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## Adrenergic Receptor Genotypes Influence Post-operative Outcomes in Infants in the Single Ventricle Reconstruction Trial

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**Conflict of Interest statement:**

The authors have no conflicts of interest.

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## Abstract

**Objectives**—Adrenergic receptor (ADR) genotypes have been associated with adverse outcomes in heart failure. Our objective was to evaluate the association of ADR genotypes with post-Norwood outcomes in infants with hypoplastic left heart syndrome (HLHS).

**Methods**—Infants with HLHS participating in the Pediatric Heart Network Single Ventricle Reconstruction Trial underwent genotyping for four single nucleotide polymorphisms in three ADR genes: *ADRB1\_231A/G*, *ADRB1\_1165G/C*, *ADRB2\_5318C/G* and *ADRA2A\_2790C/T*. The association of genotype with freedom from serious adverse events (SAE) (death, transplant, extra-corporeal membrane oxygenation, cardiopulmonary resuscitation, acute shunt failure, unplanned re-operations, or necrotizing enterocolitis) during 14 months follow-up was assessed using Cox regression and the association with post-Norwood complications was assessed using Poisson regression. Models were adjusted for clinical and surgical factors.

**Results**—The study included 351 eligible patients (62% male; 83% White). The mean age at Norwood procedure was  $5.6 \pm 3.6$  days. 152 patients had SAEs during 14-month follow-up including 84 deaths and 10 transplants. *ADRA2A\_2790CC* genotype had lower SAE-free survival compared to *CT/TT* genotypes during follow-up (Log rank test,  $p=0.02$ ) and this association was independent of clinical and surgical risk factors (Adjusted Cox regression. HR 1.54 [1.04, 2.30]  $p=0.033$ ). Post-Norwood complication rate did not differ by genotype.

**Conclusions**—Infants with HLHS harboring ADR genotypes that are associated with higher catecholamine release or sensitivity had lower event-free survival after staged palliation. Excess catecholamine activation may adversely affect cardiovascular adaptation after the Norwood procedure. Future studies should explore if targeting adrenergic activation in those harboring risk genotypes can improve outcomes. ([ClinicalTrials.gov number, NCT00115934](https://clinicaltrials.gov/ct2/show/study/NCT00115934))

Single ventricle lesions comprise over 7% of all congenital heart defects with hypoplastic left heart syndrome (HLHS) being the most common lesion.<sup>1, 2</sup> In HLHS, a single right ventricle (RV) acts as a systemic ventricle while the left ventricle (LV) is hypoplastic or rudimentary. Patients with HLHS require multiple palliative surgeries to separate the pulmonary and systemic circulations with the first staged palliation i.e. the Norwood procedure performed in the newborn period. Maintenance of systemic cardiac output after the Norwood procedure requires a balance between the systemic and pulmonary circulations and adequate RV function. Abnormal systemic and pulmonary vascular resistance and ventricular dysfunction are major contributors to maladaptation to a single ventricle physiology. In one analysis, up to 40% of patients with staged palliation were noted to have some degree of heart failure.<sup>3</sup>

Single ventricle patients have fragile hemodynamics and are especially vulnerable after cardiac surgery. The post-operative physiological stress response involves a surge in endogenous catecholamine production which can have profound effects on cardiac function

and vascular tone mediated by adrenergic receptors. Adrenergic receptors include presynaptic  $\alpha_2a$  and  $\alpha_2c$  receptors that inhibit norepinephrine release, cardiac  $\beta_1$  receptors that mediate chronotropic and inotropic response to norepinephrine, and  $\beta_2$  receptors that mediate vascular smooth muscle relaxation and response to  $\beta$ -blockers.<sup>4</sup> Adrenergic upregulation is an important mechanism of cardiovascular adaptation during stress particularly in the infant where the circulation is highly catecholamine-dependent.<sup>5, 6</sup> While acute upregulation is compensatory, chronic upregulation can have detrimental effects by increasing systemic and pulmonary vascular resistance, and increasing myocardial oxygen demands, resulting in adverse cardiac remodeling and ventricular dysfunction.<sup>4, 7–12</sup> In children with dilated cardiomyopathy,  $\alpha_2$  and  $\beta_1$  upregulation and  $\beta_2$  downregulation genotypes were associated with worse ventricular function, heart failure progression and acute hemodynamic decompensation.<sup>13</sup> The impact of adrenergic receptor genotypes on adaptation to hemodynamic and surgical stress in infants with HLHS undergoing staged palliation is not known. We hypothesized that genetic modulation of receptor activity would alter catecholamine response and thereby influence post-operative clinical course. The purpose of our study was to evaluate if genetic variants that upregulate adrenergic receptor activity adversely impact post-Norwood outcomes in infants with HLHS enrolled in the single ventricle reconstruction (SVR) trial.

