Diastolic Blood Pressure Alleles Improve Congenital Heart Defect Repair Outcomes

By

Joseph H. Breeyear

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Approved:

Todd L. Edwards, Ph.D.

Digna Velez-Edwards, Ph.D.

Douglas M. Ruderfer Ph.D.

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ABBREVIATIONS

Body Mass Index (BMI), Congenital Heart Defect (CHD), Diastolic Blood Pressure (DBP), Polygenic Risk Score (PRS), ICU Length of Stay (ICU-LOS), In-hospital Mortality (HM), Length of Stay (LOS), Society for Thoracic Surgeons-European Association for Cardio-Thoracic Surgery (STAT) Category, Systolic Blood Pressure (SBP), Vasoactive-Inotropic Score (VIS)

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CHAPTER 1
INTRODUCTION

Congenital malformations are the leading cause of infant mortality in the United States.[1] Congenital heart defects (CHD) represent the most frequent congenital malformation, affecting 40,000 US births per year.[2, 3] Approximately half of the children with CHD will require one or more surgical interventions, resulting in roughly $1.5 billion in hospital costs yearly.[4] Advances in interventional techniques have reduced operative complications, but post-operative complications still cause significant morbidity.[5] Furthermore, individuals that survive congenital heart surgery have a significantly higher risk of cardiovascular morbidity and mortality than the age- and sex- standardized United States population.[6, 7] Better understanding of the basis for clinical variability in related morbidity and mortality has the potential to improve clinical management and outcomes.

Polygenic risk scores (PRS) are models that summarize genetic liability for complex traits. Polygenic risk score performance is sometimes attenuated when these models are applied to populations with genetic ancestry different than the ancestry of the population used to develop the models. It has not been widely evaluated to what extent PRS are portable between adults and children, or how much pleiotropy should be expected for PRS that are developed in adults when looking at pediatric cohorts. Our goal was to evaluate whether the genetic determinants of blood pressure in adults influence CHD surgery outcomes in children.

Recent studies of pediatric outcomes that leverage results derived from large-scale genetic studies in adults have observed relationships between genotypes and pediatric outcomes that are distinct from the adult outcomes that led to the detection of the risk alleles. Kachuri et al. recently reported that alleles associated with blood cell traits in adults are also causally associated with childhood acute lymphoblastic leukemia through increases in lymphocyte counts.[8] Additionally, a recent study by Gopel et al. has reported that a PRS for increased blood pressure derived from genome-wide association studies in adults was associated with improved survival in preterm infants with a birth weight below 1.5kg.[9] We sought to determine if this association was generalizable to outcomes of other significant health stressors in a pediatric CHD population. Our observations support those previously reported and further suggest that the wellness of infants with a genetic tendency towards high blood pressure is observed in several aspects of recovery and survival from CHD repair surgery.

CHAPTER 2

METHODS

Study Design

This study utilized an ongoing prospective observational cohort study of children enrolled at the time of cardiac surgery for CHD. The study included any neonates through young adults undergoing their first surgery for CHD at the Monroe Carell Jr. Children's Hospital at Vanderbilt University Medical Center (n_{max} = 2,498) between September 2007 and July 2020 who consented to the study.[10] All CHD diagnoses and counts are presented in **Table 1**.

Table 1. Primary Congenital Heart Defect Diagnosis with Counts.

Subject Primary Diagnosis	Count	Subject Primary Diagnosis	Count
Anomalous Coronary Artery	25	Interrupted Aortic Arch	21
Aortic arch hypoplasia	50	Intracardiac Mass	4
Aortic coarctation and VSD	11	Mitral valve prolapse	11
Aortic Stenosis	78	Other Single Ventricle Anomalies	120
AP window	6	Partial Anomalous Pulmonary Venous Return	75
Arrhythmia only	19	Patent Ductus Arteriosus	12
Atrial Septal Defect	262	Pulmonary artery sling	3
Atrioventricular Septal Defect	229	Pulmonary Atresia w/ VSD (TOF type)	22
AVSD and TOF	8	Pulmonary atresia with IVS	35
Cardiac disease NOS	17	Pulmonary atresia with VSD	32
ccTGA	13	Pulmonary Valvar Stenosis	35
Cleft MV	2	Restrictive CM	4
Coarctation of the Aorta	198	Rheumatic carditis	2
Complex Congenital Heart Disease	21	Shone's Complex	21
Congenital heart disease NOS	33	Shone's complex/HLHS variant	8
Cor Tritriatum	5	Subaortic stenosis	72
Coronary artery fistula	3	Supravalvar AS	18
Critical Pulmonary Stenosis	7	Supravalvar PS	8
Dilated CM	18	Tetralogy of Fallot	321
DORV (TOF type)	20	Tetralogy of Fallot w/ absent Pulmonary Valve	11
Double Chamber RV	15	TOF with pulmonary atresia	31
Double Inlet Left Ventricle	49	Total Anomalous Pulmonary Venous Return	35
Double Outlet Right Ventricle	106	Transitional/partial AVSD	56
Ebstein's Anomaly	26	Transposition of the Great Arteries	143
Heterotaxy	4	Tricuspid Atresia	59
Hypertrophic CM	7	Truncus Arteriosus	55
Hypoplastic Left Heart Syndrome	196	Vascular Rings	59

