

# Abnormal embryonic karyotype is the most frequent cause of recurrent miscarriage

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**BACKGROUND:** We previously found that a normal karyotype in a previous miscarriage is a predictor of subsequent miscarriage. However, the prevalence of recurrent miscarriage caused by an abnormal embryonic karyotype has not yet been reported, since embryonic karyotype is not typically analyzed during conventional examinations.

**METHODS:** A total of 482 patients who underwent both embryonic karyotype determination and conventional examinations for recurrent miscarriage were enrolled in this study. The distribution of the causes and the live birth rate for each cause were examined.

**RESULTS:** The total percentage of subjects in whom conventional causes of recurrent miscarriage could be detected was 29.5%. The prevalence of the abnormal embryonic karyotype was 41.1% in the subjects in whom no conventional causes of miscarriage could be identified. The prevalence of recurrent miscarriage of truly unexplained cause, that is, of subjects without conventional causes in whom the embryonic karyotype was ascertained to be normal, was 24.5%. Among the patients in whom the first determination revealed an abnormal embryonic karyotype, 76.2% (32/42) showed an abnormal embryonic karyotype in the repeat determination as well. The cumulative live birth rate (71.9%) in women with recurrent miscarriages caused by the abnormal embryonic karyotype was significantly higher than that (44.7%) in women with recurrent miscarriages associated with the embryonal euploidy.

**CONCLUSION:** An abnormal embryonic karyotype was found to represent the commonest cause of recurrent miscarriage, and the percentage of cases with recurrent miscarriage of truly unexplained cause was limited to 24.5%. The two groups should be distinguished for both clinical and research purposes.

**Key words:** embryonic karyotype / live birth rate / prevalence / recurrent miscarriage

## Introduction

Established causes of recurrent miscarriage include the presence of antiphospholipid antibodies (aPLs), uterine anomalies and abnormal chromosomes, particularly translocations, in either partner, (Farquharson *et al.*, 1984; Sugiura-Ogasawara *et al.*, 2004; Sugiura-Ogasawara *et al.*, 2010). According to previous reports, in about half of the cases seen at research centers, the cause of recurrent miscarriage remains unexplained despite conventional examinations conducted to identify the cause (Clifford *et al.*, 1994; Stephenson, 1996).

A majority of miscarriages that occur before 10 weeks of gestation are due to chromosomal aneuploidies arising from new non-disjunctive events, such events being more frequent in very early

miscarriages (Sierra and Stephenson, 2006). We found that the abnormal embryonic karyotype rate was as high as 51% in subjects with recurrent miscarriages, even though it was significantly lower than that of 76.3% in patients with sporadic miscarriages (Ogasawara *et al.*, 2000). Many reports have suggested that the abnormal embryonic karyotype contributes to not only sporadic, but also to recurrent miscarriage (Stern *et al.*, 1996; Carp *et al.*, 2001; Stephenson *et al.*, 2002; Sullivan *et al.*, 2004). A recent review recommended chromosomal analysis of the products of conception in addition to the conventional tests in the evaluation of women with recurrent miscarriage (Branch *et al.*, 2010). However, to the best of our knowledge, there are no reports of the precise distribution of all causes of recurrent miscarriage, because embryonic karyotype analysis cannot be performed in all centers, including research centers. Patients wishing to undergo investigation

for recurrent miscarriage usually visit hospitals while they are not pregnant.

Information about the prevalence of an abnormal embryonic karyotype in women with a history of recurrent miscarriage and their future prognosis is still limited. Therefore, the present study was conducted to assess the subsequent live birth rate due to various causes, in women presenting with a history of recurrent miscarriage.

## Methods

### Patients

We studied 482 patients with a history of two or more (2–21) consecutive miscarriages who completed a systematic conventional examination and whose embryonic karyotype was ascertained at least once and documented in our medical records. Patients wishing for a second opinion after undergoing an examination at another hospital or who requested specific treatment were excluded to avoid a selection bias in the present study. The mean [standard deviation (SD)] age and number of previous miscarriages were 32.4 (4.45) and 3.04 (1.38), respectively.

