A novel chromosomal translocation and heteromorphism in a female with recurrent pregnancy loss—a case study

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Abstract

Purpose To evaluate the clinical, biochemical and cytogenetic analyses of a couple with reproductive failure.

Methods A couple with a history of recurrent pregnancy loss was referred to the Institute of Genetics for cytogenetic evaluation. Chromosomal analysis of the phenotypically normal parents was done to ascertain the role of chromosomal abnormalities and offer appropriate genetic counseling. Further, advanced karyotype analysis by spectral karyotyping was also carried out in the couple and parents of the female partner.

Results Clinical and hormonal profile of the couple revealed normal phenotypes. The ultrasound scan of the female showed normal uterus and ovaries. Chromosomal analysis of the couple revealed a normal 46, XY karyotype in the male spouse, and a unique balanced reciprocal translocation 46, XX, t(12;13)(q13;q33) + 15pst+ chromosomal constitution in the female partner. Cytogenetic analysis of her parents revealed a similar translocation between chromosomes 12 and 13 in the father and 15pst+ in the mother. Further, corroboration of the chromosome abnormalities was carried out by spectral karyotyping.

Capsule A unique and novel familial transmission of paternally derived balanced reciprocal translocation and maternally derived heteromorphism in a female was associated with the history of recurrent pregnancy loss.

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Conclusion A unique and novel familial transmission of paternally derived balanced reciprocal translocation and maternally derived heteromorphism in a female with the history of recurrent pregnancy loss was reported as an original investigation.

Keywords Recurrent pregnancy loss · Balanced translocation · Heteromorphism · Spectral karyotype · Familial transmission · Hormonal profile · Chromosomal defect · Miscarriage

Introduction

Recurrent pregnancy loss (RPL) is the most common complication of pregnancy, responsible for significant emotional distress to the couple desiring children. The cause being multifactorial is mostly associated with the endocrine dysfunction, autoimmunity, genetic abnormalities, advanced maternal and paternal age, infectious diseases, environmental toxins, congenital and structural uterine anomalies etc. [1].

Approximately 15–20 % of all pregnancies in females end up in spontaneous abortions with 50 % of these being associated with chromosomal abnormalities. Although the cause is obscure, transmission of parental chromosomal abnormality may be one of the possibilities for recurrence of miscarriage in the first trimester of pregnancy [2]. It is estimated that 7 % of couples report at least two spontaneous abortions, with one parent being carrier for a balanced chromosome rearrangement [3]. Such rearrangements may result in meiotic errors and nondisjunction leading to production of unbalanced gametes. Usually these imbalances are lethal to the developing embryo/fetus culminating to spontaneous abortions. Cytogenetic heteromorphisms are described as heritable variations at specific regions with no proven impact on the phenotype. A frequently observed

variant is a change in size of the short arm sequences of acrocentric chromosomes [4].

In the present study, a novel translocation in a female with history of recurrent pregnancy loss associated with co-inheritance of balanced chromosomal translocation and heteromorphism from both the parents based on conventional and spectral karyotyping is reported.

Case report

A couple who were second cousins (consanguineous marriage) was referred for chromosome analysis because of a history of spontaneous abortions in the first-trimester. The wife was 22 years old, and the husband 30 years. There was no known exposure to recognized teratogens. They were healthy and phenotypically normal (Male height- 172 cm and weight 66 kg, Female height- 155 cm and weight 49 kg). The present study was approved by the Institutional Ethical Committee. An informed consent was obtained from the couple before investigation. Reproductive history of the female revealed six, first trimester miscarriages during the past 4 years, all being consecutive repeated abortions. The cause and genetic status of the miscarriages was not known.

The uterine cavity was evaluated by three dimensional ultrasonography and revealed normal size (6.0X3.5X3.0 cm) and echo pattern. Both the ovaries were found to be normal in size with right and left ovaries measuring 2.2X2.0 cm and 2.1X1.8 cm respectively.