The Institutional Review Board at Vanderbilt University Medical Center approved the study and written consent was obtained from parents or legal guardians. Peri-operative data were obtained from the patients'

electronic medical record, including age at surgery, weight at surgery, height at surgery, sex, Society for Thoracic Surgeons-European Association for Cardio-Thoracic Surgery (STAT) category (a standard scoring tool designed to analyze the risk for mortality associated with CHD surgeries, ranging from category 1 to 5), and in-hospital mortality (HM). Pre-operative blood pressure measurements (up to 48 hours prior to surgery) were captured from individuals' electronic health records. Post-operative blood pressure measurements (up to 48 hours post-surgery) were captured from individuals' electronic health records. Blood pressure measurements pre- and post- surgery were routinely manipulated through pharmacological means and did not reflect native blood pressures in these patients. The intensive care unit (ICU) length of stay (ICU-LOS) was defined as days between surgery and ICU discharge, excluding those individuals experiencing in-hospital mortality. The post-operative length of hospital stay (LOS) was defined as days between surgery and hospital discharge, excluding those individuals with experiencing in-hospital mortality. Subjects' preoperative, operative, and postoperative data were obtained from the patients' electronic medical record in a systematic fashion in order to minimize observer bias, maintained in a REDCap database.

Vasoactive-inotropic scores were calculated via electronic health record data for each patient for each hour of the first 48 hours after admission to the pediatric cardiac intensive care unit as previously published.[11, 12] The vasoactive-inotropic score quantifies the pharmacological cardiovascular support required by infants undergoing surgical procedures. The score is a summation of adjusted doses of dopamine, dobutamine, epinephrine, milrinone, vasopressin, and norepinephrine, and their respective coefficients to produce physiologically equivalent values. The maximum vasoactive-inotropic score was used for analyses to capture the intensity of treatment necessary to manage blood pressure, **Equation 1**.

$$VIS = dopamine\ dose\ (\mu g/kg/min) + dobutamine\ dose(\mu g/kg/min) + 100 \times epinephrine\ dose\ (\mu g/kg/min) + 10 \times milrinone\ dose\ (\mu g/kg/min) + 10{,}000 \times vasopressin\ dose\ (U/kg/min) + 100 \times norepinephrine\ dose\ (\mu g/kg/min)$$

Sample Collection & Genotyping Quality Control

Anticoagulated blood, saliva, or buccal swabs were collected from each subject. Blood samples were not obtained after recent blood transfusion. If a transfusion was necessary, lab samples obtained prior to transfusion

were utilized, or a sample was obtained in a follow-up at least four months after any transfusion. Genomic DNA was extracted through the Vanderbilt Technologies for Advanced Genomics (VANTAGE) Core laboratory. Genotyping was conducted on the Axiom™ Precision Medicine Research Array and Axiom™ Precision Medicine Diversity Array according to manufacturer protocols at the Children's Hospital of Philadelphia DNA core. Quality control filtering was conducted on individuals and SNPs. SNPs were removed for genotype call rate < 98%, or if minor allele frequency was >20% different from 1000 Genomes phase 3 European reference populations. Individuals were removed if they had a genotype call rate < 98%, the genetically estimated sex differed from the sex assigned in the database, or if they were 1st or 2nd degree relatives of another sample in identity-by-descent analysis. Genotype data was imputed to the TOPMed reference panel r2 using the TOPMed Imputation Server.[13-15] We calculated principal components from the genetic correlation matrix to account for potential bias caused by population substructure.

