Mutations in the ANKRD1 gene encoding CARP are responsible for human dilated cardiomyopathy

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Aims
Dilated cardiomyopathy (DCM) is familial in ~30% of cases, and mutations have been identified in several genes. However, in a majority of familial cases, the responsible genes are still to be discovered. The ANKRD1 gene is over-expressed in heart failure in human and animal models. The encoded protein CARP interacts with partners such as myopalladin or titin, previously shown to be involved in DCM. We hypothesized that mutations in ANKRD1 could be responsible for DCM.

Methods and results
We sequenced the coding region of ANKRD1 from 231 independent DCM cases. We identified five missense mutations (three sporadic and two familial) absent from 400 controls and affecting highly conserved residues. Expression of the mutant CARP proteins after transfection in rat neonate cardiomyocytes indicated that most of them led to both significantly less repressor activity measured in a reporter gene assay and greater phenylephrin-induced hypertrophy, suggesting altered function of CARP mutant proteins.

Conclusion
On the basis of genetic and functional analysis of CARP mutations, we have identified ANKRD1 as a new gene associated with DCM, accounting for ~2% of cases.

Keywords
ANKRD1 • Dilated cardiomyopathy • Gene • Cardiomyocyte • CARP • Mutation

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Introduction
Dilated cardiomyopathy (DCM) is defined by the presence of left ventricular dilatation and left ventricular systolic dysfunction in the absence of abnormal loading conditions (hypertension, valve disease) or coronary artery disease sufficient to cause global systolic impairment.¹ It is one of the most frequent causes of heart failure with systolic dysfunction and the leading indication for heart transplantation. About 30% of DCM cases are familial (FDCM),² indicating a very likely monogenic origin. To date, 15 responsible genes are identified in phenotypically isolated forms and up to 25 if additional traits including myopathy, arrhythmias, or more complex syndromes are taken into account (reviewed in 3). Transmission is mainly autosomal dominant with incomplete and age-dependent penetrance.⁴ There is no major gene identified in FDCM, and, overall, a causative mutation has been identified in only a small fraction of the cases. A major aim is to identify additional genes responsible for DCM, leading to the identification of new pathophysiological pathways, and also to better clinical outcomes for more families who will benefit from early diagnosis through genetic testing and counselling.⁵
All the known or suspected genes are encoding proteins important for cardiomyocytes structure or function such as the sarcomeric apparatus, cyto-architectural proteins, nuclear envelope or Ca\(^{2+}\) cycle regulation, and ionic exchanges. These genes were mostly identified by candidate gene approach on the basis of their known physiological effects. Given that the majority of genes and mutations responsible for FDCM are still to be identified, it is tempting to speculate that other less predictable mechanisms are at work. Cardiac response defect to mechanical load and subsequent increase in cellular length and ventricular wall thickening constitute an attractive DCM causing hypotrophic mechanism. Mutations in the titin gene have been reported in DCM. This giant protein plays a central role in cardiomyocyte mechanosensing through its cardiac-specific N2A elastic region. Moreover, we recently identified DCM-associated mutations in the gene encoding myopalladin, one of the protein partners involved in the titin-associated stretch-sensing complex. Another partner of myopalladin is cardiac ankyrin repeat protein (CARP), encoded by the ANKRD1 gene. CARP is a member of the MARP (muscle ankyrin repeat proteins) family of muscular proteins which also includes Ankrd2/Arpp and DARP involved in muscular stress response. In addition to its localization to the myopalladin/titin complex, it is tempting to speculate that other less predictable mechanisms are at work. Cardiac response defect to mechanical load and subsequent increase in cellular length and ventricular wall thickening constitute an attractive DCM causing hypotrophic mechanism. Mutations in the titin gene have been reported in DCM. This giant protein plays a central role in cardiomyocyte mechanosensing through its cardiac-specific N2A elastic region. Moreover, we recently identified DCM-associated mutations in the gene encoding myopalladin, one of the protein partners involved in the titin-associated stretch-sensing complex. Another partner of myopalladin is cardiac ankyrin repeat protein (CARP), encoded by the ANKRD1 gene. CARP is a member of the MARP (muscle ankyrin repeat proteins) family of muscular proteins which also includes Ankrd2/Arpp and DARP involved in muscular stress response. In addition to its localization to the myopalladin/titin N2A region, CARP has been shown to be modulated in response to numerous cardiac stimulations leading to heart failure (reviewed in 10). CARP is localized either at the sarcomeric I-band or at the nucleus upon stretch stimulation where it is believed to be a transcriptional repressor. The CARP nuclear factor expression is involved in rat (constriction of abdominal aorta, spontaneously hypertensive and Dahl salt-sensitive) and dog models of left ventricular hypertrophy, human heart failure, DCM, and adriamycin-induced cardiomyopathy. These features support the hypothesis for a role of CARP as a nuclear-signalling protein modulating gene expression in response to mechanical load sense at sarcomeres. Our hypothesis was, therefore, that mutations in ANKRD1, encoding the CARP factor, could be responsible for DCM.

