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ARTICLE

Exploration and Genetic Counseling of Using Multiple Genetic Techniques to Detect Derived Chromosomes in Prenatal Diagnosis

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ABSTRACT

Purpose: Derivative chromosomes, resulting from complex structural rearrangements, pose significant challenges in prenatal diagnosis due to their unpredictable inheritance patterns and potential phenotypic consequences. This study evaluates the efficacy of multiple genetic technologies—including karyotyping, fluorescence in situ hybridization (FISH), chromosomal microarray analysis (CMA), and next-generation sequencing (NGS)—in detecting and characterizing derivative chromosomes in prenatal samples. **methodology:** We analyzed 150 cases of suspected chromosomal abnormalities, comparing detection rates, resolution, and clinical utility across these methods. **Results:** Our findings demonstrate that integrated multi-technology approaches significantly improve diagnostic accuracy, with NGS-based structural variation analysis achieving the highest detection sensitivity (99.2%) for cryptic rearrangements. **Conclusions:** Additionally, long-read sequencing (PacBio/Oxford Nanopore) enabled precise breakpoint mapping in 92% of cases, facilitating more accurate genetic counseling. Clinically, this approach enhances risk assessment for fetal anomalies, guides pregnancy management, and improves parental counseling for recurrence risks.

1. Introduction

Derivative chromosomes arise from unbalanced translocations, inversions, or insertions, often leading to partial aneuploidy, gene disruption, or imprinting disorders. In prenatal settings, their detection is critical because 30% of balanced carrier parents unknowingly transmit unbalanced rearrangements.Cryptic genomic imbalances may cause developmental delays, congenital anomalies, or pregnancy loss.Precise breakpoint characterization is essential for phenotype-genotype correlation.Traditional karyotyping has limitations in resolution (5-10 Mb), while modern technologies (CMA, NGS) detect submicroscopic aberrations (<100 kb). This study evaluates how multi-technology integration improves prenatal diagnosis and counseling.Compare detection rates of karyotyping, FISH, CMA, and NGS in identifying derivative chromosomes.Assess clinical utility in prenatal risk stratification.Develop a decision-making algorithm for genetic counseling.

2. Materials and Methods

2.1 Study Cohort

150 pregnant women with,Abnormal ultrasound

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findings (n=70),Family history of chromosomal rearrangements (n=50),Recurrent pregnancy loss (n=30),Samples: Amniotic fluid (n=100), chorionic villi (n=50).

2.2 Genetic Technologies Compared

2.2.1 Conventional Karyotyping (G-Banding) for Derivative Chromosome Detection

G-banding karyotyping remains a fundamental technique in prenatal cytogenetic diagnosis, operating on the principle of differential Giemsa staining patterns along metaphase chromosomes. The protocol involves:(1) Sample Preparation:Amniocytes/chorionic villi cultured in Chang Medium® for 10-14 days.Synchronization using thymidine block (0.3 mg/mL for 17h).Metaphase arrest with colcemid (0.1 μg/mL for 45min).(2)Chromosome Processing:Hypotonic treatment (0.075M KCl at 37°C for 20min).Triple fixation (methanol:acetic acid 3:1). Droplet technique on pre-chilled slides.(3)Banding and Analysis:Trypsin-Giemsa banding (0.025% trypsin for 45-90s).550-band resolution standard (ISCN guidelines). Minimum 20 metaphases analyzed, 5 karyotyped.

Performance Characteristics in Derivative Chromosome Detection Validation studies demonstrated,Sensitivity 68% for rearrangements >5Mb.Resolution limit 3-5Mb for unbalanced material.Mosaicism detection threshold ≥20% abnormal cells.Derivative Chromosome Identification Reciprocal translocations 92% detection rate.Robertsonian translocations 100% detection.Complex rearrangements 45% complete characterization.Banding quality score (BQS) ≥6/10 for diagnostic interpretation.Mitotic index >5 metaphases/1000 cells.Culture success rate 98.5% (amniocytes), 94.2% (CVS).

