Molecular diagnostic approaches to CMs.

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INTRODUCTION

Approximately 50-60% of hypertrophic cardiomyopathies (HCM) and 20% dilated cardiomyopathies (DCM) are inherited as an autosomal dominant trait caused by mutations in cardiac sarcomere protein genes (1, 2). Although current clinical statements recommend routine genetic testing of patients with HCM, DCM and ARVC, its use in everyday clinical practice has been limited by the cost and complexity of conventional sequencing technologies. Massive parallel sequencing (or next-generation sequencing) can overcome these obstacles, but may also pose new challenges (in particular, in case of rare variants). According to guidelines a molecular genetic diagnosis may be useful in families with cardiomyopathies (CMs) in situations in which clinical diagnosis is not possible (*) and in which the diagnosis will change the management of the patient (&). (*) families with extremely high incidence of sudden cardiac death (SCD) or heart failure in young individuals (&) at highly athletic members of families with family history (3).

We used the Haloplex Cardiomyopathy (Agilent Technologies, Santa Clara, USA) as a next generation sequencing target enrichment panel designed specifically for inherited forms of CMs. Focused genes are described in ***Table n.1***.

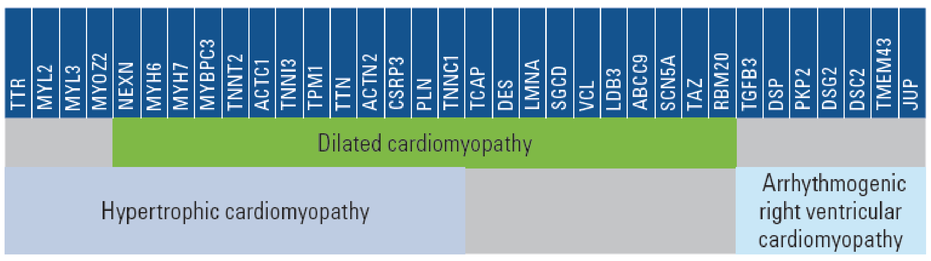

***Table n. 1**: Mutations in some genes are responsible for a special type of CM, but other can cause both, DCM or HCM.*

Our patients with different kinds of CMs were selected with regard to two parameters: family history and yearly onset manifestation of the disease. All patient provided written informed consent, received genetic counselling. DNA was isolated from peripheral blood lymphocytes using DNA extraction kit (Qiagen, Valencia, CA, USA).

CONFIRMATION, QUANTIFICATION OF THE LIBRARY AND NGS SEQUENCING

Principle of target enrichment and library preparation is presented in ***Figure n. 1***.. PCR afficacy was confirmed by using microchip electrophoresis MultiNA MCE®-202 (Shimadzu, ***Figure n. 2A and B***), the library concentration was measured on Fluorometer DQ300 (Hoefer, San Francisco, CA, USA) and accuracy of molarity was determined by Kapa library quantification kit (Kapa Biosystems, Wilmington, MA, USA). The NGS library was mixed with PhiX DNA (20%), its final concentration (6pM) was sequenced using paired-end, 150-cycle chemistry on the Illumina MiSeq 2000 (Illumina, San Diego, CA). Approximately 90% of bases had base call quality scores >Q30, with next characteristics: 850K clusters/mm2 and 94% of reads filter passed (600Mb data). Data reads were aligned and processed with NextGENe® Software trial version (SoftGenetics, USA).

