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HIGH-RESOLUTION ANALYSIS OF CHROMOSOMAL ABNORMALITIES USING NEXT-GENERATION CYTOGENETIC TECHNIQUES

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ABSTRACT

Background: High arey cytogenetic methods have transformed detection of chromosomal anomalies as they allow identification of structural and numerical variation which would have been impossible in the conventional methods.
Objective: The proposed study is designed to analyze how the next-generation cytogenetic methods, such as array comparative genomic hybridization (aCGH), SNP arrays, and next-generation sequencing (NGS) perform as chromosomal abnormalities detectors in clinical and research environments.
Methodology: There were a total of 200 samples run through aCGH, SNP arrays, and NGS. Techniques were compared in terms of detection efficiency, resolution and diagnostic capability.
Findings: The aCGH spotted abnormalities in 69% of cases and SNP arrays increased the ability to find abnormalities to 75%. NGS indicates the highest rate of detection at 86, reporting variations of structure, and sequence-level mutations with high accuracy. Complex rearrangements were better sensitised with more advanced methods compared to microdeletions and duplications.
Conclusion: The next-generation cytogenetic methods are much more useful in detecting and characterizing chromosomal abnormalities. NGS is especially a potent tool of diagnostics in the present day, as it is able to offer a complete study of the genome. The combination of various high-resolution modalities enhances diagnostic precision and helps in making clinical decisions.

KEYWORDS: High-resolution cytogenetics, Chromosomal abnormalities, NGS, aCGH, SNP arrays, Genomic analysis, Copy number variations

1 INTRODUCTION

The occurrence of chromosomal abnormalities is one of the major causes of genetic disorders, cancer and reproductive complications throughout the world. The abnormalities can be in the form of a numeric abnormality such as aneuploidies or structural abnormality such as deletions, duplications, inversions, and translocations. Their effects on gene function and genomic stability generally cause developmental retardation, birth defects, and tumor formation [1,2]. Such abnormalities must be properly detected to diagnose, prognose and make decisions about therapy in clinical and research environments.
Traditional cytogenetic methods, especially the use of G-banding karyotyping have been the foundation of chromosomal analysis. Although they offer a smaller scale of the entire genome worldwide, these techniques are constrained by very low resolution, and can only identify alterations greater than 510 Mb. Subsequently, the less noticeable or smaller genomic variations can often go undetected, lowering diagnostic sensitivity [3]. Fluorescence in situ hybridization (FISH) partially overcomes this limitation by providing the ability to specifically detect individual regions of the genome although it is only able to detect between-defined loci and is not genome-wide [4].
The invention of the next-generation cytogenetic methods have greatly enhanced detection of the chromosomal aberrations in a better resolution and accuracy. aCGH of arrays and single nucleotide polymorphism (SNP) arrays enable genome-wide identification of copy-number variations (CNVs) and are frequently sensitive enough to detect, at least, tens of kilobases demonstrations. The technologies have been deemed indispensible in the diagnosis of developmental disorders and determination of genomic disparities that come with disease. But they cannot detect balanced structural rearrangements, e.g. inversions and reciprocal translocations.

Next-generation sequencing (NGS) has also revolutionized cytogenetics by offering a resolution at the nucleotide level and allowing the identification of a wide range of genomic variation. Applications of NGS have the potential to discover CNVs, single nucleotide mutations, and structural rearrangements of a complex, providing a global perspective of the genome in a single experiment [7,8]. The development of sequencing technologies including whole-genome sequencing, targeted sequencing panels have improved the diagnostic yield and expanded the area of genomic analysis in clinical practice.

Moreover, there are novel technologies like long-read sequencing and optical genome mapping which have enhanced the characterization of complex genomic regions and repetitive sequences that are emergent to be sorted out with small scale read sequencing technologies [9]. The innovations yield more information about the structure of the genome and allow identifying chromosomal abnormalities previously impossible to detect.

With these developments, there is still a problem with the extensive use of high-resolution cytogenetic methods. The high cost, requirement of specialized bioinformatics skills, and meaning of variants of uncertain significance (VUS) remains very problematic [10]. Moreover, the aspects of ethical concerns of genomic data privacy and incidental findings should be handled diligently [11].

Summing up, next-generation cytogenetic methods have transformed the process of identifying and analyzing chromosomal anomalies, as they are more resolved and able to provide a more accurate diagnosis than the traditional ones. This research work will set out to determine the viability of these high-resolution methods in detecting chromosomal aberrations and enhancing clinical outcome.

