REVIEW ARTICLE

Genetic Analysis of 1p36 Deletions for Six Aborted Fetuses

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ABSTRACT

Context • Chromosomal abnormalities in embryos are the most common cause of early spontaneous abortions. Chromosome 1p36 deletion syndrome (OMIM 607872) is the most common subtelomeric, terminal microdeletion syndrome.

Objective • The study intended to analyze miscarriage samples using chromosomal microarray analysis (CMA), to explore the mechanism of chromosomal aberrations, and to identify the recurrence risk and a prenatal diagnostic strategy for couples experiencing spontaneous abortions.

Design • The research team performed a narrative review by searching PubMed databases. The search used the keywords 1p36 deletion, CMA, karyotype analysis, FISH and aborted fetus. The team also conducted case studies using genetic analyses.

Setting • The study took place at Wuxi Maternity and Child Health Care Hospital in Wuxi, Jiangsu, PR China.

Participants • Out of 673 abortion samples, six had 1p36 deletions (0.89%). Participants were the six families who had had those spontaneous abortions.

Outcome Measures • The research team evaluated the fetal samples using: (1) CMA, (2) karyotype analysis, and (3) novel fluorescence in-situ hybridization (FISH).

Results • The CMA showed that: (1) fetus 1 had a 1.75 MB microdeletion at the 1p36.32p36.31 band, which testing didn't detect in fetus 1's parents, but the research team couldn't exclude the possibility that one of the parents was a carrier of a chromosomal insertional translocation; and (2) fetus 2 had a 5.10 MB microdeletion at the 1p36.13p36.12 segment, and fetus 3 had a 9.21 MB deletion at the 1p36.33p36.22 band, and the high-resolution karyotype analysis and FISH of the parents of both fetuses appeared normal, indicating that the chromosomal abnormalities were de novo; (3) fetus 4 had a 9.28 MB deletion at 1p36.33p36.22, although the high-resolution karyotype analysis of fetus 4's parent was normal; (4) fetuses 5 and 6 had a 7.64 MB microdeletion at 1p36.33p36.23 and a 4.45 MB deletion at 1p36.33p36.32, respectively, although the parents of both fetuses waived further testing.

Conclusions • This study provides the first report of recurrent spontaneous and sporadic abortions with 1p36 deletion syndrome. The CMA combined with a reasonable family-pedigree investigation can detect cryptic chromosomal aberrations in miscarriages and can determine the mechanism of the chromosomal variations. It thus is invaluable in assessing recurrence risk and providing appropriate prenatal diagnostic strategies for affected families. (*Altern Ther Health Med.* 2024;30(10):384-390).

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INTRODUCTION

Spontaneous abortion occurs in about 15-25% of pregnancies, with 80% of those being early abortions at 6-12 weeks.[1] Many reasons exist for such abortions, including embryological, maternal, genetic, environmental, immunological, and endocrinal factors. Chromosomal abnormalities in embryos are the most common cause of early abortions, accounting for about 50-60%, with 86% of them being chromosome-number abnormalities, 6% being structural aberrations, and 8% involving mosaicism and other abnormalities.[1-3]

Chromosomes

The chromosomal subtelomeric region is composed of complex and varied DNA sequences that interact with related proteins to play an important role in maintaining the stability of the genome and the fidelity of chromosome replication. Key biological activities that DNA terminals mediate include

cell-life-cycle regulation, cell senescence and immortalization, intracellular movement and chromosomal localization, and the transcriptional regulation of subtelomeres.[4]

Chromosomal rearrangement often occurs in the terminal subtelomere region, resulting in mental retardation and congenital birth defects. Moreover, most chromosomes' ends are in the shallow G-banding zone, which is prone to hidden rearrangements not easily found through common karyotype analysis.[5]

Chromosome 1, the largest human chromosome, is nearly six times the length of the smallest human chromosomes—21, 22, and Y. It contains 3141 genes, 991 pseudogenes, and many overlapping coding regions, accounting for about 8% of human DNA information and relating to more than 350 human diseases.[6]

Genetic Evaluation

Genetic methods for evaluation of clinical abortions include traditional G-banding karyotype analysis, novel fluorescence in-situ hybridization (FISH), real-time fluorescence quantitative PCR (QF-PCR), multiple ligation-dependent probe amplification (MLPA) and other rapid detection techniques.

