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Copy Number Variant Analysis in Next-Generation Sequencing Panels for Germline Disorders

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A copy number variant (CNV) analysis is widely performed in next-generation sequencing (NGS) tests for germline disorders, such as NGS panels or whole exome sequencing. The CNVs provide a high proportion of positive results and the frequency of the CNVs varies depending on the NGS panel categories. To provide accurate CNV results from NGS data, we need to select appropriate analysis methods and manage quality control (QC) metrics of NGS testing. This review briefly introduces the CNV analysis methods associated with NGS panels, investigates the frequency of CNVs among the different NGS panel categories, and suggests factors that need to be considered before performing a CNV analysis as part of an NGS test.

Key words: Next-generation sequencing panel, Copy number variant, Germline disorder

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INTRODUCTION

Importance of copy number variant detection in next-generation sequencing panels for germline disorders

The primary purpose of next-generation sequencing (NGS) panel testing for hereditary diseases is to detect sequence variants in target genes. However, advances in the bioinformatics analysis pipeline and quality control (QC) management of the wet bench process mean that it is now possible to accurately detect copy number variants (CNVs), such as deletions or duplications in genomic regions, by statistically analyzing sequencing depth data [1,2]. Currently, such analysis pipelines are almost routine. They allow a CNV analysis to be actively conducted even in clinical laboratories that perform NGS panel testing. The literature on CNV analyses of targeted NGS panels suggests that CNVs are detected in about 1%–2% of all NGS panel tests. However, the CNVs account for a higher proportion, at approximately 9%–10%, among the cases reported as positive due to pathogenic variants [3,4]. The distribution of reported CNVs also varies depending on the type of panel used. For example, neurologic disease panels show a high reporting rate of 30%–40% [3]. Thus, a CNV analysis of a NGS panel will provide useful complementary information, especially for cases where making a diagnosis is challenging.

Copy number variant detection methods used in NGS tests for germline disorders

Statistical data analysis methods for target gene regions

Deletions or duplications in the coding regions of target genes can be identified using a statistical analysis if there are 10 or more samples in a batch. Representative statistical indicators include the z-score and z-ratio [5]. Algorithms, such as

VisCap, are also frequently used in NGS panels to detect CNVs [6]. One of the recently developed CNV detection tools such as CABANA shows highly accurate intragenic CNVs according to base-level normalization [7].

CNV detection methods in whole exome sequencing tests

Most hereditary disease NGS panel tests now use whole exome sequencing (WES), which means that it is possible to perform a CNV analysis based on WES data, including NGS panel test data. Algorithms commonly used in WES tests include XHMM, CODEX, and Conifer [8-10] and the results of the CNV analysis can be visualized on a genome-wide basis using z-score and z-ratio plots. This allows the identification of large-scale deletions and duplications at similar levels to a chromosomal microarray (CMA).

Off-target analysis

Sequencing data from off-target regions can be utilized in CNV analyses of data from targeted NGS panels or WES tests. This method, known as an off-target CNV analysis, is particularly useful for detecting large deletions or duplications. Additionally, it offers the advantage of being able to estimate breakpoints to some extent [11].

Analysis using the Integrative Genomics Viewer program

When a CNV is suspected after a statistical analysis, the Integrative Genomics Viewer (IGV) program can, in some cases, be used to identify the split reads. This enables the precise localization of deletions or duplications at the base pair level. Additionally, though rare, it is also possible to detect genomic rearrangement [12].

Distribution of CNVs in NGS panels for hereditary disease

The NGS panel with the highest detection rate for CNVs is the Charcot-Marie-Tooth disease panel, where pathogenic CNVs have been identified in approximately 50% of total positive cases [3]. This is because the major causative factor for this disease is a heterozygous duplication in the PMP22 gene region. Additionally, other tests in which CNVs are frequently detected include panels for neurodevelopmental disorders such as epilepsy, where CNVs are found in over 10% of all positive cases [3]. Furthermore, in panels for hereditary cancer, cardiomyopathy, arrhythmia, and connective tissue disease, CNVs are detected in approximately 5%–10% of the total positive cases [3,4]. Interestingly, in hereditary cancer and dyslipidemia panels, most reported CNVs are small and occur at the exon level. However, in panels for autism, epilepsy, and skeletal dysplasia, large CNVs—such as several hundred kilobase CNVs associated with microdeletion syndromes or several megabase CNVs that are related to chromosomal unbalanced translocations—are frequently reported [4,13].

Special considerations

QC management

The primary objective of NGS panel tests for germline disorders is the analysis of sequence variants. However, sufficient average coverage is required for a sequence variants analysis and the CNV analysis requires additional QC management. For efficiency reasons, the sequencing depth should be uniformly distributed across all target regions. This is referred to as coverage uniformity and is assessed using various indicators, such as the coefficient of variation [14]. In particular, coverage uniformity must meet a certain threshold and values such as the median absolute pair-wise difference (MAPD) must be within an acceptable range [15]. However, these QC values may fall outside the acceptable range due to specimen quality issues or other unknown factors. Consequently, maintaining the QC for CNV analyses as high as that achieved for a CMA is challenging and can sometimes lead to difficulties in performing accurate CNV analyses.

Confirmation test for detected CNVs in NGS tests

Even if CNVs detected through NGS tests have been reported, additional confirmatory testing may still be required in some cases. Confirmation using an alternative method is recommended, particularly when a CNV is suspected in cases where there has been a single exon deletion or where CNVs have been found in regions that are difficult to analyze by NGS, such as homologous regions or areas with insufficient coverage. Multiplex ligation-dependent probe amplification (MLPA) is commonly used as a confirmatory test, but it cannot confirm all genes due to limitations in the available probes. An alternative is a CMA-based test, such as the CytoScan Xon array, which can detect exon-level regions [16,17]. In clinical practice, the validation of the CNVs in NGS result by MLPA or CMA is important to confirm the genetic diagnosis.

CONCLUSION

A CNV analysis is often performed concurrently in current NGS-based genetic testing for germline disorders because CNVs play a significant role in genetic diagnosis and substan-

tially improve the diagnostic yield. Most NGS panels for hereditary diseases are performed using WES and CNV analyses based on WES data have become increasingly accurate. Notably, CNVs can be detected across a broad range, from the single exon level to Mb-scale large deletions. In particular, accurate interpretation and reporting of CNV—supported by the interpreting specialist's extensive clinical experience and analytical expertise—greatly enhance the diagnostic accuracy for hereditary diseases.

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