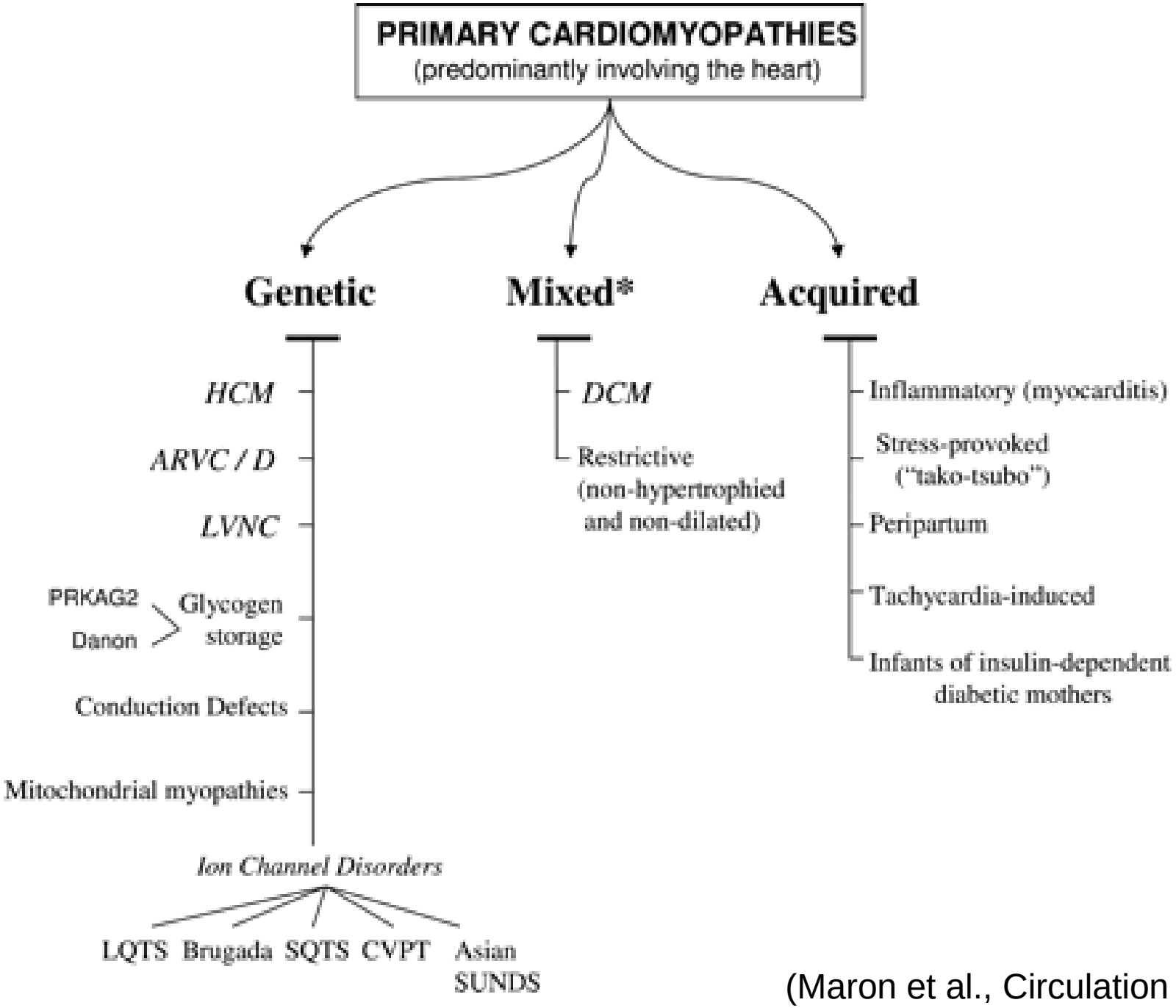
Restrictive Cardiomyopathy (RCM) Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)

Prof. Ralph Knöll

Lecture 4



(Maron et al., Circulation 2006)

Restrictive Cardiomyopathy

Primary restrictive cardiomyopathy as defined here is a **rare** form of heart muscle disease and a cause of heart failure that is characterized by **normal or decreased volume of both ventricles associated with biatrial enlargement**, normal LV wall thickness and AV valves, impaired ventricular filling with restrictive physiology, and normal (or near normal) systolic function. Both sporadic and familial forms have been described, and in 1 family, a troponin I mutation was responsible for both restrictive cardiomyopathy and HCM.

Restrictive Cardiomyopathy

- Characteristics:
  Restrictive, diastolic ventricular dysfunction
  Systolic function normal.

- Pathology: heart weight normal or slightly increased, atria enlarged
  Thrombi present
  possibly interstitiel fibrosis (but not always!)
  Endokardial fibrosis.

- Quite often in Africa, Asia and South America

Differential Diagnosis

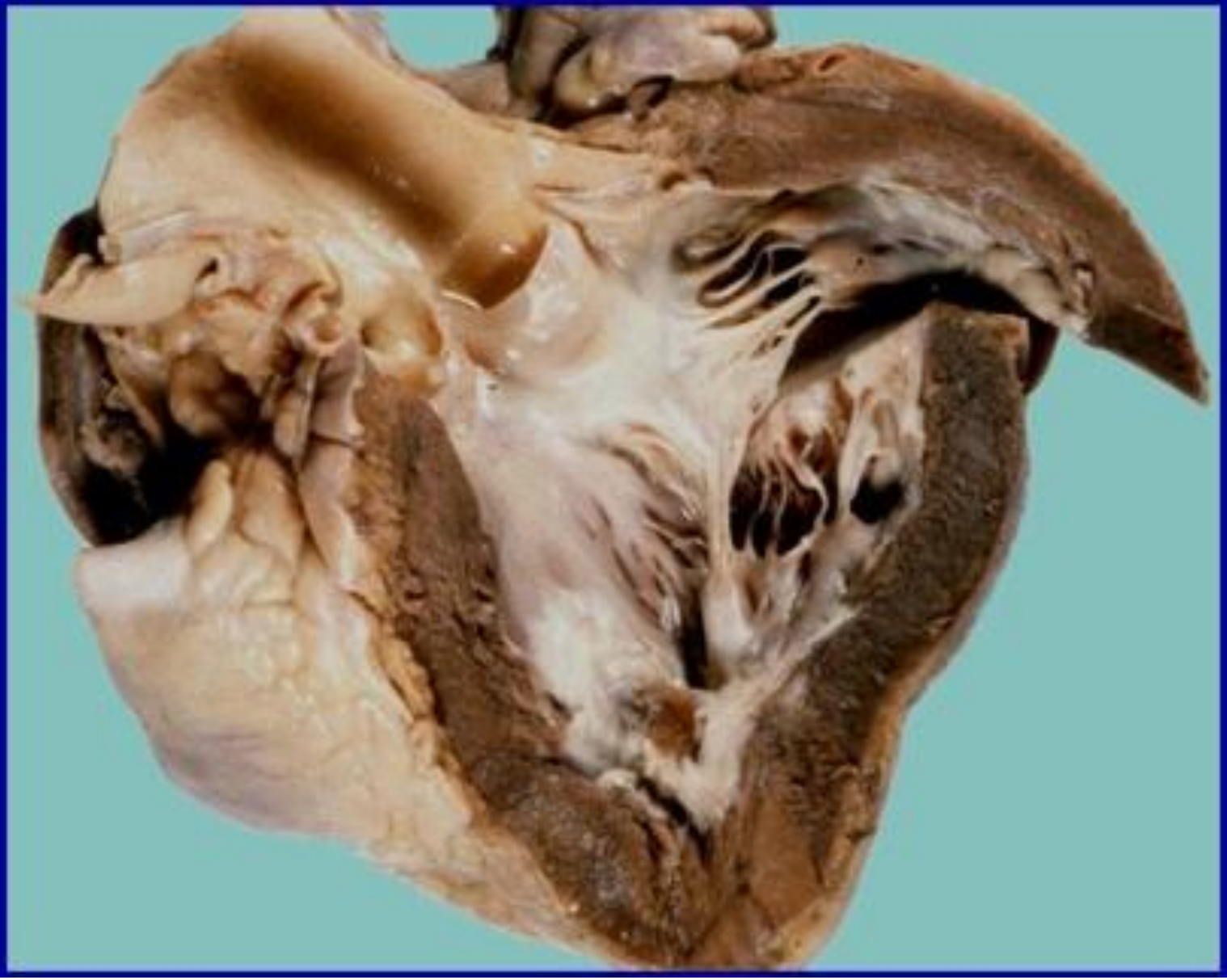
Differential Diagnosis:

Constrictive Pericarditis

Storage disease: Amyloidosis (quite often), Hemochromatosis.

Löffler´sche Endocarditis with eosinophilie (thickening of the endocardium, affects the valves as well)

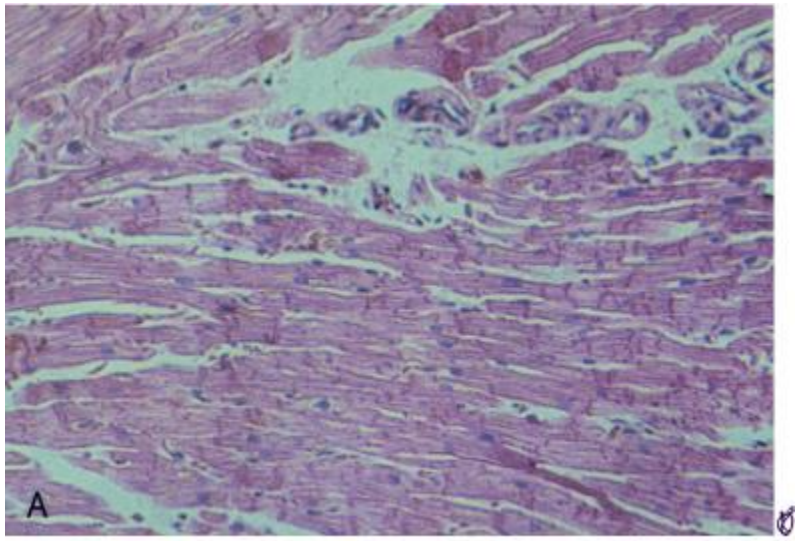
RCM – endocarditis Loeffler

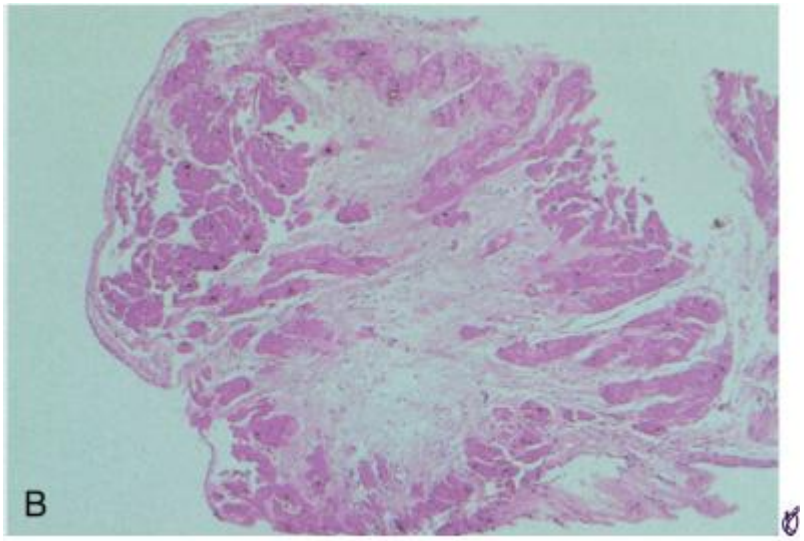


Loeffler endocarditis

- A form of endocarditis (and a form of hypereosinophilic syndrome)
- It is a restrictive cardiomyopathy characterized eosinophilia and eosinophilic penetration leading to the fibrotic thickening of portions of the heart (similar to that of endomyocardial fibrosis) and commonly has large mural thrombi. Common symptoms include edema and breathlessness. It is commonly found in temperate climates, and is rapidly fatal.

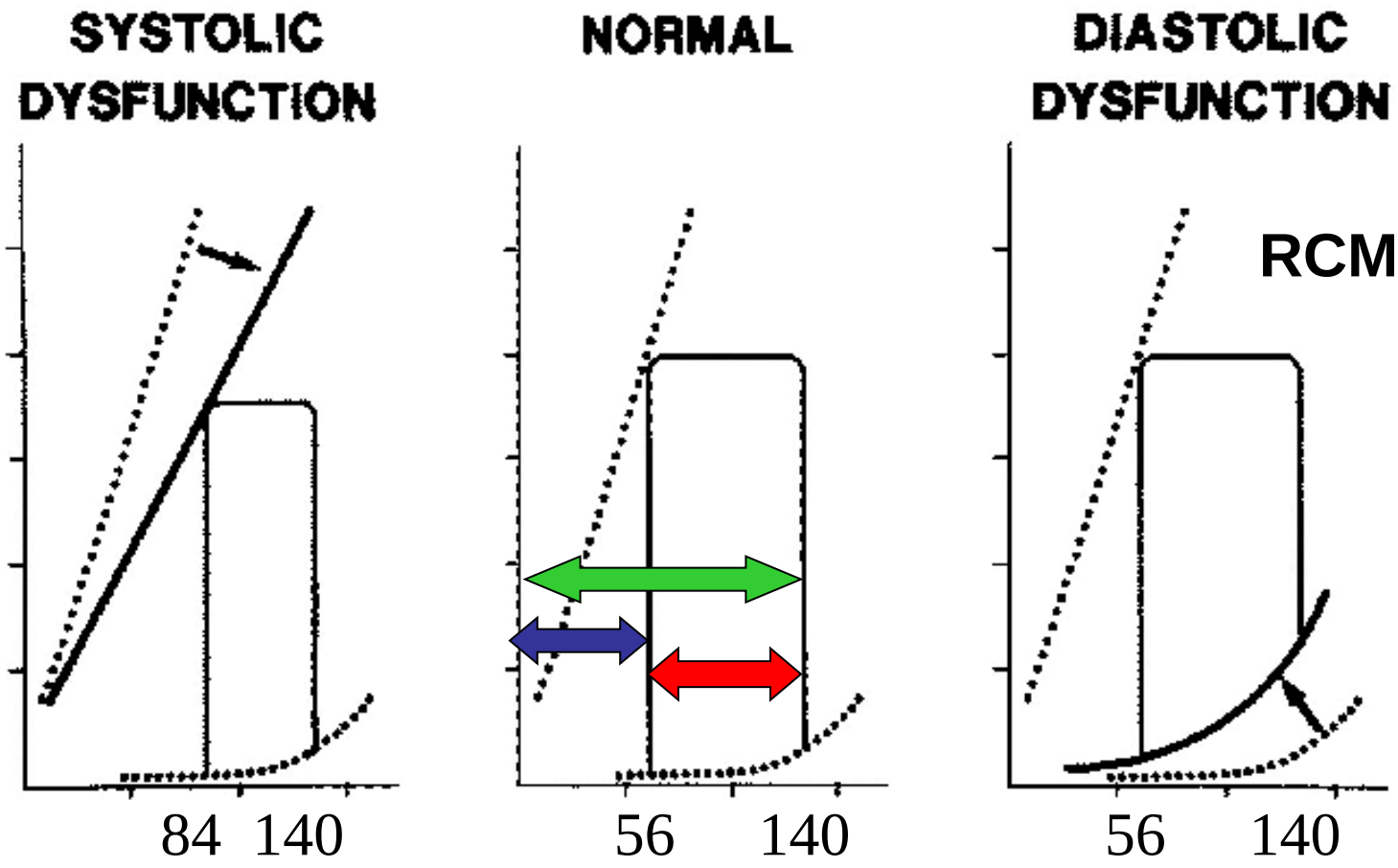
RCM - Histology





Endomyocardial biopsy specimens from patients with idiopathic restrictive cardiomyopathy. **A,** This histological specimen (hematoxylin and eosin, ×250) shows myocytes with slight hypertrophy but is otherwise normal. **B,** Another specimen (hematoxylin and eosin, ×40), from another patient, shows marked interstitial fibrosis, which may also occur in idiopathic restrictive cardiomyopathy.

