Journal of Electrocardiology 53 (2019) 40–43

Contents lists available at ScienceDirect

Journal of Electrocardiology

journal homepage: www.jecgonline.com

Double de novo mutations in dilated cardiomyopathy with cardiac arrest☆

Ilenia Monaco, MD [a], Rosa Santacroce, MD [a], Graziapia Casavecchia [a], Michele Correale, MD [a], Dario Bottigliero, MD [a], Giorgia Cordisco, MD [a], Angelica Leccese, MD [a], Matteo Di Biase, MD [b], Maurizio Margaglione [a], Natale Daniele Brunetti, MD, PhD [a,*]

[a] University of Foggia, Department of Medical & Surgical Sciences, Foggia, Italy
[b] GVM Care & Research, Bari, Italy

ARTICLE INFO

Keywords:
Dilated cardiomyopathy
Sudden cardiac death
Mutation
NKX2-5
RBM20
Genetic screening

ABSTRACT

Here we report the identification of two novel mutations in a previously asymptomatic young man who suffered an out-of-hospital sudden cardiac arrest. During following evaluation, diagnosis of early stage dilated cardiomyopathy was established, while electrocardiogram monitoring showed frequent complex ventricular arrhythmias, incomplete right bundle branch block and prolonged QT duration. No reversible causes explaining the clinical presentation were established and an automatic implantable cardioverter defibrillator was therefore implanted. Heterozygous mutations in human protein coding genes NKX2-5 and RBM20 are associated with a wide array of pathological phenotypes some of which are sudden cardiac death, unexplained syncope and either combined or isolated congenital heart diseases such as dilated cardiomyopathy.

Background

Dilated cardiomyopathy (DCM) is a major cause of progressive heart failure (HF) and sudden cardiac death (SCD) [1]; DCM is defined as left ventricular (LV) dilation and systolic dysfunction in the absence of abnormal loading conditions or coronary artery disease sufficient to cause global systolic impairment [2] DCM is inherited in about 30–40% of all cases and more than 50 nuclear encoded or mitochondrial genes have been found as causes in humans so far [3,4] with mutations in genes encoding cytoskeletal, nucleoskeletal, mitochondrial, and calcium-handling proteins. Potentially pathogenic genetic mutations are found in at least 20% of adults with DCM and between 10 and 20% of relatives have evidence for disease on clinical screening [5].

Sarcomere and desmosomal protein gene mutations are the most common, but mutations in lamin A/C and desmin are frequent in patients with conduction diseases [6].

Diagnosis of DCM is probable if there is one major criterion (unexplained decrease of LVEF or LV dilation) plus at least one minor criterion (complete left ventricular bundle branch, AV block, unexplained ventricular arrhythmia, segmental wall motion abnormalities in the left ventricle in the absence of intraventricular conduction defect, late enhancement of non-ischemic origin on cardiac magnetic resonance imaging, evidence of non-ischemic myocardial abnormalities, and others) or one major criterion plus carrying the causative mutation identified in the proband [7].

The study of DCM genetics has been recently improved by the introduction of next-generation sequencing (NGS) technologies, which allows the quick evaluation of more than 40 different genes associated with DCM and a comprehensive genetic analysis in shorter time and with minor costs than Sanger.

Case report

We report the case of a 26-year-old man without history of heart disease admitted to acute cardiac care unit after out-of-hospital cardiac arrest while walking. The patients was early defibrillated, intubated and admitted to intensive care unit. At admission, hemodynamic conditions were stable and physical examination unremarkable. Admission electrocardiogram showed sinus tachycardia, complete right bundle branch block and mild ST-T elevations in V1-V3. Echocardiography showed mild left ventricular enlargement and low-normal systolic function with global hypokinesis. Chest x-ray showed a normal cardiac silhouette and no signs of pulmonary venous congestion. CT scan excluded aortic dissection or acute neurological disorders.

Laboratory exams, troponin levels and drug test were negative; coronary angiography, immediately performed, was normal. The patient was cooled for 24 h and weaned without complications.

