*Slov. Antropol., 21(1):6-10, 2018*

# CARDIOMYOPATHIES: CLINICAL DIAGNOSTICS AND MOLECULAR-GENETIC APPROACH

**Iveta Boroňová[1], Jarmila Bernasovská[1], Ján Kmec[2], Michaela Zigová[1], Eva Petrejčíková[1], Ivan Bernasovský[3], Daniela Grejtáková[1], Soňa Mačeková [1], Iveta Tóthová[1]**

[1] Faculty of Humanities and Natural Sciences, Department of Biology, University of Prešov, 17. november street 1, 081 16 Prešov, Slovakia, e-mail: iveta.boronova@unipo.sk
[2] Faculty of Health Care, University of Prešov, Partizánska 1, 080 01 Prešov, Slovakia, e-mail: kmecj@fnsppresov.sk
[3] Center of Languages and Cultures of National Minorities, University of Prešov, 17. november street 1, 081 16 Prešov, Slovakia, e-mail: ivan.bernasovsky@unipo.sk

**Abstract:** Cardiomyopathies with known genetic cause include hypertrophic (HCM), dilated (DCM), restrictive (RCM), arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) and left ventricular noncompaction (LVNC). Recent advances in understanding of genetic basis of cardiomyopathies present new challenges for cardiologists and clinical practice. The objective of the present study was identification of familial form of cardiomyopathies in the survey of eight patients (six males, two females) from East Slovakia with clinical diagnosis of cardiomyopathy and report of valid reccommmendations for genetic evaluation of cardiomyopathies for practical application in clinical practice. We identified one case of familial form of cardiomyopathy. In patient with familial cardiomyopathy, heterozygous variant in exon 33 of myosin-binding protein-3 (*MYBPC3*) gene – potencially pathogenic variant, six variants of uncertain significance in *TTN* and *LMNA* genes and three novel variants of *BRAF*, *MYH7* and *TAZ* genes were detected. Interdisciplinary approach to diagnosis and treatment of cardiomyopathies anticipates implementation of HFSA Guideline Approach to Medical Evidence for Genetic Evaluation of Cardiomyopathy recommendations.

**Key words:** cardiomyopathies, next generation sequencing, genetic counselling, recommendations, East Slovakia, Central Europe

## Introduction

Cardiomyopathies comprise a relatively small group of related but clinically distinguishable primary diseases of the heart muscle. Cardiomyopathies with hypertrophic, dilated, restrictive, left ventricular noncompation and arrhytmogenic right ventricular phenotypes can be cardiac conditions with inherited status (Watkins, Ashrafian and Redwood 2011). These conditions are characterized by a high risk of heart failure and they can contribute substantially to mortality before the fifth decade of life.

Cardiomyopathies have known genetic etiologies. The genetic diagnostic procedures including genetic testing are the best way how to effective and soon estimate the pattern of inheritance, detect the causal mutations, elucidate the clinical overlaps among cardiomyopathy conditions or provide the genetic counselling in affected families (Oliveira et al. 2015). In this regard the HFSA established the recommendations as Guideline approach to medical evidence for genetic evaluation of cardiomyopathy. The strengths of recommendations in this Guideline are identical to those in the general Guideline.

This guideline describes the approach and expertise needed for the genetic evaluation of cardiomyopathies. Based on listed recommendations, clinical applications with consideration of molecular-genetic examinations results is possible.

**Subjects and methods**

The patients were diagnosed by cardiologist according to criteria provided by the World Health Organization and ESC Guidelines. Written, informed consent was obtained from all probands and all institutional ethics requirements were met.