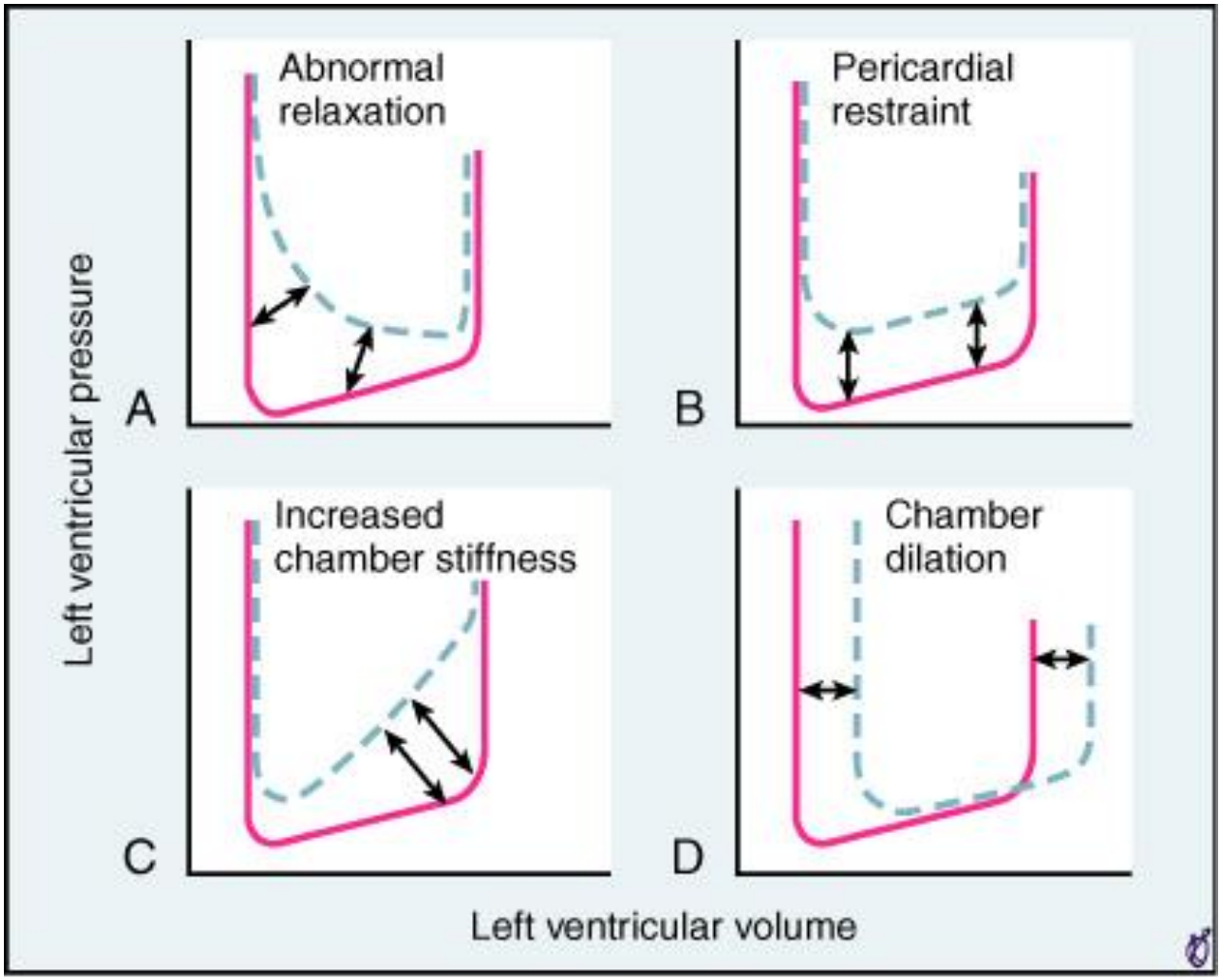
Systolic and diastolic heartfailure



Ejektionsfraktion (EF) = SV/EDV oder EDV-ESV/EDV

Hier: EF = 140 – 56 / 140 = 84 / 140 = 0,6 oder 60%

Types of heart failure



RCM - Causes

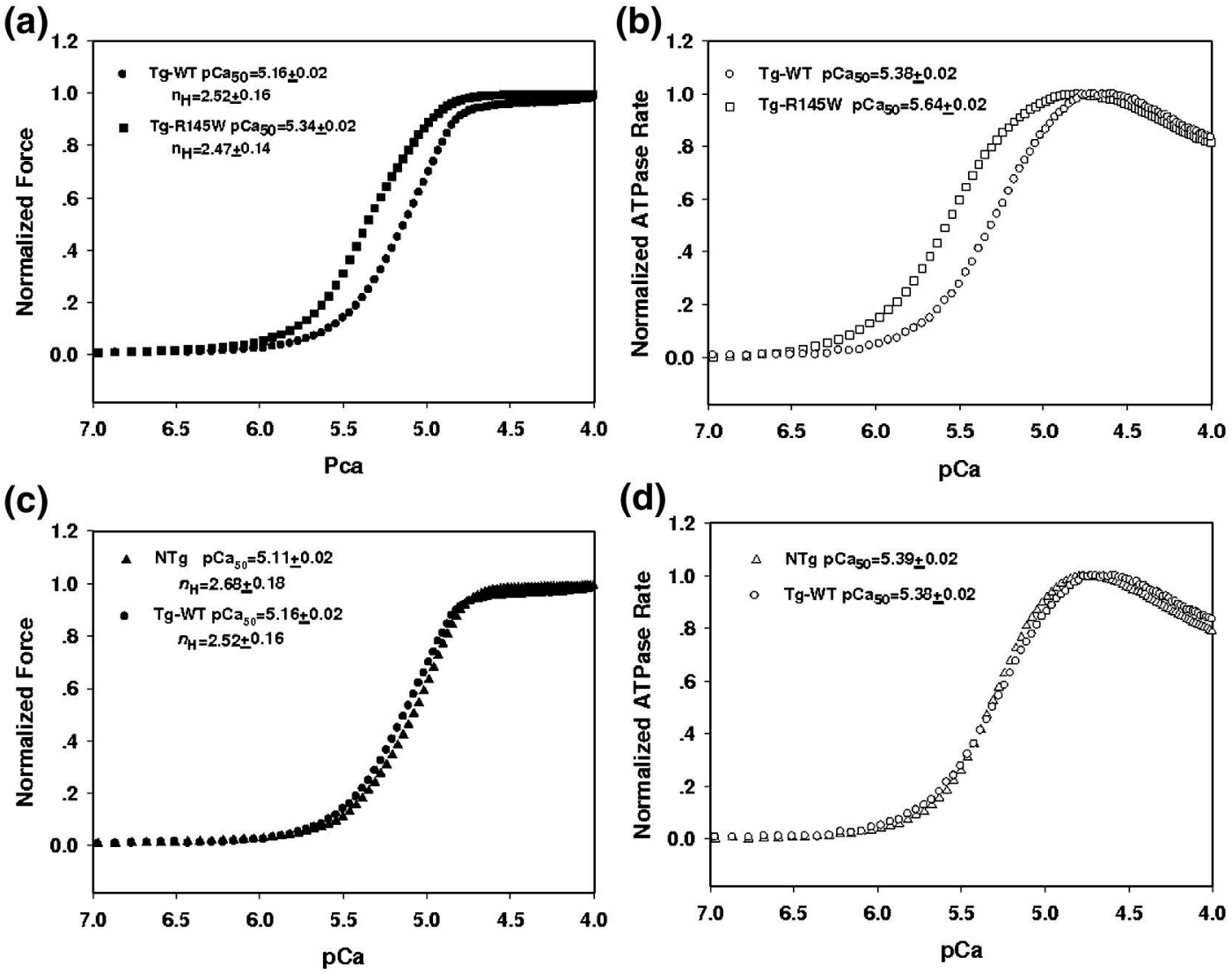
- Genetic: Mutations in Troponin I (such as the R145W troponin I mutation)
- But how does this mutation cause a „restrictive“ phenotype?
- Wen et al., Journal of Molecular Biology 2009 used a transgenic mouse model to analyze the effects in vivo.

RCM - Causes

Tg-R145W fibers showed an 13–16% increase in maximal Ca^{2+}-activated force and ATPase activity compared to hcTnI wild-type transgenic mice.

Also, the Tg-R145W fibers showed a large increase in the **Ca^{2+} sensitivity** of both force development and ATPase.

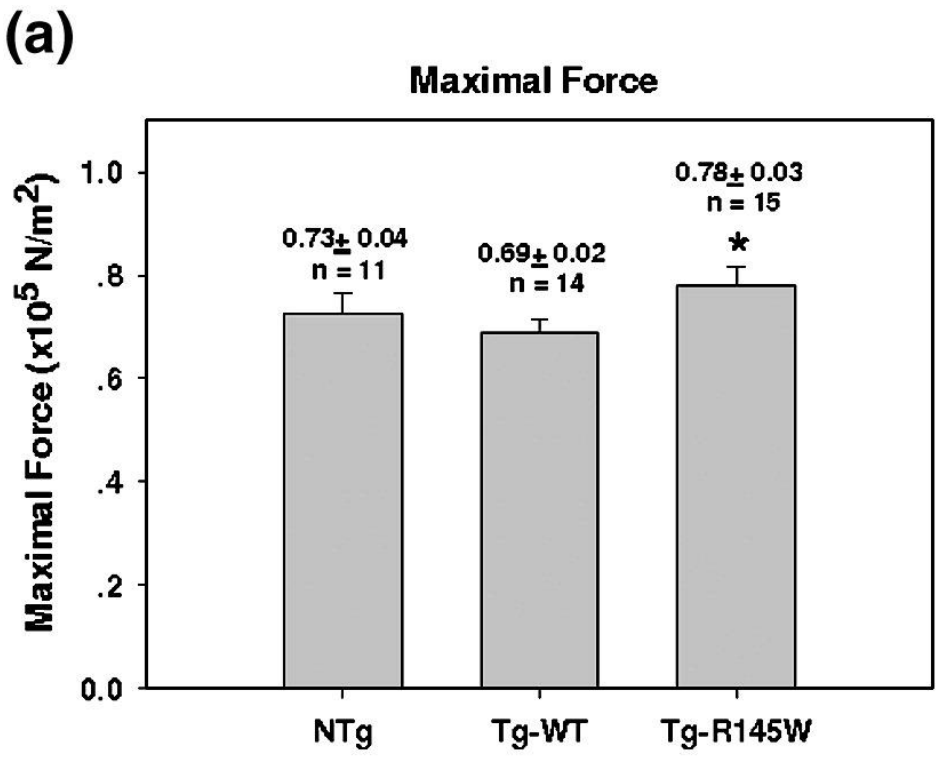
R145W Troponin I

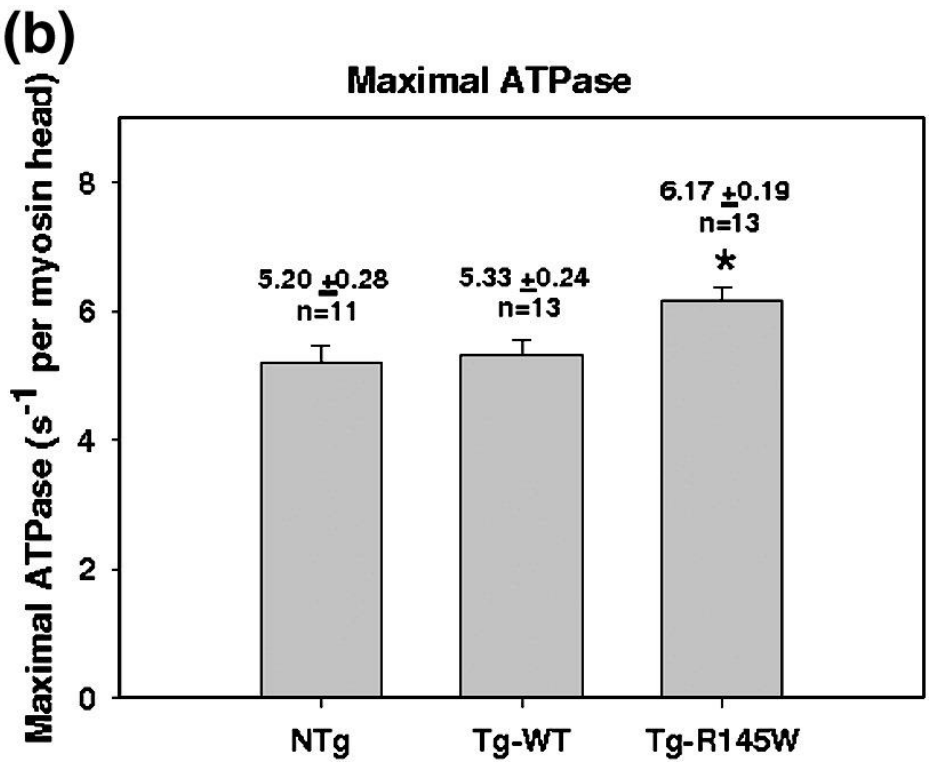


R145W troponin I

The effects of the hcTnI R145W mutation on the Ca^{2+} sensitivity of **steady-state force** and ATPase activity measured simultaneously in isometric muscle fibers. The force–pCa relationship between Tg-R145W fibers (*n* = 15) and Tg-WT fibers (*n* = 13) (a), and between NTg fibers (*n* = 11) and Tg-WT fibers (*n* = 13) (c). The ATPase–pCa relationship between Tg-R145W fibers (*n* = 14) and Tg-WT fibers (*n* = 13) (b), and between NTg fibers (*n* = 11) and Tg-WT fibers (*n* = 13) (d). The pCa values and Hill coefficients for force curve and the pCa values for ATPase curve are shown in the graphs.

R145W troponin I





R145W troponin I

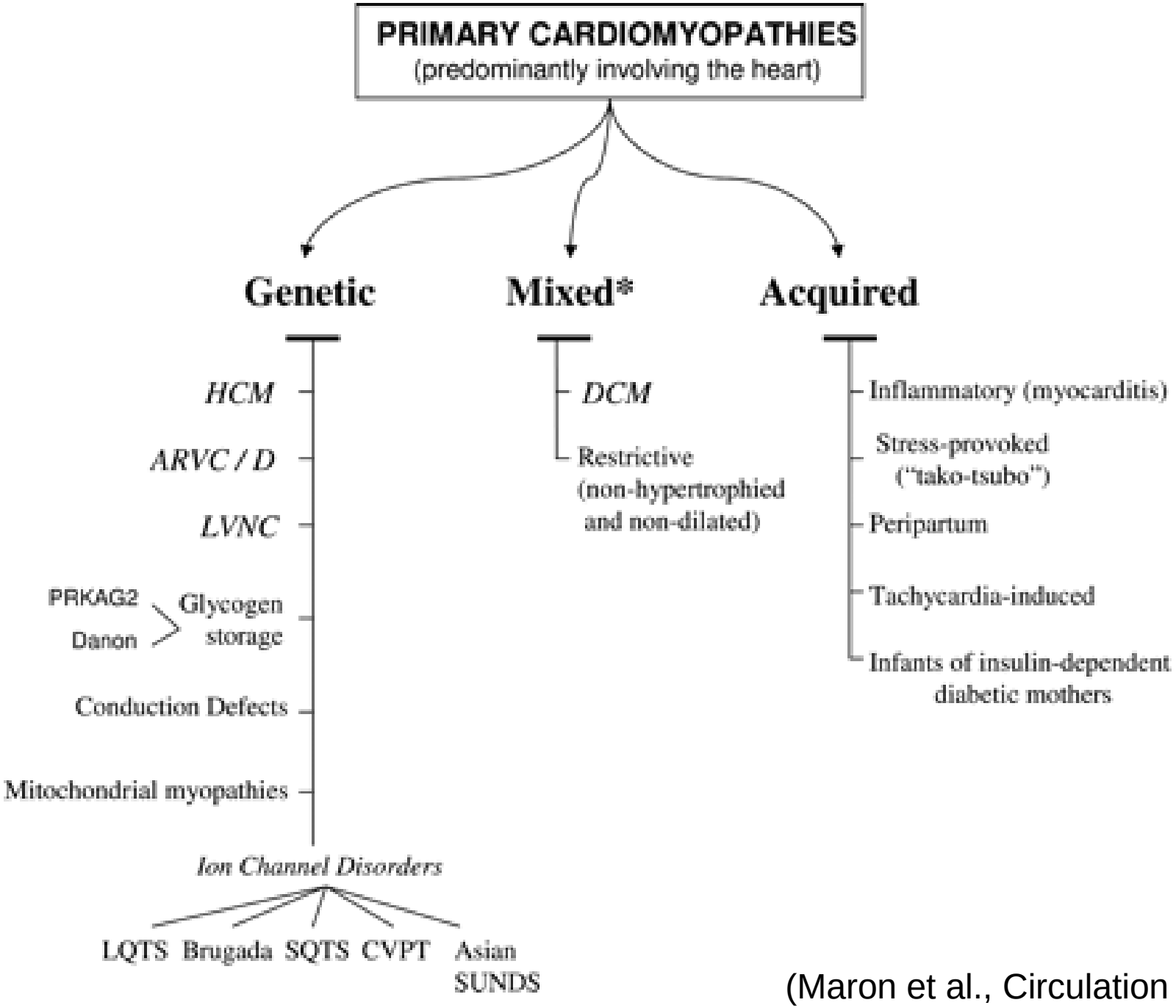
- The effect of the hcTnI R145W mutation on maximal force (10^5 N/m^2) and maximal ATPase activity (s^{-1} per myosin head) in transgenic papillary muscle fibers. (a) The maximal Ca^{2+}-activated force is 13% higher in Tg-R145W fibers than in Tg-WT fibers. (b) The maximal Ca^{2+}-activated ATPase is 16% higher in Tg-R145W fibers than in Tg-WT fibers. No statistically significant difference in both maximal force and ATPase between NTg and Tg-WT fibers ($P > 0.05$). Statistically significant difference ($P < 0.05$). Data are expressed as the mean of *n* experiments ± SE

RCM - Causes

In intact fibers, the mutation caused prolonged force and intracellular [Ca^{2+}] transients and increased time to peak force. Analysis of force and Ca^{2+} transients showed that there was a 40% increase in peak force in Tg-R145W muscles, which was likely due to the increased Ca^{2+} transient duration.

RCM - Causes

(1) there would be an increase in resistance to ventricular filling during diastole resulting from the prolonged force and Ca^{2+} transients that would result in a decrease in ventricular filling (diastolic dysfunction); and (2) there would be a large (approximately 53%) increase in force during systole, which may help to partly compensate for diastolic dysfunction.



(Maron et al., Circulation 2006)

Arrythmogenic right ventricular cardiomyopathy (ARVC)

ARVC/D is an uncommon form of inheritable heart muscle disease (estimated 1:5000) with a relatively recent description (20 years ago).

