SCPC diverts superior vena cava flow directly to the lungs, thus partially unloading the ventricle.2 It is not until after the Fontan procedure that there is a complete separation of the pulmo-
nary and systemic circulations. The single ventricle therefore remains exposed to chronic hypoxia and increased volume load throughout infancy. This is usually associated with an increase in ventricular mass that is disproportionate to the increase in volume. This can have a detrimental effect on cardiac function by causing an imbalance in myocardial oxygen demand and supply with resultant myocardial damage. Ultimately, the burden of the abnormal physiology, coupled with the maladaptive response of the ventricular muscle, contributes to poor long-term outcomes including growth impairment, heart failure, and reduced survival.

Clinical Perspective on p 2362
An important mediator of the ventricular response to hemodynamic load is the renin-angiotensin-aldosterone system (RAAS). Persistent RAAS upregulation can have a detrimental effect by causing peripheral vasoconstriction as well as promoting cellular apoptosis and fibrosis. Although angiotensin-converting enzyme (ACE) inhibitor therapy has shown beneficial antihypertrophic effects in patients with pressure overload–induced hypertrophy, the recently concluded Pediatric Heart Network randomized trial failed to show a beneficial effect of ACE inhibition on the 14-month outcomes of ventricular mass or somatic growth in infants with single ventricle. Variations in RAAS genes are an important determinant of the ventricular hypertrophic response in physiological and pathological states. These variations can also influence the response to ACE inhibitor or angiotensin receptor blocker therapy in patients with other conditions, like systemic hypertension. The Pediatric Heart Network therefore undertook a pharmacogenetic substudy to investigate if polymorphisms in RAAS genes influence the cardiac phenotype and the response to ACE inhibitor therapy in infants with single ventricle.

The primary objective of this analysis was to investigate associations between RAAS gene polymorphisms and the ventricular remodeling response to enalapril in infants with single-ventricle lesions. The 5 RAAS genes included angiotensinogen (AGT), angiotensin-converting enzyme (ACE), angiotensin II type 1 receptor (AGTR1), aldosterone synthase (CYP11B2), and cardiac chymase A (CMA1). We also studied the potential influence of RAAS genotypes on somatic growth and measures of renal function.

Methods

Study Design
The study was performed as part of a randomized, double-blind, placebo-controlled clinical trial by the Pediatric Heart Network comparing the effects of enalapril versus placebo on somatic growth in infants with single ventricles. Patients were randomly assigned to enalapril (target dose 0.4 mg · kg⁻¹ · d⁻¹) or placebo and followed up from enrollment until 14 months of age to assess the effects on growth for at least 6 months after the SCPC surgery. Informed consent was obtained for the genetic study before the SCPC surgery. The study protocol was approved by local institutional review boards, and written informed consent was obtained from a parent/guardian.

Renin-Angiotensin-Aldosterone System Genotyping
A blood sample was obtained and genomic DNA was isolated from whole blood using PureGene kits (Gentra Systems). Patients were genotyped for polymorphisms in 5 RAAS genes: (1) a G/A missense variant at position –235 in angiotensinogen (AGT) (rs11568053); (2) a 287 bp intron 16 deletion variant of angiotensin-convert enzyme (ACE); (3) an A/C substitution at position 1166 in the 3’ untranslated region of angiotensin II type 1 receptor (AGTR1) (rs15816); (4) a C/T polymorphism at position –344 in aldosterone synthase (CYP11B2) (rs1799998); and (5) an A/G polymorphism at position –1903 of cardiac chymase A (CMA1) (rs1800875). Renin-angiotensin-aldosterone system genotypes were determined by pyrosequencing assays for AGTR1, CYP11B2, AGT, and CMA1 and electrophoresis of polymerase chain reaction products for the ACE assay, as previously described. Polymorphisms were selected on the basis of previous association studies, functional effects, and population allele frequencies. Alleles previously associated with RAAS upregulation were classified as risk alleles with high risk defined as homozygosity for the risk alleles. The clinicians caring for the patients were unaware of patient genotypes.