A total of eight patients (six males and two females, mean age 53.13±13.25 years) with clinical diagnosis of cardiomyopathy recruited from Cardiocenter at Faculty Hospital of J.A Reyman in Prešov (Slovakia) were subjected to next generation sequencing analysis. Peripheral whole-blood samples of patients were collected during examination and DNA was extracted using RepliaPrep$^{TM}$BloodgDNA isolating kit (Promega, Madison, USA) following the manufacturer's instructions. An amount of at least 2 μg of genomic DNA at a minimum concentration of 50 ng/μl extracted from patients was used for exome sequencing based on bridging amplification after library preparation and reversible dye terminator for sequencing purposes. Next Generation Sequencing (NGS) of patient´s with clinical diagnosis of cardiomyopathy included 90 cardiomyopathy-associated genes: *ABCC9, ACTC1, ACTN2, ADRB1, ADRB2, ADRB3, AGL, ANK2, ANKRD1, BAG3, BRAF, CALR3, CAV3, CBL, CRYAB, CSRP3, CTF1, DES, DMD, DSC2, DSG2, DSP, DTNA, EMD, EYA4, FHL1, FHL2, FKTN, FLNC, FXN, GAA, GLA, HRAS, ILK, JPH2, JUP, KRAS, LAMA4, LAMP2, LDB3, LMNA, MAP2K1, MAP2K2, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOT, MYOZ2, MYPN, NEBL, NEXN, NRAS, PDLIM3, PKP2, PKP4, PLEC, PLN, PNN, PRKAG2, PSEN1, PSEN2, PTPN11, RAF1, RBM20, RPSA, RYR2, SCN5A, SDHA, SGCD, SHOC2, SLC25A4, SOS1, SPRED1, SYNE1, SYNE2, TAZ, TCAP, TGFB3, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, VCL*. Exome data from cardiomyopathy-associated genes were analyzed using an algorithm that filtered results on genotype quality, frequency, and database information. The goal of our study also was identification of familial form of cardiomyopathies which allow application of the HSFA recommendations (Fig. 1).

**Results**

NGS analyses of selected cardiomyopathy-associated genes declosed 51 nucleotide variants. Online bioinformatics software (*PolyPhen-2, SIFT* and *MutationTaster*) was used to predict the functional effects of the altered proteins in patients with cardiomyopathy. Using next-generation sequencing non-synonymous variants possibly acting as disease modifiers were identified in 37.5% of patients with cardiomyopathy. We identified one case of familial form of cardiomyopathy. In patient with familial cardiomyopathy, heterozygous variant in exon 33 of myosin-binding protein-3 (*MYBPC3*) gene – probably pathogenic variant, six variants of uncertain significance in *TTN* and *LMNA* genes and three novel variants of *BRAF, MYH7* and *TAZ* genes were detected. The downstream analysis of the patient's sample identified the presence of several sequence variants. One heterozygous nucleotide variant was identified in exon 7 of the *LMNA* gene. The available information at this time is not sufficient to associate this variant with the clinical features described in the patient. Therefore it is classified as a „variant of uncertain significance". The patient´s sample presents next heterozygous variants in exon 305, exone 296, exone 29, exone 6 and exone 155 of the *TTN* gene, also. It is necessary to confirm the presence of these variants with an additional methodology (Sanger sequencing). By analyzing patient´s family anamnesis it has be found that patient´s daughter with cardiomyopathy subject to sudden cardiac death at the age of 29 years old. As far as this case is concerned, at this time in cooperation with a cardiologists we continue in analyses of patient´s relatives regarding of the clinical, biochemical and molecular-genetic examinations. Based on „HFSA Guideline Approach to Medical Evidence for Genetic Evaluation of Cardiomyopathy" we recommended extending the co-segregation analysis of the variant to other

family members including affected and healthy individuals in order to establish if this variant co-segregates with the pathology. We recommended beginning the familial studies by parents to rule out if the variant has been inherited or if it has appeared as a consequence of a „*de novo"* event and following to offer a genetic counselling in a familial context.

**Discussion**

Exome and whole genome tests are increasingly applied in clinical medicine. In our study we have successfully applied NGS technology to the simultaneous analysis of genes related to cardiomyopathies. The analyses were focused on a restricted of genes that encode proteins involved in heart functions or that might be related to the development of cardiomyopathies and and detection of familial form of cardiomyopathies.

Cardiomyopathies are genetically heterogeneous disorder caused by mutations in multiple genes. The genetic diagnosis of cardiomyopathy relies on completely sequencing the coding regions of cardiomyopathy genes since most pathogenic variation is rare or private. Common variation is atypical in inherited cardiomyopathy, although common variants may modify disease phenotype. Over the past years, NGS methods have dramatically improved, allowing now the analysis of gigabases of sequence information in one single run (Wheeler et al. 2008). In contrast to Sanger sequencing, NGS techniques are efficient, fast and cost-effective (Herman et al. 2009, Sikkema-Raddatz et al. 2013).