The hormonal profile of the female partner demonstrated normal values for luteinizing hormone (LH) 5.5 mIU/ml (1.5–9.3 mIU/ml), Prolactin-8.5 ng/ml (3–21 ng/ml) and TSH 2.5 UIU/ml (0.35–5.5 UIU/ml). Follicle stimulating hormone (FSH) was determined on the 3rd day of the cycle and was in normal limits [8.5 mIU/ml (1.4–18.1 mIU/ml)]. Similarly, hormonal profile of the male spouse was within the normal range.

Materials and methods

Cytogenetic analysis was carried out based on phytohaemagglutinin-stimulated peripheral blood lymphocyte cultures, of the couple and parents of the female partner. Lymphocyte culturing and GTG-banding were performed following standard protocols as described by the AGT Cytogenetics Laboratory Manual [5, 6]. Karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN 2005).

Spectral karyotyping

Spectral karyotyping (SKY) was also performed according to the manufacturer's procedure [Applied Spectral Imaging (ASI)

Ltd, Migdal Ha'Emek, Israel] [7], to revalidate conventional cytogenetic findings. This technique employs combined Fourier spectroscopy, charge-coupled device (CCD) imaging, and optical microscopy. In brief, slides were denatured in 70 % formamide/2 X standard saline citrate at 70°C for 1 min and taken through a cold alcohol series and air-dried. The SKY probe mix was composed of chromosome-specific composite libraries, generated by polymerase chain reaction (PCR) from flow-sorted human chromosomes.

The chromosome specific probes were conjugated to a specific combination of five different fluorophores: Cy2, Spectrum green, Cy3, Texas Red, and Cy5. Denatured SKY Paint probe was added (1 ml) to the slide with metaphase spreads, covered with a 6 × 6 mm coverslip and sealed with rubber cement. Hybridization was carried out at 37°C in the dark, moist chamber for 48 h. Post-hybridization washes were done as per manufacture's protocol without modifications. The fixed metaphase plates were examined using an Olympus BX 60 epifluorescence microscope equipped with a triple band pass fluorescence filter set (SKY-1; Chroma Technology). SKY Vision spectral karyotyping software (Applied Spectral Imaging) was used to capture and analyze the metaphases obtained from the excitation of the five fluorochromes present in the SKY probe mixture.

Results

Chromosomal analysis of the wife revealed a balanced translocation between chromosomes 12 and 13 and a heteromorphism of chromosome 15 indicating the karyotype to be 46, XX, t(12;13)+15 pstk+. A major part of the chromosome 12 was dislodged below the centromere and translocated to chromosome 13 at the distal end of chromosome 13, indicating a balanced translocation (Fig. 1). Chromosomal analysis of the phenotypically normal parents of the proband was done to ascertain the origin of abnormal chromosome. A similar translocation was seen in the paternal karyotype (Fig. 2), while a heteromorphism of 15 pstk+ was observed in the maternal karyotype (Fig. 3). The karyotype of the husband was found to be normal with 46, XY chromosomal constitution.

Spectral karyotyping was performed in the lymphocyte cultures of the female partner and her parents. After performing SKY, it was revealed that a balanced reciprocal translocation between chromosomes 12 and 13 with the break-points localized to q13 and q33 respectively and the karyotype was determined as 46, XX, t(12;13) (q13;q33) +15pstk+ (Fig. 4). The father showed similar translocation between chromosome 12 and 13 with identical break points, while the mother showed heteromorphism of chromosome 15. Thus, the proband inherited the balanced reciprocal translocation from her father and heteromorphism of 15 from her mother, which is a rare event. To the best of our knowledge this is a

Fig. 1 Karyotype of the female partner with 46, XX, t(12;13) (q13;q33)+15pstk+ chromosomal constitution

unique and novel familial transmission of balanced reciprocal translocation and heteromorphism in a female with recurrent pregnancy loss.