Methods

Patients and families

Patients and relatives gave written informed consent to participate in the study in accordance with the Declaration of Helsinki and a protocol was approved by the local Ethics Committee. Patients fulfilled the diagnostic criteria for DCM according to Mestroni et al. Patients were enrolled from the Paris registry described elsewhere or from European centres involved in the EUROGENE Heart Failure programme (see Appendix). Patients were classified as familial cases when at least two first-degree relatives in the same family were affected after familial investigation.

Molecular genetics

Genomic DNA was extracted from whole blood at Généthon (Evry, France) using a standard protocol (phenol/chloroform extraction). As MYH7 has been found mutated in up to 10% of DCM cases, all included DNAs were previously screened for MYH7 exonic mutation and none carried potential causative variant. Moreover, 45% (103/231) of them have also been screened for mutation in eight other DCM/responsible genes (ACTC, DES, TTN, LMNA, SCG2, TNNT2, PLN, MYPN). Each of the exons and exon–intron boundaries of the CARP gene (NM_014391,2) was PCR-amplified and directly sequenced on one strand by the BigDye v3.3 terminator sequencing method on an Applied Biosystem genetic analyzer (ABI3100). PCR primers are listed in Supplementary material online, Table S1. Sequence variants were identified by multiple alignments and confirmed by sequencing on the other strand of an independent PCR product and genotyped in 400 ethnically matched control DNA (800 alleles) using a PCR-RFLP-based method (see Supplementary material online, Table S2).

Cloning of CARP cDNA in frame with V5 epitope was performed into the pcDNA3.1D/V5-His-TOPO (Invitrogen) using RT–PCR amplification from human heart total RNA with primers (CARP-clo.F 5' cccatgtgtaactgaatgag 3' and CARP-clo.R 5' gaatgtcatctgga-gagt 3'). The cloned cDNA was transfected into pEGFP-N3 vector (Clontech) using KpnI/Apol restriction digest to obtain C-terminally GFP-tagged CARP (CARP-NGFP vectors).

The wild-type vectors, that is pcDNA3.1 and pEGFP backbones with CARP cDNA inserts, were used as template for site-directed mutagenesis (QuickChange, Stratagene) to obtain the two wild-type and mutant vector series: WT/MUT-CARP-NGFP and WT/MUT-CARP-V5.

For luciferase assay, four CARP-responsive MLC2v minimal promoters were excised from the adenovirus Ad.Kv4.3 (a generous gift from R.J. Hajjar) with BglII and HindIII and cloned in the multiple cloning site of pGL3basic (Promega) S' to the firefly luciferase cDNA to obtain p(MLC2v)+Luc reporter plasmid.

All construct inserts were sequenced to ensure that no random mutations arose. Two different plasmid preparations were randomly used to avoid technical artefacts associated with preparation quality.

Cells culture and transfection

Luciferase assay

Rat neonate cardiomyocytes (RNC) were isolated and grown for 24 h as described previously before transfection. Cells were co-transfected in 35 mm dishes with 10 μL lipofectamine 2000 (Invitrogen), 1 μg of p(MLC2v)+Luci, and 1 μg of empty pcDNA3 or WT/MUT-CARP-V5 expression vector.

Rat neonate cardiomyocyte hypertrophy experiments

Cells were isolated essentially as described in Duboscq-Bidot et al. with slight modification. To avoid serum-dependent hypertrophy before transfection, cells were plated in 5% foetal bovine serum (FBS) instead of 10% horse serum and 5% FBS. Cells were transfected with 10 μL lipofectamine 2000 (Invitrogen), 2 μg of WT/MUT-CARP-NGFP or pEGFP control expression vectors for 5 h. After transfection, the culture medium was replaced but supplemented with only 1% FBS for a 12 h recovery period. Then, the cells were starved with medium containing 0.4% FBS during 24 h. Cells were then put back to 1% FBS and hypertrophy induced with 100 μM norepinephrine chloride (Sigma Aldrich) for 48 h before immunolabelling.

