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Phenotypic heterogeneity of sarcomeric gene mutations:

a matter of gain and loss?

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After several decades of intense research and various attempts of definition and classification, cardiomyopathies still remain disorders of remarkable and intriguing complexity. Once more, this aspect is elicited by the recent discovery that mutations in the cardiac ankyrin repeat protein or CARP, a protein functionally part of the sarcomere, can cause different types of cardiomyopathies, as reported in this issue (1,2), as well as a congenital heart disease (3).

ANKRD1 in normal heart and disease

CARP is a 36 kD protein encoded by the cardiac ankyrin repeat domain 1 gene *ANKRD1*, which maps on chromosome 10. *ANKRD1* is a member of a conserved gene family, coding for muscle ankyrin repeat proteins (MARPs), involved in muscle stress response such as stretch, injury and hypertrophy (4). CARP is a nuclear transcription co-factor, a signaling molecule predominantly expressed in the heart. CARP is found in the sarcomere, where it co-localize with the N2A domain of titin and myopalladin in the I-band of the Z disk (Figure 1), and in the nucleus (4). The expression of CARP is controlled, at list in part, by the titin-based mechano-transduction signaling pathway, and it is increased in heart development and conditions of injury and stress. In heart development, CARP acts as transcriptional repressor of myocyte contractile elements. In heart failure, CARP is overexpressed, suggesting a role in the “fetal gene program” characteristic of the molecular remodeling of the failing heart (5).

Since titin was previously found to be associated to both HCM and DCM (6-8), also CARP, as part of the titin complex, was hypothesized to play a role in cardiomyopathies. In this issue, two reports confirm this hypothesis and show that in fact *ANKRD1* mutations can cause both DCM and HCM (1,2).

In the first article, Arimura et al. (2) report the results of the *ANKRD1* mutation screening in a large HCM population collected in Japan and in USA. In 384 index patients, they found 3

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missense mutations (*ANKRD1* Pro52Ala, Thr123Met and Ile280Val), accounting for ~1% of HCM cases. Interestingly, they also investigated the N2A CARP-biding domain of titin, and found two additional mutations (*TTN* Arg8500 and Arg8604Gln) in their HCM cohort. In the second report, Moulik et al (1) investigated a series of 208 DCM index patients of Japanese and USA origin, and found 3 missense mutations (*ANKRD1* Pro105Ser, which was recurrent in 2 families, Val107Leu, and Met1841Ile) accounting for in 2% of DCM cases, further supporting a role of the titin mechano-transduction complex in the pathogenesis of cardiomyopathies. But, how to explain two different cardiomyopathies with opposite patho-physiology caused by the same gene?

Phenotypic heterogeneity in cardiomyopathies

Phenotypic heterogeneity (also called “allelic variants” in OMIM (9)) is a well known and common phenomenon in genetics, referring to the occurrence of more than one phenotype caused by allelic mutations at a single locus (10): examples familiar to cardiologists are Duchenne and Becher muscular dystrophies caused by the same dystrophin gene, laminopathies ranging from progeria to lipodystrophy due to lamin A/C gene, LQT syndrome and congenital conduction defect caused by the cardiac sodium channel gene *SCN5A*.

The reason for the clinical variability in allelic disorders lies in the different function of the mutant proteins. In the case of sarcomeric genes, it appears that a “gain” of function usually results in increased energy demand, inefficient ATP utilization and hypertrophy, whereas a “loss” of function in decreased contractility (Table). The two studies published in this issue seem to follow the rule. Arimura et al. (2) show that *ANKRD1* mutations in HCM increase binding of CARP to titin and myopalladin, and that titin mutations at the CARP-binding site have the same effect. On the other hand, Moulik et al. (1) show that *ANKRD1* mutations in DCM cause a loss of CARP binding to talin 1, potentially leading to loss of stretch-sensing, disruption of the link between titin complex and cytoskeletal network, and transcriptional deregulation of genes involved in cell cycle and other pathways.

However, gain and loss are not the only mechanism involved in the phenotypic heterogeneity of *ANKRD1*. Indeed, a recently publication by Cinquetti et al. (3) reports the identification of increased CARP expression or protein stability in 3 cases with total anomalous pulmonary venous return (TAPVR), a rare congenital heart defect characterized by failure of the pulmonary veins to connect to the left atrium during development. In this case, CARP overexpression or its increased activity are believed to repress normal cardiac gene expression leading to abnormal heart development.

