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The analysis of chromosomal abnormalities in patients with recurrent pregnancy loss, focusing on the prognosis of patients with inversion of chromosome (9)

Taro Nonaka[1] | Makiko Takahashi[1] | Chika Nonaka[1] | Takayuki Enomoto[1] | Koichi Takakuwa[2]

[1]Department of Obstetrics and Gynecology, Niigata University Medical and Dental Hospital, Niigata, Japan

[2]Center for Perinatal, Maternal and Neonatal Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan

Correspondence
Koichi Takakuwa, General Center for Perinatal, Maternal and Neonatal Medicine, Niigata University Medical and Dental Hospital, Niigata City, Japan.
Email: obgy@med.niigata-u.ac.jp

Abstract

Purpose: Inversion of chromosome 9 (inv[9]) is considered to be a normal variant, and the inv(9) in patients or husbands with recurrent pregnancy loss (RPL) is believed to be harmless. However, there are few reports concerning the outcomes of pregnancy in patients with RPL when the patient or their partner has inv(9). In this study, we analyzed the outcomes of pregnancy in this patient population.

Methods: Chromosomal karyotyping was performed for 2006 couples with RPL (two or more consecutive early pregnancy losses including non-visualized cases) with their informed consent. The frequency of various chromosomal abnormalities in the patient population was then analyzed, and the outcomes of pregnancy in patients with inv(9) were investigated.

Results: The frequency of inv(9) in the overall population was 2.6% (52/2006). Thus far, 32 patients have conceived repetitively, resulting in live births in 23 cases and early pregnancy losses in nine cases. Four of the nine cases obtained a good outcome in the subsequent pregnancy. Thus, a successful outcome was obtained in 27 of the 32 (84.4%) cases.

Conclusions: This study suggests that inv(9) has no adverse influence on subsequent pregnancy.

KEYWORDS

chromosomal abnormality, inversion of chromosome 9, normal variant, perinatal prognosis, recurrent pregnancy loss

1 | INTRODUCTION

Recurrent pregnancy loss (RPL), which is typically defined as 2 or more consecutive early pregnancy losses, occurs in up to 5% of reproductively active couples.[1] There are several risk factors for RPL, including chromosomal anomalies, Mullerian anomaly, hormone deficiency, metabolic disorder, infectious diseases, and autoimmune abnormalities, represented by positive antiphospholipid antibodies. Although there is a little evidence of relevance to RPL among these, chromosomal anomalies, which can affect the woman or her partner, are one of the most relevant risk factors for RPL.

The frequency of chromosomal anomalies among couples with RPL is estimated to be 2%-8%,[2-6] with the most common being balanced translocations between two chromosomes in one individual.

It has been reported that this anomaly occurs in approximately 5% of couples with RPL,[7-9] and couples in which one partner carries this anomaly are at increased risk of infertility, RPL, and the delivery of chromosomally abnormal offspring because erroneous chromosome distribution might occur in meiosis.

The balanced pericentric inversion of chromosome 9 (inv[9]) is the most common inversion in the human karyotype and occurs in 1%-3% of the normal population.[10-14] It is considered to be a normal variant and has been reported to be a harmless anomaly in couples with RPL.[14] However, there are some reports concerning the frequency in couples with RPL, in which a patient or a husband possesses the inv(9), and it is reported that the frequency of inv(9) is increased among couples with RPL.[11,13,15] Furthermore, there are few reports regarding the outcomes of subsequent pregnancy in these couples.

Simple inversion consists of a double break point fusion event involving just 1 chromosome where the interstitial segment is reinserted in a 180° orientation. In cases of chromosomal inversion including inv(9), it is considered that circularized configuration between normal and inverted chromosomes is formed at the pachytene stage of meiosis I. Subsequently duplicated or deficient recombinant chromosomes are made, causing various unbalanced karyotypes to emerge in the gametes (oocytes or sperms). The increase in the abnormal karyotype in the fertilized eggs is followed by RPL.

