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Analysis of the clinical features of pericentric inversion of chromosome 9

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Abstract

Objective: The pericentric inversion of chromosome 9 (inv9) is one of the most common structural balanced chromosomal variations, and it is considered to be a normal population variant. The aim of this study was to re-evaluate the clinical impact of patients with inv9.

Methods: We studied the karyotypes from 4853 patients at a single center and retrospectively reviewed their clinical data.

Results: There were 67 inv9 patients among 2988 adults, and 62 of them showed different clinical features, including male and female infertility, oligoasthenozoospermia, and azoospermia. Thirty-one cases of inv9 were found in 1865 fetuses, including two cases in chorionic villus (6.90%) and 29 in amniotic fluid (1.67%), but there were no cases in umbilical cord blood. The rates of fetal phenotype abnormal and adverse pregnancy outcome with inv9 in the chorionic villus were 100.00% (2/2), while only 17.24% (5/29) in the amniotic fluid showed abnormalities, among which 60.00% (3/5) had adverse pregnancy outcomes.

Conclusions: Although there is no clear evidence that inv9 is pathogenic, the genetic counseling on inv9 in early pregnancy and adults needs to be given more attention.

Keywords

Chromosome karyotype analysis, inversion of chromosome 9, fetal abnormality, pregnancy outcome, infertility, genetic counseling

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Introduction

Chromosomal variants of constitutive heterochromatin were usually reported in the human karyotype, especially chromosomes 1, 9, 16, and Y.[1] The pericentric inversion of the heterochromatin region of

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chromosome 9 (inv9), including inv9 (p11q13) or inv9(p12q13), is the most common pericentric inversion that is found in 1% to 3.57% of the general population.[2,3] The inv9 can be found in routine cytogenetic analysis, and it follows a Mendelian inheritance pattern of transmission with low mutation rates between generations.

The inversion of chromosome 9 used to be considered a normal variant with no clinical phenotypic effect.[2] However, some studies using classical cytogenetics have recently shown that inv9 could be associated with infertility,[4,5] recurrent miscarriages,[6] and idiopathic reproductive failure.[7] Various studies on inv9 have also showed an association with azoospermia, congenital anomalies, growth retardation, and, rarely, with an abnormal phenotype.[8,9] In this study we re-evaluated the clinical features of patients with inv9 and reviewed the literature.

Materials and methods

Clinical data

We retrospectively analyzed 2988 adults and 1865 fetuses who were referred to the cytogenetic department at the six affiliated hospitals of Guangzhou Medical University from April 2014 to May 2019. Venous blood was drawn from adults for chromosome karyotype analysis. Chorionic villus was cultured for fetal chromosome analysis in early pregnancy (9–13 weeks), while amniotic fluid was used in mid-pregnancy (16–24 weeks), and umbilical cord blood was used in late pregnancy ($\geq$24 week). The prenatal case series for karyotype analysis showed clinical indications such as multiple fetal abnormalities, increased Down syndrome risk, an abnormal single index for serum screening, and advanced maternal age (>35 years). All fetuses were followed-up at birth. The fetus phenotypes were also recorded at induction or at birth. All patients signed the informed consent form. Informed consent for the fetal participants was obtained from their parents. All procedures that were performed in studies involving human participants were approved on the basis of the ethical standards at the six affiliated hospital of Guangzhou Medical University and by the research committee (No. 20130430).

Patients who had lived with their wives for at least 1 year but were unable to conceive or produce an offspring were defined as having male infertility. The man whose wife had a bad obstetric history was defined as a bad spousal obstetric history. A male whose wife had a history of spontaneous abortion was defined as a spousal history of spontaneous abortion. In accordance with the seminal fluid analysis criteria of the WHO,[10] men whose seminal fluid had no sperm three times on testing were diagnosed as having azoospermia; those whose sperm concentration was lower and the ratio of sperm progressive motility was less than 32% were diagnosed as having oligoasthenozoospermia. The other group for men includes spousal biochemical pregnancy, abnormal fetal development, and *in vitro* fertilization (IVF) failure.

Primary infertility was defined as women who had not become pregnant after at least 1 year of having regular intercourse without using birth control methods.[11] Secondary infertility was defined as women who had been able to get pregnant at least once, but who were now unable to become pregnant.[11,12] A bad obstetric history meant that a woman had a previous poor pregnancy outcome in childbearing. Spontaneous abortion was defined as the natural death of an embryo or fetus before 20 weeks gestation.[13,14] The other group for women mainly referred to biochemical pregnancy, abnormal menstruation, IVF failure, and uterine hypoplasia. Adult patients with normal physical examination for

standardized karyotyping during this period served as a control group, including 98 men and 74 women.

