Frontiers in cardiovascular medicine

The research venture in arrhythmogenic right ventricular cardiomyopathy: a paradigm of translational medicine†

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Arrhythmogenic right ventricular cardiomyopathy is a recent discovery in the field of non-ischaemic myocardial diseases. It represents a unique example on how it is possible in few years to move from the identification of a new lethal morbid entity at the anatomical theatre towards the unveiling of the genetic aetiology, thus allowing early detection of carriers with effective strategies for premature death prevention.

Keywords

Arrhythmogenic right ventricular cardiomyopathy • Genetics • Pathology • Sudden death

Introduction

I feel particularly honoured that my lecture is dedicated to the memory of René Laennec (Figure 1). He invented the stethoscope on the base of clinico-pathologic correlations, employing the method introduced in my University by Giovanni Battista Morgagni, the discoverer of organ pathology.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a recent discovery in the field of non-ischaemic myocardial diseases. It represents a unique example on how it is possible in few years to move from the identification of a new lethal morbid entity at the anatomical theatre towards the unveiling of the genetic aetiology, thus allowing early detection of carriers with effective strategies for premature death prevention.

I took part to this venture since the beginning and I would like to tell this journey, which is really a paradigmatic instance of translation in Cardiovascular Medicine.

History of the disease

First descriptions of ARVC date back centuries ago, much earlier than modern observations.

The first report of ARVC as heredo-familial disease was published posthumously in 1736 by Giovanni Maria Lancisi. In the book entitled "De Motu Cordis et Aneurysmatibus, Caput V, De Hereditaria ad Cordis Aneurysmata Constitutione: De Cordis Prolapse (an hereditary predisposition to cardiac aneurysm and bulgings) he described the history of a family with disease recurrence in four generations, presenting with palpitations, dilatation and aneurysms of the RV, heart failure and sudden death.

The first detailed pathological description was made by the same Laennec in his book 'De l’auscultation médiate ou traité du diagnostic des maladies des poumons et du cœur'. In chapter XV on the accumulation of fat about the heart, he wrote 'In medical writing we find many examples of the heart being overloaded with fat [... ] and even the sudden death [... ] The fatter the heart is, the thinner [... ] are its walls. Sometimes these are extremely thin, being reduced almost to nothing, especially at the apex of the heart and the posterior side of the right ventricle. [...] On examining ventricles [...] the scalpel seems to reach the cavity without encountering almost any muscular substance [...].'

This is confirmed by the protagonist Dr Lydate in Middlemarch of George Eliot, published in 1871, who, while talking to his patient, said ‘You are suffering from what is called fatty degeneration of the heart, a disease which was first described by Laennec... it is my duty to tell you that death from the disease is often sudden. [...]’

William Osler reported a case of 40 years old man who died suddenly while climbing up to a hill. The heart specimen, which is now
part of the Abbot Collection in Montreal and was reviewed by Segall, disclosed a biventricular massive myocardial atrophy, which Osler had named 'parchment heart'.

The case observed by Uhl at the Johns Hopkins Hospital in Baltimore and published in 1952 is still controversial and a source of misconceptions. It dealt with an 8-month old female infant with 'almost total absence of myocardium in the right ventricle in the absence of fatty tissue', who died due to congestive heart failure without arrhythmias. 'Examination of the cut edge of the ventricle wall reveals it to be paper-thin with no myocardium visible...', clearly a congenital absence as emphasized in the title itself. The eponym Uhl's anomaly has been employed also in adult with parchment RV free wall, clearly a misnomer since the papyraceus appearance of the ventricles in adults is the end stage of an acquired progressive, genetically determined loss of the myocardium. The parchment heart patients, reported then in the literature as Uhl's anomaly, were 17 to 81 years old and died either by congestive heart failure or arrhythmic cardiac arrest like the Osler patient. Obviously the infant reported by Uhl was affected by a structural defect present at birth, i.e. a congenital heart disease. We cannot exclude that myocardial loss could have occurred in the fetal life.

The research venture in Padua started in the 60’s when Professor Sergio Dalla Volta, the founder of the modern cardiology at our University, published cases with ‘auricularization of the right ventricle’ to emphasize the hemodynamic dysfunction, namely the absence of an effective systolic contraction of the RV with the blood pushed from the right atrium to the pulmonary artery. Dalla Volta concentrated the attention to hemodynamic features, although ventricular arrhythmias were also present. Thirty years later, one of these patients underwent cardiac transplantation at the age of 65, presenting with parchment like RV and still intact left ventricle.

