Original Article

Pericentric inversion of chromosome 9[inv(9)(p12q13)]: Its association with genetic diseases

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BACKGROUND: The chromosomal polymorphism of short arms of acrocentric chromosomes and heterochromatin variation of Chromosomes 1, 9, 16 and Y have been reported in humans. The pericentric inversion of Chromosome 9 is commonly seen in normal humans and the frequency estimated to be 1 to 3% in general population and inherited in mendalian fashion or might occur spontaneously without any clinical significance.
AIM: The aim of the study was to study the frequency of inv(9) and its clinical correlation with human genetic diseases.
MATERIALS AND METHODS: The chromosomal analysis using GTG-banding was carried out in 3,392 cases suspected with genetic diseases.
RESULTS: The pericentric inversion frequency of different chromosomes in our study was 1.24% and frequency of inv(9)(p12q13) was high (64.29%) compared to other pericentric inversions in our study. A high frequency (9.33%) of inv(9)(p12q13) was detected in children with dysmorphic features and congenital anomalies.
CONCLUSION: As a high frequency of inv(9)(p12q13) detected in children with dysmorphic features, the inv(9) definitely have a role in the abnormal phenotype development. During inversion event there might be loss or suppression of euchromatin chromosome region and hence detailed chromosomal break point study is important to understand the clinical significance of the pericentric inversion of Chromosome 9.

Key words: Chromosome aberrations, dysmorphic features, genetic diseases, inv(9), pericentric inversions

Introduction

The frequency of human chromosome abnormalities (numerical, structural) reported to be 7.5% in general population.[1] Advancement in cytogenetic technology using fluorescence *in situ* hybridization (FISH), comparative genomic hybridization (CGH) and recently array-based CGH provided to detect submicroscopic rearrangements in various human diseases.[2] The chromosomal polymorphism of short arms of acrocentric chromosomes and heterochromatin variations of chromosomes 1, 9, 16 and Y also have been reported in humans.[3] The pericentric inversion of Chromosome 9 or inv(9) is commonly seen in normal humans and the frequency estimated to be 1 to 3% in general population.[4-7] The inv(9)(p12q13) also been reported in various human diseases such as couples with repeated spontaneous abortions, bad obstetric history, infertility and congenital anomalies.[8-10] In our study for the first time we have correlated inv(9)(p12q13) with various human disease conditions.

Materials and Methods

During a five year period (2000-2005), cytogenetic study was carried out in 3,392 patients suspected with genetic diseases. The patients clinical details, age, sex, consanguinity, income, living environment etc. were recorded in the pro forma. The pedigree analysis was done up to at least three generations.

Peripheral blood cultures were set up in F-10 nutrient media and with 20% fetal bovine serum. The cultures were stimulated with phytohaemagglutinin (PHA-M) and incubated for 72h at 37^0C. The cultures were arrested with colchicine (10 mg/ml) at 68^{th} h and treated with 0.075 M KCl. The cultures were fixed with cornoy fixative (methanol: Acetic acid, 3:1). The chromosomes were prepared on prechilled slides and stored for three days at room temperature for ageing of the slides. The chromosome preparations were subjected to GTG-banding using standard procedure. Briefly, the slides treated with trypsin-EDTA in Sorensen's buffer for 30 seconds and stained with

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giemsa stain. At least 30 well-spread and banded metaphases were analyzed under microscope and karyotyped according to ISCN 2000.

Results

The cytogenetic screening of 3,392 patients suspected with genetic diseases revealed 12.23% chromosome aberrations [Table 1]. The chromosome abnormalities were numerical (7.43%), structural (3.21%), inversions (1.24%) and deletions (0.35%). Among 42 pericentric inversions, inv(9)(p12q13) was detected in 27 (64.29%) patients [Table 2] [Figure 1]. The frequency of inv(9) in different human diseases is presented in Table 3. A high frequency (9.33%) of inv(9) was detected in children with dysmorphic features and congenital anomalies. Parental origin of inv(9)(p12q13) was detected in maternal (18.52%), paternal (18.52%) and *de novo* origin was 70% [Table 3].

Table 1: Frequency of chromosome abnormalities in patients suspected with genetic diseases

Type of aberration	No.	%
Numerical	252	(7.43)
Translocations	109	(3.21)
Deletions	12	(0.35)
Inversions	42	(1.24)
Normal karyotypes	2977	(87.77)
Total	3,392	(12.23)

Table 2: Pericentric inversions frequency in human diseases

Type of inversion	No.	%
inv(9) (p12q13)	27	64.29)
inv(Y) (p11q13)	10	(23.81)
inv(6) (p12q23)	2	(4.76)
inv(5) (p14q11)	1	(2.38)
inv(7) (p12p22)	1	(2.38)
inv(7) (p12q23)	1	(2.38)
Total	42	(100)

Figure 1: Partial karyogram showing inv(9)(p12q13)

Discussion

Chromosome abnormalities are responsible for at least half of spontaneous abortions or miscarriages and are an important cause of congenital malformations.[11-13] More than 0.5% of new born children are born with significant abnormalities of autosomes or sex chromosomes.[14] Among these, the most common and best known serious chromosomal disorders are trisomy 21 or the Down syndrome and the fragile X syndrome. The frequency of chromosome abnormalities in our study is similar to the frequencies reported in the literature.[1] Among the nonacrocentric human chromosomes, Chromosome 9 represents with the highest degree of morphological variations. The mechanisms of origin of inversions 9 are highly complex.[15] In our study the pericentric inversion of different chromosomes was 1.24% and the frequency of the inv(9), inv(Y) and others were 64.29%, 23.81%, 11.90% respectively [Table 2]. However inv(9) alone associated with different clinical conditions such as children with dysmorphic features and couples with repeated spontaneous abortions [Table 3]. The inv(9) is believed to be a frequent occurrence in