Statistical Analysis

Polygenic Risk Score Development

The data utilized for the systolic blood pressure (SBP) and diastolic blood pressure (DBP) PRSs were obtained from a published GWAS (n_{max} = 760,226 subjects).[16] The SBP and DBP PRSs were validated in the BioVU resource (n_{max} = 37,132, SBP PRS $p = 4.18 \times 10^{-132}$, DBP PRS $p = 7.63 \times 10^{-113}$) for association with blood pressure measurements, adjusting for age, sex, body mass index (BMI), and the top ten principal components.[17] The BioVU genetic data were pruned for linkage disequilibrium at an r^2 threshold of 0.1 at a maximum distance of 250 kilobases from associated SNPs ($p < 1\text{x}10^{-5}$) in the blood pressure summary statistics. Weighted scores were calculated in PLINK.[18] Polygenic risk score effects are presented per standard deviation increase of the respective PRS. Bonferroni Corrections for the peri-operative outcomes are reported as "nominally" ($0.05 > p > 6.3\text{x}10^{-3}$) or "statistically" ($p < 6.3\text{x}10^{-3}$) significant, to account for eight independent tests. Bonferroni Corrections for the associations including the interaction between the blood pressure PRSs and the vasoactive-inotropic score are reported as "nominally" ($0.05 > p > 8.3\text{x}10^{-3}$) or "statistically" ($p < 8.3\text{x}10^{-3}$) significant, to account for six independent tests.

Peri-Operative Outcomes

Polygenic risk score associations with pre-operative blood pressure were modeled using linear regression in STATA 16, adjusting for age, sex, body mass index, and the top ten principal components of ancestry, including individuals older than 14 days of age with at least two blood pressure measurements during the 48 hours prior to surgery.[19, 20] The analyses were conducted in the multi-ancestry cohort as well as non-Hispanic White and non-Hispanic Black subsets of the CHD cohort.

The developed PRSs were tested for association in the multi-ancestry cohort (n_{max} = 2,498) as well as non-Hispanic White (n_{max} = 1,957) and non-Hispanic Black (n_{max} = 253) subsets of the CHD cohort. For right-skewed outcomes that could be affected by extreme observations, we calculated DFBETAs and removed observations that were greater than the suggested 1.[21] For outcomes with potential heteroscedasticity, we used robust standard errors, which utilizes the Huber Sandwich Estimator, for statistical inference.[22] Analyses for in-hospital mortality and post-operative blood pressure were not conducted in the non-Hispanic Black subset as there were too few events and measurements for stable estimation of unknown parameters. Polygenic risk score associations with post-operative outcomes were modeled using linear or logistic regression in STATA 16, adjusting for age, sex, body mass index, STAT category, and the first ten principal components of ancestry.[19]

Vasoactive-Inotropic Score Interaction

Systolic and diastolic blood pressure variances, for each individual (n_{max} = 2,054), were calculated utilizing blood pressure measurements within a 48-hour window after CHD surgery to assess relationships between risk factors and the ability to maintain target blood pressure. Polygenic risk score associations with the post-operative blood pressure variance were modeled using linear regression in STATA 16, allowing for an interaction between blood pressure PRSs and vasoactive-inotropic score, adjusting for age, sex, BMI, STAT category, and the top ten principal components of ancestry.[19]

Associations with HM, allowing for interaction between the DBP PRS and vasoactive-inotropic score, were modeled using logistic regression in STATA 16, adjusting for age, sex, BMI, STAT category, and the top ten principal components of ancestry.[19] The combined effect of the DBP PRS and the interaction between the

DBP PRS and vasoactive-inotropic score were modeled with the *lincom* function in STATA 16 to address the linear combined of the two predictors.[19]

CHAPTER 3

RESULTS

The CHD cohort was 52% male. Race/ethnicity included non-Hispanic White (79%), non-Hispanic Black (10%), Hispanic (6%), and Other (5%) as seen in **Table 2**.

Table 2. Demographic and Clinical Variables of Children in the Congenital Heart Defect Cohort

Characteristics	**Multi-Ancestry N = 2,498**	**Non-Hispanic White N = 1,957**	**Non-Hispanic Black N = 253**
Age at Surgery (Days) (Median) (IQR)	201 (43 – 1,764)	196 (6.5 – 1,797)	194 (69 – 1,616)
Male	1,293 (52%)	1,025 (52%)	135 (53%)
Race/Ethnicity			
Non-Hispanic White (N)	1,957 (79%)	1,957 (100%)	0 (0%)
Non-Hispanic Black (N)	253 (10%)	0 (0%)	253 (100%)
Hispanic (N)	143 (6%)	0 (0%)	0 (0%)
Other (N)	122 (5%)	0 (0%)	0 (0%)
Body Mass Index (kg/m^2) (Median) (IQR)	15.2 (13.5 – 17.3)	15.2 (13.5 – 17.2)	15.1 (13.6 – 17.3)
Pre-Op Systolic BP (mmHg) (Mean) (SD)	95.63 (18.77)	95.69 (19.08)	94.01 (17.73)
Pre-Op Diastolic BP (mmHg) (Mean) (SD)	55.43 (12.25)	55.48 (12.30)	54.17 (12.22)
STAT Category			
1	871 (35%)	673 (34%)	98 (39%)
2	512 (20%)	401 (20%)	48 (19%)
3	263 (11%)	215 (11%)	23 (9%)
4	459 (18%)	346 (18%)	48 (19%)
5	172 (7%)	144 (7%)	13 (5%)
Vasoactive-Inotropic Score (Mean) (SD)	6.77 (8.26)	6.87 (8.45)	6.09 (6.54)
ICU Stay (Days) (Median) (IQR)	3 (1 – 7)	3 (1 – 7)	3 (1 – 7)
Hospital Stay (Days) (Median) (IQR)	7 (4 – 15)	7 (4 – 15)	7.5 (4 – 19)
In-hospital Mortality (N)	89 (3.6%)	72 (3.7%)	6 (2.4%)