## METHODS

### Study population

This is an ancillary study that derives from the Pediatric Heart Network (PHN) SVR Trial, in which infants with HLHS (n=555) were enrolled from 15 North American centers between May 2005 and July 2008. The details of the study design, inclusion criteria, study assessments as well as trial results have been previously published.<sup>14, 15</sup> In brief, inclusion criteria for the SVR trial included a diagnosis of HLHS or a related single morphologic RV anomaly, planned Norwood procedure, and absence of a genetic or medical condition that would affect transplant-free survival. Infants were randomized to receive a modified Blalock-Taussig shunt (MBTS) versus a right ventricle – pulmonary artery shunt (RVPAS) as part of their Norwood procedure and were followed for 14 months with serial assessment. The study was approved by the local institutional review boards and informed consent for study participation was obtained from the parents or legal guardians. Data collection included patient demographics and baseline characteristics, operative variables, hospitalization course, clinical outcomes, and echocardiographic assessment of RV ejection fraction (RVEF). All echocardiograms were reviewed centrally by an independent observer at the echocardiography core laboratory.<sup>14, 15</sup>

### Genotyping

Buccal swab epithelial cells were collected at enrollment or during Norwood hospitalization using CytoSoft cytology brushes (Medical Packaging Corporation, Camarillo, CA) after obtaining informed consent. Methods have been reported previously.<sup>16</sup> Genomic DNA was extracted using a PureGene kit (Gentra Systems, Inc, Minneapolis, Minn) according to the manufacturer's protocol. Samples were retrieved from the PHN Biorepository for genotyping. Participants in the SVR trial who did not consent to future testing of their

samples were not included in the ancillary study. The ancillary study was approved by the PHN Ancillary Study Review Committee and by the University of Michigan Institutional Review Board.

Adrenergic receptor single nucleotide polymorphisms (SNPs) were selected on the basis of previous association studies, functional effects, and population allele frequencies.<sup>13, 17,24</sup> Genotyping (Sequenom SNP) was performed for four SNPs in three adrenergic receptor (ADR) genes: a A/G missense variant at position 231 in *ADRB1* resulting in a serine to glycine substitution (rs1801252), a C/G missense variant at position 1165 in *ADRB1* resulting in a glycine to arginine substitution (rs1801253), a C/G missense variant at position 5318 in *ADRB2* resulting in a glutamine to glutamic acid substitution (rs1042714) and a C/T 3' untranslated region (UTR) variant at position 2790 in *ADR2A* (rs553668). Patients carrying a single copy of the variant allele or SNP (also known as minor allele) at the genetic locus were defined as having a heterozygous genotype, those with two copies of the variant allele as having a homozygous genotype, and those without a variant allele i.e. carrying only the normal allele (also known as major allele) were defined as having a wild-type genotype.

Since variants in the renin-angiotensin-aldosterone signaling (RAAS) pathway genes have been previously associated with adverse ventricular remodeling in infants with single ventricle lesions, genotyping was also performed for four SNPs and one insertion-deletion variant in five RAAS genes. The following were considered RAAS-upregulation genotypes based on our previous work: *AGT\_CC*, *ACE\_DD*, *AGTR1\_CC*, *CYP11B2\_CC*, and *CMA1\_AA*. The *CYP11B2* and *CMA1* variants were genotyped using Sequenom SNP genotyping and the *AGTR1*, *AGT* and the *ACE* insertion/deletion polymorphisms were genotyped using TaqMan primers and probes. For samples and SNPs which did not yield a valid result by Sequenom or TaqMan analysis, PCR amplification followed by Sanger sequencing was performed. The detailed genotyping methods, primers and probes for the Sequenom SNP analysis, the Taqman analysis and for the PCR amplification or Sanger sequencing are listed in Supplemental Methods and Supplemental Table 1.

### Statistical Analysis

Allele and genotype frequencies were determined to calculate Hardy-Weinberg equilibrium. All demographic and clinical data were expressed as frequencies, mean with standard deviations, or medians with interquartile ranges (IQR). The primary outcome was a composite of any serious adverse event (SAE) during a 14-month follow-up after the Norwood procedure and included death, transplant, need for post-operative extra-corporeal membrane oxygenation (ECMO), postoperative cardiopulmonary resuscitation (CPR), acute shunt failure, unplanned re-operations or necrotizing enterocolitis.<sup>25, 26</sup> Secondary outcomes included frequency of post-Norwood complications up to hospital discharge from stage 2 surgery, and RVEF during 14 months follow-up. Complications were defined as adverse events using the common terminology criteria for adverse events version 3.0 developed by the National Cancer Institute, NIH, but that did not meet criteria for SAEs.<sup>26</sup> Since post-Norwood hospital and intensive care unit (ICU) length of stay were highly correlated with each other and with Norwood complication rate when analyzed using bootstrap Spearman rank correlation (data not shown), these outcomes were not analyzed separately.

ADR genotype associations were analyzed by comparing outcomes in homozygous or heterozygous SNP carriers versus wild-types using a dominant model. Freedom from the primary outcome was described using the Kaplan-Meier method with stratification by genotype. The log-rank test was used to assess between-stratum differences. Cox proportional hazard models adjusted for covariates of interest were used to test the association of the genotypes with the primary outcome. The proportional hazards assumption was verified using cumulative Martingale process plots. Associations were quantified by hazard ratios (HRs) and reported along with their 95% confidence intervals (CIs). P-values were calculated based on Wald's statistics. Poisson regression adjusted for over-dispersion and for exposure time (number of days of hospitalization for Norwood procedure) was used to model the association of risk genotype with frequency of post-Norwood complications and results were presented as incidence rate ratios (IRR) with 95% confidence intervals. A mixed model was used to assess for association of genotype with RVEF during 14 months follow-up. All analyses were adjusted for gender, race, birth weight, gestational age, presence of aortic atresia or obstructed pulmonary venous return, presence of a genetic syndrome, age at Norwood procedure, total Norwood cardiopulmonary bypass time, deep hypothermic circulatory arrest time, type of shunt, and presence of a RAAS-upregulation genotype.<sup>27</sup> The covariates selected were not found to exhibit significant collinearity. In regression models, when patients had missing data on the presence of identifiable syndromes, it was assumed that identifiable syndromes were not present. Observations with missing data in other variables were excluded from the models and this represented a very small percentage of the total cohort. All statistical analyses were performed using SAS v9.4.

