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MIXED GONADAL DYSGENESIS WITH 45,X/46,X,IDIC(Y)/ 46,XY,IDIC(Y) KARYOTYPE

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Summary: *Mixed gonadal dysgenesis with 45,X/46,X,idic(Y)/46,XY,idic(Y) karyotype:* To present the new karyotype with mixed gonadal dysgenesis, the aetiologic approach and difficulties in genetic counseling in mosaic sex chromosome disorders. We report a fourteen-year-old boy presented with slightly ambigious genitalia. Cytogenetic and fluorescence *in situ* hybridization investigations were carried out on his peripheral lymphocytes. As a result, three cell lines, 45,X, 46,X,idic(Y)(q11.2) and 46, XY were observed. A markedly higher percentage of Y-containing cells was observed in the blood (68%), which was not considered to be the major reason why the case did not have distinct ambiguous genitalia. We suggest that study of cytogenetic and molecular mosaicism involving sex chromosomes may help to further unravel the mysterious process in mixed gonadal dysgeneic patients.

Key-words: Mixed Gonadal Dysgenesis – Sex Chromosome Mosaicism – Isodicentric Y.

INTRODUCTION

One of the most common structural changes of the Y chromosome is dicentric Y. As dicentric chromosomes are inherently unstable and a mosaic monosomic X (45,X) cell line is frequently present. Mosaicism for 45,X/46,XY exhibits wide variability ranging from Turner syndrome to mixed gonadal dysgenesis to normal males (3, 13, 14). With 45,X/46,XY mosaicism, the frequency of 45,X and 46,XY cells in peripheral leucocytes often does not correlate with the phenotype. Sexual phenotype is thought to be related to the ratio of normal testicular tissue which induces virilization. Particularly those with the presence or absence of the sex determining gene, SRY, on the structurally abnormal Y chromosome. However, studies on gonadal tissue are hindered by the fact that it is rarely available for analysis, and a more easily-accessible tissue is usually studied. Also, in patients with MGD, molecular analyses have failed to demonstrate the presence of mutations in the *SRY* gene (1, 2). Therefore, previous studies have suggested that these individuals may bear mutations in other genes involved in the testis-determining pathway such as azoospermia factor (*AZF*) a, b and c genes, desert hedgehog (*DHH*) gene, etc. (1, 2). Here, we present a mosaic 45,X/46,X, + marker chromosome boy with an slightly ambiguous male genitalia, whose abnormal Y-containing marker chro-

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mosome was later to be delineated as an idic(Y)(p11.3). Mosaic sex chromosome disorder is a difficult issue in genetic counseling. We thus find our case interesting because of the extensive laboratory work, and also because of the late diagnose time.

CASE REPORT

The patient is a 14 year old male referred to the genetics clinic for evaluation of ambiguous external genitalia. He was the first child in a sibship of two of a nonconsanguineous marriage with uneventful birth history, and his developmental milestones were normal. The parents had signed the birth certificate designating the infant a male and this was never later questioned by the family. No medical records are available from his childhood, but the family reports that he was delayed in his development and shorter than his peers. At 7 years of age he entered public school, and he was succesfull. He had no serious illnesses and was on no medication until this medical evaluation at 14 years of age. His physical examination showed normal physical measurements. The external genitalia comprised a 2 cm phallus with a redundant ventral foreskin. Hypospadia was found. The labioscrotal folds were fused with minimal rugation and hyperpigmentation. No gonadal structures were palpable. At the base of the phallus, there was a 2 mm orifice that was not probed. Pelvic ultrasonography was not detect any gonadal tissue. Laboratory findings included normal levels of 17-hydroxy progesterone (2.5 nmol/L) (normal range: 0.6–3.6 nmmol/L), thus excluding simple virilising congenital hyperplasia. Laparoscopic exploration revealed a bilaterally streak testes and uterus and residual mullerian structures. He underwent bilateral gonadectomy. Histopathology showed the left gonad to contain streak ovarian elements, while the right gonad consisted mainly of immature testicular tissue with some rudimentary ovarian tissue in the adjacent connective tissue. The cardiac examination was normal.

