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Single Cell Abnormality in Couples with Bad Obstetric History and Repeated Fetal Loss: Occurrence and Clinical Outcome

Frenny Sheth, Jhumur Pani, Manisha Desai and Jayesh Sheth

Institute of Human Genetics, FRIGE House, Jodhpur Gam Road, Satellite, Ahmedabad 380 015.Gujrat, India
Telephone: 91-79-26921414/65122802, Fax: 91-79-26921415;
E-mail: fshethad1@googlemail.com

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ABSTRACT The occurrence of chromosomal aberration in a single metaphase plate is defined as single cell abnormality (SCA). Its causes of origin and significance are not clearly understood. Hence, 389 individuals having clinical history of RFL/BOH were enrolled during April, 2008 - March, 2009 for chromosomal analysis. SCA was observed in 28 patients (7.2 percent). Of these, 17 were females (60.7 percent) giving an overall frequency of 4.4 percent as compared to 2.83 percent in males. Structural rearrangements were observed in 22 cases as compared to numerical anomalies seen in 6 subjects. Reciprocal translocation in 22 patients indicates its definitive role in the event of early pregnancy loss or its contribution in causing deleterious effects leading to fetal loss, fetal growth retardation or multiple birth defects. Genomic instability could attribute to the cause of origin of SCA.

INTRODUCTION

A single cell abnormality (SCA), by definition is an isolated metaphase of a sporadic cell with gross chromosomal rearrangements, in an individual with an otherwise normal chromosomal constitution (Higgins and Palmer 1987; Reddy and Thomas 1985; Hustinx et al. 1979). A few studies have attempted to establish the breakpoint regions and occurrence frequency in order to examine the probable cause of their origin and further investigate its potential clinical implications (Higgins and Palmer 1987; Hustinx et al. 1979; Dewald et al. 1986; Russo et al. 1989; Zech and Huglaund 1978; Devi and Sayee 2005). Nonetheless, the same is still not determined as the patients require long term follow-up studies and the sheer number is not statistically large enough to draw firm conclusions. Hence, the present study was carried out to know the occurrence of SCA in cases with repeated fetal loss and/or bad obstetric history (RFL/BOH) as an attempt to correlate any tangible information on the occurrence of various chromosomes involved and its role in genomic instability, fetal loss and congenital birth defects.

Address for Correspondence:
Frenny Sheth
Institute of Human Genetics,
FRIGE House
Jodhpur Gam Road, Satellite,
Ahmedabad 380 015, Gujrat, India
Telephone: 91-79-26921414/65122802
Fax: 91-79-26921415
E-mail: fshethad1@googlemail.com

MATERIAL AND METHODS

A total of 389 individuals (194 couples and 1 female) in the age range of 26yrs to 37yrs with mean of 31.5yrs were referred for RFL and/or BOH have been included in this study during April 2008 to March 2009 after approval from the ethical committee of the Institute. Analysis was also performed on a female enrolling for assisted reproductive technology (ART). Detailed family history was taken for all the individuals enrolled and all of them had more than one miscarriage with/without normal child (n= 10 and n=28 respectively). Other parameters such as immunological assays, hormonal assays, and infections were also checked and were found to be in the normal range. None of them had any teratogenic or radiation exposure in near past. Cases having constitutional chromosomal anomalies were not included in the present study. Peripheral blood samples were collected after taking consent and processed according to the standard 72-hour, phytohaemaglutinin (PHA) stimulated culture set-up. GTG–banding technique was applied to all metaphases encompassing minimum 400-band resolution. In each case, a minimum of 30 metaphases were thoroughly scanned and upon identifying any ab-

normality, total 100 metaphases were scored; however, no abnormalities were detected. This rule out mosaicism from SCA but not among different tissues. Blind scoring was carried out in all the cases by two individuals independently, irrespective of the clinical history. The International System for Human Cytogenetic Nomenclature was referred and applied to identify breakpoints (ISCN 2009). Digital images were captured using Olympus BX-51 equipped with CCD camera and Adobe Photoshop software.

RESULTS AND DISCUSSION

In the present study, of the 389 individuals investigated, SCA was observed in 28 (7.2 percent) patients (11 males, 17 females). RFL and BOH were observed in 18 and 10 cases respectively. Our results are in concurrence with the early report and the frequency increases up to 30 percent in cases of RFL and up to 50 percent in cases having BOH elucidating a plausible, currently unidentified underlying cause and effect between SCA and BOH (Devi and Sayee 2005; Domínguez et al. 1999).

It was observed that 79 percent of SCA involved reciprocal balanced translocation between two chromosomes (Table 1). Translocation solely involving #7 and #14 were predominantly observed in 8 patients (28 percent). However, overall involvement of #7 and #14 were observed in 12 and 10 cases respectively. It has been observed that cases of SCA involving t(7;14) in individuals not susceptible to malignancy or augmented chromosomal breakage is a consequence of viral exposure, change in DNA repair mechanism or due to PHA stimulated growth conditions (Reddy and Thomas 1985; Devi and Sayee 2005; Domínguez et al. 1999; Dave and Shetty 2010).) These SCA when systematically studied, were not detected in amniocyte cultures, bone marrow or unstimulated blood cultures. It has no correlation with patients' age or sex, any month or season of the year (Dewald et al. 1986)

The detection of chromosomal translocations, especially between chromosome 7 and 14 occurring as nonrandom *in vitro* events in peripheral blood lymphocyte cultures is quite well known (Reddy and Thomas 1985; Dewald et al. 1986; Russo et al. 1989; Zech and Huglaund 1978; Devi and Sayee 2005). Break point regions involved on #7 and #14 were found to be 7p13, 7p15, 7q32 and 14q11.2 (Table 2) in genes for gamma (7p13-15), beta and alpha chains of the human T-cell receptors correspondingly. These translocations may be related to the site specific rearrangements occurring during somatic development of the T-cell receptor unit near 7p15 which is responsible for normal development of the immune system.

Even though the study involved a rather small number of cases, various chromosome involved in SCA are shown for the first time to the best of our knowledge. We report additional breakpoint regions, its frequency and chromosomes involved in SCA to expand the databank of the

Table 1: Cases presenting with SCA

Structural				*Structural*			
S. No.	*Karyotype*	*SCA*	*Break point region*	*S. No.*	*Karyotype*	*SCA*	*Break point region*
1	46,XY	46,XY, t(7;14)	(p15;q11.2)	17	46,XX	46,XX,t(7;10)	(p22;p11.2)
2	46,XX	46,XY, t(7;14)	(p15;q13)	18	46,XY	46,XY,t(1;8)	(p32;q21.2)
3	46,XY	46,XY, t(7;14)	(q35;q11.2)	19	46,XY	46,XY,t(6;10)	(q15;q24)
4	46,XX	46,XY, t(7;14)	(q32;q11.2)	20	46,XY	46,XY,t(4;22)	(p16;q13)
5	46,XY	46,XY, t(7;14)	(q32;q22)	21	46,XX	46,XX,del(1q)	(q21-qter)
6	46,XX	46,XY, t(7;14)	(p13;q11.2)	22	46,XY	46,XY,i(11)(q10)x2	(q10)
7	46,XY	46,XY, t(7;14)	(p13;q11.2)	*Numerical*			
8	46,XY	46,XY, t(7;14)	(q32;q13)	*S. No.*	*Karyotype*	*SCA*	
9	46,X,inv(Y)	46,X,inv(Y),t(9;20)	(p13;13.3)				
10	46,XX	46,XX, t(12;14)	(q13;q22)	1	46,XX	47,XXX	
11	46,XX	46,XX, t(5;7)	(q13;q32)	2	46,XX	49,XXXXX	
12	46,XX	46,XX, t(7;7)	(p15;q34)	3	46,XX	47,XXX/49,XXXXX	
13	46,XX	46,XX, t(2;14)	(q33;q11.2)	4	46,XX	47,XXX	
14	46,XX	46,XX, t(1;10)	(q12;11.2)	5	46,XY	47,XXY	
15	46,XX	46,XX, t(2;7)	(p23;q22)	6	46,XX	47,XXX	
16	46,XX	46,XX, t(5;13)	(p15.3;q14)				

Table 2: Frequency of breakpoints on chromosome 7 and 14

Chromosome 7	*Twice*	*Thrice*	*Once*	*Once*	*Thrice*	*Once*	*Once*
	p13	p15	p22	q22	q32	q34	q35
Chromosome 14	*6 Times*	*Twice*	*Twice*				
	q11.2	q13	q22				

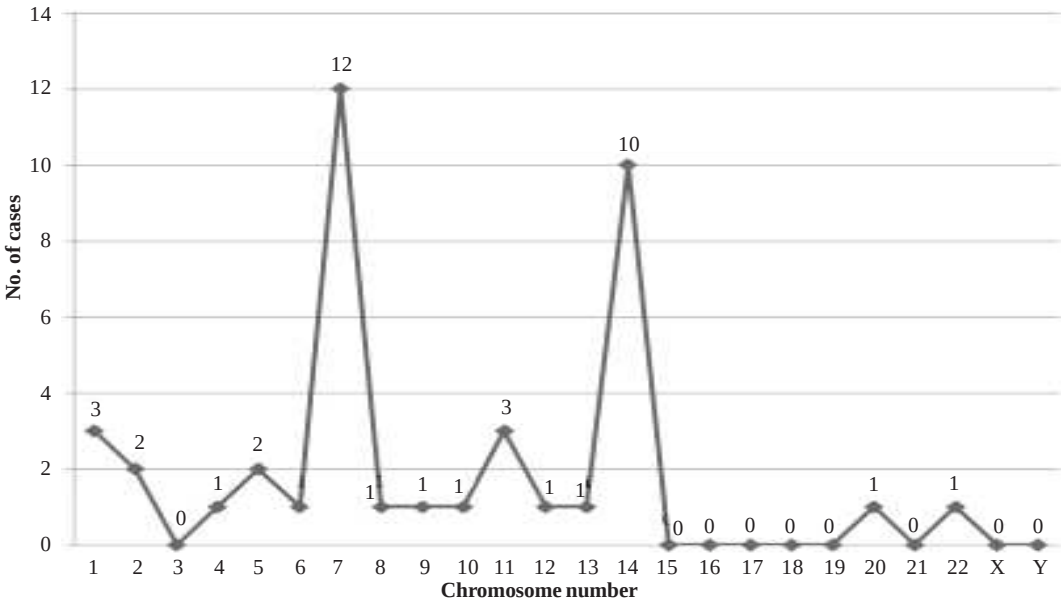

Fig. 1. Incidence of chromosome involved in SCA

Table 3: Chromosomes and breakpoint region involved in formation of SCA

Chromosome involved	*Breakpoint region*	*Frequency*
#1	p32	1
	q12	1
	(q21→qter)	1
#2	p23	1
	q33	1
#4	p16	1
#5	p15.3	1
	q13	1
#6	q15	1
#7	p22	1
	p15	3
	p13	2
	q22	1
	q32	3
	q34	1
	q35	1
#8	q21.2	1
#9	p13	1
#10	p11.2	1
	q11.2	1
	q24	1
#11	q10	1
#12	q13	1
#13	q14	1
#14	q11.2	6
	q13	2
	q22	2
#20	q13.3	1
#22	q13	1
#X	+1	5
	+3	2

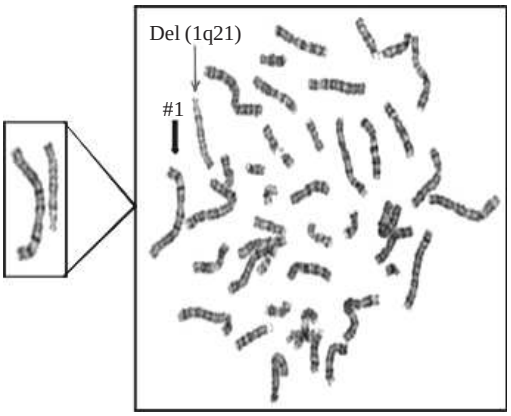

Fig. 2. Karyotype showing deletion at 1q21qter

same (Table 3). Chromosomes involved in SCA besides #7 and 14, were #1, 2, 4, 5, 6, 8, 9, 10, 12, 13, 20, and 22. These chromosomes have taken part in translocations not more than thrice (Fig. 1). X-chromosome was involved only in

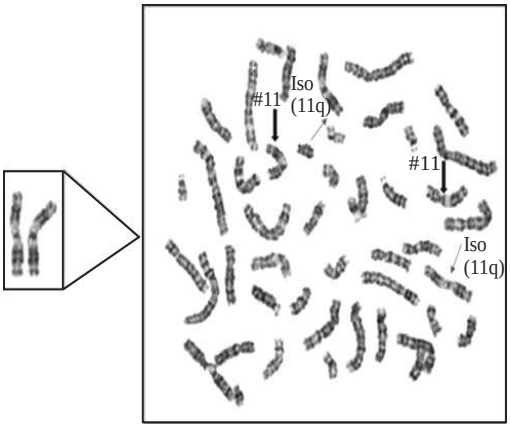

Fig. 3. Karyotype showing isochromosome of #11(q10)

numerical SCA in 6 cases (Table 1). Other than reciprocal translocations, deletion at 1q21m→ qter and isochromosome of #11(q10) was seen in one each (Fig. 2 and 3). Structural anomalies were three times the numerical anomalies.

In an attempt to uncover the plausible explanation for SCA (except t(7;14) due to PHA stimulated conditions), the observed cases were analyzed for a rather universal underlying cause. The role of hormonal, immunological, or infectious parameters and exposure to teratogens or radiation in recent past was ruled out by obtaining detailed medical history. SCA is not specifically linked with age or number of miscarriages as per clinical history and are in concordance with the reported cases in the literature (Higgins and Palmer 1987; Reddy and Thomas 1985; Zech and Huglaund 1978). Hence in the present study, observation of higher number of SCA can be predominantly be attributed to the good, elongated metaphases and eye karyotyping by the experts which still remain the gold standard in the field of cyto-genetic where fully automated systems have taken over.

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