## RESULTS

Of 555 patients in the SVR trial, 436 provided DNA and 351 had complete genotype data (i.e. genotyping results for all 9 SNPs) and were included in the analysis (Figure 1). ADR genotype frequencies are shown in Table 1. All genotypes were in Hardy Weinberg equilibrium. Patient clinical characteristics and outcomes in the group with complete versus incomplete genotypes are shown in Table 2. The only significant difference was a higher incidence of death in the completely genotyped cohort compared to the incompletely genotyped cohort. Incomplete genotyping was the result of technical issues such as low quality or quantity of DNA. The results hereby focus on the 351 subjects with complete genotype data.

We evaluated the association of ADR genotypes with the primary outcome of composite SAEs. Sixty-four percent patients had the *ADRA2A\_2790CC* genotype (wild-type). Kaplan-Meier analysis showed that patients with the *CC* genotype had lower SAE-free survival during 14 month follow-up compared to *CT/TT* genotypes (log rank test:  $p=0.02$ ) (Figure 2). This association was independent of RAAS genotype, clinical and surgical risk factors (Adjusted Cox regression: HR 1.549 [1.04, 2.30]  $p=0.033$ ). There was no synergistic adverse effect of multiple risk genotypes on outcomes.

Tables 3 and 4 describe the incidence rate of Norwood complications by genotype groups. Overall, 79 percent patients had a post-Norwood complication with a median [95% CI] of 2

[1–4] complications per patient (Table 2). Complications by systems are shown in Table 5. The list of complications included for this trial have been previously published.<sup>26</sup> Cardiac complications included arrhythmias, pericardial effusion, hypotension or hypertension, RV dysfunction, valvar insufficiency, and other. We evaluated the association of ADR genotypes with the rate of complications after the Norwood procedure using Poisson regression. All regression models were adjusted for demographic, clinical and surgical risk factors as well as for RAAS genotypes and the Poisson regression was adjusted for exposure time. There were no significant differences in incidence rates of Norwood complications by genotype (Figure 3). ADR genotypes were not associated with significant difference in RV function during 14 month follow-up (data not shown) as assessed by mixed effects models adjusted for clinical and surgical factors.

## DISCUSSION

In an era of precision medicine, there is a growing recognition of the importance of identifying not just clinical predictors but also genetic predictors of outcomes that can be used to individualize care and improve the safety and efficacy of medical and surgical interventions based on the unique genome and phenome of a patient. Previous studies have identified genetic variants that increase susceptibility to neurodevelopmental outcomes and adverse ventricular remodeling in single ventricle patients undergoing staged palliation as well as in patients with other types of congenital heart disease undergoing surgical repair.<sup>16, 28, 29, 30, 31</sup> In this study, we evaluated the association of genetic variants that increase ADR signaling with post-Norwood outcomes in infants with HLHS. Using genetic material collected during the trial, we demonstrated that the ADR genotype is an important predictor of outcomes of stage 1 palliation in infants with HLHS with lower serious adverse event-free survival in patients harboring ADR risk genotypes. Although further study is required, these findings may have implications for individualizing peri-operative management in this cohort based on genotype.

We evaluated two SNPs in the *ADRB1* gene and one in the *ADRB2* gene. The *ADRB1\_389GG* genotype has been previously associated with pediatric and adult heart failure and the *ADRB1\_231AA* genotype is known to be associated with lower basal adenylate cyclase activity but increased sensitivity of the  $\beta_1$  receptor to norepinephrine with enhanced sympathetic blood pressure response.<sup>17, 18</sup> Neither *ADRB1* genotype nor the *ADRB2* genotype were associated with post-operative complications or SAEs in our study.<sup>10</sup>

The ADR2A receptor is important in vascular tone as well as metabolic responses like insulin release from pancreatic cells and adipocyte metabolism in humans. Genetic variations lead to alterations in G-protein coupling and in agonist-promoted receptor phosphorylation and desensitization thereby modulating response to catecholamines.<sup>12</sup> The *ADRA2A\_2790CC* genotype (wild-type) has been reported to cause reduced feedback inhibition of norepinephrine and failure to inhibit sympathetic tone. While we did not measure sympathetic tone, we found that patients with the *CC* genotype had lower freedom from SAEs during 14 month follow-up compared to *CT/TT* genotypes. One explanation for this finding is that a milieu of high circulating catecholamines in patients with the *CC* genotype may have resulted in increased vasomotor tone and an adverse effect on systemic

output and organ perfusion, thereby increasing the risk of post-Norwood SAEs. The event-free survival curves diverged relatively sharply after the early postoperative period, at around 15 days (Figure 1). This suggests that while early events were more likely secondary to surgical complications which were similar in both genotype groups, later events were more likely influenced by genetic differences in adrenergic system-mediated adaptation to the Norwood circulation as well as recovery from complications. Overall, our findings suggest that genotypes associated with increased adrenergic neurohormonal activation and responsiveness may have a detrimental effect in the post-Norwood circulation.

