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EDITORIAL

TGFβ3 mutations cause arrhythmogenic right ventricular dysplasia type 1 and open the door to understanding the biological role of TGFβ3 (where there's a will, there's a way)

EXPERT'S PERSPECTIVE

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This editorial refers to an article by G. Beffagna *et al.*[10] published in *Cardiovascular Research* in 2005. It is accompanied by a retrospective editorial by one of the authors of that original article, A. Rampazzo, pp. 191–194, this issue, as part of this Spotlight on Landmark Papers in *Cardiovascular Research*.

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is an inherited myocardial disease characterized by progressive fibrofatty replacement of the right ventricular myocardium, leading to right ventricular failure and arrhythmias.[1,2] The left ventricle can also be involved, and in some patients, it can be the predominant site of involvement. The estimated prevalence of the disease in the general population ranges from 1:1000 to 1:5000.[1,2] ARVC/D is a leading cause of sudden cardiac death (SCD), especially in young adults and athletes.[1,2] The current diagnostic criteria are based on the presence of major and minor standardized criteria including ECG, ventricular arrhythmias, right ventricular function and morphology, histopathology, and family history.[3]

ARVC/D is a familial disease in at least 50% of cases, usually transmitted as an autosomal dominant trait, with reduced penetrance and highly variable clinical expression.[1,2,4] ARVC/D has been associated with mutations in genes encoding cardiac desmosomal proteins involved in cell-to-cell interaction, including plakophilin-2 (*PKP2*), desmoplakin (*DSP*), desmoglein-2 (*DSG2*), desmocollin-2 (*DSC2*), and plakoglobin (*JUP*).[1–4] In addition, two complex cardiocutaneous disorders with autosomal recessive inheritance, the Naxos disease and the Carvajal syndrome, in which ARVC/D is associated with palmoplantar keratoderma and woolly hair, are also associated with homozygous mutations in *JUP* and in *DSP*, respectively. These findings confirmed that desmosomal dysfunction might be the final common pathway in the pathogenesis of ARVC/D. Desmosomes are protein complexes in the intercalated disk that are responsible for mechanical coupling of cardiac myocytes. They also play an important role in direct intracellular signalling and proliferation/differentiation as well as in anchoring and regulation of ion channel function.[4] How mutations of desmosomal protein genes cause the ARVC/D remains uncertain. It has been hypothesized that desmosomal protein defects impair cell–cell adhesion and/or intermediate filament function, leading to accelerated apoptosis of cardiomyocytes with fibrofatty replacement of the myocardium.[1–5] However, additional mutations have also been identified in extra-desmosomal genes encoding transforming growth factor-β3 (*TGFβ3*), cardiac ryanodine-2 receptor (*RyR2*), and transmembrane protein 43 (*TMEM43*) in patients with ARVC/D1, ARVC/D2, and ARVC/D5, respectively.[1–4]

In 1994, Rampazzo *et al.*[6] performed linkage studies in two Italian families spanning over four generations (82 subjects, 19 affected) with ARVD1 and mapped the involved gene to chromosome 14q24.3. Among genes mapped in this chromosome and expressed in the myocardium, *TGFβ3*, which encodes the TGFβ3, appeared to be a very promising candidate. TGFβ3 is a cytokine member of the TGFβ family (TGFβs) synthesized and released from cardiac myocytes and fibroblasts that regulates many physiological processes, including cardiac development, cell adhesion, proliferation, differentiation, and apoptosis and modulates myocardial fibrosis and remodelling.[7,8] However, Rampazzo *et al.*[9] failed to detect causative mutations in exonic sequences of *TGFβ3* and three other candidate genes included in the critical region of 14q23–q24 (*POMT2*, *TGFB3*, *KIAA1036*, and *KIAA*) in two families with ARVD1. Therefore, Beffagna *et al.*[10] extended mutation screening to the promoter and untranslated regions (UTRs) of *TGFβ3* in a large ARVD1 family, which included 38 members in four generations and 30 unrelated probands with a diagnosis of ARVC/D. A single-nucleotide substitution (c.-36G>A) in the 5′ UTR of the *TGFβ3* gene was identified in all affected and in three asymptomatic relatives. Subsequent screening of 30 unrelated ARVC probands, in which mutations in the known

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ARVD genes were excluded, identified an additional mutation (c.1723C>T) in the 3′ UTR in a young man with spontaneous sustained ventricular tachycardia whose brother died suddenly at the age of 16 and was found to have ARVD at autopsy. This patient and another patient with the 5′ UTR mutation presented sustained ventricular tachycardia with left bundle branch block morphology and extensive fibrofatty replacement in an endomyocardial biopsy. However, neither of the nucleotide changes was detected in 300 control subjects from the same population. Finally, luciferase reporter activity was ~2.5-fold higher in C2C12 cells transfected with mutant constructs when compared with those transfected with wild-types. Thus, after 11 years of intense research, the study of Beffagna *et al.*,[10] which was published in 2005 in *Cardiovascular Research*, identified *TGFβ3* as the disease gene involved in ARVD1, being an example that 'if there's a will, there's a way'. This study represents a breakthrough as it was the first demonstration of an association of *TGFβ3* mutations with inherited human diseases and the starting point for a better understanding of the pathophysiology of ARVD1 and the biological role of TGFβ3 in cardiovascular function and development.[10,11] Furthermore, these findings have the potential for further discoveries about the molecular basis of ARVD1 and the biological functions of *TGFβ3*.