☆ Authors have no potential conflict of interest to disclose.

* Corresponding author at: Viale Pinto 1, 71122 Foggia, Italy.
E-mail addresses: rosa.santacroce@unifg.it (R. Santacroce), maurizio.margaglione@unifg.it (M. Margaglione), natale.brunetti@unifg.it (N.D. Brunetti).

https://doi.org/10.1016/j.jelectrocard.2018.12.015
0022-0736/

After weaning, the patient reported no symptoms anticipating his collapse, and excluded any possible case of hypoglycemia or dehydration. The man also reported smoke habit, but no episode of chest pain or syncope, no drug therapy, no alcohol abuse. However, there was family history of early coronary disease (father with acute myocardial infarction when 42-year old), but not of sudden cardiac death.

Electrocardiogram monitoring showed frequent premature ventricular beats and runs of non-sustained ventricular tachycardia (max 23 beats), despite drug therapy with amiodarone, metoprolol and lidocaine. An earlier electrocardiogram was characterized by atrial enlargement, incomplete right bundle branch block and mild prolongation of QT (Fig. 1).

Cardiac magnetic resonance showed mild left ventricular dilation and mildly impaired ejection fraction (50%); right ventricular dimensions and function, instead, were normal. Neither edema nor fibrosis was found. Bicameral ICD was therefore implanted in secondary