Whole-genome assessment in single assay.Detection of balanced rearrangements (invisible to CMA).Low technical failure rate (<2%).but 14-day turnaround time limits urgent cases.12% of derivatives require FISH confirmation.Cannot detect: Submicroscopic CNVs (<5Mb),Uniparental disomy,Low-level mosaicism (<20%).

Protocol Optimization for Enhanced Detection, Extended Culture Conditions.Prolonged incubation (up to 21 days) increases yield for,Mosaic cases (detection threshold ↓ to 10%),Poor-growing samples.Alternative Banding Techniques,Reverse banding (R-banding) improves,Telomere region visualization,Detection of terminal deletions.Microscopy Advancements,Automated metaphase finders (e.g., Metafer) increase,Screening efficiency (200 metaphases/hour),Abnormal cell detection sensitivity.

This comprehensive analysis confirms karyotyping's continued relevance as a first-line test, particularly for balanced rearrangements, while highlighting the necessity for supplemental technologies in complete derivative chromosome characterization.

2.2.2 Fluorescence In Situ Hybridization (FISH) for Targeted Derivative Chromosome Analysis

Our laboratory has developed an enhanced FISH protocol specifically optimized for prenatal derivative chromosome detection:(1)Probe Selection Strategy:Primary panel (24h) 13/18/21/X/Y aneuploidy probes,Secondary panel (48h) 15q11.2, 22q11.2, 1p36, 4p16.3,Custom probes (72h) For known familial rearrangements.(2)Hybridization Enhancement:Slide pretreatment,Pepsin digestion (0.1mg/mL in 0.01N HCl, 10min),Post-fixation (1% formaldehyde/PBS, 5min),Dehydration series (70%/85%/100% ethanol).73°C ± 0.5°C on calibrated heat block.Formamide concentration titration (65-75%).(3)Signal Amplification:Tyramide signal amplification (TSA) for Low-copy number targets,Archived specimens,Poor-quality samples,Multi-layer fluorophore stacking (up to 7 colors).Intra-slide CV 2.1-3.8%,Inter-operator CV 3.5-5.2%,Inter-lot reagent variation 4.1%.Mosaicism 5% abnormal cells (95% CI),Minimal abnormal cells scored 50 nuclei,Signal-to-noise ratio threshold ≥3:1.

Table 1. Analytical Sensitivity

Target Size	Detection Rate	False Positive Rate
>3 Mb	99.8%	0.1%
1-3 Mb	97.5%	0.5%
500 kb-1 Mb	89.2%	1.2%

Sample Processing Timeline:Day 0 Sample receipt and slide preparation,Day 1 Primary panel hybridization (overnight),Day 2 Secondary panel initiation,Day 3 Custom probe applications,Day 4 Final analysis and reporting.Daily Probe validation with control slides,Equipment calibration checks.Weekly Technologist proficiency testing, Environmental monitoring.Monthly Reagent lot validation,Procedure competency assessment. Weak signals:Repeat denaturation (increased time/temperature),Additional protease treatment,Probe concentration adjustment.High background: Increased stringency washes,Formamide concentration optimization,Additional ethanol dehydration.

Multi-color FISH Simultaneous WCP and locus-specific probes,Breakpoint mapping within 50kb resolution,Derivative chromosome origin determination. Interphase/metaphase correlation 200-cell scoring

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minimum,Tissue-specific differences analysis,Confined placental mosaicism identification.3D-FISH Chromosome territory mapping,Translocation partner proximity studies,Gene positioning effects assessment.

This optimized FISH protocol demonstrates superior performance in targeted derivative chromosome analysis, providing critical diagnostic information within clinically relevant timeframes while maintaining rigorous quality standards. The tiered probe approach balances comprehensive assessment with practical workflow considerations, making it an essential component of modern prenatal genetic diagnosis.

2.2.3 Chromosomal Microarray Analysis (CMA) for Genome-wide Detection of Derivative Chromosomes

Our CMA protocol utilizes the latest high-resolution SNP-array technology for comprehensive derivative chromosome analysis:

(1)Platform Specifications:Chip design Illumina CytoSNP-850K array featuring 850,000 markers (300kb median spacing),Enhanced coverage in 500+ clinically relevant regions,SNP CNV ratio of 4:1 for optimal call accuracy.

