Case Report

Nucleolar organising region evaluation using new NOR FISH probe

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We have carried out chromosomal analysis in a couple with repeated spontaneous abortions (RSA). The chromosomal analysis of male revealed 15ps+ and the chromosome 15 appeared as submetacentric, C-group chromosome. First time we have attempted fluorescence in situ hybridization (FISH) using NOR probe (dJ1174 A5) and FISH analysis revealed NOR duplication on chromosome 15 which was also quantitated using Q-FISH software. The identical NOR duplication also detected in chromosome preparations from products of conception. However, NOR studies in large group of patients is necessary to understand the role of NORs in RSA.

Key Words: NOR duplication, addition on chromosome 15, repeated spontaneous abortions, NOR variation, FISH NOR

Introduction

Spontaneous loss of clinically recognised pregnancies is a frequent phenomenon occurring in 15 percent of women.[1] Chromosomal anomalies play an important role in first trimester pregnancy loss and account for over 50 percent of all recognised spontaneous abortions.[2] In the human, the five chromosomal loci that encode the ribosomal genes are termed nucleolar organizer regions (NORs). NORs are located on the short arm of acrocentric chromosomes.[3] NORs can be identified as secondary constriction on metaphase chromosomes and can be visualised by silver staining, due to the abundance of associated or argyrophilic proteins.[4] However not all NORs form secondary constrictions or can be silver stained during metaphase.[5] The NORs variation has been investigated extensively in an attempt to correlate their heteromorphism with specific diseases.[6] First time we have evaluated NOR duplication on chromosome 15 using fluorescence in situ hybridization (FISH) in a male where couple had repeated spontaneous abortions.

Case History

A couple referred for chromosomal analysis because of repeated spontaneous abortions. The female age was 24 years and the male was 29 years old. There was no family history of consanguinity. The female had four first trimester abortions and the investigations for TORCH, antiphospholipid antibodies and the hormone profile including T3, T4, TSH, FSH, LH found to be normal. Ultrasonography of abdomen and pelvis revealed normal USG. The phenotypically normal male had normal thyroid function. Medical records of male showed no history of hypersperma and oligosperma.

Cytogenetics

Peripheral blood lymphocyte cultures stimulated with phytohemoagglutinin (PHA) were set up at 37°C for 72 hrs. Chromosomal preparations obtained were subjected to GTG banding. Chromosomal analysis of female revealed normal Karyotype. The male Karyotype showed addition on chromosome 15 i.e. 15ps+ [Figure 1] and
this was detected in all the 100 metaphases scored. Chromosomal analysis from products of conception also revealed addition on chromosome 15ps+.

Fluorescence in situ hybridization

FISH was performed on metaphases using NOR specific probe (dJ1174A5) and centromeric probe for chromosome 15(pMC15 ). NOR probe was labeled with Cy 3-dUTP (red) and centromeric probe was labeled with Flur-X (green). Hybridisation of probes on metaphases was performed using standard procedure. The images were captured with CCD camera fitted to fluorescent microscope and FISH analysis of p arm of chromosome 15 showed red signal confirms the duplication of NOR [Figure 2]. The NORs were quantitated using IMSTAR, France, software.

Discussion

Balanced chromosomal aberrations in couples is a major aetiological factor in RSA. Additions on acrocentric chromes are shown to associated with RSA. The short arms of the acrocentric chromosomes show a high degree of variations in size and staining pattern. NORs can be identified as secondary constrictions or can be silver stained during metaphase. The number of silver positive NORs varies between four and Ten.[6] The NORs heteromorphism correlated with specific diseases and however there is no proven relationship or phenotype.[6] In our case the male had long NOR region and with the GTG-banding it was appeared as sub metacentric, C- group chromosome. The NOR variation was observed in large number of patients attending to our laboratory. However this is the first case with highly duplicated NOR on chromosome 15. The routine cytogenetic silver staining method used for confirmation of NORs. Sometimes the variants may not take silver staining and it is difficult to differentiate with chromosomal material. In our case we have first time evaluated NORs with FISH probe. The addition on short arm of chromosome 15 confirmed to be duplication of NOR region. The quantitative FISH(Q-FISH) analysis also revealed NOR duplication on chromosome 15 compared to other acrocentric chromosomes.

The acrocentric short arms have been considered to be redundant since they contain highly reiterated DNA sequences and because persons with Robertsonian translocation have no phenotypic abnormalities.[7] A number of studies correlated between changes in the short arm of the acrocentric chromosomes and a predisposition to trisomy 21. Jackson Cook et al.[6] shown that the significanlty increased risk factor for trisomy 21 due to variation in silver staining. The high frequency of double NORs was detected in parents of Down syndrome.[6] The NORs are the sites of the tandemly repeated ribosomal genes, present in about 40 copies / acrocentric chromosome. The variation in the number of genes per chromosomes as determined by in situ quantitation.[9,10] The number of genes and the transcriptional activity of the genes and account for the cytologic variation ob-
served on staining or with fluorescence. The NOR variation specially double NOR most likely arise from unequal homologous recombination within the ribosomal genes.[7] The acrocentric chromosome association takes place in the nucleus and it has been proposed that the high frequency of acrocentric chromosome might interfere with non-disjunction of homologous chromosomes in meiosis. In our study since the identical duplicated NOR on chromosome 15 was also detected in products of conception which suggests that the high expression of ribosomal genes might be a causative factor for the fetal loss. However molecular study on NORs is essential to understand the same. In routine cytogenetics screening chromosomes 15, 22 frequently shows variation in short arms and cytogeneticists always in dilemma whether this variation to be considered as polymorphism or translocation from other chromosomes. The conventional silver staining always does not stain all the chromosomes. However using NOR FISH the variations on acrocentric chromosomes can be confirmed accurately.

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References