The median (IQR) age at surgery for the multi-ancestry cohort, non-Hispanic White subset, and non-Hispanic Black subset was 201 (43 – 1,764), 196 (6.5 – 1,797), and 194 (69 – 1,616), days respectively. The median (IQR) intensive care unit (ICU) length of stay for the multi-ancestry cohort and both subsets were 3 (1 – 7) days. The median (IQR) length of hospital stay for the multi-ancestry cohort, non-Hispanic White, and non-Hispanic Black subsets were 7 (4 – 15), 7 (4 – 15), 7 (4 – 15), and 8 (4 – 19), days respectively. The mean (SD) vasoactive-inotropic score for the multi-ancestry, non-Hispanic White, and non-Hispanic Black subsets was 6.77 (8.26), 6.87 (8.45), and 6.09 (6.54), respectively. The subsets of the multi-ancestry cohort, only including individuals who were parentally identified as non-Hispanic White (n = 1,957) or non-Hispanic Black (n = 253), possessed similar median values of measured clinical variables. In non-Hispanic White participants, we observed stronger associations between blood pressure PRSs and clinical outcomes than in multi-ancestry

analyses. We observed no statistically significant associations between blood pressure PRSs and clinical outcomes in the non-Hispanic Black subset.

Peri-Operative Outcomes

We observed significant associations between the SBP PRS and pre-operative blood pressure in the multi-ancestry cohort as well as in the non-Hispanic White subset (**Table 3**).

Table 3. Polygenic Risk Score Associations with Pre-Operative Systolic and Diastolic Blood Pressure.

CHD Cohort Trait	Population	N	PRS	β* (SD)	*p* - value
Pre-Operative Diastolic Blood Pressure	Multi-Ancestry	1,323	Diastolic	0.37 ± 0.27 mmHg	0.17
	Non-Hispanic White	1,035	Diastolic	0.42 ± 0.33 mmHg	0.19
	Non-Hispanic Black	151	Diastolic	1.21 ± 1.64 mmHg	0.46
Pre-Operative Systolic Blood Pressure	Multi-ancestry	1,323	Systolic	1.28 ± 0.37 mmHg	**0.00051**
	Non-Hispanic White	1,035	Systolic	1.64 ± 0.42 mmHg	**0.000093**
	Non-Hispanic Black	151	Systolic	0.38 ± 1.63 mmHg	0.82

*adjusted for age, sex, body mass index, and the top ten principal components of ancestry.

For each standard deviation increase in SBP PRS, the pre-operative SBP was estimated to rise by 1.28 ± 0.37 mmHg, $p = 5.1 \times 10^{-4}$, in the multi-ancestry cohort. For each standard deviation increase in SBP PRS, we estimated the pre-operative SBP to rise by 1.64 ± 0.42 mmHg, $p = 9.3 \times 10^{-5}$, in the non-Hispanic White subset. The DBP PRS was not significantly associated with pre-operative DBP in any population.

In-Hospital Mortality

We observed statistically and nominally significant associations between the DBP PRS and HM in the multi-ancestry cohort as well as in the non-Hispanic White subset as seen in **Table 4**.

Table 4. Polygenic Risk Score Associations with In-Hospital Mortality

CHD Cohort Trait	Population	N	PRS	Standardized* Odds Ratio (95% CI)	*p* - value
In-Hospital Mortality	Multi-Ancestry	2,277	DBP	0.66 (0.48 – 0.90)	$\mathbf{8.5 \times 10^{-3}}$
			SBP	1.14 (0.82 – 1.58)	0.43
	Non-Hispanic White	1,779	DBP	0.57 (0.39 – 0.82)	$\mathbf{2.2 \times 10^{-3}}$
			SBP	1.24 (0.87 – 1.78)	0.24

*adjusted for age, sex, body mass index, STAT category, and the top ten principal components of ancestry presented as per standard deviation increase in PRS.

