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Case Report

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A Case With 46,X, +mar (Y), inv (Y) (p11.2;q11.23)? Karyotype

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ABSTRACT

Pericentric inversion of Y chromosome has an estimated frequency of the one per thousand. This inversion is always inherited but also is possible a de novo presentation. Sometimes this inverted chromosome is associated with the Down, Klinefelter and other chromosomal syndromes. For the carriers of pericentric inversion the risk of the mental retardation or multiple abortion is not apparently increase and there is not relation with abnormal phenotypic features. The pericentric inversion of Y chromosome is only a rare chromosomal heteromorphism. Chromosome analysis of the father is advisable to determine whether or not the inversion is familial in order to be able to provide genetic counselling. Cytogenetic analysis from amniotic fluid cells and parents' peripheral blood lymphocyte cultures were prepared by routine methods. Fluorescence in situ hybridization (FISH) was performed on cultured amniotic fluid cells to confirm the presence of Y-bearing cells. We performed molecular genetic analysis for Y chromosomal loci (SRY, ZFY, SY84, SY86, SY127, SY134, SY254, SY255). In cytogenetic analysis, the karyotype of male fetus, other brother's and his father's were exhibited as 46,X, +mar (Y), inv (Y) (p11.2;q11.23)?. His mother's was 46, XX. In these cases, it was concluded that there was no clinical significance because the same abnormality was found in other members of the family. All of them have normavl phenotypic features. This finding suggests that the pericentric inversion of the Y chromosome affects neither the phenotype nor reproductive performance. *©2008, Firat University, Medical Faculty.*

***Key words**: Prenatal diagnosis, SRY, marker Y chromosome, FISH, inversion*

ÖZET

46,X, +mar (Y), inv(Y) (p11.2;q11.23)? Karyotipli Bir Olgu

Y kromozomunun perisentrik inversiyonunun görülme sıklığı 1/1000'dir. Bu inversiyon daima kalıtılmaktadır ama de novo olarak da meydana gelmesi mümkündür. Bazen bu inverted kromozom Down, Klinefelter ve diğer kromozomal sendromlarla ilişkili olabilir. Perisentrik inversiyon taşıyıcılarında mental retardasyon veya çoklu düşük riskinde artış görülmez ve anormal fenotipik özelliklerle ilişkisi yoktur. Y kromozomunun perisentrik inversiyonu, sadece nadir kromozomal bir heteromorfizmdir. Genetik danışmanlık verebilmek için inversiyonun ailesel olup olmadığını saptamada babanın kromozomal analiz yaptırması tavsiye edilebilir. Sitogenetik analiz amniyotik sıvı hücrelerinden ve ebeveynlerin periferik kan lenfosit kültüründen rutin metodlara göre yapıldı. Floresans in situ hibridizasyon (FISH), Y taşıyan hücrelerin varlığını tespit etmek için kültürü yapılmış amniyotik sıvı hücrelerinden yapıldı. Y kromozom bölgeleri (SRY, ZFY, SY84, SY86, SY127, SY134, SY254, SY255) için moleküler genetik analiz yapıldı. Sitogenetik analizde; erkek fetüsün, kardeşinin ve babasının karyotipinin 46,X, +mar (Y), inv(Y) (p11.2;q11.23)? olduğu görüldü. Probandın annesinin karyotipi 46,XX'di. Bu vakalarda, bu karyotipin klinik bir öneminin olmadığı kararına varıldı. Çünkü aynı anormallik ailenin diğer üyelerinde bulundu. Onların hepsi de normal fenotipik özelliklere sahiptiler. Bu bulgular, Y kromozomunun perisentrik inversiyonunun ne fenotipe ne de üretkenlik perfonmansına herhangi bir etkisinin olmadığını ileri sürmektedir. *©2008, Fırat Üniversitesi, Tıp Fakültesi*

***Anahtar kelimeler:** Prenatal tanı, SRY, marker Y kromozom, FISH, inversiyon.*

Pericentric inversion of the human Y chromosome has an estimated frequency of the one per thousand. This inversion is always inherited but also is possible a de novo presentation. Sometimes this inverted chromosome is associated with the Down, Klinefelter and other chromosomal syndromes. For the carriers of pericentric inversion the risk of the mental retardation or multiple abortion is not apparently increase and there is not relation with abnormal phenotypic features. For some authors the pericentric inversion of the human Y chromosome is only a rare chromosomal heteromorphism (1, 2).