(2)Sample Processing Workflow:DNA extraction Automated Maxwell® RSC system (Promega),Minimum input 50ng (amniocytes), 100ng (CVS),QC threshold A260/280 = 1.7-2.0, concentration ≥10ng/μL.Whole-genome amplification (28 cycles),Enzymatic fragmentation (37°C, 1h),Hybridization (20h at 48°C),BeadChip scanning (iScan system).

(3)Data Analysis Pipeline:Primary analysis GenomeStudio v2.0 with CNV Partition 3.2.0,B-allele frequency (BAF) and log R ratio (LRR) calculation. Secondary analysis Nexus Copy Number 10.0 with FASST2 algorithm,Population frequency filtering (DBVAR/ClinVar),Gene content analysis (OMIM/ClinGen).

Table 2. Resolution and Detection Capabilities

Variant Type	Effective Resolution	Detection Rate
CNV >100kb	99.5%	98.7%
CNV 50-100kb	95.1%	92.3%
UPD regions	N、A	100%
Mosaicism ≥20%	N、A	94.8%

3.8× higher detection rate for pathogenic findings,10× better resolution for CNVs,Genome-wide coverage vs targeted approach,87% reduction in need for reflex testing.Sample QC metrics Call rate ≥98.5%,SD of LRR ≤0.25,BAF drift ≤0.01.Median CV for control samples <5%,Cluster separation score >0.4.

Comprehensive Characterization,Unbalanced rearrangements, Precise breakpoint mapping (±50kb), Gene content analysis of affected regions, Detection of concomitant CNVs,Marker chromosomes,Genomic content identification, Presence of pathogenic loci, Mosaicism level estimation.Specialized Applications, Uniparental disomy detection, Runs of homozygosity analysis, SNP pattern recognition, Imprinting disorder risk assessment,Low-level mosaicism, Digital pattern recognition, BAF deviation analysis, Sensitivity to 10% mosaicism.

Interpretation Framework and Reporting Standards, Variant Classification, Five-tier system, Pathogenic (ACMG Class 5),Likely pathogenic (Class 4),Variant of uncertain significance (Class 3), Likely benign (Class 2),Benign (Class 1).Clinical Correlation Guidelines,Phenotype-matched analysis,HPO term-driven gene prioritization,Ultrasound anomaly correlation,Known syndrome matching.Reporting Timeline,Standard 10-14 days (95% of cases),Rapid 5-7 days (critical findings),Extended 21 days (complex rearrangements).

This comprehensive CMA protocol provides unprecedented resolution for derivative chromosome analysis, delivering clinically actionable results with significantly higher diagnostic yield than conventional methods. The integration of SNP data enables unique applications including UPD detection and mosaicism assessment, making it an indispensable tool in modern prenatal genetics.

2.2.4 Next-Generation Sequencing (NGS) in Derivative Chromosome Analysis

Our institute has implemented a comprehensive NGS pipeline specifically optimized for prenatal derivative chromosome detection:(1)Sequencing Platforms:Short-read sequencing Illumina NovaSeq 6000 (2×150bp),Minimum 30× coverage (50× for mosaicism),500ng DNA input requirement.Long-read sequencing,PacBio HiFi (15kb reads, ≥Q30),Oxford Nanopore PromethION (Ultra-long >100kb),Specialized library prep for high-molecular-weight DNA.(2)Library Preparation Protocols:Whole genome sequencing PCR-free TruDNA prep (reduce GC bias),350bp insert size optimization,Dual-indexed barcoding (384-plex). Targeted approaches,Custom capture panel (1.5Mb clinically relevant regions),Mitochondrial DNA enrichment protocol.(3)Quality Control Metrics:Pre-sequencing,DNA integrity number (DIN) ≥7.0,Fragment analyzer profile,Qubit quantification (CV<5%).Post-sequencing,≥80% bases ≥Q30,>95% on-target rate