Cytogenetic analysis of the peripheral blood showed the presence of three cell lines. Of the 100 cells analyzed, the karyotype was determined to be 45, X [30]/46,X, + mar [68]/46,XY,mar [2] by conventional G-banding analysis (Fig. 1).

FISH analysis for centromeric Y sequences (DYZ3; Vysis, Downer's Grove, IL, USA) was performed and the marker chromosome was found to be dicentric. An isodicentric Y chromosome was thus highly suspected. FISH analysis of at least 20 interphase nuclei as well as 20 metaphase spreads by use of the whole painting probe for Yq (WCPYq; Vysis) and a locus-specific probe for SRY (Yp11.3; Vysis) were perfor-

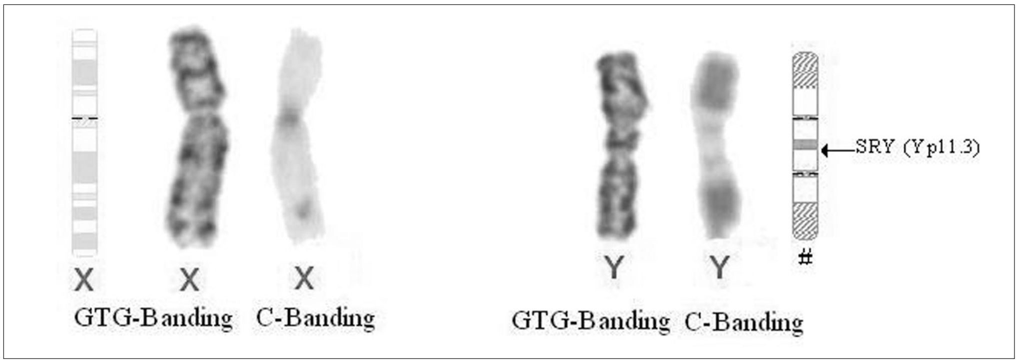

Figure 1: Patient's partial karyotype with GTG and C-banding techniques. Ideograms showing the normal X and the idic(Y) (p11.3). The idic(Y) is indicated by an arrow.

med and the results showed that the derivative Y chromosome was an idic(Y) (p11.3), with only one signal of the FISH probe aimed at the SRY locus seen, both in interphase and metaphase cells (Figs 2, 3). The karyotype of the peripheral blood was thus to be further designated as 45,X[30]/46,X,idic(Y)(p11.3) ish idic(Y)(SRY + , DYZ3+ +)[68]/ 46,XY, idic(Y)(p11.3)[2]. The ideogram of the idic(Y) chromosome is proposed in figure 1.

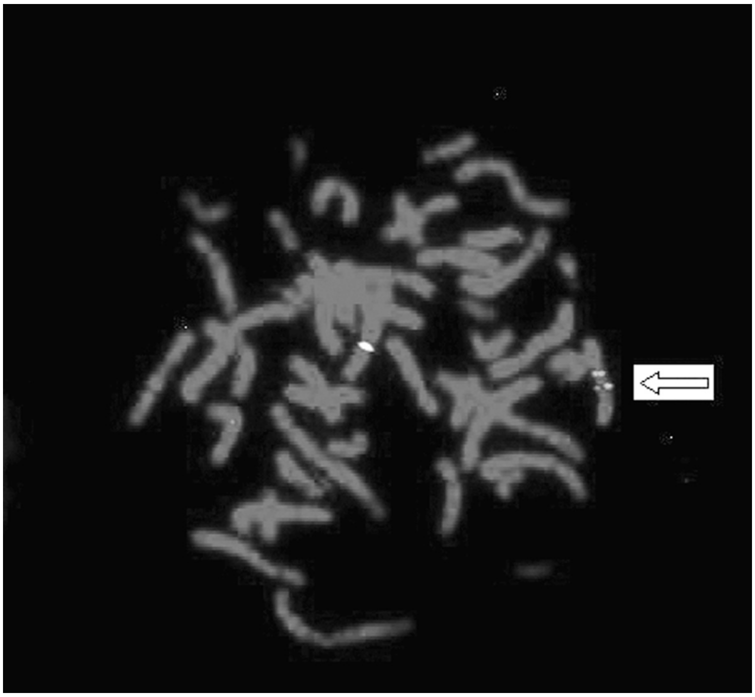

Figure 2: Fluorescence *in situ* hybridization (FISH) probe for centromeric Y sequence DYZ3 (Spectrum Orange, Vysis, Downer's Grove, IL, USA) was successfully hybridized to the marker chromosome and two signals are seen, suggesting the marker chromosome is dicentric.