In the multi-ancestry cohort, an increasing DBP was nominally associated with decreased risk of HM (OR = 0.66 (0.48 – 0.90), $p = 8.5 \times 10^{-3}$). In the non-Hispanic White participants, an increasing DBP PRS was significantly associated with decreased risk of HM (OR = 0.57 (0.39 – 0.82), $p = 2.2 \times 10^{-3}$).

Intensive-Care Unit Length of Stay

We observed statistically and nominally significant associations between the DBP PRS and ICU LOS in the multi-ancestry cohort, the non-Hispanic White subset (**Table 5)**. In the multi-ancestry cohort, an increasing DBP PRS was nominally associated with decreased ICU LOS (-1.68 ± 0.68 days, $p = 1.3 \times 10^{-2}$).

Table 5. Polygenic Risk Score Associations with ICU Length of Stay

CHD Cohort Trait	Population	N	PRS	Standardized* β (SD)	*p* - value
ICU Length of Stay	Multi-Ancestry	2,235	DBP	-1.68 ± 0.68 days	$\mathbf{1.3 \times 10^{-2}}$
			SBP	0.86 ± 0.76 days	0.26
	Non-Hispanic White	1,743	DBP	-2.41 ± 0.86 days	$\mathbf{5.4 \times 10^{-3}}$
			SBP	1.14 ± 0.92 days	0.22
	Non-Hispanic Black	227	DBP	1.97 ± 0.89 days	$\mathbf{2.9 \times 10^{-2}}$
			SBP	-1.22 ± 1.27 days	0.34

*adjusted for age, sex, body mass index, STAT category, and the top ten principal components of ancestry per SD increase in PRS

In the non-Hispanic White participants, an increasing DBP PRS was significantly associated with decreased ICU LOS (-2.41 ± 0.86 days, $p = 5.4 \times 10^{-3}$). In the non-Hispanic Black participants, an increasing DBP PRS was significantly associated with increased ICU LOS (1.97 ± 0.89 days, $p = 3.8 \times 10^{-2}$).

Length of Hospital Stay

We observed nominally significant associations between the DBP PRS and LOS in the non-Hispanic White subset (**Table 6**).

Table 6. Polygenic Risk Score Associations with Length of Hospital Stay					
CHD Cohort Trait	**Population**	**N**	**PRS**	**Standardized* β (SD)**	***p* - value**
Length of Hospital Stay	Multi-Ancestry	2,277	DBP	-2.03 ± 0.98 days	$\mathbf{3.8 \times 10^{-2}}$
			SBP	0.56 ± 0.97 days	0.56
	Non-Hispanic White	1,779	DBP	-2.88 ± 1.26 days	$\mathbf{2.2 \times 10^{-2}}$
			SBP	0.76 ± 1.17 days	0.51
	Non-Hispanic Black	230	DBP	2.54 ± 1.51 days	0.095
			SBP	-2.22 ± 1.90 days	0.25
*adjusted for age, sex, body mass index, STAT category, and the top ten principal components of ancestry per SD increase in PRS					

In the non-Hispanic White participants, an increasing DBP PRS was nominally associated with decreased LOS (-2.88 ± 1.26 days, $p = 2.2 \times 10^{-2}$). In the multi-ancestry cohort, an increasing DBP PRS was nominally associated with decreased LOS (-2.03 ± 0.89 days, $p = 3.8 \times 10^{-2}$).

Vasoactive-Inotropic Score

We observed nominally significant associations between the DBP PRS and vasoactive-inotropic score in the multi-ancestry cohort as well as in the non-Hispanic White subset. In the multi-ancestry cohort, an increasing DBP PRS was nominally associated with decreased vasoactive-inotropic score (-0.49 ± 0.25, $p = 4.9 \times 10^{-2}$) (**Table 7**).

Table 7. Polygenic Risk Score Associations with Vasoactive-Inotropic Score.					
CHD Cohort Trait	**Population**	**N**	**PRS**	**Standardized* β (SD)**	***p* - value**
Vasoactive-Inotropic Score	Multi-Ancestry	2,106	DBP	-0.49 ± 0.25	$\mathbf{4.9 \times 10^{-2}}$
			SBP	0.36 ± 0.23	0.12
	Non-Hispanic White	1,641	DBP	-0.54 ± 0.31	8.2×10^{-2}
			SBP	0.36 ± 0.27	0.18
	Non-Hispanic Black	213	DBP	-0.45 ± 0.49	0.36
			SBP	0.25 ± 0.55	0.64
*adjusted for age, sex, body mass index, STAT category, and the top ten principal components of ancestry per SD increase in PRS					

Vasoactive-Inotropic Score Interaction

We investigated the effect of the blood pressure PRSs and vasoactive-inotropic score on the post-operative blood pressure variance. We observed significant and nominal associations between the blood pressure PRSs and post-operative blood pressure variance, while allowing for interaction with vasoactive-inotropic score (**Table 8**).