ARVC/D involves predominantly the right ventricle with progressive loss of myocytes and fatty or fibrofatty tissue replacement, resulting in regional (segmental) or global abnormalities.

Arrythmogenic right ventricular cardiomyopathy (ARVC)

Although frequently associated with myocarditis (enterovirus or adenovirus in some cases), ARVC/D is not considered a primary inflammatory cardiomyopathy.

In addition, evidence of LV involvement with

fibrofatty replacement, chamber enlargement, and myocarditis is reported in up to 75% of patients.

Arrythmogenic right ventricular cardiomyopathy (ARVC)

- ARVC/D has a broad clinical spectrum, usually presenting clinically with ventricular tachyarrhythmias (eg, monomorphic ventricular tachycardia).
- **A recognized cause of sudden cardiac death in the young, it is also regarded as the most common cause of sudden death in competitive athletes in Italy.**

Arrythmogenic right ventricular cardiomyopathy (ARVC)

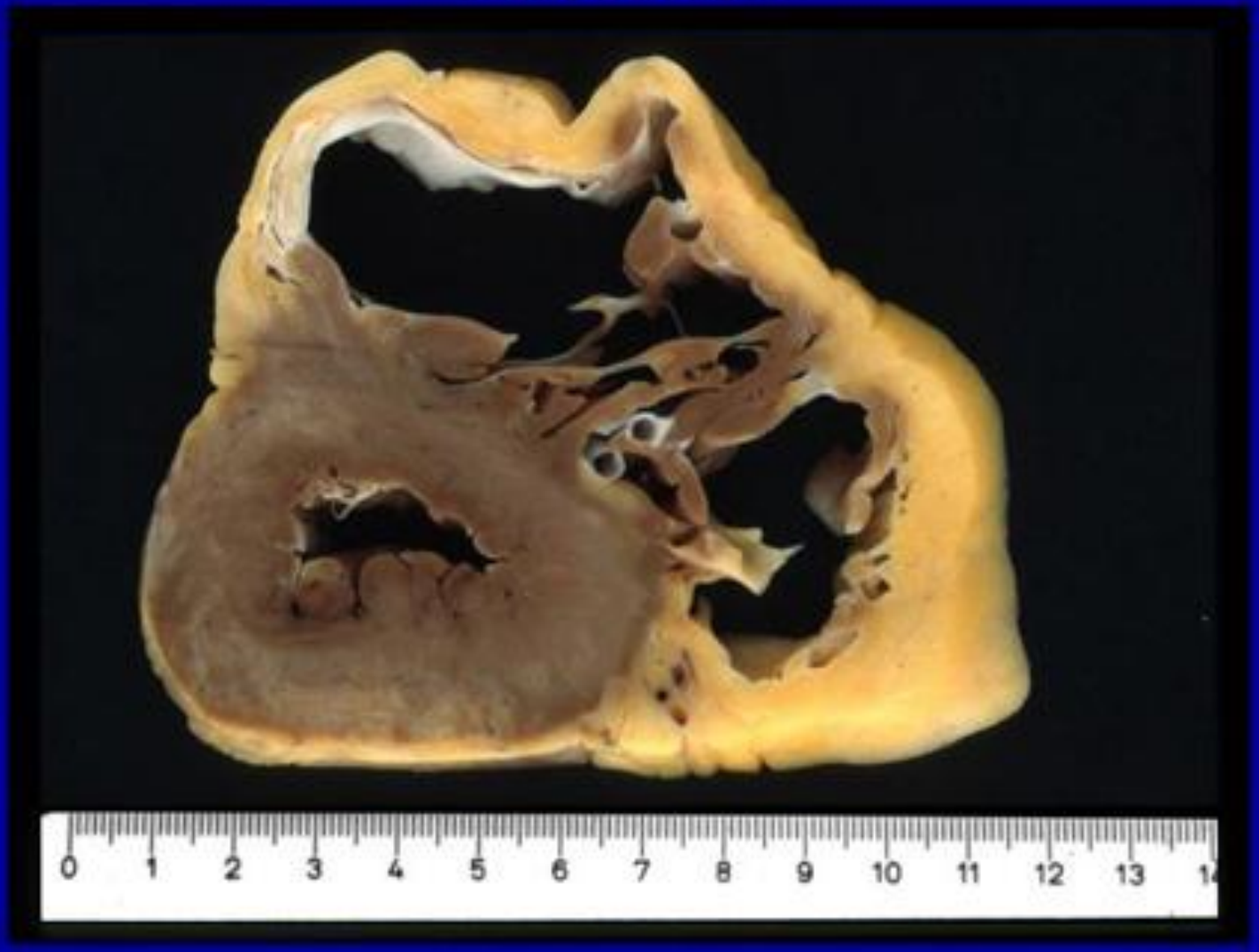
Characteristics:

Thinning of right ventricular walls, contractility defects, and arrhythmogenesis

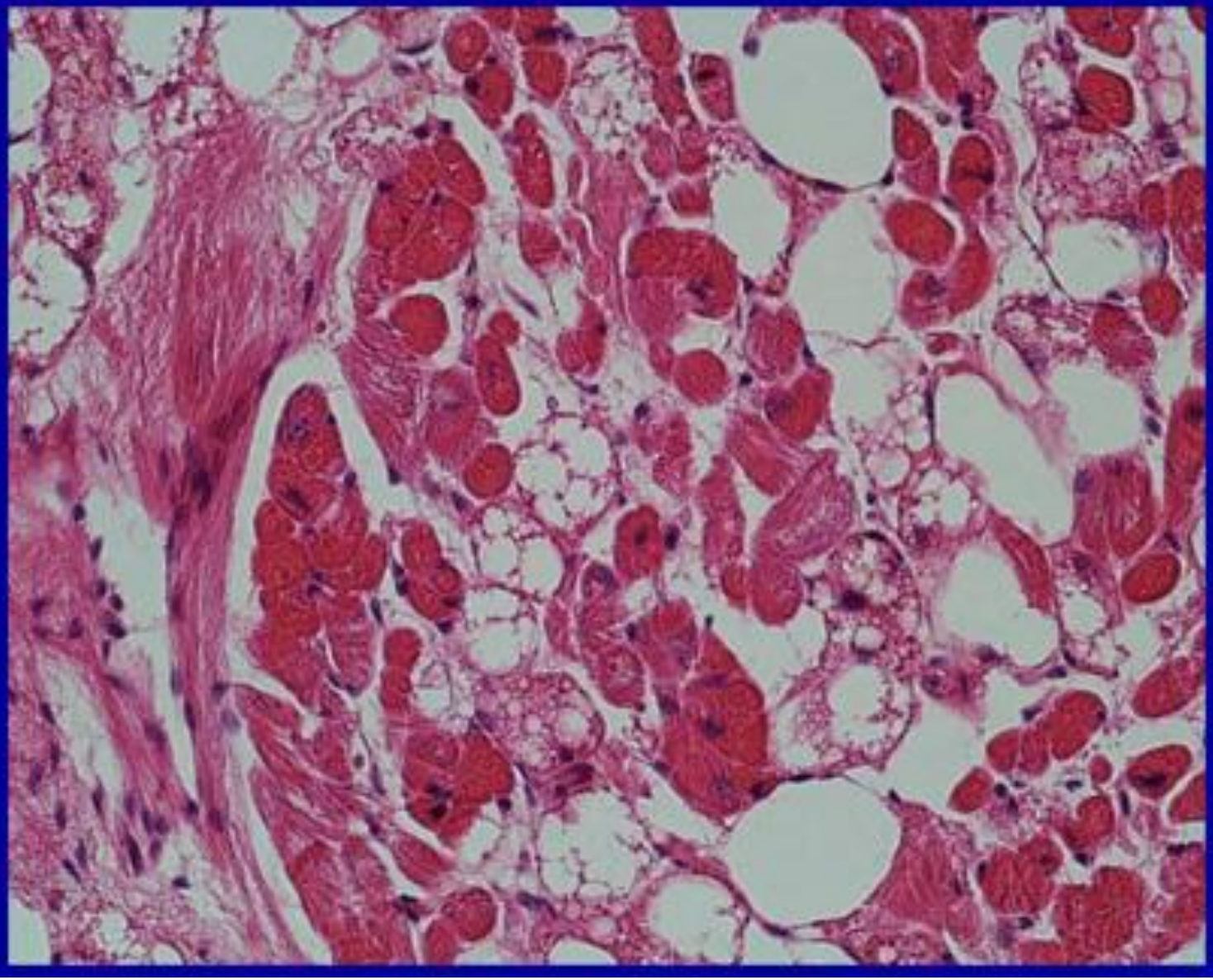
Death via arrhythmogenic events

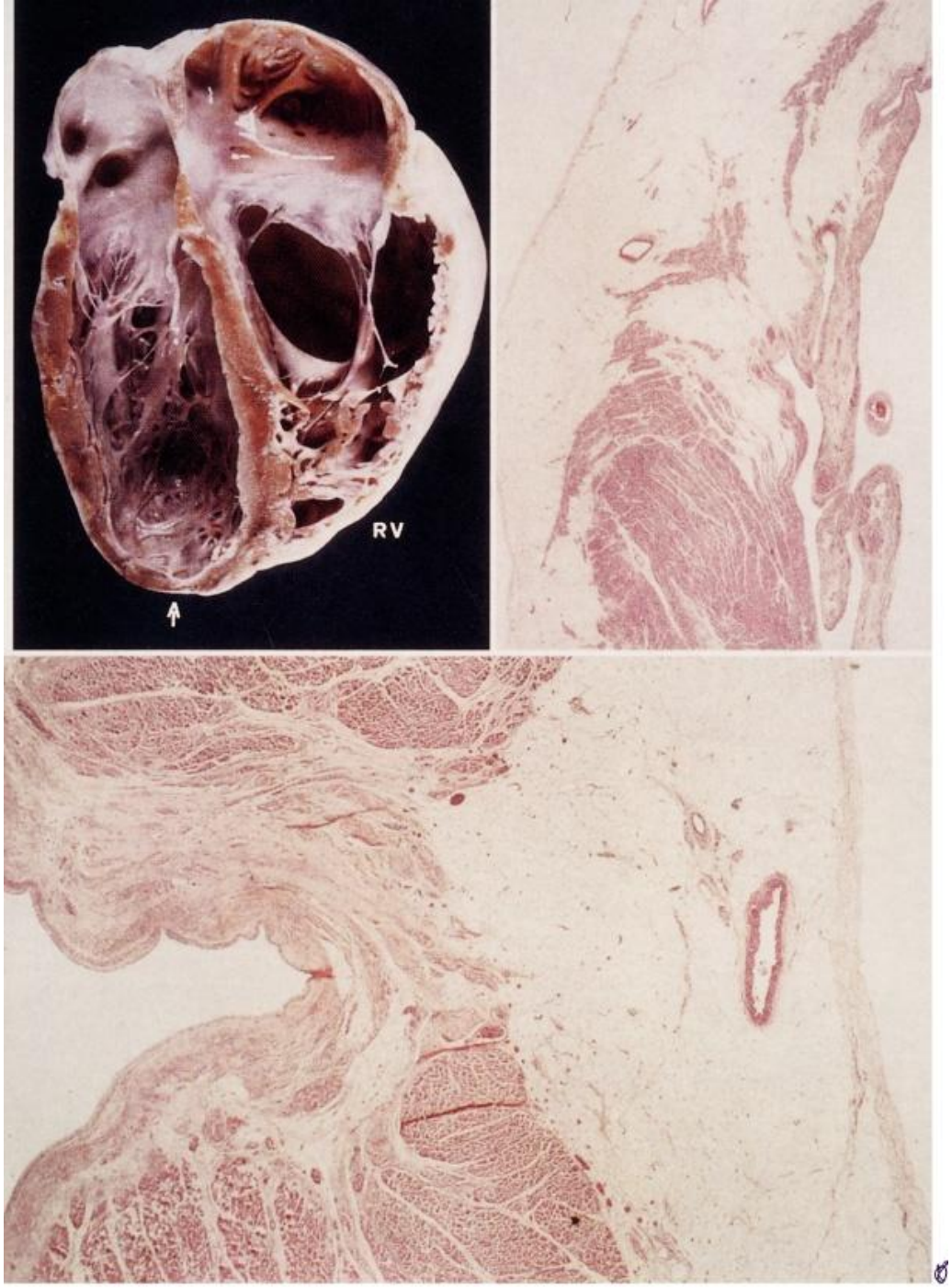
Pathology: fibrofatty replacement

ARVCM



ARVCM





Top left, Postmortem pathological section of heart (four-chamber) in a patient with arrhythmogenic right ventricular cardiomyopathy and biventricular involvement. Severe widespread fatty infiltration of right ventricular (RV) wall is present; an apical aneurysm is present at the left ventricular level (arrow). **Top right,** Histological section at level of RV inflow (hematoxylin and eosin, ×2.5). Severe transmural fibrofatty infiltration of RV wall is present, compatible with RV dysplasia. **Bottom,** Histological section at the level of the left ventricle (outflow) (hematoxylin and eosin, ×2.5) shows focal severe fibrofatty infiltration with myocellular atrophy, compatible with left ventricular involvement.

ARVC/D - Causes

- Mutations in plakophilin 2 (up to 25% of all patients affected by ARVC/D)
- Plakoglobin
- Desmoplakin
- Ryanodin Receptor
- TGF beta

Table. Summary of Desmosomal Genes and Encoded Proteins Implicated in Inherited Human Disease

Gene Superfamily	Gene	Chromosomal Location	Encoded Protein	Mutation-Associated Phenotypes
Armadillo	*JUP*	17q21	Plakoglobin	Naxos disease (AR arrhythmogenic cardiomyopathy with PPK and woolly hair)
				AD arrhythmogenic cardiomyopathy
	PKP1	1q32	Plakophilin-1	Ectodermal dysplasia and skin fragility syndrome
	PKP2	12p11	Plakophilin-2	AD arrhythmogenic cardiomyopathy
				AR arrhythmogenic cardiomyopathy
Desmosomal cadherin	*DSG1*	18q12	Desmoglein-1	Striate PPK
	DSG2	18q12	Desmoglein-2	AD arrhythmogenic cardiomyopathy
				? AR arrhythmogenic cardiomyopathy
	DSG4	18q12	Desmoglein-4	Inherited hypotrichosis
Desmosomal cadherin	*DSC2*	18q12	Desmocollin	Arrhythmogenic cardiomyopathy
				Arrhythmogenic cardiomyopathy with palmoplantar keratoderma and woolly hair
Plakin	*DSP*	6p24	Desmoplakin	Striate PPK
				Keratoderma, keratin retraction, skin fragility and woolly hair/alopecia
				AD arrhythmogenic cardiomyopathy
				Carvajal syndrome
				Lethal acantholytic epidermolysis bullosa

AD indicates autosomal dominant; AR, autosomal recessive.

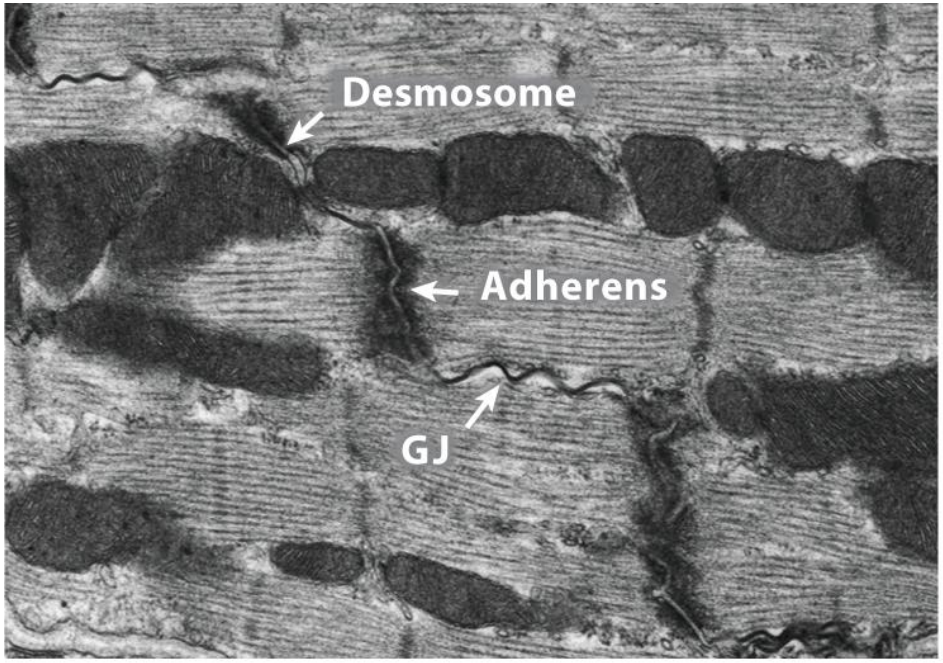
Chromosomal loci and disease-causing genes in arrhythmogenic right ventricular cardiomyopathy (ARVC)

Designation (pattern of inheritance)	Chromosomal locus	Gene mutation
ARVC1 (AD)[a]	14q23–q24	*TGFβ3*
ARVC2 (AD)	1q42–q43	*RyR2*
ARVC3 (AD)	14q12–q22	?
ARVC4 (AD)	2q32.1–q32.3	?
ARVC5 (AD)	3p23	*TMEM43*
ARVC6 (AD)	10p12–p14	?
ARVC7 (AD)	10q22	?
Naxos disease (AR)	17q21	*JUP*
ARVC8 (AD)	6p24	*DSP*
ARVC9 (AD)	12p11	*PKP2*
ARVC10 (AD)	18q12.1	*DSG2*
ARVC11 (AD)	18q12.1	*DSC2*
ARVC12 (AD)	17q21	*JUP*

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; DSC2, desmocollin 2; DSG2, desmoglein 2; DSP, desmoplakin; JUP, plakoglobin; PKP2, plakophilin 2; RyR2, cardiac ryanodine receptor; TGFβ3, transforming growth factor β3; TMEM43, transmembrane 43.

