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SHORT COMMUNICATION

The Discordance between G-Banding Karyotyping and Microarray in Structural Abnormality

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SUMMARY

Background: **Cytomolecular genetic laboratory techniques have developed from conventional G-banding karyotyping to whole genome sequencing. Although resolution has greatly increased, various cytogenetic techniques have their advantages and limitations in detecting genomic variations.**
Methods: **We compared the chromosomal abnormalities detected by G-banding karyotyping and SNP-based microarray testing in 62 patients from July 2020 to December 2022. We analyzed their difference according to chromosomal abnormalities, including numerical and structural and others.**
Results: **Of the 62 patients, 28 patients showed chromosomal aberration detected in one or more of the two test methods. Aneuploidy was detected in both methods, while gain and loss less than 3 Mb were only detectable by the microarray. G-banding karyotyping is fundamental to detect structural chromosome rearrangement such as inversions, ring chromosomes, and translocations, but additional breakpoint or unknown origin materials information obtained from microarray. Loss of heterozygosity was only detectable in microarray, and mosaicism had limitations in both G-banding karyotyping and microarray.**
Conclusions: **Various disease cause genomic structural variants, it is very important to detect this. We showed discordance between G-banding karyotyping and SNP based microarray in clinical laboratory. It can be helpful to clinical physicians to decide which diagnostic tool to use.**
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KEYWORDS

chromosome, chromosome microarray, karyotyping, loss of heterozygosity, mosaicism

INTRODUCTION

Since the discovery that humans have 46 chromosomes in 1956, cytomolecular genetic techniques have advanced significantly from conventional G-banding, fluorescence in situ hybridization (FISH), microarray, and next generation sequencing [1-3].
Regardless of the time of development, various cytomolecular genetic technologies have differences in detection ability depending on the variation of genomic mutations, and these differences are important points that can increase the diagnostic accuracy in clinical settings. One of the differences in detection between karyotyping

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and microarray is structural abnormalities. Structural abnormalities of chromosomes is defined as genomic alterations that involve part of DNA that are larger than 1 kb [2]. In cases of translocation, inversion, and large-scale copy number variants, they can be detected through G-banding karyotyping, but copy number variants less than 50 kb resulting from deletions can only be detected through array techniques [2,3]. Understanding the genetic mechanisms of a disease can lead to more accurate prognosis, treatment, and genetic counselling, so it is very important to understand the testing methods. In this study, we aimed to investigate the frequency of detecting various numerical and structural abnormalities and analyze their differences by conducting karyotyping and SNP-based microarray simultaneously on 62 patients at a single tertiary hospital.

MATERIALS AND METHODS

G-banding karyotyping

Karyotyping using standard G-banding techniques on heparinized peripheral blood were performed at Samkwang medical laboratory (Seoul, Republic of Korea). At least 5 metaphases were analyzed whenever possible. Clonal abnormalities were defined as two or more cells with the same gain or structural rearrangement, or at least three cells with the same chromosome deletion. Karyotypes were recorded according to the International System for Human Cytogenetic Nomenclature (ISCN) 2020 [4].

SNP-based chromosome microarray (microarray)

Peripheral blood samples were collected in EDTA tubes. All procedures were performed at Green Cross Laboratories (Yongin, Republic of Korea) and performed with CytoScan Dx Assay (Affymetrix Inc., Santa Clara, CA, USA). Human Genome Build 19 was used to perform the description of genomic variants. CNVs were classified into five categories according to the American College of Medical Genetics and Genomics guidelines into pathogenic (P), likely pathogenic (LP), uncertain significance (VUS), likely benign (LB), and benign (B) [5].

RESULTS

From July 2020 to December 2022, G-banding karyotyping and microarray were simultaneously performed on a total of 62 patients. Among them, normal findings were observed in both tests in 34 (54.8%) patients, and genomic variations were observed in one of the two tests in the remaining 28 patients. These variations were classified as numerical chromosome and structural chromosome abnormalities and were further analyzed by classifying them as loss of heterozygosity (LOH) or mosaicism (Figure 1). The differences between these testing methods are described in Supplementary Table 1.

Numerical chromosome abnormalities

Trisomy 21 was detected in one patient, and this was detectable in both G-banding karyotyping and microarray.