Table 8. Interaction effect between Diastolic and Systolic Blood Pressure PRS and Vasoactive-Inotropic Score on Post-Surgical Systolic Blood Pressure Variance.

CHD Cohort Trait	Population	N	PRS	β* (SD)	*p* - value
Post-Operative Diastolic Blood Pressure Variance	Multi-Ancestry	2,054	DBP PRS	1.88 ± 0.92	**0.041**
			Interaction	-1.91 ± 0.86	**0.026**
	Non-Hispanic White	1,600	DBP PRS	2.50 ± 1.10	**0.024**
			Interaction	-2.47 ± 1.03	**0.017**
Post- Operative Systolic Blood Pressure Variance	Multi-Ancestry	2,054	SBP PRS	3.70 ± 1.78	**0.038**
			Interaction	-3.12 ± 1.88	0.096
	Non-Hispanic White	1,600	SBP PRS	3.95 ± 2.04	0.053
			Interaction	-4.48 ± 2.20	**0.041**

In the non-Hispanic White subset, for each standard deviation increase in DBP PRS, we estimated the post-operative DBP variance to rise 2.50 ± 1.10, $p = 2.4 \times 10^{-2}$. The interaction term between DBP PRS and vasoactive-inotropic score was estimated to lower the DBP variance (-2.47 ± 1.03, $p = 1.7 \times 10^{-2}$) as DBP PRS and vasoactive-inotropic score increased. We observed a similar trend with the associations between the SBP PRS and post-operative SBP, while allowing for interaction with vasoactive-inotropic score. In the non-Hispanic White subset, the effect of the SBP PRS was not significant (3.95 ± 2.04, $p = 5.3 \times 10^{-2}$), however, the interaction term between SBP PRS and vasoactive-inotropic score was estimated to lower the SBP variance (-4.48 ± 2.20, $p = 4.1 \times 10^{-2}$) as the SBP PRS and vasoactive-inotropic score increased.

We investigated the interaction between the DBP PRS and vasoactive-inotropic score on HM. We observed statistically and nominally significant associations in the multi-ancestry cohort and non-Hispanic White subset (**Table 9**).

Table 9. Interaction effect between per SD increase in Diastolic Blood Pressure PRS and Vasoactive-Inotropic Score Tertiles on In-Hospital Mortality.					
CHD Cohort Trait	**Population**	**N**	**Variable**	**Standardized* Odds Ratio (95% CI)**	***p* - value**
In-Hospital Mortality	Multi-Ancestry	2,106	DBP PRS	1.39 (1.18 – 1.64)	$\mathbf{9.2 \times 10^{-5}}$
			VIS Tertile 2 Interaction	0.40 (0.23 – 0.69)	$\mathbf{9.4 \times 10^{-4}}$
			VIS Tertile 3 Interaction	0.52 (0.89 – 0.71)	$\mathbf{2.8 \times 10^{-5}}$
	Non-Hispanic White	1,641	DBP PRS	1.31 (1.09 – 1.59)	$\mathbf{5.1 \times 10^{-3}}$
			VIS Tertile 2 Interaction	0.46 (0.24 – 0.88)	$\mathbf{1.9 \times 10^{-2}}$
			VIS Tertile 3 Interaction	0.48 (0.34 – 0.68)	$\mathbf{2.8 \times 10^{-5}}$
*adjusted for age, sex, body mass index, STAT category, and the top ten principal components of ancestry per SD increase in PRS					

In the non-Hispanic White subset, for each standard deviation increase in DBP PRS was estimated to increase risk of HM (1.39 (1.18 – 1.64), $p = 5.1 \times 10^{-3}$). However, the interaction between the DBP PRS and vasoactive-inotropic score reduced risk of HM (0.48 (0.34 – 0.68), $p = 2.8 \times 10^{-5}$) (**Figure 1**).