Saffitz, Annual Reviews of Pathology 2011

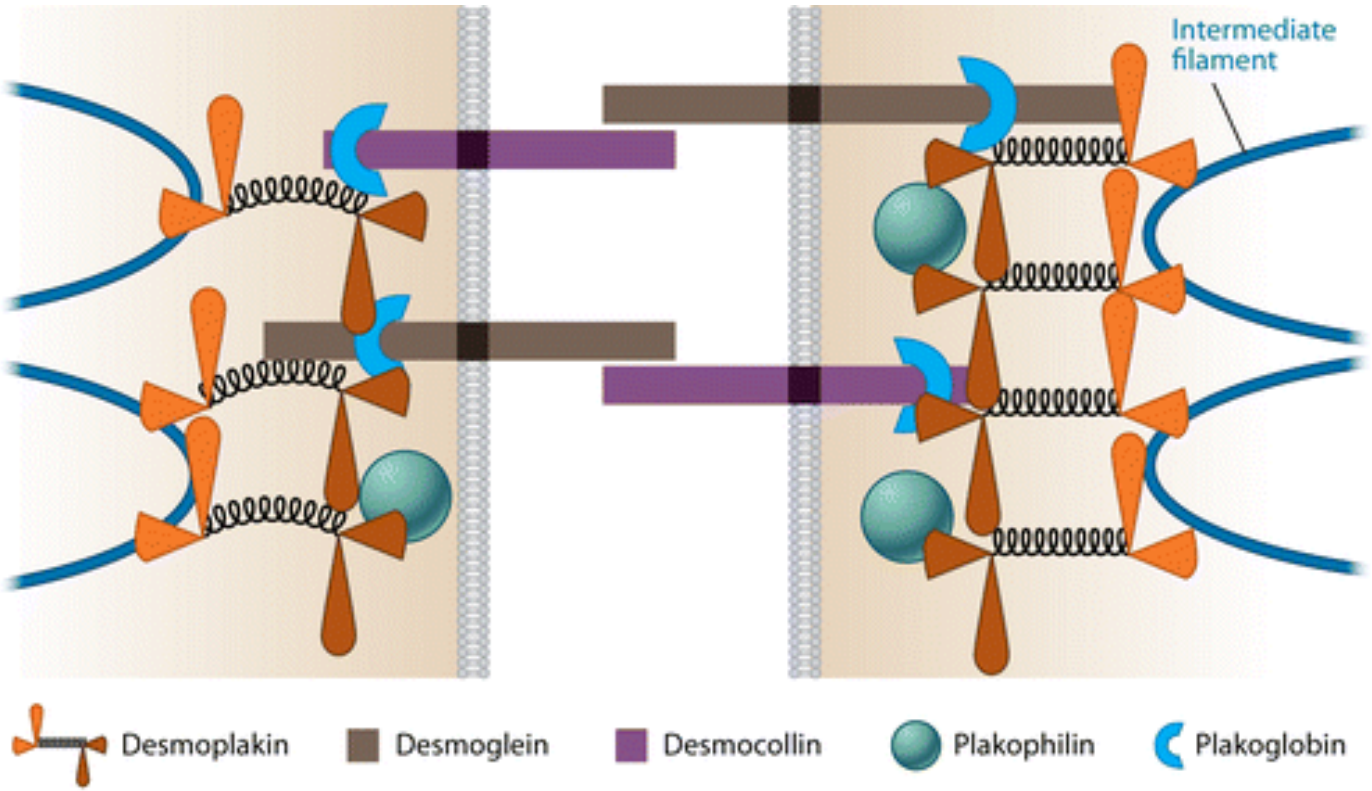
Desmosomes (ARVC/D)



An electron micrograph of an intercalated disc connecting two ventricular myocytes. This large cell-cell junction complex contains the two major types of adhesion junctions (desmosomes and adherens junctions), as well as electrical (gap) junctions (GJ).

Saffitz JE. 2011.
Annu. Rev. Pathol. Mech. Dis. 6:299–321

Desmosomes (ARVC/D)



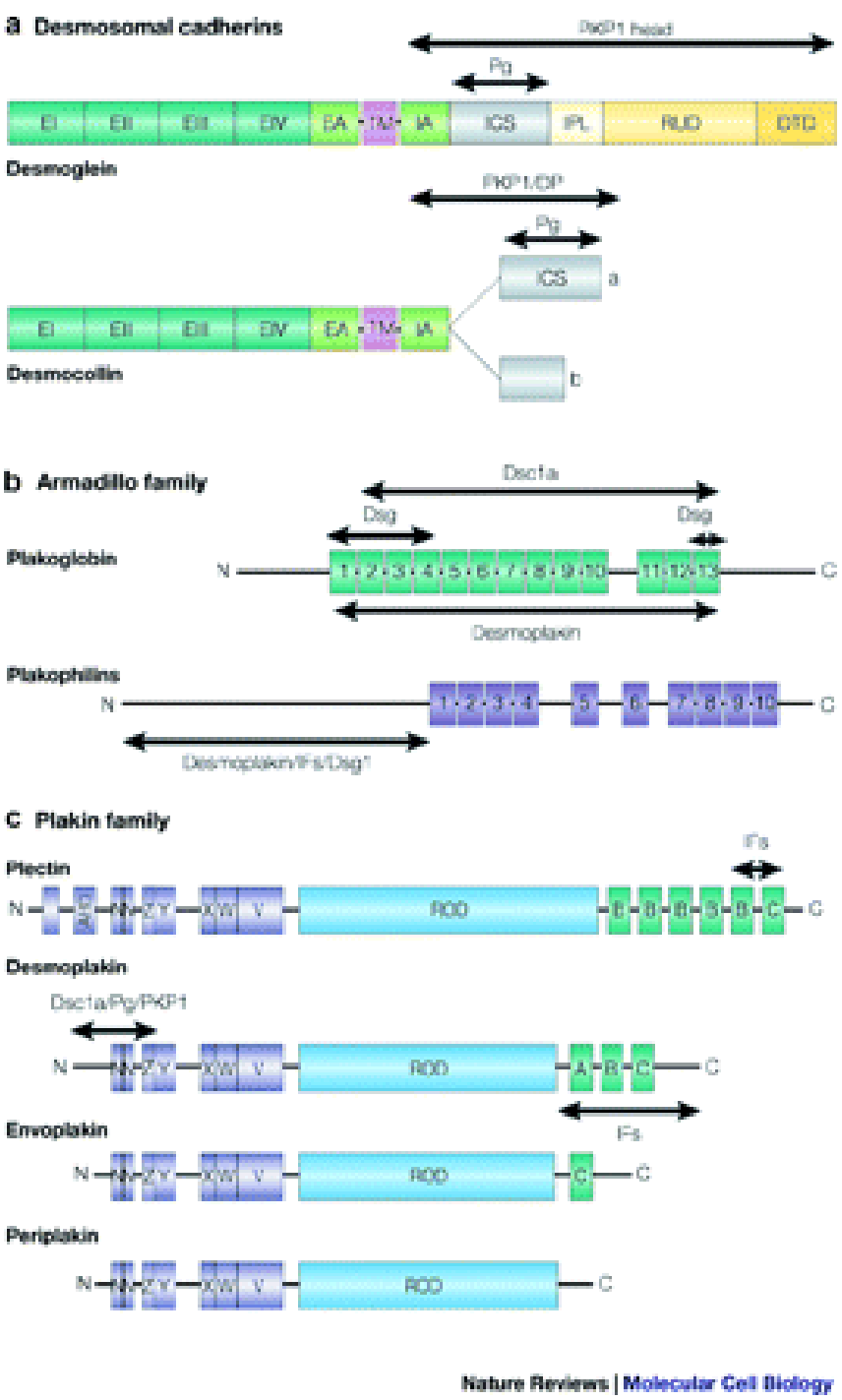
The molecular composition of the desmosome showing the three major types of molecules:

1. desmosomal cadherins (desmogleins and desmocollins),
2. intracellular linker proteins (desmoplakin, plakoglobin, and plakophilin), and
3. intermediate filaments (desmin)

ARVC/D

- A disease of the desmosome ?

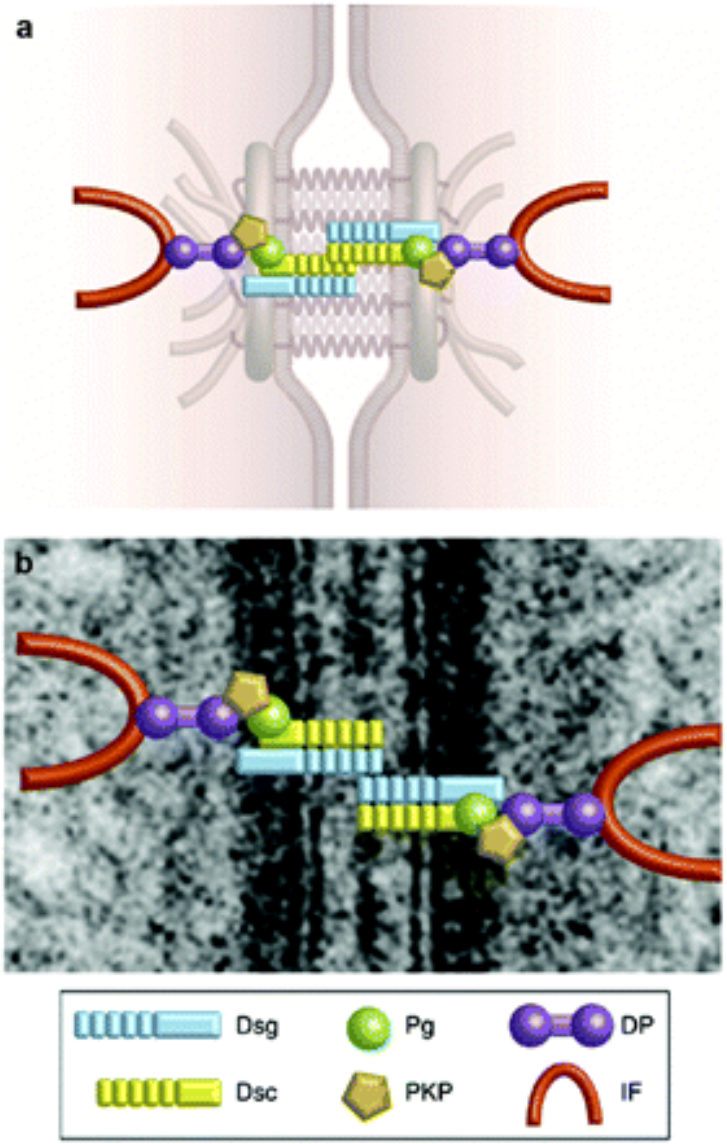
  (Desmosomes, together with gap junctions (exchange of ions) and adherens junctions are important for cell – cell interactions) – not only in the myocardium, but also in a variety of different other tissues and organs such as the skin.



Nature Reviews | Molecular Cell Biology

Schematic structure of principal desmosomal proteins

ARVC/D



Please note: the intermediate filaments (IF) are linked to the desmosomes (such as desmin). As such it is no surprise to find desmin mutations associated with ARVC/D.

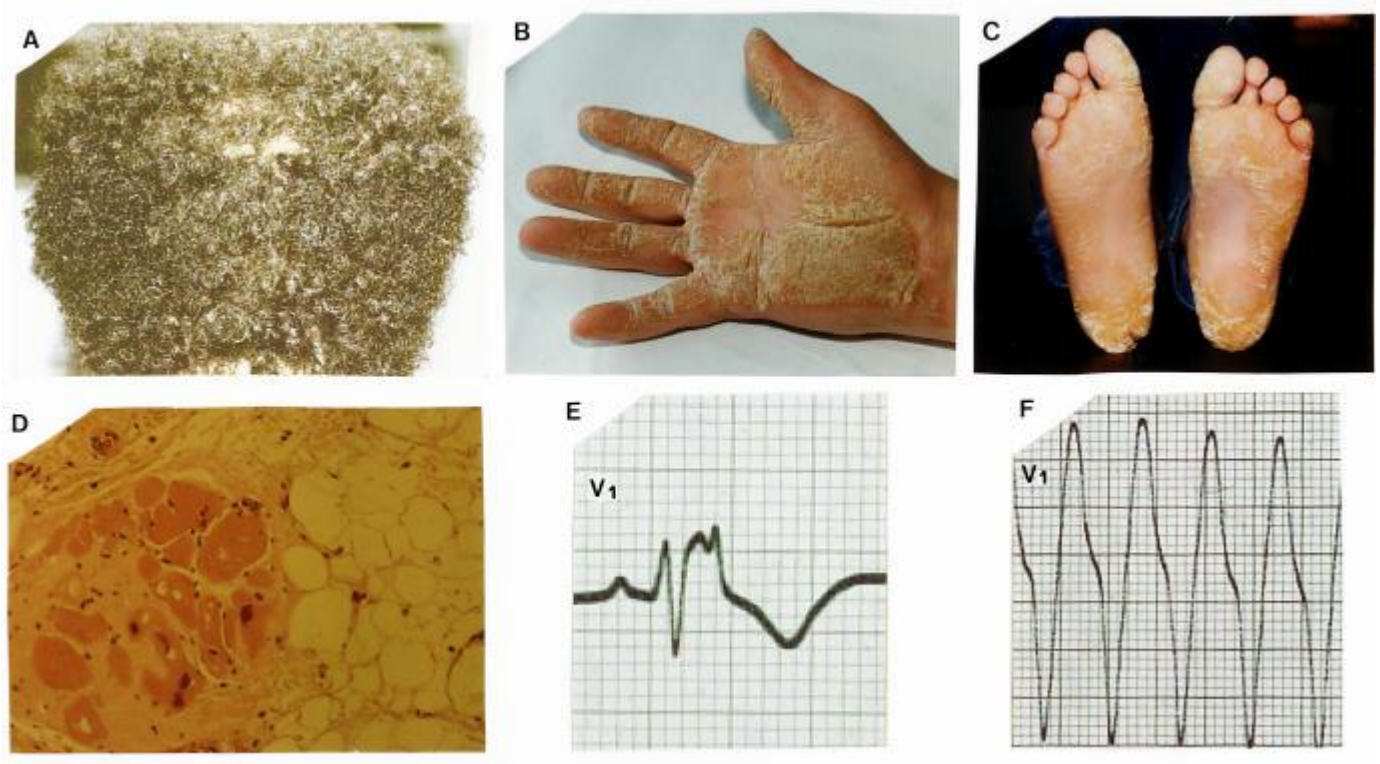
Naxos Disease

- Since 1995, according to the World Health Organisation's classification of cardiomyopathies, Naxos disease has been considered as the recessive form of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC/D). It is a stereotype association of ARVC/D with a cutaneous phenotype, characterised by woolly hair and palmoplantar keratoderma.

Epidemiology

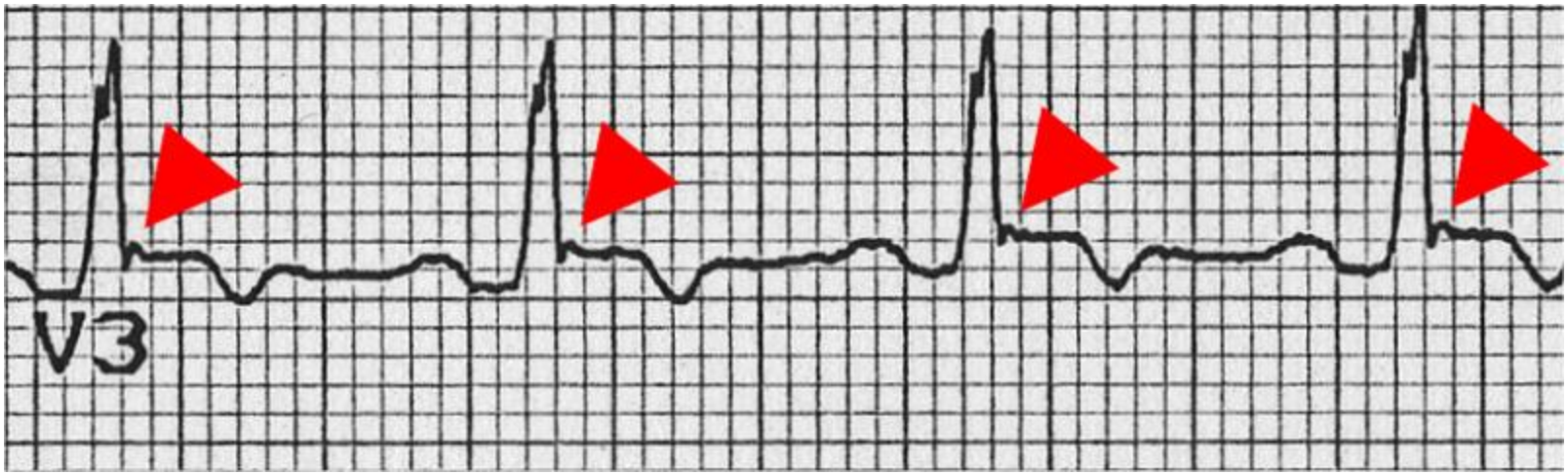
- Naxos disease was first reported in 1986 by Protonotarios et al in patients originating from the Hellenic island of Naxos. Apart from Naxos, cases have also been reported from other Hellenic islands, as well as from Turkey, Israel and Saudi Arabia. The prevalence of the disease in Hellenic islands reaches 1:1000. A variety of Naxos disease presenting at a younger age with more pronounced left ventricular involvement has been described in families from India and Ecuador (**Carvajal syndrome**).