When I was still in training, my mentor in Pathology Professor Vito Terribile performed an autopsy of a woman with a history of palpitations and congestive heart failure, who died of pulmonary thromboembolism. The heart showed a severe dilatation of the RV with mural thrombosis, 'adipositas cordis' and fibrosis of the left ventricular free wall, in keeping with biventricular ARVC (Figure 2).

The arrhythmic features of the disease were discovered by Guy Fontaine in the '70 s, by demonstrating the origin of non-ischemic ventricular tachyarrhythmias from the RV with left bundle branch block morphology. He observed a delayed repolarization at the end of the QRS complex (post-excitation syndrome), which he named epsilon wave.

Knowledge on the disease made progress in Padua thanks to Andrea Nava, a true pioneer and leader of our group, of whom I have been a pupil during my fellowship in Cardiology. Nava was the one to realize the heredo-familiar nature of the disease with mendelian dominant transmission and to demonstrate the onset of phenotype in childhood. Meanwhile, Marcus and Fontaine, by reporting a series of adult patients with ventricular arrhythmias and left bundle branch block morphology, in keeping with origin from RV, introduced the term 'dysplasia' because the bizarre histologic features of the myocardium at histology, which they considered a maldevelopmental defect. The term was then abandoned and ARVC
introduced definitively in the WHO nomenclature and classification of cardiomyopathies.\textsuperscript{17}

**ARVC is a cause of sudden cardiac death in the young: pathology**

At the late ’70s we started a research program, collecting and studying all the cases of juvenile sudden cardiac death (SCD) (<35 years, sudden infant death syndrome excluded) occurring in the Veneto Region, a northern-east part of Italy with nearly 5 million inhabitants. We were able to cover the whole phenomenon, thanks to the collaboration of forensic and anatomic pathologists. The project is still ongoing and has no equivalent worldwide, since all the hearts are forwarded to our Pathology Core Lab. Among the first 60 consecutive cases, 12 (20\%) were found to be affected by ARVC and most of them were athletes. Thanks to the availability of the ECG, which is compulsory in Italy for eligibility to sport activity, it was possible to realize that the subjects presented inverted T-wave in the right precordial leads and apparently benign premature ventricular beats of the left bundle branch block morphology (Figure 3). The paper was published in the *New England Journal of Medicine* on a January issue 1988.\textsuperscript{18} It was a shock for the scientific cardiovascular medicine community to realize that not only hypertrophic cardiomyopathy is a cause of sudden death in the young and athletes.\textsuperscript{19} Genetic variability or, more probably, misdiagnosis at postmortem may have accounted for the discrepancy of the prevalence in United States vs. Italy. As usual, you see what you know.

Pathology was then reported in detail.\textsuperscript{20} The phenomenon consists of a transmural fibro-fatty replacement in the RV wall, with aneurysms in 50\% of cases, located in the inferior, apical and infundibular regions (’triangle of dysplasia’). The disease could be segmental and equally malignant. Left ventricular free wall involvement was observed in nearly half of the cases (Figure 4), whereas location in the ventricular septum was rare. Even hearts with isolated left ventricular involvement were reported, thus the term arrhythmogenic cardiomyopathy was advanced.\textsuperscript{21} A glance at the light microscope allowed to see cell death, followed by fibro-fatty infiltration. This observation was in keeping with an ongoing myocardial injury and repair phenomenon and with a genetically determined cardiomyopathy characterized by an acquired, progressive loss of myocardium with fibro-fatty scarring, well in agreement with the concept of myocardial dystrophy\textsuperscript{9,20} (Figure 5). At electron microscopy and Tunel staining, apoptosis appeared to be the mode of the ongoing myocyte death.\textsuperscript{22}

Focal myocardial inflammation was observed in about 75\% of autopsy cases and might account for worsening of electrical instability and onset of life-threatening arrhythmias.\textsuperscript{20,23,24} Whether the inflammatory cells are a reaction to cell death or the consequence

*Figure 2* The autopsy record of a female patient with arrhythmogenic right ventricular cardiomyopathy, first postmortem case studied at the University of Padua. Note the ‘adipositas cordis’ in the right ventricle and the ‘myocardial sclerosis’ in the left ventricle.
of infective or immune mechanisms has been a source of controversy. Superimposed viral infections were ruled out by molecular investigations.25

Concerning the more prominent pathology of the RV, mechanical stress is the most plausible explanation for. The RV cavity is larger and the free wall thinner than the left ventricle. According to Laplace’s law, wall tension is particularly high in the RV free wall and a role of mechano-transduction has been postulated to convert mechanical stimuli to biochemical intracellular signals,26,27 especially in the setting of genetically determined fragility of the intercellular junctions.