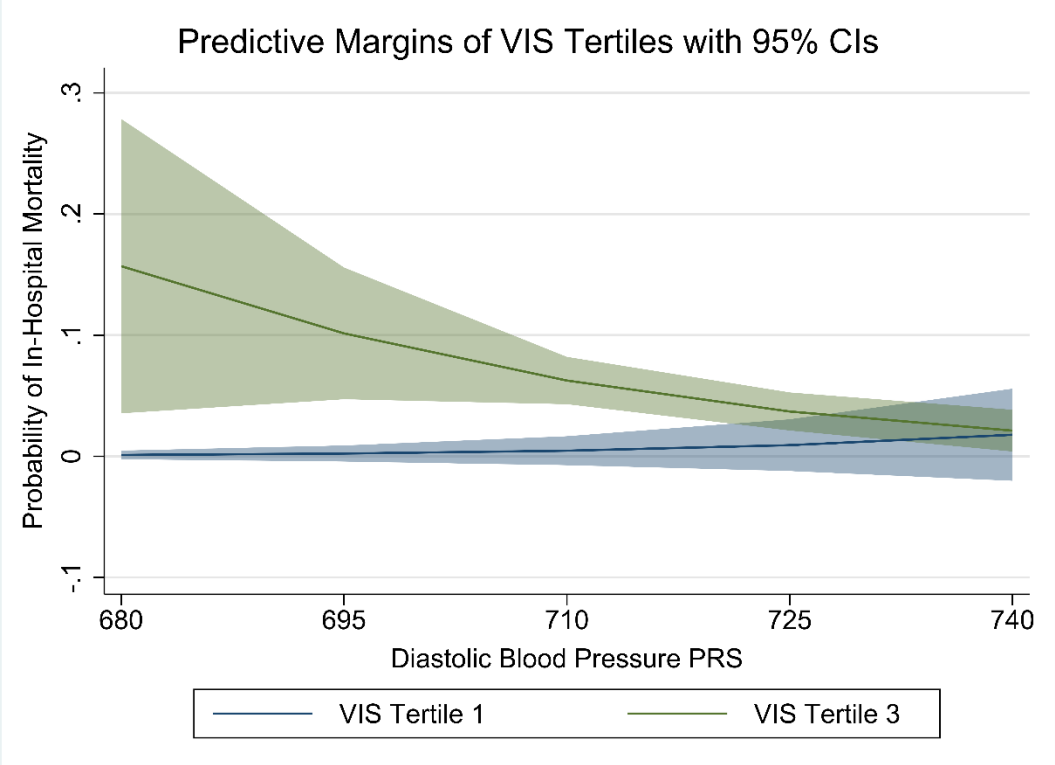

Figure 1. Predicted Margins of Vasoactive-Inotropic Score Teriles by Diastolic Blood Pressure PRS on Probability of In-Hospital Mortality.

The combined effect of the DBP PRS and the interaction between the DBP PRS and vasoactive-inotropic score estimated that those in the upper tertile of vasoactive-inotropic score, with a DBP PRS one standard deviation above the mean, have a 37% (16% – 53%) lower risk compared to those in the upper tertile of vasoactive-inotropic score, with a DBP PRS at the mean. However, those in the upper tertile of vasoactive-

inotropic score, with a DBP PRS one standard deviation below the mean, have a 58% (19% – 110%) higher risk compared to those in the upper tertile of vasoactive-inotropic score, with a DBP PRS at the mean (**Table 10**).

Table 10. Combined Linear Effect of the Interaction between Diastolic Blood Pressure PRS and Vasoactive-Inotropic Score Top Tertile on In-Hospital Mortality compared to the mean diastolic blood pressure PRS.

CHD Cohort Trait	Population	N	SD from DBP PRS Mean	Odds Ratio* (95% CI)	*p* - value
In-Hospital Mortality	Multi-Ancestry	2,106	+2	0.53 (0.32 – 0.86)	**0.010**
			+1	0.73 (0.57 – 0.93)	
			-1	1.38 (1.08 – 1.76)	
			-2	1.90 (1.17 – 3.10)	
	Non-Hispanic White	1,641	+2	0.40 (0.23 – 0.41)	**0.0016**
			+1	0.63 (0.47 – 0.84)	
			-1	1.63 (1.19 – 2.11)	
			-2	2.51 (1.42 – 4.43)	

*adjusted for age, sex, body mass index, STAT category, and the top ten principal components of ancestry

CHAPTER 4
DISCUSSION

Our findings present evidence that pediatric CHD patients, from neonates to young adults, with a genetic predisposition to adult high blood pressure have a reduced risk of HM following CHD repair surgery. For each standard deviation increase in DBP PRS, in adjusted models we estimated the odds of in-hospital mortality risk was reduced by 43%. The results of this study support and expand upon the findings of Gopel et al., which reported that pre-term infants, under 2.5kg, with a higher genetic burden of blood pressure increasing alleles have a survival advantage.[9] Furthermore, a genetic predisposition for adult high blood pressure was also shown to be associated with improved post-surgical recovery. For each standard deviation increase in DBP PRS we estimated the ICU LOS was reduced by 2.41 days, resulting in a reduction of an individual's cost of care by $5,456.[23] These findings, along with those from Gopel et al., suggest that the genetic predictors of adult blood pressure have a pleiotropic effect on HM risk, both for individuals undergoing CHD surgical repair and for those born prematurely. Many alleles have been shown to affect more than one phenotype. These alleles are considered to have pleiotropic effects when they influence two or more phenotypes. We demonstrate pleiotropy for the genetic determinants of adult blood pressure, as they also are associated with risk of in-hospital mortality following surgery for CHD repair.