In this paper, we report a rare male case with no malformation and a 46,X,+mar (Y), inv(Y) (p11.2;q11.23)? karyotype, in whom the marker chromosome appears by molecular analysis to have been derived from the Y chromosome. The importance of banding studies for precise identification of structurally abnormal chromosomes and the need for chromosome study of family members for the peroper counseling of prenatal diagnosis of such a variant chromosome are discussed. In the presentation of this case, it has been aimed to resolve the socio-psychologic problems at the patients and his/her relatives and to be a guide to doctors about this subject.

CASE REPORT

Cytogenetic analysis was performed with GTG-banding. Cytogenetic analysis analyses from amniotic fluid cells and parents' peripheral blood lymphocyte cultures were prepared by routine methods. For each case at least 25 metaphases were evaluated. Structural anomalies were recorded according to the International System for Human Cytogenetic Nomenclature. Fluorescence in situ hybridization (FISH) was performed on cultured amniotic fluid cells to confirm the presence of a Y-bearing cell line and determine its distribution in the fetal tissue

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examined. The cases were analysed by also interphase FISH technique using dual prob (Vysis, Ullinois, USA) to exhibit exist Y chromosome. Hybridization was performed in according to the manufacturer's instructions. Dual-labeling hybridization was performed using 10µl of the hybridization mixture containing fluorescein direct labeled chromosome Y alpha-satellite probe and rhodamine direct-labelled SRY gene probe. Molecular analysis of sex-reversed patients led to the discovery of the SRY gene (sex-determining region on Y). We performed molecular genetic analysis for Y chromosomal loci (SRY, ZFY, SY84, SY86, SY127, SY134, SY254, SY255) blood leukocytes with Y Chromosoma Deletion Kit (Dr.Zeydanlı Life Science, Ankara, Turkey).

Genomic DNA was extracted from peripheral leukocytes collected from a venous blood sample. Genomic DNA was extracted by standard methods from peripheral leukocytes of all patients (3). The DNA was amplified for 30 cycles with denaturation at 94°C for 5 min, at 94°C for 1 min, at 54°C 1 min, at 72°C for 2 min and extension at 72°C for 6 min using a PTC-100 thermal cycler (MJ Research). The PCR products were separated by electrophoresis on 2 per cent agarose gel containing ethidium bromide and photographed using Gel Doc system (Hero Lab, Germany). Product was obtained 947 bp for Y. Health male and female samples were used as positive and negative control.

A pericentric inversion of chromosome Y was detected in an unborn baby by a second-trimester amniocentesis for prenatal diagnosis because of advanced maternal age. Cytogenetic investigations of the fetal cells revealed a male karyotype with a pericentric inversion of the Y chromosome. Cytogenetic analysis from amniotic fluid cells showed 46,X,+mar (Y) inverted Y (p11.2;q11.23)? (Figure 1). Chromosome analysis of the proband's father and other elder brother was also undertaken in order to decide whether this inversion was inherited or of de novo origin. The identical pericentric inversion was found in the phenotypically normal proband's

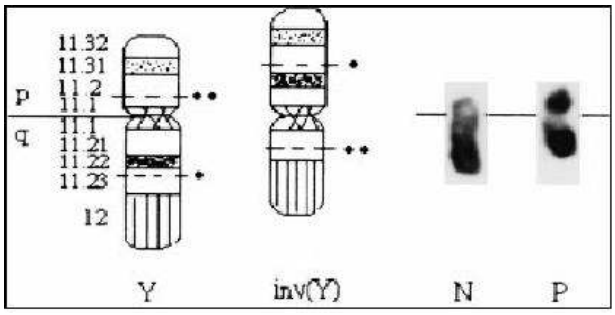

Figure 1 Pericentric inversion of the Y chromosome in this family. Left, diagram showing a normal Y (Y) from a healthy individual and the common inv(Y)(p11.2;q11.23) chromosome [inv(Y)] of the male family members. Right, high resolution G banding of a normal Y (N) and the Y chromosome of case (P).

father and a elder brother. In this case of familial pericentric inversion, the parents were assured that their unborn baby was anticipated to be normal.

The existence of Y chromosome was determined by interphase FISH besides a number of metaphase analysis. It was detected SRY gene and centromeric signals in patients with 46,X,+mar(Y), inverted Y(p11.2; q11.23) karyotype (Figure 2). The formation of testes can be considered as existence of SRY (sex-determining region of Y) as a testis-determining factor. The sequence tagged sites (STS) primers SY14, ZFY, sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc) were used for each case. In all cases the SRY gene was present. No mutations were identified in this gene in any of the patients (Figure 3).

Figure 2. The existence of SRY gene was determined by interphase FISH.