A limitation of our study is that only a small number of candidate SNPs were analyzed. The DNA in the trial was acquired via buccal swabs which required PCR amplification and did not yield sufficient quantity or quality of DNA for a more unsupervised approach using whole exome sequencing. Nonetheless, the SNPs were carefully selected based on prior associations with outcomes in other pediatric cardiac studies. Also, RV myocardial samples and detailed hemodynamic data were not collected during the Norwood procedure for the trial precluding assessment of tissue adrenergic receptor expression, and of potential influence of the genotypes on metabolic and hemodynamic function. Additionally, we were unable to evaluate the pharmacogenetic influences of the ADR genotypes with  $\beta$ -blocker response since less than 5% patients were receiving  $\beta$ -blocker therapy during follow-up. Also, the sample size of this study was reduced due to insufficient DNA or incomplete genotyping in the trial cohort. Nonetheless, the baseline characteristics of the genotyped and non-genotyped cohorts were similar excluding a survival bias in our study cohort.

In summary, ADR genotypes that enhance catecholamine levels and/or sensitivity increase the risk of serious adverse events in patients who survive beyond the first two weeks after the Norwood procedure. Further studies are needed to delineate the biological and hemodynamic effects of these genotypes and to validate these findings in additional cohorts. Pharmacologic modification of adrenergic receptors has been shown to positively influence outcome in other heart failure cohorts.<sup>21,22, 23</sup> While there is no evidence to support empiric  $\beta$ -blocker use in HLHS patients, studies are needed to determine if pre-operative identification of these genetic subtypes can be used to target  $\beta$ -blockers and/or other forms of more aggressive systemic vasodilation post-Norwood to the subset with these risk genotypes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

See Appendix (Supplementary Material) for a complete list of the Pediatric Heart Network Investigators.

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## Glossary of Abbreviations

<b>ADR</b>	Adrenergic receptor
<b>CI</b>	confidence interval
<b>ECMO</b>	Extra-corporeal membrane oxygenation
<b>HLHS</b>	Hypoplastic left heart syndrome
<b>HR</b>	Hazard ratio
<b>LV</b>	left ventricle
<b>MBTS</b>	modified Blalock- Taussig shunt
<b>OR</b>	Odds ratio
<b>PHN</b>	Pediatric Heart Network
<b>RAAS</b>	Renin- angiotensin- aldosterone system
<b>RV</b>	Right ventricle
<b>RVEF</b>	right ventricular ejection fraction
<b>RVPAS</b>	Right ventricle to pulmonary artery shunt
<b>SVR trial</b>	Single Ventricle Reconstruction Trial

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**Perspective Statement**

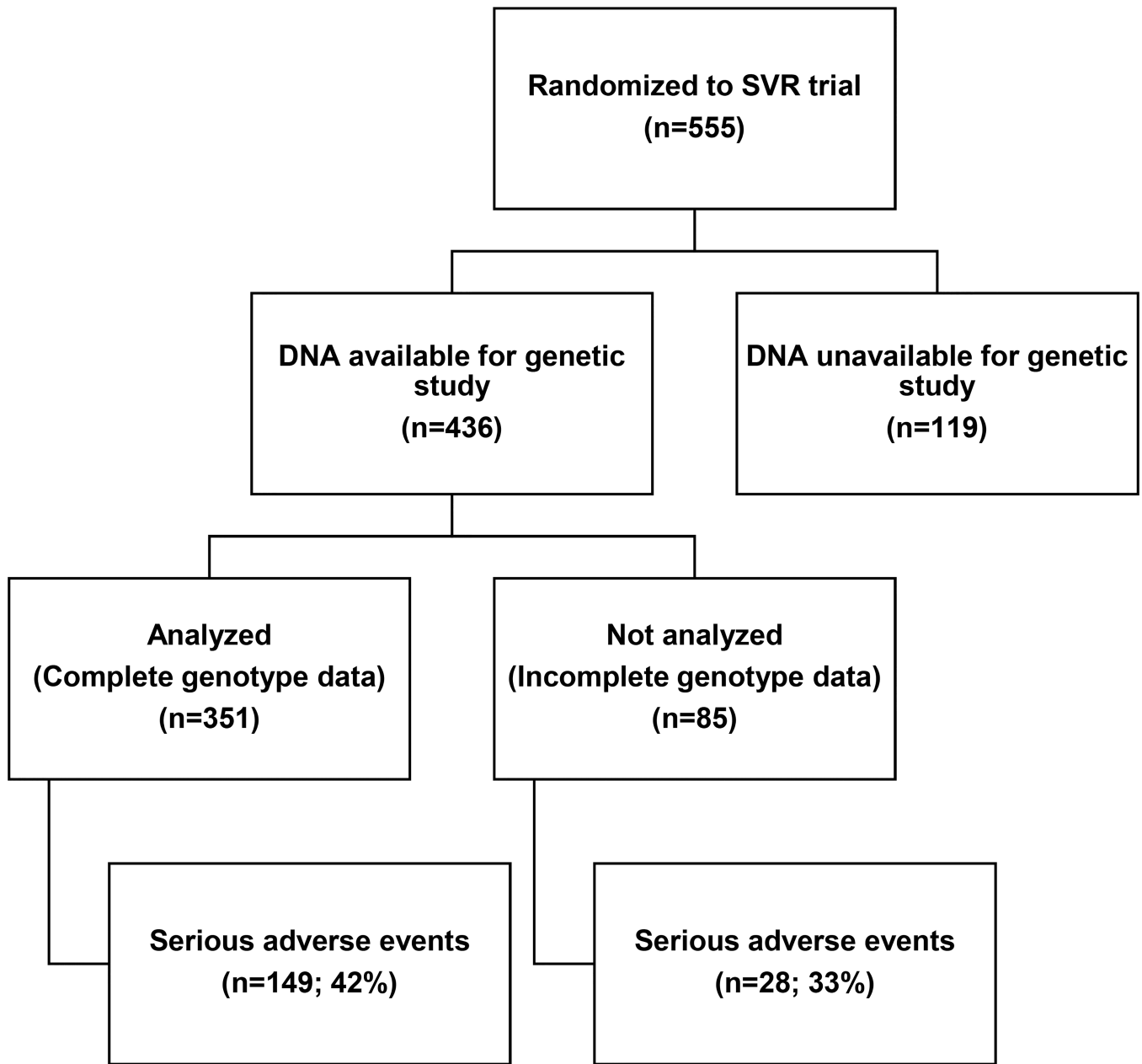
Staged palliation for single ventricle lesions is associated with significant morbidity and mortality. Patients with adrenergic receptor genotypes associated with augmented catecholamine signaling had lower freedom from serious adverse events after Stage 1 palliation. Future studies should explore if targeting adrenergic activation in those harboring risk genotypes can improve outcomes.