Another explanation involves the origin of the adipocytes from progenitor cells of the second heart field, which give rise to the bulbus cordis and pulmonary infundibulum. According to this hypothesis, the progenitor cells of the second heart field switch to adipogenesis because of suppressed wnt–catenin β1 signalling as a result of the translocation of junction plakoglobin to the nucleus.28

Because ARVC is now considered to be a biventricular disease, subepicardial progenitor cells are more likely than those from the second heart field to have a role in fibrofatty replacement. Indeed, subepicardial progenitor cells spread into both ventricles, and generate the myocardium and interstitial cells. The wave-front extension of the fibrofatty tissue from the epicardium towards the endocardium supports this alternative theory.29

**ARVC is a genetically determined cardiomyopathy: desmosomal disease**

In the ’90s we had no idea where to look for the gene mutations. Certainly neither into sarcomeric genes, like in hypertrophic cardiomyopathy, since there was no evidence of hypertrophy or disarray, not into cytoskeleton dystrophin complex ones, like in dilated cardiomyopathy, since mechanical contractility was fairly preserved in the left ventricle. The inspiration came from a letter to the Editor submitted to the New England, following the publication of our paper,18 by a group of Greek scholars from the Naxos island.30 They drew readers’ attention to the existence of a recessive cardiocutaneous syndrome, combining ARVC phenotype and palmoplantar keratosis with woolly hair.31 Since both epidermal cells and myocytes possess desmosomes, ensuring mechanical adherence, genes coding proteins of cell junctions became candidate.
A German group from Heidelberg showed that knock out transgenic mice of the JUP (plakoglobin) gene (γ-catenin) resulted in severe myocardial injury with disappearance of desmosomes and spontaneous fetal cardiac rupture. The human plakoglobin gene is located on chromosome 17q21, a region not yet identified in human cardiomyopathy patients they said.32

Thus, JUP became a candidate gene for the Naxos disease. Linkage analysis was carried out in Naxos affected families, identifying the gene defect exactly in the locus 17q2133. Thereafter it was proved through gene sequencing that the molecular defect was a deletion in plakoglobin gene.34

In the late 90s Luis Carvajal Huerta, a dermatologist from Equador, had reported another recessive cardio-cutaneous syndrome, featured by dilated cardiomyopathy, woolly hair and palmoplantar keratosis.35 The defect was found in DSP (desmoplakin) gene, encoding a major protein of the desmosome apparatus.36 By studying with Jeff Saffitz in St Louis the heart specimen of one original child with Carvajal syndrome, who had died with congestive heart failure, I realized that it was undoubtfully a biventricular ARVC, with aneurysms in the RV, typically located in the triangle of dysplasia, and with extensive dilatation of the left ventricle with mural thrombosis.37

This experience was inspiring in so far as DSP immediately became a candidate gene also for the dominant variant of ARVC. The genetic screening of families, followed-up by Nava in Padua, led to the identification of a missense mutation of DSP gene.38 Genotype-phenotype correlations demonstrated that in vivo imaging by cardiac magnetic resonance of genotyped DSP patients have frequently a biventricular or even predominant left ventricular involvement.39

All the other genes encoding desmosomal proteins were soon investigated in non syndromic ARVC and missense mutation in PKP2 (plakophilin-2), DSG2 (desmoglein-2), DSC2 (desmocollin-2) were discovered. Thus, both recessive and dominant variant of ARVC were attributed to cell junction defects and the disease overall named desmosomal disease44,45 (Figure 6).

Electron microscopy in genotyped patients affected by ARVC revealed ultrastructural abnormalities of the desmosomes, which appeared less numerous, short, pale, fragmentary, and we postulated that disruption of intercalated disc might be the final common pathway of the genetically determined myocyte death.46

Additional non-desmosomal-related genes have been associated with ARVC, such as RYR2 (ryanodine receptor 2), TGFB3 (transforming growth factor β3), TMEM43 (transmembrane protein

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**Figure 4** A 14-year-old boy died suddenly during a soccer game: at postmortem, including magnetic resonance, biventricular fibro-fatty replacement was observed.