Chromosome karyotype analysis

Chromosome karyotype analysis was detected using the G-banding technique. Peripheral blood and the samples of prenatal diagnosis were collected, and lymphocytes were cultured in RPMI-1640 medium with fetal bovine serum for 72 hours. Prenatal testing samples involved collecting chorionic villus, amniotic fluid, or umbilical cord blood that were cultured in RPMI-1640 medium for 10 to 14 days. Cells were harvested after colcemid treatment for 2.5 hours. More than 20 G-banded meta phase chromosomes were detected for each patient. Chromosomal disorders were described in accordance with the International System for Human Cytogenetic Nomenclature.[15]

Literature review

Relevant studies involving inv9 case series were collected from PubMed using the following terms: "inv9, pericentric inversion of chromosome 9 and variant on chromosome 9". Cases with inversion of 9p11-12 and 9q13-21.1 regions on chromosome 9 were enrolled, while other regional abnormalities of chromosome 9 or inv9 with other chromosomal abnormalities were excluded. Twelve studies were collected, and the reported phenotypes that were associated with inv9 were variable during the different human growth stages.

Statistical analysis

Data were analyzed using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). Differences between subgroups and the control group were analyzed using the Pearson Chi-square test or the Fisher's exact test. $P < 0.05$ was considered to be statistically significant.

Results

Sixty-seven inv9 cases were found among 2988 adult patients, with a total detection rate of 2.24%. The inv9 detection rate of male patients with clinical features was 2.26% (31/1373), while that of female patients was 2.15% (31/1443) and the control group was 2.91% (5/172). There were no significant differences in inv9 detection rates between clinical characteristics of the subgroups in men and women compared with the control group (Table 1). Among the 67 inv9 cases, only five patients did not show any clinical features, and the remaining 62 inv9 adults exhibited different clinical features, including male and female infertility, oligoasthenozoospermia, azoospermia, bad obstetric history, and spontaneous abortion (Table 1).

Fetuses with clinical indications of prenatal diagnosis underwent cytogenetics analysis. Among the 1865 prenatal cases, there were 321 patients (17.21%) who had chromosomal abnormalities. The common trisomy 21, 18, 13, sex chromosome aneuploides, and mosaicism were detected in 62 (3.32%), 22 (1.18%), eight (0.43%), 21 (1.13%), and 18 (0.97%) cases, respectively. Chromosome translocations, duplications/deletions, and variants were found in 13 (0.70%), 16 (0.86%), and 83 (4.45%) cases, respectively.

There were 31 cases of inv9 among 1865 fetuses, two of which were found in the chorionic villus (2/29, 6.90%), 29 were found in amniotic fluid (29/1736, 1.67%), and there were no inv9 cases found in umbilical cord blood samples. Two cases of inv9 (2/2, 100.00%) from chorionic villus exhibited an abnormal phenotype and adverse pregnancy outcomes, while only 17.24% (5/29) of inv9 in amniotic fluid had fetal abnormalities, among which 60.00% (3/5) had

Table 1. Frequencies of inversion of chromosome 9 (inv9) in different adult subgroups compared with control.

Clinical manifestations	No. inv9 (Rate)	*P* value
Control group	5/172 (2.91%)	
Men (groups)	Total: 31/1373 (2.26%)	N.S.
Male infertility	2/203 (0.99%)	N.S.
Bad obstetric history of spouses	12/425 (2.82%)	N.S.
Oligoasthenozoospermia	3/96 (3.13%)	N.S.
Azoospermia	3/117 (2.56%)	N.S.
Spouse history of spontaneous abortion	9/293 (3.07%)	N.S.
Others[a]	2/239 (0.84%)	N.S.
Women (groups)	Total: 31/1443 (2.15%)	N.S.
Primary infertility	11/392 (2.81%)	N.S.
Secondary infertility	0/40 (0)	N.S.
Bad obstetric history	7/288 (2.43%)	N.S.
Spontaneous abortion	5/349 (1.43%)	N.S.
Others[b]	8/374 (2.14%)	N.S.