Clinical Phenotype
All subjects underwent assessment of weight, height, head circumference z-scores, ventricular mass, volumes, and ejection fraction on 2-dimensional echocardiography, Ross heart failure class, systolic and diastolic blood pressure (BP) at echocardiography visits, and B-type natriuretic peptide (BNP) levels pre-SCPC and at 14 months. For each of the height, weight, and head circumference measurements, the z-score represents the number of SDs from the mean value for age compared with normative values published by the World Health Organization and the Centers for Disease Control. Serial serum creatinine was used to estimate glomerular filtration rate (eGFR) using the Schwartz equation, the only validated method for the pediatric population. Because the Schwartz equation can underestimate renal insufficiency, we analyzed change scores in eGFR for our study. Echocardiograms were analyzed by a single core-laboratory observer as detailed previously. Ventricular mass, volume, and mass:volume ratio were expressed as z-scores relative to body surface area. B-type natriuretic peptide concentration was measured in a core laboratory. End points were measured at the study visit immediately before the SCPC surgery (mean age, 5.1 ± 1.6 months) and at the final study visit (mean age, 14.1 ± 0.9 months).

Statistical Analysis
We ascertained if the genotype frequencies were in Hardy-Weinberg equilibrium using the Pearson χ² test. We combined risk genotypes in order to analyze the compound effect of multiple risk genotypes within the same biological pathway. Because of sample size limitations, for the primary analysis we divided subjects into 2 prespecified subgroups: a high-risk group with ≥2 homozygous risk genotypes and a low-risk group with <2 risk genotypes. This classification was based on our previous reports that showed an association of ≥2 RAAS high-risk homozygous genotypes with ventricular hypertrophy in cardiomyopathy and transplantation patients. The following outcomes were compared by risk group at the pre-SCPC and final study visit: z-scores for weight, height, head circumference, ventricular mass, ventricular end-diastolic volume (EDV), mass:volume ratio, ejection fraction, systolic and diastolic BP, Ross heart failure score, BNP levels, and eGFR. The change in outcomes between the 2 time points was also compared. Mean z-scores were compared with the normal mean (zero) using a 1-sample t test or Wilcoxon signed rank test. We also analyzed the interaction of systemic ventricular morphology with genotype on ventricular mass by ANOVA.
significant associations by risk group were found, we assessed the effect of each individual genotype using the recessive and dominant models. We examined association among treatment, outcomes, and level of genetic risk by fitting the number of high-risk genes (0, 1, 2, and 3) as a linear term. We used linear regression with a treatment assignment and high- versus low-risk interaction term to assess whether there was a differential effect of treatment (enalapril versus placebo) on outcome by risk subgroup. Statistical analyses were performed using SAS Statistical Software v.9.2 (SAS Institute, Cary NC) and S-Plus 8.0 (Insightful Corp, Seattle, WA). We estimated effect size for the 2-factor interaction (treatment×genetic risk) at a power of 0.80 for \( P \leq 0.05 \) assuming equal variance for \( z \)-scores (variance=1.16). The minimum effect size at 80% power for the primary outcome measure of weight \( z \)-score was 0.61\( z \) between high- and low-risk genotype groups, 0.97\( z \) between high-risk enalapril versus placebo, and 0.63\( z \) between low-risk enalapril versus placebo.33,34

### Results

#### Genotype Frequencies

Of 230 subjects enrolled in the trial, 31 died or underwent cardiac transplantation. Of the remaining subjects, 195 were approached, 164 (84\%) consented for the genetic substudy, 159 submitted a blood sample, and 154 had adequate DNA samples for genotyping. As samples were obtained before SCPC, the study cohort was biased toward patients who survived beyond stage 1 palliation, as seen by the higher incidence of death/transplantation in the nongenotyped versus genotyped patients (33\% versus 4\%, \( P<0.001 \)). The allele and genotype frequencies are shown in Table 1, and were comparable to the general population.17,21,22,27 Genotype frequencies were in Hardy-Weinberg equilibrium, with no gender-, race-, or ethnicity-based differences. Forty-six patients (30\%) had no homozygous risk genotypes, 70 (45\%) had one, 27 (17\%) two, 10 (7\%) three and 1 (1\%) had four risk genotypes. Thirty-eight patients (25\%) were classified as high-risk (\( \geq 2 \) homozygous risk genotypes), and 116 patients as low-risk (<2 homozygous risk genotypes).