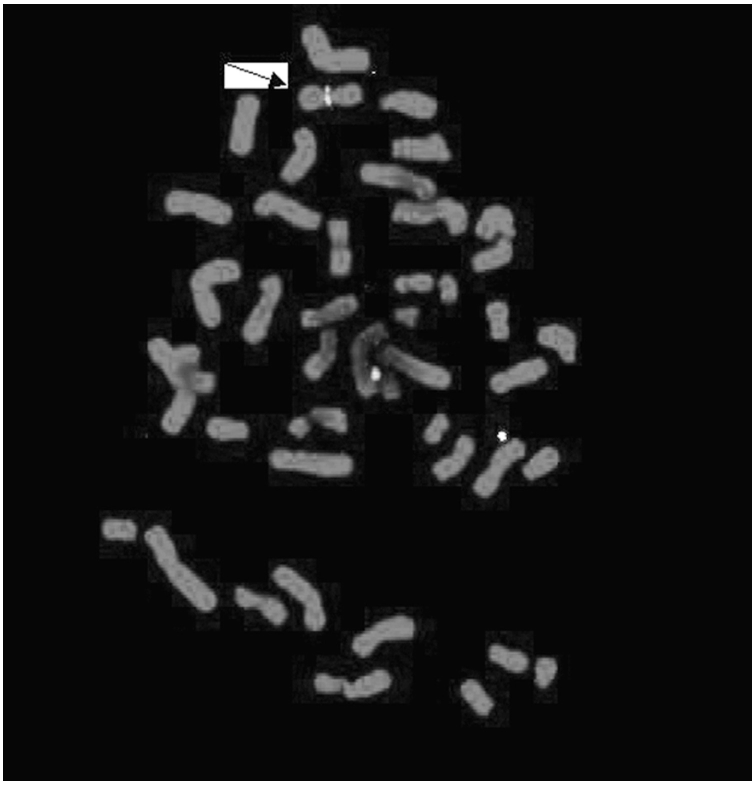

Figure 3: Fluorescence *in situ* hybridization (FISH) probes for SRY (Yp11.3, Spectrum Orange, Vysis, Downer's Grove, IL, USA) were used. Isodicentric Y shows one copy of the SRY (red signals).

Generally mosaicism in the lymphocytes did not reflect the same proportion of 45,X and 46,XY cells as in the gonads. The decision to remove the gonads has been based on the possibility of the presence in the gonads of a Y chromosome with a subsequent risk of gonadoblastoma. Therefore, all streak gonads and all dysgenetic testes were removed.

DISCUSSION

Mixed gonadal dysgenesis (MGD) comprises an heterogeneous group of diverse chromosomal, gonadal and phenotypic abnormalities which are characterized by the presence of a testis on one side and a contralateral streak or an absent gonad. Most patients have a 45,X/46,XY chromosomal mosaicism and germ cell tumours, such as gonadoblastoma or dysgerminoma, which develop in about one third of patients with this syndrome (6, 11). Gonadoblastoma is an unusual mixed germ cell–sex cord–stromal tumor that has the potential for malignant transformation.

To date, the aetiology of MGD has not been completely elucidated; traditionally, it has been proposed that in MGD predominance of XY or X0 gonadal cells, would determine gonadal differentiation into a testis or a streak gonad. However, in various cases previously reported there has not been concordance between the gonadal karyotype and the differentiation of the gonad (5, 7, 12). These reports have demonstrated that the theory which states that gonadal sex determination is due to the predominance of a specific cell type is not always confirmed.