For this reason, we analyzed the incidence of various chromosomal abnormalities in 2006 couples with RPL over a 27- year period, as well as the frequency and outcomes of pregnancy among those with inv(9).

2 | MATERIALS AND METHODS

From January 1990 to December 2016, 2337 couples with RPL who had experienced 2 or more consecutive early pregnancy losses, including non-visualized cases, were referred to our hospital. Among those couples, 2006 couples (2006/2337 = 85.8%) underwent chromosome analyses; the 331 couples who did not undergo these analyses did so of their own volition. Routine examinations for RPL, such as congenital uterine anomaly, luteal disfunction, hormonal deficiency, metabolic disorder, infection disease, autoimmune disorder (antinuclear antibody, rheumatoid factor and antiphospholipid antibody), and abnormal blood coagulation, were performed for these couples. The patients were diagnosed with luteal disfunction if the value of progesterone was <10 ng/mL in their luteal phase. With regard to antiphospholipid antibody, lupus anticoagulant (LAC), anti-cardiolipin IgG antibody (aCL-IgG), and anti-cardiolipin beta2 glycoprotein I antibody (aCL-β2-GPI) were examined. LAC was estimated by the diluted Russell viper venom test (dRVVT) provided by Gradipore, LTD., and the cutoff value was 1.3. The aCL-IgG was estimated by the MESACup™ cardiolipin test provided by Medical and Biological Laboratories Company, LTD., and the cutoff value was 10 units/mL. The aCL-β2-GPI was estimated using the Yamasa EIA kit provided by Yamasa Company, LTD., and the cutoff value was 3.5 units/mL. Regarding abnormal blood coagulation, protein S, protein C, and coagulation factor XII activity were examined, with cutoff values of 60% for protein S, 82% for protein C, and 50% for factor XII. The chromosome analysis was performed after obtaining informed consent from all individuals. Chromosome preparations were obtained from peripheral blood lymphocyte cultures, the staining of which included G-banding, according to a previously reported method.[16,17]

First, the frequency of various chromosomal abnormalities in the couples was analyzed retrospectively. Second, the number of couples in which one partner carried inv(9) was analyzed, and the pregnancy outcomes were investigated in the cases that were able to achieve a term pregnancy. In cases in which early pregnancy loss occurred again, villi samples were subjected to a chromosomal analysis

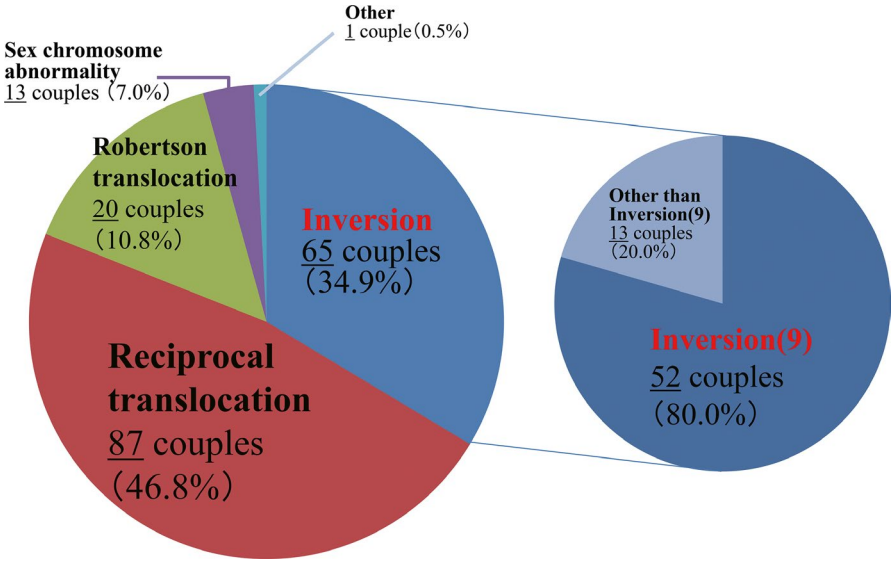

FIGURE 1 Chromosomal abnormalities were detected in 186 couples with RPL. Among these 186 couples, inversions were observed in 65 couples (65/186 = 34.9%). Among these 65 couples with inversion, inv(9) was detected in 52 couples (52/65 = 80.0%)

to clarify the cause of pregnancy loss. The outcome of the pregnancy was also analyzed. Finally, we revealed the success rate of pregnancy in the patient population with inv(9) and compared it to that in the patients with RPL but without inv(9).