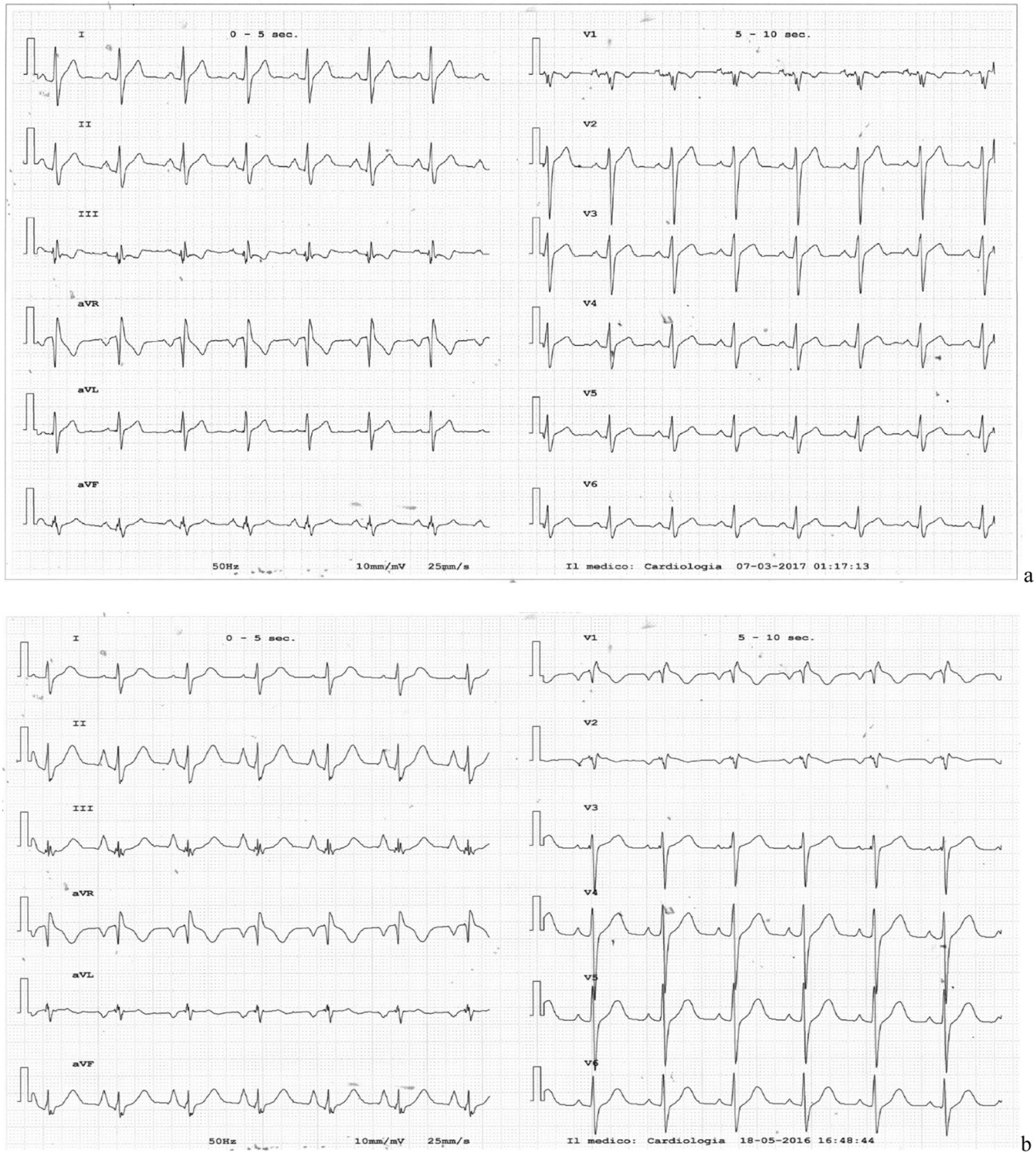

Fig. 1. Admission electrocardiogram showing mild intraventricular conduction delay. b) One year earlier electrocardiogram showing incomplete right bundle branch block, atrial enlargement and mildly prolonged QT (465 msec).

prevention according to 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Final diagnosis was dilated cardiomyopathy.

Using next-generation sequencing (NGS) (TruSight Cardio Sequencing Kit for MiSeq System- Illumina) and subsequent mutation confirmation with traditional capillary Sanger sequencing analysis, two possible causative non-synonymous mutations were identified in the patient's DNA: double heterozygosity for NKX2-5 exon 2 (c.809 G>A, p.Cys270Tyr) and RBM20 exon 2 (c680. G>T, p.Gly227Val) mutations (Fig. 2).

The two variants have not been previously described in the literature and have not been previously reported in the Human Gene Mutation Database (HGMD).

For these mutations, "in silico" analysis, which takes into consideration straightforward physical and comparative parameters, was performed to investigate the impact of amino acid substitution on the protein structure and function. The prediction software reported that this both variants should be classified as VUS ambiguous or unknown significance. The in silico pathogenicity prediction and determination of the putative effect of amino acid substitutions on protein structure were performed by using established available bioinformatics tools (Table 1).

The patient was discharged 10 days after admission in therapy with atenolol 50 mg; two-year follow up was uneventful. Echocardiography was unchanged. Device interrogation revealed frequent premature ventricular contractions, without ventricular arrhythmias.

Discussion