Mosaicism is defined as the presence in the same individual of two or more cell lines derived from a single stem line but with different chromosomal complements. In most cases of 45,X/46,XY mosaicism, the cause is considered to be the loss by nondisjunction of the Y chromosome after normal disomic fertilization. In the monozygotic patients described in previous reports, they have distinct sexual phenotypes due to different ratios of mosaicism (8).

Molecular-based approaches to study mosaicism are difficult, especially when three cell lines exist (one cell line does not contain Y, one cell line contains a normal Y, one cell line contains an idic [Yp]). FISH-based technology is more useful in studying mosaic genotypes (12). We successfully used FISH. Previous reports on Y-containing derivative chromosomes in mosaic 45,X cases revealed that the derivative Y chromosomes could be idic(Yp), idic (Yq), or Y microdeletions (3). Remarkably, the only one SRY signal as noted in figure 3 may actually represent that one SRY was deleted.

Isodicentric Y chromosomes appear to be formed by a single break in one of the Y chromatids, followed by a fusion of the broken ends of sister chromatids and the loss of the acentric fragment during gametogenesis before formation of spermatids. Such rearrangements are generally unstable and an additional 45,X cell line is frequently present (10).

The peripheral blood lymphocytes showed 68% of cells with an idicY chromosome. As gonadal tissues are not easily available, there are few reports on such comparisons, and most studies correlate phenotype with peripheral lymphocyte karyotypes, which leads to the current uncertainty with predicting the phenotype resulting from this mosaic karyotype (4, 9). Formation of the testis from the undifferentiated embryonic gonad depends on the presence of the short arm of the Y chromosome, containing SRY-sequences. Testosterone production stimulates development of the Wolffian system and induces male development of the external genitalia, failing which, differentiation proceeds along female lines and Müllerian structures are formed.

There seems to be the necessity of a minimal amount of SRY to be present for the undifferentiated gonad to become a testis. Apparently, in our case, the threshold of SRY-containing cells (68%) required for the development of the embryonic gonad into a testis was obviously adequate to enable complete differentiation of the right gonad into a testis.

In conclusion, the cells predominantly present in the gonads seem to be influencing the phenotypic sex, irrespective of the percentage of Y-bearing cells in the blood or buccal mucosa. The 45,X cell line may exert a more dominant effect on sexual differentiation, only if it is predominant in the gonads. The presence of testes in these individuals obviously suggests that the Y cell line with adequate SRY product must have been present in the gonadal tissue, enabling differentiation along male lines, though infertility may be a distinct possibility. It is, however, clear that the percentages of the Y-bearing cell line in the peripheral blood remains an unreliable indicator of the sexual phenotype. This is exemplified in our proband where the presence of only the 45,X cell line leads to the formation of ovarian tissue in the left streak gonad, whereas the mosaic presence of the idic(Y) cell line together with the 45,X cell line, leads to formation of testicular tissue inthe right streak ovotestis. In our patient, the 70% Y cells in the peripheral blood are not a suitable indicator for the resulting phenotype. Genetic counseling was given the family according to literature and we reported here due to it's an exceptional mosaic nature and also because of the late diagnose time.