Naxos Disease



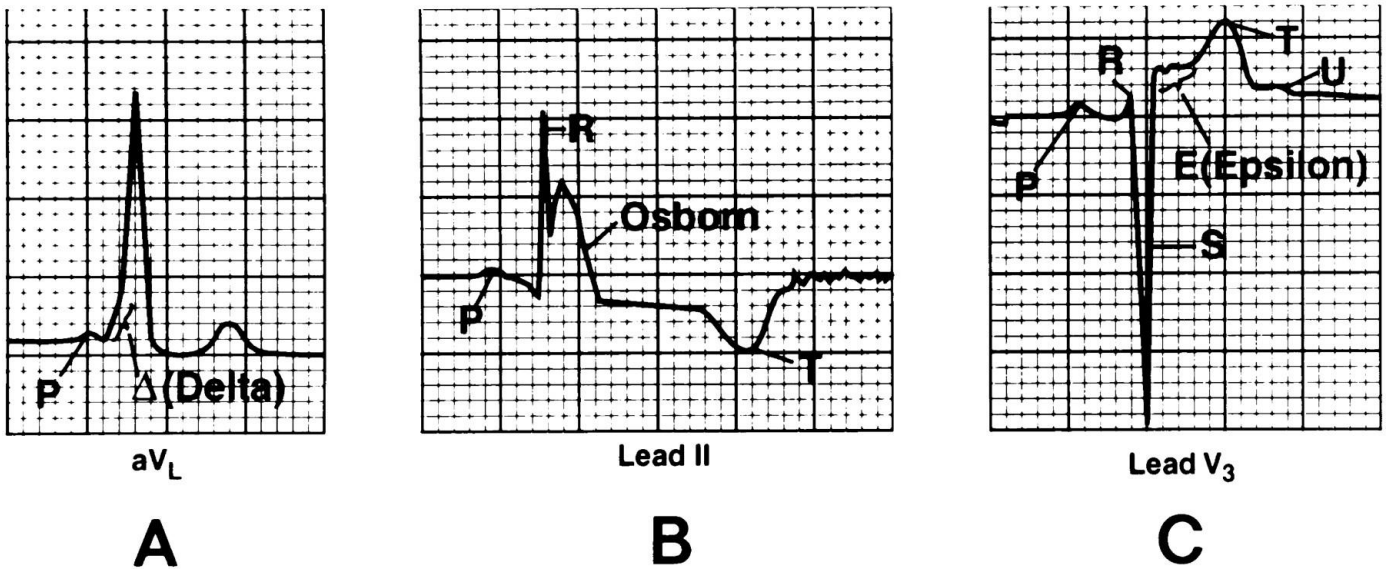
Naxos disease phenotype of the skin and heart. Woolly hair (**A**) and palmoplantar keratoderma (**B** and **C**) are the principal cutaneous abnormalities. The disease is expressed in the heart as arrhythmogenic right ventricular dysplasia. Myocardial loss and fibrofatty replacement of right ventricular myocardium (**D**) results in delayed activation (epsilon waves on surface ECG) (**E**) predisposing to reentrant ventricular arrhythmias (**F**).

Epsilon Wave



The **epsilon wave** is found in about 50% of those with ARVD. This is described as a terminal notch in the QRS complex. It is due to slowed intraventricular conduction. The epsilon wave may be seen on a surface EKG; however, it is more commonly seen on signal averaged EKGs.

A, Delta wave, named by Segers, Lequime, and Denolin (see text and Reference 17)



Hurst, J. W. Circulation 1998;98:1937-1942

Figure 4. A, Delta wave, named by Segers, Lequime, and Denolin (see text and Reference 17). It is caused by preexcitation of the ventricles via a congenital bypass tract. Adapted from Hurst JW, Myerburg RJ. *Introduction to Electrocardiography.* 2nd ed. 1973:185. B, The term "Osborn wave" designates the spike-and-dome shape of the QRS complex. The QT interval is prolonged. The abnormal deflection is commonly found in patients during extreme hypothermia. From Trevino A, Razi B, Beller BM. The characteristic ECG of accidental hypothermia. *Arch Intern Med* 1971;127:472. (Reprinted with permission.) C, The **epsilon wave** is common in patients with arrhythmogenic right ventricular dysplasia and is also seen in other diseases of the right ventricle. This figure was sent to the author by Dr Guy Fontaine; the recording is from a 27-year-old man who had episodes of palpitation.

Circulation

Mutations in the plakophilin 2 gene ARVC/D

Table 1 Clinical status and *PKP2* mutations of 32 index individuals with ARVC

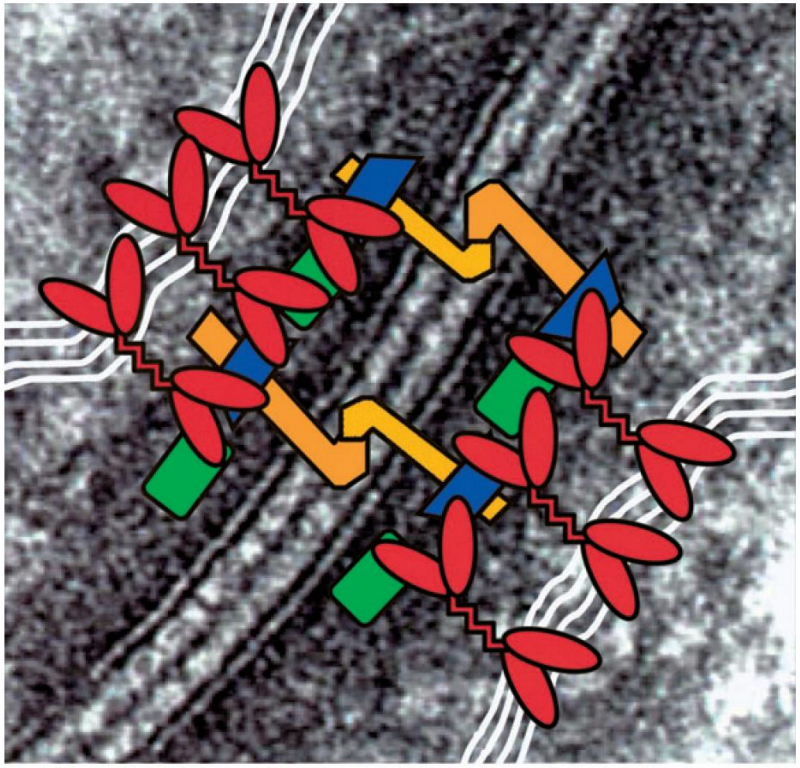
Individual	Sex	Clinical status	Exon	Nucleotide change	Amino acid change
1	M	VT, RV, LV, H	1	145_148delCAGA	S50fsX110
EPF17	F	RV, H	1	216insG	Q74fsX85
3	M	VT, RV, H	2	235C→T	R79X
4	M	VT, RV	2	235C→T	R79X
5	M	S, CA, VT, RV	2	235C→T	R79X
6	M	CA, VT, RV, LV	2	235C→T	R79X
7	M	VT, RV	2	235C→T	R79X
8	M	VT, RV	2	235C→T	R79X
9	F	VT, RV	3	419C→T	S140F
10	M	VT, RV	3	534_535insCT	C179fsX190
11	M	VT, RV, LV	5	1369_1372delCAAA	Q457X
12	M	VT, RV	7	1631_1632insTT	L544fsX563
13	M	S, VT, RV, LV	7	1642delG	V548fsX562
14	M	S, VT, RV, LV	9	1844C→T	S615F
15	M	S, VT, RV	9	1912C→T	Q638X
16	M	S, VT, RV, LV, H	9	1948delA	V650fsX655
17	M	VT, RV, LV, H, HTX	9	1951C→T	R651X
18	M	S, VT, RV	9	1960A→C	K654Q
A100/161	M	VT, RV, H	10	2076_2077delAA	C693fsX741
20	M	S, RV	10	2088insA	K696fsX742
21	M	S, VT, RV	10	2095C→T	Q699X
22	M	CA, VT, RV, H	11	2146–1G→C	Mutant splice product*
23	M	S, VT, RV, LV	11	2146–1G→C	Mutant splice product*
24	M	VT, RV	11	2176C→T	Q726X
25	F	VT, RV, LV, H	11	2203C→T	R735X
26	M	S, VT, RV	11	2203C→T	R735X
27	M	VT, RV	12	2386T→C	C796R
28	F	VT, RV	12	2393_2401delCATTGAACA	N798fsX879
29	M	S, SCD, VT, RV, LV, H	12	2424insA	E809fsX826
30	F	VT, RV	12	2489+1G→A	Mutant splice products*
31	M	S, VT, RV, LV	13	2490–1G→C	Mutant splice products*
32	M	VT, RV, LV	13	2509delA	V837fsX930

M, male; F, female; RV, right ventricular involvement; LV, left ventricular involvement; H, positive family history; S, syncope; CA, cardiac arrest; HTX, heart transplantation. *See **Supplementary Figure 2** online. Numbering of the nucleotides starts at the ATG codon of *PKP2*.

ARVC – genetic causes

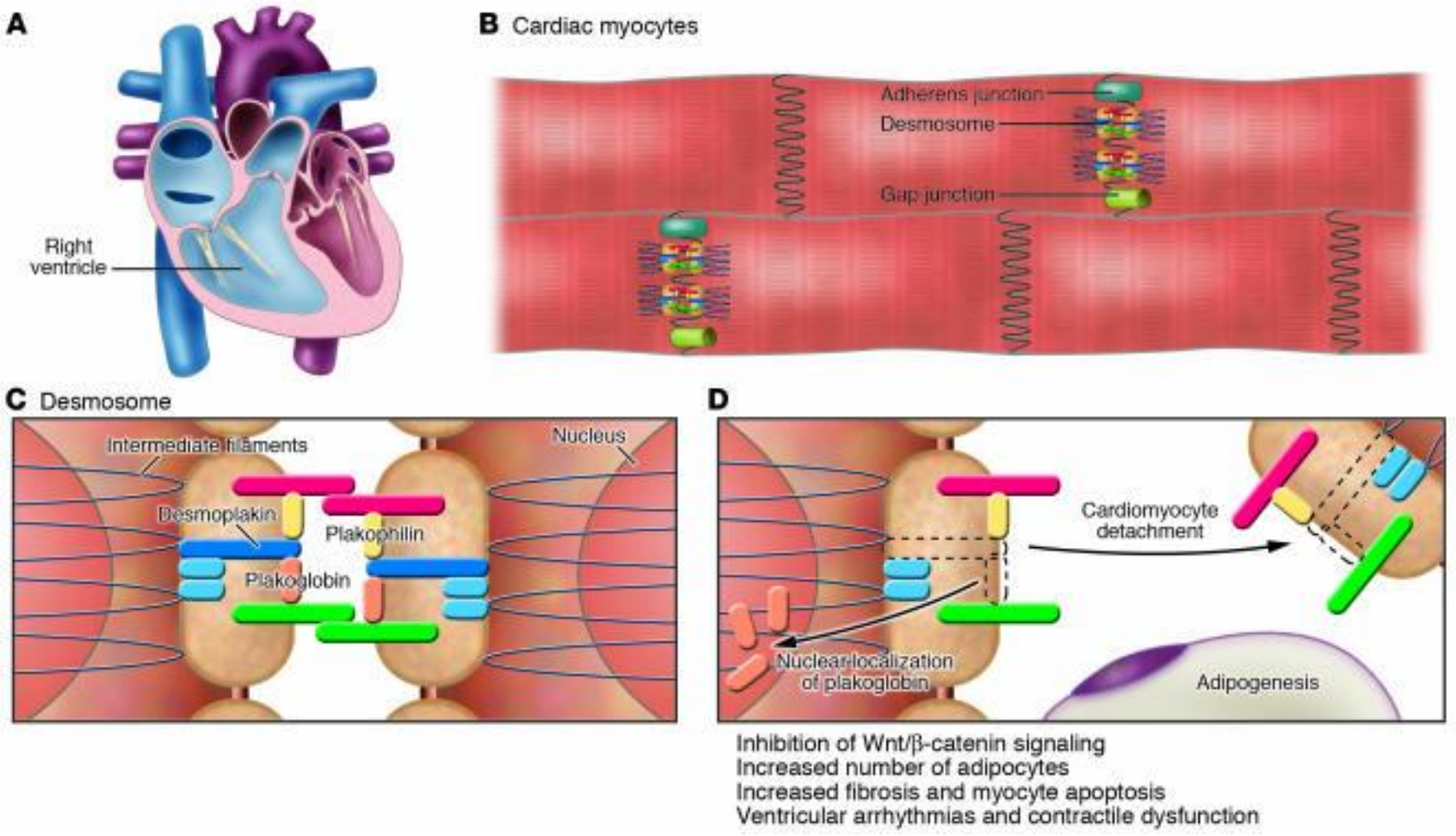
Identified genes / mutations:

- Desmoplakin (*DSM* 6p24)
- Plakoglobin (*JUP* 17q21)
- Plakophilin-2 (*PKP2* 12p11)
- Desmoglein-2 (*DSG2*)
- Ryanodin-Receptor (*RyR2* 1q42)
- TGFβ3 (*TGFβ3* 14q24.3)



Aus: J. A. McGrath, *Australasian Journal of Dermatology* 46 (2005)

Desmosomes (ARVC/D)



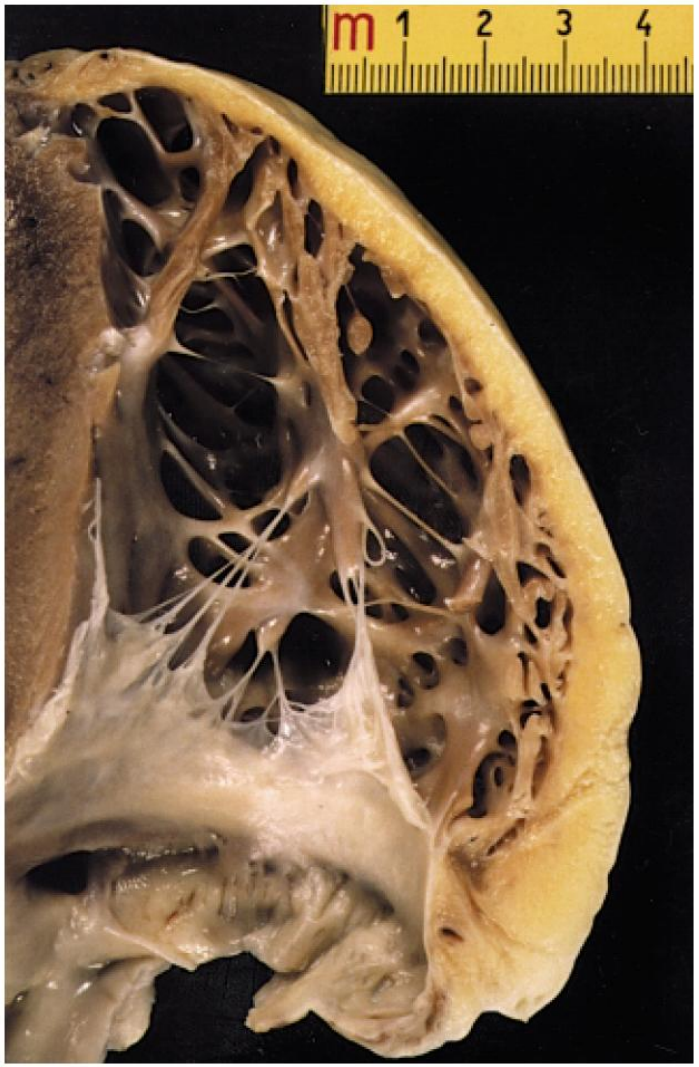
ARVC – experimental proof

- So how do we proof, that a single mutation is indeed disease causing?

- Linkage analysis

- Introduce the mutant gene into an experimental animal (i. E. „Koch trias“)

- Use of stem cells and create cell types useful for analysis.



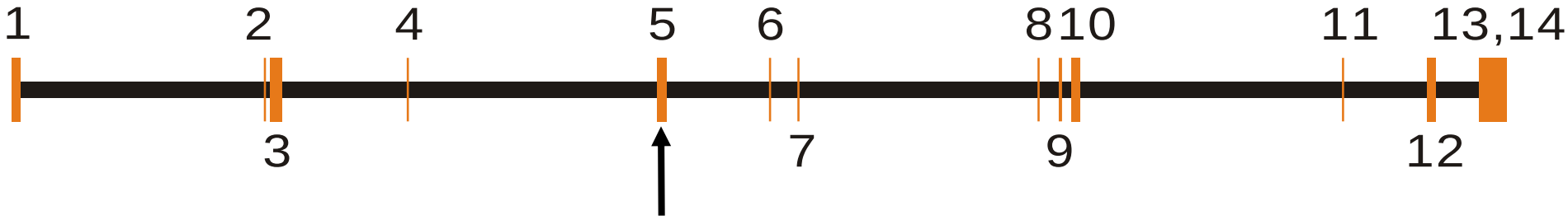
Aus: G. Thiene, *Herz* 25 (2000)

Index - patient: characteristics

63 year old patient :

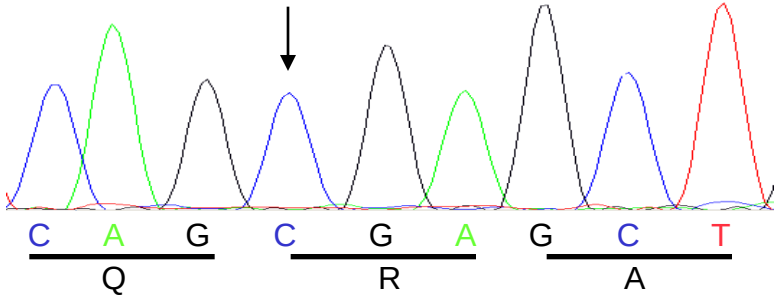
- Reason for hospitalization: new CD-shock during VT
- Since the age of 16 years collaps after sport
- In 1974 documented VT and hypotonus
- In 1994 electrophysiological analysis of the son -> LQT-syndrome ?
- On Dec. 19, 1995 ICD-Implantation (V.a. LQT)
- In 1996 following RV-Angiographie - ARVC ?