Structural chromosome rearrangements

In microarray, a total of 8 gains and 6 losses were observed. The detection sizes of these varied from 449 kb to 2.7 Mb, which are sizes that are difficult to detect with G-banding karyotyping. As a result, no cases were detected using chromosomal analysis. Normal variable chromosome feature, 46,XX,inv(9)(p12q13) is a finding of a pericentric inversion on chromosome 9. This variation was detected in two patients by G-banding karyotyping but was not detected by microarray.

In two patients, microarray results showed simultaneous detection of one gain and one loss finding. Only one of these results was readable by G-banding karyotyping. The derivative chromosome 8 was detected resulting from the rearrangement between 8p23 and 16q22 (Figure 2A). Initially, it was analyzed as a normal karyotype, but reanalysis was done regarding with microarray result. The second case involved a suspected translocation between 5p15.2 and 15q13.3, but it could not be detected even after G-banding karyotyping reanalysis. The affected chromosome size in both cases was less than 3 Mb, which is below the resolution that can be analyzed in microscopic karyotyping. A ring chromosome 18 was observed in one patient, and this was observed in the form of loss at the 18p11.32 and 18q22.2q23 region in microarray. Additionally, the finding of an 18p11.32p11.32 duplication was also detected. (Figure 2B). The other additional material of unknown origin detected in chromosome karyotyping was identified as a duplication at 9p24.3p13.1 by microarray (Figure 2C).

Other

LOH findings that could only be detected by microarray were found in four patients. The sizes ranged from 10.9 - 24.1 Mb. Among them, there were regions that included the 15q11.2 region, which can cause Prader-Willi or Angel man syndrome through uniparental disomy. Mosaicism was detected in four patients. Two were mosaic for sex chromosomes and the other two were mosaic for autosomes. The mosaic ratios were 9.6%, 10.7%, 26.0%, and 76.7%, and microarray was detected in only two of them. Interestingly, in the case of 47,XX, +psu(9)(q12)[6]/46,XY[56], despite the low level of the mosaic clone, copy number variant was relatively high at four copies due to idic (9) and was detected as arr(9p)x2~3 in microarray (Figure 2D).

DISCUSSION

Both methods, G-banding karyotyping and microarray, have their own strengths and limitations in detecting chromosomal aberration. In the case of inversions, translocations, and ring chromosomes, the whole struc-