REFERENCES

1. *ALVAREZ-NAVA F., SOTO M., BORJAS L., ORTIZ R., ROJAS A., MARTINEZ S., REVOL A., BARRERA H., ALVAREZ Z:* Molecular analysis of SRY gene in patients with mixed gonadal dysgenesis. *Ann. Genet.,* 2001, *44*, 155–159.
2. *CANTO P., GALICIA N., SODERLUND D., ESCUDERO I., MENDEZ J.P:* Screening for mutations in the *SRY* gene in patients with mixed gonadal dysgenesis or with turner syndrome and Y mosaicism. *Eur. J. Obstet. Gynecol. Reprod. Biol.,* 2004, *15*, 55–58.
3. *HSU L.Y:* Phenotype/karyotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. *Am. J. Med. Genet.,* 1994, *53*, 108-140.
4. *MARCUS-SOEKARMAN D., HAMERS G., MULDER A.L., OFFERMANS J., ENGELEN J., VAN LENT-ALBRECHTS J.C., ROBBEN S.G., DE MUINCK KEIZER-SCHRAMA S., WOLFFENBUTTEL K.P., LOOIJENGA L.H., OOSTERHUIS J.W., SCHRANDER-STUMPEL C., NIJHUIS J:* Sonographic genital ambiguity in a fetus due to a mosaic 45,X/46,X,idic(Y)(qterp11.32::p11.32-qter) karyotype. *Prenat. Diagn.,* 2005, *25*, 279-282.
5. *MAILHES J.B., PITTAWAY D.E., RARY J., CHEN H., GRAFTON W.D:* H-Y antigen-positive male pseudohermaphroditism with 45, X/46, XYq- mosaicism. *Hum. Genet.,* 1979, *53*, 57–63.
6. *MENDEZ J.P., ULLOA-AGUIRRE A., KOFMAN-ALFARO S., MUTCHINICK O., FERNANDEZ DEL CASTILLO C., REYES E., PEREZ-PALACIOS G:* Mixed gonadal dysgenesis: clinical, cytogenetic, endocrinological, and histopathological findings in 16 patients. *Am. J. Med. Genet.,* 1993, *46*, 263–267.
7. *MENDEZ J.P., CANTO P., LOPEZ M., DE LA*

CHESNAYE E., TORRES L., CHAVEZ B., ULLOA-AGUIRRE A., KOFMAN-ALFARO S: Scant XYqh-testicular cells with normal SRY was enough to differentiate bilateral testes in a 45,X/46,XYqh patient. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 1999, *87*, 159–162.

8. *NONOMURA K., KAKIZAKI H., FUKUZAWA N., FUJIEDA K., HARADA N., NIIKAWA N., KOYANAGI T:* Monozygotic twins with discordant sexual phenotypes due to different ratios of mosaicism of 47,X,idic(Y),idic(Y)/46,X, idic(Y)/45,X. *Endocr. J.*, 2002, *49*, 497-501.
9. *QUEIPO G., NIETO K., GRETHER P., FRIAS S., ALVAREZ R., PALMA I., ERANA L., PENA Y.R., KOFMAN-ALFARO S:* Unusual mixed gonadal dysgenesis associated with Müllerian duct persistence, polygonadia, and a 45,X/46,X,idic(Y)(p) karyotype. *Am. J. Med. Genet.,* 2005, *136*, 386-389.
10. *QUILTER C.R., NATHWANI N., CONWAY G.S., STANHOPE R., RALPH D., BAHADUR G., SERHAL P., TAYLOR K., DELHANTY J.D:* A comparative study between infertile males and patients with Turner syndrome to determine the influence of sex chromosome mosaicism and the breakpoints of structurally abnormal Y chromosomes on phenotypic sex. *J. Med. Genet.,* 2002, *39*, 80.
11. *ROBBOY S.J., MILLER T., DONAHOE P.K., JAHRE C., WELCH W.R., HASELTINE F.P., MILLER W.A., ATKINS L., CRAWFORD J.D:* Dysgenesis of testicular and streak gonads in the syndrome of mixed gonadal dysgenesis: perspective derived from a clinicopathologic analysis of twenty-one cases. *Hum. Pathol.* 1982, *13*, 700–716.
12. *SUGARMAN I.D., CROLLA J.A., MALONE P.S:* Mixed gonadal dysgenesis and cell line differentiation. Case presentation and literature review. *Clin. Genet.,* 1994, 46, 313–315.
13. *TUCK-MULLER C.M., CHEN H., MARTINEZ J.E., SHEN C.C., LI S., KUSYK C., BATISTA D.A., BHATNAGAR Y.M., DOWLING E., WERTELECKI W:* Isodicentric Y chromosome: cytogenetic, molecular and clinical studies and review of the literature. *Hum. Genet.,* 1995, *96*, 119-129.
14. *WANG B.B., YU L.C., PENG W., FALK R.E., WILLIAMS J:* Prenatal identification of i(Yp) by molecular cytogenetic analysis. *Prenat. Diagn.,* 1995, *15*, 1115–1119.

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