Transfection efficiency was maximized by adjusting DNA/lipofectamine ratio and reached 12–15% for RNC as measured by GFP-positive cell vs. DAPI-labelled nucleus counting.

Immunolabelling of rat neonate cardiomyocytes

Fixation and immunolabelling of the RNC were done as described previously except that sarcomere-specific labelling was obtained with a monoclonal antibody directed against M-band rat myosin at 1/300
luciferase assay

Transfected RNC were scrapped after 4 days and the firefly luciferase activity was measured with a Lumat LB9507 luminometer (Berthold Technologies) as described by the provider (Luciferase Assay System, Promega). The mean values of eight independent experiments were measured in duplicate for each expressing vector. Results were normalized to total protein content measured by the Bradford colorimetric assay to take into account variation in cell number between experiments.

Rat neonate cardiomyocyte hypertrophy quantization

Individualized RNC areas were measured using the ImageJ software (Supplementary material online figure). The mean values of at least 10 cell areas from five independent experiments were measured for each vector.

Statistical methods

To compare luminometric signals or cellular areas, we used non-parametric one-way analysis of variance for repeated measures (Friedman's test) to take into account that each independent experiment was performed on separate RNC culture isolates. A Dunn's post hoc analysis of specific pairs of conditions was used for comparisons of WT- vs. MUT-CARP constructs. The statistics were calculated with GraphPad Prism® v5.01 software, and P < 0.05 was considered significant.

Results

Population studied

The ANKRD1 gene was analysed in 231 consecutive and unrelated index cases (158 cases with familial, and 73 with sporadic DCM) mainly from European descent (93%). Eighty-five per cent of cases had isolated DCM phenotype, and the remaining patients presented with associated conduction defects (10.9%) or mild skeletal muscular dystrophy (2.6%) or isolated mild CPK elevation (1.7%). Among the patients, 55.5% were from France, 28.1% from Germany, 13.8% from Italy, and 2.6% from other European countries.

Genetics

We identified five heterozygous mutations in ANKRD1-coding sequence (Supplementary material online, Table S3). All were missense mutations leading to mononucleotide substitutions (Figure 1). All carrier patients were of Caucasian origin, and mutations were absent from 400 ethnically matched healthy control subjects, indicating they are not frequent polymorphisms. Formally, ANKRD1 mutations were involved in 1.3% (2/158) of familial DCM (mutations p.Thr116Met and p.Ala276Val) and in 4% (3/73) of sporadic cases (mutations p.Glu57Gln, p.Arg66Gln, and p.Leu199Arg). However, premature sudden deaths were observed in first-degree relatives of two sporadic cases (families G108-R66Q and G19-L199R), suggesting they may have familial disease. The familial mutations were present in two affected relatives with DCM, including a father-to-son transmission in one family (G47-T116M). It was also transmitted to a young adult (family F17390, III.01, 27 years of age) with a normal cardiac examination and echocardiography at that age, suggesting reduced penetrance (Figure 1).

Detailed phenotypic data about heterozygous subjects carrying a mutation are reported in Table 1. The phenotype of patients was characterized by isolated DCM, without associated conduction defect or skeletal myopathy (except one patient with skeletal muscle abnormalities related to an acquired cause: Macroscopic myofasciitis). Mean age at diagnosis was 42.6 years (±12.9). Prognosis was characterized by one cardiac death during the prospective follow-up of mutation carriers (sudden death at 42 years of age) and four premature deaths (including one sudden death) in relatives without DNA analyses (at 36, 38, 40, 42 years of age). Mean age at death was 39.6 years (±2.6). A phenocopy was present in family G19-L199R as a patient with acute heart failure and systolic dysfunction (II.01) rapidly evolved towards an important recovery of heart function, after immunoglobulin therapy (because endomyocardial biopsy exhibited parvovirus B19 persistence) and stopping alcohol. The patient was, therefore, supposed to have myocarditis and/or alcohol-induced DCM.

All mutated amino acid residues were located in protein domains or motifs predicted or experimentally described to interact with a known molecular partner (Figure 2B). Molecular alignment of sequences surrounding mutations with CARP from other species showed very high conservation of the mutated residues through evolution for Glu57, Thr116, and Ala276 to high conservation for Arg66 and Leu199, suggesting selection pressure and functional importance of the residues (Figure 2C).

