Burden of potentially pathologic copy number variants is higher in children with isolated congenital heart disease and significantly impairs covariate-adjusted transplant-free survival

Daniel Seung Kim, BS,[a,b,c] Jerry H. Kim, MD, MPH,[d] Amber A. Burt, MS,[a] David R. Crosslin, PhD,[a,b] Nancy Burnham, MSN,[e] Cecilia E. Kim, BS,[f] Donna M. McDonald-McGinn, MS,[g] Elaine H. Zackai, MD,[g] Susan C. Nicolson, MD,[h] Thomas L. Spray, MD,[e] Ian B. Stanaway, BS,[b] Deborah A. Nickerson, PhD,[b] Patrick J. Heagerty, PhD,[c] Hakon Hakonarson, MD, PhD,[f] J. William Gaynor, MD,[e] and Gail P. Jarvik, MD, PhD[a,b]

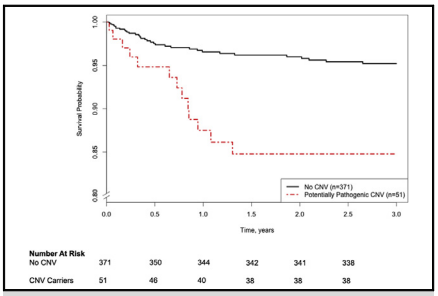
ABSTRACT

Objectives: Copy number variants (CNVs) are duplications or deletions of genomic regions. Large CNVs are potentially pathogenic and are overrepresented in children with congenital heart disease (CHD). We sought to determine the frequency of large CNVs in children with isolated CHD, and to evaluate the relationship of these potentially pathogenic CNVs with transplant-free survival.

Methods: These cases are derived from a prospective cohort of patients with nonsyndromic CHD (n = 422) identified before first surgery. Healthy pediatric controls (n = 500) were obtained from the electronic Medical Records and Genetic Epidemiology Network, and CNV frequency was contrasted for CHD cases and controls. CNVs were determined algorithmically; subsequently screened for >95% overlap between 2 methods, size (>300 kb), quality score, overlap with a gene, and novelty (absent from databases of known, benign CNVs); and separately validated by quantitative polymerase chain reaction. Survival likelihoods for cases were calculated using Cox proportional hazards modeling to evaluate the joint effect of CNV burden and known confounders on transplant-free survival.

Results: Children with nonsyndromic CHD had a higher burden of potentially pathogenic CNVs compared with pediatric controls (12.1% vs 5.0%; $P = .00016$). Presence of a CNV was associated with significantly decreased transplant-free survival after surgery (hazard ratio, 3.42; 95% confidence interval, 1.66-7.09; $P = .00090$) with confounder adjustment.

Conclusions: We confirm that children with isolated CHD have a greater burden of rare/large CNVs. We report a novel finding that these CNVs are associated with an adjusted 2.55-fold increased risk of death or transplant. These data suggest that CNV burden is an important modifier of survival after surgery for CHD. (J Thorac Cardiovasc Surg 2016;151:1147-51)



Covariate-adjusted transplant-free survival by potentially pathogenic CNV in CHD cases.

Central Message

Isolated CHD cases have greater CNV burden. We also report a novel finding that CNVs are associated with an increased risk of death.

Perspective

Large genomic copy number variants (CNVs) are rare and potentially pathogenic. We found that children with isolated congenital CHD have a greater burden of large/rare CNVs, and that these CNVs are associated with an increased risk of death. These data suggest that CNV burden is an important modifier of survival after surgery for CHD and has the potential for use in risk stratification.

See Editorial Commentary page 1152.

From the [a]Division of Medical Genetics, Department of Medicine, Departments of [b]Genome Sciences, [c]Biostatistics and [d]Anesthesiology and Pain Medicine, University of Washington, Seattle, Wash and the Divisions of [e]Cardiothoracic Surgery, [g]Genetics and [h]Cardiothoracic Anesthesiology, and [f]Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, Pa.

This work was supported by a grant from the Fannie E. Rippel Foundation, an American Heart Association National Grant-in-Aid (9950480N), National Institutes of Health (NIH) Grant HL071834, and a Washington State Life Sciences Discovery Award to the Northwest Institute for Genetic Medicine. The Children's Hospital of Philadelphia site of the eMERGE network was supported by NIH Grant U01HG006830. DSK was supported by NIH Grants 1F31MH101905-01 and T32HL007312 and a grant from the American Heart Association Western States Affiliate (16POST27250048).

Read at the 95th Annual Meeting of The American Association for Thoracic Surgery, Seattle, Washington, April 25-29, 2015.

Received for publication April 20, 2015; revisions received Aug 14, 2015; accepted for publication Sept 14, 2015; available ahead of print Dec 15, 2015.

Address for reprints: Gail P. Jarvik, MD, PhD, Medical Genetics, Box 357720, University of Washington, Seattle, WA 98195-7720 (E-mail: pair@u.washington.edu).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2015.09.136

Abbreviations and Acronyms

CHD = congenital heart disease
CHOP = The Children's Hospital of Philadelphia
CNV = copy number variant
CPB = cardiopulmonary bypass
DHCA = deep hypothermic circulatory arrest
ECMO = extracorporeal membrane oxygenation
eMERGE = electronic Medical Records and Genetic Epidemiology
LOS = length of stay

Supplemental material is available online.