Karyotype analysis is still the gold standard for prenatal cytogenetic diagnosis. A high-resolution karyotype can reach 3-5 MB. This method, however, can't detect the submicroscopic structural variations of some pathogenic chromosomes, finding only large-fragment abnormalities that are greater than 10 MB and excluding large-fragment structural rearrangement at the whole chromosome level. In addition, cells' biological activity in abortion samples is low, and karyotype analysis requires cell cultures of abortion tissues, which is time consuming and easily contaminated and often results in the absence of a test report.

Rapid detection techniques such as FISH, QF-PCR, and MLPA, which don't require cell cultures, can quickly detect abnormal numbers of some chromosomes, such as chromosomes 13, 16, 18, 21, 22, X, and Y.[7] Because of the limitations of various genetic testing methods, such as karyotype analysis, however, the resolution of image can be low. Also, FISH can provide dose and location rearrangement information for abnormal fragments, but testing can't determine the size of the abnormal fragments.

Chromosomal microarray analysis (CMA), a new molecular diagnostic technique, can detect submicroscopic chromosomal variations, providing the scientific basis for determining an abortion's cause and a risk assessment in regard to the next pregnancy.

CMA

CMA can help obtain a comprehensive assessment of abortion samples with chromosomal abnormalities and avoid misdiagnosing submicroscopic structures of abnormal chromosomes. This technology can perform genome-wide scans and is able to detect chromosome euploidy, aneuploidy, large-fragment structural abnormalities, mosaicism, and other chromosomal abnormalities. It also has the ability to discover problems not identifiable by conventional karyotype analysis, including microdeletions and microduplications at the genome level, loss of heterozygosity (LOH), and uniparental disomy (UPD).

The CMA's resolution is nearly 1000 times higher than traditional karyotype analysis, and CMA is able to detect at 50-100 kB for copy-number variations (CNVs), using fewer samples than other methods with high accuracy and a short reporting period and without cell culturing.[8]

CMA provides a high level of accuracy, but it can't detect balance changes, including dose-free distortions such as insertions, translocations, and inversions. For chromosomal abnormalities that CMA suggests, physicians should select appropriate genetic analysis techniques according to the type and size of the abnormal segments to solve clinical problems both economically and efficiently.

Chromosome 1p36 Deletion Syndrome

Chromosome 1p36 deletion syndrome (OMIM 607872) is the most common subtelomeric, terminal microdeletion syndrome, and it's a congenital disease that the microdeletion of the short-arm end of chromosome 1 causes. Chromosome 1p36 deletion syndrome has an estimated frequency of 1 in 5000-10 000 live births.[9]

The missing chromosomal pieces are between 1p36.13-1p36.33. The syndrome's probability is about 1/5000-1/10000 per pregnancy. Such fetal patients generally have typical clinical characteristics, including physical or mental retardation; hypotonia, epilepsy, visual impairment, hearing loss, congenital heart defects, cardiomyopathy, renal dysfunction; eye abnormalities, behavioral abnormalities, smallness in stature; and abnormal brain development as well as facial abnormalities, such as microcephaly, a large fontanelle, the fontanelle's delayed closing, frontal bossing, straight eyelashes, sunken eyes, epicanthus, a short palpebral fissure, a wide or flat nose, midfacial dysplasia, and a pointed chin and ear malformation.[10,11]

Patients with 1p36 deletion syndrome can have four types of chromosome rearrangement: simple terminal deletions (67.2%), unbalanced translocation-derived chromosomes (16.4%), simple intermediate deletions (9.7%) and complex rearrangements (6.7%).[12] Due to the different sizes and locations of deletion fragments and the different types and quantities of the genes of patients with 1p36 deletion syndrome, different haploid-dose-sensitive genes can produce different genetic effects, can have different gene imprinting effects, or can expose some recessive mutant alleles, so that patients' clinical phenotypes present individual differences and diversifications.[13]

The 1p36 zone contains many important genes associated with the typical symptoms of patients with 1p36 deletion syndrome. Many researchers have reported studies about 1p36 microdeletion syndrome. For example, Yee JX, Rastani A and Soden ME found that the "potassium voltage-gated channel subfamily A regulatory beta subunit 2" (KCNAB2)

and "gamma-aminobutyric acid receptor subunit delta" (GABRD) genes located in the 1p36.3 zone were candidate genes for epilepsy.[14]