We found in this study for two mutations hypothetically responsible for dilated cardiomyopathy and sudden cardiac arrest: the variants c.809 G>A in the exon 2 (p.Cys270Tyr) of NKX2-5 and c.608 G>T in the exon 2 (p.Gly227Val) of RBM20. The in silico analysis performed suggests that these new variants may be harmful.

Although it has been estimated that a portion of cardiomyopathies, at least 20% to 35%, has a Mendelian basis of distribution in affected families, however not all family members carrying the same mutation are affected [8]. Actually, poligene non-Mendelian models for a series of diseases, including cardiomyopathies, have been proposed [9,10].

We herein provide evidence that the propositus carries for at least 1 other rare variant contributing to his cardiomyopathy.

Modern sequencing techniques and knock-out animal subjects have been utilized to analyze the complex mechanisms involved in such phenotypic manifestations. NKX2-5 typically is a cardiac transcription factor with a leading role in cardiac dysmorphogenesis. So far more than 50 different mutations have been identified for this gene, including missense, synonymous and nonsense mutations and only a few have been functionally characterized [11]. NKX2-5 (NK2 Homeobox 5) is a protein coding gene, located in the long (q) arm of chromosome 5 at position 35.1 (Cytogenetic Location: 5q35.1) [12]. NKX2-5 belongs to the NK2 family of homeobox genes and is a homolog of the "tinman" gene found in *Drosophila melanogaster* [13]. It represents a key regulator in cardiac morphogenesis, modulating the transcription of various genes involved in the process; in particular, NKX2-5 has been shown to interact with GATA4 and TBX5, depending on the JAK-STAT pathway, to work along with MEF2, HAND1 and HAND2 transcription factors to direct heart looping during early heart development, controlling cardiomyocite differentiation. The gene has been show to play a role in the heart's conduction system, after birth [14,15].

