

Effect of Reciprocal Translocations on Phenotypic Abnormalities

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ABSTRACT The chromosomal disorders make a significant contribution to human mortality and morbidity. Karyotyping allows the identification of various chromosomes involved in a rearrangement. Chromosomal aberrations occur in approximately 1 in 200 live – born infants and the incidence of reciprocal translocations (rcpts) occur as 1/500 live births. Balanced reciprocal translocations can lead to a variety of unbalanced products. In this study, undertaken at the Division of Human Genetics of the Department of Anatomy of St. John's Medical College, Bangalore, 58 cases of reciprocal translocations were collected from the existing data and the results were compiled. The most important observations noted in this study were:

1. The frequency of rcpts was 4.2% among the chromosomal abnormalities identified in the laboratory.
2. The common chromosomes involved in rcpts were chromosomes 1,2,3,5,7,9 and 22. The comparative site of the breakpoints showed preference at 1q, 2p, 5q, 3q, 7p and 9p.
3. Comparison between the distribution of parental carrier status and the cases, which were *de novo*, was explained. Parental carrier status was seen in 18 cases (31.03%) and *de novo* status was seen in the remaining 40 cases (68.97%).
4. Determination of the sex-ratio and the incidence among the affected male/female cases was 1.14:1 showing predictable maternal carrier predominance.
5. A higher clinical correlation between Bad Obstetric History and MR/MCA to various types of rcpts were identified in these individuals.

All these results were correlated and serve as a basis for predictive genetic counseling to these affected individuals and provide clues to the positioning of important genes that may be responsible for human malformations, thus indicating important developmental genes being disrupted during segregation. This study has highlighted for the first time, a profile on rcpts in the Indian population.

INTRODUCTION

The chromosomal disorders make a significant contribution to human mortality and morbidity. Karyotyping allows the identification of various chromosomes involved in a rearrangement. Chromosomal aberrations occur in approximately 1 in 200 live – born infants and the incidence of reciprocal translocations (rcpts) occur as 1/500 live births. Balanced reciprocal translocations can lead to a variety of unbalanced products.

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Rest of the chromosome (including the centromere) is the centric segment. The rearranged chromosome is called derivative (der) chromosome.

The term "*genotype*" is generally used to refer the genetic make-up of an individual. The term "*phenotype*" on the other hand, describes the form and functioning of an individual (Gardner and Sutherland 1996). The complexity of the phenotype reflects largely but not entirely the complexity of the genotype. In this study, retrospective data on the individuals referred to Division of Human Genetics and found to have a confirmed rcpt by cytogenetics analysis, have been checked for a possible repeated expression of phenotypic abnormalities (phenotype) in relation to the reciprocal translocation (genotype).

The reciprocal arrangement could be *de novo* or transmitted from carrier parents, resulting in disruption of important developmental genes.

Aims and Objectives

The aims of this research work were:

1. To state the frequency of reciprocal translocations (rcpts).
2. To identify the chromosomes involved in rcpts.
3. To group the various chromosomes involved in the rcpts
4. To identify the subjects based on retrospective data.
5. To determine the parental carrier status and to observe the various phenotypic effects arising from the meiotic mode of segregation of the reciprocal translocations.
6. To study the comparative effects of the common chromosomes involved the site of the breakpoints, the frequency and its outcome in the phenotype of the segregants.
7. To highlight the reciprocal translocation profile on Indian population and probable sex-ratio.

The mode of genetic counseling, to the family with affected and carrier members, depends on the rcpt, segregation of chromosomes, sex of the affected individuals/carriers and the cause of the premeiotic or meiotic balanced translocation.

MATERIAL AND METHOD

Material: These observations were collected from the existing data in the Division of Human

Genetics, Department of Anatomy, St. John's Medical College, Bangalore, since 1976. The major areas of referral have been cases of Bad Obstetric History, Fertility failure, Mental retardation with or without multiple congenital abnormalities, Amenorrhoea (primary or secondary), and genital ambiguity. Based on the cytogenetic findings, the patients and their families have been appropriately counseled and the possible management options were explained to them.