### Investigations for maternal causes of recurrent miscarriage

All patients completed conventional examinations, such as hysterosalpingography, chromosomal analysis of both partners, determination of aPL, including lupus anticoagulant, by diluted activated partial thromboplastin time, diluted dilute Russel viper venom time and  $\beta$ 2glycoprotein I-dependent anticardiolipin antibody methods (Ogasawara et al., 1996) and blood tests for hypothyroidism and diabetes mellitus (DM), before a subsequent pregnancy. Transvaginal ultrasonography was performed to examine the morphology of any polycystic ovaries.

Conventional causes included antiphospholipid antibody syndrome (APS), occasional aPLs, abnormal chromosomes in either partner and uterine anomalies, excluding arcuate uterine and endocrine abnormalities. Hypothyroidism, DM and polycystic ovary syndrome (PCOS) were included as endocrine abnormalities.

APS was diagnosed according to the criteria of the International Congress on Antiphospholipid Antibodies (Miyakis et al., 2006). Patients with APS were treated with low-dose aspirin plus heparin (after 1995, Cowchock et al., 1992) or low-dose aspirin plus prednisolone (before 1995, Farquharson et al., 1984). Occasional aPL-positive cases were included as a separate group, because it was found that the live birth rate in these patients could be improved by treatment with low-dose aspirin alone (Sugiura-Ogasawara et al., 2008).

### Karyotyping the aborted conceptus

Karyotyping of the conceptus was performed routinely and not in response to some indication in the Nagoya City University hospital. Gestational age was calculated from basal body temperature charts. Ultrasonography was performed once or twice a week from 4 to 8 weeks of gestation. Dilution and curettage was performed on all patients diagnosed as having miscarriage. Part of the villi was cultured, and the cells were harvested after 6–22 days of cultivation to analyze the chromosomes. A total of 635 aborted conceptuses could be karyotyped using a standard G-banding technique. The 234 aborted conceptuses and 18 patients reported in our previous study were included in the present analyses (Ogasawara et al., 2000; Mizutani et al., 2011). In this paper, abnormal embryonic (fetal) karyotype patient refers to patients without conventional causes of recurrent miscarriage whose embryonic (fetal) karyotype was abnormal. Mixed patient refers to patients without conventional causes

who had miscarried both normal embryos/fetuses and embryos/fetuses with an abnormal karyotype. Unexplained patient refers to patients without conventional causes whose embryonic (fetal) karyotype was normal.

The study was conducted with the approval of the Research Ethics Committee at the Nagoya City University Medical School.

### Statistics

In the present study, we examined the distribution of the causes of recurrent miscarriage, including the abnormal embryonic karyotype, the cumulative live birth rate for each cause of recurrent miscarriage and the distribution of causes in cases with secondary versus primary recurrent miscarriage and in women over 40 years old versus those under 40 years of age.

The analysis was carried out using the SPSS for Windows, Version 19.0.  $P < 0.05$  was considered to denote statistical significance.

## Results

Of the 635 aborted products, normal and abnormal karyotypes were 44.9% ( $n = 285$ ; euploid 280, translocation 5) and 55.1% ( $n = 350$ ; trisomy 199, double trisomy 22, monosomy 25, polyploidy 38, tetraploidy 9, derivative of translocation 34, others 23), respectively.

The distribution of the causes and the characteristics of the subjects are shown in Table I and Figure 1. The total percentage of cases in which conventional causes of miscarriage could be detected was 29.5%. The prevalence of the abnormal embryonic karyotype was 41.1% in the subjects in whom no conventional causes of miscarriage could be identified. The prevalence of recurrent miscarriage of truly unexplained cause, in women without conventional causes whose embryonic karyotype was ascertained to be normal, was limited to 24.5%.

Details of abnormal embryonic abnormalities were included in Table I. The frequency of derivative was 47.9% (34/71) in aborted conceptus of patients with translocation.

The cumulative live birth rates in patients with abnormal embryonic karyotype, abnormal chromosome in either partner, uterine anomalies and miscarriages of truly unexplained cause were 71.2, 58.0, 65.2 and 52.5%, respectively. The mean ages at pregnancy of the patients with abnormal chromosomes in either partner, uterine anomaly and unexplained causes were significantly younger than that of the patients with an abnormal embryonic karyotype. The mean total numbers of losses of patients with occasional aPL, abnormal chromosomes in either partner, and unexplained and mixed causes were significantly higher than that of the patients with an abnormal embryonic karyotype. These results suggest that the prognosis of patients with abnormal chromosomes in either partner and unexplained causes is poor.

