Concordance between variants detected by clinical exome, gene panel and Sanger sequencing

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Introduction

Exome sequencing (ES) is becoming a preferred methodology for detecting DNA changes in genetic diseases with no known molecular cause or no definitive diagnosis. This results from the fact that next-generation sequencing technology allows a greater number of bases to be sequenced at an increasingly lower cost. However, sequencing a high number of genes requires an evaluation of the analytical performance of ES before it is used in the clinical setting.

Methods

Fifteen genomic DNA samples were used to prepare sequencing libraries with the TruSight One Sequencing Panel (Illumina) consisting of 4813 disease-associated genes ('clinical exome'), according to the manufacturer's procedures. Libraries were sequenced on the MiSeq (Illumina) and the results were analyzed using the MiSeq Reporter and IGV. Variants identified in ES were compared with those validated previously in a subset of genes using the TruSight Cancer gene panel (Illumina) and Sanger sequencing. This study was conducted in 2 phases. In the first, the clinical exome of 9 samples was sequenced and the variants obtained were compared with known variants in 8 genes. In the second phase, 6 samples were sequenced and the variants in 8 genes were analyzed without prior knowledge of the results obtained in the other methods. Furthermore, it was not known that one of these samples had been sequenced in the first phase of the study.

Results

In the first phase, ES identified all the exonic (n=41) and intronic flanking (n=15) variants validated in the *MSH2*, *MLH1*, *APC*, *MUTYH*, *BRCA1*, *BRCA2*, *STK11* and *TP53* genes, while no additional changes have been detected. In the second phase, ES detected a total of 50 variants in *MSH2*, *MLH1*, *APC*, *BRCA1*, *BRCA2*, *TP53, CDH1* and *ATM* genes which were found to include each of the 46 variants previously validated and 4 additional changes located outside the genomic regions defined in the gene panel. The same 15 exonic variants were identified in the sample independently processed and sequenced in both phases. Taken together, 87 variants were independently identified using different sequencing approaches.

Discussion

The results of this work showed a complete agreement between variants identified by clinical exome, gene panel and Sanger sequencing. Moreover, these results support the notion that the clinical exome panel can also be used as a set of sub-panels of genes applicable to different genetic diseases.