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Prenatal Diagnosis and Genetic Analysis of *De Novo* Isodicentric Y Chromosome

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Abstract

Objective: Isodicentric Y chromosome [idic(Y)] is the most common structural abnormality of the Y chromosome. Different breakpoints and fusions may lead to different clinical phenotypes. In this study, multiple techniques were used to accurately diagnose a case of prenatal idic(Y).

Methods: Using karyotype analysis, high-throughput sequencing, Short Tandem Repeat (STR) analysis, Fluorescence *in situ* Hybridization (FISH), multiplex PCR, and other detecting techniques were used to make accurate prenatal diagnosis.

Results: Amniotic fluid and umbilical cord blood karyotype results were 46,XY; high-throughput sequencing Copy Number Variation (CNV) analysis revealed two copies of the Y chromosome, FISH probes detected two centromeres of the Y chromosome, Fluorescent Quantitative PCR (QF-PCR) revealed the Y chromosome STR double peak at the site, and Sequence-Tagged Sites of Y chromosome (STS) displayed Azoospermia Factor (AZF)a+b+c(+), Sex-Determining Region Y gene (SRY) (+), and SY160(-). The karyotype analysis of the parents was normal. The above test results indicated that the karyotype of the fetus was *de novo* 46,X, idic(Y)(q12).

Conclusion: Combining multiple cytogenetic and molecular techniques may improve the accuracy of prenatal diagnosis for complex chromosomal aberrations. It will play an important role in accurate genetic counseling and reducing the birth-defect rates.

Keywords: Isodicentric; Prenatal diagnosis; FISH; High-throughput sequencing; QF-PCR

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Introduction

The Isodicentric Y Chromosome [idic(Y)] is one of the most common Y chromosome structural abnormalities [1-2] that may arise from intrachromosomal recombination after Y chromosome breakage or fusion between sister chromatids [3]. Since the broken Y chromosome is extremely unstable during cell division, numerous cell lines can be produced; therefore, most reported patients (90%) have chromosomal mosaicism with 45,X cell line [4]. Individuals with idic(Y) have various somatic, genital, and gonadal phenotypes that depend on the location of the breakpoints and the type of mosaicism [5]. As idic(Y) affects the patient's phenotype significantly, prenatal diagnosis is crucial. Several approaches, including cytogenetic and molecular techniques, are usually combined for prenatal diagnosis of idic(Y) chromosomes. These methods can complement each other according to their advantages and limitations [6].

This article describes a case of *de novo* idic(Y) in prenatal diagnosis. The unique feature of this case is that the breakpoint was located in the long-arm heterochromatin region (Yq12), resulting in complete Y chromosome duplication, and there were no 45,X, or other cell lines, which is rare in previous reports.

Case Introduction

It was the first pregnancy for the 23-year-old woman. The fetus's Nuchal Translucency (NT) of 1.2 mm in the first trimester at 12+3 weeks of pregnancy. Non-Invasive Prenatal Testing (NIPT) results indicated high risk for sex chromosome aneuploidy. There was no obvious abnormality in B-ultrasound. Prenatal diagnosis was then performed at 24+3 weeks of pregnancy. Amniotic fluid was collected by transabdominal amniocentesis in a sterile environment and subjected to karyotyping, high-throughput sequencing CNV assay and QF-PCR detection. Umbilical cord blood samples were also collected aseptically during delivery for karyotype analysis, FISH, STS, and QF-

PCR testing.

Methods

Cell chromosome karyotyping

G-banding chromosomes were prepared from general cultures of amniotic fluid cells and cord blood cells. Using the ZEISS MetaClient system, we counted 40 amniotic fluid metaphase cells and analyzed 5 karyotypes and 100 cord blood metaphase cells and analyzed 10 karyotypes. Karyotypes were described according to the standards of the International System for Human Cytogenomic Nomenclature (ISCN2020).

Fluorescence *in situ* hybridization

FISH was performed using prenatal chromosome detection kits from the Beijing Jinbojia Company. A multicolor probe panel containing CEP 18 (D18Z1) (blue), X(DXZ1) (green), and Y (DYZ3) (red) was used to detect duplicates of the centrioles of chromosomes 18, X, and Y. Specific hybridization with centromeric regions showed fluorescence of different colors.