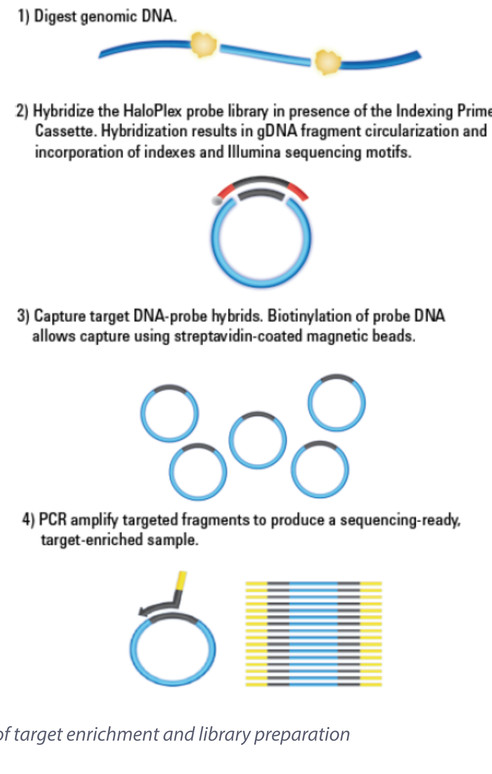

***Figure n. 1**: Principle of target enrichment and library preparation*

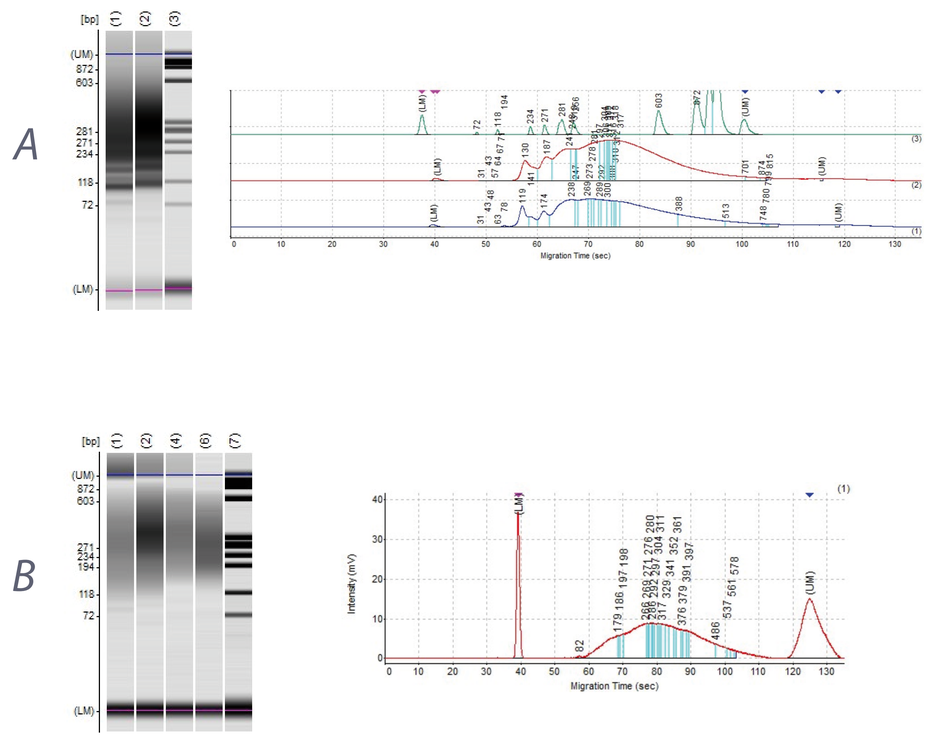

***Figure n. 2**: Confirmation and quantification of the library (A-before purification, B-after purification. The aim of purification is removal of unwanted primer-dimers and primer-adapter dimers).*

REVEALED CAUSAL MUTATIONS

GENE	TYPE OF CM	MUTATION
MYH7	HCM	L961R http://genepath.med.harvard.edu/~seidman/outdated-mutdb/muts/MYH7_mutations_TOC.html
TTN	DCM	c. 53848_53849insC, p. Fs17950L
TTN	HCM	FS: 7687V indel (del12ins1)
TTN	DCM, HCM	R8500C R8500H described by Arimura et al. 2009 (4) as a mutation that causes higher binding capacity of titin to CARP.

All pathogenic variants were confirmed by conventional Sanger sequencing by using BD v.3.1 chemistry. Predictive diagnostics for family members in risk were also completely performed.

CONCLUSION

In the study we identified a large number of novel TTN variants (non synonymous SNPs-nsSNPs). The significance of nsSNPs is difficult to assess. Novel missense variants were predicted in silico (SIFT and Polyphen2) to decide if they are pathogenic. All missense variants were also evaluated considering the database LOVD, which include data from 400 healthy individuals. Twenty one percentages of nsSNPs were predicted as probably demaging and it is assumed that they serve at least as modifiers of phenotype and their effect can be potentiated by the presence of rare variants in other genes associated with cardiomyopathies.

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