Kandaswamy R, McQuillin A, Curtis D and Gurling H found that for the atypical protein kinase C zeta (PRKCZ) gene, which encodes for protein kinase C, is essential for the process of the wingless-related integration site (Wnt)-signal-mediated regulation of axon differentiation and that an insufficient dosage of the haploid gene can affect the brain's growth and development.[15]

Bassiouni W, Ali MAM and Schulz R found that the expression level of the matrix metalloproteinase 23 (MMP23) gene is related to the fontanel's closure and that a single insufficient dose of this gene can cause a delay in its closure, while excessive expression can lead to premature closure of the cranial suture.[16]

Battaglia et al used karyotype analysis and FISH with sixty patients with 1p36 microdeletion syndrome and found that they experienced developmental and language delays; had straight eyebrows, deep eye sockets, facial hypoplasia, wide nose bridges, long philtrum and pointed chins, and a short flexion finger or short feet.[17] These researchers also found that other symptoms that included congenital heart defects (71%), microcephaly (65%), epicanthus (50%), late closure of fontanelles (77%), ear deformities (40%), eye/visual abnormalities (52%), nerve deafness (28%), skeletal malformations (41%), genitalia malformations (25%), kidney malformations (22%), central nervous system abnormalities (88%), epilepsy (44%), hypotonia (95%), and behavior abnormalities (47%).

Jacquin C, Landais E, Poirsier C, Afenjar A, Akhavi A, et al. used CMA and FISH to study 50 patients with 1p36 monomer syndrome and found that the types and sizes of the missing fragments were diverse among patients: 38 patients had simple terminal microdeletions (76%), including 3 with chimerism; seven had unbalanced translocation (14%), and five had intermediate microdeletions (10%).[10] These researchers also performed a genotypic-phenotypic correlation analysis and found that the key genes for facial abnormalities and mental retardation were located in the 1.8-2.1 and 1.8-2.2 MB regions, respectively, of the band 1p36. They also found that patients with missing fragments larger than 6.2 MB were generally immobile, suggesting that inadequate haploid doses of the "potassium voltage-gated channel subfamily A regulatory beta subunit 2" (KCNAB2) and the "chromodomain-helicase-DNA-binding protein 5" (CHD5) genes located 6.2 MB from the telomere could cause severe neurodevelopmental delays. The "PR domain containing 16" (PRDM16), PRKCZ, and "arginine-glutamic acid dipeptide repeats" (RERE) genes may be candidates for such complications, although the relationship between congenital heart defect phenotypes and patients' genotypes is unclear.

Ji et al and Wu et al found that physicians, at present, have diagnosed 17 cases of 1p36 deletion syndrome in the prenatal period, and their clinical indications mainly included brain dysplasia and heart defects, including eight cases of lateral ventriculomegaly, four cases of hydrocephalus, four cases of corpus callosum dysplasia, two cases of cerebellar vermis absence, one case of small ventricular expansion, and eight cases of cardiac dysplasia with ventricular septal and atrial septal defects. In terms of the deletion fragment types, five cases were purely terminal deletions and 12 were derived chromosome-1p36 deletions.[18,19]

At present, the most commonly studied gene is SKI, located in region 1p36.33. This gene is a proto-oncogene that promotes cell generation, and an insufficient single dose can lead to such effects as hypotonia, mental retardation, motor retardation, heart defects, cleft lips or palates, and limb deformities.[20-23]

Current Study

The current study intended to analyze miscarriage samples using chromosomal microarray analysis (CMA), to explore the mechanism of chromosomal aberrations, and to identify the recurrence risk and a prenatal diagnostic strategy for couples experiencing spontaneous abortions.

METHODS: LITERATURE REVIEW

Procedures

The study took place at Wuxi Maternity and Child Health Care Hospital in Wuxi, Jiangsu, PR China.

Search strategy. The research team performed a narrative review by searching PubMed databases. The search used the keywords 1p36 deletion, CMA, karyotype analysis, FISH and aborted fetus. The review includes articles published in the PubMed language for English.

Inclusion and exclusion criteria. The research team included studies if: Early spontaneous abortion patients in Wuxi Maternity and Child Health Care Hospital.

The research team excluded studies if: Non-early abortion pregnant women.

Literature screening and quality evaluation. Whether to include or not is decided by the relevance of the literature. It is finally decided by Fangbo Qian.