The embryonic karyotypes in 95 of 482 women could be analyzed at least twice (Table II). The abnormal embryonic karyotype was the cause of miscarriages in 32 women with repeated miscarriages, while 38 women with repeated miscarriages showed embryonic euploidy. In all, 73.7% (70/95) of the women were found to show repeated miscarriages of the same cause (Table II). In 76.2% of (32/42) the patients in whom the first determination revealed an embryonic karyotype was abnormal, the second determination also revealed an abnormal embryonic karyotype.

**Table 1** Distribution of the causes, the subject characteristics and the cumulative live birth rate

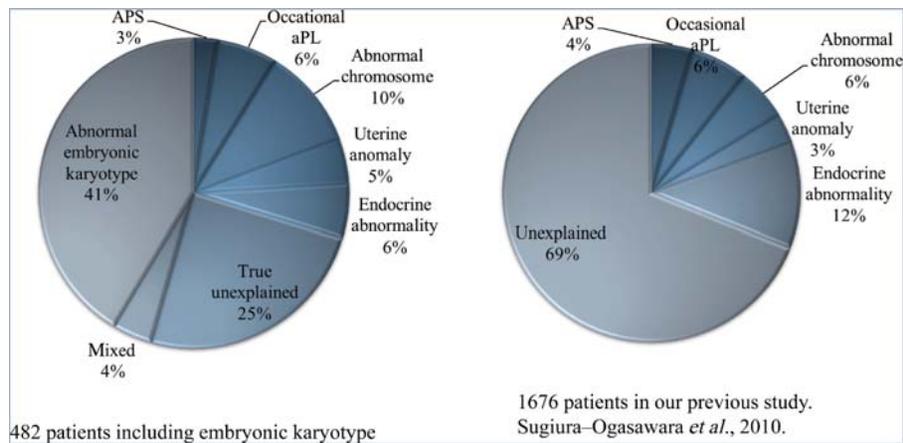
	Conventional causes					No conventional causes		
	APS	Occasional aPL	Abnormal chromosome in either partner	Major uterine anomaly	Hypothyroidism, DM, PCOS	Unexplained	Mixed	Abnormal embryonic karyotype
Prevalence [% (n)]	2.5 (12)	6.2 (30)	10.4 (50)	4.8 (23)	5.6 (27)	24.5 (118)	4.1 (20)	41.1 (198)
Mean (SD) age at pregnancy	33.2 (4.7), NS	32.9 (4.6), NS	30.4 (4.5) <sup>a</sup> , <i>P</i> < 0.0001	31.2 (4.4) <sup>a</sup> , <i>P</i> = 0.04	33.7 (3.6), NS	31.3 (4.3) <sup>a</sup> , <i>P</i> = 0.0002	32.1 (3.9), NS	33.2 (4.4)
Mean (SD) number of losses	3.7 (1.2), NS	4.8 (2.0) <sup>a</sup> , <i>P</i> = 0.0006	4.9 (2.0) <sup>a</sup> , <i>P</i> < 0.0001	5.0 (2.6), <i>P</i> = 0.056	4.5 (1.6), NS	4.9 (3.1) <sup>a</sup> , <i>P</i> = 0.001	5.8 (2.7) <sup>a</sup> , <i>P</i> = 0.005	3.9 (1.2)
The prevalence [% (n)] of women with at least one stillbirth	25.0 (3)	6.7 (2)	4.0 (2)	4.3 (1)	3.7 (1)	8.5(10)	15.0 (3)	4.0 (8)
The prevalence [% (n)] of women with at least one previous live birth	8.3 (1)	13.3 (4)	18.0 (9)	4.3 (1)	11.1 (3)	16.9 (20)	10.0 (2)	18.7 (37)
% of embryos with abnormal karyotype <sup>b</sup>	46.7 (7/15)	41.9 (18/43)	73.2 (52/71)	13.8 (4/29)	44.4 (16/36)	0 (0/158)	44.9 (23/49)	100 (229/229)
The prevalence [% (n)] of women with at least one abnormal karyotype	50.0 (6)	53.3(16)	86.0 (43)	17.4 (4)	48.1(13)	0 (0)	100 (20)	100 (198)
Karyotype (n)	Trisomy 4 Double trisomy 1 Triploidy 1 Tetraploidy 1	Trisomy 10 Double trisomy 1 Monosomy 4 Triploidy 2 Other 1	Derivative 34 <sup>c</sup> Trisomy 10 Double trisomy 3 Monosomy 1 Triploidy 2 tetraploidy 1 Other 1	Monosomy 2 Triploidy 1 Tetraploidy 1	Trisomy 11 Double trisomy 2 Tetraploidy 1 Other 1		Trisomy 14 Triploidy 7 Other 2	Trisomy 149 Double trisomy 1 Monosomy 16 Triploidy 25 Tetraploidy 5 Other 18
Cumulative live birth rate [% (n)]	50.0 (6)	60.0 (18)	58.0 (29)	65.2 (15)	70.4 (19)	52.5 (62)	65.0 (13)	71.2 (141)