A total of 58 cases were selected from the existing data of 1350 cases identified with chromosomal abnormality studied at Division of Human Genetics.

Method: Rooney et al. (2001); A complete history and details of physical examination of the subjects were recorded in the consent of the proforma. Patients or the parents (in case of children) for the investigation was taken for every case (Appendix 1).

For the cytogenetic analysis the blood samples were processed employing routine lymphocyte cultures.

Collection of Samples: About 3-5ml of venous blood was collected from the couple and children in sterile disposable syringe containing heparin and was mixed well by tilting the syringe, 2-3 times. Heparin prevents coagulation without diminishing the quality of the preparation. Peripheral blood is a plasma-based suspension of erythrocytes, platelets and leukocytes; only the leukocytes are nucleated and therefore divide.

Culture: The lymphocytes were stimulated by the use of mitogen. The mitogen transforms the lymphocytes into mitotically active cells. PHA (Phytohaemagglutinin) a mucoprotein was added to the lymphocytes in the culture. The constituents of the lymphocyte culture include RPMI 1640 basal medium, supplements such as serum (usually fetal bovine serum) that contains various growth factors essential for cellular proliferation. Antibiotics were added to control/prevent the microbial infection. PHA was added to stimulate lymphocyte proliferation.

Harvesting Lymphocyte Culture: After 72 h of incubation of the lymphocyte culture, it was harvested by first adding colchicine, which arrests the dividing cells in the metaphase, when the chromosomes are maximally condensed and easiest and are easy for analysis. After exposure to colcemid, the cells are subjected to a hypotonic solution such as KCl. This solution causes swelling of cells, rupture of nucleus and better separation of the individual chromosomes.

Centrifugation of the sample results in supernatant and the pellet is seen at the bottom of the test tube. The supernatant is removed and the pellet that contains the lymphocytes is "fixed". "Fixation" of the cell pellet is a process whereby the cells are killed, nuclear proteins removed and the chromosomal morphology is preserved. The fixative consists of 3 parts of methanol and one part of acetic acid. After fixation the pellet is dropped from a height onto a labeled slide and allowed to dry. These slides were later stained using Giemsa stain (GTG banding)

Staining of Slides: The slides were aged for 3-4 days at room temperature and then stained. These slides were first dipped in trypsin and subsequently stained with Giemsa. The banding pattern reflects both structural and functional components of the chromosomes. Dark bands are AT rich regions containing few active genes as compared to the light bands.

Analysis and Karyotyping: ISCN (2005): Chromosomes spreads are analysed under the microscope. The banding pattern helped to analyse the structural variations and the numerical variations. Fifteen metaphase spreads were counted, 5 drawn, 3 spreads captured using CCD camera and printed, one of these prints was chosen for karyotyping and results were recorded.

Karyotyping is a display of the chromosomes according to their length and position of the centromere of the chromosome (metacentric, submetacentric, and acrocentric). Thus, cytogenetic abnormalities, were detected, interpreted and results were correlated with the clinical presentations, in order to indicate their implications.

RESULT AND DISCUSSION

Frequency of rcpts

In this study, the frequency of the rcpts has been determined in 58 out of 1350 with chromosomal abnormality. The percentage

Table 1: List of reciprocal translocations (n 58):

1;2 1;2 1;3 1;3 1;7 1;14 1;15 1;21 1;21	10;11 10;14
2;4 2;9 2;9 2;11 2;13 2;18 2;21	11;16
3;7 3;8 3;11 3;2 3;15	13;17
4; 6;15 4;12 4;21	15;17 15;20 15;22 15;22
5;6 5;7 5;8 5;7;10;13 5;8 5;13	18;20
6;8	19;20
7;8 7;11 7;13 7;13 7;18	20;22
8;11	X;12 X;14 X;22
9;10 9;12 9;14 9;15 9;22	Y;22 Y;Y

frequency is 4.2% as against the overall 3 to 4% of structural rearrangement reported in literature (Mueller and Young 2001). The difference may be because of the bias in the sampling.

