New Insight into the Variable Expression of Arrhythmogenic Right Ventricular Cardiomyopathy Provided by the Analysis of a Plakophilin-2 Splice Mutation

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy mostly caused by heterozygous desmosomal gene mutations. Plakophilin 2 (*PKP2*) is the major disease-causing gene, accounting for 50–90% of mutation-positive patients [1, 2]. Nevertheless, the genetic cause remains unknown in half of the affected families [1]. The identification of genetic causes of ARVC has significantly improved the familial management of patients and has changed our perception of the natural history of the disease. Although probands usually display a severe phenotype with a high rate of ventricular arrhythmias, ARVC is often associated with mild presentation in relatives, suggesting that only a minority of mutation carriers develop symptoms and complications. Familial studies have indeed clearly shown that desmosomal gene mutations are associated with reduced penetrance, as only 34–39% of the first-degree relatives carrying a mutation display a definite diagnosis of ARVC based on the 2010 International Task Force Criteria [2–4]. In the latest issue of *Cardiology*, van der Smagt et al. [5] provide additional evidence of reduced penetrance associated with *PKP2* mutations. They performed a large clinical familial analysis of a frequent heterozygous splice-site mutation (c.2489+4A>C) identified in four different

Dutch families. A founder effect was identified by haplotype sharing tests. This variant was associated with aberrant *PKP2* splicing, demonstrated through transcriptional analysis. They identified ten additional mutation carriers in relatives, which were predominantly asymptomatic. Only four (40%) displayed a definite diagnosis of ARVC according to the Task Force Criteria, one (10%) had a borderline diagnosis and four (40%) displayed a normal phenotype (all women), confirming the reduced penetrance associated with this *PKP2* mutation. Incomplete penetrance of desmosomal mutations partly explains why almost 50% of ARVC are apparently sporadic and has important consequences for the familial management of ARVC. Thus, a negative clinical evaluation does not exclude the possibility that a relative can be an asymptomatic mutation carrier, highlighting the usefulness of predictive genetic screening in this cardiomyopathy. The identification of such asymptomatic carriers is highly important to assess their arrhythmic risk and to set up an adequate preventive strategy and cardiac follow-up. In addition, asymptomatic mutation carriers can transmit the causative mutation to their offspring that can further develop a more severe phenotype and arrhythmic complications. In absence of a positive genetic

test in the proband (which is the case in half of the families), the clinical identification of possibly affected relatives remains challenging and requires a long-lasting cardiac follow-up, due to age-related penetrance [3]. The identification of the remaining genetic causes of the disease is then crucial to improve the management of ARVC families. The clinical management of asymptomatic mutation carriers remains difficult in the absence of comprehensive prognostic data in these patients. Some data suggest that their cardiac risk is probably low [3] but long-term prospective studies are needed to determine the natural history of the disease and to assess the sudden cardiac death risk in these patients.

The understanding of factors underlying the incomplete penetrance and variable expressivity of ARVC remains poorly understood. Nevertheless, their identification is highly important for the diagnosis, risk stratification and prognosis of relatives in ARVC families and remains an important challenge. Reduced penetrance has been shown to be sex- and age-related, with an overrepresentation of men in probands [3, 6]. Other environmental factors such as intensive practice of sports or virus have been suggested [7], and genetic factors are also involved. The presence of multiple mutations/genetic variants is associated with an increased penetrance and a more severe phenotype [1, 8]. Nevertheless, the role of genetic modifying factors, especially rare desmosomal missense variants, remains poorly understood and still needs to be further investigated in larger population studies [9]. These variants are indeed frequent in the general population (5% of Caucasians) and their pathogenic role is still debated in the absence of a proven functional effect [10]. The genetic heterogeneity of desmosomal gene mutations, the strong genetic background of rare missense variants in the general population and the small size of families make the study of putative genetic modifying factors difficult. The identification in the Dutch population of frequent mutations with a founder effect provides a more homogenous population, with a large number of individuals carrying the same mutation that could be a useful tool to study such factors. In addition, the reduced penetrance observed as associated with this mutation [5] is in line with a founder effect of a mutation that is supposed to have limited consequences on reproductive fitness and life expectancy, and can be transmitted for more than seven generations.

Van der Smagt et al. [5] also discuss the mutational mechanism associated with splice site *PKP2* mutations. *PKP2* mutations are predominantly truncating, leading to a putative haplo-insufficiency mechanism. However,

there is a lack of functional data on the mechanisms underlying splice-site and missense *PKP2* mutations. Although splice-site mutations can lead to an abnormal mRNA escaping the nonsense-mediated messenger RNA decay, there is no evidence that the abnormal protein could have a dominant negative effect. Some data suggest that haplo-insufficiency could be the major ARVC-causing mutation mechanism, including nontruncating mutations. A functional study of a missense *PKP2* mutation has shown that the mutated *PKP2* presented no dominant negative effect on mature desmosomes but displayed an increased turnover and a premature degradation suggesting, at least partly, a haplo-insufficiency mechanism [11]. Other data from a knock-in animal model homozygous for a mutated *DSG2* lacking the extracellular domain showed a decrease of total *DSG2* amount, suggesting degradation of the abnormal protein [12]. We could hypothesize that abnormal *PKP2* proteins secondary to aberrant splicing could also lead to premature *PKP2* degradation. The identification of the mutational mechanism of such mutations needs further in vivo investigations with the characterization of the mutated *PKP2* in cardiac tissue, including Western blot analyses. However, the access to heart tissue from mutation carriers remains limited. The recent possibility of obtaining induced pluripotent stem cells from human fibroblasts (from a skin biopsy for instance) could provide a useful human heterozygous in vitro model to study the consequences of mutations on protein expression and function. The identification of the mutational mechanisms associated with desmosomal gene mutations is indeed critical for a better understanding of ARVC physiopathology and for the development of future therapeutic strategies.

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