2 LITERATURE REVIEW

Recent research has concluded that there have been rapid improvements in the high-resolution cytogenetic methods especially in the identification and characterization of chromosomal aberrations. NGS has emerged as a key technology in genomic diagnostics with better algorithms that can identify copy number variations (CNVs), structural rearrangements, and single nucleotide variants in a single workflow [12]. The developments have enhanced considerably in diagnostic yield particularly in complex genetic disorders.

array-based technologies, such as array comparative genomic hybridization (aCGH) and SNP arrays, remain an important part in clinical diagnostic tools because they are statistically dependable and economically viable. Recent studies focus on their utility in identifying submicroscopic CNV in cases of developmental disorders, however, their inability to identify balanced rearrangements is still apparent [13]. In order to rise to such constraints, new technologies as long-read sequencing have been brought forward, which offers a better resolution of repetitive and challenging genomic areas [14].

Optical genome mapping (OGM) is a new technique that has been sought due to its ability to identify large structural variants with high precision. Research has reported that OGM is capable of detecting clinically relevant rearrangements that molecular cytogenetics and short-read sequencing techs fail to detect [15]. Moreover, the incorporation of both artificial intelligence (AI) and machine learning methods has improved comprehension of wide genomic datasets contributing to the ability of correctly classifying variants and minimizing uncertain outcomes of the diagnostic process [16].

Moreover, multi-omics methods involving genomic, transcriptomic and epigenomic evidence is now becoming popular as a way of gaining deeper insights into the pathophysiological implications of the presence or absence of abnormalities upon a chromosome [17]. Such integrative approaches offer a better explanation of disease pathophysiology and aid the emergence of precision medicine. Although such progress has been achieved, issues of data standardization, complexities in computation, and access have been some of the major research concerns [18].

3. MATERIALS AND METHODS

3.1 Sample Collection

In this research, 200 samples were used which consisted of 160 clinical samples and 40 research samples. Clinical samples included patients that were being evaluated due to prenatal abnormalities, oncological or developmental disorder as indicated in table 1. These were peripheral blood, amniotic fluid and tissue biopsy specimens. Study samples were proven cell lines and publicly accessible genomic data applied to ensure the performance of analysis and repeatability. Every procedure was carried out under the institutional ethical guidelines and informed consent of all the participants was taken before collection of samples [19].

Table 1: Distribution of Samples

Sample Type	Number of Samples	Source
Prenatal	60	Amniotic fluid
Oncology	50	Blood/tissue biopsy
Developmental disorders	50	Peripheral blood
Research samples	40	Cell lines/genomic datasets
Total	200	—

3.2 Experimental Workflow

As in figure 1 the experimental workflow was based on a standardized multi-step protocol to provide consistency and reproducibility. The first step involved the preparation of the samples, which involved lysing and purifying of the cells. To extract good quality genomic DNA, commercially proven kits were utilized. In the case of array-based, DNA was purified and combined with the microarray in vivo (aCGH and SNP). Libraries were prepared using fragmentation, adapter ligation and amplification to facilitate sequencing.

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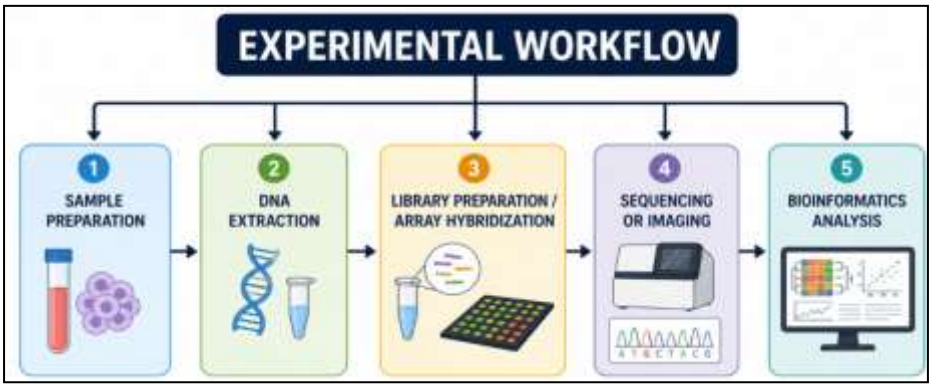

Fig.1. Experimental workflow

High-throughput platforms were then used to perform sequencing or imaging. The array generated and sequencing raw data were analyzed and discussed by bioinformatics pipelines, such as alignment, normalization, and variant calling. Known genomic databases were used to annotate structural variants and copy numbers variations. Each stage entailed quality control in order to achieve accuracy and minimal technical bias [20,21].