The protocol of our study was approved by the Institutional Medical Ethical Review Committee of Niigata University School of Medicine. The management of the patients was in accordance with the provisions of the Declaration of Helsinki. Written informed consent was obtained from the patients and their husbands for the publication of these features of their cases. Their anonymity has been completely preserved.

3 | RESULTS

Chromosomal abnormalities were detected in 186 of 2006 (9.3%) couples (Figure 1). Balanced translocation was observed in 107 of the 186 (57.5%) couples. Among these 107 couples, reciprocal translocation was observed in 87 couples, and Robertsonian translocation was observed in 20 couples. Inversion was observed in 65 of the 186 (34.9%) couples. Among these 65 inversion couples, inv(9) was detected in 52 couples (52/65 = 80.0%). Thus, the frequency of inv(9) in the whole population was 2.6% (52/2006). Among the 52 couples with inv(9), inversion was found in the female partner in 28 cases and the male partner in the remaining 24 cases. Among these 65 inversion couples, other inversions were also observed in 13 couples (13/65 = 20.0%). Among these 13 couples, the detected inversions of chromosome in patients or husbands were all balanced pericentric or paracentric, and they were observed on chromosomes 1, 3, 4, 5, 6, and 11. No patients in the present study had overlapping chromosome abnormalities.

Other risk factors for RPL and their frequency were detected in some of the 52 couples with inv(9) (Table 1). Data on the patient couples without inv(9) are also presented in the table. As treatments for such risk factors, low-dose aspirin (LDA; 81 mg/d) was administered as anti-coagulation therapy to patients who were positive for antinuclear antibody (ANA) or rheumatoid factor (RF). Moreover, to treat patients who were positive for antiphospholipid antibody (APA), we administered the Japanese traditional herbal medicine Sairei-to (Tsumura; 9 g/d), which may possess similar pharmacologic effects to adrenal corticosteroid hormone as immunosuppressive therapy in combination with LDA according to our previously reported protocol.[18-20] Immunotherapy with paternal lymphocytes was administered to patients who were diagnosed with an allogeneic immune disorder. These patients were negative for blocking antibodies as evaluated by a mixed lymphocyte culture reaction between spouses (MLR-Babs). Informed consent was obtained from all of the patients before the immunotherapy, and the protocol for the immunotherapy was approved by the Institutional Review Board of Niigata University School of Medicine. Before the immunotherapy, the husbands were confirmed to be negative for syphilis, hepatitis viruses, HIV and HTLV-1. Each husband's lymphocytes were obtained from

TABLE 1 Comparison of other risk factors

	Luteal dysfunction	ANA[a] or RF[b]	APA[c]	Abnormal blood coagulation	Muellerian anomaly	Allogeneic immune disorder	Balanced translocation	None	Total
Inversion (9) Female	1 (3.6%)	14 (50.0%)	4 (14.3%)	3 (10.7%)	1 (3.6%)	1 (3.6%)	0 (0%)	9 (32.1%)	28
Inversion (9) Male	1 (4.2%)	7 (29.2%)	7 (29.2%)	3 (12.5%)	0 (0%)	1 (4.2%)	0 (0%)	6 (25.0%)	24
couples with RPL[d] without Inversion (9)	111 (5.7%)	728 (37.3%)	206 (10.5%)	95 (4.9%)	35 (1.8%)	116 (5.9%)	107 (5.5%)	735 (37.6%)	1954

[a]Antinuclear antibody.
[b]Rheumatoid factor.
[c]Antiphospholipid antibody.
[d]Recurrent pregnancy loss.

heparinized blood irradiated with 30 Gy of X-rays in order to prevent any graft-versus-host disease (GVH) reaction.