#### Patient Characteristics

There were no differences in demographic characteristics at enrollment between the genotype groups, except for an older age (mean, 24 versus 20 days) and lower weight and height \( z \)-scores at enrollment in the high-risk group (Table 2). Clinical and echocardiographic characteristics at the pre-SCPC and final study visits are shown in Table 3. The average age at SCPC surgery was not different between the high- and low-risk groups (5.4±1.7 versus 5.6±1.6 months, \( P=0.72 \)). There were no differences in echocardiographic ventricular volumes, ejection fraction, incidence of moderate-severe atriointerventricular valve regurgitation, BNP levels, systolic and diastolic BP, Ross heart failure score, and incidence of death/transplantation between the 2 groups at the pre-SCPC and final study visits. Ventricular mass was higher in the high-risk group at the final study visit.

#### Renin-Angiotensin-Aldosterone System Genotype and Response to Volume Unloading

Ventricular mass, volume, and mass/volume ratio were significantly elevated compared with normative values for age in both groups at the pre-SCPC visit (Table 3). Ventricular EDV \( z \)-score decreased by an average of 1.2 \( U \) in the low-risk group from pre-SCPC to 14 months (\( P<0.001 \)) but not in the high-risk group (\( P=0.02 \) versus low-risk group, Figure 1A). Similarly, ventricular mass \( z \)-score decreased after the SCPC surgery in the low-risk (\( P<0.001 \)) but not the high-risk group and remained elevated above normal at the final study visit (\( P=0.049 \) versus low-risk group; Figure 1B). Ventricular mass/volume ratio remained significantly elevated compared with normative values for age in both groups pre-SCPC and at 14 months (Table 3). There was a positive association between number of RAAS-upregulation genotypes and ventricular mass \( z \)-score at 14 months (0.55±0.24 increase in \( z \)-score per risk genotype, \( P=0.015 \)). When analyzed by individual genotypes, this association was significant for the AGTR1 risk genotype (Figure 2). The mass/volume ratio at 14 months also showed a positive correlation with a higher number of risk genotypes (0.50±0.27 increase in \( z \)-score per risk genotype, \( P=0.05 \)). There was no significant interaction

### Table 1. Frequency of High-Risk Alleles and Risk Genotypes (n=154)

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>High-Risk Allele</th>
<th>Homozygous High-Risk Genotype Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT (%)</td>
<td>C (0.56)</td>
<td>CC (0.32)</td>
</tr>
<tr>
<td>ACE</td>
<td>D (0.54)</td>
<td>DD (0.27)</td>
</tr>
<tr>
<td>AGTR1</td>
<td>C (0.27)</td>
<td>CC (0.05)</td>
</tr>
<tr>
<td>CYP11B2</td>
<td>C (0.44)</td>
<td>CC (0.20)</td>
</tr>
<tr>
<td>CMA1</td>
<td>A (0.44)</td>
<td>AA (0.18)</td>
</tr>
</tbody>
</table>

\( AGT \) indicates angiotensinogen; \( ACE \), angiotensin-converting enzyme; \( AGTR1 \), angiotensin II type 1 receptor; \( CYP11B2 \), aldosterone synthase; and \( CMA1 \), chymase.