Conventional causes: APS, occasional aPL, abnormal chromosome in either partner, major uterine anomaly and endocrine abnormality including hypothyroidism, DM and PCOS. Three patients with CAM and one patient with protein C deficiency were excluded.

<sup>a</sup>The comparison was performed between patients with an abnormal embryonic karyotype and the others.

<sup>b</sup>The number of abnormal karyotypes/the number of conceptuses karyotyped.

<sup>c</sup>The frequency of derivative was 47.9% in aborted conceptus of patients with translocation.



**Figure 1** Comparison of the distribution of causes between our 482 subjects including those with the abnormal embryonic karyotype and the 1676 subjects of a previous study.

**Table II** The live birth rate and characteristics of the 95 patients in whom the embryonic karyotype could be analyzed at least twice.

Embryonic karyotype	Abnormal	Mixed	Normal	P-value <sup>a</sup> , odds ratio (95% CI)
No. of patients	32	25	38	
Mean (SD) age at pregnancy	34.0 (4.8)	32.2 (3.9)	30.4 (3.9)	0.001
Mean (SD) number of losses	5.0 (1.5)	5.8 (2.6)	7.2 (4.4)	0.007
The prevalence [% (n)] of women with at least one stillbirth	12.5 (4)	12.0 (3)	15.8 (6)	NS
The prevalence [% (n)] of women with at least one previous live birth	25.0 (8)	8.0 (2)	10.5 (4)	NS
Live birth rate (%)	71.9 (23)	64.0 (16)	44.7 (17)	0.02, 1.6 (1.072–2.532)

<sup>a</sup>Comparison was performed between patients with abnormal and normal embryonic karyotypes.

The mean age and previous number of losses in the women with recurrent miscarriages associated with abnormal embryonic karyotype were significantly higher and fewer, respectively, than those in the women with recurrent miscarriages associated with the embryonic euploidy ( $P = 0.001$  and  $0.007$ ). The cumulative live birth rate (71.9%) in women with recurrent miscarriages caused by the abnormal embryonic karyotype was significantly higher than that (44.7%) in women with recurrent miscarriages associated with the embryonic euploidy ( $P = 0.02$ , odds ratio 3.2, Table II).

