Dear Colleagues,

Dear Members of the Working Group,

Please find enclosed the 30th issue of our Newsletter.

Read the answer to the previous challenging ‘case of the month’!

At the end of this year, I would like to thank all the contributors and visitors of our website. I wish everyone a Peaceful Christmas and a Happy New Year.

Tiina Heliö
Identification of Novel Mutations in RBM20 in Patients with Dilated Cardiomyopathy
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Presented by Dr. Abdelaziz Beqqali and Prof. Dr. Yigal Pinto, Heart Failure Research Center, University of Amsterdam, The Netherlands.

Summary
To date, the genetic cause of the majority of dilated cardiomyopathy (DCM) cases remains unresolved despite the fact that mutations in more than 30 genes have been shown to be disease causing or disease associated [1, 2].

Most of the genes in which mutations cause DCM, encode sarcomeric proteins involved in contraction, or cytoskeletal proteins important for cell structure or force transduction. Brauch et al. previously identified a novel non-sarcomeric, non-cytoskeletal gene underlying DCM [3]. Using genetic linkage analysis, the authors first mapped the disease locus to chromosome 10q25, then screened positional candidate genes within this locus and identified missense mutations in exon 9 of RNA-binding motif protein 20 (RBM20), in each of the linked families as well as in multiple probands. Where available, family members of these probands were tested, revealing further evidence that RBM20 mutations are associated with DCM and suggesting the pathogenicity of these mutations. Ultimately, heterozygous missense mutations in exon 9 of RBM20 were found in 3% of all DCM cases tested, and in over 13% of those with a history of sudden death [3].

RBM20 is predominantly expressed in the heart according to the gene expression omnibus (GEO) database. A "Conserved Domain Data" base search of the translated reference RBM20 cDNA indicated homology to an RNA Recognition Motif 1 Superfamily domain (RRM-1) spanning exons 6 and 7 and a arginine/serine (RS)-rich [4] domain (in exon 9) prototypical of spliceosome proteins that regulate alternative pre-messenger RNA splicing [5].

Li et al recently reported the discovery of novel mutations in RBM20 in patients with dilated cardiomyopathy [6].

To further evaluate the role of RBM20 in DCM pathogenesis and the DCM clinical characteristics caused by RBM20 mutations, the authors genetically screened a cohort of 312 DCM probands, and their family members when a mutation was identified.

While Brauch et al. screened all exons of RBM20, Li et al. focused their study on the most conserved regions of RBM20. Bidirectional DNA sequencing was conducted for the region of RBM20 showing strong conservation across species, including exon 6 through exon 9. Both the RNA recognition motif region 1 (RRM-1) and the arginine/serine (RS) rich domains are also located within this region.

Six unique missense mutations were found in six unrelated probands, four of which were novel (V535I, R634W, R636C, and R716Q) and two were previously described by Brauch et al. (R634Q and R636H) [3]. These variants were predicted to alter highly conserved amino acids, none of which were present in dbSNP or 450 Caucasian control DNAs.
Consistent with Brauch et al., four of the six mutations were located in the same mutation hotspot in the RS-rich region in exon 9 and modified two amino acids (R634 and R636). The two other novel mutations (V535I and R716Q) were found in domains outside of the hotspot region. V535I was found in exon 6 in the strictly conserved RRM-1. R716Q was located in exon 9 approximately 60 residues downstream of the RS-rich region (residues 632–654). All available DNA specimens from family members of the probands with RBM20 mutations were also sequenced for the family mutation. Seventeen family members from three unrelated probands tested positive for the family mutations.

These RBM20 mutations were clinically manifested as mild-to-severe DCM, including heart failure, or the need for heart transplantation or an implantable cardiac defibrillator (ICD). The average age of DCM onset for the RBM20 mutation carriers was 37.1 years, and the mean LVEF was 31%. Six mutation carriers also had an ICD or pacemaker implanted, and several subjects had supraventricular and/or ventricular arrhythmias, suggesting that RBM20 mutations in addition to disturbing cardiac contraction may also adversely impact cardiac conduction and rhythm. No patient who carried an RBM20 mutation had left ventricular hypertrophy by echocardiography, with left ventricular septal and posterior wall measurements within normal limits. Two RBM20 mutations (V535I, and R634Q) had sporadic DCM, while the other four (R634W, R636C, R636H, and R716Q) had familial DCM consistent with autosomal dominant inheritance.

Comments

The function of RBM20 and thereby the mechanism through which RBM20 mutations may cause DCM remains to be elucidated. The functional relevance of the RRM-1, the RS and the U1 zinc-finger domain can be readily deduced from their strict conservation among vertebrates. The RRM-1 domain is thought to bind the target transcript precursor and regulate splicing [4, 7]. Therefore one could speculate that the RBM20 mutation V535I residing in the highly conserved RRM-1 region may interfere with its RNA-binding capacity.

Four RBM20 mutations (R634Q, R634C, R636C, and R636H) altered two highly conserved arginine residues in its RS-rich region. Since the RS-rich domain is predicted to be involved in protein–protein interaction [4, 7], those mutations may affect the ability of RBM20 to interact with other spliceosome proteins, thus disrupting the normal RNA splicing process.