Sequence-tagged sites of Y chromosome

Deletions in AZFa, AZFb, and AZFc regions of the Y chromosome were detected using the Y chromosome Microdeletions Test Kits (STS) (Shenzhen Ya Neng Biotechnology Co., Ltd.), and the results were interpreted in strict accordance with the standards manual. The subregion encompassed six sequence tags for detecting microdeletions in the AZF region, eight sequence tags for determine whether the entire region was completely missing, and one hetero-chromosomal tag, SY160. The internal references used were SRY and ZF X/Y.

QF-PCR

Fluorescent PCR capillary electrophoresis was used for qualitative detection of specific Short Tandem Repeat (STR) loci on X and Y chromosomes. The specific SRY locus on the Y chromosome, the AMXY locus that reflects the number ratio of X and Y chromosomes, and four specific loci, DXS1187, DXS6809, DXS8377, and DXS981 on the X chromosome, were selected for detection.

High-throughput sequencing

DNA was extracted using reagents from Hangzhou Berry Genomics Company, and then low-depth whole genome sequencing was performed by "reversible termination sequencing". Chromosome aneuploidy and genomic Copy Number Variation (CNV) above 100 kb were detected.

Results

The fetal amniotic fluid chromosome karyotype result was 46, XY (Figure 1a); FISH probe indicated two Y chromosome centromeres (Figure 1b); the high-throughput sequencing CNV results showed two Y chromosome copies (Figure 1c); QF-PCR results revealed double peaks of Y chromosome STR loci (Figure 1d); Y chromosome microdeletion detection (STS) suggested AZFa+b+c(+), SY160(-), and SRY(+) (Figure 1e). The father's peripheral blood chromosome analysis for G-banding (Figure 1f) was normal as 46,XY. His C-banding (Figure 1g) of the Y heterochromatin region showed deeply stained. The same mutation as the fetus was not found. Based on the above test results, we diagnose the fetus Y chromosome change was *de novo* with a 46,X,idic(Y)(q12) karyotype.

After thorough genetic counselling and consideration, the couple decided to continue the pregnancy. At 40 week's pregnancy, a normal baby boy of 49 cm in height and 2,700 g in weight was born. The external genitalia are normal.

Discussion

The idic(Y) is the most common Y chromosome aberration, occurring in 15% to 30% of infertile men with abnormal Y chromosomes [7]. The mechanism of idic(Y) formation remains unclear. However, the prevailing view is intrachromosomal recombination or fusion between sister chromatids following the chromosomal break of the Y chromosome [8]. The clinical phenotype is based on various factors, including the Y chromosome breakpoint, the proportion of idic(Y) cell lines in tissues such as gonads, and the presence of SRY and AZF genes [9].

Due to the presence of two centromeres, idic(Y) is rather unstable, which leads to various mosaic phenomena. The most common is the 45,X cell line, which is probably due to the loss of idic(Y) during mitosis. Then it is followed by the 46,X,idic(Y) cell line. Mosaicism induces highly diverse clinical phenotypes ranging from partial virilization and genital malformations at birth to an exclusively female or male phenotype. Sex determination in these mosaic patients depends on the predominant cell lineage in the undifferentiated gonads. Specifically, the presence of the 45,X cell lineage leads to Turner syndrome, 46,XY cell lineage leads to a male phenotype, and the presence of both cell lineages leads to incomplete mixed gonadal development [10]. Most previous case reports were chimeric.

We collected all non-chimeric idic(Y) case reports and summarized them in Table 1.