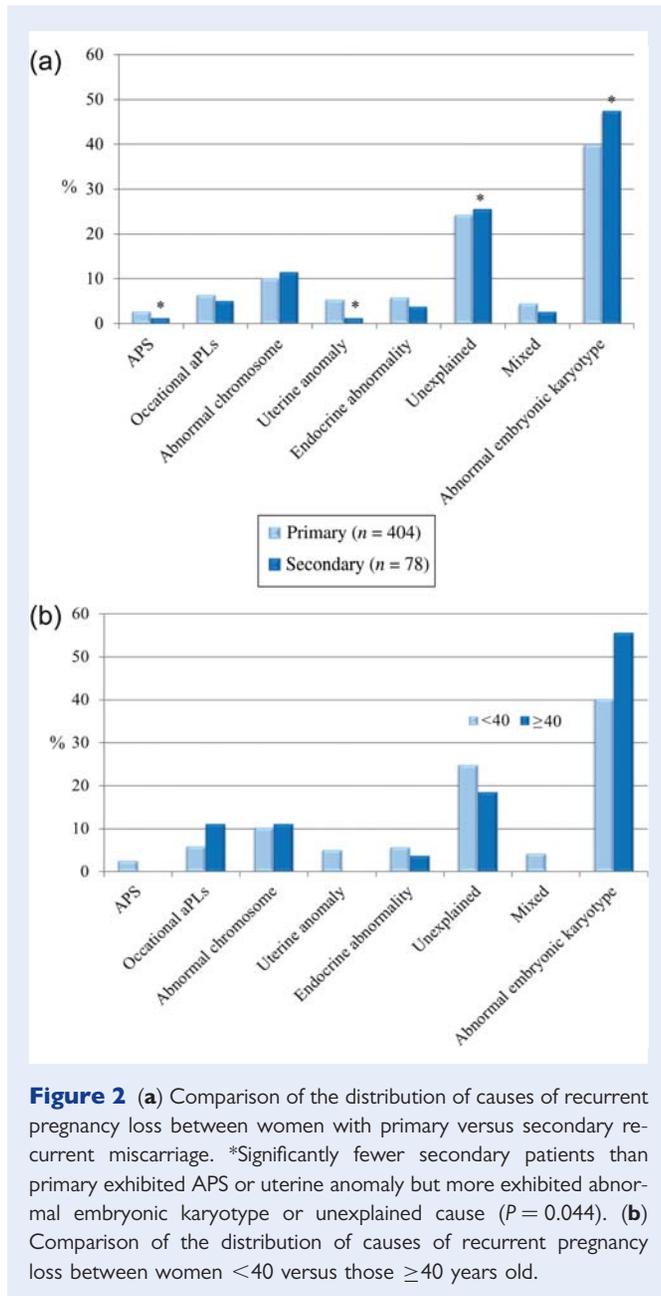
Comparison between the 404 patients with primary and 78 patients with secondary recurrent miscarriages revealed that compared with primary (primary versus secondary; Fishers' exact probability test,  $P = 0.044$ , Fig. 2a) fewer secondary patients exhibited APS (2.7, versus 1.3%) or uterine anomalies (5.4 versus 1.3%) but more exhibited abnormal embryonic karyotype (39.8 versus 47.4%) or unexplained cause (24.3 versus 25.6%). Comparison of the 455 subjects who were under 40 years old and 27 subjects who were over 40 years old ( $<40$  versus  $\geq 40$ ; Fishers' exact probability test, NS, Fig. 2b) revealed that older patients appeared less likely to exhibit APS (2.6 versus 0%) or uterine anomalies (5.1 versus 0%) but more likely to exhibit abnormal embryonic karyotype (40.2 versus 55.6%) or unexplained cause (24.8 versus 18.5%), although these differences

were not statistically significant. APS or uterine anomalies were seldom found in patients with secondary recurrent miscarriages or who were over 40 years old. In all 5 patients with secondary recurrent miscarriages who were  $>40$  years old, the cause was abnormal embryonic karyotype.

## Discussion

This is the first study to show the prevalence of the abnormal embryonic karyotype as a cause of recurrent miscarriage. In the present study, the prevalence was 41.1%. An abnormal embryonic karyotype was detected as the commonest cause of recurrent miscarriage. The subjects in the present study can be regarded as representative Japanese patients with recurrent miscarriage because patients wishing for a second opinion after undergoing an examination at another hospital or who requested specific treatment were excluded; thus, a selection bias in the distribution of each cause was avoided.

The prevalence of abnormal embryonic karyotypes was reported to be 57% (Stern et al., 1996), 29% (Carp et al., 2001), 46% (Stephenson et al., 2002), 25.4% (Sullivan et al., 2004) and 55.1% (present study). The difference depends on women's age and the number of miscarriages. The abnormal rate increases as the women's age increases



**Figure 2** (a) Comparison of the distribution of causes of recurrent pregnancy loss between women with primary versus secondary recurrent miscarriage. \*Significantly fewer secondary patients than primary exhibited APS or uterine anomaly but more exhibited abnormal embryonic karyotype or unexplained cause ( $P = 0.044$ ). (b) Comparison of the distribution of causes of recurrent pregnancy loss between women <40 versus those  $\geq 40$  years old.

and the previous number of miscarriages decreases (Ogasawara et al., 2000).

