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Case Report

Genetics Section

Balanced Autosomal Translocations in Two Women Reporting Recurrent Miscarriage

BRINDHA ARUMUGAM[1], CHANDRA R SAMUEL[2], SANTHIYA SATHIYAVEDU THYAGARAJAN[3]

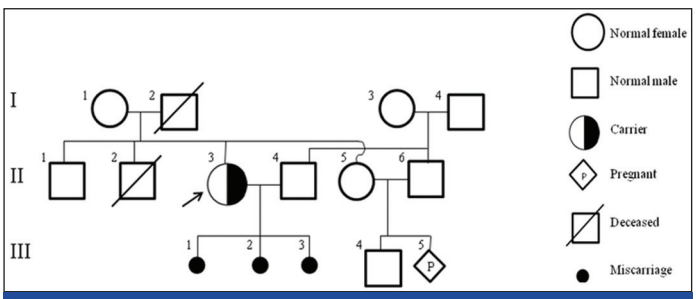
ABSTRACT

Spontaneous abortion or loss of fetus prior to 20 weeks of gestation is observed in 15-20% of clinically recognized pregnancies. Recurrent Miscarriage (RM) is defined as three or more consecutive pregnancy losses and it affects 1-2% of women. Parental chromosomal rearrangements account for 2-5% of RM. This report describes two couples with a clinical history of RM who were subjected to conventional cytogenetic analysis to ascertain the chromosomal aetiology. Analysis of GTG-banded metaphases obtained from cultured lymphocytes at approximately 500-band resolution revealed balanced translocation in the female spouses as 46,XX,t(8;11)(p11.2;q23.3) in BR27W and 46,XX,t(5;7)(p15.1;q32) pat in BR49W. Both the male partners exhibited 46,XY karyotype. Fluorescent In Situ Hybridization (FISH) analysis was subsequently carried out to confirm the balanced translocation using suitable whole chromosome paint probes. These balanced chromosomal abnormalities in the parents could be responsible for the repeated fetal losses. Hence, karyotype analysis should be a mandatory etiological investigation for couples with RM towards genetic counselling. Disruption of critical genes through these rearrangements could also underlie the pregnancy outcome.

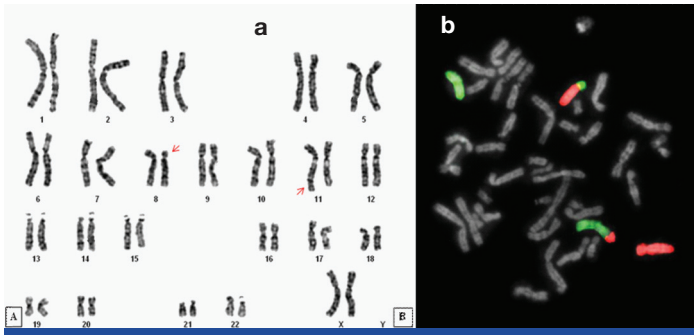
Keywords: Chromosomal rearrangement, Fluorescent in situ hybridization, Genetic counseling

CASE REPORT 1 - BR27W

A 29-year-old female (proband) was referred from the Institute of Obstetrics and Gynecology, Chennai with a history of three first trimester miscarriages. She presented no family history of either miscarriages or genetic disorders [Table/Fig-1]. Her age at menarche was 15 years and had regular menstrual cycles. Ultrasound imaging revealed bilateral polycystic ovaries (RO: 4.3 x 2.3cm; LO: 4.4 x 2.2cm) and normal uterus (7.4 x 4.1 x 4cm). Hormonal profiles were normal (FSH 7.93mIU/ml; LH 3.97mIU/ml; Estradiol 77.9pg/ml). Laboratory investigations were carried out to exclude TORCH infection and anti-phospolipid syndrome. The study was approved by the Institutional Human Ethical Committee (UM/IHEC/06-2014-II). After obtaining written informed consent from the couple, cytogenetic analysis was performed on GTG-banded metaphases at 400-500 band level resolution using Applied Spectral Imaging Systems karyotyping software (BandView version 6.0) as per standard protocols. The abnormalities were designated following ISCN 2013 [1] guidelines. The husband exhibited a normal 46,XY karyotype while the female partner (BR27W) showed a balanced translocation as 46,XX,t(8;11)(p11.2;q23.3) [Table/Fig-2a]. Fluorescence In Situ Hybridization (FISH) analysis using Whole Chromosome Paint (WCP) probes for chromosomes 8 and 11 with Applied Spectral Imaging Systems FISH software (FISH View version 6.0) confirmed the chromosomal re-arrangement [Table/Fig-2b]. The proband's family denied consent for further study aimed to resolve the origin of the translocation.



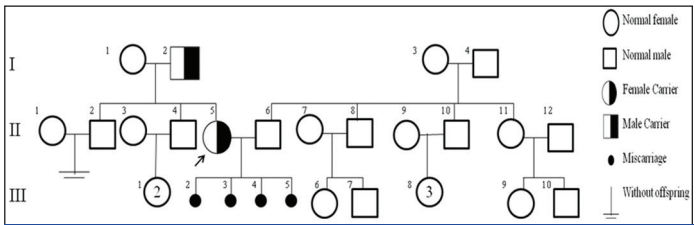
[Table/Fig-1]: Pedigree of Case Report 1 (BR27W).



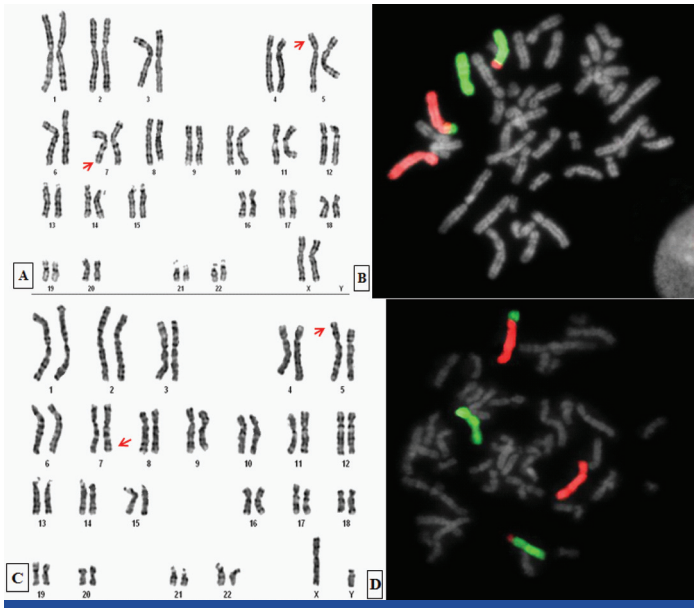
[Table/Fig-2]: a) GTG-banded karyogram showing the translocation t(8;11) (indicated by arrows) in the wife BR27W; b) FISH using WCP8 (red) and WCP11 (green) probes confirmed the translocation.

CASE REPORT 2 - BR49W

A 26-year-old female gave a history of four miscarriages, all in second trimester, although there was no family history of abortions [Table/Fig-3]. She experienced regular menstrual cycles since, the age of 16 years. Endocrine profiling revealed slightly elevated level of TSH (7.12µIU/ml) and normal total T3 (201ng/dl) and total T4 (12.66ug/dl). Investigations ruled out TORCH infection and anti-phospholipid syndrome. Written informed consent was obtained from the couple. A balanced translocation 46,XX,t(5;7)(p15.1;q32) observed in the wife (BR49W) [Table/Fig-4a] was confirmed through FISH employing WCP probes for chromosomes 5 and 7 [Table/Fig-4b]. Extended chromosomal analysis of her parents established her father also to be a carrier of the denoted translocation [Table/Fig-4c,d]. Of her two brothers, one was infertile (II-2) and the other has two daughters (II-4) but they did not cooperate for this study. Her husband and mother had normal karyotype.



[Table/Fig-3]: Pedigree of Case Report 2 (BR49W).



[Table/Fig-4]: GTG-banded karyogram showing the translocation t(5;7) (arrows) (a) in the patient BR49W which was confirmed by FISH using WCP5 (red) and WCP7 (green) probes (b); GTG-banded karyogram (c) and metaphase FISH using WCP probes (d) from her father exhibiting the same translocation.

DISCUSSION

Recurrent Miscarriage (RM) is defined as three or more consecutive pregnancy losses prior to 20 weeks of conception and is observed in 1-2% of women [2]. However, two pregnancy losses also prompt the diagnosis of RM as recurrence rates and risk factors are fairly similar [3,4]. Several etiologies besides age such as genetic, anatomical, endocrine, immunologic, environmental and life-style risk factors contribute to ~50% of the cases [4]. Fetal chromosomal abnormalities cause about 70% of sporadic miscarriages while they lead to only a relatively smaller fraction of pregnancy losses in RM couples [3].

Parental structural chromosomal rearrangements have been reported in 2-5% couples with RM [2,4]. These anomalies include reciprocal and Robertsonian translocations, inversions, insertions and mosaicism [2]. Robertsonian translocations account for 35% of the cases carrying a translocation, while 65% are of reciprocal type [5]. Reciprocal translocations, found in one out of 500 people, do not produce any phenotypic effects but usually result in RM, offspring with chromosomal abnormalities or infertility in the carriers [6]. When a parent carries a balanced translocation, there is a 4% risk of unbalanced translocation in the fetus [5]. The chromosome arms 2q, 5q, 7p, 7q, 12q, 13q, 17q, 18q and 22q were preferentially involved in RM [6]. In addition to size of the chromosomal segment, the position and frequency of the break points also play a critical role [7]. The risk of RM doubles when one partner is a carrier [8]. Low maternal age, history of RM and family history of RM increase the probability of carrier status [9].

Karyotypic analysis forms a part of diagnostic work-up of couples with RM [4]. Chromosomal anomalies including polymorphic variants have been recorded in 2-12.5% of couples with RM [10,11]. These anomalies trigger meiotic unequal crossing over leading to formation of genetically imbalanced gametes that is lethal to the embryo, causing miscarriage [11]. At meiosis, chromosomes involved in reciprocal translocations form quadrivalent complexes that segregate by alternate, adjacent-1, adjacent-2, 3:1 or 4:0 modes to form gametes with either balanced or imbalanced chromosome complements. Eventually of the 32 possible zygotes only two are genetically normal or balanced [6]. Parents as carriers have 2.9% chance of their offspring born with an imbalanced karyotype and congenital anomalies [10]. There also occurs a gender influence over clinical pathology as inherited balanced chromosomal rearrangements in males cause sterility [7]. The woman BR49W reporting RM in this paper had inherited the translocation from her father.

Varied translocation break points involving the same chromosomes as observed in our probands have been described in couples with RM earlier [Table/Fig-5] [12-16]. Further, chromosomal rearrangements depicting identical loci have also been implicated in RM [Table/Fig-6] [6,13,16-19]. Molecular characterization of these break points would elucidate not only the critical genes responsible for the clinical condition but also unravel the mechanism of inactivation through interruption or by position effect [19]. List of genes located at the translocation breakpoints and their likely role are given in [Table/Fig-7] [20]. Some of them

Karyotype	Reference
46,XX,t(5;7)(p15.1;q32)	Present study
46,XY, t(5;7)(p13;p15)	Stephenson and Sierra, [12]
46,XY,t(5;7)(p13;p15)	Chaithra et al., [13]
46,XY,t(5;7)(q13;q22)	Sheth et al., [14]
46,XX,t(5;7)(q13;q32)*	Sheth et al., [15]
46,XX,t(8;11)(p11.2;q23.3)	Present study
46,XX, t(8;11)(q11.23;q24.2)	Stephenson and Sierra, [12]
46,XX,t(8;11)(q11.23;q24.2)	Chaithra et al., [13]
46,XX,t(8;11)(q21;q13)	Sugiura-Ogasawara et al., [16]

[Table/Fig-5]: Published cases of t(5;7) and t(8;11) associated with Recurrent Miscarriage [12-16]
* Single cell abnormality.

Band	Karyotype	Phenotype	Reference
5p15	46,XX,inv(5)(p15.3q15)	RM	Ghazaey et al., [17]
	46,XY,inv(5)(p15.3q35)	RM	Goddijn et al., [18]
	46,XY,inv(5)(p15.2p13)	Male infertility	Bugge et al., [19]
	46,XY,t(5;12)(p15.1;p12.2)	RM	Chaithra et al., [13]
	46,XX,t(5;12)(p15.1;q22)	RM	Goddijn et al., [18]
	46,XX,t(X;5)(q22;p15.2)	Female infertility	Bugge et al., [19]
	46,XY,t(4;5)(q25;p15.2)	RM	Ghazaey et al., [17]
7q32	46,XX,t(1;7)(q32.1;q32)	RM	Chaithra et al., [13]
	46,XX,t(2;7)(q37.1;q32)	RM	Ghazaey et al., [17]
	46,XX,t(3;7)(q22;q32)	RM	Ghazaey et al., [17]
8p11	46,XX,inv(8)(p11.22q13.1)	RM	Goddijn et al., [18]
	46,XY,t(8;15)(p11.2;q25)	RM	Sugiura-Ogasawara et al., [16]
11q23	46,XX,inv(11)(q21q23)	RM	Goddijn et al., [18]
	46,XX,t(11;22)(q23.3;q11.2)	RM	Ogilvie and Scriven [6]
	46,XY,t(11;22)(q23.3;q11.2)	RM	Ogilvie and Scriven [6]
	46,XX,t(11;22)(q23.3;q11.2)	RM	Ghazaey et al., [17]
	46,XX,t(11;22)(q23;q11)	RM	Sugiura-Ogasawara et al., [16]
	46,XY,t(11;22)(q23;q11)	RM	Ghazaey et al., [17]

[Table/Fig-6]: Phenotypes reported in cases with identical breakpoints [6,13,16,17,18,19].

regulate basic cellular processes like response to DNA damage, microtubule assembly, chromosome segregation (*CEP164*), cell differentiation (*PAX4*), cell-cell interaction, sperm–egg interaction (*ADAM2*), cell adhesion (*C1QTNF5* and *PVRL1*), cell senescence (*ETS1*) and apoptosis [20]. Genes encoding transcription factors (*PAX4*, *ETS*), if disrupted could manifest a cascade effect affecting a specific pathway involving an array of genes [Table/Fig-7]. Future efforts integrating studies on skewed X-chromosome inactivation, copy number variations, parental- and tissue- specific imprinted genes and microRNA expression profiling for RM-related tissues would aid in the identification of biomolecular risk factors for RM [3]. It could be speculated that disruption of critical genes through chromosomal rearrangements and their consequent functional impairment possibly results in RM. Therefore, molecular characterization of the breakpoints in future might reveal the candidate genes underlying RM.

Gene	Band	Role	Phenotype
ANKH	5p15.1	Multipass transmembrane Protein	Craniometaphyseal dysplasia - AD
PAX4	7q32	Transcription Factors • Critical role in fetal development and cancer growth • Differentiation of insulin producing beta cells	
STAR	8p11.2	Acute regulation of steroid hormone synthesis	Congenital adrenal hyperplasia
ERIIN2	8p11.2	SPFH domain-containing family of lipid raft-associated proteins	Spastic paraplegia-18
ADAM2	8p11.2	Cell-cell interaction & cell-cell matrix interaction • Fertilization, muscle development and neurogenesis • Sperm-egg interaction	
CBL	11q23.3	Proto-oncogene	Noonan syndrome-like disorder
APOC 3	11q23.3	Apolipoprotein C III	Hypertriglyceridemia due to increased expression
ETS1	11q23.3	*ETS* family of transcription factors (activator/repressor) • Stem cell development • Cell senescence and death • Tumorigenesis	
HMBS	11q23.3	Hydroxymethlylibane super family	Acute intermittent porphyria – AD
PVRL1	11q23.3	Adhesion protein	Cleft lip and palate/ ectodermal dysplasia 1 syndrome
TMPRSS4	11q23.3	Serine protease family	Multiple human diseases and disorders due to malfunction
C1QTNF5	11q23.3	Cell adhesion	Late onset retinal degeneration
DPAGT1	11q23.3	Catalytic enzyme	Congenital disorder of glycosylation type Ij
CEP164	11q23.3	Centrosomal protein • Microtubule organization • DNA damage response • Chromosome segregation	
ARCN1	11q23.3	Intra-cellular protein	Multiple disease-associated chromosome translocations

[Table/Fig-7]: List of genes at the translocation breakpoints reported in this study [20].

CONCLUSION

Karyotype analysis is mandatory for couples with RM to ascertain the chromosomal etiology towards genetic counseling. Further, prenatal diagnosis in every subsequent pregnancy and oocyte or sperm donation followed by preimplantation genetic diagnosis are recommended when a parent proves to be a carrier of chromosomal rearrangement. Alteration of expression of critical genes through these rearrangements could also underlie the pregnancy outcome.

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PARTICULARS OF CONTRIBUTORS:
1. Research Scholar, Department of Genetics, Dr. ALM PG IBMS, University of Madras, Chennai, Tamilnadu, India.
2. Associate Professor, Department of Genetics, Dr. ALM PG IBMS, University of Madras, Chennai, Tamilnadu, India.
3. Professor (Retd.), Department of Genetics, Dr. ALM PG IBMS, University of Madras, Chennai, Tamilnadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Santhiya Sathiyavedu Thyagarajan,
Department of Genetics, Dr. ALM PG IBMS, University of Madras, Taramani, Chennai-600113, Tamilnadu, India.
E-mail: v_santhiya63@hotmail.com

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