Based on the collected cases, non-Chimeric Muric idic(Y) is rare. In this case, we counted 40 amniotic fluid cells and 100 umbilical cord blood cells in metaphase and tested amniotic fluid CNV; no 45,X, or other karyotypes were found. We diagnosed the fetus as homozygous 46,X, idic(Y) karyotype. In 2005, Wolfram Heinritz reported an adult male with a Klinefelter phenotype of 47,XX, +idic(Y)(q12). FISH analysis and the results of molecular studies were consistent with those described for an individual with a karyotype of 48, XXYY, which also supports the finding that isodicentric Y chromosomes are often accompanied by other sex chromosome abnormalities [11]. DesGroseilliers et al. described two patients with non-chimeric idic(Y) who had a karyotype of 46,X, idic(Y)(q11.21). As a result, most Yq was lacking, and the Y centromere (DYZ3) and SRY were positive, but Yq heterochromatin (DYZ1) was negative. Both patients had normal external genitalia, with only slight language and growth retardation [12]. Cauwenberghe et al. reported a non-chimeric patient with a 46,X, idic(q11.22) karyotype. The Y chromosome microdeletion detection test revealed deletion of the AZFbc gene, and the patient demonstrated azoospermia [14]. Yang et al. reported a case of prenatal diagnosis of a fetus with a karyotype of 46,X, idic(Y)(p11.3).ishidic(Y) (p11.3)(SRY++). The patient decided to continue the pregnancy and gave birth to a phenotypically normal male [6]. The clinical phenotype of non-chimeric idic(Y) is strongly associated with the breakpoint and the loss of SRY and AZF genes.

Moreover, identifying the breakpoint is the focus of idic(Y) diagnosis. FISH, CNV, Sequence-Tagged Site (STS), and other techniques must be combined for a comprehensive evaluation. Two important genes on the Y chromosome, the SRY gene located in the short arm (p11.32), are critical for the development of male secondary sexual characteristics [17]. However, when other cell lines

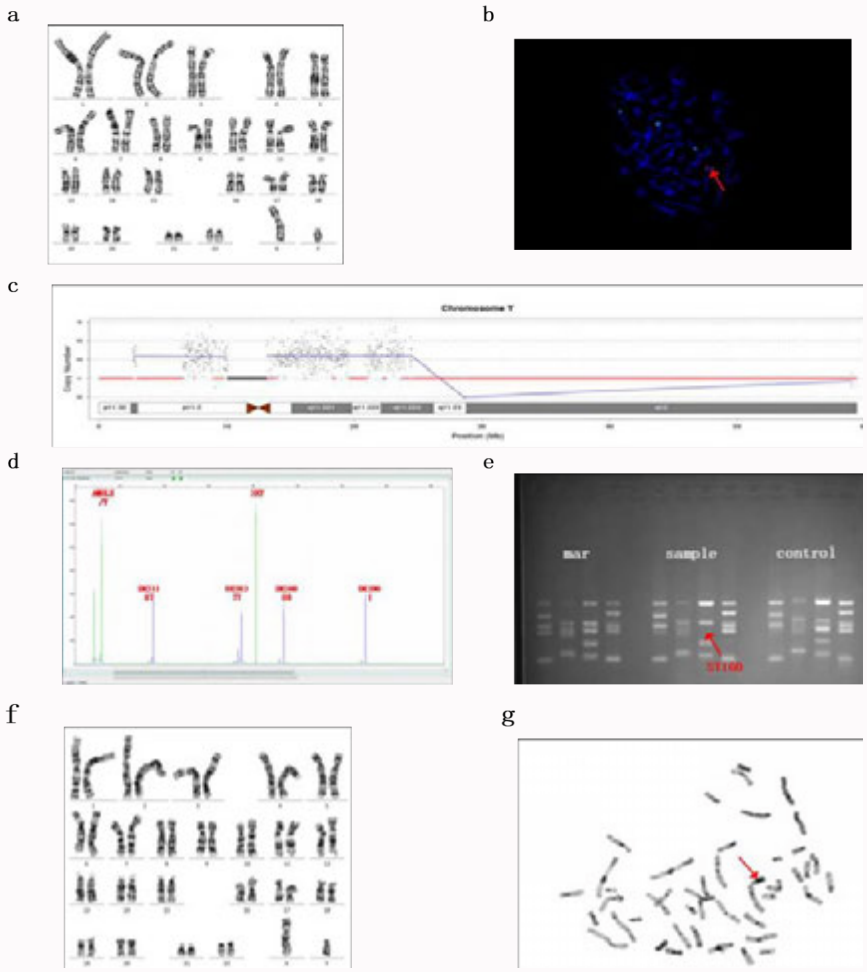

Figure 1: a) G-banding of the chromosomes. b) FISH analysis of the Y chromosome. The two red signals represent isodicentric Y chromosome. c) CNV shows the duplication of Y chromosome euchromatic region. d) QF-PCR shows the duplication of SRY. e) STS shows AZF a+b+c(+),SY160(-),SRY(+). f) Father's G-banding of the chromosomes. g) Fathers C-banding of the chromosomes.

