# Patient Genotypes Impact Survival After Surgery for Isolated Congenital Heart Disease

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*Background.* **Survival after cardiac surgery in infancy requires adaptive responses from oxidative stress management and vascular regulation pathways. We tested the hypothesis that genetic variation in these pathways influences postoperative survival in nonsyndromic congenital heart disease children.**

*Methods.* **This is an analysis of a cohort of nonsyndromic congenital heart disease patients who underwent cardiac surgery with cardiopulmonary bypass before 6 months of age (n = 422). Six single nucleotide polymorphisms (SNPs) in six genes involved in oxidative stress and vascular response pathways, identified through a priori literature search, were tested for effects on transplant-free survival. Survival curves, adjusting for confounding covariates, were calculated using the Cox proportional hazard models.**

*Results.* **Long-term survival was strongly associated with vascular endothelial growth factor A gene SNP rs833069 ($p = 7.03\times10^{-4}$) and superoxide dismutase 2 gene SNP rs2758331 ($p = 0.019$). To test for joint effects of the two SNPs on transplant-free survival, the genotypes were grouped to form a risk score reflecting the cumulative number of risk alleles (0 to 4 alleles per patient). A higher risk score based on the *VEGFA* and *SOD2* SNP genotypes was associated with worse transplant-free survival ($p = 3.02\times10^{-4}$) after confounder adjustment. The total burden of risk alleles was additive; subjects with the highest risk score of 4 (n = 59 subjects, 14.2% of the cohort) had a total covariate-adjusted hazard ratio of 15.64 for worse transplant-free survival.**

*Conclusions.* **After cardiac surgery, infants who are homozygous for the high-risk alleles for both the *VEGFA* and *SOD2* SNPs have an approximately 16-fold increased risk of death or heart transplant, suggesting that genetic variants are important modifiers of survival after surgery for congenital heart disease.**

**(Ann Thorac Surg 2014;■:■–■)**


Congenital heart defects (CHDs) are the most common human birth defect. Approximately one third of CHD cases require surgical intervention, with a majority involving cardiopulmonary bypass (CPB). Long-term mortality in the postoperative stages remains considerable, especially for the more severe heart defects [1].

Oxidative stress is considered to be a major factor after cardiac surgery with CPB owing to postoperative organ dysfunction [2]. The importance of oxidative stress in postoperative outcomes has been demonstrated through the finding that allopurinol, which blocks free radical formation and its resulting oxidative stress, is associated with decreased cardiac event rate after surgery with CPB in high-risk infants with hypoplastic left heart syndrome (HLHS) [3].

Studies of postsurgical outcomes in pediatric patients have successfully identified several genetic variants involved in vascular response pathways that affect long-term outcomes. First, endothelin-1 missense variant (*EDN1* G5665T) has been associated with transplant-free

Accepted for publication March 5, 2014.

Presented at the Fiftieth Annual Meeting of The Society of Thoracic Surgeons, Orlando, FL, Jan 25–29, 2014. Winner of the J. Maxwell Chamberlain Memorial Award for Congenital Heart Surgery.

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Ms McDonald-McGinn discloses a financial relationship with Natera.

The Appendix can be viewed in the online version of this article [http://dx.doi.org/10.1016/j.athoracsur.2014.03.017] on http://www.annalsthoracicsurgery.org.


Published by Elsevier Inc

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2014.03.017

**Abbreviations and acronyms**

CHD = congenital heart defect
CPB = cardiopulmonary bypass
EA = European ancestry
EDN1 = endothelin-1
HLHS = hypoplastic left heart syndrome
MI = myocardial infarction
SNP = single nucleotide polymorphism
SOD2 = superoxide dismutase 2
TGA = transposition of the great arteries
TOF = tetralogy of Fallot
VEGFA = vascular endothelial growth factor A

survival in patients with functional single ventricle CHD, with the greatest effects in children with the most severe phenotype, HLHS [4]. More recently, a randomized clinical trial reported that missense mutations that upregulated the renin-angiotensin-aldosterone system were associated with impaired ventricular remodeling, renal function, and somatic growth in infants with functional single ventricle postcardiac surgery, highlighting the role of vascular response genes on a wide spectrum of postsurgical outcomes [5].

