Editorial
Diagnostic Genetics

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Toward Comprehensive Clinical Genomics: Integrating Exon-Level Analysis for Detecting Monogenic Copy Number Variations

Copy number variations (CNVs), which result from deletions or duplications that affect an entire single gene or specific exons within a gene, have increasingly been recognized as substantial etiological contributors to Mendelian inherited disorders. Such single-gene or intragenic CNVs can lead to gene dosage imbalance, functional disruption, or haploinsufficiency, thereby playing a critical pathogenic role in monogenic conditions previously attributed primarily to sequence variants [1].

A recent study involving the assessment of next-generation sequencing (NGS) data from a cohort of 143,515 patients with genetic disorders, employing simultaneous assessment of nucleotide sequence variants and CNVs, revealed that intragenic CNVs represented up to 10% of all the positive diagnostic results [2]. Another recent study utilizing genome-wide microarray in large-scale clinical cohort (n=13,648) reported that CNVs affecting a single gene accounted for approximately 10% of all identified pathogenic and likely pathogenic CNVs [3]. Notably, 26.3% of these single-gene CNVs involved a single exon, underscoring the clinical relevance of high-resolution, exon-level CNV analysis in routine genetic diagnostics.

Efforts have been made to develop diagnostic platforms capable of reliably detecting exon-level CNVs to improve diagnostic yield of clinical genetic testing. Multiple ligation-dependent probe amplification (MLPA) assay is used as the prototype technique to detect exon-level CNVs targeting well-established disease-causing genes, whereas genome-wide approaches include the use of NGS and chromosomal microarray. However, in the context of CNV detection, these platforms (namely MLPA, NGS, and chromosomal microarray) continue to face substantial technical limitations that hamper reliable resolution and identification of CNVs restricted to individual exons or small exonic regions.

MLPA represents a targeted, quantitative method for assessing CNVs within specific genes or genomic loci, offering sufficient resolution to reliably detect exon-level dosage. However, the commercial MLPA kits (MRC Holland, Amsterdam, The Netherlands), which are typically designed for assessing well-established disease-causing genes, impose constraints on flexibility, particularly when investigating novel CNVs in underrepresented or newly implicated genes [4]. Additionally, a critical limitation arises from the presence of sequence variants [e.g., single-nucleotide polymorphisms (SNPs) or small insertions/deletions] at probe hybridization or ligation sites, which can lead to false-positive results as these variants could be inaccurately identified as deletions even when no true deletion exists [5].

NGS-based CNV detection relies on computational algorithms that analyze read depth, normalized coverage patterns, or paired-end mapping signatures derived from NGS data. Although this approach has become a powerful tool for genome-wide CNV identification in clinical diagnostics, its performance is highly dependent on the size of the target region; detection sensitivity and reliability decline dramatically when the affected re-

gion spans a small number of exons. Moreover, exon-level CNVs involving only one or two exons are associated with a markedly elevated false-positive rate [6, 7].

Chromosomal microarray analysis (CMA) offers high sensitivity and reliability in detecting large-scale, genome-wide CNVs; however, probe density in CMA is generally insufficient to capture single or multiple exon-level variants. CNVs smaller than several tens of kilobases and exon-level copy number changes are difficult to detect using the currently standard CMA platforms, given the technical limitations [1].

To address the limitations of MLPA, NGS, and standard CMA, high-resolution array-based comparative genomic hybridization (array CGH) platforms have been developed. These platforms feature densely tiled, high-density oligonucleotide probes specifically targeting the exonic regions of clinically relevant disease genes, enabling sensitive and accurate detection of single- and multi-exon CNVs [1, 6, 8].

In this issue of *Annals of Laboratory Medicine*, Kim *et al*. [9] present a laboratory-based evaluation of a recently developed, commercially available SNP-array platform specifically designed for exon-level resolution. The platform, CytoScan XON assay (Thermo Fisher Scientific, Waltham, MA, USA), integrates high-density oligonucleotide and SNP probes to achieve whole-genome coverage while targeting the exons of more than 7,000 clinically relevant genes [7]. Using a cohort of patients with previously confirmed exonic CNVs identified through orthogonal methods, including MLPA, the authors demonstrated 100% concordance for multi-exon CNVs and 82.6% concordance for single-exon CNVs in targeted regions. The finding that all discordant cases were single-exon events underscores a residual technical limitation precisely at the smallest interval where array-based methods remain most challenged. Notably, the authors uniquely addressed mosaic CNV detection (historically a difficult area for MLPA and exome-depth algorithms) and found superior resolution of low-level or complex CNVs using array-based data. In non-targeted regions, the authors were able to significantly reduce false-positive CNV calls to < 0.01 per gene per person by applying practical filtering strategies. Compared with four established genome sequencing–based CNV callers (CNVkit, CNVpytor, Delly, and Manta), the platform showed a markedly lower false-positive rate.