We hypothesized a mechanistic link between the genetic predisposition to adult high blood pressure and CHD repair-related HM may be due to either increased sensitivity to or reduced need for vasopressors. These drugs are commonly used in the care of children during and after cardiac surgery, allowing for preservation of end-organ perfusion and mitigating the low cardiac output state commonly seen after cardiopulmonary bypass. The observed association between the DBP PRS and vasoactive-inotropic score, that captures vasopressor treatment intensity, supports this hypothesis. For each standard deviation increase in the DBP PRS we estimated the vasoactive-inotropic score was reduced by 0.49 (4.9 μg/kg/min dose-equivalents of milrinone). This suggests that patients with a higher DBP PRS require less pharmacological intervention to maintain target blood pressure after surgery. These results are consistent with a model where people with a genetic predisposition to high blood pressure have more constricted blood vessels and higher average blood pressure compared with

people with lower DBP PRS. While this becomes a pathology in adults, in young children recovering from CHD repair surgery it may proxy for vasopressive treatment and thereby reduce the need for vasopression and improve survival and recovery.

The outcomes we studied are the result of the complex interplay between naturally occurring biological processes and pharmacological interventions that target elements of those processes. As such, we used interaction terms to evaluate context dependencies between blood pressure PRSs and vasopressor treatment intensities. Our observation that the interaction between the DBP PRS and vasoactive-inotropic score lowered blood pressure variance supports the concept that individuals with a higher DBP PRS allows individuals to maintain a more stable blood pressure (lower variance) at a given vasoactive-inotropic score. Additionally, the same interaction term in an analysis of HM revealed that risk decreases in patients with high vasopressor treatment intensity as the DBP PRS increases and is similar to HM in patients with low vasopressor treatment intensity at high percentiles of the PRS. These relationships suggest that patients who require more vasopressor support experience better control of DBP and less HM if they have a relatively high level of alleles that increase blood pressure in adults.

Variability in surgical outcomes including mortality and complications still exist between individuals undergoing CHD repair. Current risk models for mortality, including the STAT category, incorporate multiple chromosomal abnormalities and syndromes, however, they do not consider other genetic information.[24, 25] Improvement of risk models for mortality after CHD surgical repair can lead to the modification of management strategies. Our results suggest that the inclusion of the DBP PRS could boost the prediction accuracy of the STAT category or other risk models. Specifically, the knowledge of the interaction between the blood pressure PRS and vasoactive-inotropic score could help clinicians decide when to incorporate vasopressor treatment for hypotensive individuals.

The results suggest a genetically determined treatment and survival advantage association with blood pressure increasing alleles in CHD repair recovery. Genetic predisposition to elevated blood pressure in children who survive CHD surgery may also result in the observed increase in hypertension in adults with CHD.[7] The evidence of reduced HM in patients with higher burdens of blood pressure increasing alleles

motivates further study into adaptive processes that contribute to the high prevalence of hypertension in the global population.

One limitation of our study is the lack of long-term outcomes. We focus on short-term outcomes as they are directly related to clinical treatment, however, long-term outcomes such as survival and cardiovascular morbidities could improve our understanding of the progression of disease. Additionally, while we test for the association between pre- and post-operative blood pressure and blood pressure PRSs, over 65% of the individuals blood pressures were manipulated through pharmacological means. This was a single center study without an external replication cohort. Furthermore, the lack of associations in the non-Hispanic Black population may be due to limited sample size, resulting in underpowered tests. Further work with collaborators with more diverse studies of CHD will investigate if the risk factors identified in the non-Hispanic White population generalize to the non-Hispanic Black population. Larger studies of blood pressure genetics in recent African ancestry populations would also greatly aid these studies.

Leveraging the observations made in adults, such as here and in Gopel et al., is an effective way to evaluate heritable factors with pleiotropic biological effects throughout life for the benefit of populations that are inaccessible to large-scale genomics research. Cumulatively, these findings suggest that genetic variants that increase risk of high blood pressure later in life influence the degree of pharmaceutical support needed and have beneficial effects in pediatric post-surgical recovery as well as a protective effect from post-operative in-hospital mortality.[9] These findings in combination with the Gopel et al. results potentially support the contention that the high prevalence of hypertension in global populations is the result of positive selection for alleles that improve survival of premature and injured infants. Elucidating potential drivers of the individual differences in surgical outcomes including mortality, through the inclusion of blood pressure PRS screening, may potentially lead to the improvement of clinical management and outcomes of pediatric CHD surgery patients.

CHAPTER 6

APPENDIX

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CHAPTER 7

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