METHODS: CASE STUDIES

Participants

Participants were families who had spontaneous abortions and whose fetuses had 1p36 deletions. Out of 673 abortion samples, six had 1p36 deletions. We obtained informed consent forms from all mothers involved in the study, including for taking blood samples and using their data for fetal and parental analysis. The research was approved by the Ethics Committee of Wuxi Maternity and Child Health Care Hospital.

Procedures

Abortion samples. The research team used whole-genome Affymetrix Cytoscan 750K (Santa Clara, California, America), to detect genomic abnormalities in abortion samples and identified cases of 1p36 deletions. The research team clinically diagnosed these 1p36 deletions using a CMA test with villus samples taken from all the mothers during the abortions. The CMA of the samples' genomic DNA included digestion, amplification, purification, fragmentation, labeling, chip hybridization, washing, scanning, and data analysis in strict accordance with the standard operating procedures of

Affymetrix. The research team also performed karyotyping analysis and FISH. The research team determined the provenance of the deletions through genetic analysis of the fetuses' families.

Sample collection. The research team obtained the chorionic villus during uterine curettage and then washed it in sterile saline multiple times to remove the relevant blood components that could affect cell attachment.

Single nucleotide polymorphism (SNP)-array assay. The research team extracted the genomic DNA from the aborted villus using a tissue-extraction kit (QIAGEN, Duesseldorf, Germany). The CytoScan 750K chip has probes for both CNVs and SNPs. The CNV probes cover the whole genome on average, and the detection rate of genomic structural variation is as high as 99%. The SNP probe can detect not only the microdeletions and duplications of the genome but also polyploids, mosaic, loss of heterozygosity (LOH), and uniparental disomy (UPD).

Provenance. To investigate the origin of the 1p36 deletions in the fetuses, the research team obtained appropriate pedigree analysis from the couples, based on the quantity of copy-number variations (CNVs) in each case and according to the families' wishes.

CMA. Includes the following processes of digestion, amplification, purification, fragmentation, labeling, chip hybridization, washing and scanning.

Digestion: Add 10X Nsp I Buffer, 100X BSA, DNA and Nsp I enzyme to wells, load the plate onto the thermal cycler and run the CytoScan Digest program.

Amplification: Transfer samples to the PCR plate. Add 10X TITANIUMTM Taq PCR Buffer, dNTP Mixture, PCR Primer and Taq DNA Polymerase. Run the CytoScan PCR program.

Purification: Add Purification Beads, centrifuge the tubes, discard the supernatant, add Purification Wash Buffer, Place the tubes on the magnetic stand until all beads are pulled to the side, transfer eluted sample to the appropriate well of a fresh 96-well plate.

Fragmentation: Add Fragmentation Master Mix to each sample, spin down in a pre-chilled centrifuge, load the plate onto the thermal cycler and run the CytoScan Fragment program.

Labeling: Add TdT enzyme, 5X TdT Buffer and 30 mM DNA Labeling Reagent. Load the plate onto the thermal cycler and run the CytoScan Label program.

Chip hybridization: Add Hybridization Master Mix to each sample. Hybridize the arrays 16 to 18 hours.

Washing and scanning: Load the samples and select the appropriate Protocol.

The research team performed data analysis using ChAS software (Affymetrix, Santa Clara, CA, USA) and related bioinformatics methods. The reported threshold applied in this study was more than 200 kB for microdeletions and more than 500 kB for duplications.

Karyotyping analysis. The research team took 5 mL of peripheral blood from the parents of fetuses 1, 2, 3, and 4 for lymphocyte culture. For fetus 1's parents, the team performed the conventional karyotype analysis, using 400-450 bands, while for the parents of fetus 2, 3 and 4, the team performed high-resolution karyotyping, using 550-650 bands.

