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Inter Chromosomal Effect (ICE) Resulting in Increased Abnormal Pregnancies in an Infertile Female with a Rare Robertsonian Translocation (13;21)(p10;p10) and SRY Gene carrier with 9 Consecutive Abortions

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ABSTRACT To provide information for the first time about an infertile female with a rare 45, XX rob (13;21)(p10;p10) and translocated SRY gene on X chromosome and to make an attempt to detect any inter chromosomal effect (ICE) if any in the production of increased number of abnormal pregnancies. One infertile female proband with rob (13;21) and 9 consecutive abortions and 4 unaffected family members were taken up for the study. The hormonal levels in the proband were measured by using Radio Immuno Assay (RIA). Karyotyping of G-banded chromosomes from leukocyte cultures for all the subjects were made and analysed using IKaros software (Metasystems). The DNA isolation and PCR analysis were undertaken for identification of SRY gene. The proband exhibited a karyotype of 45, XX rob (13;21)(p10;p10), her husband, father and brother showed 46, XY and mother had normal 46, XX. All the hormonal levels in proband were with in the normal range. She was positive for SRY gene and her mother was negative, her husband, father and brother were positive. The unaccountable increased number of abortions in the proband, an infertile female with a rare 45, XX rob (13;21)(p10;p10) and with a SRY gene may be due to the inter chromosomal effect (ICE) associated with rob itself or due to the presence of SRY gene, or may be due to the combined effect of both.

INTRODUCTION

Robertsonian translocations (rob) are one of the most common structural chromosomal rearrangements and occur in approximately 2–3% in infertile men (Hatakeyama et al. 2006). Translocation between chromosomes 13 and 14 is the most frequent one in humans, estimated to be approximately 75% of all rob. The t(14; 22) and t(13;21) are two rare rob, comprising about only 1.2 and 2% of all detected rob, respectively (Therman et al. 1989). Further only one report is available in the literature on rob (13;21) in male (Hatakeyama et al. 2006) and till date nothing in female with that translocation is available. Hence in this study for the first time an infertile female with a rare rob (13;21) and SRY gene on her X chromosome with her normal husband having a normal semen count is reported. Further she had five consecutive spontaneous abortions and four abortions after In Vitro Fertilization (IVF) treatment. In general, male carriers of rob are phenotypically normal, but do have more frequent fertility problems. The rob carriers produce increased numbers of unbalanced gametes resulting in abnormal pregnancies ending in repeated abortions (Chen et al. 2007). The reported female rob in this study too has those characteristic features.

In general, rob may adversely affect the meiotic segregations of other uninvolved chromosomes pairs too. It is attributed to the phenomenon, inter chromosomal effect (ICE). Some reports supported the inter chromosomal effect (ICE) in rob (Shi and Martin 2001; Anton et al. 2004; Ogur et al. 2006). However, others demonstrated no evidence of this phenomenon (Syme and Martin 1992; Acar et al. 2002; Hatakeyama et al. 2006). Most of the ICE studies associated with the production of the abnormal

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gametes were conducted only in male rob. Practically no such study is available in female rob and this is the case with the infertile female rob (13;21) in this study also. This may be due to the rare availability of female rob and the inherent limitations in them to obtain an optimal number of her gametes to study the nature of meiotic segregation not only in the translocated chromosome but also in other uninvolved chromosomes. Hence in this study an alternate method was planned to look into the meiotic pairing of the X chromosomes in this unique infertile female proband with rob and with SRY gene at a molecular level to detect any structural difference in them that may contribute to the abnormal pairing resulting in abnormal gametes that would indicate an ICE. It would be predicted that the presence of the illegitimately translocated SRY gene would result in a change of sequence homology between the pair of sex chromosomes that would result in pairing defects causing an increased meiotic segregation irregularities resulting in an increased number of abnormal gametes indicating an ICE.

MATERIALS AND METHODS

Case Report

A couple with 36-year-old woman and her 37-year-old husband were referred for karyotyping analysis due to the history of 5 repeated spontaneous first trimester abortions and another 4 abortions after IVF.

Investigations

Physical Parameters The phenotype of the proband is feminine without any dysmorphic feature and with normal secondary sexual characters. Her height is 157 cm and weight is 55 kg. External genitals are clearly feminine.

Magnetic resonance imaging (MRI Scan) No sign of Wolffian derivatives. Mullerian structures (uterus and fallopian tubes) are in normal status. The pelvic region shows uterus regularly connected to the vaginal cervical channel.