NKX2-5 knockout RATS showed early embryonic death, while mice with a deficit of only one copy exhibit defects in the electrical conduction system of the heart at various levels (AV node and bundle of His) with anomalies of cellular density and gap junction proteins. Mutations of NKX2-5 transcriptor factors are also known to cause ventricular noncompaction, tetralogy of Fallot, hypothyroidism non-goitrous type 5, syncope and sudden death [16].

Functional analysis of non-coding variants of NKX2-5 gene regulatory regions revealed enhanced transcriptional activity of the NKX2-5 gene promoter altering the expression levels of the aforementioned gene affecting cardiac morphogenesis [8].

RBM20 (RNA Binding Motif Protein 20) is a protein coding gene, that binds RNA and regulates splicing regulating alternative splicing of a number of genes, including TTN, CAMK2D and MYH7 [17,18]. It is located in the long (q) arm of chromosome 10 at position 25.2 (Cytogenetic Location: 10q25.2) and it is expressed mainly in striated muscle and in cardiac muscle tissue [19]. RBM20 expression is involved with sarcomere structuration, regulating cardiac isoform expression during

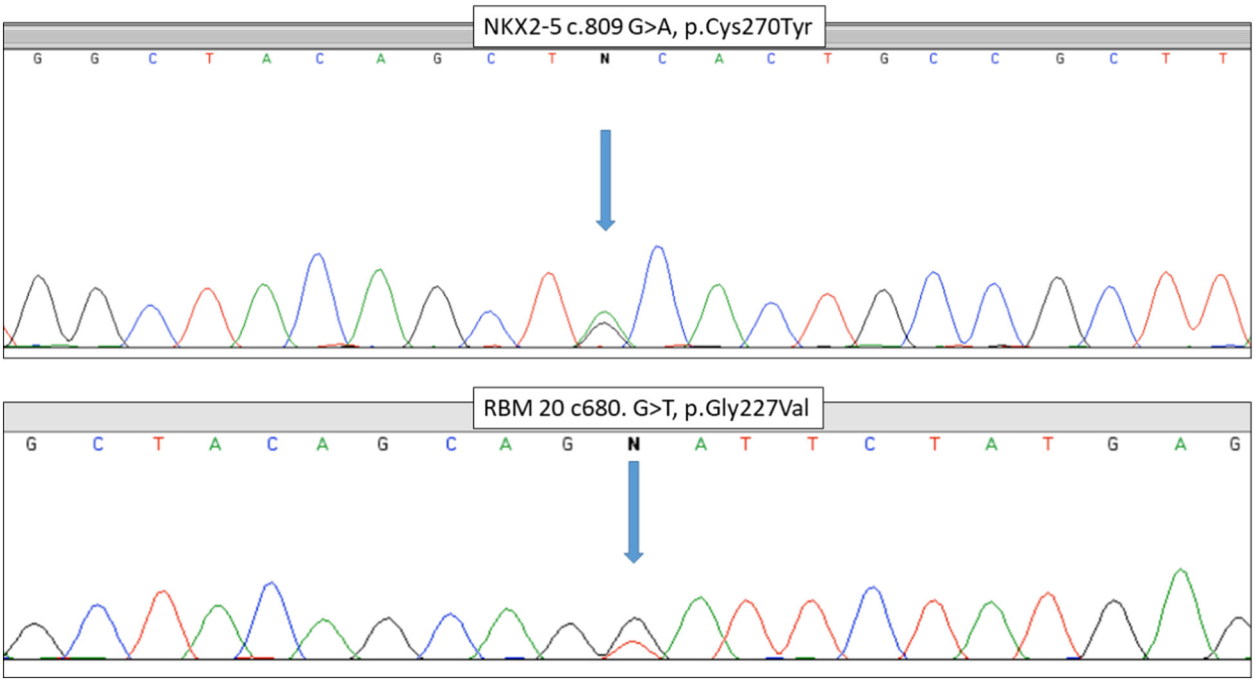

Fig. 2. Sequencing of the exon 2 of gene and exon 2 of gene confirming the novel mutations sequencing of the exon 2 of the NKX2-5 gene and the exon 2 of the RBM20 gene confirming the identification of the two new mutations p.Cys270tyr and p.Gly227Val respectively, in a condition of heterozygosis (indicated by arrows).

Table 1
In silico prediction of the effect of amino acid substitution of variations occurring in NKX2-5 and RBM20.

Variant	SIFT	Polyphen-2	CADD	REVEL	METALR	gnomeAD frequency
NKX2-5 c.809 G>A (p.Cys270Tyr)	Deleterious score 0	Benign score: 0.078	Benign	Benign score: 0.485	Deleterious score: 0.659	0.00008437
RBM20 c.608 G>T (p.Gly227Val)	Deleterious score 0	Probably damaging score: 0.775	Benign	Benign score: 0.438	Deleterious score: 0.745	0.001215

myoblasts differentiation [20]. Despite TTN is the main gene associated with DCM, TTN gene variants were searched for but no pathologic variant was found.

Mutations in RBM20 are highly penetrant and often linked with serious diseases, above all familial DCM [21,22]. RBM20 mutations are found in 1.9–3% of individuals with idiopathic dilated cardiomyopathy. Subjects with RBM20 missense mutations present cardiomyopathy with fibrosis and arrhythmia with a higher risk of sudden death [23]. RBM20 knock out rats show defects in the correct splicing of the sarcomeric gene titin; that results in a shift to different titin isoforms, affecting normal increases of stroke volume in response to an increase in ventricular volume [20,24].

RBM20 deficiency may lead to left ventricular dilatation in rats, increased diastolic diameter with unchanged systolic dimensions or contractility indices [21,23].

Interestingly, a prior electrocardiogram showed sign suspected for prolonged QT-syndrome and/or Brugada Syndrome no more present at last examinations and largely unexplained. Further studies are warranted to identify the exact role of such mutations in the development of dilated cardiomyopathy and sudden cardiac arrest.

Conclusions

Two mutations hypothetically responsible for dilated cardiomyopathy and sudden cardiac arrest are reported: the variants c.809 G>A in the exon 2 (p.Cys270Tyr) of NKX2-5 and c.608 G>T in the exon 2 (p.Gly227Val) of RBM20.

It was not possible to carry out a study to evaluate the presence of a family segregation. Therefore, the pathogenic role for both variants is yet to be defined; however, in silico studies seem to suggest a possible involvement of such variants with the patient's clinical status. We herein provide evidence that the propositus carries at least 1 other rare variant contributing to his cardiomyopathy.

Compliance with ethical standards

Authors have no conflict of interest to disclose.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

