Since mutation of the myosin heavy chain-coded gene in human hypertrophic cardiomyopathy (HCM) was first demonstrated in 1990,3 more than 17 causative genes that are responsible for the occurrence of HCM have been reported.3,4 In addition, causative gene mutations have been found in other Mendelian cardiovascular diseases, such as inherited arrhythmic syndrome or familial hypercholesterolemia.5 As for clinical implications, the presence of a gene mutation could be related to the possible occurrence of adverse cardiac events in HCM.5,6 Under these conditions, the dideoxy chain termination reaction known as direct DNA sequencing, or “Sanger sequencing”,5 has been an indispensable method for identifying causative gene mutations in patients with HCM and other Mendelian diseases.6 However, there are patients typically diagnosed as HCM who do not have an identifying gene mutation, even in the presence of condensed family history,7 suggesting a limitation of Sanger sequencing.

DNA sequencing was revolutionized when the Solexa sequencing-by-synthesis and 454 pyrosequencing technology, a next-generation sequencing (NGS) method, was first released in 2005. In contrast to Sanger sequencing, NGS has enabled handling of huge genomic data in a very short amount of time. To date, NGS has become widely used for genome-wide association study, whole-exome and whole-genome sequencing,8 and the number of projects using NGS has been steadily increasing. NGS is no longer “next” generation, but “now” generation sequencing.9

When a new diagnostic tool is applied to clinical medicine, there are 2 additional issues to overcome: cost and reliability. The sequencing cost, including all direct and indirect items, has significantly decreased beyond the Moore’s law estimation with the rise of NGS (Figure 1);10 as of April 2014, whole-genome sequencing (×30) cost US$4,920 and whole-exome sequencing (50Mb, ×50) only US$137 per individual. As for reliability, one of the next-generation sequencers, MiSeq Dx™ (Illumina, San Diego, CA, USA) has been approved by the US Food and Drug Administration (FDA) for clinical diagnosis of cystic fibrosis,11 because of its extremely high accuracy rate, which is precise enough to replace Sanger sequencing. However, the FDA has not yet approved its use to clinically diagnose other diseases, because the reliability of using NGS for other diseases, including HCM, is still ambiguous.

In this issue of the Journal, Gómez et al12 highlight the efficacy and cost-effectiveness of amplicon sequencing with pooled samples using a semiconductor NGS (Ion Torrent PGM™, Life Technologies, NY, USA) in HCM. Amplicon sequencing is a target resequencing method, which enriches targets of specific DNA regions by multiplex PCR (Figure 2). An Ion AmpliSeq™ custom panel was used in this study to perform the process (especially generating each target primer of 9 HCM genes) with as little as 10ng of DNA for each sample. They were individually barcoded and pooled together in a couple of tubes simultaneously, thus resulting in significant reduction of sequencing costs. After validating traditional direct sequencing, known and novel variants in MYH7 (myosin heavy chain), MYBPC3 (myosin binding protein C), TNNI3 (troponin T), TNNT2 (troponin I), MYL3 (regulatory myosin light chain), MYL2 (essential myosin light chain), TPM1 (tropomyosin) and ACTC1 (actin alpha cardiac muscle) genes were successfully identified with sensitivity of 100%, specificity of 97% (single nucleotide variants: SNVs) and that of 90% (insertions and deletions [indels]). Novel variants were evaluated by certain damaging score calculators such as SIFT and Polyphen 2 to validate its deleteriousness in silico analysis.

The most impressive finding of the present study was that this amplicon sequencing method for pooled HCM samples demonstrated high accuracy rates (sensitivity 100%, specificity 97%) with extremely low cost as for the detection of SNVs. Sekkema-Raddatz et al13 also reported that the discordant rate between target resequencing and Sanger sequencing in HCM patients using MiSeq™ sequencer (Illumina) with SureSelect™ Target Enrichment Kit (Agilent Technologies, Santa Clara, CA, USA) was only 0.00315%, and estimated sensitivity was 97.7–100% at 95% confidence interval. As for a cost problem, the authors mentioned that the cost for library preparation was significantly decreased from €40,800 (Sanger sequencing) to €2,400 (amplicon sequencing) per whole-sample sequencings. Taking these points into consideration, NGS-driven target resequencing such as amplicon sequencing for identifying SNVs could have almost the same reliability with...
is the only system approved by the FDA for diagnosis of cystic fibrosis. It needs a little more time for NGS to completely replace Sanger sequencing in the clinical setting.

The most fundamental but most important method of identifying disease-causing variants in the NGS era is still detailed medical interview and clinical examination. NGS is one of the methods for research objectives, even though it has tremendous potential to identify causative mutations for many diseases. However, there are still some limitations to using this method for clinical diagnosis. First, as the authors mentioned in the present article, Ion Torrent PGM still has a tendency to produce homopolymer-associated indel errors, which could affect the accuracy of detecting indel evaluation. Second, this system has not been authorized for use as a tool for clinical diagnosis (research use only). To date, Illumina MiSeqDx is the only system approved by the FDA for diagnosis of cystic fibrosis.

Figure 1. Transition of sequencing costs before and after the next-generation sequencing (NGS) era. Since the NGS-derived cost in 2008, the sequencing costs have dramatically decreased even compared with Moore's law estimation. Latest data (April, 2014) show the cost per human genome (30x) and human exome (50Mb, 50x) as US$4,920 and $137, respectively. The costs include all the production costs of sequencing instruments, library constructions, wages and salaries, informatics activities, submission of data to a public database, and indirectly that which relates to previous items (Data generated by NHGRI).

Figure 2. Multiplex amplification method in targeted gene regions (amplicon sequencing) utilizes the power of PCR to enrich target gene regions from only a small sample of DNA (Ion AmpliSeq needs only 10 ng). Samples are applied to barcoded array-based multiplex amplification, pooled together, and sequenced and analyzed simultaneously.
NGS to Replace Sanger for Screening Variants?

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References


Inaccurate phenotype information will produce nothing, or will be even harmful to validation of true genotype-phenotype correlation. Therefore, we once more need to go back to basics and reconsider the importance of exact phenotypic evaluation, in order to bring out the full potential of this promising NGS methodology.