Discussion

Recurrent pregnancy loss is defined as three or more consecutive spontaneous miscarriages [8]. Known causes of recurrent miscarriage include chromosomal anomaly, uterine malformations, primary endometrial defects and endocrinological abnormalities [9]. In the present case, the female partner had no

endocrinological and uterine abnormalities as confirmed by hormonal profile and 3D-ultrasound scan.

Reciprocal (non-Robertsonian) translocations are one of the most frequently occurring human chromosomal aberrations. Balanced reciprocal translocations occur in about 1 in 600 persons in the general population, whereas they have a frequency of about 7 % in couples with recurrent miscarriages [10]. These rearrangements are twice more common in females than males. In most cases, carriers of balanced reciprocal translocations have a normal phenotype but may experience reproductive issues such as infertility or multiple miscarriages. Interestingly, by empirical studies, 6 % of

Fig. 2 Karyotype of the father of female partner with 46, XY, t(12;13) (q13;q33) chromosomal constitution

Fig. 3 Karyotype of the mother of female partner with 46, XX+15pstk+chromosomal constitution

apparently balanced *de novo* translocations are associated with clinical abnormalities [11]. Recently, it has been shown by molecular analyses (e.g., array comparative genomic hybridization) that up to 40 % of the apparently balanced

reciprocal chromosome translocations in patients with an abnormal phenotype are accompanied by a chromosome imbalance [12–14]. The present study revealed a unique balanced translocation along with heteromorphism in a

Fig. 4 Spectral karyotype of the female partner of the couple with balanced reciprocal translocation and 15 pstk+

female with reproductive failure. The translocation between chromosomes 12 and 13 traced to the paternity, while heteromorphism to maternal lineage.

Most of chromosomal abnormalities can be readily diagnosed with standard cytogenetic analysis. However, further refinements like subtle chromosome rearrangements and intrachromosome exchanges can be identified by advanced molecular cytogenetic techniques like spectral karyotyping which opened a new era of interpretation of deletions, insertions, or visible but subtle translocations [15]. Hence, spectral karyotyping carried out in the female partner and her parents was useful in validating the findings by conventional cytogenetic analysis. The break points of the balanced reciprocal translocation between chromosomes 12 and 13 were localized to q13 and q33 respectively in the female partner of the couple with SKY.

Couples with balanced reciprocal translocation have a 50 % chance of having spontaneous abortions and a 20 % risk of having children with abnormal genetic makeup [16]. The formation of normal, balanced and unbalanced gametes is dependent on the basis of the chromosomes involved and on the location of break points. The configuration of the quadravalent at the pachytene stage may alter the pattern of segregation, resulting in normal and balanced and unbalanced gametes. Balanced chromosomal translocations may also lead to sequence rearrangements of the functional genes which may result in the reproductive errors accompanied by repeated abortions [17]. Further analysis of the break points and molecular characterization of genes involved might enlighten the understanding of the basis of repeated abortions.

Earlier studies have shown higher frequency of heteromorphism in infertile couples compared to normal population. Polymorphic heterochromatic regions were found to alter the synapsis of homologous chromosomes during meiosis. These regions are the last to synapse, thereby disturbing the timing of the whole division and leading to probable meiotic defects eventually resulting in infertility [18]. The present report reveals the presence of heteromorphism along with the balanced reciprocal translocation in the female partner resulting in meiotic errors and associated miscarriages.

The present study thus delineates a unique and novel case report of balanced reciprocal translocation and heteromorphism which could result in the generation of unbalanced gametes due to meiotic errors associated with first trimester recurrent pregnancy loss. Cytogenetic analysis is therefore mandatory for all the couples with reproductive failure to delineate the causal effect of recurrent pregnancy loss. Thus, the carriers of such abnormalities should be informed about the risk of the birth defects in their offspring due to *de novo* submicroscopic rearrangements. Adequate genetic counseling strategies should also be offered which could allow the

parents/couples to make an informed reproductive decision regarding subsequent pregnancies.

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Conflict of Interest Nil.

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