Beffagna *et al.*[10] hypothesized that mutations in *TGFβ3* increased myocardial fibrosis and modulated the expression of desmosomal genes. In fact, *TGFβ3* is a profibrotic cytokine that induces the expression of extracellular matrix genes and the phenotypic transformation of fibroblasts into myofibroblasts, stimulates pro-collagen and collagen synthesis in human dermal fibroblasts,[12] and suppresses the activity of matrix metalloproteinases (MMPs).[7,13] Moreover, *TGFβ3* overexpression increased collagen production in the skin of patients with scleroderma.[14] Furthermore, TGFβ3 also increased the expression of desmosomal genes *JUP* in human cardiac fibroblasts[13] and *DSP* I and II in bronchial epithelial cells.[15] In primary cultured Sertoli cells, TGFβ3 perturbed the cell tight-junction barrier and reduced the production of occludin, N-cadherin, and zonula occludens-1 via the p38 MAPK signalling pathway.[16] TGFβs also play a key role in epithelial-to-mesenchymal transition, a process characterized by the disintegration and disassembly of cell–cell junctions, including tight junctions, desmosomes, adherens junctions, and gap junctions. Thus, overexpression of TGFβ3 adds further support to the hypothesis that desmosomal dysfunction is the 'final common pathway' in the development of ARVD1.

There are controversial data regarding changes in TGFβ3 expression in different cardiac pathologies. Thus, TGFβ3 expression decreased during the development of pressure overload-induced hypertrophy in rats following aortic constriction,[17] and in patients with aortic stenosis, reduced circulating TGFβ3 levels are associated with the reverse cardiac remodelling process that follows aortic valve replacement.[18] Conversely, TGFβ3 expression markedly increased in rats after coronary artery ligation, and this increase correlated positively with the expression of type I and type III collagen, MMP-2, and tissue inhibitor of MMP-2.[19] An increase in TGFβ3 expression also plays a role in the pathogenesis of canine chronic mitral valvular disease by inducing myofibroblast-like differentiation of valvular stromal cells and extracellular matrix secretion.[20,21] Moreover, TGFβ3 rs3917187 polymorphism is associated with abnormal left ventricular structure and function in hypertensive patients.[22] Overall, TGFβ3 overexpression caused by *TGFβ3* mutation promotes desmosomal dysfunction and abnormal cardiac remodelling (hypertrophy, fibrosis), thus creating an arrhythmogenic substrate for the development of life-threatening arrhythmias leading to SCD.

However, a scientific breakthrough frequently raises more questions than answers, and the study of Beffagna *et al.* was not an exception. In fact, a number of questions remain to be answered. Are the reported *TGFβ3* gene mutations the only cause of ARVD1? What is the role of *TGFβ3* in the regulation of cardiovascular structure and function and in the pathogenesis of cardiovascular diseases? Is fibrofatty replacement in right ventricular myocardium a direct or a secondary consequence of *TGFβ3* overexpression? Why, despite the widespread tissue expression of *TGFβ3*, is there an apparently selective cardiac phenotype of ARVD1? Finally, are mutations in genes encoding other members of the TGF family also involved in ARVC/D?

Adult mouse models mimicking *TGFβ3* mutations will be useful in understanding cardiac TGFβ3 signalling pathways and in establishing a clear cause-and-effect relationship between the *TGFβ3* mutations and ARVD1. Unfortunately, $TGF\beta3^{-/-}$ mice died at birth due to a cleft palate and delayed pulmonary development, precluding functional studies in adults.[23] However, very recently, Doetschman *et al.*[24] generated $TGF\beta3^{cko/cko}$ mice harbouring conditional null alleles of *TGFβ3*. These mice may provide an exciting opportunity to study the pathophysiological role of *TGFβ3* in adult cardiovascular tissues as well as the mechanisms underlying *TGFβ3* dysregulation in ARVC/D1.

Beffagna *et al.* should be congratulated because their novel and exciting findings have had three main consequences. First, they demonstrated that regulatory mutations in *TGFβ3* cause arrhythmogenic ARVC/D1, adding a new brick in the wall to better understand the molecular mechanisms underlying the pathogenesis of ARVC/D in particular and the genetic causes of SCD in general.[10] Secondly, they opened a new field of research on the biological function of TGFβ3 and the contribution of specific TGFβ ligands or their combinations to the TGFβ signalling in heart development as well as in adult cardiovascular function and remodelling. This information will allow a better understanding of its role in cardiac physiology and pathophysiology of cardiovascular diseases. Thirdly, a better insight into genetic causes of ARVC/D and SCD holds the promise to provide new approaches to the diagnosis, and potential therapeutic interventions based on the genetic underlying cause of disease may be developed, resulting in better long-term care and survival for patients with ARVC/D.

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