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Molecular Cytogenetics Reveals A Novel Complex Three Way Translocation Involving Chromosomes 4, 10 And 16 In A Woman With Reproductive Failure

Aswathy C.G.[1], Suresh Kumar R.[1], Krishna Chandran R.[1], Preethi G.N.[1], Priya G.[1], Sangeetha V.[1], Sreelatha S.[2], Hariharan S.[1,*]

[1] *(Laboratory of Cytogenetics & Molecular Diagnostics, Research Division, Regional Cancer Centre Thiruvanathapuram, Kerala, India)*

[2] *(Department of Obstetrics and Gynaecology, Sree Avittom Thirunal Hospital, Thiruvanathapuram, Kerala, India)*

Abstract: *Complex Chromosomal Rearrangements (CCRs) involving three or more chromosomes are often detected in phenotypically normal female patients with an adverse obstetric history. Here, we report a 27-yr-old phenotypically normal female with a history of multiple pregnancy failures and carrying a complex chromosomal rearrangement involving chromosomes 4, 10, and 16. CCRs are difficult to interpret using routine cytogenetic studies with GTG banding. FISH was used to clarify and for the correct interpretation of the CCR.*

Key words: *complex chromosomal rearrangement, FISH, cytogenetics, chromosomes 4, 10 and 16, infertility*

I. Introduction

Complex Chromosomal Rearrangements (CCRs) are defined as rare structural rearrangements with 3 or more break points and exchange of genetic material between two or more chromosomes (1). Around 15 to 20% of pregnancies end up in spontaneous abortions (SABs) in humans. The incidence of chromosomal rearrangements in those abortions is as high as 50% and a majority of the rearrangements may be of balanced type, often not associated with any phenotypic abnormalities. These balanced type rearrangements may sometimes lead to reproductive problems including infertility, multiple miscarriages and stillbirths (2,3,4). Detection of such a rearrangement aids in the diagnosis of infertility, the following treatment, the evaluation of the risk for the future child and the appropriate management of the pregnancy to be obtained. The preparation of karyotype may reveal most of these arrangements but it is impossible for detection of subtle rearrangements with less than 5-10 Mb resolution in CCR carriers by cytogenetic techniques. Hence molecular cytogenetic technique, fluorescent in-situ hybridization (FISH with whole chromosome specific painting probes) allows exploring chromosome rearrangements in greater detail (5,6,7). In the current report, we present a case of a CCR involving chromosomes 4, 10 and 16 in a woman with a clinical history of three pregnancy failures. To the best of our available knowledge, this seems to be the first report on translocation among these chromosomes in an individual with infertility.

II. Methodology

2.1 Case

A couple, a 31 year old man and a 27 year old woman presented in this case report was initially investigated at Sree Avittom Thirunal (SAT) hospital, Trivandrum, for she had three pregnancies resulting in one perinatal loss of a small-for-date baby and further a delay in conception and the next two resulted in early pregnancy failure during the couple's 6 years of marriage. The female was antiphospholipid antibodies (APLA) negative and not found any comorbidities associated with reproductive failure and were referred for karyotyping to the Cytogenetics Lab of Regional Cancer Centre (RCC). The physical examinations showed both to be phenotypically normal. The family history indicated no evidence of genetic disease or other inherited disorders.

2.2. Conventional cytogenetics and fluorescent in-situ hybridization (FISH)

2ml peripheral blood samples were collected from both husband and wife with their informed consent. Metaphase spreads obtained from 72 hour PHA-stimulated peripheral blood lymphocyte cultures were GTG-banded according to standard procedures (8). Twenty metaphases were karyotyped using Cytovision Software (Cytovision, USA), and the karyotype was designated according to International Standard for Chromosomal Nomenclature (ISCN 2013).The husband had a normal karyotype showing 46,XY. However, metaphases from the wife revealed a terminal deletion of 16q and not any other rearrangements could be karyotypically determined (Fig.1). Additionally, an extra band seemed to be present on 10p15, but was not confirmed.

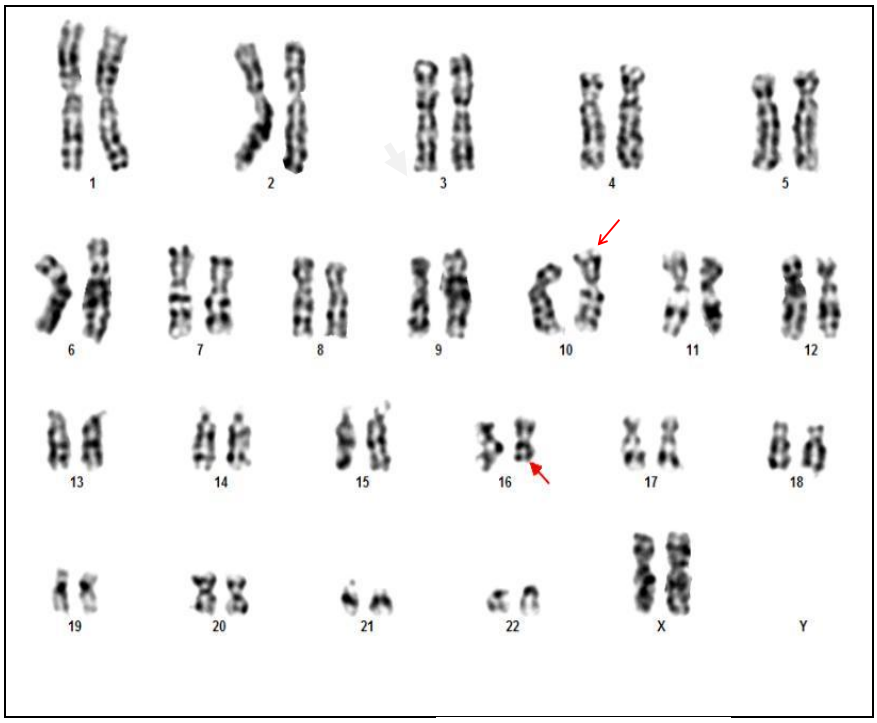

Fig.1: Karyotype showing 46,XX,del(16)(q22) pattern

In order to check whether the deleted portion of 16 has been deleted or translocated i.e; to identify the chromosomal rearrangement, FISH was carried out using the Vysis CBFB Break Apart FISH Probe which has been used to detect chromosomal rearrangements at the CBFB locus on chromosome 16q22, according to standard protocol (9). The chromosome 16 probing of metaphase spreads identified one normal chromosome 16 and confirmed the insertion of a segment from the other chromosome 16 to be surprisingly found on the p terminal region of a large submetacentric chromosome (B group: chromosome 4 or 5) (Fig.2), but it could not be karyotypically determined. To identify that, both Whole Chromosome Probe (WCP) of 4 and 5 was used along with CBFB Break Apart probe of chromosome 16, and revealed it to be present at the terminal P region of chromosome 4 and surprisingly, an additional chromosomal rearrangement was revealed consisted of the portion of chromosome 4 has been present on another chromosome (Fig.3). As the karyotype showed an extra band on chromosome 10; FISH was carried out using WCP of 4, 10 and CBFB Break Apart Probe of 16 and the result confirmed that the insertion in the chromosome 10 was derived from chromosome 4 (Fig.4). So, the karyotype of the wife was confirmed as 46,XX,t(4,10,16)(p15;p15;q22).

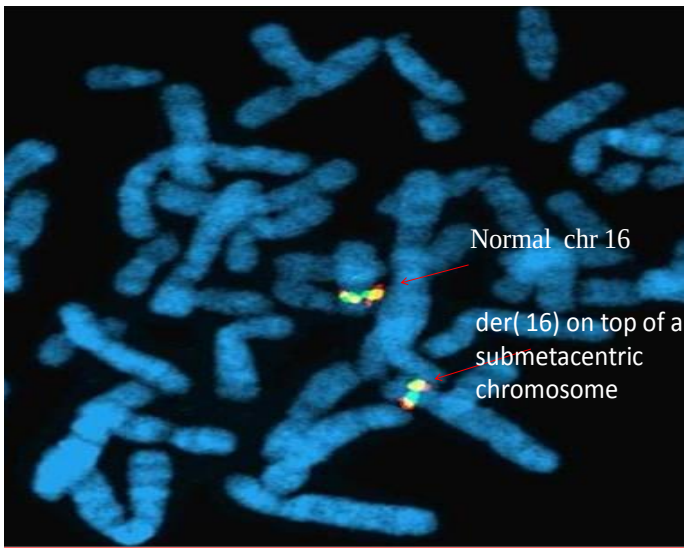

Fig.2: Metaphase FISH showing one normal chr 16 and the deleted portion of the other 16 on top of a large submetacentric chromosome. (chr: chromosome)

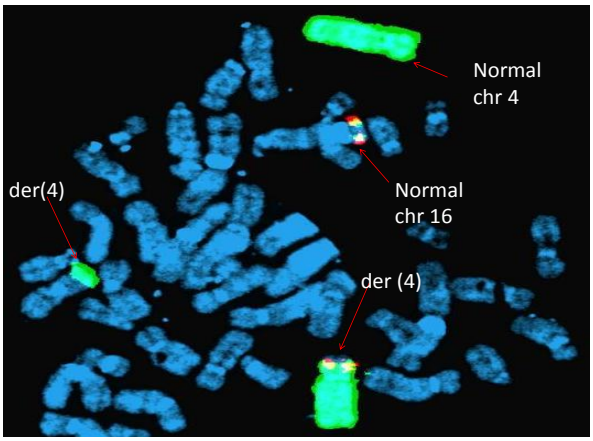

Fig.3: Metaphase spread showing normal chr16 and the deleted portion of other 16 on terminal p arm of chr 4, one normal chr 4 and a portion of other chr 4 on terminal end of one Submetacentric chromosome.

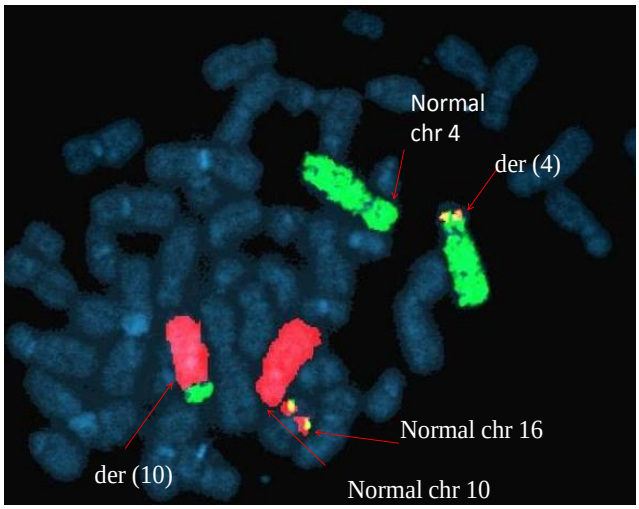

Fig.4: Metaphase spread showing normal chr 16, deleted portion of other chr 16 on terminal p arm of chr 4, one normal chr 4, deleted segment of chr 4 on top of chr 10 and normal chr 10.

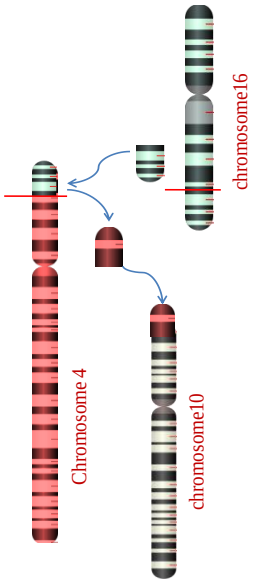

Fig.5: Diagrammatic representation of the three way translocation involving chromosomes 4, 10 and 16.

III. Results And Discussion

CCRs are rarely found in phenotypically normal individuals and are usually detected in connection with reduced fertility, i.e., in- or subfertility in male carriers or recurrent miscarriages in female carriers (10). If fertility is maintained, the birth of a child with malformations can indicate familial occurrence of a CCR (11,12). When present in the germinal lineage, chromosomal abnormalities can be segregated in gametes and transmitted to the offspring, while in other cases they can hamper meiosis up to the arrest of gametogenesis, or may give rise to unbalanced gametes (13,14,15,16,17,18,19). Most diagnosed CCRs are three-way rearrangements, and only a minority consists of highly complex aberrations (20, 21). When the number of breakpoints (22) and the number of chromosomes implicated increase, the correct characterization of the rearrangement by the cytogeneticist becomes increasingly difficult (23). Conventional banding techniques alone may not be sufficient for the interpretation of CCRs; hence FISH with chromosome specific DNA probes allows to explore chromosomal rearrangements in greater detail (5,7,24). In the present case also, karyotyping alone was inadequate to explain all the abnormalities as the der(16) occupied the deleted portion of chromosome 4, it was unrevealed on karyotyping moreover the extra band on chr 10 was also undetectable, hence FISH was necessary as an adjunct to conventional cytogenetic analysis. Using CBFB break apart probe of chromosome 16 and whole chromosome painting probes (WCP4 &WCP 10), the derivative chromosome 4 showed hybridization signals along the entire euchromatic length, with the exception of a part in the distal region of the short arm which was occupied by the CBFB break apart probe of chromosome 16 and the other normal chromosome 16 was also found out. One additional signal of chromosome 4 origin was detected in the distal short arm region of the derivative chromosome 10 along with the normal chromosome 10 (Fig.3).

In the index case with a CCR and clinical history of multiple abortions, the complex karyotype may be attributed to several factors involving genes in the vicinity of the breakpoints. These include (i) disruption of a dosage-sensitive gene at the breakpoints or expression of a recessive gene; (ii) position effect with variable expression of genes near the translocation breakpoint; (iii) uniparental disomy with structurally balanced chromosomes and a functional imbalance; and (iv) additional unbalanced submicroscopic rearrangements (25,26,27).

Table 1: Literature citations of certain CCRs involving chr 4/chr10/chr16 in phenotypically normal females with miscarriages

Case	Complex chromosome Rearrangements / Karyotype	References
1	46,XX,t(1;7;16)(p32.1;q22;q13)	Hanjoon Kim et al.2013[28]
2	46,XX,t(3;4;7;9;17)(q22; p14;q34; p13;q13)	Kuechler et al.2005[29]
3	46,XX,t(4;10;11)	Migliori et al.2004[30]
4	46,XX,(5;16;10;18)(q13;q22;q11.2;q21)	Lee MH et al.2002[31]
5	46,XX,t(7;10;21)(q11;q22;q22)	Gorski et al.1986[32]

Certain previously described CCRs (Table 1) in female cases were also ascertained by recurrent miscarriages were all phenotypically normal, it is likely that these chromosomal breakpoints might not include genes or gene regulatory regions whose disruptions may give rise to physical dysfunction and clinical phenotypes. It has been thought that the number of chromosomes involved or the location of the breakpoints may also play a role in the reproductive condition of CCR carriers. Giardino et al. (33) have noted that chromosomes 2, 3, 4, 7, and 11 are more frequently implicated in CCRs. Moreover, breakpoints on chromosomes 2, 6, 7, 8, 10 and 11 were reported more to be present in females with recurrent miscarriages and chromosomes 6, 7, 8, 11 and 16 have frequently been reported in men with recurrent miscarriages. All of the chromosomes, except for chromosomes 17, 20 and 22 were also reported in females having recurrent miscarriages.

Detection of couples with chromosomal abnormalities can undoubtedly help to prevent the birth of grotesque generations. Banded chromosomal studies are recommended for couples with repeated abortions. The occurrence of CCRs is rare, and its mechanism remains mysterious. Detailed cytogenetic analysis is essential for predicting the success of assisted reproductive procedures in those having decreased reproductive fitness. Assisted reproductive procedures may have a limited role in management of couples with CCRs, due to the high rate of unbalanced gametes and possibility of apparently balanced gametes with functional abnormalities in the offspring of females with CCR. It is difficult but important to provide adequate genetic counseling and alternative of donor ova or adoption may be recommended for such cases.

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