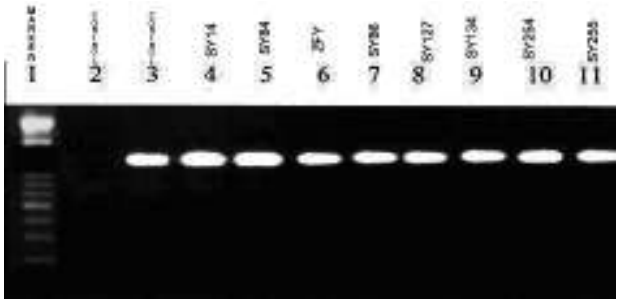

Figure 3. Gel photograph showing Y microdeletion. Lane 1, molecular weight marker; lane 2 and 3 negative control (female and male samples), lane 4,5,6,7,8,9,10 and 11: SY14, sY84, ZFY, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc).

The amplified loci by PCR in each patient are indicated in the Table 1. The present report illustrates the importance of FISH and molecular techniques as a complement to cytogenetic methods for accurate identification and characterization of chromosome rearrangements in prenatal diagnosis.

Table 1. Amplified loci by PCR in two patients with 46,X,inv(Y)

Primer	Map position	∗Patient	∗∗Patient 2	Controls	
				Male	Female
SRY	**Yp11.32**	+	+	+	-
ZFY	**Yp11.32**	+	+	+	-

∗Proband's father

∗∗A elder brother

DISCUSSION

We are reporting three subjects with apparently the same chromosomal constitutions (46,X,mar(Y)) as demonstrated by cytogenetic and molecular analyses performed in peripheral blood lymphocytes and amniotic fluid cells. All patients had the same Y-chromosome specific sequences. FISH hybridization signals on marker chromosomes confirmed that they are derived from Y-chromosome in all patients. Furthermore, PCR analysis with Y-chromosome specific sequences allow us to conclude definitively that these marker chromosomes were Y-derived.

Prenatal diagnosis has become the major focus of genetic counselling and for this, several important areas of technology have evolved. Especially cytogenetic prenatal diagnosis using analysis of cultured cells from the amniotic fluid at mid-trimester was introduced in 1966 by Steele and Breg (4). Most cases with structural aberrations of sex chromosomes are associated with abnormalities of the external genitalia at birth or lack of secondary sexual characteristics at puberty (5). The molecular study of individuals with abnormal gonadal differentiation and sex chromosome anomalies has led to the assignment of specific regions for sexual differentiation and gonadal function on the Y chromosome (6-8). Recently, investigation with DNA probes has allowed the generation of a physical deletion map dividing the Y chromosome into eight intervals (9), and more detailed deletion mapping has been reported (10). Many individuals with sex chromosome abnormalities remain undiagnosed because usually their phenotype falls within the limits of 'normality,' although the karyotypic change has an effect on most non-mosaic cases. Even now, when many abnormalities of the sex chromosomes have been defined and their clinical consequences reported in detail, the precise role of the sex chromosomes in sex differentiation and in the genetic control of gametogenesis still remain obscure. FISH is a rapid method for the detection of specific DNA target sequen ces in metaphase and interphase chromosomes. These techniques, combined with conventional G-banding, have a clear diagnostic and prognostic value, and contribute to genetic counselling (11).

In 1999, Acar et al were reported conventional and molecular cytogenetic studies in a patient with multiple anomalies who is a carrier of a pericentric inversion on chromosome Y and a chromosome 15p+. His parents were phenotypically normal. The father is a carrier of a pericentric inversion of chromosome Y, and the mother carries a large chromosome 15p+ variant (12).

In our study, the inverted Y was found to be of paternal origin. Maternal chromosomal pattern was normal 46,XX. Cytogenetic investigation of a healthy couple with 2 spontaneous abortions revealed a pericentric inversion of the Y chromosome. In these cases, it was concluded that there was no clinical significance because the same abnormality was found in two other members of the family. All of them have not anormal phenotype. Chromosome analysis of the father is advisable to determine whether or not the inversion is familial in order to be able to provide genetic counselling. This finding suggests that the pericentric inversion of the Y chromosome affects neither the phenotype nor reproductive performance. After reviewing the literature, it was concluded that an inverted Y chromosome does not impede the production of normal sperm and does not predispose to non-disjunction of other chromosomes in the progeny. Thus, the earlier concept of nondisjunction was rejected, and it is suggested that aberrant cases with aneuploidy and an inverted Y are fortuitous. The prevalence of males with pericentric Y inversion in the general population is approximately 1 per 1000. It is suggested that a pericentric inversion of the Y chromosome is a rare chromosomal heteromorphism and should be called type III. The pericentric inverted Y is inherited from generation to generation and has no clinical significance.

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