Hormones Blood sample was obtained for determination of serum concentration of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), 17beta-Estradiol (17β-E2), Prolactin (PRL) and Dehydroepiandrosterone (DHEA) levels by using Radio Immuno Assay (RIA)

Cytogenetic Analysis

The proband, her husband, father, mother and brother were subjected to karyotyping to find out the origin of rob in the proband. Chromosome preparations were obtained from PHA-stimulated peripheral blood lymphocytes by using modified method of (Hungerford 1965). At least fifty well spread metaphase plates were scored by direct microscopic analysis. Well spread metaphases were photographed under oil immersion objective lens (100X) of Leica DM2000 microscope with Metasystems camera and the photomicrographs of banded spreads were karyotyped using automatic IKaros software (Metasystems). The karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN 2005).

Molecular Analysis

The molecular study was carried out to detect the presence of SRY gene in the proband, her husband, father, mother and brother.

DNA Extraction, Quantification and PCR Analysis: 9ml of intravenous blood was sampled from the patient, her husband, parents and brother by using EDTA coated Vaccutainer. The genomic DNA was extracted from peripheral blood by using modified method of (Lotery et al. 2000) and standardized at Biomedical Genetics Research Lab at VIT University. Qualitative analysis of DNA was carried out by 0.8% Agarose Gel Electrophoresis and quantification of DNA by using Biophotometer (Eppendorf). Dilutions of DNA were made up to 10ng/µl concentration by using TE buffer, pH-8.0. The 10 ng/µl of concentrated DNA solution was checked on 0.8% agarose gel.

The SRY gene residing in Y chromosome was amplified with a pair of primers (F -5'-CCCGAATTCGACAATGCAATCATATGCTTCTGC-3', R (5' -CTGTAGCGGTCCCGTTGCTGCTG CGGTG -3'), covering 609bp around HMG box of the gene. Polymerase chain reaction consisted of 10µl PCR reaction mixture and included 1.0µl PCR buffer (10X), 1.0µl MgCl2 (25 mM), 0.8µl deoxynucleotide tri-phosphates (10 mM), 0.5pM of each primer, 1unit of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, Calif) and 20ng of genomic DNA. Polymerase Chain Reaction conditions for SRY gene consisted of initial denaturation at 94°C for 12 minutes followed by 35 cycles of denaturation at

94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. To confirm the amplification of PCR product of SRY gene, the PCR products were checked by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 mg/ml) and the bands visualized under UV illumination.

RESULTS

The cytogenetic analysis of the proband showed a karyotype of 45, XX rob (13;21) (p10;p10) (Fig.1). The karyotype of her husband, father and brother showed a normal 46, XY male type and the mother showed a normal 46, XX female karyotype. These studies indicate a de

