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Biallelic Truncating Mutations in *ALPK3* Cause Severe Pediatric Cardiomyopathy

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ABSTRACT

BACKGROUND Cardiomyopathies are usually inherited and predominantly affect adults, but they can also present in childhood. Although our understanding of the molecular basis of pediatric cardiomyopathy has improved, the underlying mechanism remains elusive in a substantial proportion of cases.

OBJECTIVES This study aimed to identify new genes involved in pediatric cardiomyopathy.

METHODS The authors performed homozygosity mapping and whole-exome sequencing in 2 consanguineous families with idiopathic pediatric cardiomyopathy. Sixty unrelated patients with pediatric cardiomyopathy were subsequently screened for mutations in a candidate gene. First-degree relatives were submitted to cardiac screening and cascade genetic testing. Myocardial samples from 2 patients were processed for histological and immunohistochemical studies.

RESULTS We identified 5 patients from 3 unrelated families with pediatric cardiomyopathy caused by homozygous truncating mutations in *ALPK3*, a gene encoding a nuclear kinase that plays an essential role in early differentiation of cardiomyocytes. All patients with biallelic mutations presented with severe hypertrophic and/or dilated cardiomyopathy in utero, at birth, or in early childhood. Three patients died from heart failure within the first week of life. Moreover, 2 of 10 (20%) heterozygous family members showed hypertrophic cardiomyopathy with an atypical distribution of hypertrophy. Deficiency of alpha-kinase 3 has previously been associated with features of both hypertrophic and dilated cardiomyopathy in mice. Consistent with studies in knockout mice, we provide microscopic evidence for intercalated disc remodeling.

CONCLUSIONS Biallelic truncating mutations in the newly identified gene *ALPK3* give rise to severe, early-onset cardiomyopathy in humans. Our findings highlight the importance of transcription factor pathways in the molecular mechanisms underlying human cardiomyopathies. (J Am Coll Cardiol 2016;67:515–25) © 2016 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

DCM = dilated cardiomyopathy

DNA = deoxyribonucleic acid

HCM = hypertrophic cardiomyopathy

ICD = implantable cardiac-defibrillator

IVS = interventricular septum

LV = left ventricular

LVPW = left ventricular posterior wall

PCR = polymerase chain reaction

RNA = ribonucleic acid

RV = right ventricular

Cardiomyopathies represent a clinically and genetically heterogeneous group of disorders affecting the ventricular myocardium. Among children <18 years of age, overall incidence of cardiomyopathy is 1.13 cases per 100,000 annually in the United States (1). Pediatric cardiomyopathy is associated with significant morbidity and mortality: up to 40% of affected children die or undergo cardiac transplantation within 5 years of diagnosis (2,3). Cardiomyopathy can be classified into 5 clinical phenotypes based upon morphological and functional characteristics: hypertrophic cardiomyopathy (HCM); dilated cardiomyopathy (DCM); restrictive cardiomyopathy; arrhythmogenic right ventricular (RV) cardiomyopathy; and unclassified cardiomyopathy, including left ventricular (LV) noncompaction (4). Extremely diverse, particularly in the pediatric population, the etiology of cardiomyopathy encompasses both nongenetic and genetic causes, including myocarditis, neuromuscular diseases, inborn errors of metabolism, malformation syndromes, and familial forms caused by mutations in genes encoding the specialized, often structural, components of cardiomyocytes. Because of the routine incorporation of genetic testing in the diagnostic evaluation of patients with cardiomyopathy, a causal diagnosis can be identified in more than 70% of children (5). Interestingly, the same genetic causes that result in cardiomyopathy in adults are prevalent in the pediatric population (e.g., sarcomeric or cytoskeletal gene mutations) (6).

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Despite recent advances in understanding the genetic etiologies of pediatric cardiomyopathy, a substantial number of cases remain unsolved, suggesting that other genes await discovery. Establishing an underlying genetic cause for cardiomyopathy allows pre-symptomatic identification of family members at risk and facilitates reproductive decision making. Genetic and genomic studies continue to provide new insights into the pathophysiological processes contributing to cardiomyopathy and will ultimately facilitate development of patient-specific prevention and treatment strategies.

To identify new genes contributing to pediatric cardiomyopathy, we used a combined approach of homozygosity mapping and whole-exome sequencing.

METHODS

We studied 4 individuals with pediatric cardiomyopathy from 2 consanguineous families of Dutch and Moroccan descent, respectively (**Figures 1A and 1B**). A third family of Turkish descent was identified by subsequent cohort screening (**Figure 1C**). Patients underwent extensive clinical investigations including high-resolution prenatal ultrasound, physical examination, 12-lead electrocardiography, transthoracic echocardiography, and post-mortem examination. Mutation screening of up to 48 known cardiomyopathy-related genes per individual was negative. A complete overview of the genetic and metabolic tests performed before this study is provided in the Online Appendix. All asymptomatic siblings and parents underwent echocardiographic screening. HCM was defined by the presence of increased LV wall thickness (>2 SD above the mean for body surface area in children or ≥13 mm in adult relatives) in the absence of hemodynamic stresses sufficient to account for the degree of hypertrophy. DCM was defined by the presence of LV dilation (LV end-diastolic dimension >2 SD above the mean for body surface area) and systolic dysfunction (fractional shortening or LV ejection fraction >2 SD below the mean for age) in the absence of abnormal loading conditions sufficient to cause global systolic impairment (4). A cohort of 60 unrelated patients with idiopathic pediatric cardiomyopathy from diverse ethnic backgrounds was available for mutational screening of candidate genes. These patients had previously been screened for mutations in 8 to 55 known cardiomyopathy-related genes. The medical ethical committees of the University Medical Center Groningen and the Erasmus University Medical Center approved this study. Written informed consent was obtained from all participants or their legal guardians.

HOMOZYGOSITY MAPPING. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood samples (A-VIII:1, VIII:2, IX:1, and IX:2; B-III:1, IV:3, and IV:4), buccal swabs (A-IX:3 and IX:4), amniotic fluid (B-IV:1), or fibroblasts (B-IV:2) (**Figure 1**). Genome-wide

paper to disclose. Drs. Almomani and Verhagen contributed equally to this work. Drs. Jongbloed and van de Laar are joint senior authors.

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