Thus far, of the 52 couples, 32 patients conceived repetitively, which resulted in a good outcome (live birth) on the next pregnancy in 23 cases and early pregnancy loss in nine cases (Tables 2 and 3). A chromosomal analysis was performed in 7 of these nine cases. Although the remaining two cases were scheduled to undergo an operation for miscarriage and have a chromosomal analysis performed, they underwent natural loss of the products of conception before that point. There was thus no information available regarding the

TABLE 2 Prognosis of pregnancy female partners showed inversion (9)

Case	Chromosome (Female)	Chromosome (Male)	Positive	Treatment	Pregnancy loss	Successful pregnancy
1	46XX, inv(9)	46XY	Luteal dysfunction Allogeneic immune disorder	Luteal support Immunotherapy[a]	2	0
2	46XX, inv(9)(p12q13)	46XY	ANA	LDA[b]	0	1
3	46XX, inv(9)	46XY	ANA, APA	Sairei-to[c] + LDA	0	2
4	46XX, inv(9)	46XY	None	No medication	0	2
5	46XX, inv(9)(p12q13)	46XY	APA	Sairei-to + LDA	1	1
6	46XX, inv(9)(p + q-)	46XY	ANA	Sairei-to + LDA	0	1
7	46XX, inv(9)	46XY	ANA, RF, APA	Sairei-to + LDA	0	1
8	46XX, inv(9)	46XY	ANA	LDA	0	1
9	46XX, inv(9)	46XY	ANA	LDA	0	1
10	46XX, inv(9)(p13q13)	46XY	ANA	No medication	0	1
11	46XX, inv(9)(p12q13)	46XY	None	No medication	0	1
12	46XX, inv(9)(p12q13)	46XY	None	No medication	1	0
13	46XX, inv(9)(p12q13)	46XY	ANA, RF	LDA	0	1
14	46XX, inv(9)(p12q13)	46XY	None	No medication	1	0

[a]Injection of paternal lymphocytes.
[b]Low-dose aspirin (LDA; 81 mg/d).
[c]Japanese traditional herbal medicine (Tsumura; 9 g/d).

TABLE 3 Prognosis of pregnancy male partners showed inversion (9)

Case	Chromosome (Female)	Chromosome (male)	Positive	Treatment	Pregnancy loss	Successful pregnancy
1	46XX	46XY, inv(9)	APA	Sairei-to + LDA	2	1
2	46XX	46XY, inv(9)	Low Protein S	Sairei-to + LDA	0	2
3	46XX	46XY, inv(9)(p12q13)	APA	Sairei-to + LDA	0	1
4	46XX	46XY, inv(9)	Luteal dysfunction	Luteal support	0	1
5	46XX	46XY, inv(9)	None	No medication	2	0
6	46XX	46XY, inv(9)	APA	Sairei-to + LDA	1	1
7	46XX	46XY, inv(9)(p12q13)	APA	Sairei-to + LDA	1	0
8	46XX	46XY, inv(9)(q22q34)	APA	Sairei-to + LDA	1	1
9	46XX	46XY, inv(9)	ANA	LDA	1	1
10	46XX	46XY, inv(9)	None	No medication	0	1
11	46XX	46XY, inv(9)(p + q-)	Allogeneic immune disorder	Immunotherapy	0	2
12	46XX	46XY, inv(9)	None	No medication	0	2
13	46XX	46XY, inv(9)	ANA	No medication	0	1
14	46XX	46XY, inv(9)	None	No medication	0	1
15	46XX	46XY, inv(9)(p12q13)	ANA, APA	Sairei-to + LDA	1	1
16	46XX	46XY, inv(9)(p12q13)	APA	Sairei-to + LDA	0	1
17	46XX	46XY, inv(9)(p12q13)	APA	Sairei-to + LDA	0	1
18	46XX	46XY, inv(9)(p12q13)	ANA Allogeneic immune disorder	LDA Immunotherapy	0	1

karyotype of those two pregnancy losses. The results were as follows: normal karyotype (n = 2) and autosomal trisomies (n = 4), inv(9) (n = 1). Four of the 9 cases subsequently obtained a good pregnancy outcome. Thus, 27 of the 32 (84.4%) patients ultimately obtained a successful outcome.