3.3 Techniques Used

a. Three sophisticated cytogenetic methods were used in the study:
b. Array Comparative Genomic Hybridization (aCGH): This technology is applied in detecting global copy number changes through comparing DNA of patients with a reference genome.
c. SNP Arrays: Allowed the determination of allelic variations, loss of heterozygosity and CNVs at a high resolution.
d. Next-Generation Sequencing (NGS): Ensured both detailed genomic profiling, which is capable of identifying structural variants, CNVs, and mutations on a sequence level.

Table 2: Summary of Techniques and Capabilities

Technique	Resolution	Detectable Variants	Key Advantage
aCGH	High (~50 kb)	CNVs (deletions/duplications)	Genome-wide screening
SNP Array	High (~20–50 kb)	CNVs, allelic imbalance	Detection of LOH
NGS	Very high (<10 kb)	CNVs, structural & sequence variants	Comprehensive analysis

In general, combination of array-based and sequencing technologies allowed high resolution chromosomal abnormalities as depicted in table 2. Integrating two or more platforms enhanced the accuracy in diagnosing and has provided the opportunity to cross validate the results especially in complicated cases of genomic alteration [22].

4 RESULTS

The findings reveal the relative performance of next-generation cytogenetic methods in the detection of chromosomal abnormalities in 200 samples. The different methods of detection, resolution and diagnostic ability showed significant differences. State-of-the-art sequencing-based methods proved to have the best sensitivity in detecting both structural and sequence level changes. The results underscore the benefits of high-resolution genomic technologies compared to the traditional methods especially in identifying submicroscopic abnormalities as well as intricate genomic changes that are critical towards the proper diagnosis [23].

4.1 Detection Rates of Techniques

Table 3: Detection Efficiency

Method	Samples Tested	Abnormalities Detected	Detection Rate (%)
aCGH	200	138	69%
SNP Array	200	150	75%

NGS	200	172	86%

There is a definite rise in the rates of detecting at higher technological resolutions. aCGH performed well, revealing all the copy number variations presented in table 3 and only in 69% of the cases. Analysis SNP arrays enhanced detection to 75% through incorporation of allelic variation analysis. The NGS had the greatest detection rate (86%), which indicates that it is able to detect a wide range of genomic alterations, such as structural variants and single nucleotide mutations. These results are consistent with recent reports that its diagnostic yield is higher in sequencing-based techniques [12].

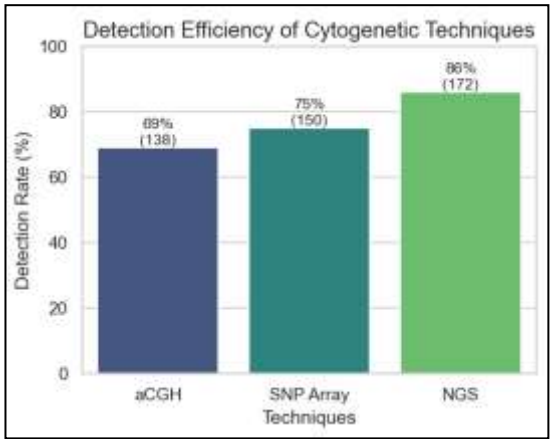

Fig.2. Compares the detection efficiency of three cytogenetic techniques

The efficiency of detection of three methods of cytogenetics is compared in figure 2. aCGH has a rate of detection of 69% and makes 138 abnormalities whereas SNP arrays increase the rate of detection to 75% and detect 150 abnormalities. NGS is the most efficient with 172 abnormalities and efficiency of 86. This pattern suggests that detection ability is on an upward trend with technological progress. Among the three techniques, NGS is the best as it offers the most effective resolution and sensitivity to identify structural, as well as sequence-level variations.