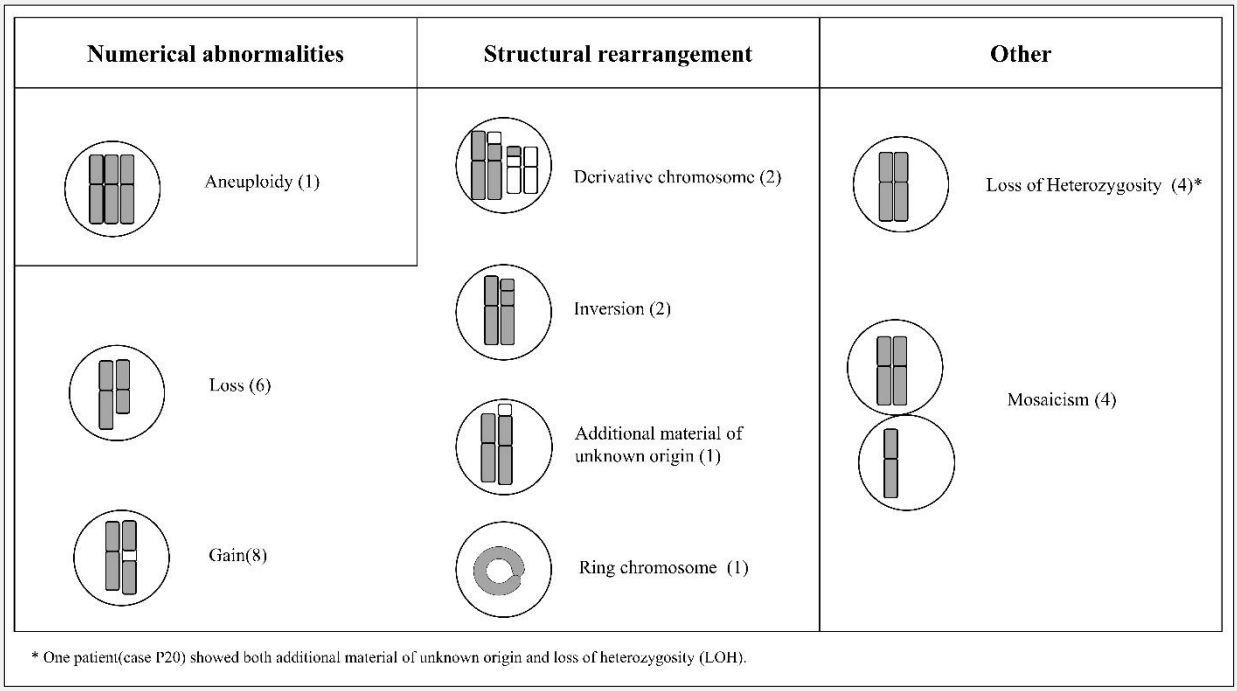


Figure 1. Frequency of the genomic variation detected using karyotyping.

Of the total 62 patients, genomic variations were detected in 28 patients. Among them, one had a numerical abnormality, 20 had structural abnormalities, 4 showed loss of heterozygosity, and 4 had mosaicism.

ture of the chromosome can be identified through G-banding karyotyping. On the other hand, microarray showed additional findings that cannot be detected using G-banding karyotyping due to low resolution.

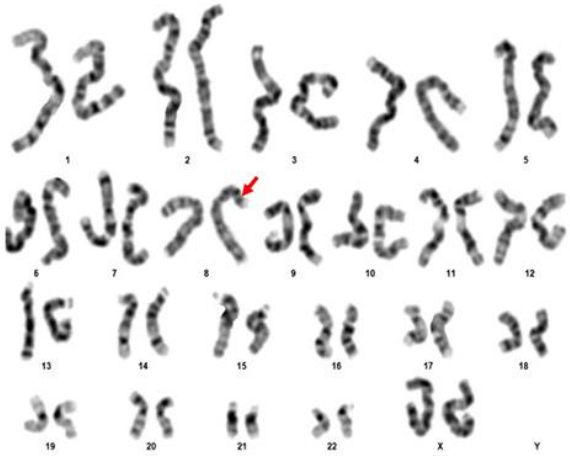
In the case of derivative chromosome resulting from rearrangement like the case of P16 (Figure 2A), microarray techniques can only find paired loss and gain regions, so G-banding karyotyping is still necessary for structural abnormalities [1]. Meanwhile, for Robertsonian translocation (case P17), which cannot be detected using G-banding karyotyping because of low resolution, it has been found that other techniques such as microarray or FISH. Interestingly, in the case of ring chromosomes (case P21, Figure 2B), it is known that ring chromosome have fused on the terminal p and q arms usually missing, but in our case where there is also additional partial duplication, it can be detected by microarray. The duplication of 18p11.32p11.31(1,205,753 - 6,112,310) region in this patient contains important clinical genes such as *EMILIN2* that is classified as a likely pathogenic variant present with intellectual disability/developmental delay [6]. So, if only karyotyping was performed, it would not have been possible to detect it. In addition, the case of additional material (case P20, Figure 2C) also emphasizes the importance of microarray in accurately identifying affected chromosomal aberration.

The case demonstrates the advantage of SNP-based microarray in maximizing the detection of LOH. Uniparental duplication/deletion of part of chromosome can cause imbalance dosage of gene involved in genomic imprinting and homozygosity of a recessive inherited genetic disease [7]. Among the three patients where LOH was observed, the region of LOH was observed in chromosome 11 and 15 (case P22, 23), which are well known to be associated with uniparental disomy diseases. However, there were no phenotype and symptoms consistent with chromosome 11 UPD disease (Silver-Russel syndrome and Beckwith-Widemann syndrome), and chromosome 15 UPD disease (Prader-Willi syndrome and Angelman syndrome). According to a previous large cohort study which analyzed 32,067 clinical exome trio sequencing, that showed whole-chromosome UPDs were detected in 0.31% of cases, and only 0.14% of these resulted in a clinical diagnosis [8].