Identification of Chromosomes Involved in rcpts / Comparative Site of the Break Points, Frequency and its Outcome

The observed chromosomes involved in rcpts are tabulated in table 1.

The chromosomes involved in the rcpt have been arranged as per the group to which they belong and also as per the ascending order ISCN (2005). The chromosomes belonging to A, B and C groups are found to be more commonly involved in rcpt.

Inference

1. The most commonly involved chromosomes that have entered into the rcpt configurations were: Chr. 1 (9 times), 2 (7 times), 5 and 22 (6 times), 3,7 and 9 (5 times each)
2. The chromosomal arms implicated in the exchanges showed that preferentially break points have occurred at: 1q,2p,5q,3q,7p, and 9p.

In literature, the preferentially involved chromosomes in bad obstetric history / infertility and in giving rise to karyotypically abnormal live births with mental retardation and or multiple congenital abnormalities are: 2p, 5q, 7p, 7q, 12q, 13q, 17q, 18p, 22q and 4,7,9,11,18,21,22 (de Braekeleer and Dao 1990).

In the present study, the chromosomes involved in rcpts in the parents having bad obstetric history / infertility, were 2,3,7 and 22 and in giving rise to genotypic and phenotypic abnormalities (mental retardation and or multiple congenital abnormalities) in live births were 1,3,5,9.

Determination of Parental Carrier Status

In the present study, the comparison between

Table 2: Parental origin-rcpt

<i>Maternal</i>	<i>Paternal</i>
1. 46,XY, t (1;7) mat	1. 46, XX, -9 + der (9) pat
2. 47,XX, t (1;21) mat	2. 46, XY, t (2;21) pat
3. 47,XX, t (1;21) mat	3. 46, XY, partial Trisomy 15(pat)
4. 46, XY, + der(8) t(3;8)mat	4. 46, XX (5p+)pat
5. 46, XX, t (4;12) mat	5. 46, XX (18p+)pat
6. 46, XY (5p+) partial Trisomy 7q (mat)	6. 46, XY (9p+)pat
7. 46, XX, t (7; 11) mat	
8. 46, XY, der (12) partial Trisomy 9p(mat)	
9. 46, XX, t (9; 15) mat	
10. 46, XX, t (10; 14) mat	
11. 46, XX, t (1; 15) mat, pat	
12. 46, XY, der 5, del 22 (mat, pat)	

the distribution of parental carrier status and the cases which were *de novo* were:

Parental status seen in 18 cases (31.03%)

De novo status seen in 40 cases (68.97%)

Out of the 18 cases, who showed the carrier status (the translocation heterozygote status), in 12 (66.6%) the mother was the carrier and in 6 (33.45%) paternal carrier status was determined. It may be noted that in 2 cases both maternal / paternal carrier status were observed.

Break down as per the parental origin and the carrier status are given in table 2.

The maternal carrier predominance has been shown in literature from 74.3 to 93.5%. The proposed reason for the striking difference between the maternal versus paternal carrier predominance in rcpts is proneness to the un-successful fertilization for the aneuploid sperm and / or the selection for the euploid sperm; thereby the likelihood of non-viability is increased (Davies et al. 1985).