In our study, possible causative non-synonymous mutations were identified in 37.5% of patients with clinical diagnosis of cardiomyopathy. In analysed survey of patients with cardiomyopathy, we identify one case of familial form of disease. In this patient, pathogenic heterozygous variant in exon 33 of *MYBPC3* gene was identified. *MYBPC3* encodes the cardiac isoform of myosin-binding protein C. Myosin-binding protein C is a myosin associated protein found in the cross-bridge-bearing zone (C region) of A-bands in striated muscle. *MYBPC3*, the cardiac isoform, is expressed exclusively in heart muscle. Regulatory phosphorylation of the cardiac isoform in vivo by cAMP-dependent protein kinase (PKA) upon adrenergic stimulation may be linked to modulation of cardiac contraction. Mutations in *MYBPC3* are associated with familial hypertrophic cardiomyopathy, dilated cardiomyopathy and left ventricular non-compaction cardiomyopathy. In patients with familial cardiomyopathy, subsequent analyzes confirmed the presence of heterozygous variant in exon 33 of myosin-binding protein-3 (*MYBPC3*) gene – probably pathogenic variant, six variants of uncertain significance in *TTN* and *LMNA* genes and three novel variants of *BRAF, MYH7* and *TAZ* genes. Once confirmed, it is recommended to perform a co-segregation analysis of these changes in affected and unaffected relatives of the patient in order to further investigate their possible involvement in the patient's condition.

Next generation sequencing technology is widely practiced in medical research. Whole genome sequencing and target sequencing can identify the genetic cause of a disease (primary results), but it can also identify pathogenic variants underlying diseases that are not being sought (secondary or incidental results). A major controversy has developed surrounding the return of secondary results to research participants. Estimating disease risk from an individual's genetic profile for disease prevention and treatment is the objective of personalized medicine. Although genomic medicine may appear within reach, the development and finetuning of the analyses and clinical interpretation of sequencing data remains a major hurdle. New sequencing technologies are redefining the understanding of genotype-phenotype relationships, even if the interpretations of the numerous identified variants pose several challenges. Although sequence variants within disease genes can be reliably detected by NGS, proving their disease causality by cosegregation or functional genomics is key to the genetic diagnosis of cardiomyopathies.