### Table 2. Patient Characteristics at Enrollment (n=154)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>High Risk</th>
<th>Low Risk</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>38</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>Age at randomization, d</td>
<td>24±11</td>
<td>20±9</td>
<td>0.05</td>
</tr>
<tr>
<td>Gestational age at birth, wk</td>
<td>38.3±1.5</td>
<td>38.4±1.4</td>
<td>0.58</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.21±0.51</td>
<td>3.28±0.52</td>
<td>0.67</td>
</tr>
<tr>
<td>Birth weight for gestational age percentile</td>
<td>45.7±29.1</td>
<td>50.2±29.1</td>
<td>0.37</td>
</tr>
<tr>
<td>Male, %</td>
<td>74</td>
<td>66</td>
<td>0.43</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>Hispanic</td>
<td>8</td>
<td>19</td>
<td>0.20</td>
</tr>
<tr>
<td>Hypoplastic left heart syndrome, %</td>
<td>61</td>
<td>58</td>
<td>0.07</td>
</tr>
<tr>
<td>Weight for age ( z )-score</td>
<td>-1.70±1.40</td>
<td>-1.14±1.24</td>
<td>0.02</td>
</tr>
<tr>
<td>Height for age ( z )-score</td>
<td>-1.44±1.39</td>
<td>-0.94±1.20</td>
<td>0.03</td>
</tr>
<tr>
<td>Head circumference for age ( z )-score</td>
<td>-2.11±1.61</td>
<td>-1.65±1.38</td>
<td>0.09</td>
</tr>
</tbody>
</table>
difference in weight and head circumference between the z-scores compared with the low-risk group at enrollment. Infants with high-risk genotypes had lower weight and height and Somatic Growth

Renin-Angiotensin-Aldosterone System Genotype

0.35). SCPC (P = 0.53) or at the 14-month visit (P = 0.05). There were no differences between systolic and diastolic BP (or BP z-scores), and genotype on ventricular mass z-score; systemic vascular resistance was not measured. The frequency of recurrent coarctation was also similar between the 2 risk groups (5.3% in the high-risk and 5.2% in the low-risk group). There was no significant interaction of risk-genotype group and ventricular morphology on the ventricular mass z-score before SCPC (P = 0.53) or at the 14-month visit (P = 0.35).

Table 3. Patient Characteristics at Pre-SCPC and Final Study Visits

<table>
<thead>
<tr>
<th></th>
<th>Pre-SCPC</th>
<th>Final Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High Risk</td>
<td>Low Risk</td>
</tr>
<tr>
<td>n†</td>
<td>38</td>
<td>116</td>
</tr>
<tr>
<td>Weight z*</td>
<td>-1.9±1.0</td>
<td>-1.5±1.2</td>
</tr>
<tr>
<td>Height z*</td>
<td>-1.6±1.3</td>
<td>-1.1±1.2</td>
</tr>
<tr>
<td>Head Circumference z*</td>
<td>-1.5±1.3</td>
<td>-1.3±1.2</td>
</tr>
<tr>
<td>Median BNP, pg/ml (IQR)</td>
<td>80 (44–155)</td>
<td>71 (30–161)</td>
</tr>
<tr>
<td>EDVz*</td>
<td>1.7±2.6</td>
<td>2.1±2.4</td>
</tr>
<tr>
<td>ESVz*</td>
<td>2.7±3.8</td>
<td>3.3±3.6</td>
</tr>
<tr>
<td>EF%</td>
<td>59±9</td>
<td>58±10</td>
</tr>
<tr>
<td>Mass z*</td>
<td>4.1±3.5</td>
<td>4.2±2.8</td>
</tr>
<tr>
<td>Mass/vol z*</td>
<td>1.3±0.55</td>
<td>1.2±0.48</td>
</tr>
<tr>
<td>Mod AVVR</td>
<td>27%</td>
<td>25%</td>
</tr>
<tr>
<td>O2 sats, %</td>
<td>75±8</td>
<td>76±7</td>
</tr>
<tr>
<td>EDP, mm Hg†</td>
<td>8±3</td>
<td>8±4</td>
</tr>
<tr>
<td>Stroke vol</td>
<td>13±5</td>
<td>14±6</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>118±18</td>
<td>123±19</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>88±11</td>
<td>88±14</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>43±13</td>
<td>46±12</td>
</tr>
<tr>
<td>DBPz</td>
<td>-0.11±1.4</td>
<td>0.18±1.29</td>
</tr>
<tr>
<td>Ross HF score, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>68</td>
<td>50</td>
</tr>
<tr>
<td>II</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>III</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

High Risk Low Risk

* z-score differs from zero, P < 0.05.

†At the pre-SCPC visit, sample size varies from 30 to 38 for the high-risk and 107–116 for the low-risk group. At the final study visit, sample size ranges from 33–37 in the high-risk and 105 to 110 in the low-risk group, with the exception of BNP, which had group sizes of 27 and 85, respectively.