**Figure 5** Arrhythmogenic right ventricular cardiomyopathy is a progressive myocardial dystrophy. From Basso et al.29
Recapitulation of the disease in transgenic mice

We generated, in collaboration with Professor Bezina in Amsterdam, a transgenic mouse overexpressing in the heart the mouse homologue of a desmoglein-2 gene mutation, previously identified in Padua in an ARVC proband who underwent cardiac transplantation for severe RV dilatation.55

The findings of the recapitulated disease in mice were astonishing: dilatation and aneurysm formation at both ventricles with fibrous replacement of the myocardium, prolonged ventricular activation at epicardial electrophysiology, tachyarrhythmias, and SCD. Oddly enough, the hearts appeared normal at birth and cell death was documented at histology and electron microscopy with features of necroptosis.55 Necrotic myocyte death was demonstrated to be the initiating event in the myocardial injury and repair process. This was the confirmation that the disorder is a genetically determined cardiomyopathy and that the pathologic substrate occurs with time (Figure 5), due to a dose-dependent dominant-negative effect of the mutation.

Subsequent study in the same transgenic model revealed the occurrence of delayed electrical ventricular activation time and arrhythmias inducibility, before the onset of myocyte death and replacement fibrosis, as a consequence of reduced Na⁺ current density, probably due to intercalated disc widening seen at electron microscopy.56 In other words, ventricular electrical instability appears before structural remodelling, due to cross talk between cell junction and sodium channel complex. The evidence of an in vivo interaction between desmoglein-2 and sodium current under-scores the functional link of electrical disturbances during the early ARVC stages.

Advances in diagnosis

Diagnostic criteria have been dictated in 1994, as to make possible identification of patients at risk through non-invasive (ECG, echo) and invasive procedures [angiography, endomyocardial biopsy (EMB)].57 Cardiac magnetic resonance (CMR) revealed to be an extraordinary diagnostic tool in terms of both morphofunctional study (ventricular dilatation, dyskinesia, aneurysms) and tissue characterization. Although not yet included in the 2010 updated version
of the diagnostic criteria,\textsuperscript{58} contrast-enhanced CMR is now routine employed in tertiary centres. In particular, since ARVC is frequently a biventricular disease, contrast enhanced CMR with gadolinium may easily detect fibro-fatty scarring in the LV, which can be considered the ‘mirror’ of RV involvement.\textsuperscript{59} Electroanatomic voltage mapping (EVM) revealed to be a quite sensitive tool for detecting decreased electrical activity in the areas of fibro-fatty replacement and for differential diagnosis of segmental infundibular ARVC with RV outflow tract tachycardia (a ventricular arrhythmia without substrate and no genetic background) as well as myocarditis.\textsuperscript{60,61} By the way, as far as detection of fibro-fatty scarring in RV free wall, bipolar EVM is much more sensitive than contrast-enhanced CMR due to the thin wall\textsuperscript{59} (Figure 8). Recent data demonstrated that unipolar EVM recording provides a larger antenna than bipolar-EVM to accurately detect fibro-fatty substrate involvement confined to the epicardium, which commonly occurs in ARVC patients.\textsuperscript{62} Moreover, the arrhythmic risk increases with the amount of RV myocardium replaced by electroanatomic scar. However, since also cardiac sarcoidosis may mimic ARVC, employment of EMB is sometimes needed for differential diagnosis.\textsuperscript{63} Morphometric criteria were put forward for ARVC diagnosis at EMB\textsuperscript{64} and were included within 2010 diagnostic criteria.\textsuperscript{58}

Differential diagnosis with cross-over arrhythmogenic syndromes, such as Brugada syndrome and idiopathic RV outflow tract tachycardia, may be challenging.\textsuperscript{65} While the latter condition is usually benign and non-familial, without ECG repolarisation and depolarisation abnormalities, Brugada syndrome is an inherited channelopathy presenting with a characteristic ECG morphology, i.e. coved ST-segment elevation in the right precordial leads, life-threatening ventricular arrhythmias, and high risk of SCD.\textsuperscript{65} In both instances, the demonstration of the absence of RV structural abnormalities, including normal contrast-enhanced CMR, EVM as well as EMB findings, is of utmost value. Recent molecular studies suggest that ARVC and Brugada syndrome are not completely separate entities, but ‘bookends’ in a continuum of variable sodium current deficiency and structural changes, thus explaining the partial clinical overlap.\textsuperscript{66}

\textbf{Risk stratification: prevention of sudden death is possible}

The application of diagnostic criteria greatly contributed to the detection of ARVC in young subjects. In particular, the observation of inverted T waves in right precordial leads,\textsuperscript{67} QRS widening and epsilon waves at pre-participation ECG screening resulted in disqualification of athletes and sharp decline of SCD during sport activity.\textsuperscript{68}

