# CLINICAL AND CYTOGENETIC OF THREE CASES WITH ISODICENTRIC Y CHROMOSOME

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**ABSTRACT**

*Isodicentric Y is one of the most common structural abnormalities of the Y chromosome. It is unstable during cell division, can generate various types of cell lines. Most reported patients are chromosomal mosaics, generally including a 45,X cell line [1]. Clinical manifestations of people carrying isodicentric Y chromosomes are usually: Turner syndrome in women, infertility in men, ambiguous genitalia, gonadal disorders, short stature... We report 3 cases: 2 females and 1 patient of unknown gender with characteristics short stature and abnormal genitalia. All 3 cases have a mosaic mosaic karyotype between the two cell lines: 46, X,idic(Y) and 45,X, have been diagnosed in cytogenetic and molecular genetic techniques at the Vietnam National Children's Hospital.*

***Keyword:*** *genital abnormalities, isodicentric Y, 46,X,idic(Y)(p11.32), 46,X,idic(Y)(q11.2).*

## I. INTRODUCTION

The isodicentric Y chromosome [idic(Y)] was first reported in 1966 by Jacobs and colleagues [2]. This is one of the most common structural abnormalities of the Y chromosome. The formation of the idic(Y) chromosome is believed to result from intrachromosomal recombination or fusion between sister chromatids after Y chromosome breakage. The locations of breaks and fusions in the Y chromosome are very different such as: p11.32, q11.21, q12... The location of the break point of idic(Y) will determine which material of the Y chromosome is maintained, leading to different duplication or deletion of the Y. The idic(Y) chromosome is structurally unstable due to the existence of two centromeres. Depending on the location of the Y chromosome breakage and recombination, different cell lines will be created, the most common being the 45,X cell line. Patients with the idic(Y) chromosome have diverse clinical manifestations such as: Turner syndrome in women, infertility in men, unclear external genitalia, sexual dysfunction, short stature... [3]

Therefore, we present 3 patients of idic(Y) chromosome disease that were diagnosed by karyotyping techniques from peripheral blood cells and Fluorescence in situ hybridization (FISH) techniques, PCR to diagnose testicular differentiation factor (TDF) at the National Children's Hospital.

## II. CASE REPORTS

### *2.1. Case reports*

We report 3 cases: 2 patients with female phenotype and 1 patient of unknown gender with clinical and paraclinical characteristics described in table 1.

### *2.2. Genetic testing*

Chromosome examination: peripheral blood samples were cultured in RPMI medium (Gibco) in the presence of Phytohemagglutinin (PHA - Sigma) to stimulate the growth of T lymphocytes. Chromosomes were stained with Giemsa (Merk) and 30 metaphases were

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analyzed. We discovered that all 3 patients had mosaic Y chromosome abnormalities in the form of isodicentric Y (Figure 1). The karyotypes of 3 patients are:

Patient 1 has mosaicism of 2 cell lines: cell line 46,X,idic(Y)(p11.32) accounts for 86.6%, cell line 45,X accounts for 13.4%; karyotype: 46,X,idic(Y)(p11.32)[26]/ 45,X[4].

Patient 2 has two mosaic cell lines, 45,X and 46,X,+mar. After doing a fluorescence in situ hybridization test, we determined that the marker was the idic(Y) chromosome (p11.32). Therefore, patients with mosaicism of 2 cell lines: cell line 46,X,idic(Y)(q11.23) accounts for 70%, cell line 45,X accounts for 30%; karyotype: 46,X,idic(Y)(q11.23)[21]/ 45,X[9].

Patient 3 has mosaicism of 2 cell lines, cell line 46,X,idic(Y)(p11.32) accounts for 73%, cell line 45,X accounts for 27%; karyotype: 46,X,idic(Y)(p11.32)[22]/ 45,X[8].