SCPC indicates superior cavopulmonary connection; HC, head circumference; BNP, B-type natriuretic peptide; EDV, end-diastolic volume; ESV, end-systolic volume; Mod AVVR, moderate atrioventricular valve regurgitation; EF, ejection fraction; O2 sats, oxygen saturation; EDP, end-diastolic pressure (only available at pre-SCPC cardiac catheterization); HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HF, heart failure; and IQR, interquartile range.

Renin-Angiotensin-Aldosterone System Genotype and Somatic Growth

Infants with high-risk genotypes had lower weight and height z-scores compared with the low-risk group at enrollment (P < 0.05) (Figure 3). Weight and head circumference z-scores increased in both groups during study follow-up (P < 0.01 for both), so that by 14 months there was no difference in weight and head circumference between the groups. Even though change in height z-scores was similar between the 2 groups from pre-SCPC to 14 months, height z-scores at 14 months remained significantly lower in high-risk compared with low-risk patients (−1.3±1.1 versus −0.8±1.1, P = 0.01).

Renin-Angiotensin-Aldosterone System Genotype and Response to Enalapril

The proportion of subjects assigned to enalapril was 47% in the high-risk and 54% in the low-risk group (P = 0.57; mean dose, 0.31±0.13 mg·kg⁻¹·d⁻¹). There were no differences in 14-month outcomes of ventricular mass, volume, function, BNP concentration, or Ross heart failure class between the enalapril and placebo groups regardless of genotype. However, high-risk patients treated with enalapril had lower weight, height, and head-circumference z-scores pre-SCPC compared with those assigned to placebo (interaction
After adjusting for baseline z-scores, this difference remained significant for height, with a lower mean height z-score at both time points in high-risk patients on enalapril ($P < 0.05$) (Figure 4A and B; interaction $P < 0.05$).

Renin-Angiotensin-Aldosterone System Genotype and Renal Function

Table 4 shows the renal characteristics of enrolled subjects. Estimated glomerular filtration rate at enrollment was $53 \pm 15 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ in high-risk and $54 \pm 16 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ in low-risk patients ($P = 0.75$). Estimated glomerular filtration rate increased during study follow-up to $98 \pm 36 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ (77% of normal) in high-risk, and $105 \pm 26 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ (83% of normal) in low-risk patients at 14 months. $P < 0.001$) but not for the high-risk group (change score, $12 \pm 40 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$, $P = 0.13$) ($P = 0.47$ between groups) (Figure 5A). The change in eGFR was independent of enalapril (interaction $P = 0.71$; Figure 5B; Table 4).

Discussion

Our study suggests that RAAS-upregulation genotypes in infants with single ventricles are associated with unfavorable remodeling and a deleterious effect of enalapril on growth. Patients with high-risk RAAS genotypes showed persistent elevation in ventricular mass and volume, less improvement in renal function, and persistent impaired somatic growth despite volume-unloading surgery. Growth impairment was exacerbated by enalapril treatment in high-risk genotype patients. These findings have important clinical implications for genetic risk stratification and pharmacotherapy in this cohort.
The first important finding is that SCPC surgery was associated with a failure to decrease mass and volume in single-ventricle patients with RAAS-upregulation genotypes. Multiple studies have reported an association of individual RAAS genotypes with cardiac hypertrophy, although a recent meta-analysis of genome-wide association studies did not identify an association between single-nucleotide polymorphisms in the RAAS pathway and cardiac phenotype.36 Our study was unique because we evaluated the compound effect of single-nucleotide polymorphisms in multiple genes in the same pathway. Also, our study cohort consisted of single-ventricle patients during their most vulnerable phase, (ie, infancy) when the systemic ventricle is exposed to dynamic shifts in hemodynamic loading conditions, which may enhance the influence of variations in RAAS signaling on cardiac remodeling. The association of a higher number of high-risk RAAS genotypes with an incremental increase in ventricular mass at 14 months suggests a gene-dosage effect of multiple polymorphisms in the RAAS pathway. Importantly, although the RAAS genotypes have been associated with hypertrophic response to pressure and volume load, this is the first study to report an association of RAAS genotypes with a failure to achieve volume unloading and reverse remodeling after a volume-unloading procedure.16–18 The failure of effective unloading was not related to a difference in incidence of atrioventricular valve regurgitation, additional sources of pulmonary blood flow, differences in BP, or

Figure 3. A, Weight; B, height; and C, head circumference z-scores at baseline (ie, enrollment, pre-SCPC, and final study visit by genotype). Low risk (black, n=116); high risk (red, n=38). The offset at the different time points between the genotype groups is for better visualization of SEs. Squares indicate mean value; whiskers, SE; and SCPC, superior cavopulmonary connection. *P<0.05 from low-risk group.