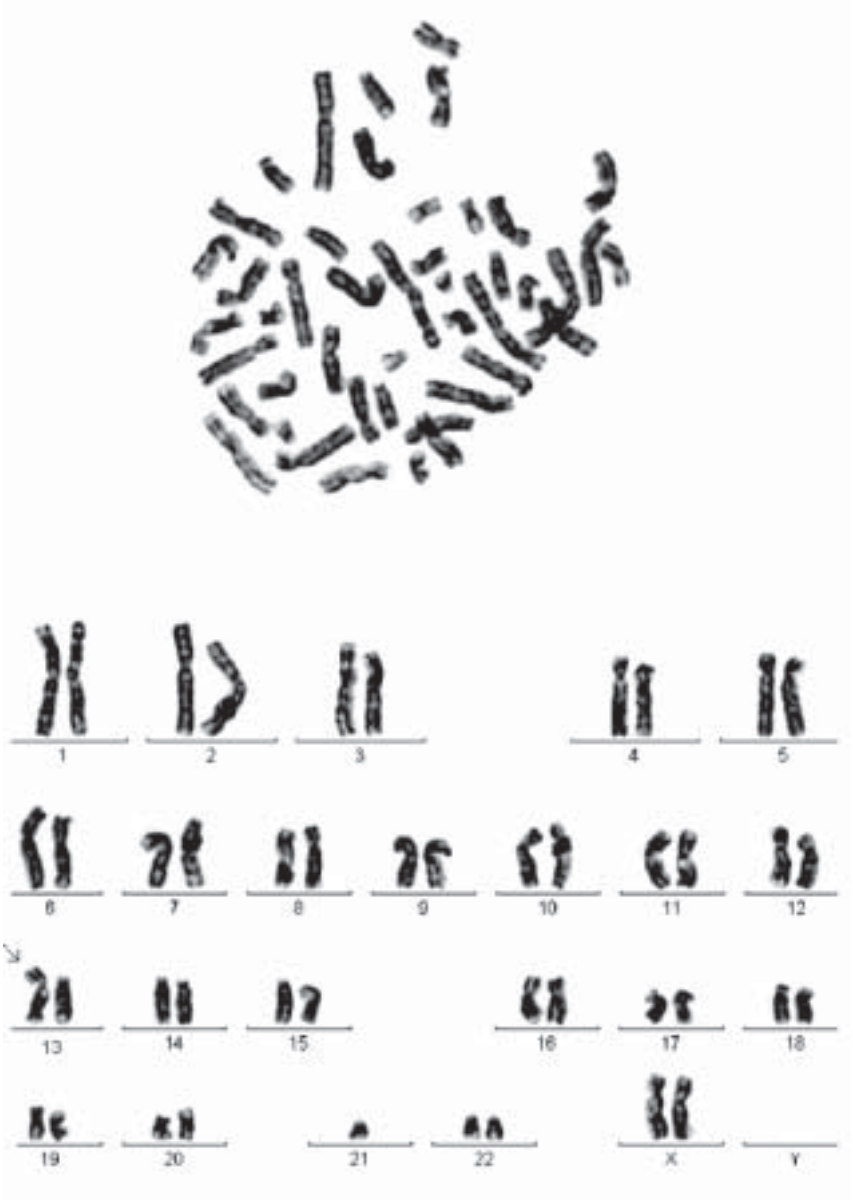

Fig. 1. Karyotype of patient with balanced 45, XX rob (13;21)(p10;p10).

Table 1: Serum hormonal level of patient with 45, XX rob (13;21)(p10;p10)

Hormone	*Patient's hormonal value*	*Day*	*Normal female range value*	*Normal male range value*
FSH	9.41 mU/ml	7th Day	2.5–10.2mU/ml	1.4–18.1 mU/ml
LH	8.7 mU/ml	7th Day	1.9–12.5 mU/ml	1.5–9.3 mU/ml
Testosterone	58.23 ng/dl	-	14–80 ng/dl	240–850 ng/dl
17â-E2	6.01 pg/ml	7th Day	10–147 pg/ml	6–72 pg/ml
PRL	4.57 ng/ml	-	0.6–29.0 ng/ml	2.0–43 ng/ml
DHEA	11 ng/ml	7th Day	2.2–12 ng/ml	2.5–9.5ng/ml

novo origin of rob 13;21 in the proband. The pregnancy history of the proband showed severe infertility, giving an indication of an increased number of abnormal gametes, all resulting in abortions. FSH, LH, 17-beta-E2, Prolactin and Dehydroepiandrosterone hormone levels in proband were within normal range and is presented (Table 1). Her husband has normal sperm parameters.

Genetic Analysis of the SRY gene

In order to confirm that the X-Y chromosomal interchange involved euchromatic DNA and to further localize the breakpoint on Y, the genomic DNA from the proband and her family members were analysed. Using the above mentioned primer combination, the HMG-box of the SRY gene was successfully amplified in proband, her husband, father and brother (Fig.2 Lane 3, 4, 6, 7). As expected, no band was visible in the patient's mother (Fig.2 Lane 5) showing the absence of the SRY gene. The SRY locus is proximal to the pseudoautosomal region on Yp (Yp11.3).

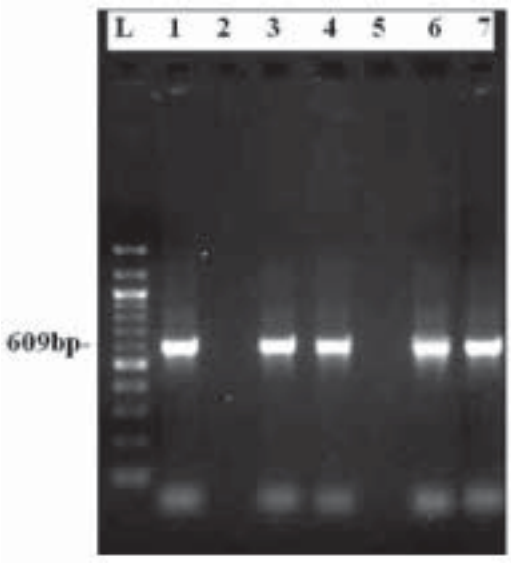

Fig. 2. Genomic PCR analysis of the HMG box of the human SRY gene. A 609-bp PCR product encompassing the HMG-box of the human SRY gene was amplified. All family members of the infertile female with a rare (13; 21) Robertsonian translocation and 9 consecutive abortions were investigated. L-100 bp DNA molecular weight Marker, 1- positive male control, 2- negative female control, 3 - proband, 4- husband, 5- mother, 6 father, 7-brother.