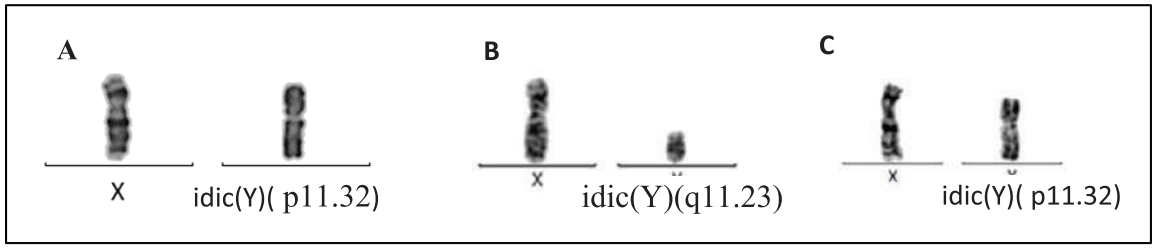


**Figure 1.** X and idic(Y) chromosomes of 3 patients.
A. Patient 1: idic(Y)(p11.32)
B. Patient 2: idic(Y)(q11.23)
C. Patient 3: idic(Y)(p11.32)

To confirm abnormalities of the Y chromosome, we performed fluorescence in situ hybridization (FISH) on cultured peripheral blood cells when the chromosomes were in metaphase (metaphase FISH). We use the 2-color probe "CEP X SpectrumGreen/Y SpectrumOrange Direct Labeled Fluorescent DNA Probe Kit" from Vysis to mark the DXZ1 gene at the centromere position Xp11.1-q11.1 of chromosome X (green) and Y chromosome (red) at the centromere position Yp11.1-q11.1 in the DYZ3 gene region. The results revealed that all 3 idic(Y) chromosomes have 2 centromeres. The mosaic rate of FISH results of 3 patients is as follows:

**Patient 1:** ish (DXZ1x1,DYZ3x0)[28/99],(DXZ1x1,DYZ3x2)[71/99]. Cell line 1 X signal accounts for 28.3%; cell lines with 1 X signal, 02 Y center signals idic(Y)(p11.32) accounts for 71.7%.

**Patient 2:** ish (DXZ1x1,DYZ3x0)[169/446],(DXZ1x1,DYZ3x2)[277/446]. Cell line 1 X signal accounts for 38%; cell lines with 1 X signal, 02 Y center signals idic(Y)(q11.23) accounts for 62%.

**Patient 3:** ish (DXZ1x1,DYZ3x0)[ 149/358 ]/(DXZ1x1,DYZ3x2)[ 209/358 ]. Cell line 1 X signal accounts for 41.6%; cell lines with 1 X signal, 02 Y center signals idic(Y)(q11.23) accounts for 58.4%.

