Editorial

Identification of a mutation causing hypertrophic cardiomyopathy using whole exome sequencing: A proof-of-concept

Hypertrophic cardiomyopathy (HCM) is caused by an autosomal dominant mutation in genes that encode sarcomere proteins. The accumulated evidence indicates that mutations in 8 sarcomere protein genes definitively cause HCM: cardiac β-myosin heavy chain (MYH7), cardiac myosin binding protein-C (MYBPC3), ventricular regulatory myosin light chain (MYL2), ventricular essential myosin light chain (MYL3), cardiac troponin T (TNNT2), cardiac troponin I (TNNT3), α-tropomyosin (TPM1), and cardiac actin (ACTC). These sarcomere protein genes have stood the test of time and now underpin genetic tests for HCM. Truncating mutation of titin gene (TTN), which is responsible for 20% of DCM cases, is rarely identified in HCM patients [1]. The present guidelines [2,3] recommend genetic testing for HCM patients. When a definite pathogenic mutation is identified in an index patient, the genetic status of each family member can be readily ascertained, which facilitates the identification of family members at risk for developing HCM (i.e. cascade genetic screening). Sarcomere protein gene mutations are known to have a strong class effect on clinical presentation, cardiac left ventricular morphology, and survival [4]. At present, however, due to the lack of robust data on specific genotype-phenotype correlation, the impact of genetic testing on clinical management is limited. From a clinician's viewpoint, identifying the cause of the disease would be highly appreciated, even if it does not necessarily contribute directly to the management of the disease at present. Currently, at least 8 sarcomere protein genes should be sequenced in HCM patients.

In this issue of the Journal of Cardiology, Nomura et al. [5] performed whole exome sequencing (WES) in a large HCM family to successfully identify the previously reported Arg94His variant in MYL3 [6,7] as a definite HCM-causing mutation. HCM-causing mutations in MYL3 are very few in contrast with those in MYH7 and MYBPC3. In the systemic screening of mutations in these 8 sarcomere protein genes in 112 unrelated Japanese patients with familial HCM, mutations in MYBPC3, MYH7, and TNNT2 were found in 19.6%, 10.7%, and 8.9% of cases, respectively, while analysis of MYL2, MYL3, and ACTC did not reveal any mutations [8]. It can be said that a comparably rare HCM-causing mutation was successfully identified using WES in this study [5]. In hindsight, however, the same result could have been obtained even using the traditional Sanger sequencing of 8 sarcomere protein genes. Given that, the success story that WES worked remarkably well as a powerful tool for detecting the HCM-causing mutation, rather than the identified mutation by itself, seems noteworthy. This study clearly showed that the WES combined with integrated variant annotation prediction could contribute to the identification of HCM-causing mutations. This proof-of-concept study can be considered as a milestone toward the realization of clinical sequencing for HCM patients. However, before the clinical sequencing is routinely performed, a significant number of issues as described below must be resolved.

First, we should establish and standardize the data-processing (annotation/filtering) methods for interpreting the pathogenic status of each sequence variant in the massive amount of WES data so that we can efficiently and reliably identify the HCM-causing mutation [9,10]. Determining whether the identified sequence variant is a pathogenic disease-causing change or a benign variant of no clinical significance is a key step in the genetic testing process, which remains a major challenge. At present, a variant is considered to be causative of HCM based on the following criteria: (i) a missense variant with an amino acid change at a highly conserved position among species, (ii) a variant altering protein structure and function (e.g. insertions/deletions causing a reading frameshift, nonsense variants, and splice-site variants), (iii) previously reported as an HCM-causing mutation, (iv) co-segregation with disease in the HCM family, and (v) absence of the variant or in <1% among unrelated and ethnically matched controls [11]. Most importantly, even when interpreting the WES data, segregation analysis remains the gold standard for the acquisition of robust evidence for pathogenicity/causality [12]. Actually, in this study [5], genotype–phenotype matching (co-segregation pattern) enabled the number of putative variants to be narrowed down from 3439 to only 13. In order to efficiently and correctly define the causative mutation, the sequencing of DNA from relatives as well as the index patient is thought to be essential. Also, the prediction of in silico pathogenicity for variants using the Combined Annotation-Dependent Depletion (CADD) prediction software [13] as well as the high heart expression gene

---

**Keywords:** Hypertrophic cardiomyopathy, Mutation, Whole exome sequencing, Clinical sequencing

---

[DOI of original article: http://dx.doi.org/10.1016/j.jjcc.2015.09.003](http://dx.doi.org/10.1016/j.jjcc.2015.09.003)
data [14] played an important role in bioinformatics filtering in this study [5]. Further improvement in prediction tools and relevant databases should contribute to the more successful identification of the causative mutation.

Along with them, in order to identify the causative mutation efficiently and reliably, detailed information on the frequency of variants both in healthy control subjects and HCM patients is essential. Next-generation, high-throughput sequencing methods are providing extensive catalogs of human variation in the general population (i.e. healthy control subjects). The extent of rare (<1%) variation with an amino acid change in individual genomes turned out to be massively greater than anticipated. Of note, a low allele frequency is a necessary but in no way sufficient criterion for a plausible disease-causing mutation; that said, the rarity of a variant does not necessarily mean it is pathogenic. Recently, many variants previously described as novel and disease-causing have been found to be present in these extensive catalogs, implying that some of them might actually be benign rare variants of no clinical significance. In the Exome Sequencing Project, the prevalence of previously reported HCM-associated variants was shown to be more than 100 times higher than expected from the general population (1:4 versus 1:500) [15]. Considering these findings, whether the identified sequence variant was previously reported as an HCM-causing mutation or not should not be emphasized in the annotation/filtering following WES. Now, construction of an updated extensive catalog of truly HCM-causing mutations is essential for the realization of clinical sequencing for HCM patients. We should perform the large-scale WES research project with the standardized annotation/filtering methods in HCM patients registered nationwide, and establish a robust database of the overall picture of HCM-causing mutations. Importantly, periodic reassessment of this mutation data based on newly available genomic information is needed [11].

Next, we must strive to understand clearly the genotype-phenotype correlation so that we can predict the prognosis (e.g. sudden cardiac death risk) and the response to therapy using the genetic information. In this regard, a nationwide prospective database of the genotype-phenotype correlation in HCM patients should be established.

Finally, before the routine use of clinical sequencing, its clinical usefulness should be also verified. For example, it should be explored whether clinical sequencing could provide us with additional information that is useful for diagnosis/management beyond the present diagnostic methods (e.g. echocardiography, cardiac magnetic resonance imaging). In addition, ethical, legal, and social issues as well as profit-cost balances should be assessed.

It will take years of effort to solve these many issues in genetic research before clinical sequencing is routinely performed. Where should we go from here? In HCM patients and their extended family members, the target resequencing analysis of a predefined panel of commonly implicated 8 sarcomere protein genes should be performed using next-generation sequencing. If no causative mutation is found in sarcomere genes, participation in the WES research project (shown above) is recommended for clarifying the full repertoire of HCM-causing mutations. These causative mutations have to be recorded in combination with the clinical phenotype. We are just taking the first step toward clinical sequencing for HCM patients. In order to achieve routine clinical sequencing in everyday cardiology practice, further large efforts are needed.

**References**


Hiroyuki Morita (MD, PhD, FJCC) Department of Cardiovascular Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

*Correspondence to: Department of Cardiovascular Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Honjo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: +81 3 5800 9170; fax: +81 3 5800 9171 E-mail address: hmrtn-tky@umin.net (H. Morita).*