Impact of *ANKRD1* mutations discovery in clinical care

The discovery of *ANKRD1* mutations in cardiomyopathies has several implications. Firstly, it contributes to fill the gap of the large number of patients in whom the cause of cardiomyopathy is still unknown, approximately 40% of cases in HCM and probably around 70% in DCM (11). Secondly, it expands our knowledge on the mechanisms leading to hypertrophy and heart failure to include abnormal stretch-based signaling in response to force: this appears to be another “common pathway” for HCM and DCM, which could be targeted by novel therapeutic strategies. Finally, it raises the question of clinical genetic testing of *ANKRD1* in HCM and DCM patients. Although the low prevalence of mutations may currently limit the routine screening of *ANKRD1* gene, we may expect that the implementation in resequencing technology will allow a systematic screening of rare cardiomyopathy genes in the patient population in the near future.

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Abbreviations

CARP, Cardiac Ankyrin Repeat Protein; ANKRD1, Cardiac Ankyrin Repeat Domain 1 Gene; DCM, Dilated cardiomyopathy; HCM, Hypertrophic cardiomyopathy.

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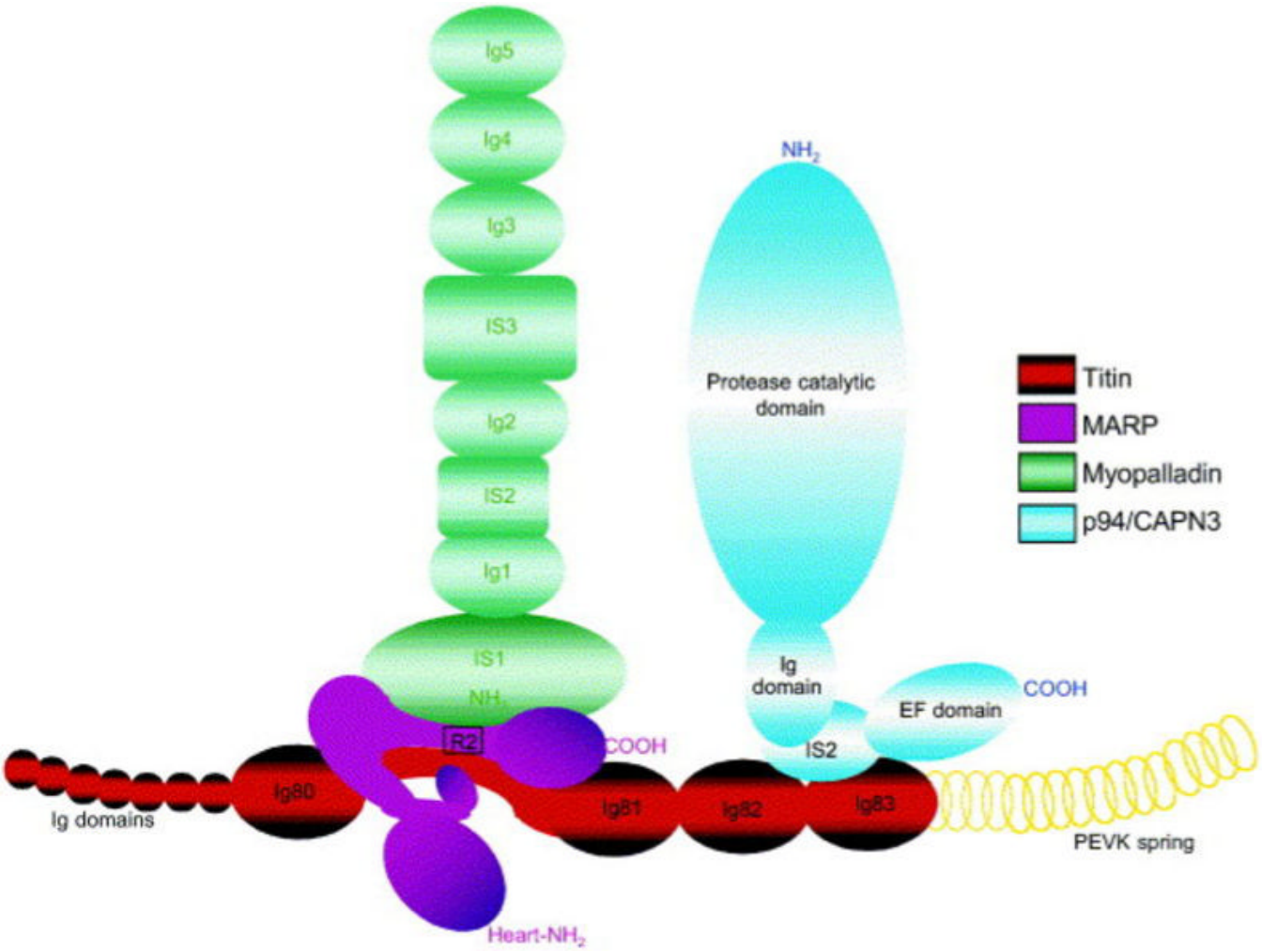