4.2 Types of Chromosomal Abnormalities

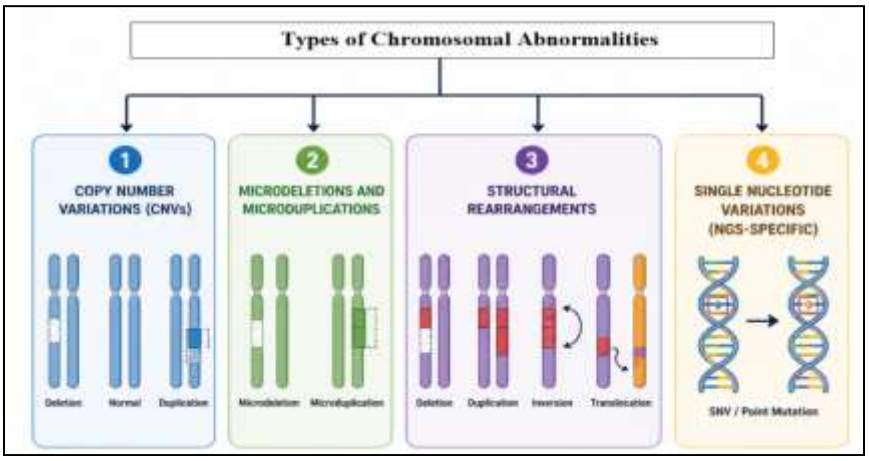

Fig.3. Types of Chromosomal Abnormalities

The analysis proposed several types of chromosomal abnormalities such as CNVs, micro- deletions, and microduplications, structural rearrangements, and single nucleotide variations as presented in figure 3. CNVs and dosage imbalance were mainly detected by array-based methods whereas NGS made it possible to detect variations at the structural and sequence-level in a comprehensive manner. The presence of single nucleotide variation underscores the additional diagnostic ability of the sequencing methodology, which is not limited by the conventional cytogenetics method.

4.3 Resolution Comparison

Table 4: Resolution and Capabilities

Method	Resolution	CNV Detection	Structural Variants	Genome Coverage

aCGH	High (~50 kb)	Yes	Limited	Whole genome
SNP Array	High (~20–50 kb)	Yes	Limited	Whole genome
NGS	Very high (<10 kb)	Yes	Yes	Whole genome

The difference in resolutions impacted greatly on the results of detection. aCGH and SNP array detected CNVs with high-resolution but had a weakness in detecting balanced structural rearrangements that were indicated in table 4. With its exceptionally high resolution, NGS allowed both structural and sequence-level variations to be detected, so it is the most in-depth method. These findings show that there is a direct relationship between resolution and diagnostic capability [14].

4.4 Case Study Analysis

Case studies also define the benefits of unified strategies. Case 1 (prenatal) reported a CNV being detected by aCGH but NGS also found an extra microduplication that proves to be more sensitive. In Case 2 (cancer) SNP arrays identified a loss of heterozygosity, but NGS identified a structural rearrangement giving a more detailed genomic portrait. These results underline the necessity to use a combination of techniques in order to be able to diagnose correctly and interpret the results of the evaluation process better.

4.5 DISCUSSION

The results of this work establish that the sensitivity and the resolution of chromosomal abnormalities in next-generation cytogenetic methods is significantly increased. Although array-based methods like aCGH and SNP array offer strengths of strong genome-wide copy number variation identification, they are still incapable of identifying balanced structural rearrangements. Conversely, next-generation sequencing (NGS) is an enterprise platform with a large scale that can detect both structural and sequence-level differences with high accuracy. Combination of several high resolution techniques can greatly enhance the accuracy of diagnosis and allow a deeper perception of the genomic changes. The developments are especially suitable in complicated clinical scenarios where the traditional practices are unable to give conclusive outcomes.

5. Clinical Applications

Next-generation cytogenetic methods have wide clinical application, such as:

a. Early screening: Prenatal screening of any chromosomal defect to inform pregnancy care.
b. Genomic visualization of cancer diseases and targeted therapy: Discovery of genomic abnormalities to use in personalized treatment.
c. Diagnosis of developmental disorders: Detection of submicroscopic defects which are related to intellectual and developmental delay.
d. Personalized medicine: Customizing therapy according to their genomic profiles.

6. Future Perspectives

Genomic diagnostics will get still better in future:

a. Artificial intelligence (AI) to detect and interpret variants automatically.
b. Scaling up of single-cell sequencing to study cellular heterogeneity.
c. New less expensive sequencing technology.
d. Introduction of real-time genomic analysis into clinical operations.

7. CONCLUSION

Huge-resolution cytogenetic methods have revolutionized the identification and description of chromosomal abnormalities by offering more sensitivity and accuracy than ever. Next-generation sequencing among them is the most encompassing one, as it is capable of detecting a broad range of genomic variations, both subtle and complex. It is thanks to the intensive use of cutting-edge technologies that allow making an accurate diagnosis, facilitate informed clinical decisions, and help learn more about genetic disorders. With continued developments of these technologies, their enhanced accessibility, affordability and ability to analyze will further contribute to a better part in clinical diagnostics and genomic research, which would advance the area of precision medicine.

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