Molecular diagnostic approaches to CMs.

Cardiomyopathy 2015-new ESC guidelines on hypertrophic cardiomyopathy
London, Great Britain, May 29, 2015

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KEYWORDS: cardiomyopathy enrichment panel, next generation sequencing

FUNDING: The study was supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00179906.

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 diagnostic test 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 (2). (*) families with extremely high risk of sudden cardiac death (SCD) or heart failure in young individuals (5). (2) Atheltic members of families with family history (3).

We used the Haplokom Cardiomyopathy (Agilens 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.7.

CONFIRMATION, QUANTIFICATION OF THE LIBRARY AND NGS SEQUENCING

Principle of target enrichment and library preparation is presented in Figure n. 1. PCR efficacy was confirmed by using microchip electrophoresis (Agilent MCE-202: Omniscript). Figure n. 24 and B, the library concentration was measured on FemtoDx2080 (Hamilton, San Francisco, CA, USA) and accuracy of molarity determination 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 filtered passed (600Mb data).

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

<table>
<thead>
<tr>
<th>GENE</th>
<th>TYPE OF CM</th>
<th>MUTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH7</td>
<td>HCM</td>
<td>R8500H</td>
</tr>
<tr>
<td>TTN</td>
<td>DCM</td>
<td>p. F8176del (del12ins1)</td>
</tr>
<tr>
<td>TTN</td>
<td>HCM</td>
<td>p. M6548K (del12ins1)</td>
</tr>
<tr>
<td>TTN</td>
<td>DCM, HCM</td>
<td>R8500C</td>
</tr>
</tbody>
</table>

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 SNP-mSNP). The significance of mSNPs is difficult to assess. Novel missense variants were predicted in silico (SIFT and PolyPhen-2) 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 impact can be potentiated by the presence of rare variants in other genes associated with cardiomyopathies.

REFERENCES


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-adaptor dimers).