Taken together, these studies suggest that oxidative stress and vascular response play important roles in injury repair and long-term survival in the pediatric CHD population. We sought to examine the effects of specific genetic variants implicated in oxidative stress management and organ recovery on long-term survival in a cohort of children with nonsyndromic CHD. Secondarily, we performed an analysis using a genetic risk score, reflecting the number of deleterious alleles each patient has, to determine if the observed genotype effects were independent and additive.

## Patients and Methods

### *Study Design*

This is an analysis of a previously described prospective cohort [6–8] of 550 subjects collected to study neurodevelopmental dysfunction after CHD palliation. This specific study sought to identify gene regions related to oxidative stress and vascular response potentially affecting survival in infants after cardiac surgery with nonsyndromic CHD. We note that no genome-wide association analyses have been attempted on the phenotype of long-term survival; this is solely a candidate gene study.

Of the 550 original subjects, 56 were removed owing to likely genetic syndrome and an additional 72 were removed owing to lack of high-quality genotype data, leaving a total of 422 subjects available for analyses. Additional information on data collection (including inclusion/exclusion criteria), operative management, genotyping, and analyses not presented in the main manuscript can be found in the online-only Appendix.

### *Single Nucleotide Polymorphism Selection*

To preserve statistical power, we selected six candidate single nucleotide polymorphism (SNPs) at six different genes involved broadly in oxidative and ischemic stress response (Table 1) a priori based on a systematic literature review of published evidence from other investigators, reporting that variants in these genes have a functional impact potentially relevant to the outcomes

*Table 1. Description of Single Nucleotide Polymorphisms Studied*

| SNP | Gene[a] | Variant Type | Chr:Position[b] | Major/Minor Allele | Minor Allele Frequency | Gene Name and Description |
|---|---|---|---|---|---|---|
| rs1051740 | *EPHX1* | Tyr113His | 1:224,086,256 | A/G | 0.291 | *Epoxide hydrolase 1*. Converts epoxides to nontoxic forms |
| rs5370 | *EDN1* | Lys198Asn | 6:12,404,241 | T/G | 0.187 | *Endothelin 1*. Pro-peptide of endothelin 1, a potent vasoconstrictor |
| rs833069 | *VEGFA* | Intronic[c] | 6:43,850,557 | A/G | 0.346 | *Vascular endothelial growth factor A*. Growth factor mediates vascular permeability and endothelium growth/apoptosis inhibition |
| rs2758331 | *SOD2* | Intronic[d] | 6:160,025,060 | T/G | 0.436 | *Superoxide dismutase 2*. Mitochondrial protein; converts superoxide byproducts to hydrogen peroxide |
| rs10776686 | *(CYP2E1)* | Intergenic | 10:135,182,921 | A/G | 0.051 | *Cytochrome P450 2E1*. Endoplasmic reticulum-associated enzyme, involved in varied processes |
| rs1001179 | *CAT* | 5′UTR | 11:34,416,807 | T/C | 0.159 | *Catalase*. Key antioxidant heme enzyme present in peroxisome |

[a] Intergenic single nucleotide polymorphisms (SNPs) are represented in parentheses naming the nearest gene, for example *(CYP2E1)*. [b] Position information and annotation from reference assembly 36.3. [c] Intronic SNP, rs833069, is in strong linkage disequilibrium (LD [$r^2 = 0.97$]) with *VEGFA* 5′UTR SNP rs2010963, which has been reported to increase expression levels of *VEGFA*. [d] Intronic SNP, rs2758331, is in strong LD ($r2 = 0.93$) with superoxide dismutase 2 (*SOD2*) Val16Ala missense SNP rs4880.

Chr = chromosome; 3′UTR = 3 prime untranslated region of a gene; 5′UTR = 5 prime untranslated region of a gene; SNP = single nucleotide polymorphism.