A pathogenic link between genetic disruption of alternative splicing-regulating RS proteins of the spliceosome and DCM has been established earlier in mouse models [8, 9]. Furthermore, the general alternative splicing factor/splicing factor 2 (ASF/SF2) has been directly implicated in the transition between fetal and adult gene programs within the heart [9]. Whether RBM20 acts in a similar manner remains to be investigated, but it will be interesting to define the transcripts of which splicing is regulated by RBM20. This information will elucidate the downstream pathways involved in the etiology of DCM and may eventually help to explore possible therapies.

Clinical implications and prospects

The results described in Li et al., confirm that RBM20 mutations should be considered in familial DCM. It further corroborates that RBM20 mutations are frequently associated with a clinically aggressive form of DCM. This includes severe heart failure, arrhythmia, and the need for cardiac transplantation at ages younger than for sporadic DCM, suggesting that this may be an important cause of familial DCM. This independent confirmation after the initial report in 2009 by Brauch et al. [3] more firmly suggests RBM20 as a novel candidate gene to the list of DCM associated genes. However, its precise frequency and pathophysiological role still needs to be determined. Most importantly, close examination of the described families reveals that penetrance may be variable and in the only large pedigree described by Li et al, variations in other DCM related genes were found as well. A robust pathophysiological role for RBM20 in DCM therefore still needs to be established,
Given the suggested malignancy of RBM20 mutations, finding mutations would suggest a closer clinical follow up, careful attention to coexistent modifiable risk factors, and earlier application of therapies proven to inhibit the process of heart failure and decrease the risk of sudden death.

References

The clinical case of the month: *What is your diagnosis?*

*Answers will be given in the next newsletter and on the web site*

Presented by Dr. Philippe Charron, Department of Cardiology, Pitié-Salpêtrière Hospital and Paris 6 University, Paris, France.

**Dilated cardiomyopathy and familial inquest**

A 42-year-old man was addressed to the department because of dyspnoea (class II NYHA) and a recent diagnosis of dilated cardiomyopathy (LV EDD 61 mm, LVEF 38%, IVS 9 mm on Echocardiography). Sinus rhythm was present on ECG, with incomplete LBBB, PR interval was 200 ms). Holter was characterized by 253 PVB/24h including one triplet, no conduction defect. After medical treatment including ACE inhibitor and betablocker, no significant PVB was observed (only few isolated PVB). Biology was unremarkable, including CK plasma level. Then an episode of near-fainting led to electrophysiological testing that demonstrated mild sinus dysfunction and mild nodal AV block. Evolution under medical treatment was satisfactory.

DCM was previously diagnosed in the father of the patient (diagnosis at 40 years of age, heart transplantation required at 52 years, non-cardiac death few days later). No cardiac examination was performed in the other members of the family (see the pedigree, Figure 1). Genetic testing was performed in the 42-year-old patient.

![Figure 1. Pedigree of the family.](image)

1/ Which cardiac examination do you recommend at that stage in the family members?

2/ Genetic testing identified a heterozygous mutation of the *LMNA* (lamin A/C) gene (p.Arg190Trp) in the propositus. **Which additional management can you propose to the family members?**
The 37-year-old sister of the propositus underwent multidisciplinary outpatient consultation and predictive genetic testing was performed. She carried the mutation (two independent molecular analyses). She had no symptoms except infrequent and short episodes of palpitations (few seconds). Cardiac examination was normal (BP 110/70 mmHg). Two-dimensional echocardiography exhibited normal LVED diameter (49 mm, 29 mm/m2) and LVEF (60%). Pulse TDI Doppler was normal. ECG indicates a normal rhythm (81/mn), normal PR interval (140 ms) (Figure 2). Exercise test (120W, 90% of maximal theoretical heart rate) was normal (especially without PVB). Holter ECG showed only 12 PVB/24h but one short run of non-sustained VT (Figure 3), no conduction defect. Usual biology was normal.

**QUESTION**

Which cardiac management do you propose now to the sister?
Answer for the previous “Clinical case of the month” presented in Oct. –Nov. issue

“A 39-year old male with complicated hypertrophic obstructive cardiomyopathy”

by Dr. Barbara Pfeiffer and Prof. Hubert Seggewiss, Medizinische Klinik 1, Leopoldina Krankenhaus Schweinfurt, Germany.

Recapitulation

The 39-year old male patient suffered from symptomatic hypertrophic obstructive cardiomyopathy (IVS max. 27 mm, MYBPC3-mutation) with dyspnea class III, palpitations and an unexplained syncope in 2001. The area of LV-obstruction was midventricular to subaortic with an intracavitatory gradient of 120 mmHg at rest and increase at Valsalva manoeuvre (Fig. 1 and 2). SAM-related moderate mitral regurgitation with SAM II-III was detected (Fig. 3). Left atrium was dilated with 56 mm.