References

[1] Mestroni L, Maisch B, McKenna WJ, Schwartz K, Charron P, Rocco C, et al. Guidelines for the study of familial dilated cardiomyopathies. Collaborative Research Group of the European Human and Capital Mobility Project on Familial Dilated Cardiomyopathy. Eur Heart J 1999;20:93–102.
[2] Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J 2008;29:270–6.
[3] Kayvanpour E, Katus HA, Meder B. Determined to fail-the role of genetic mechanisms in heart failure. Curr Heart Fail Rep 2015;12:333–8.
[4] Watkins H, Ashrafian H, Redwood C. Inherited cardiomyopathies. N Engl J Med 2011;364:1643–56.
[5] Sweet M, Taylor MR, Mestroni L. Diagnosis, prevalence, and screening of familial dilated cardiomyopathy. Expert Opin Orphan Drugs 2015;3:869–76.
[6] Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, et al. Atlas of the clinical genetics of human dilated cardiomyopathy. Eur Heart J 2015;36:1123–35.
[7] Pinto YM, Elliott PM, Arbustini E, Adler Y, Anastasakis A, Böhm M, et al. Proposal for a revised definition of dilated cardiomyopathy, hypokinetic non-dilated cardiomyopathy, and its implications for clinical practice: a position statement of the ESC working group on myocardial and pericardial diseases. Eur Heart J 2016;37:1850–8.
[8] Hershberger RE, Siegfried JD. Update 2011: clinical and genetic issues in familial dilated cardiomyopathy. J Am Coll Cardiol 2011;57:1641–9.
[9] Li L, Bainbridge MN, Tan Y, Willerson JT, Marian AJ. A potential oligogenic etiology of hypertrophic cardiomyopathy: a classic single-gene disorder. Circ Res 2017;120:1084–90.
[10] Norton N, Siegfried JD, Li D, Hershberger RE. Assessment of LMNA copy number variation in 58 probands with dilated cardiomyopathy. Clin Transl Sci 2011;4:351–2.
[11] Chung IM, Rajakumar G. Genetics of congenital heart defects: the NKX2-5 gene, a key player. Genes 2016;7(2).
[12] https://www.omim.org/.
[13] Harvey RP. NK-2 homeobox genes and heart development. Dev Biol 1996;178:203–16.
[14] Snyder M, Huang XY, Zhang YY. Stat3 directly controls the expression of Tbx5, Nkx2.5, and GATA4 and is essential for cardiomyocyte differentiation of P19CL6 cells. J Biol Chem 2010;285:23639–46.
[15] Vincentz YW, Barnes RM, Firulli AB. Hand factors as regulators of cardiac morphogenesis and implications for congenital heart defects. Birth Defects Res A Clin Mol Teratol 2011;91:485–94.
[16] van Engelen K, Mommersteeg MT, Baars MJ, Lam J, Ilgun A, van Trotsenburg AS, et al. The ambiguous role of NKX2-5 mutations in thyroid dysgenesis. PLoS One 2012;7(12).
[17] MAF Khan, Reckman Yolan J, Aufiero Simona, van den Hoogenhof Maarten MG, van der Made Ingeborg, Beqqali Abdelaziz, et al. RBM20 regulates circular RNA production from the titin gene. Circ Res 2016;119:996–1003.
[18] https://www.genenames.org/.
[19] Wyles SP, Li X, Hrstka SC, Reyes S, Oommen S, Beraldi R, et al. Modeling structural and functional deficiencies of *RBM20* familial dilated cardiomyopathy using human induced pluripotent stem cells. Hum Mol Genet 2016;25:254–65.
[20] Li S, Guo W, Dewey CN, Greaser ML. Rbm20 regulates titin alternative splicing as a splicing repressor. Nucleic Acids Res 2013;41:2659–72.
[21] Mendirichaga R, De La Cruz Luque C, Smith J, Cardoso R, Bishopric N. Extended remission and late re-decompensation of dilated cardiomyopathy associated with a novel ribonucleic acid binding motif protein 20 (Rbm20) mutation. J Am Coll Cardiol 2017;69:847 (Suppl).
[22] Beqqali A, Bollen IA, Rasmussen TB, van den Hoogenhof MM, van Deutekom HW, Schafer S, et al. A mutation in the glutamate-rich region of RNA-binding motif protein 20 causes dilated cardiomyopathy through missplicing of titin and impaired Frank-Starling mechanism. Cardiovasc Res 2016;112:452–63.
[23] Guo W, Schafer S, Greaser ML, Radke MH, Liss M, Govindarajan T, et al. RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. Nat Med 2012;18:766–73.
[24] Smart N, Riegler J, Turtle CW, Lygate CA, DJ McAndrew, Gehmlich K, et al. Aberrant developmental titin splicing and dysregulated sarcomere length in Thymosin β4 knockout mice. J Mol Cell Cardiol 2017;102:94–107.