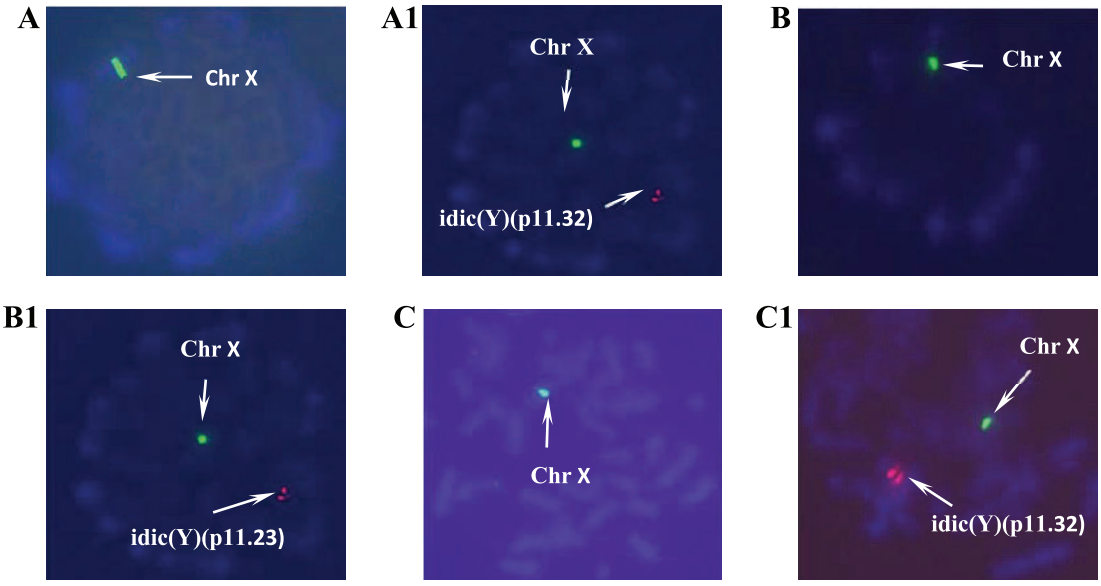


**Figure 2.** FISH results
Patient 1. A: 1 X signal, A1: 1 X signal, 2 Y idic(Y) signals (p11.32)
Patient 2. B: 1 X signal, B1: 1 X signal, 02 Y signals idic(Y)(q11.23)
Patient 3. C: 1 X signal, C1: 1 X signal, 02 Y signals idic(Y)(p11.32)

PCR test to diagnose testicular differentiation factor, results: all 3 patients have testicular differentiation gene.

**Table 1.** Clinical characteristics of 3 cases

| Case | Age at diagnosis | Sex | Phenotype | Subclinical | Genetic results |
|---|---|---|---|---|---|
| 1 | 15 year olds | Female | Delayed puberty, short stature 141.5cm(-2.8SD), 48kg, female external genitals | - Abdominal ultrasound showed a uterus with normal shape, size 26x8 mm, left ovary 6x15 mm, right ovary not visible,<br>- Bone age X-ray is equivalent to 14 years old. | 46,X,idic(Y)(p11.32) [26]/45,X[4] ish(DXZ1x1,DYZ3x0)[28/99], (DXZ1x1,DYZ3x2)[71/99] There is a gene for testicular differentiation |
| 2 | 18 months | Female | Female phenotype, height 86 cm, weight 9.8 kg within normal range, female external genitalia | Abdominal ultrasound showed a flattened uterus measuring 13x4 mm, no ovaries were seen on both sides, and no testicular tissue was observed in the inguinal canal. | 46,X,idic(Y)(q11.23) [21]/45,X[9] ish(DXZ1x1,DYZ3x0) [169/446], (DXZ1x1,DYZ3x2) [277/446] There is a gene for testicular differentiation |
| 3 | 7 months | Undefined | Weight 6.7 kg, height 65.5 cm within normal range, normal face, - Abnormal external genitalia; The scrotum has no wrinkles, resembles the labia majora, has its own urethral sinus, and the testicles are not palpable. | - Ultrasound did not show images of testicles on both sides, there was a structure behind the bladder suspected to be the uterus, and no ovarian organization was observed.<br>- The patient had inguinal hernia surgery combined with laparoscopy to explore the gonads but no testicles or ovaries were seen. | 46,X,idic(Y)(p11.32) [22]/45,X[8] Ish(DXZ1x1,DYZ3x0)[ 149/358 ]/ (DXZ1x1,DYZ3x2) [ 209/358 ] There is a gene for testicular differentiation |

## III. DISCUSSION

The Y chromosome contains about 70 genes, including important genes such as the sex determination gene SRY, spermatogenesis, growth and development genes, so deletion and duplication of the Y chromosome can cause abnormalities. often in terms of phenotypes and functional disorders such as: abnormal external genitalia, oligospermia, infertility causing risk of infertility, short stature, mental retardation...[5]

The formation of the idic(Y) chromosome is thought to be the result of intrachromosomal recombination or fusion between sister chromatids after the breakage of the Y chromosome at meiosis I and meiosis. II (Figure 3). The locations of breakage and fusion in the Y chromosome are very different such as: p11.32, q11.21, q12... The Y chromosome is more likely to be broken at AT-rich positions. Previous studies have applied conventional karyotype analysis, FISH, Southern blot, and PCR to identify idic(Y) chromosomes and breakpoints. Other techniques such as MLPA, QF PCR, sequencing of certain genes have also been used to better understand the molecular structure of the idic(Y) chromosome[2]. In this study, we used 3 methods: chromosome formulation from peripheral blood cells, fluorescence in situ hybridization (FISH), PCR to diagnose testicular differentiation factor (TDF). …., confirmed the diagnosis of Y chromosome structural abnormality as idic(Y) type abnormality, determined to have the TDF testicular differentiation gene. Due to the limitations of each technique, the combination of cellular and molecular genetic analysis will help determine the exact point of breakage and recombination on the Y chromosome and fully identify the cell lines present in the patient's body. Therefor, we can diagnose the patient's genetic abnormality and provide appropriate genetic counseling.