coexist, the copy number of the SRY gene often cannot determine a patient's phenotype. Some patients with two copies of the SRY gene in idic(Y) still have ambiguous genitalia due to mosaicism [18]. Yp11.32 has been reported to be a very common breakpoint in idic(Y), including females with certain Turner's syndrome traits, males with azoospermia, and patients with ambiguous genitalia and Mixed Gonadal Dysgenesis (MGD) [19]. The AZF gene region located on the long arm of the Y chromosome (q11.2) includes AZFa, AZFb, and AZFc subregions. Deletion of one or more of these regions can lead to sperm deficiency, and AZF gene deletion is the second leading cause of male infertility after Klinefelter syndrome [20].

Accurate prenatal diagnosis of idic(Y) is difficult using only karyotype analysis techniques. We combined molecular detection techniques, such as CNV, STR, and other analyses, to determine the presence of two copies of Y chromosomes and used FISH technology to confirm the existence of dicentrics, using CNV STS and other techniques to accurately locate the deletion and breakpoint of the SRY and AZF genes, and identified the correct karyotype as 46,X, idic(Y)(q12).SY160(-) also indicate deletion of the heterochromatin part (q12). It is widely believed that del(Y)(q12) is unrelated to infertility because the deletion is limited to heterochromatin, whereas del(Y)(q11) is associated with azoospermia or severe oligospermia because the AZF region is involved [21]. Kuan et al. reported a non-chimeric case with a prenatal diagnosis of idic(Y) (q11.23) karyotype [10]. The breakpoint is located very close to telomeres in the Yq11.23 pseudoautosomal region, which is also a hotspot for gene recombination and conversion [22]. The aCGH data revealed duplication of the entire Y chromosome, and the fetus was born with a normal phenotype. This is very similar to the case we reported. The breakpoint was in the heterochromatin region, without loss of important genes, and the external genitalia were normal after birth. The clinical phenotype of this patient is similar to that of patients with 47, XYY, including tall stature, small testicles, hypotonia and so on However, they usually exhibit normal fertility [23].

Due to the limitations of each technique, using a single method to identify idic(Y) can be challenging, especially when other cell lines coexist. Combining multiple techniques is crucial for the correct diagnosis of fetal Y chromosome structural variation and determining the deletion of important genes, enhancing the interpretation of test results [24]. In conclusion, the combination of cytogenetic and molecular analyses could not be only served as confirmation but also provided more detailed information on the derivative chromosomes for genetic counseling, which is important in prenatal diagnosis.

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Table 1: Summary of cases with non-chimeric idic(Y).

NO.	Age	Karyotype	Clinical phenotype	Document number
1	31	47,XX,+ idic(Y)(q12)	Borderline intelligence,	[11]
		SRY(+)	abnormal behavior	
2	4	46,X,idic(Y)(q11.21)	Eugenia externa	[12]
		SRY(+)	total retardation	
3	2	46,X,idic(Y)(q11.21)	Eugenia externa,	[12]
		SRY(+)	moderately language backward	
4	35	46,X,idic(Y)(q11.2)	Azoospermia	[13]
		SRY(+),AZF c(-)		
5	29	46,X,idic(Y)(q11.2)	Azoospermia	[13]
		SRY(+),AZF abc(-)		
6	30	46,X,idic(Y)(q11.222)	Azoospermia	[13]
		SRY(+),AZF bc(-)		
7	38	46,X,idic(Y)(q11.21)	Azoospermia	[13]
		SRY(+),AZF abc(-)		
8	34	46,X,idic(Y)(q12)	Azoospermia	[13]
		SRY(+),AZF abc(+)		
9	36	46,X,idic(Y)(q11.22)	Azoospermia	[14]
		SRY(+),AZF bc(-)		
10	AF	46,X,idic(Y)(q11.23)	Phenotype normal	[15]
		SRY(+),AZF abc(+)	after birth	
11	AF	46,X,idic(Y)(p11.3)	Phenotype normal	[6]
		SRY(++)	after birth	
12	33	46,X,idic(Y)(q11.2)	Azoospermia	[16]
		SRY(+),AZF bc(-)		
13	28	46,X,idic(Y)(q12)	Azoospermia	[16]
		SRY(+),AZF abc(+)		

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