4 | DISCUSSION

Inv(9), which occurs in about 1%-3% of the normal population, is considered to be one of the most common chromosomal anomalies.[10-14] A simple inversion consists of a double break point fusion event involving just one chromosome. The interstitial segment is reinserted in a 180 degree orientation. Although the inv(9) in couples with RPL is considered to be a normal and harmless variant, there are few reports concerning the outcomes of subsequent pregnancies in couples with RPL when a patient or their partner possesses inv(9). In this study, we analyzed the incidence of inv(9) in 2006 couples with RPL who were managed at a single facility over a period of almost 30 years. Inv(9) was detected in 2.6% of the 2006 couples. Thus, the frequency of inv(9) in the study population (couples with RPL) was not significantly different from that in the normal population. Moreover, it was revealed that inv(9) was the second most frequent chromosomal anomaly (after reciprocal translocation) among couples with RPL.

The frequency of inv(9) between females and males was compared. The frequency did not differ to a statistically significant extent (1.40% vs 1.20%, $P = 0.175$ by the chi-square test). Dana et al reported that the frequency of inv(9) in males and females in the infertile Romanian population did not differ to a statistically significant extent.[14] Thus, it is suggested that the frequency of inv(9) does not differ between males and females in the infertile population.

Theoretically, it is considered that circularized configuration is formed at the pachytene stage of meiosis in cases of chromosomal inversion, and that various unbalanced karyotypes would emerge in the gametes (oocytes or sperms). The increase in the abnormal karyotype in the fertilized eggs is followed by RPL. However, in this series, the karyotyping of the villi in cases of pregnancy losses did not reveal chromosomal abnormalities originating from the abovementioned circularized configuration at the pachytene stage of meiosis.

Moreover, in 27 of the 32 (84.4%) inv(9) cases, the patients ultimately obtained a successful outcome. The empirical data, as well as theoretical data, are very important for genetic consultation on chromosomal abnormalities in individuals with RPL. Thus, the data obtained from this analysis may have a clinical benefit. On the other hand, 89.5% of Japanese couples with a history of RPL have been reported to obtain good outcome on subsequent pregnancy,[21] and the pregnancy success rate of the population with inv(9) was not significantly different from that of the population with RPL without inv(9).

In conclusion, in cases in which inv(9) is detected in one partner among couples with recurrent pregnancy loss, the couple could be advised that this chromosomal anomaly has no adverse influence on subsequent pregnancies.

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DISCLOSURES

Authors: All research was performed by the authors. *Conflict of interest*: The authors declare no conflicts of interest in association with the present study. *Human and Animal Rights*: All of the procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later revisions. This study was approved by the medical ethics committees of Niigata University Medical and Dental Hospital (Approval number: 2018-****). Informed consent was obtained from all patients.