Congenital heart disease (CHD) represents the most common human birth defect, often requiring surgical intervention with cardiopulmonary bypass (CPB) or circulatory arrest soon after birth. Survival after surgery has improved, but long-term mortality remains considerable, particularly for more severe CHDs, including single-ventricle lesions.[1]

Genetic factors, particularly those that are rare and alter proteins, are hypothesized to be major contributors to human disease.[2] Copy number variants (CNVs), duplicated or deleted regions of the genome, have been reported as potential causes of sporadic CHDs.[3,4] CNVs are reportedly more frequent in children with CHD compared with controls.[5] In addition, CNVs larger than 300 kb and overlapping a gene have been reported to be more frequent in CHD cases and associated with poorer growth and cognitive outcomes.[6] Of note, previous studies reporting the prevalence of CNVs in children with CHD have included syndromic patients (eg, those with DiGeorge 22q11.2 microdeletions).

We previously used data from this cohort of children with isolated CHD to demonstrate the strong protective effects of *VEGFA* and *SOD2* genetic variants on transplant-free survival.[7] Given the protein-disrupting potential of these large, gene-overlapping CNVs, we hypothesize that such CNVs also likely affect survival. Thus, in the present study we sought to determine the frequency of these potentially pathogenic CNVs among children with CHD compared with healthy pediatric controls, and to evaluate whether these large CNVs affect transplant-free survival in the first 3 years of follow-up after surgical correction of CHD.

METHODS

Ethics Statement

Between October 1998 and April 2003, subjects were enrolled at The Children's Hospital of Philadelphia (CHOP) on a protocol approved by the Institutional Review Boards of CHOP and the University of Washington. Informed written consent was obtained from a parent or guardian of each subject.

CHD Case Population

In this analysis of a previously described prospective cohort of 550 participants enrolled in a prospective study at CHOP to study neurodevelopmental dysfunction after surgical correction for CHD (hereinafter referred to as the "CHD cases"),[8-10] patients aged 6 months or younger who underwent CPB with or without deep hypothermic circulatory arrest (DHCA) for repair of CHD were eligible for enrollment. Exclusion criteria included (1) multiple distinct congenital anomalies, (2) a recognizable genetic or phenotypic syndrome, and (3) a language other than English spoken in the home. Recognition of dysmorphic features can be difficult in neonates, however, and in some patients genetic syndromes were identified at subsequent evaluation, and these subjects were removed from the dataset before analysis.

This study examined a subset of the cohort with genetic data (n = 422) to establish the prevalence of large, gene-overlapping CNVs compared with that in healthy pediatric controls, and determine whether these potentially pathogenic CNVs were associated with differential transplant-free survival. We note that no genome-wide association analyses have been attempted using these CNV data; this is solely a study of the global burden of large, gene-overlapping CNVs and how they affect transplant-free survival in the first 3 years after surgical correction of CHD.

Of the original 550 CHD cases, 73 were excluded owing to the lack of high-quality genotype data, leaving a total of 477 subjects for analysis. An additional 55 subjects were excluded owing to the presence of DiGeorge syndrome or other chromosomal/genetic abnormalities, which would be expected to bias both the estimation of CNV prevalence and the evaluation of the effects of CNVs on survival for CHD, given that patients with genetic syndromes generally have worse survival.[11] A total of 422 patients were considered after these exclusions. Details of data collection (including further information on inclusion/exclusion criteria), operative management, and genotyping have been reported previously.[7]

Control Population

Healthy control subjects (hereinafter referred to as "controls") from the same site (CHOP) for comparisons of CNV prevalence were obtained from the Electronic Medical Records and Genetic Epidemiology (eMERGE) consortium.[12] A total of 500 healthy controls without CHD or other conditions associated with increased CNV prevalence (eg, autism, schizophrenia) were analyzed for the presence of large, gene-overlapping CNVs.

Genetic Evaluation to Exclude Subjects With Syndromic CHD

CHD cases were evaluated by a genetic dysmorphologist at the 1-year and/or 4-year examinations. Patients were classified as having no indication of genetic syndrome or chromosomal abnormality (normal, isolated CHD), suspected genetic syndrome (suspect), or a definite genetic syndrome or chromosomal abnormality (genetic). Following this classification, each CHD case's genetic record was reviewed by a second senior board-certified medical geneticist, blinded to the genetic data, to determine whether to include or exclude the subject from the current analysis, which focuses on nonsyndromic subjects. Based on this review, 55 CHD cases with a known or suspected genetic syndrome were excluded from our analysis owing to the potential for genetic confounding effects on CNV prevalence and the resulting effect on transplant-free survival within the first 3 years of follow-up after surgery.

Genotyping

Whole blood or buccal swab samples were obtained before surgery in CHD case subjects and at study enrollment in controls, and stored at