Functional studies

As CARP was described to be located in both the sarcomeric I-band and the nucleus in cardiomyocytes,9,11 we first investigated the GFP signal localization in RNC at different times after transfection of WT or MUT-CARP-NGFP (Figure 3). The time course and localization of expressed CARP were not different between WT and mutant proteins. Expression was restricted to sarcomeric structure during the first 2 days, shifting to a more nuclear expression at day 4. Under basal conditions, ~40% of GFP-positive cells displayed nuclear labelling, whereas upon phenylephrin (PE) stimulation, the expression profile was similar but more cells displayed the nuclear labelling (~70%). CARP being described as a nuclear co-repressor, we hypothesized that mutant CARP proteins could be defective in transcriptional repression activity.12,18 We tested this hypothesis by monitoring the CARP-responsive MLC2v promoter repression in response to WT or mutated CARP-V5 expression. WT-CARP expression dramatically reduced luciferase activity (31.7 ±10.2%) compared with empty vector as a standard for 100% activity (pCDNA3 empty vector), confirming the repressor activity of CARP on MLC2v promoter (Figure 4). Expression of all the mutated CARP proteins showed less marked repressor activity than WT-CARP. The Friedman test for difference between WT and mutants was highly significant (overall P-value <0.001). Mutant-associated inhibition was only 37.7 ±10.5% for p.Thr116Met compared with WT (NS; Dunn's post hoc test), 44.6 ±10.3% for p.Arg66Gln (NS), 58.0 ±21.6% for p.Glu57Gln (P <0.05), 77.7 ±22.3% for...
Ala276Val (P < 0.001), and close to absence of inhibition (89.7 ± 13.1%) for p.Leu199Arg (P = 0.001).

CARP was also described previously as over-expressed upon alpha-adrenergic-dependent hypertrophy stimulation of RNC. It was also hypothesized that CARP repressor activity was implicated in limitation of the hypertrophic response. We thus measured the effects of CARP mutant expression in RNC on hypertrophy through analysis of cardiomyocyte surface area. Compared with RNC cultured in normal trophic condition (1% FBS), untransfected (GFP-negative) RNC upon PE stimulation (100 μM) for 2 days were hypertrophied by a factor of 1.9 (mean area 1525 ± 312 vs. 2891 ± 442 μm², n = 5, Mann–Whitney P = 0.014), indicating alpha adrenergic response of RNC in our culture condition. Rat neonate cardiomyocyte expressing the GFP protein alone responded to PE stimulation in a very similar range of 1.6 (1766 ± 461 vs. 2779 ± 312 μm², n = 5, Mann–Whitney P = 0.014), indicating a non-adrenergic response of RNC in our culture condition. Rat neonate cardiomyocyte expressing the GFP protein alone responded to PE stimulation in a very similar range of 1.6 (1766 ± 461 vs. 2779 ± 312 μm², n = 5, Mann–Whitney P = 0.014), indicating an innocuous effect of GFP expression. When compared with WT-CARP-NGFP-expressing cells, the mutants were associated with an increased mean cellular surface area (Figures 3 and 5; overall P-value <0.0005). As for the effects observed with the reporter gene assay, p.Leu199Arg has the most drastic effect, with almost no CARP-dependent limitation in the hypertrophic response compared with controls (85 ± 7.9%, P < 0.001). The two other mutations have less pronounced but significant effects (p.Glu57Gln: 68 ± 10.3%, P < 0.05; p.Ala276Val: 65 ± 5.7%, P < 0.05). The increase in cell area was not significant for mutant p.Thr116Met (50 ± 11.5%) and p.Arg66Gln (51 ± 11.1%).

**Discussion**

We searched for mutation in a large population of DCM patients in the ANKRD1 gene and identified five missense variants that we considered as causal mutations on the basis of both genetic and functional analyses.

From a genetic point of view, the variants (i) affect highly conserved amino acid residues, (ii) were absent in 400 ethnically matched controls, indicating they were not common.

