Impact and cost benefits of targeted panel genetic testing by next-generation sequencing: learning and implementing bioinformatics locally

Chong YK(1), Siu WK(1), Mak CM(1), Mok NS (2), Lee KC (1)
(1) Department of Pathology, Princess Margaret Hospital (2) Department of Medicine and Geriatrics, Princess Margaret Hospital

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Introduction
Molecular genetic testing has been an established diagnostic technology available in many HA hospitals. Currently, all sequence-based genetic testing in HA are performed with the first generation Sanger sequencing. Next-generation sequencing (NGS), widely used in research as well as clinical settings world-wide, offers significant cost-reduction when compared with traditional Sanger sequencing when dealing with disorders with large genetic heterogeneity.

Objectives
To establish and validate a laboratory standard operating procedure and bioinformatics pipeline for the purpose of targeted panel genetic testing using TruSight One reagent kit (Illumina, CA) in a next-generation sequencer (MiSeq, Illumina, CA). A panel of 35 cardiac genes related to arrhythmia and cardiomyopathy were investigated and analyzed.

Methodology
Sequencing results were obtained from Illumina MiSeq sequencer, and the results generated by the bioinformatics pipeline was validated with a state-of-the-art commercially available software (NextGENe, Softgenetics, PA), as well as software supplied by manufacturer (MiSeq software, Illumina, CA). Positive genotypes were validated by Sanger sequencing. Cost effectiveness was evaluated by comparing the cost of a typical Sanger sequencing panel for cardiomyopathy (MYH7, MYBPC3; total 11,124 bp) and a typical next-generation sequencing gene panel (35 genes as used in
the research study for sudden arrhythmias death syndromes; 130,027 bp).

**Result**

A set of laboratory standard operating procedure, together with a complete bioinformatics pipeline was setup. Ten NGS results were analyzed with the established bioinformatics pipeline for the specified 35-gene panel. Coverage and quality of the data were analyzed and considered satisfactory. The established bioinformatics workflow performed at least as good as the commercially available software evaluated, and is better than the software supplied by the equipment manufacturer. It is noteworthy that the cost of Sanger sequencing for 2 cardiac genes (reagent cost, $17,750) is 2 times more than a panel of 35 cardiac genes by NGS (reagent cost, $7,120). In addition, targeted gene panel evidently offers better diagnostic efficiency and faster turnaround time. The time from planning to successful validation and implementation was four months for this project. In conclusion, for targeted panel testing using NGS, the technology has evolved such that extensive on-site bioinformatics support is no longer a necessity but a skill readily acquirable by medical and scientific colleagues, and our project proved that NGS for targeted testing is ready for clinical applications in HA hospitals.