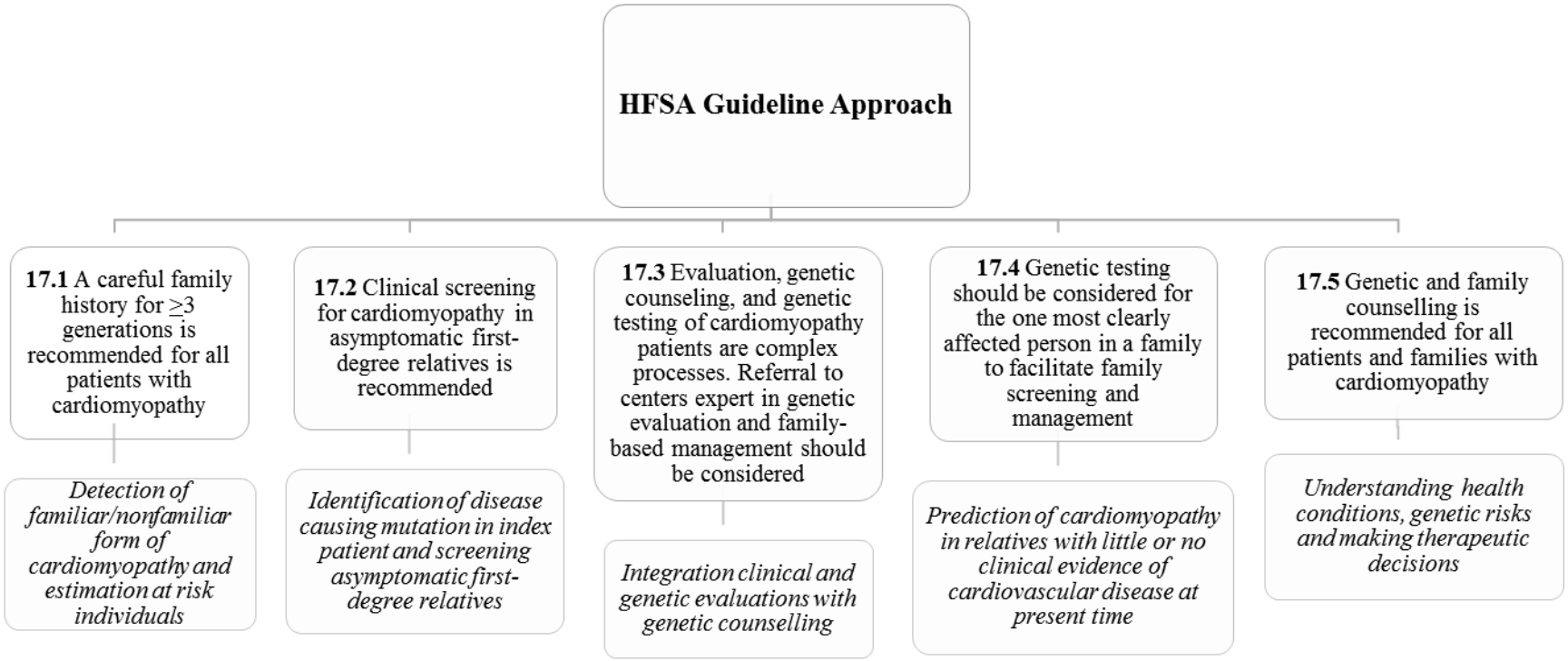


Fig. 1: HFSA Guideline Approach to Medical Evidence for Genetic Evaluation of Cardiomyopathy modified by Hershberger et al. (2009)

## Conclusion

Multi-gene testing using NGS is a highly accurate and reproducible approach to the routine molecular genetic testing of patients with cardiomyopathies. In familial forms of cardiomyopathies, it is important to search for risk individuals using FHSA recommendations. As genetic testing is a useful tool in the clinical management of disease, testing for pathogenic mutations is beneficial to the treatment of patients with cardiomyopathy and may assist in predicting disease risk for their family members before the onset of symptoms.


## Acknowledgment

This study is the result of implementation of projects APVV-0644-12 and ITMS 26220120041.


## References


HERMAN, D. S., HOVINGH, G. K., IARTCHOUK, O., REHM, H. L., KUCHERLAPATI, R., SEIDMAN, J. G., SEIDMAN, C.E., 2009: Filter-based hybridization capture of subgenomes enables resequencing and copy-number detection. *Nat. Methods*, 6(7):507-510.

HERSHBERGER, E., LINDENFELD, J., MESTRONI, L., SEIDMAN, CH. E., TAYLOR, M.R.G., TOWBIN, J. A., 2009: Genetic Evaluation of Cardiomyopathy – A Heart Failure Society of America Practice Guideline. *J. Card. Fail.*,15(2):83-97.

SIKKEMA-RADDATZ, B., JOHANSSON, L. F., DE BOER, E. N., ALMOMANI, R., BOVEN, L. G., VAN DEN BERG, M. P., VAN SPAENDONCK-ZWARTS, K.Y., VAN TINTELEN, J. P., SIJMONS, R. H., JONGBLOED, J. D., SINKE, R. J., 2013: Targeted next-generation sequencing can replace Sanger sequencing in clinical diagnostics. *Hum. Mutat.*, 34(7):1035-1042.

JACOBY, D., MCKENNA, W.J., 2012: Genetics of inherited cardiomyopathy. *Eur. Heart. J.*, 33 (3):296-304.

OLIVEIRA, T. G. M., MIGUELMITNE-NETO, L., CERDEIRA, T. J., MARSIGLIA, D. C., ARTEAGA-FERNANDEZ, E., KRIEGER, J. E, PEREIRA. A. C., 2015: Variant Detection Pipeline for Inherited Cardiomyopathy–Associated Genes Using Next-Generation Sequencing. *The Journal of Molecular Diagnostics*, 17(4):420-430.

WATKINS, H., ASHRAFIAN, H., REDWOOD, C., 2011: Redwood. Inherited cardiomyopathies. *N. Engl. J. Med.*, 364(17):1643-1656.

WHEELER, D.A., SRINIVASAN, M., EGHOLM, M., SHEN, Y., CHEN, L., MCGUIRE, A., HE, W., CHEN, Y.J., MAKHIJANI, V., ROTH, G.T., GOMES, X., TARTARO, K., NIAZI, F., TURCOTTE, C.L., IRZYK, G.P., LUPSKI, J.R., CHINAULT, C., SONG, X.Z., LIU, Y., YUAN, Y., NAZARETH, L., QIN, X., MUZNY, D.M., MARGULIES, M., WEINSTOCK, G.M., GIBBS, R.A., ROTHBERG, J.M., 2008: The complete genome of an individual by massively parallel DNA sequencing. *Nature*, 452:872-876.