Figure 4. Differences in growth z-scores between the enalapril- and placebo-treated patients in the 2 risk groups (high risk shown in red, low risk shown in black) at 2 time points: at pre-SCPC and final study visits. Data are shown as mean and 95% confidence intervals, adjusted for baseline z-scores. Mean values to the left of zero indicate lower z-scores in enalapril-treated patients (ie, placebo-beneficial); mean values to the right of zero indicate higher z-scores in the enalapril-treated patients (ie, enalapril-beneficial). The interaction \(P\) values represent the differences in treatment effect between the high- and low-risk groups. There was no treatment effect on weight, height, or head circumference in the low-risk group (black) at pre-SCPC (A), and at 14 months (B). However, high-risk patients (red) receiving enalapril had lower height z-scores at pre-SCPC, and at 14 months compared with the placebo group; n=63, enalapril-treated low risk; n=53, placebo-treated low risk; n=18, enalapril-treated high risk; n=20, placebo-treated high risk. *P<0.05 enalapril versus placebo. WTZ indicates weight z-core; HTZ, height z-score; HCZ, head circumference z-score; and SCPC, superior cavopulmonary connection.
frequency of recurrent coarctation between the 2 genotype groups. Also, there was no influence of ventricular morphology on the response of the ventricle to unloading in either risk group. The high-risk group, however, did fail to significantly increase eGFR after SCPC. This finding with respect to renal function raises the intriguing possibility that the lack of remodeling in the high-risk cohort may be related in part to persistent volume load and/or elevated systemic vascular resistance due to renal insufficiency. These cardiorenal interactions require further investigation. Other possibilities include a direct prohypertrophic effect of tissue RAAS upregulation or high myocardial oxygen demands in the high-risk group with a resulting failure to decrease stroke volume. High myocardial oxygen consumption has been previously reported in patients with high-risk RAAS genotypes, in particular the ACE DD genotype, especially during conditions with increased metabolic requirements.

The second finding of the failure of enalapril to reduce ventricular mass in either risk group is not surprising. A recent study in a rat model of eccentric left ventricular hypertrophy caused by volume overload reported that ACE inhibition did not induce reverse remodeling. Other studies have also shown the failure of ACE inhibition to reduce myocardial oxygen consumption in patients with congenital heart disease, as well as lack of efficacy of ACE inhibition in patients with complex congenital heart defects and volume overloaded ventricles. Together these findings suggest that, unlike pressure-overload hypertrophy, ACE inhibition does not have an antihypertrophic effect in volume-overload hypertrophy. Surgical unloading appears to be more effective than conventional pharmacotherapy in promoting reverse remodeling in the volume-loaded ventricle, at least in the low-risk genotype group.

The third important finding is that RAAS upregulation genotypes were associated with growth impairment including lower mean weight and height z-scores at enrollment, with persistent impairment in height at age 14 months. This impairment was most significant in the high-risk patients taking enalapril. The mechanism of height impairment in high-risk patients was not assessed in our study. However, other studies report that RAAS activation increases systemic vascular resistance and arterial afterload leading to ventricular diastolic dysfunction and fetal growth restriction. Insulin resistance as reported in newborns with an AGT M235T TT genotype is another potential mechanism for growth impairment. Impaired energy efficiency and increased whole-body oxygen consumption as reported in subjects with the ACE DD genotype also contributes to a lower anabolic response in high-risk genotype subjects. Together, these studies suggest that RAAS upregulation is associated with impaired physiological adaptation to increased metabolic demands resulting in growth impairment. The mechanism for the adverse effect of enalapril on height