The discovery of the defective genes, although still limited to 50% of ARVC patients, opened new avenues. Genetic screening, for early diagnosis of healthy carriers and reassurance of non-carriers, entails a tremendous impact on life-style, including sport activity eligibility, and on genetic counselling for disease recurrence in sibs and offsprings with primary prevention of arrhythmic risk. Risk stratification for implantable cardioverter defibrillator (ICD) has been put forward,
most based on phenotype and clinical manifestations (previous cardiac arrest, syncope, sustained tachycardia).69 – 71 Multiple compound-heterozygous mutations revealed to entail a more severe prognosis.72 Implantable cardioverter defibrillator is a miraculous life-saving tool. 25% of patients, in whom an ICD was implanted, had an appropriate electric shock at follow-up of 4 years due to ventricular fibrillation with cardioversion to sinus rhythm, which means a clear-cut decline of mortality in the natural history of the disease73 (Figure 9). Syncope should be regarded as an alarming sign for possible ICD implantation and primary prevention of SCD.74 However, ICD is associated with complications and should be employed when strictly indicated according to a precise risk stratification69 – 71 (Figure 10).

**Treatment**

There are several weapons available in the armamentarium for prevention of SCD in ARVC26,75 (Figure 11). Cardiac arrest occurs accordingly to the combination of trigger and arrhythmic mechanism, acting upon the peculiar substrate of the disease. Effort and emotion act as triggers. The risk of SCD during effort in patients with ARVC is five folds than in sedentary activity.76 Sports disqualification ‘per sé’ is a life-saving measure and the decline of SCD in athletes achieved in Italy, thanks to non-eligibility of subjects affected by ARVC, is a clear-cut evidence of its efficacy.58

Drug therapy and ablation influence the reentry arrhythmic mechanisms.69 – 71 They are both of limited efficacy, in particular catheter ablation shows a high rate of arrhythmias recurrence.77 Defibrillator, either ICD or semi-automatic external, represents a major advancement in preventing SCD. It is the most effective life-saving tool by converting ventricular fibrillation to sinus rhythm. Implantable cardioverter defibrillator is employed according to risk stratification for both secondary (after previous episodes of aborted SCD)73 or primary prevention (previous unexplained syncope, high familiarity of SCD with compound or heterozygous mutations)74. Use of semi-automatic defibrillator, made available in any sport field, together with the pre-participation ECG screening for detection and disqualification of asymptomatic carriers, represent the best synergy for prevention of SCD in athletes.

In case of heart failure or unbearable electric storms, cardiac transplantation is also available as extreme therapeutic option.69 – 71

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**Figure 8** Electroanatomic mapping is more sensitive than cardiac magnetic resonance with late enhancement to detect right ventricular involvement in arrhythmogenic right ventricular cardiomyopathy. However, the left ventricle is frequently involved, as to be considered the ‘mirror’ of the right ventricular at cardiac magnetic resonance. From Perazzolo Marra et al.59
Time for molecular therapy

All the previously mentioned pharmacologic and non-pharmacologic measures are merely palliative, in so far as they act on preventing or treating arrhythmias. They do not point to the basic pathobiology mechanisms, namely the onset and progression of cell death leading to myocardial dystrophy. We look forward to the time when arrhythmias will not be the only target to manage ARVC and to prevent SCD.75

Cellular reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) enables patient-specific in vitro modeling of human genetic disorders for pathogenic investigation and drug screening.78 Fibroblasts from skin biopsy sample of patients may be used to generate autologous cardiomyocyte in ARVC.79 Moreover, in situ reprogramming fibroblasts from the heart itself is an attractive possibility. Although so far only a small amount of reprogrammed myocytes appear beating cells, the ability to reprogram fibroblasts into cardiomyocytes could represent a revolution for the repair of fibrofatty replacement in ARVC.80

Zebrafish model may be also employed in the study of ARVC, to elucidate pathogenetic mechanism and screen drug therapy. By creating a zebrafish with cardiomyocyte-specific expression of mutated plakoglobin, a suppressor (SB21673) of ARVC phenotype was found, which normalized Na or K current density, reversed protein trafficking of intercellular disc proteins and prevented heart failure with reduced mortality.81

Engineering adeno-associated viral vector containing c-DNA of wild-type desmosomal gene, to be transferred into the heart, may
restore electrophysiological and ultrastructural abnormalities. It may represent a curative gene therapy as it has been proven in cathcolaminergic polymorphic ventricular tachycardia.\(^8\)

We are facing new avenues and time has come that molecular medicine enters the field of myocardial dystrophy in ARVC, as it was for muscular dystrophy.\(^8\)

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