After digestion and slide preparation, the team scanned the slides using the Lycra GSL-120, high-throughput, automatic chromosome scanning platform (Leica, Heidelberg, Baden-Württemberg, Germany). The team analyzed the parents' karyotypes and described the karyotypes strictly in accordance with the International System for Human Cytogenetic Nomenclature 2020 (ISCN 2020).[24]

FISH detection. The research team took the 0.5-mL cell suspension from the fixed peripheral blood culture of the parents of fetuses 2 and 3. After slide dropping, roasting, denaturing, and dehydration (Denaturation was performed in 0.25% formamide in 2×saline sodium citrate followed by overnight hybridization with a combination of probes. Post-hybridization washes included 2 minuses in 0.4×SSC/0.3% NP-40 at 72°C, followed by 1 minuse in 2×SSC/0.1% NP-40 at room temperature), the team hybridized a custom-specific FISH probe of 1p36.13 on chromosome 1 (RP11-145C4, Vysis, Chicago, Illinois, USA), using a 1p36 custom-specific combination probe with the 1pter, 1p58, and 1q25 probes (Vysis). After washing the slides, the team counterstained them with DAPI and then observed them under a fluorescence microscope (OLYMPUS BX51, Tokyo, Japan). The team performed the image analysis using CytoVision software, Version 7.3.1 (Leica, Heidelberg, Baden-Württemberg, Germany.

RESULTS: LITERATURE SEARCH

In the initial search, I found almost 100 articles. Of them, 30 articles were duplicates, and 30 articles didn't meet the study's criteria.

Outcome measures. The research team evaluated the fetal samples using: (1) CMA, (2) karyotype analysis, and (3) novel fluorescence in-situ hybridization (FISH).

RESULTS: CASE STUDIES

Participants

Out of 673 abortion samples, six had 1p36 deletions (0.89%). Participants were the six families who had had those spontaneous abortions. The six pregnant women had had no exposure to a toxic substance during pregnancy. The parents denied a family history of marriages of close relatives and had no family history of hereditary spontaneous abortion.

Pregnant woman 1. Out of three pregnancies, the woman had never given birth to a live child. The ultrasound at 70 days of the last pregnancy showed a miscarriage. Physicians at the hospital had performed drug flow and uterine curettage after consultation with the birth control department.

Pregnant woman 2. Out of two pregnancies, the woman had given birth to a live child once. An ultrasound scan at 15 weeks showed that the fetal skull aura was unclear and the brain tissue was bulging outwards. The abnormal development of the fetus required an abortion.

Pregnant woman 3. The woman's most recent pregnancy was her first, but she hadn't given birth to a live child. At 70 days of pregnancy, physicians had detected no fetal heart

buds. The patient came to the hospital for uterine curettage, with her consent and that of her family.

Pregnant women 4. The woman had had one pregnancy but hadn't given birth to a live fetus. The serological screening in the middle of her most recent pregnancy indicated a high risk of T18. The ultrasound at 24+6 weeks indicated tricuspid regurgitation; a widening of the lateral ventricle, at 12 mm; and a choroid plexus cyst. A follow-up ultrasound after two weeks showed continuous deterioration, and physicians induced labor.

Pregnant woman 5. Out of two pregnancies, the woman had given birth to a live child once, in a pregnancy of 23+5 weeks. An external hospital had referred the family for a consultation. The abnormal abortion happened due to the fetus' congenital heart defects, microcephaly, and short limbs.

Pregnant woman 6. An external hospital had referred the family. Ultrasound of the woman's current pregnancy showed fetal hydrocephalus in bilateral lateral ventricles. The ultrasound follow-up indicated a miscarriage.

CMA Tests

Table 1 shows the genetic tests' results for the six families with miscarriages.

Fetus 1. This couple had experienced recurrent abortions. Figure 1A shows that the CMA found a 1.75 MB microdeletion in the 1p36.32p36.31 band of the aborted villi, which was Arr [hg19] 1p36.32p36.31 (4,466,726-6,223,962)x1, including the *Online Mendelian Inheritance in Man* (OMIM) [15] genes nephrocystin-4 (NPHP4, 607215), KCNAB2 (601142), and CHD5 (610771). A NPHP4 gene mutation is associated with the autosomal recessive renal wasting disease (nephronophthisis 4).

This clinical phenotype includes growth retardation, renal interstitial fibrosis, polyuria, anemia, and other disease. To determine the origin of the abnormal fragment, the couple also had routine karyotype analysis and CMA testing.

Fetus 2. Figure 1B shows that this fetus had a 5.10 MB fragment missing from the 1p36.13p36.12 segment, namely, Arr [hg19] 1p36.13p36.12 (17,780,133-22,887,434)x1), which contains 36 OMIM genes, including endothelin-converting enzyme-1c (ECE1, 600423), heparan sulfate proteoglycan 2 (HSPG2, 142461), cell division control protein 42 homolog (CDC42, 116952), and Wnt family member 4 (WNT4, 603490).