**Figure 1** Mutations identified in the ANKRD1 gene. The figure presents the pedigree drawing for each family carrying an identified mutation. An electrophoregram of heterozygous sequence for each mutation is shown under pedigrees. The codon change and orientation is indicated (arrows). Pedigree symbols: square, male; circle, female; filled, dilated cardiomyopathy-affected (black) or uncertain status (grey); hatched, no phenotypic data available; slashed, deceased individual; ‘+’, heterozygous carrier; ‘−’, non-carrier. In diamond, the number of children with unknown genders and without clinical record is indicated.
Table 1  Clinical features of affected patients and mutation carriers

<table>
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<th>Subject</th>
<th>Age (year)/sex</th>
<th>Age at diagnosis (years)</th>
<th>NYHA class</th>
<th>ECG</th>
<th>LVEDD (mm)</th>
<th>EF (%)</th>
<th>IVSd (mm)</th>
<th>Muscular clin./CPK</th>
<th>Clinical status</th>
<th>Comments</th>
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<tr>
<td>II.02</td>
<td>55/M</td>
<td>45</td>
<td>I</td>
<td>SR, iLBB</td>
<td>68</td>
<td>40</td>
<td>8</td>
<td>NL/NL or mild elevation</td>
<td>Affected</td>
<td>Initial diagnosis with severe DCM (LVEF 15%, LVEDD 80 mm, normal coronarography)—mild hypercholesterolaemia with statin medical treatment</td>
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<tr>
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<td>62/M</td>
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<td>II</td>
<td>SR, LAHB</td>
<td>71</td>
<td>28</td>
<td>9</td>
<td>NL/NL</td>
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<tr>
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<td>31</td>
<td>II</td>
<td>SR, iLBB</td>
<td>79</td>
<td>25</td>
<td>8</td>
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<td>21</td>
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<td>SR, cLBB</td>
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Age is age at genetic inquest; NYHA, New York Heart Association functional class; LVEDD, left ventricular end-diastolic diameter (mm); CVA, cerebrovascular accident; SD, sudden death; EF, ejection fraction (%); IVSd, interventricular septum thickness in diastole (echography); muscular clin., abnormality in clinical muscular testing; CPK, elevated serum creatine kinase level; M, man; W, woman; SR, sinus rhythm; iLBB or cLBB, incomplete or complete left bundle branch block; LAHB, left anterior hemiblock; NL, normal; NA, not available. Clinical status is ‘affected’ when DCM was present on echocardiography, whatever the symptoms.
Figure 2 Localization of mutations on CARP gene and protein. (A) ANKRD1 gene structure. Exons are vertical bars filled blue or red (encoding ankyrin domains). Positions of the mutations are indicated on top. (B) Linear structure of CARP protein with experimentally (horizontal upper bars) and in silico-predicted (embedded motifs) sequences. CASQ, calsequestrin interactions; TTN, titin interaction; NL, nuclear localization signal. (C) Enlargement of CARP peptide sequences surrounding the mutated residues with multiple inter-species alignments generated by ClustalW. Accession numbers in brackets. Minus symbols are for identical residues compared with human (top sequence). Mutated positions are in bold characters.

Figure 3 Rat neonate cardiomyocytes expressing transfected wild-type (WT, upper panels) or mutant (Arg199, bottom panels) CARP–NGFP fusion protein (green labelling). Cardiomyocyte M-bands are labelled with anti-myosin antibody (red). Cells were compared before (day 2) and after (day 4) phenylephrin-induced hypertrophy. On these representative cells, CARP WT and mutant are localized at I-bands and nuclei. The hypertrophy response was observed with Arg199 (bottom part) but not with WT CARP (upper part). Bottom left inserts are magnification of square regions. Scale bar: 10 μm.
polymorphisms, and (iii) there was no affected family member lacking the variant, indicating segregation of the variants with the disease.

Despite the very low frequency (<1/800) of variant alleles, two of the variants have previously been described in other contexts.

First, a recent report described an association between the ANKRD1 gene variant p.Thr116Met and another cardiac disease, total anomalous pulmonary venous return (TAPVR), and was considered as a possible causal mutation.\(^{22}\) No TAPVR was observed in our DCM families (from transthoracic echocardiography) and this suggests variable phenotypic heterogeneity associated with this mutation. Second, the causal nature of the p.Ala276Val variant is, however, questionable since the screening of dbSNP database (http://www.ncbi.nlm.nih.gov/SNP; build 129) surprisingly pointed this variant in a unique heterozygous individual, without any available data on population allelic frequency (rs35550482). More in-depth observation of the SNP submission report indicated that the individual carrying the mutation has familial history of premature cardiac death (the father died at 59 years of age), with no specific mention for cardiac examination of the DNA donor.\(^{23}\) The hypothesis of DCM in this family, therefore, cannot be ruled out. Conversely, the eventuality that the variant A276V represents only a rare polymorphism remains possible. However, the clear effect we observed by functional analysis strongly suggests a role of this variant in DCM.

