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Association of Pericentric Inversion of Chromosome 9 and Infertility in Romanian Population

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ABSTRACT

Background: *One of the most common structural balanced chromosome rearrangements is pericentric inversion of chromosome 9; inv(9)(p11q12), which is consider to be the variant of normal karyotype and has been found in normal population. Although it seems not to correlate with abnormal phenotypes, there have been many controversial reports indicating that it may lead to abnormal clinical conditions such as infertility and recurrent abortions. The incidence is found to be about 1% - 3% in the general population. The aim of this study was to re-evaluate the clinical impact of inv(9)(p11q12)/(p11q13) in infertility.*

Materials and Methods: *We investigated the karyotypes of 900 infertile couples (1800 individuals) admitted in our hospital for cytogenetic analysis. The control group consists of 1116 fetuses investigated by amniocentesis. This group was considered to be a sample of the fertile population, as the fetus being karyotyped is the result of a spontaneous pregnancy. Fetal karyotyping was made according to the standard indications for prenatal diagnosis (abnormal maternal serum screening results). Chromosomes from cultured peripheral blood lymphocytes and amniotic fluid were analyzed using Giemsa Trypsin-Giemsa (GTG) banding. The results of the two groups were compared.*

Results: *1800 infertile people were submitted for cytogenetic investigation. In the control group 97.73% had normal karyotype and 2.27% showed inversion of chromosome 9, while in the studied group 96.24% had normal karyotype and 3.76% showed inversion of chromosome 9. The incidence of inversion 9 in both male and female patients is not significantly higher comparing with normal population ($p = 0.343$, $p< 0.05$).*

Conclusions: *Because a considerable proportion of patients with reproductive dysfunction had various cytogenetic abnormalities, the chromosomal analysis should be considered as a diagnostic tool in the evaluation of reproductive dysfunction (infertility in men due to spermatogenic disturbances and in recurrent spontaneous abortion in females).*

Keywords: infertility, inversion 9, subfertility

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INTRODUCTION

The study of human chromosomes plays a role in the diagnosis, prognosis, treatment and monitoring of fertility problems. Infertility is a condition of failure to conceive after 12 months of unprotected sexual intercourse without the use of birth control. It affects approximately 15% of couples in reproductive age (1,2). Infertility may be used synonymously with sterility with only sporadically occurring spontaneous pregnancies.

Subfertility generally describes any form of reduced fertility with prolonged time of unwanted non-conception. Subfertility can be primary or secondary:

Primary subfertility - a delay for a couple who have had no previous pregnancies;

Secondary subfertility - a delay for a couple who have conceived previously, although the pregnancy may not have been successful (for example, miscarriage, ectopic pregnancy).

Many factors can cause or contribute to reduced fertility.These concerns may be attributed to an issue with the woman, the man, the couple, or their lifestyle. Causes of infertility can be identified in about half of these cases, many causes are still unclear.

The major causes of subfertility can be grouped broadly as male factors (which include disorders of spermatogenesis or obstruction), ovulation disorders, tubal damage, unexplained, and other causes, such as endometriosis and fibroids. The proportion of each type of subfertility varies in different studies and in different populations. Tubal infertility is more common in those with secondary subfertility and in populations with a higher prevalence of sexually acquired infections. Less common types of male subfertility are caused by testicular or genital tract infection, disease, or abnormalities and rarely caused by endocrine deficiency. In half of the couples, causes are male-related, associated with impaired spermatogenesis (2,3).

Chromosomal aberrations, another cause of infertility, are found in 2-7% of couples with fertility problems (4). Carriers of balanced structural aberrations appear to have an increased risk of progeny with unbalanced karyotype resulting spontaneous abortion in first and second trimester.

Chromosome inversions are a relatively common structural alteration. The human chromosome 9 displays the highest degree of structural variability (4). Review of literature showed that inversions of chromosome 9 with different breakpoints could be the cause of different disturbances in carriers. The inverted region contains only the centromere and centromeric heterochromatin, so it seldom results in aberrant chromosomes after crossing-over. Pericentric inversion of chromosome 9, inv(9)(p11q12)/inv (9) (p11q13) is such a common occurrence that some cytogeneticists would consider them as normal variants, generally without phenotypic effect (5). The incidence is said to be about 1% to 3% in the general population (6). Despite the fact that it is classified as a minor chromosomal rearrangement which does not correlate with abnormal phenotypes, many reports in the literature raised conflicting views regarding the chromosomal association of this variant with abnormal clinical conditions such as infertility, recurrent pregnancy loss, deceased infants (7). It is not clear whether inv(9)(p11q12) is a normal variant or an abnormal karyotype (7). Nevertheless, this heterochromatic variant is sometimes associated with increased chromosomal instability, congenital abnormalities, and cancer (5).