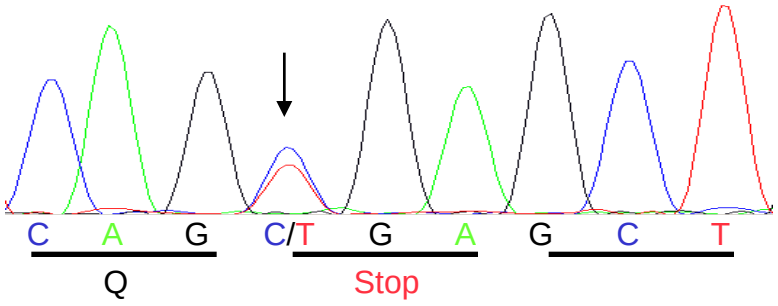
Genetic Analysis



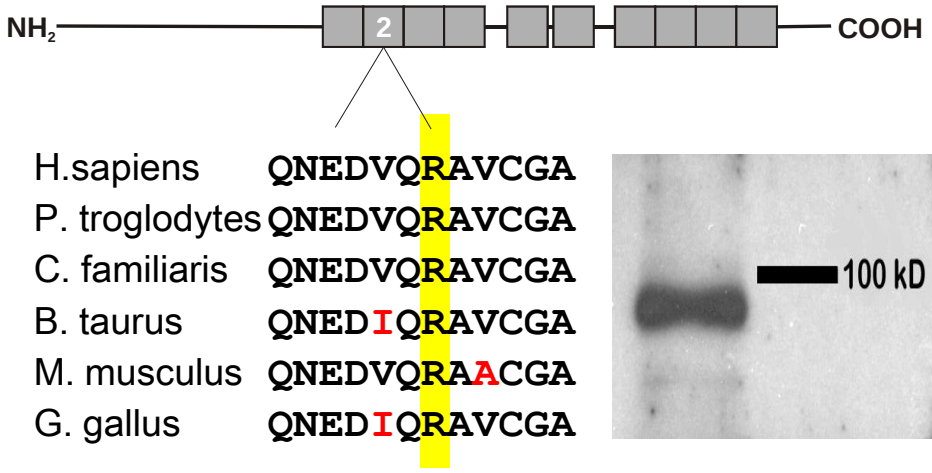
Plakophilin 2

1237C>T





R413



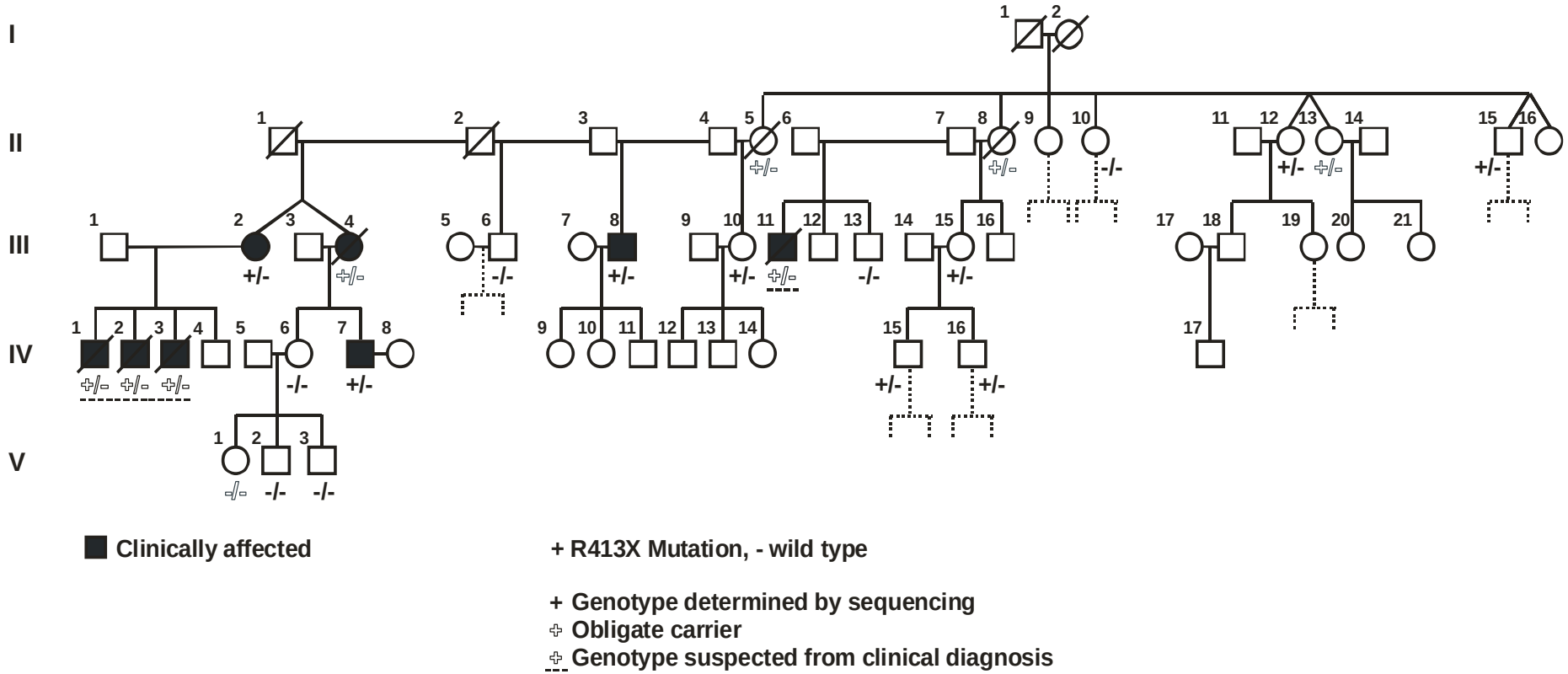
In 62 exons incl. all splice sites of the following genes

KCNQ1, HERG, SCN5A, KCNE1, KCNE2

(>85% of all known LQT mutations)

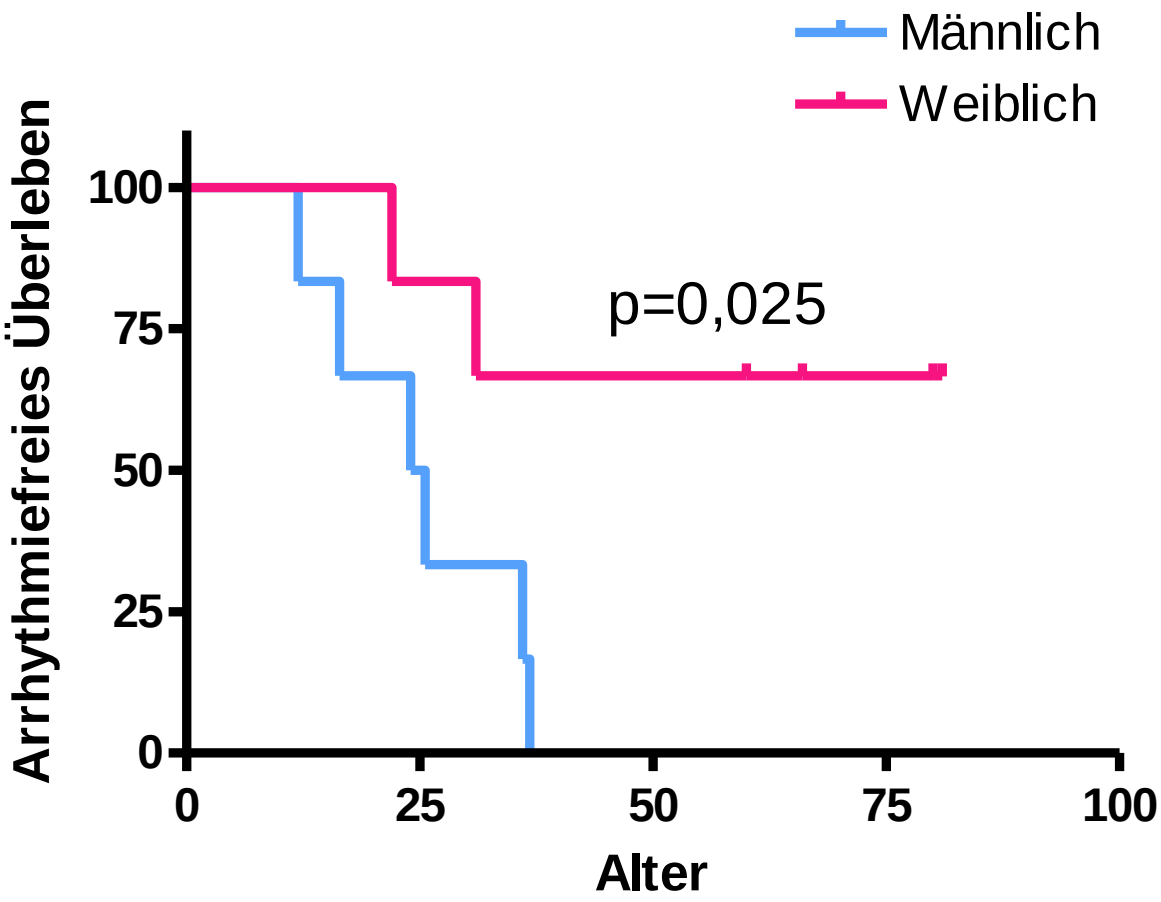
No mutation

Family analysis



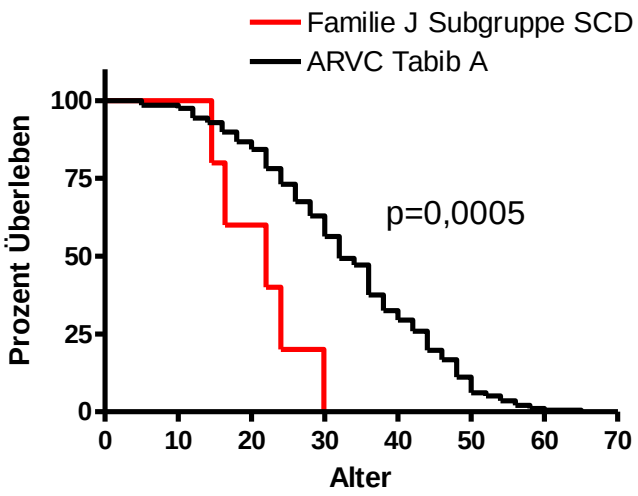
- Identification of the mutation in 8 additional family members
- 2 of them are clinically affected
- 6 individuals are clinically unaffected

Characteristics of patients

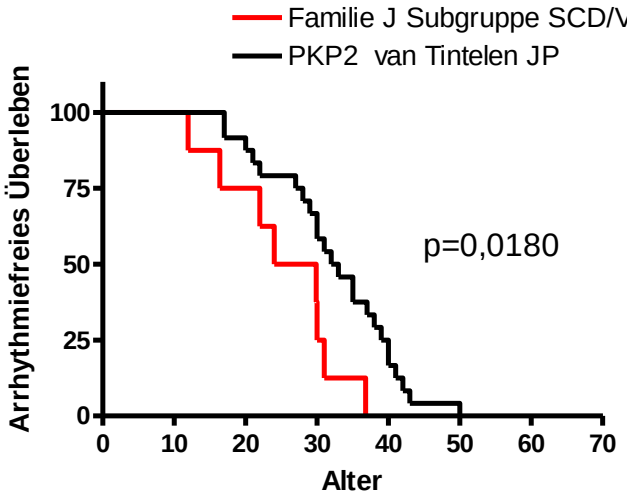


Characteristics of patients

- In comparison to the phenotypes observed in other ARVC patients (with / without PKP2-mutations) we observe with our PKP2 mutation a much more severe phenotype together with early onset of arrhythmias and SCD



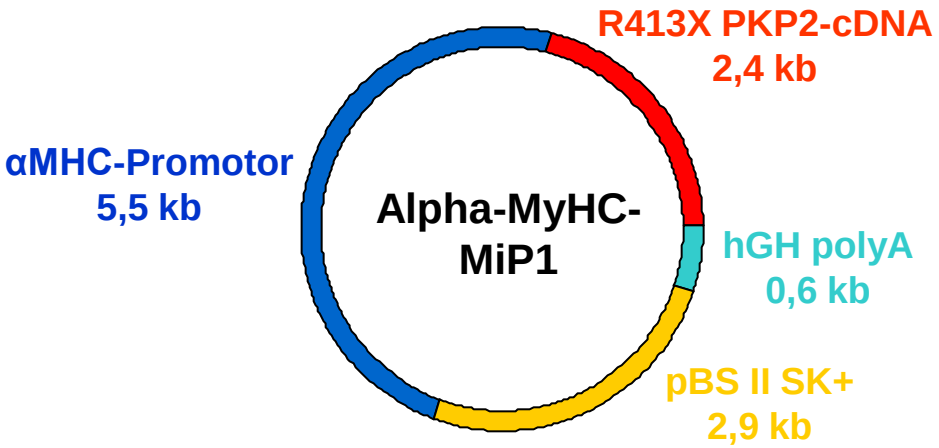
A Tabib, *Circulation* 108 (2003)

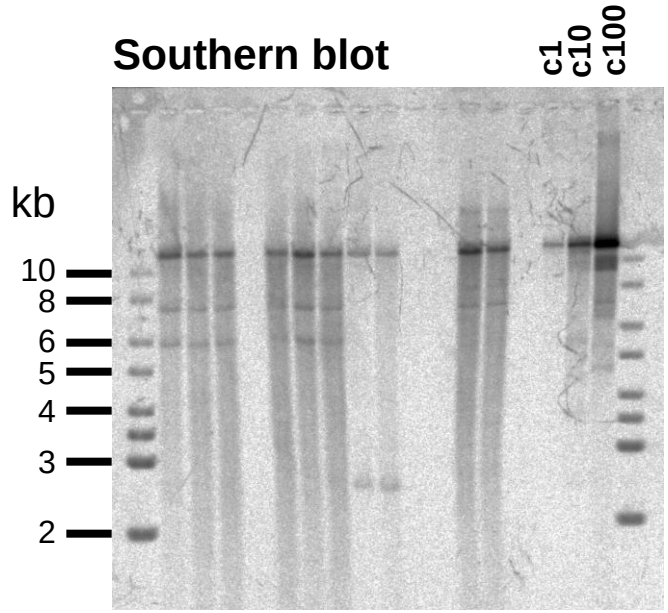


JP van Tintelen, *Circulation* 113 (2006)

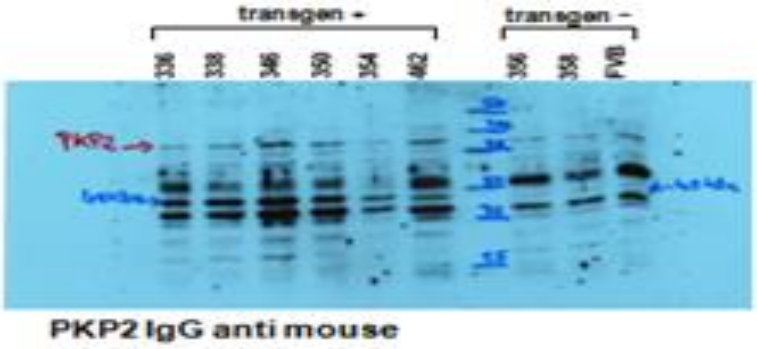
Transgenic overexpression of PKP2 R413X mutant

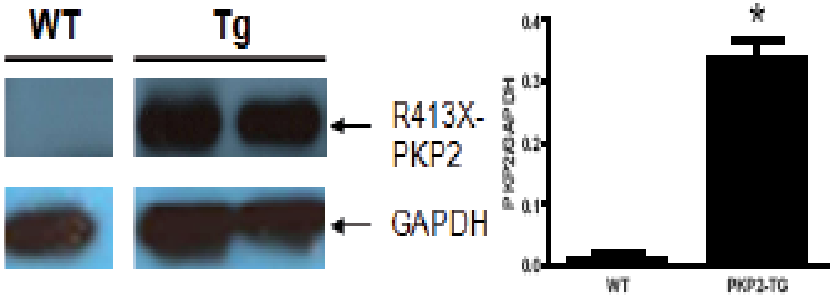
The cDNA encoding R413X was overexpressed under the control of the αMHC promotor