Several reports have suggested that the abnormal embryonic karyotype predicts subsequent live birth (Ogasawara et al., 2000; Carp et al., 2001). The cumulative live birth rate (71.2%) in women with miscarriage caused by an abnormal embryonic karyotype was found to be higher than that (52.5%) in women with recurrent miscarriage of truly unexplained cause. Patients with abnormal and normal chromosomes were found to have repeat miscarriages with abnormal and normal chromosomes, respectively. This was consistent with the results of our previous study conducted with a small sample size (Mizutani et al., 2011). Karyotype analysis should be performed at least once, because the abnormal embryonic karyotype tends to repeat itself and is also a predictor of good prognosis.

Patients with recurrent miscarriage caused by the abnormal embryonic karyotype might have gene mutations associated with aneuploidy such as SYCP3. Unexplained reasons may cause the miscarriage of a euploid embryo and may continue to cause further miscarriages. SYCP3 mutations in women were found to generate an aberrant synaptonemal complex in a dominant-negative manner and to contribute to abnormal chromosomal behavior that could potentially lead to recurrent miscarriage (Bolor et al., 2009). We found no clinical significance in the routine examination of the SYCP3 mutation because only one benign mutation was ascertained in 101 patients (Mizutani et al., 2011). However, some gene mutations, such as MLH1, may influence aneuploidy because a double trisomy can be detected in recurrent but not in sporadic, miscarriage patients (Edelmann et al., 1996; Ogasawara et al., 2000). Preimplantation screening (PGS) is performed worldwide, though it is unclear whether PGS can improve the live birth rate in patients with recurrent miscarriage (Harper et al., 2010). The cumulative live birth rate in patients with an abnormal embryonic karyotype was higher because such patients could have a euploid embryo. PGS may be useful in specific patients affected by a candidate gene mutation.

According to previous reports, about half of the women at research centers are determined as having recurrent miscarriage of unexplained cause despite receiving conventional examinations to determine the cause. There have been some reports on the distribution of the causes at individual centers. The prevalences of aPL, abnormal chromosome, uterine anomaly, endocrine abnormality (DM, both PCO morphology and mid-follicular serum LH level > 10 IU/L) and unexplained causes were 14, 3.6, 1.8, 6.8 and 73.8%, respectively, in Clifford's study (mean age, 32.9 years; median miscarriages, 4; Clifford et al., 1994). The prevalences of aPL, abnormal chromosome, uterine anomaly, endocrine abnormality and unexplained causes were 20, 3.5, 16, 20 and 42.6%, respectively, in Stephenson's study (Stephenson, 1996). These previous studies did not distinguish between truly unexplained causes and an abnormal embryonic karyotype. The prevalence of unexplained causes was 69% among our patients (Fig. 1, Sugiura-Ogasawara et al., 2010). The differences in the distribution of causes at each center may depend on age, number of previous miscarriages and methods of diagnosis for each factor. We compared the distribution of the causes between the 482 couples in the present study and 1676 couples of a previous study among whom the aborted conceptuses of all the patients were not analyzed (Sugiura-Ogasawara et al., 2010, Fig. 1). The true percentage of subjects with recurrent miscarriage of unexplained cause was limited to 24.5%. Moreover, 482 patients in our present study experienced further miscarriages after undergoing a systematic examination. The patients with APS miscarried despite anticoagulant therapy. The prevalences of abnormal chromosomes in either partner and uterine anomaly were higher than the prevalences in previous study. This finding suggests that the prognosis of abnormal chromosomes and uterine anomaly is poor.

The reported live birth rate in women with APS treated with low-dose aspirin plus heparin is 70–80% (Cowchock et al., 1992; Rai et al., 1997). The cumulative live birth rate in this group in the present study was relatively low, because women with APS gave up after the first treatment and failure. The reported prevalence range, in review articles, of APS is 5–15% (Branch et al., 2010). Several reports have indicated that about 10–15% of women with recurrent

miscarriage are diagnosed with APS (Clifford *et al.*, 1994; Rai *et al.*, 1995; Yetman and Kutteh, 1996). However, it is unclear whether the aPLs persisted according to International Criteria (Miyakis *et al.*, 2006). The prevalence of APS according to International Criteria was found to be 2.5% in the present study, presumably because the figure represents the prevalence after one treatment failure. The single positive rate of aPLs was 10.7% and the recurrent positive rate was 4.5% in our previous study (Sugiura-Ogasawara *et al.*, 2008). There are many methods used for the detection of aPLs. However, there are limited reports on which method might be most suitable for prediction of recurrent miscarriage or intrauterine fetal death. The positive rate might be large if methods with a large false-positive rate were used. The prevalence of 'true' APS might be <5%, although it depends on the age of the women comprising the study population and the method used for the detection of APS.