The abnormal clinical phenotype for this disorder includes abnormal facial features, delayed closure of the anterior fontanel, thick hair, epicanthus, short fingers or congenital flexion, short feet, developmental retardation of different degrees, and mental retardation.[12] To determine the origin and genetic models of 1p36.13p36.12 microdeletion and on the basis of the abnormal fragment size, the husband and wife both received high-resolution karyotype analyses and FISH.

Fetus 3. Figure 1C showed that this fetus had a 9.21 MB microdeletion in the 1p36.33p36.22 band, namely, Arr [hg19] 1p36.33P36.22 (849,466-10,061,193)x1, which contains 95 OMIM genes including GABRD (137163), PRDM16 (605557), and SKI (164780).

The absence of this segment is associated with facial abnormalities, intrauterine growth retardation, dystonia,

Table 1. Genetic Test Results of Six Families With Miscarriages

Families		CMA	Karyotype	FISH
Family 1	Fetus 1	46,XY,del(1p36.32p36.31),1.75 Mb	—	—
	Fat 1	46,XY	46,XY	—
	Mot 1	46,XX	46,XX	—
Family 2	Fetus 2	46,XY,del(1p36.13p36.12),5.10 Mb	—	—
	Fat 2	—	46,XY	Fish 1p36.13(RP11-145C4x2)
	Mot 2	—	46,XX	Fish 1p36.13(RP11-145C4x2)
Family 3	Fetus 3	46,XX,del(1p36.33p36.22),9.21 Mb	—	—
	Fat 3	—	46,XY	Fish 1p36(1px2,p58x2)
	Mot 3	—	46,XX	Fish 1p36(1px2,p58x2)
Family 4	Fetus 4	46,XY,del(1p36.33p36.22),9.28 Mb	—	—
	Fat 4	—	46,XY	—
	Mot 4	—	46,XX	—
Family 5	Fetus 5	46,XX,del(1p36.33p36.23),7.64 Mb	—	—
	Fat 5	—	—	—
	Mot 5	—	—	—
Family 6	Fetus 6	46,XY,del(1p36.33p36.32), 4.45 Mb	—	—
	Fat 6	—	—	—
	Mot 6	—	—	—

Figure 1. CMA results: Deletions. The CMA found: (1) for fetus 1, a 1.75 Mb deletion in band 1p36.32p36.31 (Figure 1A); (2) for fetus 2, a 5.10 Mb deletion in band 1p36.13p36 (Figure 1B); (3) for fetuses 3 and 4, a 9.21 Mb (Figure 1C) and a 9.28 Mb (Figure 1D) deletion, respectively, in band 1p36.33p36.22; and (4) for fetus 6, a 4.45 Mb deletion in band 1p36.33p36.22 (Figure 1E).

Abbreviations: CMA, Chromosomal microarray analysis

Figure 2. Karyotype Analysis. The analysis found: (1) no obvious abnormalities for fetus 2's parents (Figures 2A and 2B); (2) no chromosomal structural rearrangement for fetus 3's parents (Figures 2C and 2D); and no obvious abnormalities for fetus 4's parents (Figures 2E and 2F).