Because of the small size of the families, we were not able to perform linkage analysis and we, therefore, strengthened the genetic evidence with experimental data, demonstrating that CARP mutants did not induce the same functional effects as wild-type CARP when expressed in the RNC model.

Several lines of evidence support a role of CARP as a modulator of cardiac hypertrophy. It is proposed that CARP could counteract the effects of α-adrenergic hypertrophy stimulation.\(^{21}\) Moreover, CARP decreases the promoter activity of genes re-expressed during cardiac hypertrophy in animal models of heart failure.\(^{18}\) We thus speculate that CARP mutants could be deficient in repressing activity. We looked at the effects of CARP mutant expression at both the transcriptional level and its more downstream consequences on cardiac cells hypertrophy.

First, all but one CARP mutants showed reduced capability to repress transcription through MLC2v promoter, a known in vitro target of CARP. The pGL3basic luciferase vector, lacking promoter, did express only 2.3% of control signal (not shown), indicating that luciferase activity variation was due to the inability of CARP mutants to repress MLC2v promoter. Second, hypertrophic response of cardiomyocytes to PE was poorly controlled when mutant CARP proteins were over-expressed. Both results strongly suggested a functional alteration associated with at least three of the mutations identified in DCM cases (p.Glu57Gln, p.Leu199Arg, p.Ala276Val). The hypothesis implicating CARP as a signalling molecule shuttling from sarcomeric I-band to nucleus upon mechanical stress was proposed on the basis of multiple observations.\(^{7,24}\) CARP is localized at the I-band in a multi-protein complex interacting with the N2A elastic domain of titin. CARP is displaced to the nucleus after mechanical stretch or pressure overload, where it is believed, at least in vitro, to act as a co-repressor on gene regulated during cardiac remodelling.\(^{9,13}\) An interesting pathological mechanism supported by our observations would be that the inability of CARP mutants to translate physiological sarcomeric stretch signal into the nucleus leads to uncompensated transcriptional response and ultimately to dilation and heart failure. Accordingly, it is remarkable that animal models in which other
mechanic stress-sensing pathways are impaired in cardiac cells developed DCM.15,25 Conversely, the absence of major phenotype neither in ankrd1 KO mice, nor in MARP triple KO (ankrd1, ankrd2, ankrd23), is questioning.6,7 However, one can reconcile these observations in considering the dominant effects of the mutations, all found at the heterozygous state, although KO mice address more specifically recessive or haploinsufficiency-related effects. Our results are in accordance with the hypothesis of a dominant-negative role of CARP mutants interfering with other protein partners. Accordingly, the list of known CARP partners involved in cardiac physiology is increasing, spreading into numerous mechanisms important for cardiac function: transcriptional regulation through dimerization17 or YB1 interaction,12 sarcomeric integrity through myopalladin interaction,11 stretch sensing (titin1), or calcium homeostasis (CASQ2).28

The effects of the two other variants (p.Arg66Gln and p.Thr116Met) are not significantly revealed by our experiments. It could be indicative either that they are rare neutral polymorphisms or that their cellular effects are related to alternate mechanisms not mediated by transcriptional modulation. Further experiments would have to be performed to identify these possible alternate dominant-negative effects. Interestingly, the substituted amino acids are located in the putative dimerization and PEST domains of CARP,10,27 and the Thr116Met variant located in the PEST domain was shown to be involved in CARP degradation.22 Finally, our findings related to the identification of a new gene involved in DCM have practical implications for clinicians. Since the frequency of ANKRD1 mutations is not rare (2%), systematic screening for this gene should be considered in familial DCM to improve the management of families, namely through early diagnosis and genetic-based cardiac screening and follow-up in relatives.5 The identification of an ANKRD1 mutation in a relative at a preclinical stage, indeed, suggests age-related penetrance, which is a common finding in genetic forms of DCM.

Conclusion

Our genetic screening of the ANKRD1 gene in European DCM cases identified missense mutations in 2% of cases. Genetic data as well as experimental results obtained in cardiacocytes indicated that mutations can impair CARP nuclear function. We conclude that CARP is a new gene responsible for human DCM. A better understanding of physiopathological role of CARP mutations would benefit from animal models recapitulating the disease.

Supplementary material

Supplementary material is available at European Heart Journal online.

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Appendix

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