Table 3: rcpt and clinical correlation

	<i>Clinical condition</i>	<i>Female</i>	<i>Male</i>
1. Bad Obstetric History	46, XX, t (1; 15) 46, XX, t (2; 18) 46, XX, t (5; 13)inv9 46, XX, t (7; 8) 46, XX, t (10; 14) 46, XX, t (15; 20) 46, XX, t (15; 22)	46, XX, t (2;11) 46, XX, t (4;12) 46, XX, t (6;8) 46, XX, t (7;11) 46, XX, t (11;16) 46, XX, t (15;22) 46, XX, t (20;22)	46, XY, t (1;3) 46, XY, t (1;14) 46, XY, t (2;4) 46, XY, t (2;13) 46, XY, t (3;11) 46, XY, t (4;6;15) 46, XY, t (4;21) 46, XY, t (5;6) 46, XY, t (7;13) 46, XY, t (13;17) 46, XY, t (19;20)
2. Fertility Failure	46, XX, t (X; 14)	46, X, t (X;22)	46, XY, t (1;7) 46, XY, t (3;12) 46, XY, t (Y;Y)
3. Multiple Congenital Abnormalities (MCA)	46, XX, t (2; 9) 46, XX, t (5p+) 46, XX, t (9; 15)	46, XX, -9 +der(9) 46, XX, (18p) 46, XX, t (15;17)	46, XY, t (1;21) 46, XY, t (2;21)+der21 46, XY, t (3;7) 46, XY, +der (8) t(3;8) 46,XY,partial trisomy 15 46, XY, (5p+) 46, XY, t (5;7;10;13) 46, XY, der 5, del 22 46, XY, der (12) 46, XY, t (18;20)
4. Mental retardation	46, XX, t (1; 2) 47, XX, t(1;21)	46, XX, t(1;3)	46, XY, t (1;2) 46, XY, t (7;13) 46, XY, (9p+) 46, X +21, t (Y;22)
5. Ambiguous genitalia	46, XY, t (8;11)	46, XY, t (10;11)	
6. Amenorrhoea	46, XX, t (9;14)	46, X, t (X;12)	
7. Miscellaneous	46, XY, t (9;22)		

Sex Ratio

A greater number of males (31 cases) as compared to females (27 cases) were affected as the result of the translocations. The sex ratio was 1.14: 1.

The increased sex ratio has been directly associated to the maternal origin. The chances of male viable pregnancy seemed to be associated to the maternal carrier status than the paternal carrier status (Bourrouillou et al. 1986)

Clinical Correlation of the Type of rcpts

Based on the clinical presentations of these cases, the following results were obtained and tabulated in table 3.

Bad Obstetric History	:	25 cases
MR/MCA	:	23 cases
Fertility failure	:	5 cases
Ambiguous genitalia	:	2 cases
Amenorrhoea	:	2 cases
Miscellaneous	:	1 case

The MR/MCA severity depends on the monosomic or trisomic segment sizes. For example, on an average, GTG prepared band has 50 genes. In trisomy and monosomy imbalances, as per the number of bands involved, the trisomic imbalances (2%) are reported to be greater than the monosomic imbalance (1%)

Figure 1 and 2 represent one example each for rcpt and maternal carrier status.

Counseling

At the time of counseling, in addition to the diagnosis / prognosis, the communication includes in explaining the meiotic mechanisms. Reciprocal translocations are the most common type of translocation and can occur between any of the chromosomes and involve segments of any size. Rcpts are nearly always spontaneous that is, they occur at the time of conception and neither parent has the chromosome change. However, in one parent having a balanced translocation (father or mother), it can be passed on to the offspring. Problems arise at meiosis, because the chromosomes involved in the translocation can not pair normally to form bivalents. Instead, they form a cluster known as a pachytene quadrivalent resulting in 2:2 segregation or a 3:1 segregation. When a person carries an apparently "balanced" reciprocal

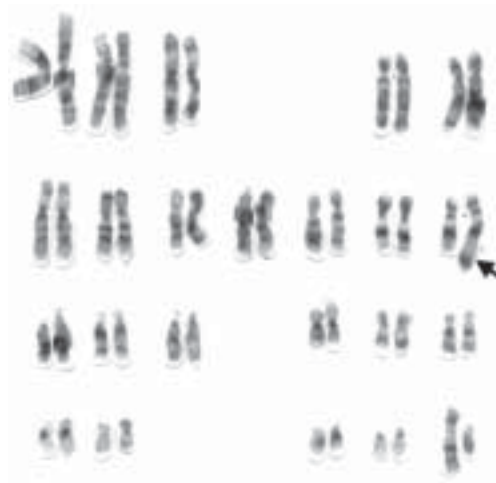


Fig. 1. 46, XY, der (12)(p11;q24)