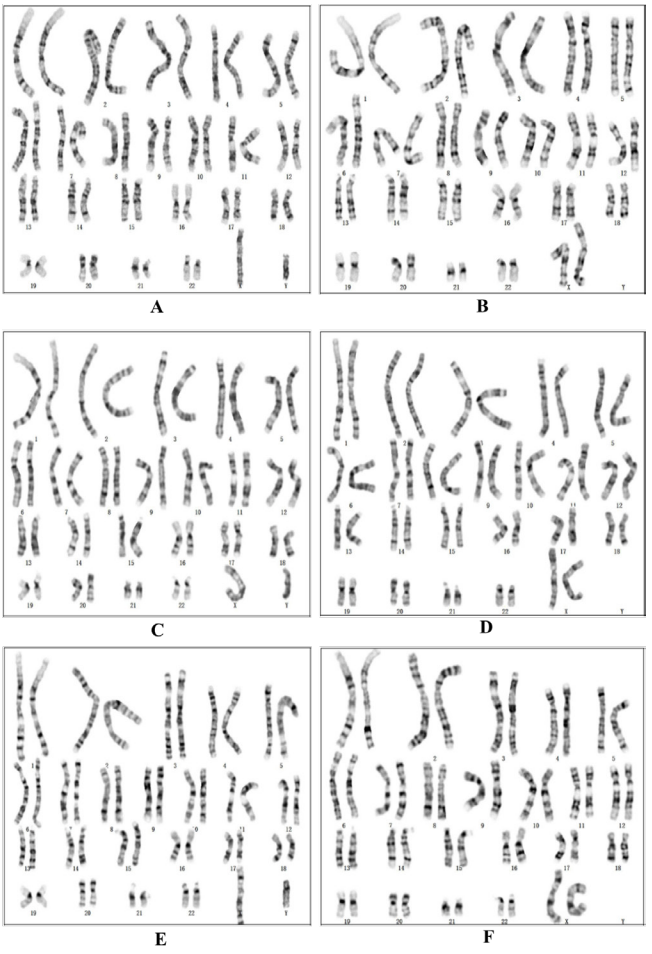
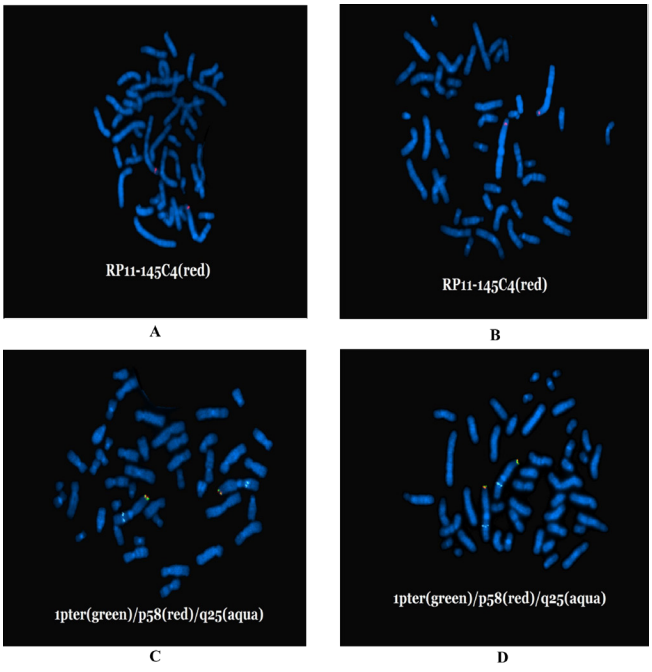


Figure 3. FISH (RP11-145C4) for Fetus 2 and FISH(1Pter, 1p58 and 1q25) for Fetus 3. FISH(RP11-145C4) for fetus 2 showed no abnormalities, such as point deletion, duplication, or translocation (Figures 3A and 3B). FISH (1Pter, 1p58 and 1q25) for fetus 3 showed no chromosome rearrangement (Figures 3C and 3D).



language retardation, intellectual retardation, epilepsy and other clinical phenotypes. The research team verified these finding using high-resolution karyotype analysis and FISH.

Fetuses 4, 5, and 6. Fetuses 4, 5, and 6 all had microdeletions of 9.28 Mb (chr 1:849,466-10,134,535) (Figure 1D), 7.64 MB (chr 1:849,466-8,490,707), and 4.45 MB (chr 1:849,466-5,300,558), (Figure 1E) in the 1p36.33p36.22, 1p36.33p36.23 and 1P36.33P36.32 segments, respectively.

In fetus 4, the 1p36 deletion syndrome involved 96 OMIM genes, such as gamma-aminobutyric acid receptor subunit delta (GABRD, 137163), PRDM16 (605557), and SKI (164780). The research team verified these finding using high-resolution karyotype analysis.

Fetuses 5 and 6 had congenital heart defects, microcephaly, short limbs and hydrocephalus in bilateral lateral ventricles. The research team suggested that the parents of both fetuses first have a high resolution karyotype analysis and then decide whether they needed further FISH testing. However, both families refused further tests, making the research team unable to effectively assess the risk for recurrence.

Karyotype Analysis

Fetus 1. Fetus 1's parents received routine, chromosomal karyotype analysis, with the results being 46, XY, and 46,XX. The test showed no abnormality; the results were normal. Because of the low resolution of the conventional karyotype analysis, it was unable to identify the 1.75 MB microdeletion at the submicroscopic level. Due to technical limitations, the CMA can detect an imbalance in copy-number variations (CNVs) but can't identify balance changes. Although the couple's peripheral blood karyotype analysis and CMA results were normal, the possibility of a 1p36.32p36 insertional translocation could still not be ruled out. The research team needed to performed a FISH to confirm it, but following a consultation, the couple declined to do further examination, so it wasn't possible to perform a risk assessment effectively for the couple to determine the risks for their next pregnancy.