The frequency of major congenital uterine anomalies has been reported to be between 3.2 and 6.9% in women with a history of recurrent miscarriage, the variation largely depending on the method of selection and the criteria used for the diagnosis (Sugiura-Ogasawara *et al.*, 2011). Uterine anomalies are encountered in miscarriages associated with euploidy (Sugiura-Ogasawara *et al.*, 2010). However, the prognosis in these cases is better than that in patients presenting with recurrent miscarriage of unexplained cause.

Recently, the mean age of the population has been increasing year by year, and the proportion of women in their 40s has been increasing in Japan. APS and uterine anomalies were found to be rare in subjects with secondary recurrent miscarriage and women over 40 years old in the present study. This should be borne in mind before evaluation of the screening tests.

The prevalence of recurrent miscarriage of truly unexplained cause, in whom the embryonic karyotype was ascertained to be normal, was found to be 24.5% in this study. Kaandorp *et al.* (2010) demonstrated, based on the results of an RCT, that aspirin plus heparin therapy has no beneficial effect in patients with two or more miscarriages. Further studies are needed in women with recurrent miscarriage of truly unexplained cause, after excluding cases with an abnormal embryonic karyotype, to confirm the conclusion.

## Limitation

The prevalence of each cause differs among centers, depending on the background of the patients, such as the mean age and previous number of miscarriages of the women in the studied population and the selected method and criteria for the diagnosis.

Subjects with thrombophilia, infection, fibroid or deficiency of progesterone were excluded from the analysis, because the contribution of these factors to recurrent miscarriage has not yet been established (Branch *et al.*, 2010).

Although the standard G-band technique is the gold standard for evaluating chromosomal abnormalities, it has several limitations, including the need for tissue culture and the possibility of maternal cell contamination. Additional analysis, such as by comparative genomic hybridization (CGH), could not be performed in the present study. Further abnormalities could be detectable and also contamination with maternal tissue could be distinguishable by CGH. A recent microarray CGH indicated that about 80% of sporadic spontaneous abortions were caused by an abnormal embryonic karyotype

(Shimokawa *et al.*, 2006). Thus, if the prevalence is similar in women with recurrent miscarriage and they have no predisposing factors, the incidence of the abnormal embryonic karyotype as a cause of recurrent miscarriage can be estimated to be  $(0.8)^n$  in patients with  $n$  consecutive miscarriages. About 51% of patients caused by an abnormal embryonic karyotype can be expected to exist in patients with three miscarriages occasionally. The incidence might increase if the candidate gene such as SYCP3 or MLH affect.

Among subjects with abnormal chromosomes in the present study, 26.8% (19/71) carried embryos that were normal or balanced (Table 1). It is possible that CGH would have revealed lack of balance, because 40% of 42 balanced translocation carriers as assessed by cytogenetic analysis were found to show a loss of balance as assessed by CGH (De Gregori *et al.*, 2007).

## Conclusion

The prevalence of the abnormal embryonic karyotype in subjects with recurrent miscarriage was found to be 41.1%. The abnormal embryonic karyotype was found to be the commonest cause of miscarriage and often recurred as the cause of subsequent miscarriages. The prevalence of recurrent miscarriage of truly unexplained cause was found to be 24.5% in the present study. This should be a target of an RCT to determine the effectiveness of a treatment. These two groups should be distinguished for both clinical and research purposes. Embryonic analysis should be added to the list of evaluation items in women with recurrent miscarriages, for both clinical and research purposes.

## Authors' roles

M.S.-O. has contributed to design the study, perform analysis and draft the article. Y.O., K.K., N.S., T.K. and E.M. have contributed to acquisition and analysis of data.

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## Conflict of interest

None declared.

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