DISCUSSION

In this study, the infertile female proband showed 45, XX rob (13;21)(p10;p10) in all the cells. Banding techniques and automated karyotyping by IKaros software (Metasystems) gave precise identification of rob (Fig.1). The theoretical possibility of gametes formation between an infertile female rob carrier and a normal male would be 6 types (4 different forms of gametes from the rob female namely (13+21), (13+t(13;21),(21) and rob (13;21) and two of same form from normal male namely (13+21) and when they combine to produce pregnancies that would be - 1) (13+21)+(13+21) normal, 2) (13+t(13;21)+ (13+21) Down, 3) (21)+(13+21) monosomy for chromosome 21 and 4) t(13;21)+(13;21) balanced rob. All the 4 types of pregnancies would be produced in equal proportion. However, monosomy 21 is lethal and the rest 3 types are viable.

However, all the 9 pregnancies of the proband (5 spontaneous and 4 after IVF failures) ended in the first trimester abortions, showing an unaccountable increased number of abnormal gamete production, resulting in abortions. The production of a high proportion of gametes with an unbalanced genetic complement is generally related to rob and causes an increased risk of spontaneous abortions and abnormal offspring. A linkage has been reported between infertility

and chromosomal translocations, which occur at a rate of 0.6% among infertile couples, a significantly higher incidence when compared with an incidence of 0.2% in the general population (Hook and Hamerton 1977). Robertsonian Translocation may adversely affect the meiotic segregation of other uninvolved chromosome pairs too. It is attributed to the phenomenon, inter chromosomal effect (ICE) (Hatakeyama et al. 2006). Several studies have found such an ICE in male rob carrier (Shi and Martin 2001; Anton et al. 2004) and some other studies did not (Syme and Martin 1992; Hatakeyama et al. 2006).

Even though there exists reports showing negative ICE effect in rob yet in majority of rob carriers the rate of production of abnormal gametes cannot be accounted only by the existence of a single abnormal rob. By the application of several probes specific for different positions along the chromosomes has shown pairing defects in translocation heterozygotes not only in chromosomes involved in the translocations but in the other uninvolved chromosomes such as sex chromosomes as well (Gianaroli et al. 2002). Thus it seems there exist an increased number of abnormal gametes in rob carriers associated with sex chromosome aneuploids and nullisomies resulting from the meiotic irregularities between the sex chromo-somes. Hence, it was thought to look into the molecular nature of sex chromosomes in the reported unique infertile female rob carrier with a translocated SRY gene on her X chromosome to detect any molecular structural difference in them that would contribute to abnormal pairing resulting in the production of abnormal gametes accounting for the increased rate of abortion. This would have been a new approach to seek for an alternate mechanism for increased abortion rate in the female proband in this study. Molecular analysis of different DNA probes located at Xp22 and Yp11 revealed that these two regions share sequence homology and suggested that homologous legitimate recombination between these sequences may be involved in the aetiology of these X:Y translocation events (Ballabio et al. 1989; Yen et al. 1991). However occasionally *illegitimate* crossing over occur outside the Pseudoautosomal region (PAR) resulting in a transfer of Y specific sequences (SRY) on to the X chromosome. Such translocations between the distal Xp and Yp occur relatively frequently (Ferguson-Smith 1966; Page et al. 1987; Sinclair et al. 1990). In this study such translocation between the maleness determining SRY gene generally located at the Y chromosome is translocated to the X chromosome of the female rob carrier with SRY gene. The presence of such an illegitimate translocation of SRY gene would result in the change of sequence homology between the pair of sex chromosomes that would result in pairing defects causing an increased meiotic segregation irregularities resulting in an increased number of abnormal gametes further resulting in increased rate of abortion indicating its own ICE effect along with the ICE effect of rob itself.

CONCLUSION

The proband an infertile female with a rare 45, XX rob (13;21)(p10;p10) and with a SRY gene had 9 consecutive abortions very much higher in number than it could be expected on theoretical basis. There is evidence to show that rob itself as well as the presence of SRY gene in a female would produce unaccountable number of abnormal gametes resulting in abortions, showing an inter chromosomal effect (ICE). Hence it is thought that in the proband the reason for the increased number of abortions may be due to rob itself, or due to the presence of SRY gene in her or due to the combination of both factors showing the (ICE) effect.

This kind of combined use of cytogenetic and molecular approaches has to be performed for proper medical management and genetic counseling for the rob carriers.

RECOMMENDATIONS

Though this study could indicate an ICE effect, yet it requires further studies to confirm the findings. For example, use of fluorescence in situ hybridization studies may be performed to confirm the presence of SRY gene in the proband. Also the PCR sequencing of the SRY gene in the proband and her family members could help in further explaining the bad obstetrics history as a consequence of rob. The presence of SRY mRNA and analysis of X chromosome inactivation using X inactivation-specific transcript (XIST) primers and methylation-specific PCR (Oktem et al. 2007) may further support this kind of study.

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