Mosaicism as a result of genetic alteration showed highly variable manifestations depending on the type of variation. It is difficult to accurately quantify the percent of mosaic cells [9,10]. In previous reports, array CGH detected mosaic cells is over 10 - 20% [11-13]. G-banding

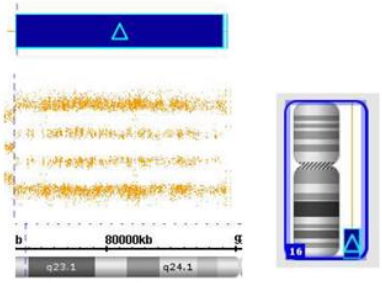
A

(a) Translocation



46,XX,der(8)t(8;16)(p23.1;q22)

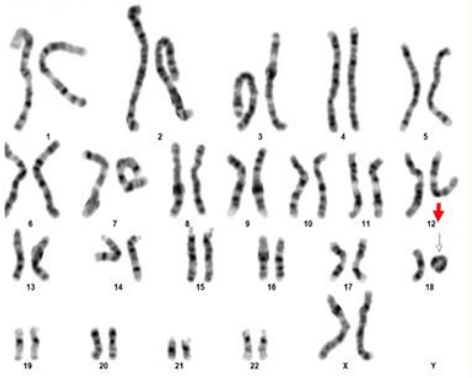
arr 8p23.3p23.1(158,048-6,999,114) X1



arr 16q22.3q24.3(73,752,981-90,155,062)X3

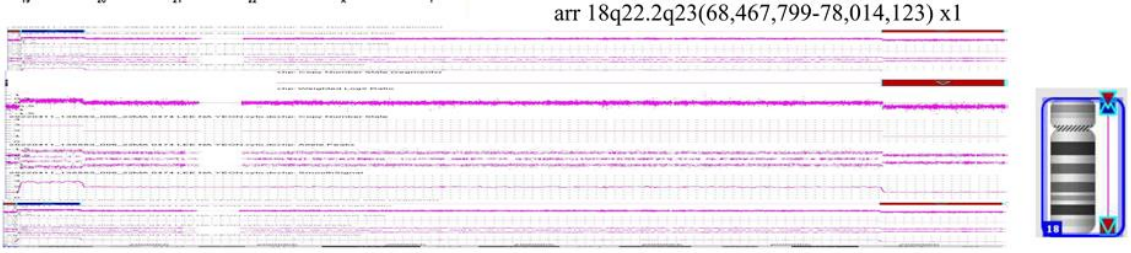
B

(b) Ring Chromosome



46,XX,r(18)

arr 18p11.32(136,226-1,164,159) x 1
arr 18p11.32p11.31(1,205,753-6,112,310)X3
arr 18q22.2q23(68,467,799-78,014,123) x1