### Table 4. Renal Function

<table>
<thead>
<tr>
<th></th>
<th>eGFR, ml·min⁻¹·1.73 m⁻²</th>
<th>High Risk/Enalapril</th>
<th>High Risk/Placebo</th>
<th>P*</th>
<th>Low Risk/Enalapril</th>
<th>Low Risk/Placebo</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n†</td>
<td></td>
<td>18</td>
<td>20</td>
<td>63</td>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrollment</td>
<td></td>
<td>55±13</td>
<td>51±156</td>
<td>0.40</td>
<td>51±14</td>
<td>57±18</td>
<td>0.03</td>
</tr>
<tr>
<td>Pre-SCPC</td>
<td></td>
<td>77±24</td>
<td>90±31</td>
<td>0.19</td>
<td>85±26</td>
<td>90±22</td>
<td>0.39</td>
</tr>
<tr>
<td>14 Months</td>
<td></td>
<td>91±41</td>
<td>104±32</td>
<td>0.40</td>
<td>107±21</td>
<td>104±31</td>
<td>0.62</td>
</tr>
</tbody>
</table>

* t test P value.
† n represents sample size at enrollment. n at 14 months was 11 (high risk/enalapril), 15 (high risk/placebo), 48 (low risk/enalapril), and 37 (low risk/placebo). eGFR indicates estimated glomerular filtration rate; SCPC, superior cavopulmonary connection.
was not assessed although we speculate that this may be related to a failure to increase cardiac output despite afterload reduction by enalapril in a single-ventricle physiology. Overall, the failure to see a benefit of enalapril in this cohort compounded by the detrimental effect on growth in the high-risk group argues against the routine use of enalapril in the management of single-ventricle lesions.

Limitations
Because this was primarily a pharmacogenetic study nested within a prospective clinical trial, a replication cohort was not available. The study may have been insufficiently powered to detect an association between individual high-risk genotypes and outcomes. Nonetheless, significant associations were seen both with linear regression analysis and with analysis dividing the cohort into 2 risk groups. This approach highlights the importance of assessing the compound effect of multiple single-nucleotide polymorphisms in a pathway rather than separate analysis of single-nucleotide polymorphisms in a single gene. Because the genetic study was biased toward patients who survived beyond the first few months of life, we were unable to assess the influence of RAAS genotypes on early mortality. Also, in light of the relatively short follow-up after the SCPC surgery, we were unable to assess if persistent ventricular hypertrophy in high-risk patients was associated with adverse outcomes, as reported previously, or whether volume-unloading surgery should be performed earlier in high-risk patients to achieve maximal benefit before progressive damage from RAAS activation.46,47

In conclusion, this is the first prospective pharmacogenetic analysis of ACE inhibition in a congenital heart disease population. We showed an important association of RAAS genotypes with cardiac and renal response to volume-unloading surgery. The findings of our study may help in identifying infants with single-ventricle lesions who are at risk for persistent elevation in ventricular mass and volume and growth impairment despite volume-unloading surgery. The failure of a beneficial effect of enalapril argues for the need to develop alternative approaches that include newer pharmacotherapies and possibly earlier surgical interventions in the high-risk cohort to prevent maladaptive ventricular remodeling.

Appendix
The following individuals comprised the Pediatric Heart Network Investigators:

National Heart, Lung, and Blood Institute: Gail Pearson, Victoria Pemberton, Mario Stylianou, Marsha Mathis

Network Chair: Lynn Mahony, University of Texas Southwestern Medical Center

Data Coordinating Center: New England Research Institutes, Lynn Sleeper (PI), Steven Colan, Lisa Virzi, Lisa Wruck*, Victor Zak, David F. Teitel, Leslie Kalish*, Patty Connell*