ORCID

Taro Nonaka https://orcid.org/0000-0001-8905-1848

REFERENCES

1. Branch DW, Gibson M, Silver RM. Clinical Practice: Recurrent Miscarriage. *N Engl J Med*. 2010;363:1740-1747.
2. Fryns JP, Van Buggenhout G. Structural chromosome rearrangements in couples with recurrent fetal wastage. *Eur J Obstet Gynecol Reprod Biol*. 1998;81:71-76.
3. Elghezal H, Hidar S, Mougou S, Khairi H, Saâd A. Prevalence of chromosomal abnormalities in couples with recurrent miscarriage. *Fertil Steril*. 2007;88:721-723.
4. Meza-Espinoza JP, Anguiano LO, Rivera H. Chromosomal abnormalities in couples with reproductive disorders. *Gynecol Obstet Invest*. 2008;66:237-240.
5. Dutta UR, Rajitha P, Pidugu VK, Dalal AB. Cytogenetic abnormalities in 1162 couples with recurrent miscarriages in southern region of India: Report and review. *J Assist Reprod Genet*. 2011;28:145-149.
6. Frenny JS, Thomas L, Pritti K, Ralph A, Harsh JS, Jayesh JS. Chromosomal abnormalities in couples with repeated fetal loss: An Indian retrospective study. *Indian J Hum Genet*. 2013;19:415-422.
7. Ostovics MK, Toth SP, Wessely JA. Cytogenetic investigation in 418 couples with recurrent fetal wastage. *Ann Genet*. 1982;25:232-236.
8. Fryns JP, Kleczkowska A, Kubien E, Petit P, Van den Berghe H. Cytogenetic survey in couples with recurrent fetal wastage. *Hum Genet*. 1984;65:336-354.
9. Portnoi MF, Joye N, van den Akker J, Morlier G, Taillemite JL. Karyotypes of 1142 couples with recurrent abortion. *Obstet Gynecol*. 1988;72:31-34.
10. Serra A, Brahe C, Millington-Ward A, et al. Pericentric inversion of chromosome 9: prevalence in 300 Down syndrome families and molecular studies of nondisjunction. *Am J Med Genet*. 1990;7:162-168.
11. Yamada K. Population studies of inv(9) chromosomes in 4300 Japanese: incidence, sex difference and clinical significance. *Jpn J Hum Genet*. 1992;37:293-301.
12. Teo SH, Tan M, Knight L, Yeo SH, Ng I. Pericentric inversion 9 incidence and clinical significance. *Ann Acad Med Singapore*. 1995;24:302-304.

13. Demirhan O, Pazarbasi A, Suleymanova-Karahan D, Tanriverdi N, Kilinc Y. Correlation of clinical phenotype with pericentric inversion of chromosome 9 and genetic counseling. *Saudi Med J*. 2008;29:946-951.
14. Dana M, Stoian V. Association of pericentric inversion of chromosome 9 and infertility in Romanian population. *J Clin Med*. 2012;7:25-29.
15. Makino T, Tabuchi T, Nakada K, Iwasaki K, Tamura S, Iizuka R. Chromosomal analysis in Japanese couples with repeated spontaneous abortions. *Int S Fertil*. 1990;35:266-270.
16. Nonaka T, Ooki I, Enomoto T, Takakuwa K. Two cases of recurrent abortion in which isodicentric chromosome 15 was observed in the husbands. *J Obstet Gynaecol Res*. 2014;40:1795-1798.
17. Nonaka T, Ooki I, Enomoto T, Takakuwa K. Complex chromosomal rearrangements in couples affected by recurrent spontaneous abortion. *Int J Gynaecol Obstet*. 2015;128:36-39.
18. Takakuwa K, Yasuda M, Hataya I, et al. Treatment for patients with recurrent abortion with positive antiphospholipid antibodies using traditional Chinese herbal medicine. *J Perinat Med*. 1996;24:489-494.
19. Takakuwa K, Ishii K, Takaki Y, et al. Effect of sairei-to combined with aspirin and prednisolone on four recurrent reproductive failure women who are positive for anti-phospholipid antibodies. *Am J Chin Med*. 2003;31:659-663.
20. Takakuwa K, Ooki I, Nonaka T, et al. Prophylactic therapy for patients with reproductive failure who were positive for anti-phospholipid antibodies. *Am J Reprod Immunol*. 2006;56:237-242.
21. Sugiura-Ogasawara M, Suzuki S, Ozaki Y, Katano K, Suzumori N, Kitaori T. Frequency of recurrent spontaneous abortion and its influence on further marital relationship and illness: The Okazaki Cohort Study in Japan. *J Obstet Gynaecol Res*. 2013;39:126-131.

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