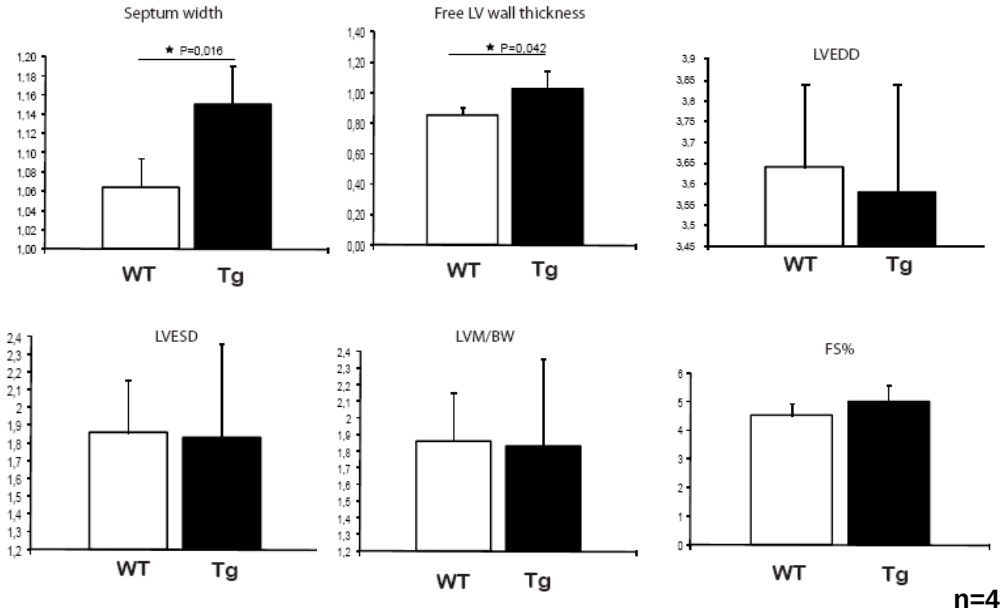




Transgenic overexpression of PKP2 R413X mutant

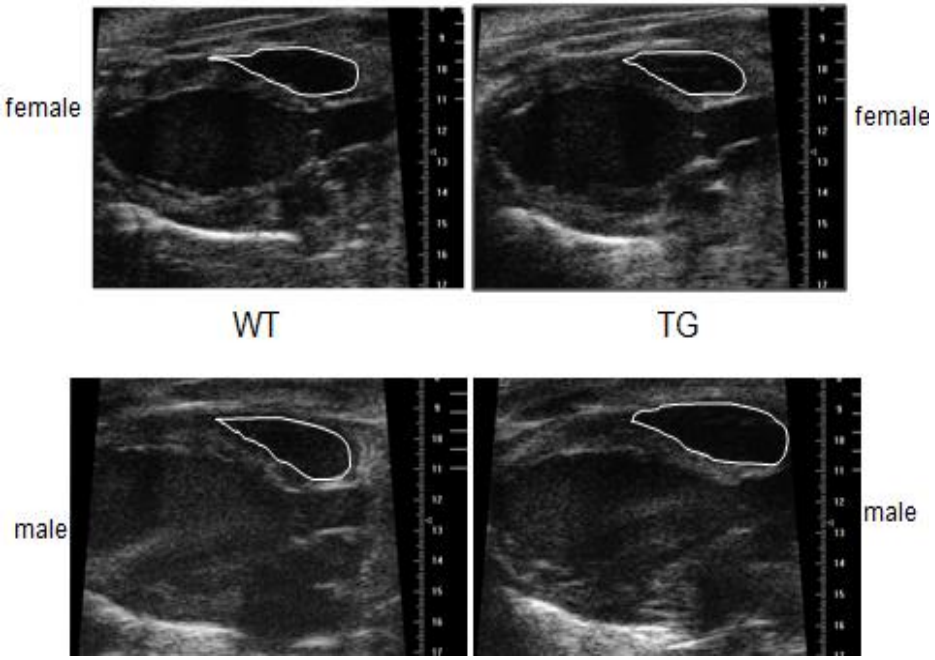


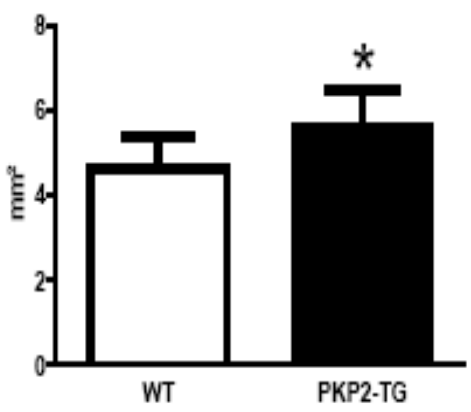


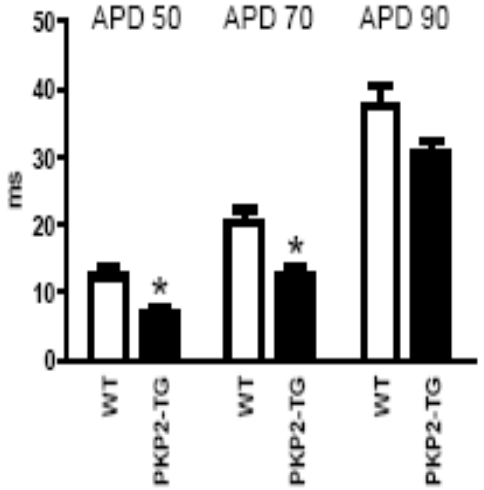


n=4

Transgenic overexpression of PKP2 R413X mutant

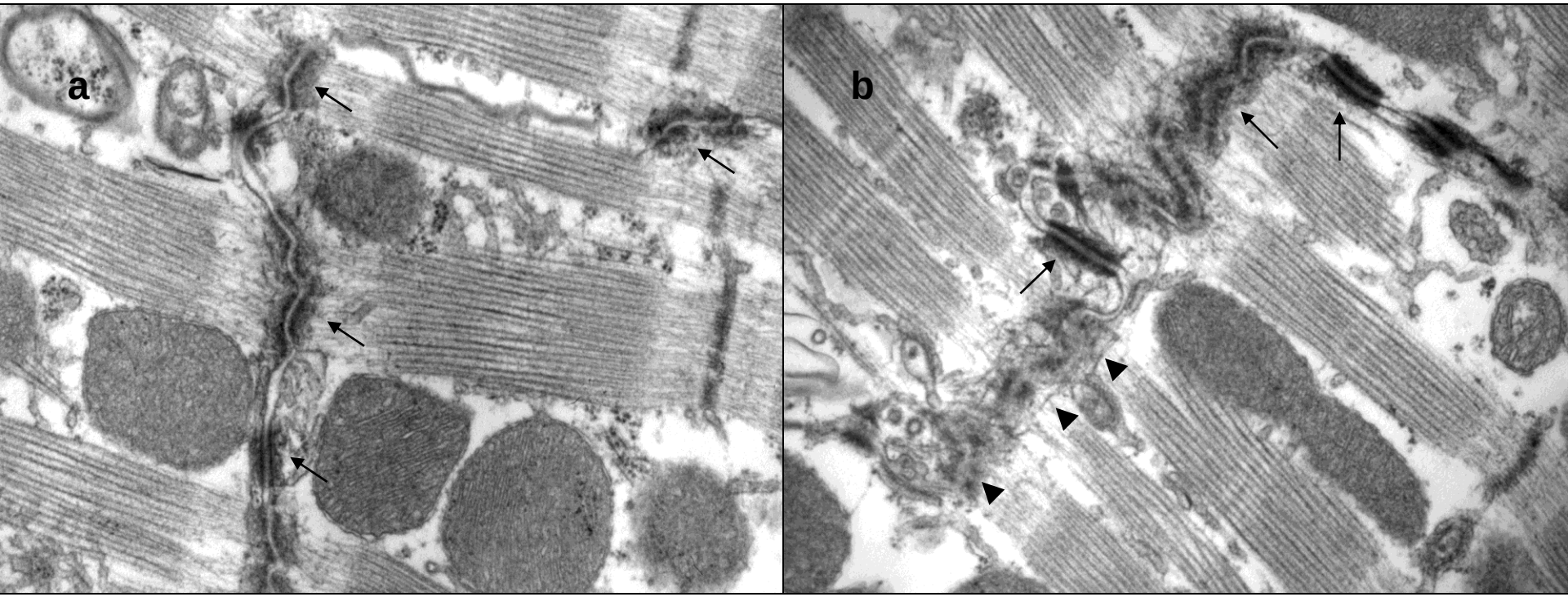






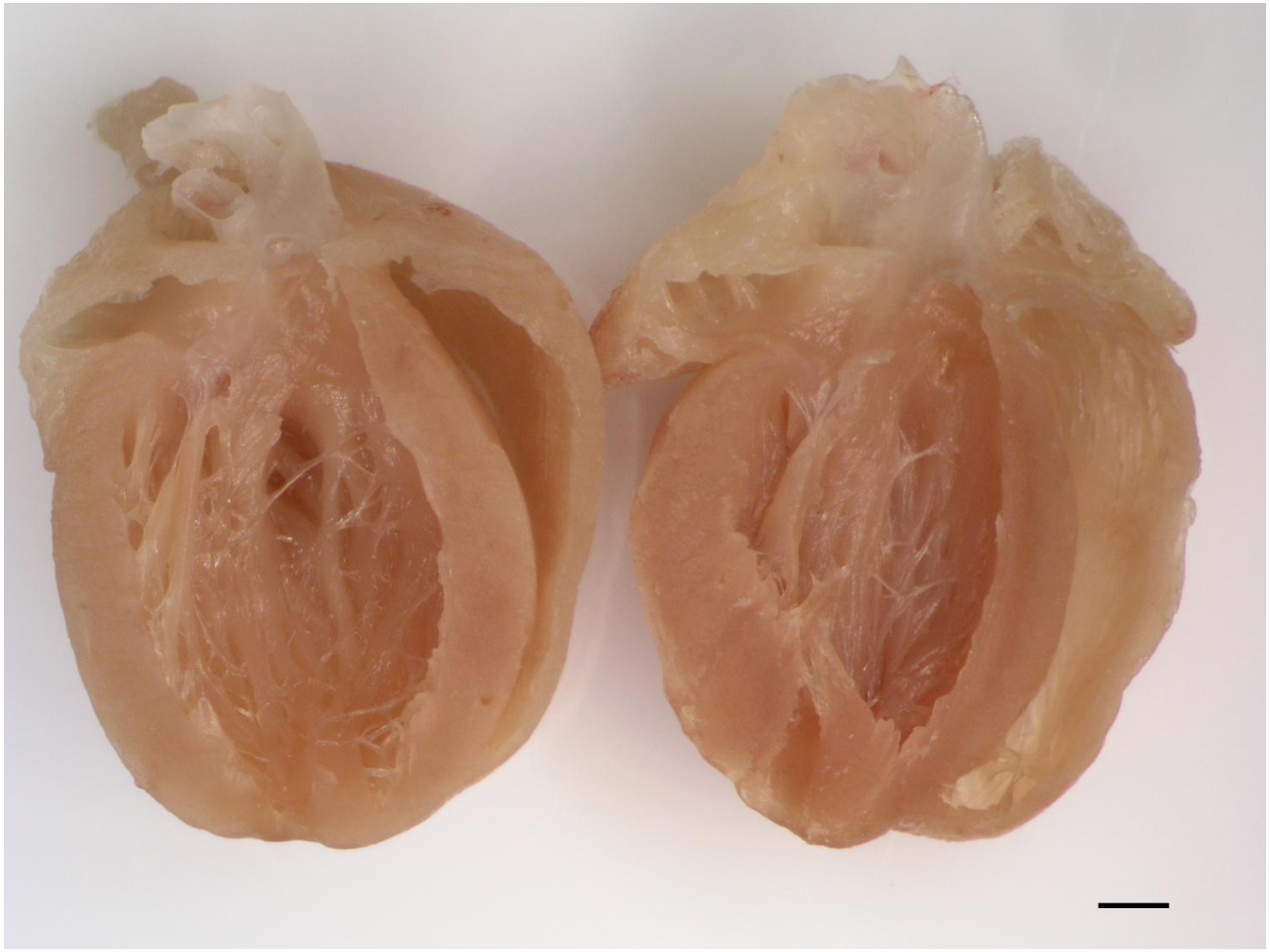
- Vergrößerung des rechten Ventrikels bei Männchen Transgenen Mäusen.
- (R413X)PKP2-Überexpressions-Mäuse haben eine signifikante Verkürzung Aktionspotentialsdauer des Herzens bei eine Reizspannung von 50 und 70 mV
  ➔ ventrikuläre Arrhythmie

Transgenic overexpression of PKP2 R413X mutant – electron microscopy



Electron microscopy of mouse heart muscles: Desmosomes (arrows) that connect the myocytes in the heart muscle to a syncytium appear loose in structure and fragmentated at the connections of myofibrillar bundles in transgenic animals (arrowheads, b) in comparison to controls (a). Magnification: x 40,000

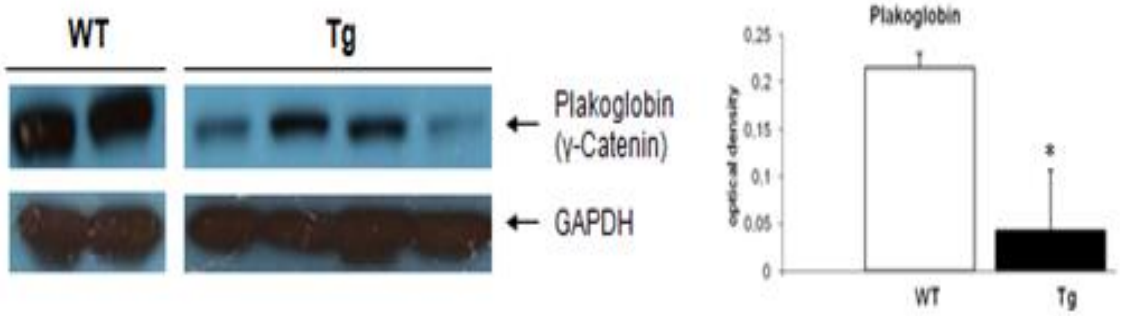
Transgenic overexpression of PKP2 R413X mutant – morpholoy



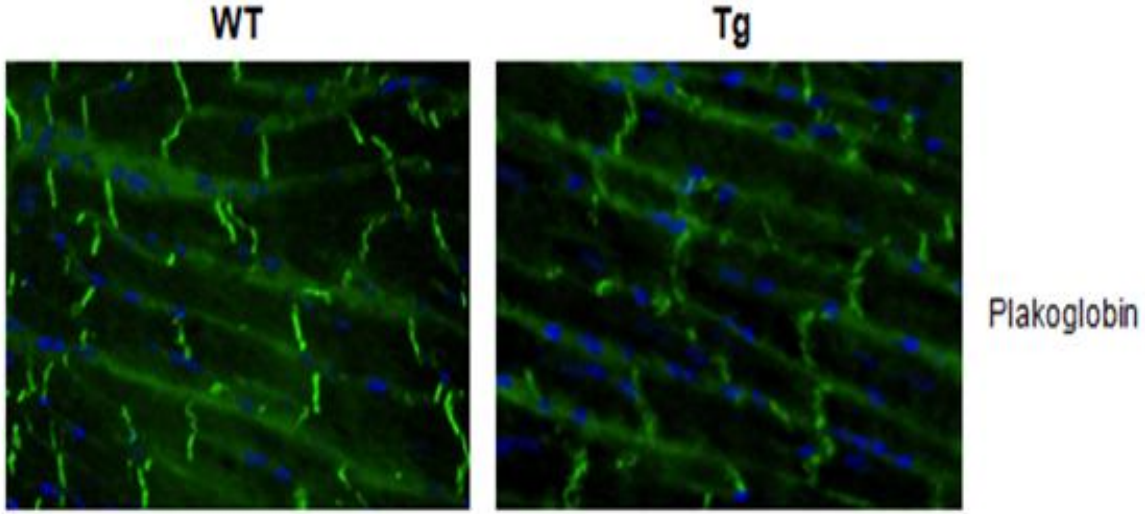
Axial sections through the heart of mice: In comparison to the control (left side) the transgenic animal shows a thinning of the right ventricle wall. (Bar = 1 mm)

Transgenic overexpression of PKP2 R413X mutant

- Mehrere Publikationen berichten über die Herunterregulierung von Plakoglobin (λ-Catenin) als Indiz für die molekulare Pathologie von ARVC.



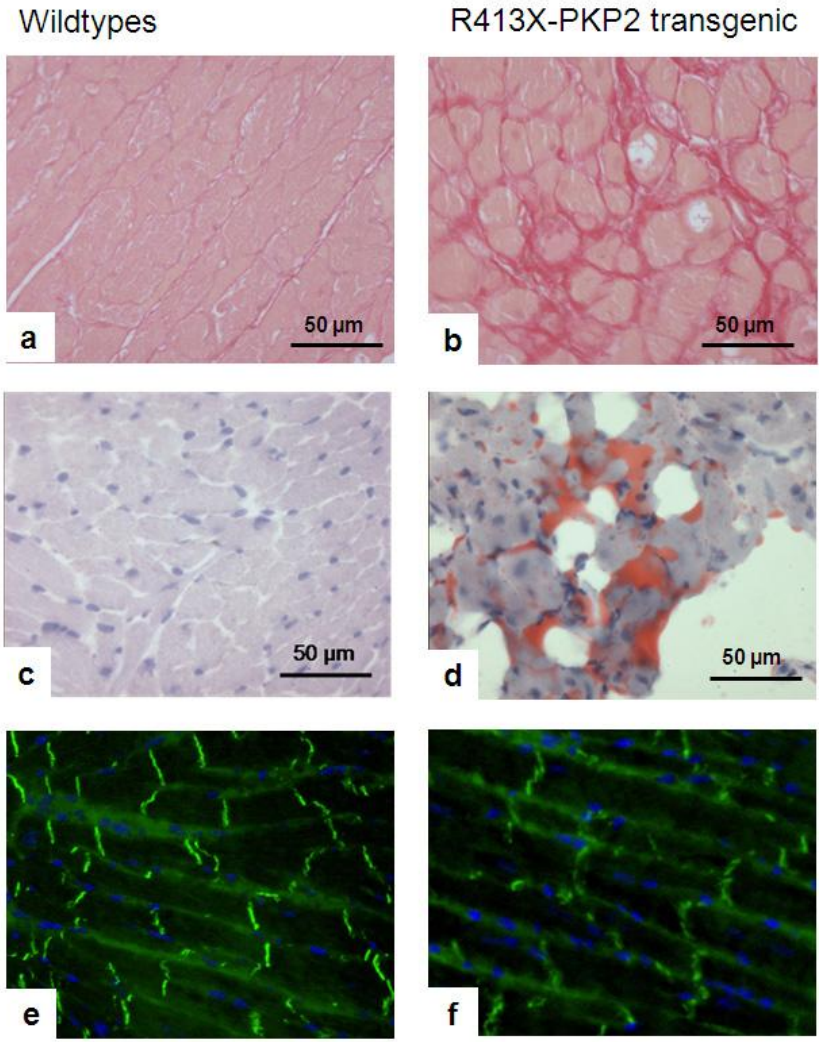
Western-Blot

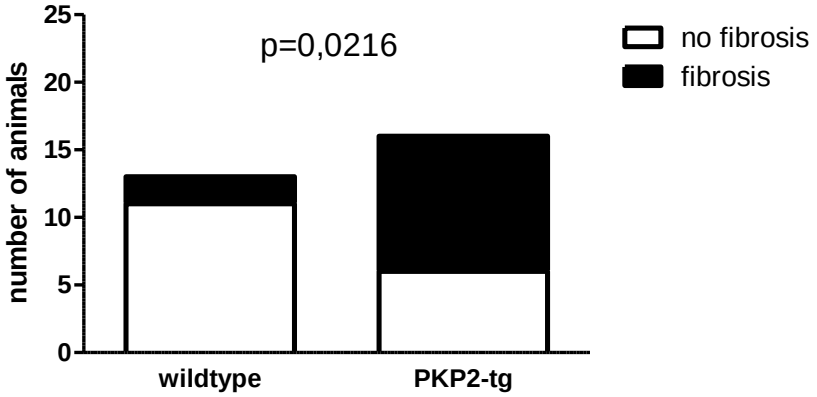


Immunohistologie

- Die signifikante Abnahme der Plakoglobin Expression bei (R413X)PKP2-transgenen Mäusen bestätigt deren ARVC Pathologie.

PKP2 R413X mutant - Histology





A, b: Picrosirius (fibrosis)
C, d: oil stain – fibroadiposis
E, f: plakoglobin (down regulated in transgenic animals

ARVC/D

- A desmosomal gene mutation may compromise either cell-cell adhesion or intermediate filament function, or both depending on its precise location and impact on protein structure and function. The right ventricle may be particularly vulnerable to impaired cell adhesion, owing to its thin walls and high distensibility and adaptation to wide physiological variations in preload.

ARVC/D

- Significant myocyte loss in either ventricle may be associated with an inflammatory response. Because the regenerative capacity of the myocardium is limited, repair by fibrofatty replacement takes place.
- Both inflammation and fibrofatty islands are potential sources of ventricular arrhythmia.

ARVC/D

- Several laboratories have also investigated how disruption of the desmosomal integrity per se can alter the electric stability of the heart, with a focus on the interaction of complexes that reside at the cardiac intercalated disc.

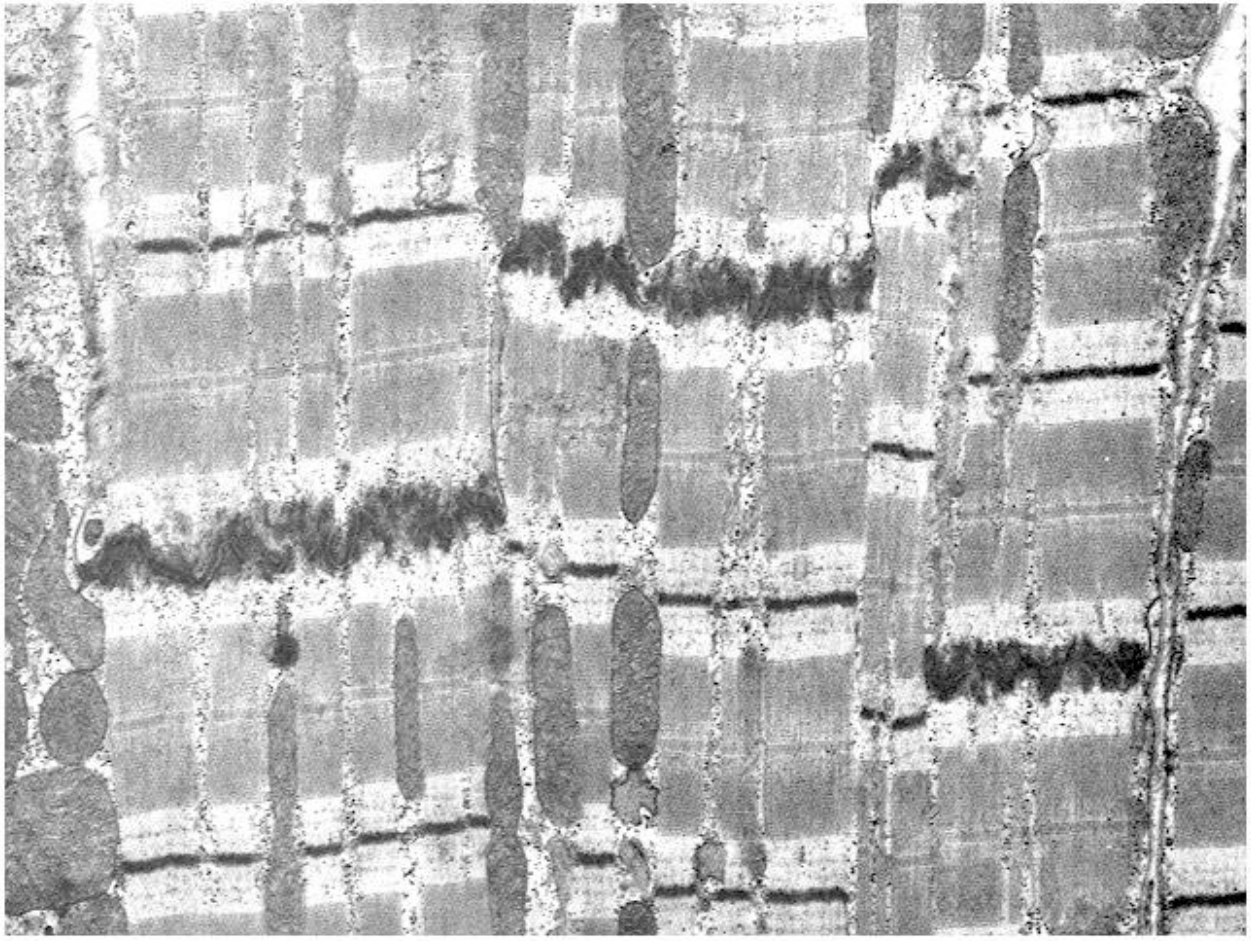
ARVC/D – intercalated disc

- An **intercalated disc** is an undulating double membrane separating adjacent cells in cardiac muscle fibers. Intercalated discs support synchronized contraction of cardiac tissue. They can easily be visualized by a longitudinal section of the tissue.
- Three types of membrane junctions exist within an intercalated disc—fascia adherens (adherens junctions, macula adherens (aka desmosomes), and gap junctions.

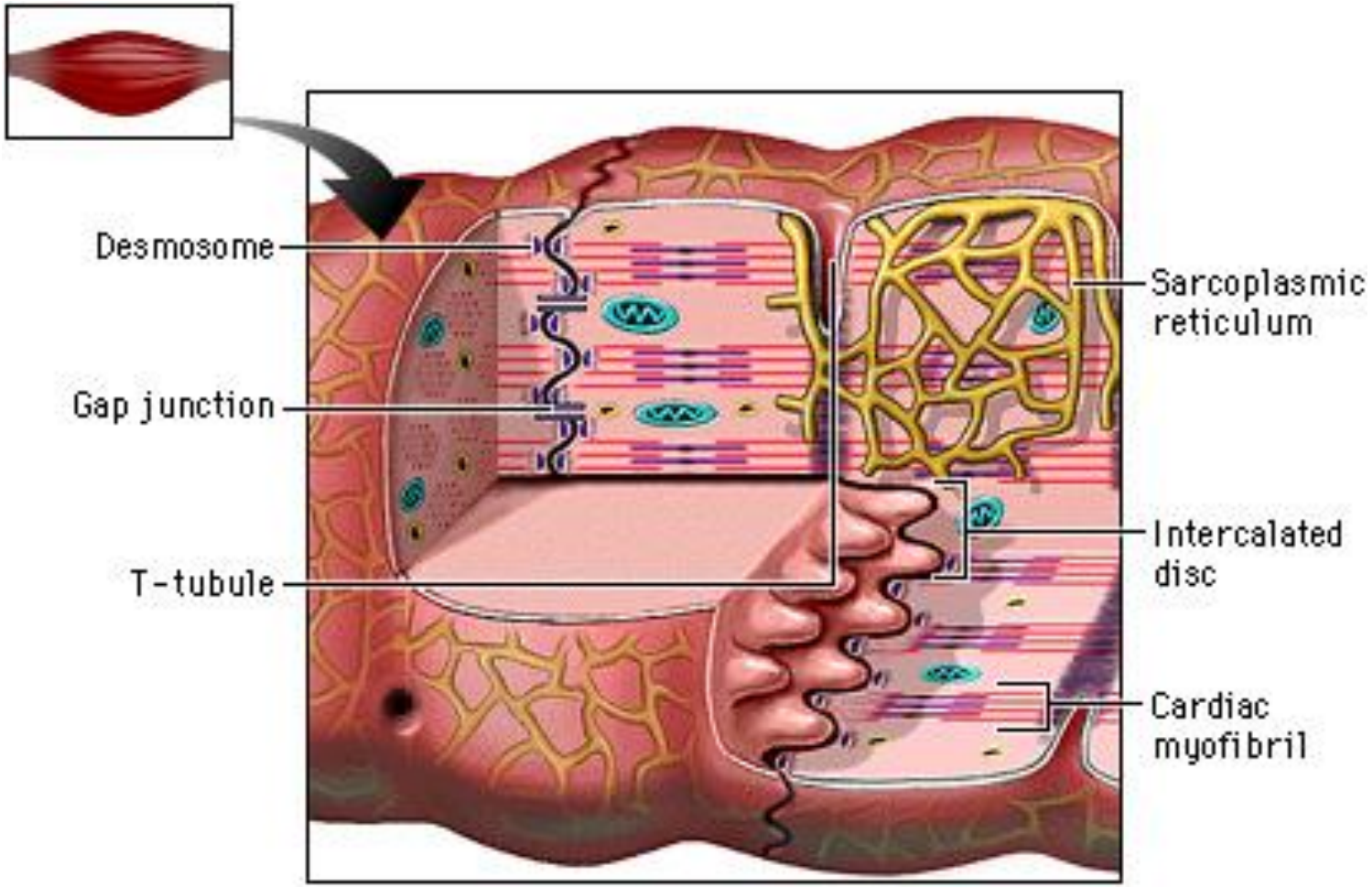
ARVC/D – intercalated disc

- *Fascia adherens (adherens junctions)* are anchoring sites for actin, and connects to the closest sarcomere.
- *Macula adherens* stop separation during contraction by binding intermediate filaments joining the cells together also called a desmosome.
- *Gap junctions* allow action potentials to spread between cardiac cells by permitting the passage of ions between cells, producing depolarization of the heart muscle.

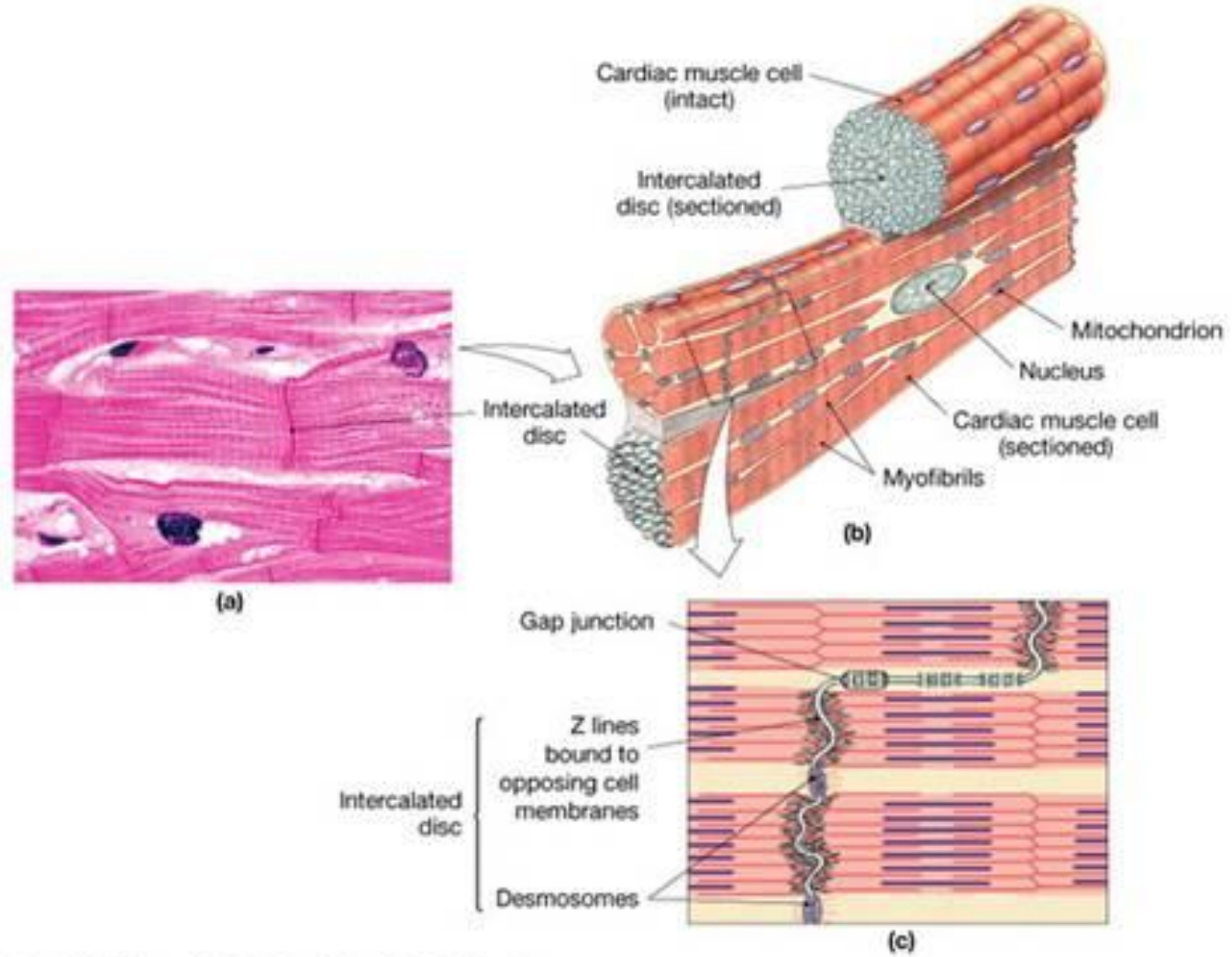
Intercalated disc



Intercalated disc



Intercalated disc



ARVC/D – intercalated disc

- *Overall, the emerging picture is that of the intercalated disc not as the summation of separate molecules with independent functions but rather an "organelle" where macromolecular complexes interact to maintain synchrony within cell populations. It is within this organelle that the desmosome resides and where most ARVC relevant mutations are found. It thus seems pertinent to consider the overall functional unit of the intercalated disc when seeking to understand the molecular pathology of ARVC.*

ARVC/D – summary

- 1. Arrhythmogenic cardiomyopathy is the most arrhythmogenic human heart disease.
- 2. Arrhythmogenic cardiomyopathy is a familial disease that is usually inherited in an autosomal dominant pattern.
- 3. Genetic penetrance and disease expression vary widely in ARVC, which suggests that there are powerful genetic, epigenetic and/or environmental modifiers of disease severity.

ARVC/D – summary

- 4. Mutations in genes encoding desmosomal proteins account for approximately 50% of cases of arrhythmogenic cardiomyopathy.
- 5. The notion that ARVC is a disease of the desmosome suggests that abnormal cell biomechanical properties play a role in disease pathogenesis.
- 6. Remodeling of gap junctions in ARVC could cause abnormal electrical conduction and arrhythmogenesis.

ARVC/D – summary

- 7. Some desmosomal proteins, such as JUP, may play a role in signaling through Wnt/β-catenin/Tcf/Lef pathways.
- 8. The pathogenesis of arrhythmogenic cardiomyopathy probably involves altered cell biomechanical behavior and altered signaling that lead to cardiac myocyte injury and death.

Thank you very much for your attention