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(capture),<5% duplicate rate (WGS).Base calling Illumina DRAGEN (v3.10),Alignment BWA-MEM (hg38 reference).Variant calling,SNVs/indels GATK HaplotypeCaller,SVs Manta, Delly, LUMPY,CNVs CNVkit, Canvas.Specialized Algorithms,Balanced rearrangement detection, Split-read analysis (GRIDSS), Discordant read pair clustering,Local assembly (SPAdes). Mosaicism analysis, BAF shift detection,Read-depth binomial testing, Machine learning classifiers.(3) Annotation and Interpretation:Variant effect prediction, Ensembl VEP, Pathogenicity assessment, ACMG/AMP guidelines, Gene constraint metrics, gnomAD pLI,Phenotype matching, HPO term analysis.

Table 3. Detection Sensitivity

Variant Type	Size Range	Detection Rate
Balanced SVs	>1kb	98.2%
Unbalanced SVs	>500bp	99.5%
Complex SVs	N、A	95.7%
Mosaicism	≥5%	89.3%

Resolution Comparison,Breakpoint precision,Short-read ±50bp,Long-read single-base,Variant size accuracy,<5% size estimation error,99% concordance with orthogonal ,methods.Turnaround Time,Rapid mode, 7 days (priority cases),Standard mode, 14 days,Comprehensive mode, 21 days (incl. long-read).Clinical Applications in Derivative Chromosome Analysis, Cryptic Rearrangement Detection,Case example,Normal karyotype/CMA,NGS detected t(5;11)(p15;q23),Disrupted regulatory elements of CDH18.Complex Chromothripsis, Resolution capability, >10 breakpoints in single event,Templated insertions,Microhomology analysis.Gene Disruption Analysis, Precise mapping, Exonic vs intronic breakpoints, Fusion gene prediction, Regulatory region impact.

Interpretation Challenges and Solutions,Variant Filtering,Population frequency, <1% in gnomAD-SV,Tissue-specific artifacts, Cultured vs direct,Technical false positives, PCR duplicates.Clinical Correlation,Gene-disease validity*: ClinGen classifications,Expression data,GTEx tissue specificity,Animal models,MGI phenotype matches.Reporting Standards,Tiered classification system,Incidental findings policy,Family studies recommendations.

This NGS approach represents a paradigm shift in derivative chromosome analysis, providing unprecedented resolution and diagnostic yield. The integration of short- and long-read technologies enables comprehensive characterization of complex rearrangements, while advanced bioinformatics facilitates clinically relevant interpretation. As the field progresses, these methods will become increasingly essential for complete prenatal genetic evaluation.

2.3 Data Analysis Pipeline

Firstly, DNA extraction and quality control of fetal cell samples were carried out to ensure the smooth progress of follow-up experiments. According to the characteristics of the derivative chromosome, choose suitable molecular markers, such as fluorescence quantitative PCR, Sanger sequencing, etc., in order to identify and evaluate the derivative chromosome in the subsequent analysis. The original data were analyzed to exclude factors that may affect the results, such as PCR amplification efficiency and sequencing quality. The sequencing data of the sample DNA is compared to the normal reference gene set to determine the derived chromosome present in the sample. Compare and analyze the results, including the copy number of derivative chromosomes, genotype, chromosomal abnormalities, etc. Based on the comparison results, the genetic characteristics of the derived chromosomes are combined to determine the type of derived cromosomes. Genetic counseling is provided to pregnant women, depending on the type and severity of the derived chromosome, to help pregnant women make reproductive decisions. In order to improve the accuracy of the analysis results, genetic linkage analysis using family samples was carried out to further confirm the source of the derivative chromosome. The specific sequences around the derived chromosome are detected by transposing enzymes to determine the type of derived cromosome. Chromosome microarray technology is used to detect the size, structure and other information of derived chromosomes to provide a basis for the diagnosis of derived Chromosomes. The above analysis results are integrated to form a detailed genetic counseling report to provide pregnant women with scientific and accurate genetic advice.

3. Results

3.1 Detection Rates by Technology

NGS outperformed karyotyping/FISH in detecting cryptic translocations (12 cases missed by CMA).Long-read sequencing resolved complex chromothripsis in 3 cases.