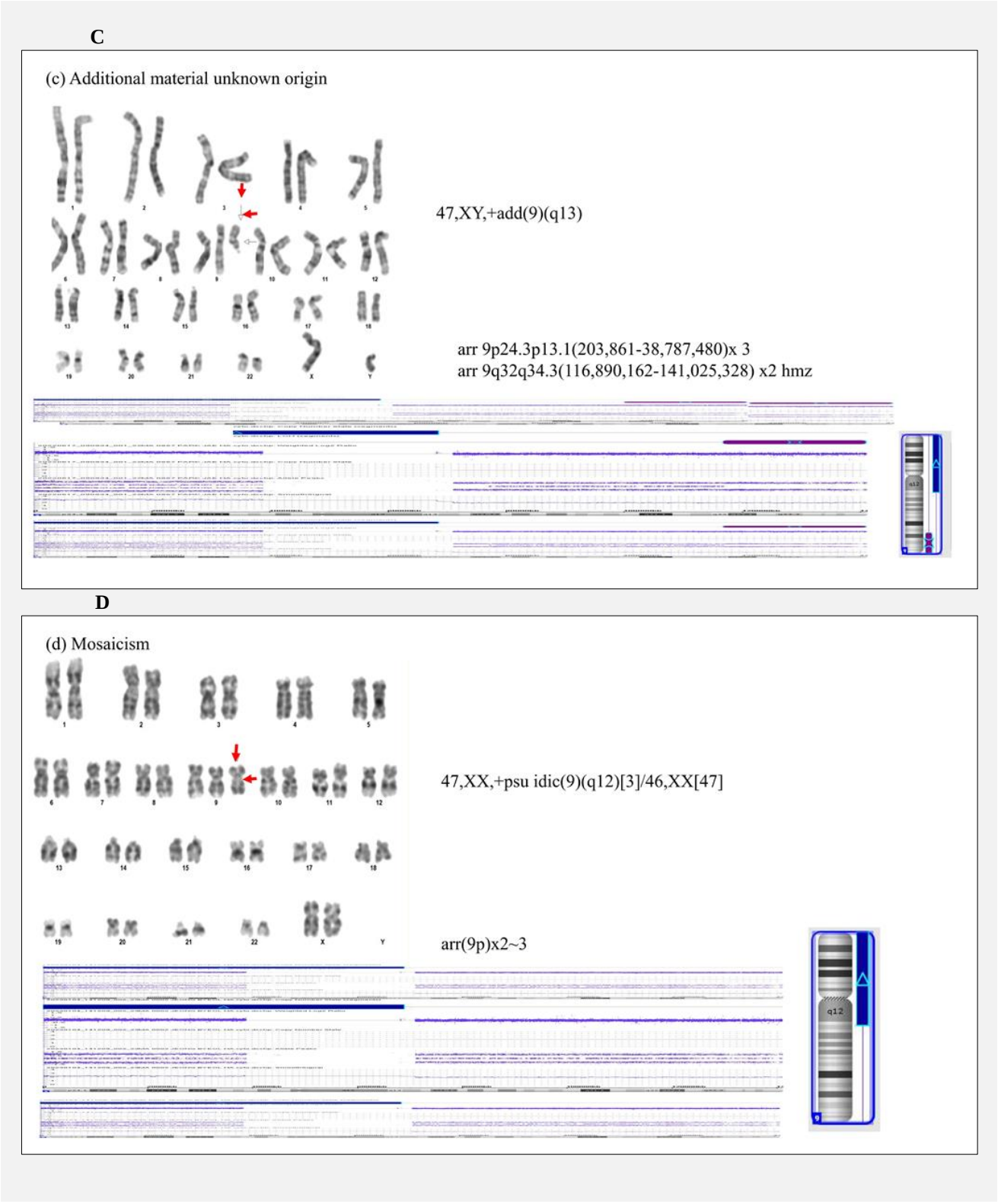


Figure 2. Discordant results between G-banding karyotyping and microarray.

(A) A pair deletion at 8p23.3p23.1 and gain at 16q22.3q24.3 were detected in microarray. Derivative 8 chromosome results in these chromosomal rearrangements, which was identified in G-banding karyotyping. (B) The microarray detected not only identified terminal deletions on the p and q arms but also provided additional findings of a gain at 18p11.32p11.31 in the ring chromosome 18. (C) The microarray provided additional genetic information on the unknown origin material attached to chromosome 9, and LOH was also detected through microarray analysis. (D) The microarray analysis suggested the possibility of mosaicism of chromosome 9, but overall structural abnormalities, pseudo isodicentric chromosome 9, were clearly identified through G-banding karyotyping.

karyotyping can also miss low-level mosaicism, which may not be detected if the laboratory's testing criteria are not met or there is low resolution [9]. Among four patients in whom mosaicism was detected in our study there was no detection in the microarray even through the proportion of mosaic cells detected in G-banding karyotyping exceeded 20%. Especially, since mosaicism is influenced by various factors such as specimen beyond differences in testing methods [14], it is recommended to perform multiple cytogenetic laboratory tests complementarily, taking into account the patient's clinical findings.

This study showed the utility in detecting various structural variants using G-banding karyotyping and microarray. Since structural variants in the genome can be the cause of various diseases, it is very important to detect them through suitable diagnostic techniques. These methods each have their own advantage and limitation, and if they are mutually complementary, they can be helpful in diagnosing patients. Although the number of cases in our study is small, it provides practical information by utilizing actual analysis in clinical practice.

Declaration of Interest:

The authors report no conflict of interest in this study.

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