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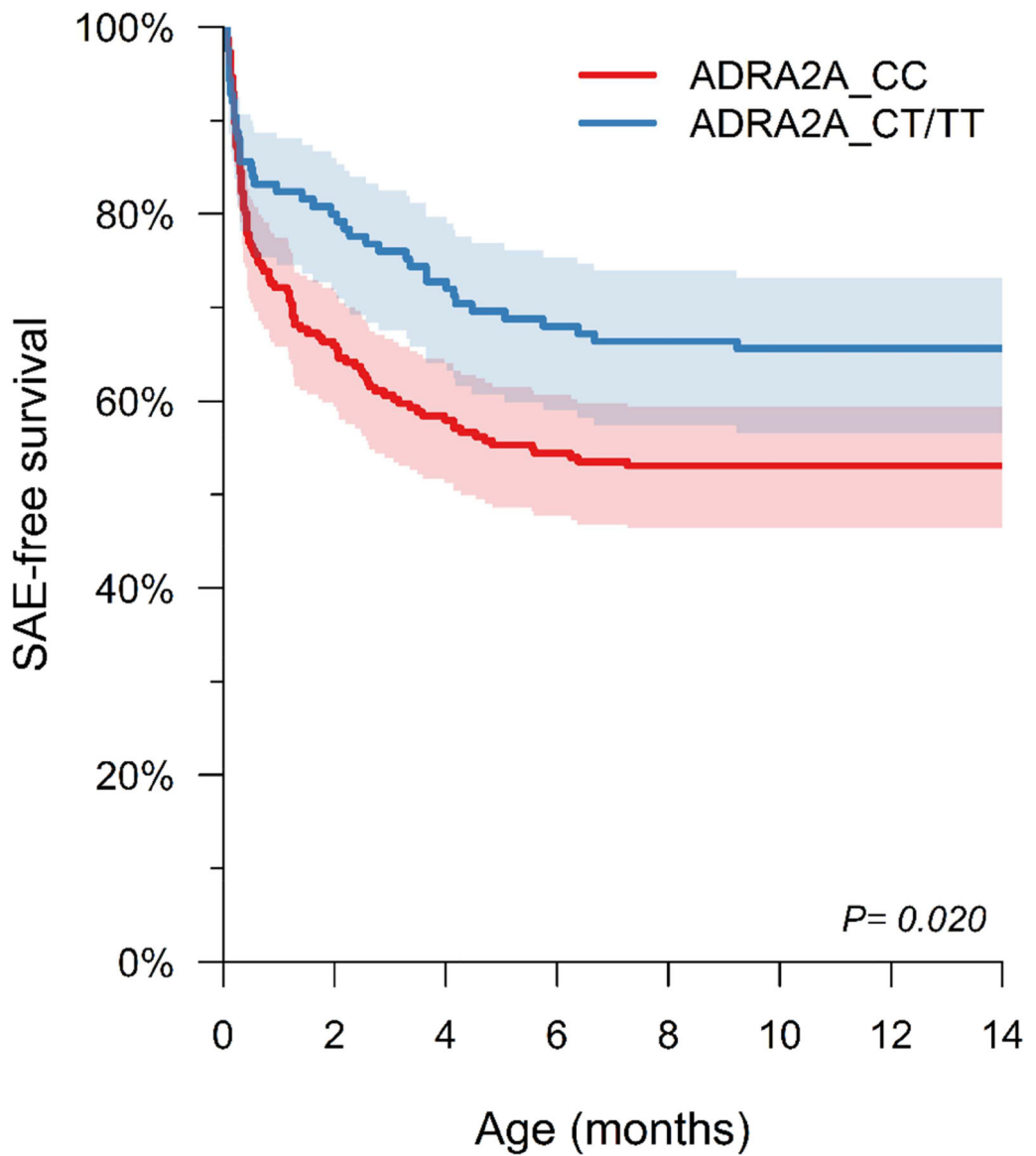
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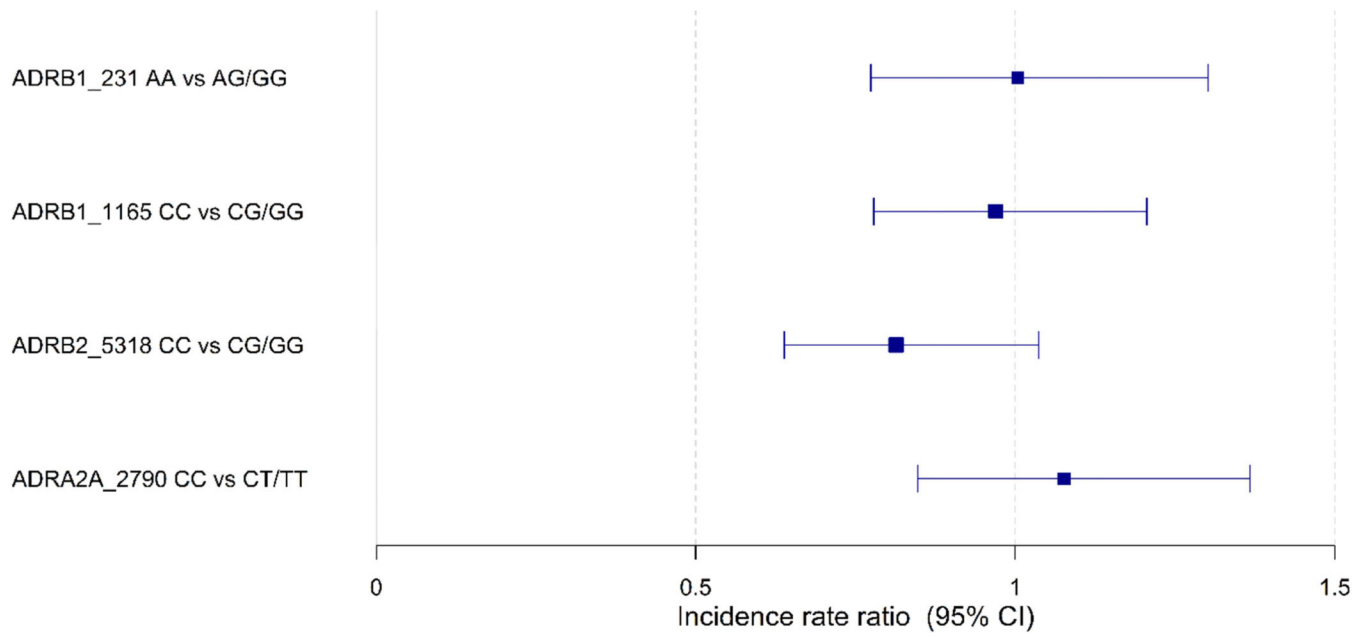
**Figure 1.**  
A consort flow diagram showing the outcomes in the complete and incompletely genotyped groups from patients randomized in the single ventricle reconstruction (SVR) trial.