Table 4. Detection Rates by Technology

Method	Detection Rate	Resolution	Turnaround Time
Karyotyping	68% (large SVs)	5-10 Mb	10-14 days
FISH	75% (targeted)	50-500 kb	1-3 days
CMA	89% (CNVs)	50-100 kb	7-10 days
NGS (short-read)	95% (all SVs)	<1 kb	10-14 days
NGS (long-read)	99.2% (precise)	Single-base	14-21 days

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3.2 Clinical Applications

3.2.1 Phenotype-Genotype Correlation

The 16p11·2 microdeletion (detected by chromosomal methylation analysis, CMA) is associated with the risk of autism.This finding provides clinicians with important information about autism risk counseling. Equilibrium translocation t (11; 22) (detected by chromosome karyotype analysis and next-generation sequencing NGS) was associated with associated disease risk. The findings could help clinicians assess a patient's risk for the disease and provide targeted genetic counseling for patients and their families.

3.2.2 Pregnancy Management Impact

Termination decision (7 cases of severe abnormality) In seven cases of fetal cases where serious abnormalities were diagnosed, the family decided to terminate the pregnancy at the family's discretion. This process involves in-depth clinical evaluation and ethical discussion. Targeted fetal surveillance (e.g., ultrasound cardiac mapping for 22q11 cases) uses targeted fetal monitoring measures for fetuses with specific derived chromosome abnormalities to better assess their health. For example, 22 q11 cases underwent echocardiography to rule out cardiac structural abnormalities. Preventive intervention (Preim) in pregnancy management takes preventive intervention measures for fetuses with derivative chromosomal abnormalities, aiming to reduce the risk of complications and improve the quality of life. Specific measures may include pregnancy care, nutritional support, etc.

3.2.3 Genetic Counseling Improvements

Common risk stratification distinguishes emerging (low risk) from hereditary (high risk) and helps doctors provide more targeted counseling to patients. Precise breakpoint analysis By analyzing the precise breakpoint of the derived chromosome, we can more accurately judge the damage of genes and provide strong support for genetic counseling. Gene destruction analysis is an important part of genetic counseling, helping to understand the effects of derived chromosomes on gene function. By analyzing the state of gene destruction, it is possible to assess the genetic risk of a patient and provide a basis for clinical decision-making. Traditional karyotype analysis can preliminarily judge whether there are derivative chromosomes by observing chromosome morphology. Gene chip technology allows a more precise determination of the presence of derived chromosomes by detecting the number of copies of genes on chromosomes. Whole-genome sequencing is the analysis of the sequences of the entire genome to find genetic variations caused by derived chromosomes. By detecting derived chromosomes, the risk of fetal genetic diseases can be assessed, and pregnant women can be provided with prenatal diagnosis and genetic counseling. Help to reduce the incidence of a disease, improve the quality of the birth population.

4. Discussion

4.1 Advantages of Multi-Technology Integration

Karyotyping + FISH Best for rapid aneuploidy screening.CMA Gold standard for CNVs in fetal anomalies.NGS Uncovers cryptic SVs, gene disruptions. Long-read sequencing Solves complex rearrangements.

4.2 Clinical Implementation Challenges

NGS VS chromosome typing analysis, the former is costly and has a long turnaround time, sequencing reading takes 3 weeks, and data analysis requires bioinformatics expertise.

4.3 Future Directions

Single-cell sequencing for mosaicism detection.AI-based SV prediction from short-read data.Non-invasive prenatal screening (NIPS) for SVs.

5. Conclusion

The integration of karyotyping, CMA, and NGS maximizes derivative chromosome detection in prenatal diagnosis. Long-read sequencing is particularly valuable for precise breakpoint mapping, improving genetic counseling accuracy. A stepwise diagnostic algorithm—combining cost-effectiveness with high resolution—optimizes clinical decision-making for at-risk pregnancies.First-line, Karyotype + CMA.Second-line, NGS if negative but high suspicion.Third-line, Long-read sequencing for complex cases.This approach ensures comprehensive risk assessment, guiding pregnancy management and family planning.

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