Inv(9)(p11q13) has been regarded as a normal familial karyotype variant, although it has also been reported in various human diseases, such as couples with congenital malformation, habitual aborts, mild growth retardation, malformations of the skull and facial (craniofacial) region, undescended testis, skeletal malformations, mental retardation, hermaphroditism, and/or cardiac defects (8,9).

The mechanism(s) of inversion formation remains unclear. A simple inversion comprises a double break points fusion event involving just one chromosome. The interstitial segment is reinserted in a 180 degree orientation. If the centromere is included, the inversion is called pericentric, and if not, it is a paracentric inversion (3,10). Pericentric inversions can be seen at all of the chromosomes except "chromosome 20". Pericentric inversions of chromosome 9 [inv(9)] are the most common pericentric inversions in humans. ❑

MATERIALS AND METHODS

During the period January, 2008 to April, 2011, 1800 infertile patients were admitted at the Department of Reproductive Medi-

cine, Life Memorial Hospital, Bucharest Romania, and were cytogenetic investigated. Among these, 430 men had azoospermia, 76 had oligospermia and 600 women had spontaneous abortions. After cytogenetic analysis numerical and structural abnormalities were highlighted (Table 1). In this study we calculated the frequency of structural abnormalities, respectively the frequency of chromosomal inversion of chromosome 9, inv(9)(p11q12)/(p11q13) in reproductive failure.

The control group consists of 1116 fetuses investigated by amniocentesis between January, 2009 and April, 2011; this group was considered to be a sample of the fertile population (spontaneous pregnancy).

Cytogenetic analysis

Chromosomes from cultured peripheral blood lymphocytes and amniotic fluid were analyzed using Giemsa Trypsin-Giemsa (GTG) banding. The results of the two groups were compared using the two – tailed Fisher's exact test and calculated online at GraphPadSoftwa rewebsite(http://www.graphpad.com/quickcalcs/contingency1.cfm8).

5 mL peripheric blood samples was taken to heparinized injectors for karyotyping the case, with informed consent provided from all patients. Peripheral blood lymphocytes were stimulated and cultured for 72 h at 37°C, in PBMax and Chromosome B medium. Colchicine was added at 4 μg/ml to the cultures 2 h before harvesting. Slides were prepared after hypotonic treatment of the cells with KCl (0.075 M) followed by fixation in methanol/glacial acetic acid (3/1 vol/vol). A concentrated suspension of the cells was dropped on slides, which were dried on a slide warmer at 60°C for a few seconds and then stained with the G-banding technique. At least 15 metaphases were analyzed for each case, and 10 metaphases were karyotyped using light microscopy. The banding resolution was 400–550 bands per haploid set (BPHS). All the patients were evaluated by a skilled medical specialist and were eliminated other causes of infertility.

Our second group of patients were fetuses whose amniotic fluid samples were obtained during the same period (n = 1116). None of the pregnancies was obtained by assisted reproductive technologies (ART) and the reasons for referral were standard indications for amniocentesis such as abnormal serum screening levels, ultrasound changes or advanced maternal age. This group was considered to be a sample of the fertile population, as the fetus being karyotyped is the result of a spontaneous pregnancy.

Amniotic fluid samples were cultured in Amniomax complete medium and all cultures are incubated in a wet, 5% CO2 incubator at 37°C. Chromosomes were analyzed G-banded after harvesting (11). At least 15 metaphases were analyzed for each case and constitutional karyotypes were described in accordance with the ISCN 2009 (12,13). ❑

RESULTS

Among 900 couples (1800 cases) studied, inversion of chromosome 9 was found in 24 males (2.73%) and 17 females (1.92%). Only seven cases had the break point of p11q13. The age of referred females ranged from 21-38 years, with a mean of 27.81 years, while the age of the males ranged from 25-42 years, with a mean of 32.09 years. The incidence of inversion 9 in male patients is not significantly higher comparing with female patients and there was no significant correlation between age and pericentric inversion of chromosome 9. Structural chromosomal abnormalities such as inversion of chromosome 9 were present in high proportion of couples with infertility problems (Table 2, Figure 1).