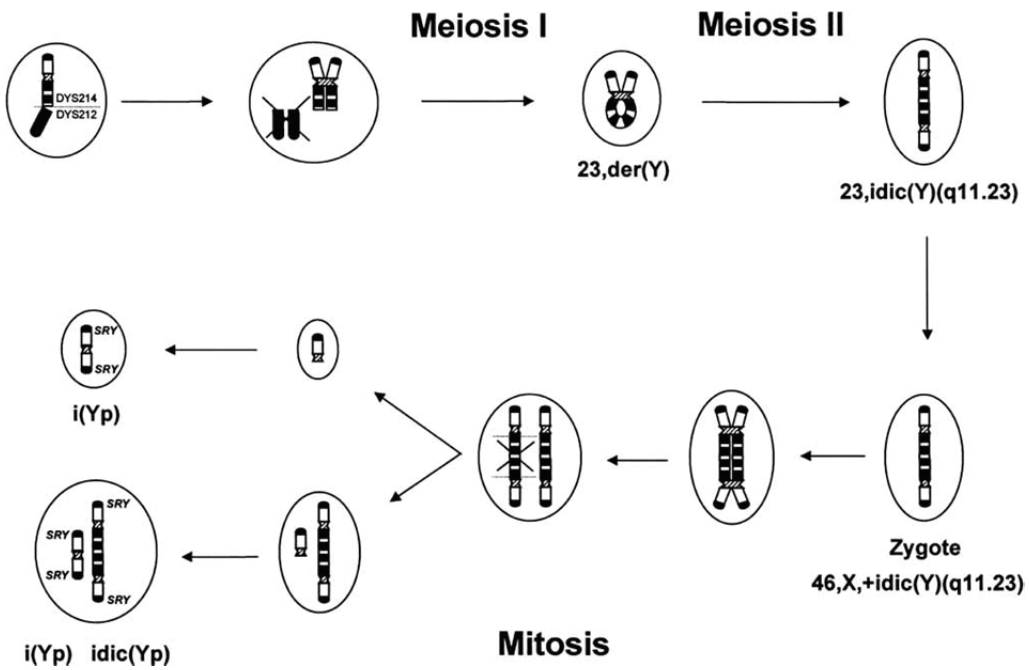


**Figure 3.** Isodicentric Y chromosome formation

In this study: 2 patients were female, 1 patient had unknown gender. Patient 1, whose age was during puberty, showed signs of delayed puberty, low height -2.8 SD, had a left ovary, and the right ovary could not be evaluated. Patient 2 has a uterus, no ovaries on either side, and no testicular tissue in the inguinal canal. Patient 3 had abnormalities in the external genitalia,

ultrasound did not show testicles on both sides, there was a structure behind the bladder suspected to be the uterus, and no ovarian organization was observed. All 3 patients have clinical features consistent with people with mosaic idic(Y) chromosomes.

Regarding the results of karyotype, all 3 patients had a mosaic chromosome formula of 46,X,idic(Y)/ 45,X, the 46,X,idic(Y) cell line accounted for more than the 45,X cell line. Patients 1 and 3 have the idic(Y) chromosome with the same break point at the short arm position p11.32. At that time, the patient will have a duplicate Y chromosome segment in the Yp11.32-q12 region, and lose the short wing of the Y chromosome in the Yp11.3-p11.32 region. Although the break is at position Yq11.32, the exact region containing the SRY testicular differentiation gene, when doing a PCR test to diagnose the testicular differentiation factor, the patient still has the SRY gene. Patient 2 has a broken idic(Y) chromosome at the long arm position Yq11.23, meaning that the Yp11.3-q11.23 gene region will be duplicated, losing the long arm of the Y chromosome region q11.23-q12. Then patient 2 will have 2 SRY testicular differentiation genes. The presence of this SRY gene was determined through testicular differentiation factor diagnostic PCR technique. Idic(Y) chromosome breaks at positions p11.32 and q11.23 are quite common and consistent with the study of Y. Yang and colleagues [3].