Single-exon CNV detection is intrinsically vulnerable across methodologies, given the probe-level signal variability and technical difficulties in regions with high GC content or repetitive sequences, all of which compromise analytical sensitivity and specificity. Exon-level arrays still require ongoing technical maintenance, including monitoring probe performance, updating disease-related gene information, and removing known positive regions to balance sensitivity with interpretive burden. Single-exon CNVs identified using microarrays may require orthogonal confirmation using targeted methods, such as MLPA or quantitative gene-dose PCR, to verify true positivity and exclude technical artifacts.

The utility of exon-level arrays extends across heterogeneous clinical phenotypes, encompassing neurodevelopmental disorders, autism spectrum disorders, and diverse Mendelian disorders [10–12]. By providing exon-level resolution, these platforms enable reliable identification of causative variants at the gene or individual exon scale in autosomal dominant, autosomal recessive, and X-linked inheritance patterns. Of particular clinical significance in recessive disorders is the platforms' ability to uncover a second-hit intragenic CNV on the opposite allele when only a heterozygous sequence variant is initially detected or when a pathogenic variant appears homozygous. Such findings often resolve compound heterozygous states that would otherwise remain undetected, thereby enabling a complete molecular diagnosis for the observed phenotype [3, 6].

The forthcoming challenge in clinical genomics lies in developing evidence-based strategies for effectively integrating genome-wide platforms into routine clinical practice. Achieving this integration will require meticulous optimization of their deployment across heterogeneous genetic disorders with diverse pathogenic mechanisms, including those driven predominantly by intragenic CNVs. Beyond sequence-variant detection, clinical genomics must continue to undergo technological and analytical evolution to improve the visibility of exon-level CNVs and facilitate their detection in routine clinical settings.

AUTHOR CONTRIBUTIONS

Conceptualization: Eul-Ju Seo. Investigation: Eul-Ju Seo. Writing – original draft & editing: Eul-Ju Seo.

CONFLICTS OF INTEREST

None declared.

REFERENCES

1. Boone PM, Bacino CA, Shaw CA, Eng PA, Hixson PM, Pursley AN, et al. Detection of clinically relevant exonic copy-number changes by array CGH. Hum Mutat 2010;31:1326–42.
2. Truty R, Paul J, Kennemer M, Lincoln SE, Olivares E, Nussbaum RL, et

https://doi.org/10.3343/alm.2026.0030

al. Prevalence and properties of intragenic copy-number variation in Mendelian disease genes. Genet Med 2019;21:114–23.
3. Chau MHK, Anderson SA, Song R, Cooper L, Ward PA, Yuan B, et al. Detection of clinically relevant monogenic copy-number variants by a comprehensive genome-wide microarray with exonic coverage. Clin Chem 2025;71:141–54.
4. Stuppia L, Antonucci I, Palka G, Gatta V. Use of the MLPA assay in the molecular diagnosis of gene copy number alterations in human genetic diseases. Int J Mol Sci 2012;13:3245–76.
5. Concolino P, De Paolis E, Rinelli M, Maneri G, Brisighelli F, Trozzi R, et al. Identification of a false-positive multiplex ligation dependent probe amplification result in BRCA1 using a copy number variation algorithm under development for a commercial next-generation sequencing-based homologous recombination deficiency assay. Ann Lab Med 2024;44:617–20.
6. Retterer K, Scuffins J, Schmidt D, Lewis R, Pineda-Alvarez D, Stafford A, et al. Assessing copy number from exome sequencing and exome array CGH based on CNV spectrum in a large clinical cohort. Genet Med 2015;17:623–9.
7. Won D, Yeom E, Shin S, Lee ST, Rak Choi J. Comparison of exon-level copy number variants in CytoScan XON assay and next-generation sequencing in clinical samples. Clin Chim Acta 2024;560:119703.
8. Dhami P, Coffey AJ, Abbs S, Vermeesch JR, Dumanski JP, Woodward KJ, et al. Exon array CGH: detection of copy-number changes at the resolution of individual exons in the human genome. Am J Hum Genet 2005;76:750–62.
9. Kim Y, Lee JS, Kim B, Kim MJ, Cho SI, Chae SW, et al. High-resolution chromosomal microarray with diagnostic potential for detecting exon-level copy number variations using targeted and non-targeted approaches. Ann Lab Med 2026;46:190-9.
10. Asadollahi R, Oneda B, Joset P, Azzarello-Burri S, Bartholdi D, Steindl K, et al. The clinical significance of small copy number variants in neurodevelopmental disorders. J Med Genet 2014;51:677–88.
11. Nagamani SCS, Erez A, Ben-Zeev B, Frydman M, Winter S, Zeller R, et al. Detection of copy-number variation in AUTS2 gene by targeted exonic array CGH in patients with developmental delay and autistic spectrum disorders. Eur J Hum Genet 2013;21:343–6.
12. Celestino-Soper PBS, Shaw CA, Sanders SJ, Li J, Murtha MT, Ercan-Sencicek AG, et al. Use of array CGH to detect exonic copy number variants throughout the genome in autism families detects a novel deletion in *TMLHE*. Hum Mol Genet 2011;20:4360–70.

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