Fig. 1: Diastolic and systolic 4- and 3-chamber-view echocardiography

Fig. 2: CW-Doppler showing midventricular and LVOT-obstruction

Fig. 3: Midventricular and LVOT-obstruction in colour Doppler and mitral regurgitation
Therapeutic strategy

As in all patients with H(O)CM therapy has to consider the two main strategies: risk stratification for the prevention of sudden cardiac death by ICD implantation and gradient reduction therapy for symptomatic improvement.

1. Risk stratification for the prevention of sudden cardiac death

According to the risk stratification model of Elliott and co-workers we identified two risk factors (unexplained exercise-induced syncope and non-sustained VT in holter-ECG) and one borderline risk factor (nearly 30mm of maximal wall thickness). Therefore, we assumed the patient being at increased risk for SCD. We discussed the risk model with the patient and recommended an implantation of a dual-chamber-ICD. The patient accepted and the implantation was done without complication.

2. Gradient reduction for symptomatic treatment

Therapeutic options for gradient reduction are medical treatment (in Germany betablocker and verapamil), surgical myectomy and septal ablation (PTSMA). Both drugs did not result in symptomatic and hemodynamic improvement. Furthermore, significant limitation of cardiopulmonary exercise testing with maximum oxygen uptake of 16.1 ml/kg/min was seen (Fig. 7). Therefore, we recommended gradient reduction. The different options of surgical myectomy and – due to the long intracavitary obstruction - stepwise septal ablation were discussed with the patient. The patient decided to undergo septal ablation.

As a first step subaortic obstruction should be treated. Coronary angiography showed an exclusion of CAD. There was a suitable septal perforator branch which splitted into two branches and the first perforator branch was selected. Myocardial-contrast-echo guided PTSMA was successfully performed with the injection of 2 cc alcohol in the proximal side branch of the first septal branch (Fig. 4) without complications, especially no AV-block. ECG changed to a right bundle branch block (Fig. 5). Maximum CK rise was 1372 U/l.

Fig. 4: Left coronary artery during first PTSMA with occlusion of the proximal side branch (arrow) of the first septal perforator.

Fig. 5: ECG before (left) and after (right) septal ablation.
At 3-months and 1 year follow-up the patient showed symptomatic improvement to class II. Echocardiography showed a mid-ventricular LV-obstruction of 70 mmHg at rest and 90 mmHg at Valsalva manoeuvre (Fig. 6) and only trivial mitral regurgitation. Basal septal thickness was reduced to 15 mm with persisting thickening of the mid-ventricular septum of 25 mm at maximum.

Maximal oxygen uptake in pulmonary exercise testing was increased to 20.9 ml/kg/min at 136 Watts (before septal ablation 16.1 ml/kg/min, 106 Watts, optimal exhaustion in both tests respectively) (Fig. 7).

As primarily intended we decided to perform a second PTSMA for reduction of the mid-ventricular obstruction. Myocardial-contrast-echo guided PTSMA was successfully performed with the injection of 2 cc alcohol in the distal side branch of the first septal branch without complications (Fig. 8). Maximum CK rise was 762 U/l.
Echocardiography after one week showed thinning of the septum and complete reduction of the LV-obstruction midventricular and subaortic (Fig. 9). Clinical follow-up after 3 months was uneventful. ICD shocks had not been detected since the first PTSMA.

**Fig. 9**: Systolic 4-chamber-view after second septal ablation

**Summary**

In this case we could demonstrate that treatment of symptomatic HOCM is complex including risk stratification for the prevention of SCD and a strategy for gradient reduction. In the last decade it could be shown that septal ablation in symptomatic patients with HOCM results in ongoing relief of clinical symptoms in more than 90% of the patients. This could be shown not only in patients with subaortic obstruction, but also in patients with mid-ventricular obstruction. Hospital mortality could be reduced to nearly 0% in experienced centres with knowledge of the special problems and complexities that can emerge in the postinterventional period. In our own series 700 consecutive patients had been treated without hospital death since February 2001. Therefore, the need for permanent pacemaker implantation in less than 10% of patients treated is in fact the most significant complication. Although in our patient we can present only mid-term follow-up without negative clinical events this case could support reported positive long-term effects after successful PTSMA. Overall, septal ablation as well as surgical myectomy should be limited to experienced centers.

**References**

List of recently published papers in the field of our WG recommended for further reading:

1. **Epsilon waves during ventricular tachycardia in a case of arrhythmogenic**

2. **Right ventricular dysplasia.**
   Menon SM, Healey JS, Morillo CA.

3. **The transcription factor GATA-6 regulates pathological cardiac hypertrophy.**

4. **Usefulness of magnetic resonance imaging to distinguish hypertensive and hypertrophic cardiomyopathy.**
   Puntmann VO. Jahnke C. Gebker R. Schnackenburg B. Fox KF. Fleck E. Paetsch I.

5. **The cardiac desmosome and arrhythmogenic cardiomyopathies: from gene to disease.**
   Delmar M and McKenna WJ.

6. **Prolactin: a new therapeutic target in peripartum cardiomyopathy.**
   Yamac H, Bultmann I, Sliwa K, Hilfiker-Kleiner D.

7. **Insights into genotype–phenotype correlation in hypertrophic cardiomyopathy.**
   Findings from 18 Spanish families with a single mutation in MYBPC3.