Core Clinical Site Investigators: Children’s Hospital Boston, Jane W. Newburger (PI), Roger Breitbart, Jami Levine, Ellen McGrath, Carolyn Dunbar-Masterson; Children’s Hospital of New York, Daphne Hsu* (Study Chair), William Hellenbrand (PI), Ashwin Prakash*, Seema Mital*, Darlene Servedo*; Children’s Hospital of Philadelphia, Victoria L. Vetter (PI), Chitra Ravishankar, Sarah Tabbutt*, Meryl Cohen, Katherine Lee, Marisa Nolan, Stephanie Piacentino, Michelle Toms; Cincinnati Children’s Medical Center, D. Woodrow Benson (PI), Catherine Dent Krawczeski, Lois Bogen- schutz, Teresa Barnard, Steven Schwartz*, David Nelson; North Carolina Consortium: Duke University, East Carolina University, Wake Forest University, Page A. W. Anderson (PI) – deceased, Jennifer Li (PI), Wesley Coviz, Kari Crawford, Michael Hines, James Jaggers, Theodore Koutlas, Charlie Sang, Jr, Lort Jo Sutton, Minglen Xu; Medical University of South Carolina, J. Philip Saul (PI), Andrew Atz, Girish Shiral, Eric M. Graham, Teresa Atz; Primary Children’s Medical Center and the University of Utah, Salt Lake City, Utah, L. LuAnn Minich (PI), John A. Hawkins, Richard V. Williams, Linda M. Lambert, Marian E. Shearror; Hospital for Sick Children, Toronto, Brian McCrindle (PI), Elizabeth Radoweski, Nancy Slater, Svetlana Khairuk, Susan McIntyre

Auxiliary sites: Children’s Hospital of Wisconsin, Nancy Ghanayem, Kathy Musatso, Michele Frommel, Lisa Young-Borkowski; University of Michigan, Albert Rocchini, Laurie Rodgers Augustynia

Echocardiography Core Laboratory: Children’s Hospital Boston: Steven Colan, Renee Margosian

Genetics Core Laboratory: Children’s Hospital of New York: Wendy Chung, Liyong Deng, Patricia Lanzano

Protocol Review Committee: Michael Artman, Chair; Judith Massicot-Fisher, Executive Secretary; Timothy Feltes, Julie John- son, Thomas Kitz, Jeffrey Krischer, G. Paul Matherne Data and Safety Monitoring Board: John Kugler, Chair; Rae-Ellen Kavey, Executive Secretary; David J. Driscoll, Mark Galantowicz, Sally A. Hunsberger, Thomas J. Knight, Holly Taylor, Catherine L. Webb

*No longer at the institution listed.

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Disclosures
None.

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vascular allometric relationships in children. J Appl Physiol. 2005;99:
445–457.
The Pediatric Heart Network conducted a pharmacogenetic study as part of a multicenter randomized, controlled trial of enalapril versus placebo in single-ventricle infants to assess if renin-angiotensin-aldosterone system (RAAS)—upregulation genotypes influence the response to enalapril. This represents the first pharmacogenetic study of enalapril in a congenital heart disease population. One-hundred fifty-four infants with single ventricle were genotyped and followed up until 14 months of age. Patients with RAAS-upregulation genotypes had persistent increase in ventricular mass and volume despite volume-unloading surgery (ie, superior cavopulmonary connection). Enalapril did not decrease ventricular mass or volume in either genotype group. Patients with high-risk genotypes had lower weight and height at enrollment, and the height impairment persisted in high-risk patients who were receiving enalapril whereas patients receiving placebo normalized their height by 14 months. The high-risk genotype group also showed mild but persistent renal dysfunction. In summary, patients with RAAS-upregulation genotypes failed to show reverse remodeling in response to volume-unloading surgery, had persistent growth abnormalities, especially with enalapril, and had persistent renal dysfunction. These patients may need earlier superior cavopulmonary connection to facilitate reversal of ventricular dilation and hypertrophy before the remodeling becomes irreversible. Because neither enalapril nor surgery showed significant benefit in high-risk genotype patients, there is a need to develop newer therapies in at-risk patients.
Renin-Angiotensin-Aldosterone Genotype Influences Ventricular Remodeling in Infants With Single Ventricle

Seema Mital, Wendy K. Chung, Steven D. Colan, Lynn A. Sleeper, Cedric Manlhiot, Cammon B. Arrington, James F. Cnota, Eric M. Graham, Michael E. Mitchell, Elizabeth Goldmuntz, Jennifer S. Li, Jami C. Levine, Teresa M. Lee, Renee Margossian and Daphne T. Hsu

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