Others[a] include spouse biochemical pregnancy, fetal development abnormal, and IVF failure.
Others[b] include biochemical pregnancy, abnormal menstruation, IVF failure, and uterine hypoplasia.
IVF, *in vitro* fertilization; N.S., not significant.

adverse pregnancy outcomes. The rest of the inv9 fetuses were normal at the follow-up visit after birth. The detailed clinical abnormal features of inv9 fetuses are shown in Table 2.

Discussion

Although pericentromeric inversion of chromosome 9 has been considered to be a heterochromatic variant, its biological and clinical significance remains controversial. In this study, we demonstrated the frequency rates of inv9 in different male and female subgroups. We also showed fetal inv9 prenatal clinical characteristics and pregnancy outcomes.

Human chromosome 9 is highly susceptible to structural chromosomal rearrangement and inv9 results from two chromosome breaks followed by reinsertion of the 180-degree rotated broken fragment. Various types of pericentric inversions 9 have been reported, but the breakpoints are preferentially located in the 9p12 or 9q13-21.1 regions.[16] The inv9(p11q13) and inv9(p12q13) cannot be distinguished on karyotyping, so "inv9" refers to these two karyotypes in this study.

Many studies have reported that inv9 was closely associated with recurrent spontaneous miscarriage, infertility, congenital anomalies, and idiopathic reproductive failure.[5,6,8,9,17] In our study, 92.54% of adults with inv9 exhibited clinical characteristics such as male and female infertility, spontaneous abortion, and bad obstetric history. In early pregnancy, 100% of fetuses with inv9 exhibited abnormal phenotypes and adverse pregnancy outcomes, while in the second trimester, the rates of abnormal phenotypes and adverse outcomes in fetuses with inv9 robustly decreased. In the third trimester, no inv9 in fetuses was detected. Whether this indicates that inv9 in fetuses had more severe phenotypes and adverse pregnancy outcomes in early pregnancy requires further confirmation.

The clinical features of growth stages in humans with inv9 were variable. The

Table 2. The detailed clinical abnormal characteristics of the fetuses with inversion of chromosome 9 (inv9).

No.	Parental age (years)	Parental clinical features	Fetus karyotypes	Gestational week	Fetus prenatal clinical features	Pregnancy outcome
1	F: 26 M: 24	Pet contact history in early pregnancy	46,XX,inv(9)(p12q13)	13	Omphalocele	13w+3 induced labor
2	F: 24 M: 22	Normal	46,XX,inv(9)(p12q13)	12	Brain deformity, abdominal fissure, right hooked hand	Induced labor
3	F: 26 M: 25	Normal	46,XY,inv(9)(p12q13)	17	NT thickening (3.6 mm)	38w+3 vaginal delivery; Fetus normal
4	F: 29 M: 23	Normal	46,XX,inv(9)(p12q13)	22	Brain deformity	20w induced labor; Abnormal brain development
5	F: 36 M: 33	Normal	46,XY,inv(9)(p12q13)	21	club foot	39w+2 cesarean delivery; club foot
6	F: 36 M: 31	Normal	46,XX,inv(9)(p12q13)	16	Increased cardiothoracic ratio	28w died in uterus
7	F: 28 M: 28	Normal	46,XY,inv(9)(p12q13)	23	Lateral ventricle widening	–

F, father; M, mother; w, week; –, no data; NT, nuchal translucency.

phenotypes that are associated with inv9 in prenatal,[18,19] newborn,[8,18,20] adolescent,[3,17,18,21] adult,[5,6,22] and elderly[23,24] stages are summarized in Table 3. There were no commonly shared clinical features within a certain period. Most of the clinical phenotypes that were associated with inv9 during the fetal period were anatomical malformations, which can be detected by prenatal ultrasound before delivery.[25,26] Some studies had reported that fetal malformations on inv9 had been observed in late pregnancy,[8,18] which suggests that the fetuses with inv9 require an ultrasound to detect these malformation in late pregnancy.

The main characteristics of adolescents with inv9 were developmental malformations or growth and mental retardation.[3,18,27] The pathogenesis that was associated with clinical manifestations and inv9 remains unclear. The effect of chromosomal variants may act upon or block the binding of certain transcription factors that are responsible for the transcription of certain genes or alter the regulation of genome-wide chromatin.[28] Indirect influences on expressed genes such as epigenetic down-regulation of gene expression or genetic imprinting are also associated with some human diseases and disorders.