Figure. Model for the titin-N2A signaling complex

N2A titin's sequence interacts with MARPs (CARP, ankrd2 or DARP). Myopalladin associates with MARP/N2A complex by interacting with the N-terminal domains of MARPs. Reprinted from Miller et al.(4) with permission from Elsevier.

Table 1

Phenotypic heterogeneity of sarcomeric genes and differential changes of mutant protein function (8,9,12-16)

Gene	Protein	DCM	Function	HCM	Function	Other allelic disorders
Thick filament						
MYH7	Cardiac β myosin heavy chain	yes	↓maximal force generation ↓contractility ↓velocity of actin sliding	yes	↑maximal force generation ↑Ca^{++} sensitivity	Laing distal myopathy, myosin storage myopathy, scapuloperoneal myopathy, left ventricular non-compaction, endocardial fibroelastosis
MYH6	Cardiac α myosin heavy chain	yes	*	yes	*	Atrial septal defect
MYL2	Regulatory myosin light chain	Not described		yes	↑Ca^{++} sensitivity	
MYL3	Essential myosin light chain	Not described		yes	↑Ca^{++} sensitivity	
MYBPC3	Cardiac myosin-binding protein C	yes		yes	Hypertrophy Diastolic dysfunction	
Thin filament						
TNNT2	Cardiac Troponin T (cTnT)	yes	↓myofibrillar function ↓Ca^{++} sensitivity	yes	↑myofibrillar function ↑Ca^{++} sensitivity	Restrictive cardiomyopathy
TNNTI3	Cardiac troponin I (cTnI)	yes	↓myofibrillar function ↓binding to cTnT	yes	↑myofibrillar function ↑Ca^{++} sensitivity	Restrictive cardiomyopathy (↑↑Ca^{++} sensitivity)
TNNC1	Cardiac troponin C (cTnC)	yes	↓myofibrillar function ↓Ca^{++} sensitivity ↓PKC effect	yes	↑myofibrillar function	
TPM1	Tropomyosin 1 alpha chain	yes	↓Ca^{++} sensitivity ↓maximum force	yes	↑myofibrillar function ↑Ca^{++} sensitivity	
ACTC1	α-Cardiac actin	yes	↓α-cardiac actinin (Z-disk) affinity ↓force transmission	yes	Impaired actomyosin binding	Restrictive cardiomyopathy
Titin filament and Z-disk						
TTN	Titin	yes	↓biding to actinin and Tcap	yes	↑biding to actinin and Tcap	Tibialis muscular dystrophy or Udd distal myopathy; hereditary myopathy with early respiratory failure (HMERF); recessive limb-girdle muscular dystrophy type 2J (LGMD2J)
TCAP	Titin-cap or telethonin	yes	↓biding to titin, MLP and myozenin-2	yes	↑biding to titin, and myozenin-2	Limb-girdle muscular dystrophy type 2G (LGMD2G)
ANKRD1	Cardiac ankyrin repeat protein (CARP)	yes	↓biding to talin1	yes	↑biding to titin and myopalladin	Total anomalous pulmonary venous return (↑expression)
LDB3	Cypher/ZASP	yes	Cytoskeletal disarray, ↓PKC affinity	Not described		Left ventricular non-compaction, myofibrillar myopathy
CSRP3	MLP	yes		yes	↓biding to actinin	
MYOZ2	Myozenin-2	Not described		yes		
OBSCN	Obscurin	Not described		yes	↓biding to titin	

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* Legend: β myosin heavy chain mutations found in HCM and DCM were studied in a myosin heavy chain of knock-in mouse models