Idic(Y) chromosomes are determined by FISH with a probe marked at the center of the X chromosome and the center of the Y chromosome: The idic(Y) chromosome is a chromosome with 2 centroid signals. All 3 patients had two mosaic cell lines, 45,X and 46,X,idic(Y), consistent with karyotypes. Although the chimera rates of the two techniques of chromosome formula and FISH are not exactly the same, they all show that all 3 patients have more 46,X,idic(Y) cell lines.

The idic(Y) chromosome carries two centromeres, so it is unstable during cell division. Researchers have noted that idic(Y) chromosomes can achieve stability through inactivation of a centromere and active centromere contraction, which becomes associated with mitotic spindles [2] [2]. However, idic(Y) chromosomes appear to be unicentric or unidirectional depending on the distance between the centroids. If the distance between centromeres is small enough, two centromeres can function as one centromere [3]. Due to the instability of idic(Y) chromosomes, they often appear as highly mosaic. In this study, all 3 patients had a mosaic karyotype and the 45,X cell line was the most common mosaic type. These findings are consistent with most reported cases of idic(Y) chromosomes [2,3,4]. There are also many unusual chromosomes that can arise from isocentric Y chromosomes, leading to very complex chromosome patterns [4]. This mosaicism may be related to the timing of idic(Y) chromosome formation during meiosis or postzygotic, mitotic instability.

The SRY gene located at position Yp11.32 plays an important role in the development of secondary sexual characteristics in men [1]. The break point at the short arm position p11.32 is very common in the idic(Y) chromosome [2]. However, the copy number of the SRY gene cannot determine the patient's phenotype due to the coexistence of other cell lines . Some patients with two copies of the SRY gene on the idic(Y) chromosome still have ambiguous genitalia resulting from mosaicism. Azoospermia and infertility observed in patients with idic(Y) chromosomes are mainly related to breakpoints in Yq, leading to deletions and rearrangements of weakly related loci azoospermic factor (AZF) (AZFa, AZFb and AZFc) [2]. These three loci are all involved in spermatogenesis and loss of any one locus causes oligospermia or azoospermia. However, some researchers reported that a patient without the AZF deletion presented with azoospermia possibly due to structural abnormalities of the Y chromosome or a mosaicism [3 ] . The Yp11.32 also contains the short stature homeobox (SHOX) gene, which is involved in the proliferation and differentiation of chondrocytes and hence growth retardation in affected patients. High incidence of 45,X cell lines induced short stature due to reduced SHOX

gene copy number during cartilage plate growth [5]. This explains the low height of patient 1.

Regarding treatment: the principle is symptomatic treatment. Patients with idic(Y) chromosome abnormalities often have diverse and complex clinical manifestations, so they need to be monitored and treated at specialized hospitals. Patients with short stature can achieve near-adult height with growth hormone therapy at an early age. Female patients with idic(Y) and gonadal chromosomes are at high risk of developing gonadoblastoma, especially after puberty; therefore, prophylactic gonadectomy is strongly recommended [3].

## IV. CONCLUSION

Idic(Y) chromosome abnormalities are one of the most common structural abnormalities of the Y chromosome. Clinical manifestations of idic(Y) chromosome carriers are: clinical features of the syndrome Turner in women, infertility in men, unclear external genitalia, genital disorders, short stature... Genetic tests are used to diagnose idic(Y) chromosome abnormalities. Identifying chimeric cell lines plays an important role in providing treatment plans, management and genetic counseling for patients and families.

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