Table 3: Frequency and inheritance of inv(9) in genetic diseases

S.No.	Diagnosis	Inheritance							
		inv(9)		Maternal		Paternal		*Denovo*	
		no.	%	no.	%	no.	%	no	%
1 2	RSA/BOH (n=315)	12	3.81	2	16.67	1	8.33	9	75.0
3	DF/CA (n=150)	14	9.33	3	21.43	4	28.57	7	50.0
4	Infertility (n=68)	1	1.4	-	-	-	-	1 1.47	
	Total	27	100	5	18.52	5	18.52	17	62.96

Table 4: Clinical features in children with inv(9)(p12q13)

Age/sex	Clinical features
12 yrs/F	Dysmorphism, short stature, broad forehead, synophosis, depressed nasal bridge, down slanting eyes, low set small ears, malochonded teeth, delayed milestones.
13 yrs/F	Short stature, lack of secondary sexual characters, absence of ovaries, hypoplastic uterus.
11 yrs/M	Facial dysmorphism, 3rd and 4th toe short, hyperactive.
5 yrs/M	Dysmorphism, autism, hyperactivity.
14 yrs/M	Congenital cataract, ophthalmic bindings, blindness of left eye, deafness.
2 m/M	Dysmorphic features, low set ears, depressed nasal bridge, low pashire hair line, ASD, VSD.
13 yrs/F	Hydronephrosis, short stature, primary amenorrhea.
13 yrs/M	Short stature, down slanting eyes, squinty, abnormal palmar crease, hypertolerism, small hands and feet, delayed mile stones.
4 yrs/M	Microcepaly, short stature, prominent forehead, hypertolerism, down slanting eyes, beak shaped nose, high arched palate.
7 yrs/F	Microcephaly, cleft lip palate, depressed nose, low set ears, delayed milestones.
1 yr 6M/M	Delayed milestones with dysmorphic features, short toes
17 yrs/F	Short stature, delayed milestones, enlarged ears, obesity
3 m/M	Microcephaly, depressed nose, large ears, delayed milestones
8 m/F	Dysmorphic features, hypertolerisms, low set ears, delayed milestones.

the general population and inherited in a Mendelian fashion or might appear for the first time in a child without any apparent phenotypic consequences.[16] The inv(9) reported to be associated with RSA/BOH, infertility and congenital anomalies.[8-10]

In our study a high frequency of inv(9) was detected in children with dysmorphic features and developmental delay and about 70% were *de novo* origin [Table 3]. The correlation of inv(9) with clinical features of children with dysmorphic features revealed that most of the children had facial dysmorphism, abnormal phenotype and delayed milestones [Table 4]. This suggests that the unbalanced inversions at different break point regions might have a role in the abnormal phenotype development. In case of RSA/BOH majority (75%) of inv(9) were *de novo* origin and 25% were inherited from their parents. The cytogenetic analysis from abortus of patients with RSA was not done and such study may give some clue to understand inv(9) association in spontaneous abortions. Few studies have been carried out to study the pericentric incersion of Chromosome 9. The molecular characterization of 9qh revealed additional alphoid sequences at inverted region of the 9th Chromosome and suggested that structural organization of Chromosome 9 are apparently breakage prone and may be associated with a higher incidence of pericentric inversions.[16] Ramesh and Verma[17] have studied inv(9) break point regions using different FISH probes and showed that the break points are variable and can be localized in the alpha or in the satellite III and beta regions or both. However clinical consequences of these variable break point regions still not fully understood. In another study Starke *et al*[18] demonstrated 12 heteromorphic patterns of inv(9) using Chromosome 9 specific probes and suggested that pericentromeric heterochromatin of Chromosome 9 have several hotspots for recombination events. Starke *et al*[18] also demonstrated that constitutional inversions affecting the pericentromeric region of Chromosome 9 carry breakpoints located preferentially in 9p12 or 9q13-21.1 regions. Human centromeric and pericentromeric regions have shown to be highly plastic[19] in sharp contrast to the relative stability of the rest of the genome.[20] Recently it was also proposed that the positional change of centromeres and formation of neocentromeres i.e., ectopic centromeres (neocentromeres) at non-alphoid containing chromosomal sites.[21-24] Transposition of centromeric sequences into a distinct centromere has been documented in a prenatal diagnosis case.[25] As the inv(9) inherited from parents there is a need to study whether this positional change of centromere in the chromosome is due to pericentric inversion or existence of neocentromere through generations or evolution.

As high frequency of inv(9) was detected in various disease conditions in our study, these inversion definitely have role in the disease development especially in cases with *de novo* inversions. During breakage reunion process there may be chance of suppression or deletion of euchromatic sequences which might be causing abnormal development. Hence there is a need to study each breakpoint region of inv(9) using molecular

cytogenetic probes and molecular biology methods to understand the disease association. The parental chromosomal analysis is essential for appropriate genetic counseling.

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