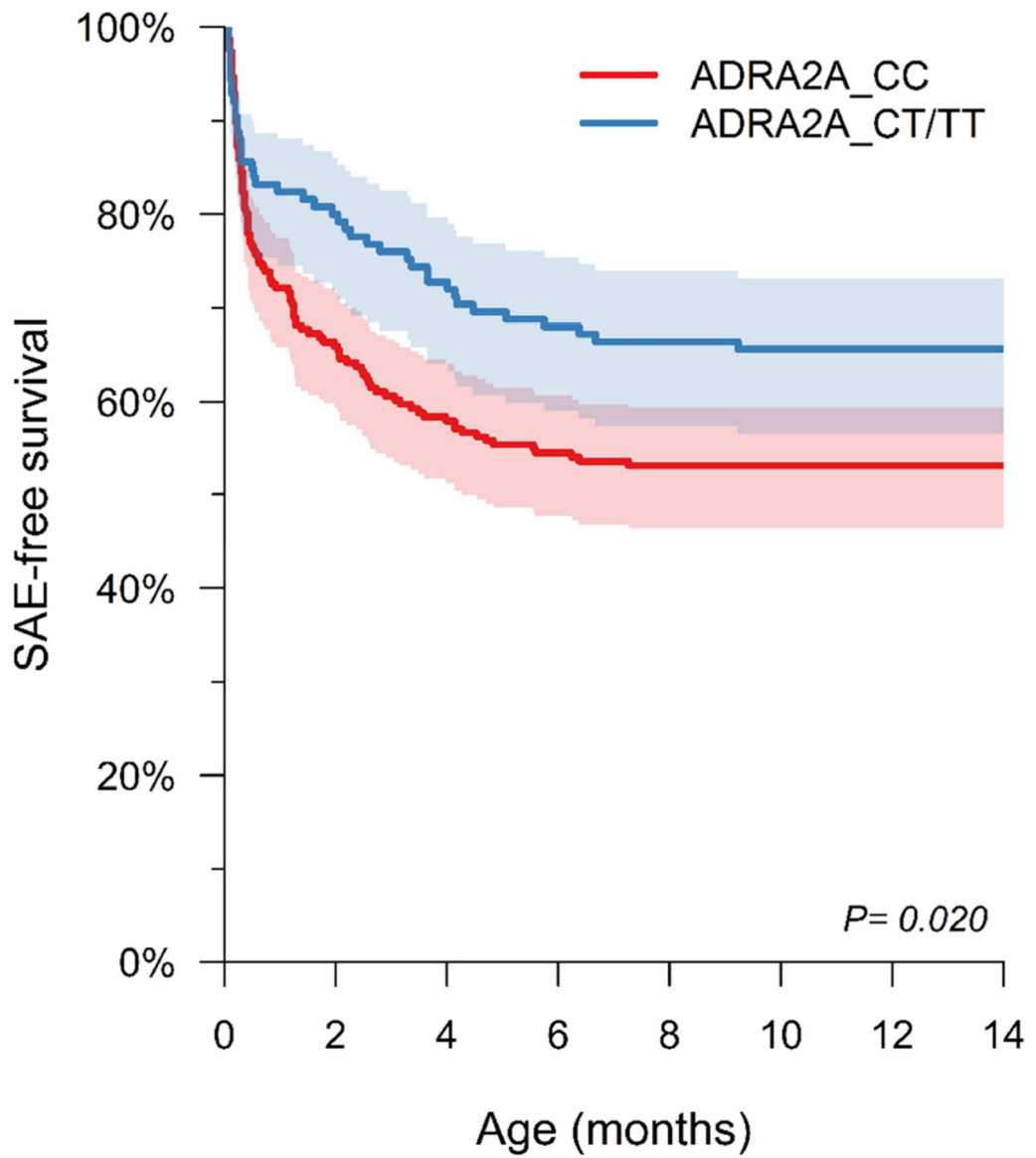
At-risk:

ADRA2A_CC:	226	149	131	123	120	120	120	120
ADRA2A_CT/TT:	125	100	91	85	83	82	82	82

**Figure 2.** Kaplan Meier survival curve showing survival free from composite primary outcome stratified by *ADRA2A\_2790* genotype. The shaded bands represent 95% confidence intervals. Subjects with *ADRA2A\_2790CC* genotype had lower survival free from serious adverse events (SAE) compared to subjects with *CT/TT* genotypes (p=0.02).



**Figure 3.** Forest plot of incidence rate ratios (IRRs) of Norwood complications by genotype. The IRR is the ratio of the expected number of complications per patient per day of Norwood hospitalization in each genotype group. There were no significant differences in the IRRs of Norwood complications by genotype.



At-risk:

ADRA2A_CC:	226	149	131	123	120	120	120	120
ADRA2A_CT/TT:	125	100	91	85	83	82	82	82

**Central Message.**

Variations in adrenergic receptor genes influence postoperative outcomes in infants with hypoplastic left heart syndrome undergoing staged palliation.

Kaplan Meier plot: Lower serious adverse event-free survival with *ADRA2A\_7790CC* genotype (log rank test p-value=0.020).

**Table 1**

Adrenergic receptor variant frequencies

Gene (SNP ID)	Nucleotide substitution	Amino acid substitution	Function by genotype	SNP carrier frequency	HWE p value
<i>ADRB1</i> (rs1801252)	231 A/G (missense)	Ser49Gly	GG: Higher basal adenylate cyclase activity and lower isoproterenol sensitivity	32%	0.717
<i>ADRB1</i> (rs1801253)	1165 C/G (missense)	Gly389Arg	CC: Increases $\beta$ 1 receptor sensitivity	44%	0.818
<i>ADRB2</i> (rs1042714)	5318 C/G (missense)	Gln27Glu	GluGlu: Enhances response to $\beta$ adrenergic receptor antagonist	62%	0.972
<i>ADRA2A</i> (rs553668)	2790 C/T (3' UTR)		CC: Increased norepinephrine release	36%	0.918

ADR, adrenergic receptor; HWE, Hardy Weinberg equilibrium; SNP, single nucleotide polymorphism



**Table 2**

Patient characteristics and outcomes

Characteristics	N	Complete genotyping	N	Incomplete genotyping	p <sup>†</sup>
Gender, Male (%)	351	220 (62.7%)	85	56 (65.9%)	0.62
Race, White (%)	351	288 (82.1%)	85	68 (80.0%)	0.44
Birth weight (kg)	351	3.13 ± 0.53	85	3.13 ± 0.53	0.96
Gestational age (weeks)	344	38 ± 1	83	38 ± 2	0.38
Identifiable syndrome (%)	150	22 (14.7%)	19	3 (15.8%)	1.00
Aortic atresia (%)	351	215 (61.3%)	85	50 (58.8%)	0.71
Obstructed PVR (%)	351	10 (2.8%)	85	1 (1.2%)	0.70
Age at Norwood (days)	351	6 ± 4	85	6 ± 4	0.12
Age at stage II palliation (days)	276	162 ± 53	75	164 ± 77	0.78
Total CPB time (mins)	351	139 ± 51	85	140 ± 43	0.86
DHCA time (mins)	324	33 ± 21	76	31 ± 18	0.27
MBTS (%)	351	170 (48.4%)	85	44 (51.8%)	0.63
RVPAS (%)	351	183 (52.1%)	85	43 (50.6%)	0.81
<b>Norwood course</b>					
Median hospital LOS (days)	351	24 (15–42)	85	22 (15–32)	0.35
Median ICU LOS (days)	255	15 (9–33)	52	13 (9–24)	0.65
Patients with complications (%)	351	278 (79.2%)	85	63 (74.1%)	0.31
Number of complications per patient	351	3 ± 3	85	2 ± 3	0.25
Pre-stage II RVEF (%)	195	44 ± 9	56	43 ± 7	0.47
<b>14 months follow-up</b>					
Deaths (%)	351	84 (23.9%)	85	11 (12.9%)	0.028
Norwood hospitalization (%)	84	36 (42.9%)	11	4 (36.3%)	
After Norwood discharge (%)	84	48 (57.1%)	11	7 (63.6%)	
Transplants (%)	351	10 (2.8%)	85	2 (2.4%)	1.00
Norwood hospitalization (%)	10	6 (60%)	2	1 (50%)	
After Norwood discharge (%)	10	4 (40%)	2	1 (50%)	

Characteristics	N	Complete genotyping	N	Incomplete genotyping	p <sup>‡</sup>
Serious adverse events (%)	351	152 (43.3%)	85	29 (34.1%)	0.14
14 months RVEF (%)	168	43 ± 8	48	42 ± 7	0.52

<sup>‡</sup> p values were calculated using either Fisher exact test or Student t test.

PVR, pulmonary venous return; CPB, cardiopulmonary bypass; DHCA, deep hypothermic circulatory arrest; MBTS, modified Blalock-Taussig shunt; RVPAS, Right ventricle to pulmonary artery shunt; LOS, Length of stay; ICU, intensive care unit; IQR, interquartile range; RVEF, Right ventricular ejection fraction.

**Table 3**

Frequency of SAEs during the first 14 months of follow-up and incidence rate of Norwood complications by ADR genotype.

Gene (SNP ID)	Proportion of subjects with at least one SAE in the first 14 months	P-value	Incidence rate of Norwood complications (Number/subject/day of hospitalization) [95% CI]	P-value
<i>ADRB1</i> (rs1801252)	AA / 108/240 (45%)	GA/GG 44/111 (40%)	AA 0.09 [0.08, 0.10] GA/GG 0.08 [0.07, 0.09]	0.38
<i>ADRB1</i> (rs1801253)	CC / 85/195 (44%)	CG/GG 67/156 (43%)	CC 0.07 [0.06, 0.09] CG/GG 0.09 [0.08, 0.1]	0.07
<i>ADRB2</i> (rs1042714)	CC / 58/133 (43%)	CG/GG 94/218 (43%)	CC 0.09 [0.08, 0.09] CG/GG 0.08 [0.07, 0.10]	0.70
<i>ADRA2A</i> (rs553668)	CC / 107/226 (47%)	CT/TT 45/125 (36.0%)	CC 0.08 [0.07, 0.10] CT/TT 0.08 [0.07, 0.10]	0.93

<sup>1</sup>Major allele: AA, CC, CC, CC genotypes at the four loci represent wild-type genotypes; remainder are polymorphic genotypes i.e. heterozygotes or homozygotes for the SNP ADR, adrenergic receptor; SAE, serious adverse event; CI, confidence interval

Comparison of occurrence of at least one SAE and incidence rate of Norwood complications during 14-month follow-up by ADR genotype

**Table 4**

Gene (SNP ID)	Genotype	Odds ratio [95% CI] of SAE in first 14 months	P-value <sup>†</sup>	Incidence rate ratio [95% CI] of Norwood complications	P-value <sup>††</sup>
<i>ADRB1</i> (rs1801252)	AA / vs. GA/GG	0.732 [0.215, 2.498]	0.62	1.004 [0.774, 1.302]	0.98
<i>ADRB1</i> (rs1801253)	CC / vs. CG/GG	0.564 [0.205, 1.555]	0.27	0.969 [0.779, 1.206]	0.78
<i>ADRB2</i> (rs1042714)	CC / vs. CG/GG	1.036 [0.346, 3.105]	0.95	0.814 [0.638, 1.037]	0.10
<i>ADRA2A</i> (rs553668)	CC / vs. CT/TT	1.785 [1.040, 3.063]	0.036	1.077 [0.875, 1.367]	0.55

<sup>†</sup> Logistic regression adjusted for covariates of interest. All subjects who did not experience an SAE were followed for at least 14 months.

<sup>††</sup> Poisson regression adjusted for covariates of interest and exposure time.

<sup>‡</sup> Major allele: AA, CC, CC, CC genotypes at the four loci represent wild-type genotypes; remainder are polymorphic genotypes i.e. heterozygotes or homozygotes for the SNP SAE, serious adverse event; CI, confidence interval

**Table 5**

Frequency of post-Norwood complications by systems

Post-Norwood complications by system	N=1025
Cardiac	239 (23.3%)
Gastro-intestinal	100 (9.8%)
Hematologic	84 (8.2%)
Infectious disease	233 (22.7%)
Neurological	41 (4.0%)
Renal	35 (3.4%)
Respiratory	249 (24.3%)
Vascular	5 (0.5%)
Other	39 (3.8%)

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