Fetuses 2, 3 and 4. The parents of these fetuses received high-resolution, chromosomal karyotype analysis, and the results were all 46,XY, and 46,XX. The test found no aberrations involving fragment deletion, repetition, or translocation rearrangement (Figures 2A-2F). Following consultation, fetus 4's parents refused to conduct a FISH verifications.

FISH Tests

Fetus 2. The research team tested fetus 2's parents with a FISH probe (RP11-145C4) from manufacturer Vysis (Chicago, Illinois, USA) and found no abnormalities, such as deletions, duplications, or translocations of the probe-site

fragment in either parent (Figures 3A and 3B). Therefore, the microdeletion of 1p36.13p36[25] in the aborted fetuses should have been de novo abnormalities, with a very low risk for recurrence in the next pregnancy.

Fetus 3. At the same time, the team used customized FISH probe groups—1Pter, 1p58 and 1q25—to examine fetus 3's parents and found no abnormalities, such as deletions, duplications, or translocations of the above probe sites, in the parents (Figures 3C and 3D). Thus, neither the husband nor wife were carriers of the 1p36.33p36.22 abnormalities. The microdeletion of this segment in fetus 3 should have been a de novo abnormality, such that the risk for recurrence was very low if the couple gave birth again.

DISCUSSION

As far as the current research team knows, previous reports of 1p36 microdeletion syndrome have primarily involved children and a small number of prenatal fetal samples. This study is the first to report six 1p36 microdeletion cases in abortion samples.

In the current study, two fetuses had 1p36 intermediate deletion. Fetus 1's family had an abortion in the early gestational weeks, and the study found no obvious ultrasound abnormality and fetus 2 showed unclear skull halo and brain tissue bulge, which was consistent with the typical brain development abnormality of 1p36 deletion syndrome.

Four fetuses had 1p36 terminal deletion. Fetus 3 didn't have a fetal bud at 70 days, which may be associated with 1p36 deletion, and the deletion influenced abnormal cardiac development, which lead to the embryo being spontaneously aborting in early pregnancy. Fetus 4 exhibited a widened lateral ventricle, tricuspid regurgitation. and choroid plexus cyst, all related to 1p36 microdeletion symptoms, including heart defects and developmental anomalies of the central nervous system. Fetus 5's deletions involved the fetal heart, bones, and multiple structural abnormalities of multiple organs. Fetus 6's ultrasound showed bilateral hydrocephalus in the fetal lateral ventricle, which are consistent with classical 1p36 microdeletion syndrome symptoms.

Thus, Chromosome 1 is prone to chromosomal rearrangement in the short-arm terminal or telomere region, with many types of associated aberrations. Among the six fetuses in the current study, the abnormalities 1p36 involved intermediate and terminal microdeletion.

The microdeletion syndrome is very harmful. It can cause recurrent or accidental abortions. The CMA's scope is whole-genome analysis, and it has the advantage of high-resolution analysis without requiring cell cultures, so it can find chromosomal submicroscopic structural aberrations in abortion samples. Conventional and high-resolution G-banding karyotype analysis, FISH, and CMA have different advantages and disadvantages. Based on this, physicians can design a reasonable family verification scheme to determine the source of chromosome variation and genetic patterns. This is very valuable in pregnancy risk assessments and prenatal diagnoses.

The number of samples in this study is not large enough and there are not many positive cases. In future work, we hope to detect more cases of 1p36 microdeletions involving intermediate and terminal microdeletions. We will further investigate the relationship between 1p36 microdeletion and embryo abortion, abnormal growth and development.

CONCLUSIONS

This study provides the first report of recurrent spontaneous and sporadic abortions with 1p36 deletion syndrome. The CMA combined with a reasonable family-pedigree investigation can detect cryptic chromosomal aberrations in miscarriages and can determine the mechanism of the chromosomal variations. It thus is invaluable in assessing recurrence risk and providing appropriate prenatal diagnostic strategies for affected families.

AUTHOR CONTRIBUTIONS

Ye Shen and Wei Zhang contributed equally to this work.

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