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ORIGINAL INVESTIGATIONS

Autosomal Recessive Cardiomyopathy Presenting as Acute Myocarditis

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ABSTRACT

BACKGROUND Myocarditis is inflammation of the heart muscle that can follow various viral infections. Why children only rarely develop life-threatening acute viral myocarditis (AVM), given that the causal viral infections are common, is unknown. Genetic lesions might underlie such susceptibilities. Mouse genetic studies demonstrated that interferon (IFN)-α/β immunity defects increased susceptibility to virus-induced myocarditis. Moreover, variations in human *TLR3*, a potent inducer of IFNs, were proposed to underlie AVM.

OBJECTIVES This study sought to evaluate the hypothesis that human genetic factors may underlie AVM in previously healthy children.

METHODS We tested the role of TLR3-IFN immunity using human induced pluripotent stem cell-derived cardiomyocytes. We then performed whole-exome sequencing of 42 unrelated children with acute myocarditis (AM), some with proven viral causes.

RESULTS We found that *TLR3*- and *STAT1*-deficient cardiomyocytes were not more susceptible to Coxsackie virus B3 (CVB3) infection than control cells. Moreover, CVB3 did not induce IFN-α/β and IFN-α/β-stimulated genes in control cardiomyocytes. Finally, exogenous IFN-α did not substantially protect cardiomyocytes against CVB3. We did not observe a significant enrichment of rare variations in TLR3- or IFN-α/β-related genes. Surprisingly, we found that homozygous but not heterozygous rare variants in genes associated with inherited cardiomyopathies were significantly enriched in AM-AVM patients compared with healthy individuals ($p = 2.22E-03$) or patients with other diseases ($p = 1.08E-04$). Seven of 42 patients (16.7%) carried rare biallelic (homozygous or compound heterozygous) nonsynonymous or splice-site variations in 6 cardiomyopathy-associated genes (*BAG3*, *DSP*, *PKP2*, *RYR2*, *SCN5A*, or *TNNI3*).

CONCLUSIONS Previously silent recessive defects of the myocardium may predispose to acute heart failure presenting as AM, notably after common viral infections in children. (J Am Coll Cardiol 2017;69:1653–65)

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ABBREVIATIONS AND ACRONYMS

AM = acute myocarditis

ARVC = arrhythmogenic right ventricular cardiomyopathy

AVM = acute viral myocarditis

CVB3 = Coxsackie virus B3

DCM = dilated cardiomyopathy

iPSC = induced pluripotent stem cell

MAF = minor allele frequency

WES = whole-exome sequencing

Acute myocarditis (AM) is an inflammatory disease of the myocardium. The estimated annual incidence of myocarditis is 1 per 100,000 children in the United States, corresponding to a prevalence of ~1/10,000 (1). The actual incidence of this heterogeneous disease is probably higher because of unrecognized cases.

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Myocarditis most typically presents with sudden onset of congestive heart failure or cardiogenic shock. Some patients recover fully and spontaneously or following symptomatic treatment. Others suffer from cardiac sequelae such as dilated cardiomyopathy (DCM) (1), requiring long-term anticongestive therapy or heart transplantation. In some cases, sudden death occurs (1). Biopsy-proven myocarditis is reported in up to 46% of children with an identified cause of DCM (2). Although myocarditis has many causes, most cases apparently result from infection with viruses, including enteroviruses, adenoviruses, and parvoviruses (1). Infections from such viruses are common, particularly during childhood; it is unclear, therefore, why only a very small proportion of children develop acute viral myocarditis (AVM). Of note, clinical severity is uncorrelated with viral infectious causes, suggesting a human genetic predisposition. Studies in inbred mice have implicated the major histocompatibility complex and other loci in genetic susceptibility to Coxsackie virus-induced myocarditis (3,4). Overall, these observations suggest that human genetic variations, rendering the heart more sensitive to common viral infections, may contribute to the progression of myocarditis, at least in some children.

Severe childhood infectious diseases, including viral diseases striking healthy children, can result from single-gene inborn errors of immunity (5,6). Some of these immunodeficiencies have tissue-specific phenotypes, which could reflect restricted viral tropism or tissue specificity of the inborn error. Herpes simplex encephalitis (HSE) and severe pulmonary influenza can be due to brain- and lung-intrinsic defects in interferon (IFN) immunity, respectively (6). Interestingly, heart, brain, and lungs are typically the only organs severely affected in patients with AVM, HSE, and severe influenza, respectively, despite the ability of the causal viruses to replicate in many tissues. Moreover, inborn errors of adaptive immunity do not predispose to any of these conditions (7).

Recently, the case of an adult patient with enteroviral AVM carrying a rare, dominant-negative toll-like receptor 3 (*TLR3*) allele was reported (8). Similarly, mice lacking genes encoding key proteins in antiviral IFN immunity, such as IFN-β and TLR3, were more susceptible to Coxsackie virus B3 (CVB3) infection and virus-induced myocardial injury (9,10). Whether variations in these human genes influence the outcome of infection by cardiotropic viruses remains unclear. To test the hypothesis that inborn errors of heart-intrinsic TLR3-IFN immunity may underlie AVM in previously healthy children, we explored the impact of mutations in TLR3-IFN-related genes on antiviral immunity of human cardiomyocytes derived from induced pluripotent stem cells (iPSCs) and searched for TLR3-IFN-related gene mutations in AVM using whole-exome sequencing (WES). As our results were inconsistent with the TLR3-IFN hypothesis, we then tested the hypothesis that hitherto silent cardiomyopathies may underlie AVM.

METHODS

Control and mutant iPSCs carrying deleterious mutations in *STAT1* (c.1928_1929insA/c.1928_1929insA) or *TLR3* (p.P554S/p.E746*) were derived from primary dermal fibroblasts of healthy donors or patients with severe viral diseases other than AM, respectively (11,12). Using a modified version of the original protocol, iPSCs were differentiated into cardiomyocytes (13). Details of cardiomyocyte differentiation and

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