As for the 1116 amniocentesis samples studied, we detected female karyotype in 573 and male karyotype in 543 fetuses. We obser-

Chromosomal abnormalities		Karyotype
Numerical abnormalities	Trisomy X Trisomy XXY Trisomy XYY Monosomy 45,X	47,XXX 47,XXY 47,XYY 45,X
Structural abnormalities	Inversion	46,XX,inv(9)(p11q13) 46,XX,inv(9)(p11q12) 46,XY,inv(9)(p11q13) 46,XY,inv(9)(p11q12) 46,XX,inv(3)(p11q11.2) 46,XY,inv(3)(p11q11.2) inv(9)(p11q13) 46,XX,inv(8)(p22q13) 46,XY,inv(1)(q23p13) 46,XX,inv(1)(q13p31) 46,XY,inv(10)(p11.2q21) 46,XX,inv(5)(pterq13)

TABLE 1. Numerical and structural abnormalities

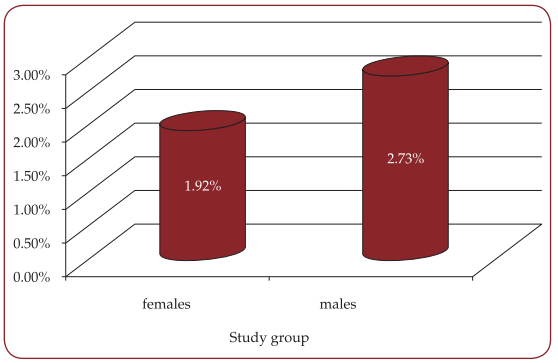

FIGURE 1. Comparison of frequency of inversion 9 in male and female infertility

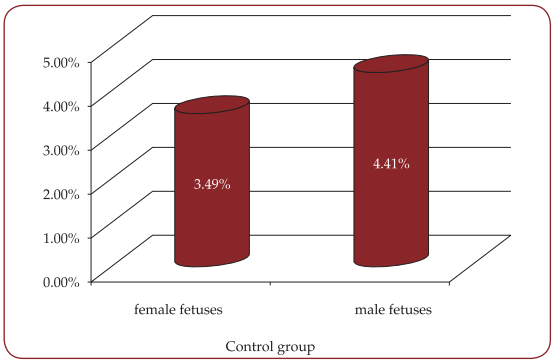

FIGURE 2. Comparison of frequency of inversion 9 in male and female fetuses

ved inversion of chromosome 9 in 20 female fetuses (3.49%) and 24 male fetuses (4.41%). The results of this second group are shown in Figure 2. ❑

DISCUSSION

A summary of reports for various abnormalities associated with pericentric inversion of chromosome 9(p11q12)/(p11q13) is shown in Table 3. In infertility cases, inversion 9 was observed in 2.27% which is similar to the earlier reports by Sasiadek et al, and Mozdarani et al, in the cases of recurrent miscarriage.

Most common clinical diagnosis among patients who presented inversion of chromosome 9 were recurrent miscarriage and azoospermia (common cause of male infertility). Comparing the incidences of pericentric inversion of chromosome 9 in male and female infertility we determined the relative risk of infertility as two-tailed p value equals 0.343 ($p < 0.05$) (Fisher's exact test). As this result was not statistically significant we haven't proved any strong association of inv(9) with specific clinical diagnosis and the clinical significance of inv(9) still remains uncertain. Inversion of chromosome nine could be acknowledged as a reason of fertility problems in these investigated couples. Rule out of other reasons for infertility or abortion in them make these possibility stronger.

Sex	Mean age	Total	Normal	inv(9)carriers	Mean frequency
Female	27.81	900	883	13(p11q12) 4(p11q13)	0.014
Male	32.09	900	876	21(p11q12) 3(p11q13)	0.023

TABLE 2. Frequency of inversion 9 in female and male infertility cases

Karyotype	Disturbance	Reference
Inv(9)(p11q12)	Infertility/recurrent abortion	Sasiadek et al, 1997(14)
Inv(9)	Male infertility	Sasagawa et al, 1998(15)
Inv(9)(p11q13)	Asperger syndrome	Pia Verri and Cimbro, 2002(16)
Inv(9)(p11q13)	Goldenhar syndrome or oculo-auriculo-vertebral spectrum	Stanojevic et al, 2000(17)
Inv(9)	Immotile/ultrastructural sperm defect	Baccetti et al, 1997(18)
Inv(9)(p11q13)	Schizophrenia-like psychosis	Miyaoka et al, 1999(19)
Inv(9)	Recurrent spontaneous first trimester abortions	Parmar and Sira, 2003(20)
Inv(9)(p11q12)	Infertility/recurrent abortion	Mustaqahamed et al, 2011(2)
Inv(9)(p11q12)	Male infertility/recurrent abortion	Mozdarani et al, 2007(21)
Inv(9)(p11q12)	Infertility/recurrent abortion	The present study

TABLE 3. Associations between the different disturbances and inversion 9 with (p11q12) or (p11q13) breakpoints as well as infertility

CONCLUSION

The results of this study support the clinical reports that inversion of chromosome 9 appear to have no phenotypic or clinical adverse effects, or any apparent association with infertility problems.

Therefore, cytogenetic tests are recommended for patients with a history of abortion, especially in olderly fertile women to identify chromosomal alterations. This study strongly point out the importance of karyotyping in couples' evaluation that need assisted reproductive technologies for genetic counseling.

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