Reproductive failure often occurs in inv9 adults.[5,6,17] Chromosomal pericentric inversion carriers usually have a normal appearance, but in gametogenesis that results from the pairing of homologous chromosomes, a unique inversion cycle will be formed during the first meiosis. The homologous chromosome exchange in the inversion circle will result in four gametes, one of which is normal, one of which is inverted, and the other two are partly duplicated or deleted. The formation of unbalanced gametes may cause miscarriage, infertility, and reproduction failure. In our study, inv9 was not found in 40 cases of female secondary infertility, which suggested that

Table 3. Clinical features with the inversion of chromosome 9 (inv9) variant from the literature that were analyzed in human different periods.

	Early pregnancy (9–13 weeks)	Mid-pregnancy (16–24 weeks)	Late pregnancy (≥24 weeks)	Birth	Adolescence	Adult	Older people
Wang et al. (2016); Keung et al. (2003)	–	–	–	–	–	Chronic myelogenous leukemia (Wang et al.); acute leukemia (Keung et al.)	
Sipek et al. (2015)	–	–	–	–	Idiopathic reproductive failure; congenital anomalies; mental retardation; impaired spermatogenesis		–
Sotoudeh et al. (2017)	–	–	–	Ambiguous genitalia	–	–	–
Demirhan et al. (2008)	Triphalangeal thumb, congenital anemia; mucopolysaccharidosis; mental retardation; development delay			Undeveloped right hand fingers; atypical face	Growth and mental retardation	Spontaneous abortion; bad obstetric history	–
Jeong et al. (2010)	–	–	Polydactyly; club foot; microtia; deafness; asymmetric face; giant Meckel's diverticulum; duodenal diaphragm; small bowel malrotation; pulmonary stenosis; cardiomyopathy; arrhythmia; intrauterine growth restriction		–	–	–
Elkarhat et al. (2019) Minocherhomji et al. (2009) Demirhan et al. (2003)	–	–	–	–	–	Recurrent spontaneous miscarriage (Elkarhat et al.); infertility (Minocherhomji et al.); schizophrenic (Demirhan et al.)	–
Malinverni et al. (2016) Caksen et al. (2019)	–	–	–	–	Hypertelorism; telecanthus; epicanthal folds; beaked nose; long philtrum; fifth finger clinidactyly; bilateral broad hallux and fifth toe clinodactyly; intellectual disability	–	–
Salihu et al. (2001)	Hydramnios; anhydramnios; hydroureter; hydronephrosis; encephalocele and prune belly syndrome			–	–	–	–
Our study	Omphalocele; brain deformity, abdominal fissure, right hooked hand	NT thickening; Joubert syndrome; club foot; cardiothoracic; lateral ventricle widening	–	Club foot	–	Male and female infertility; bad obstetric history; spontaneous abortion; oligoasthenozoospermia; azoospermia	–

–, no data; NT, nuchal translucency

abnormal gametes caused by inv9 may have caused adult reproductive failure.

Inv9 in adults and elderly people was closely related to carcinogenicity.[23,24] Multiple mechanisms were used to explain the relationship between inv9 and tumors. The breakpoints of the inversion 9 disruption of the gene or translocation from one genetic region to another may lead to neoplasia. The proximal short arm of chromosome 9 has been implicated in several malignant disorders.[29] An additional possibility was that inv9 may activate a neocentromere at 9q13 and result in cancer-related epigenetic events.[30]

The following limitations exist in this study: 1) This study only relied on limited karyotype analysis to explain the correlation between pericentric inversion of chromosome 9 and abnormal phenotype. With the application of microarray chip and next generation sequencing (NGS),[31] patients with an abnormal phenotype and inv9 should be further investigated using better detection resolution; and 2) The features of inv9 in fetuses that were *de novo* or inherited remain unknown. Excluding the pathogenic copy number variants and gene mutation, the fetus with *de novo* inv9 may be more able to explain the correlation between inv9 and phenotypes.

This study shows the frequencies of inv9 in different types of male and female subgroup and reveals the inv9 of fetuses abnormal phenotypes and adverse pregnancy outcomes in early pregnancy. The mechanisms of clinical features that are associated with inv9 need to be further clarified. However, genetic counseling on inv9 in early pregnancy and for adults requires additional attention.

Authors' contributions

Xiaolei Xie designed the study. Xiaolei Xie, Fuguang Li, and Weihe Tan collected the data and performed the data analysis. Weihe Tan performed the clinical diagnosis. Fuguang Li and Jiang Tang performed chromosome karyotype analysis. All authors read and approved the final manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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