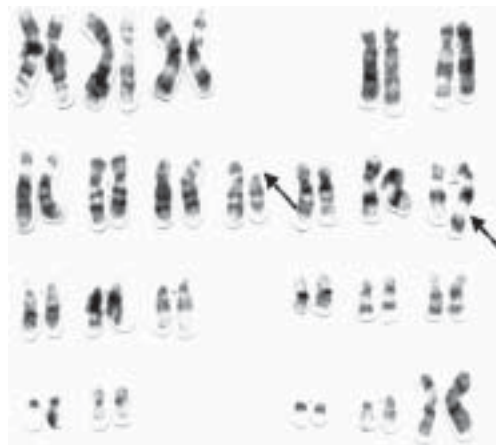


Fig. 2. 46, XX, rcpt (9; 12)(p11;q24)

translocation, even though they themselves may have no problems, there is an increased chance that there will be reproductive consequences. The nature of these consequences depends on the particular chromosomes involved and the size of the translocated material. Thus, the reproductive potential or outcome for these balanced carriers is uncertain. The imbalanced consequences of rcpts may be relatively minor or may be substantially inconsistent with fetal survival.

Hence, genetic counseling to each of these couples / individuals / families offers the possibility of predictive screening. The families are informed about determining the carrier status in the rest of the members and the utility of antenatal diagnosis is emphasized.

Recurrence risk: depending on the involvement of the certain chromosomal segments the recurrence risk of an abnormality can vary between 20 to 30%, irrespective of the parental origin Harper (1999).

CONCLUSION

1. The frequency of the rcpts, in this study, among the total number of chromosomal abnormalities (1350 cases) is 4.2% (n=58).
2. The chromosomes preferentially involved in bad obstetric history were: 2, 3, 7, 22 and in mental retardation with or without MCA were: 1, 3, 5, 9.

The maximum number of rcpts was among the autosomes (53 cases), including 2 cases of multiple autosomal translocations. The remaining 5 cases were of X – autosomal translocations.

3. The incidence of the most common chromosomal involvement in rcpts was: 1, 2, 3, 5, 7 and 9.
4. The maternal carrier predominance was determined (66.6%) and reflected the phenotypic effects arising from the predominance with the involvement of male offsprings being affected was more than the females.
5. The male to female sex ratio was found to be 1.14: 1.

SIGNIFICANCE

1. The present study has highlighted for the first time, to a large extent, a profile on rcpts in Indian population.
2. It has predicted the chromosomes that may

be vital for *in utero* survivability from the common chromosomes involved in rcpts and their common breakpoints.

3. The study has demonstrated that the chromosomal arms of the potential imbalanced segments involved in interchange, may determine the directly and or indirectly consequences of the phenotypic effects. Thus, the larger the segment involved in the translocation, the greater the MR/MCA in the probands.
4. The meiotic segregation mode (adjacent / alternate) of the imbalanced chromosomes could also be predicted, resulting in either monosomic, trisomic or carrier status in the probands.
5. A predominant transmission from maternal balanced carriers may result in a higher incidence of male offsprings with MR/MCA could be predicted.
6. The apparently balanced chromosomal translocation may be associated with a specific phenotype depending on the disrupted functional / coding genes and the altered positional effect of the coding as well as the adjacent genes, between the involved chromosomes.
7. The study has generated, clues to the possible location of human malformation genes, by correlating the chromosomal aberrations that give rise to specific phenotypes.

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APPENDIX - I

**Division of Human Genetics, Department of Anatomy, St. John's Medical College
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CONSENT FORM

We and
aged and years, residing at
..... hereby state that we have
been explained fully, by the staff of Division Human Genetics, Department of Anatomy, St. John's
medical college, Bangalore, the Probable side effects and after effects of withdrawing our / our child's
blood for cytogenetic tests.

We also agree to take our / child's photographs by the above department as part of documentation.

We also give consent that any further cytogenetic investigations may be done on the blood
taken, to help in the diagnosis.

We give our full consent to use our / our child's case details and photographs for research
purposes, scientific publications and presentations in scientific forums by the above department.

Name and Signature of the consultants / guardian:

1. Name:.....

Signature:.....

2. Name:.....

Signature:.....

Place:

Date: