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Diagnosing structural chromosome abnormalities in couples with recurrent miscarriage; the relevance of maternal age

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Background The annual number of parental karyotypes in case of recurrent miscarriage is increasing gradually in the Netherlands. Women with recurrent miscarriage and advanced maternal age are eligible for prenatal chromosome studies, in addition to parental karyotyping. The efficiency of offering parental karyotyping in couples with recurrent miscarriages has not been evaluated, especially for the group with miscarriages at advanced maternal age.

Methods A historical cohort study and nested case control study was conducted, including couples with at least two miscarriages. Data was retrieved from medical records and telephone interviews. The obstetric follow-up was recorded for at least two years after the chromosome analysis. Data was analysed to compare frequencies of carriers and non-carriers in couples in which maternal age was ≥36 years or <36 years at the time of karyotyping, at second miscarriage and at third miscarriage. A projected prevalence of carriership of a structural chromosome abnormality was calculated by extrapolating the number of included patients to the original level of the total screening population.

Results 41 couples with carriership of a structural chromosome abnormality and 76 couples without carriership were included. A trend towards a lower projected prevalence of carriership was found at higher maternal age.

Conclusions In conclusion, a relative low frequency of carriership is found in women with recurrent miscarriage who are ≥36 years at second miscarriage, without any unbalanced offspring after the detection of carriership.

Key words recurrent miscarriage, structural chromosome abnormalities, carriership, translocation, maternal age

Introduction

In the Netherlands, the annual number of parental karyotypes in case of recurrent miscarriage is increasing gradually. In 1992, karyotyping for this indication was performed 2,595 times and in 2001, 5,015 times. Between 1992 and 2001 942/38,099=2.5% carrier individuals were detected. Epidemiological evidence shows a steep increase in sporadic miscarriage rate in women of 36 years and older[1-5]. This age-related risk is due to a higher number of aneuploidies, mainly trisomies[6-8]. The age-dependent increase of trisomies, with recurrent miscarriage as its manifestation, may be due to a recurrence of sporadic chromosome abnormalities[8].

Women with recurrent miscarriage and advanced maternal age are eligible for prenatal diagnosis, in addition to parental karyotyping. The effectiveness of offering women both screening programmes - karyotyping for parental carriership and prenatal chromosome studies of the fetus- to this group of women needs to be better explored. Therefore, we investigated the yield of chromosome analysis for parental carriership in two maternal age groups in women with recurrent miscarriages.

Materials and methods

Study Design

A historical cohort study of couples who had presented themselves in the past for carriership detection after two or more miscarriages was conducted. From January 1992 until June 2000, all carriers detected in the Academic Medical Center, as well as a random subset of two non-carriers per carrier, were invited. We included men and women with two or more miscarriages up to 20 weeks gestational age, proven by a pregnancy test and/or ultrasound. In case only one parent was karyotyped with a normal karyotype result, these couples were not included in the study. Genetic diseases other than a structural chromosome abnormality were recorded. Carriers and non-carriers were contacted by mail with an invitation to participate in the study, and informed consent was obtained. Reminders were sent out in cases of non-response. Data was retrieved from medical records and telephone interviews, and focused on the parental characteristics at the time of the chromosome analysis, the previous miscarriages, obstetric history and obstetrical outcome for at least two years after chromosome analysis. Institutional Review Board approval was obtained.

Statistical Analysis

The number of carriers and non-carriers in two maternal age groups, <36 years and ≥36 years were established at the time of karyotyping, at second miscarriage, and at third miscarriage. Then Odds Ratios with their 95% CIs were calculated to establish a difference of risk on being a carrier in the two respective age-groups. Statistical significance was defined as confidence intervals did not include 1.0. Statistical analysis was performed with the Statistical Package for the Social Sciences 10.0 (SPSS Inc., Chicago, IL).
Given the total number of karyotypes performed in the same period, frequencies of carriership in the study population were calculated back to the original level of the screening population.

Results

The screening population in the inclusion period consisted of 1324 couples. All couples were referred for karyotyping because of recurrent miscarriage.
In 51 of the 1324 couples, a carrier was identified, and these couples served as cases. For the control couples, 106 couples without carriership were selected in a random, chronological way, i.e. they were karyotyped directly before and after the carrier. In 25 couples we could not objectivate the miscarriages due to non-response (unknown address, or no telephone (n=14), refusal to participate in the study (n=11)). In five couples we identified only one miscarriage, however in combination with another obstetrical adverse outcome. In four couples no pregnancy test and/or ultrasound was performed.
We excluded five non-carrier couples, because one parent or semen donor was not karyotyped and the other parent had a normal karyotype, and another couple was excluded because the father was detected to be carrier of a structural chromosome abnormality only after the birth of a child with congenital abnormalities.
A total number of 117 couples remained for analysis, which consisted of 41 couples including one carrier and 76 non-carrier couples. In 103 couples, the country of birth was the Netherlands (88%). In the 14 other couples, the remaining countries of birth were Surinam (n=4), Turkey (n=3), Curacao (n=2), Africa (n=2), and other countries (n=3). The mean age of the women at time of karyotyping was 34.3 years (range 22.3-46.4). No difference in maternal age was found between the carrier-couples (33.2 years, range 22.3-43.6) and the non-carrier couples (34.9 years, range 23.5- 46.4). Consanguinity was found in 1 carrier-

couple (nephew-niece) and in 2 non-carrier couples (nephew-niece). The couples were referred from four sources: 58 couples were referred by an academic gynecologist (49.6%), 54 couples by a gynecologist from a general hospital (46.2%), three couples by a midwife (2.6%) and two couples by a general practitioner (1.7%).

Chromosomal Outcome

The structural chromosome abnormalities of the 41 carrier couples are listed in Table 1. We found more female carriers (n=27) than male carriers (n=14). There were 26 reciprocal translocations (63.4%), five pericentric inversions (12.2%), four paracentric inversions (9.8%), three Robertsonian translocations (7.3 %), two (Y;22) translocations (4.9%) and one marker chromosome (2.4%).

Normal Variable Chromosome Features

In three non-carrier couples one partner represented normal variable chromosome features. The three karyotypes were two low-level mocaicisms: mos 45,X[1]/47,XXX[2]/46,XX[27], and mos 45,X[3]/46,XX[27], and one increased satellite: 46, XX, 21s+.

Obstetrical History

Before karyotyping, the 41 carrier couples had had 160 pregnancies, and the 76 non-carrier couples 294 pregnancies. The mean number of pregnancies per couple before karyotyping was the same for carrier couples (3.9; range 2-11) and non-carrier couples (3.9; range 2-10). Nearly half of the couples had had two miscarriages (56/117=48%), 41 couples had had three miscarriages (41/117=35%) and 20 couples had had four or more miscarriages (20/117=17%). The highest number of miscarriages was ten. The mean gestational age was 9.0 weeks in carriers (n=124 miscarriages, range 4 – 19 weeks) and 8.6 weeks in non-carrier couples (n=216 miscarriages, range 5 – 19 weeks). No statistical differences in frequencies of carriership were found between couples with two miscarriages versus couples with three miscarriages, and between couples with three miscarriages versus couples with four or more miscarriages (see Table 2).

Table 1 Type of structural chromosome abnormalities of the 41 carrier couples with recurrent miscarriage

Female Carriers (n=27)

Reciprocal Translocations

46,XX,t(1;7)(q32.1;q32)
46,XX,t(1;10)(q23;22.3)
46,XX,t(1;11)(p34.3;q13)
46,XX,t(1;16)(p35.3~p36.1;p13.3)
46,XX,t(2;8)(p15;q13.1)
46,XX,t(2;12)(p25;q13)
46,XX,t(3;6)(q25~q26;q23~q24)
46,XX,t(3;6)(q25;q23.1)
46,XX,t,(3;15)(p13;q26.1)
46,XX,t(4;6)(q31.1;q22.32)
46,XX,t(4;10)(q13.3; q24.3)
46,XX,t(5;12)(p15.1;q22)
46,XX,t(11;12)(p15.4;p13.2)
46,XX,t(11;20)(q13;p13)
46,XX, t(14;18)(q22;q11.2)
46,XX,t(16;20)(q24;p13)
46,XX,t(17;18)(q21.1;q12.2)
mos46,XX[20]/46,X,t(X;14)(p21.1;q21.1;q21)[10]

Robertsonian Translocations

45,XX,der(13;22)(q10;q10)
45,XX,der(13;14)(q10;q10)*

(Y;15 and Y;22) Translocations

46,XX,der(22)t(Y;22)(q12;p12/13)
46,XX,der(22)t(Y;22)(q12;p12/13)

Pericentric Inversions

46,XX,inv(8)(p11.22q13.1)
46,XX,inv(9)(p13q13)

Paracentric Inversions

46,XX,inv(6)(p21.3p25)
46,XX.ish inv(8)(p21p23)
46,XX,inv(11)(q21q23)

Male Carriers (n=14)

Reciprocal Translocations

46,XY,t(1;3)(q21;q25)
46,XY,t(2;13)(q35;q32)
46,XY,t(5;12)(q35.1;q24.1)
46,XY,t(5;17)(q33.1;q25.3)
46,XY,t(6;16)(q25.3;p13.3)
46,XY,t(8;12)(p23.1;p13.3)
46,XY,t(10;13)(q23.3;q13)
46,XY,t(11;21)(p15.4;q22.1)

Robertsonian Translocations

45,XY,der(13;14)(q10;q10)

Pericentric Inversions

46,XY,inv(2)(p11.2q13)
46,XY,inv(5)(p15.3q35)**
46,XY,inv(9)(p13q13)

Paracentric Inversions

46,XY,inv(12)(q15q24.1)

Marker Chromosomes

47,XY,+idic(15)(q11.2)

* The carrier of this translocation is known with Autosomal Dominant Benign Myopathy.

** The inversion was only detected in second instance after the birth of a child with congenital abnormalities (see discussion section).

Table 2 Frequency of carrier couples after different number of miscarriages

	2 miscarriages	3 miscarriages	≥ 4 miscarriages	OR	95% CI
Carriership after 2 versus 3 miscarriages	18/56	15/41		1.2	0.52 – 2.84
Carriership after 3 vs ≥4 miscarriages		15/41	8/20	1.2	0.39 – 3.46

Maternal Age

Table 3 shows the frequency of carrier couples at different points in time, i.e. at the time of karyotyping, at second and at third miscarriage for two maternal age groups, <36 years and ≥36 years. Thus, Odds Ratios could be calculated for the occurrence of carriership in the two age groups. The Odds Ratio calculated at the time of karyotyping showed no clear difference between the risk of being a carrier when maternal age is <36 years, versus a maternal age ≥36 years (Odds Ratio 1.6; 95% CI: 0.7- 3.4).

The Odds Ratio calculated at the time of second miscarriage was 2.7 (95% CI: 0.9 – 7.8) which implies that the risk of being a carrier was higher when maternal age is below 36, although a significant level was not met. The Odds Ratio calculated at the time of third miscarriage was quite similar (Odds Ratio 2.6; 95% CI: 0.5 – 13.7).

Extrapolation of data from table 3, by multiplying numbers to the original level of the screening population, provides a prevalence estimate in this population. In two carrier couples, the year in which miscarriages occurred was unknown. Therefore, at the time of second miscarriage results of 39 out of 51 carrier couples were available for analysis, and the multiplication factor was 1.3 (51/39). For non-carrier couples the multiplication factor was 17.2 (1273/74). Then, at the time of second miscarriage, a prevalence of 3.9 % carrier couples was found, 4.6 % in the maternal age group <36 years, and 1.9 % in the maternal age group ≥36 years.

About the same prevalences could be calculated at time of third miscarriage. When multiplying the number of carrier couples with 2.4 (51/21), and non-carrier couples with 34.4 (1273/37), a 4.4 % prevalence of carriership was found in the maternal age group <36 years, and 1.8 % in the maternal age group ≥36 years.

Table 3 Frequency of carrier couples at different points in time

Maternal age at measurement	Carriership <36 yrs (n/N)	Carriership ≥36 yrs (n/N)	No. of couples	OR	95% CI
At karyotyping	23/57	18/60	117	1.6	0.7 – 3.4
At second miscarriage	34/87	5/26	113	2.7	0.9 – 7.8
At third miscarriage	19/48	2/10	58	2.6	0.5 – 13.7

n=number of carrier couples in maternal age group.

N=total number of couples in maternal age group.

Obstetrical Outcome in Carrier Couples

The mean duration of follow-up for carrier couples was 71.7 months, range 25-118 months. Twenty-five out of 41 carrier couples (61%) were pregnant at time of karyotyping, with at that time unknown carrier status. Of the 25 pregnancies, 22 resulted in a healthy child (88%) and three resulted in a miscarriage (12%). In 18 of these pregnancies, prenatal diagnosis was performed. Actually, six of these cases were referred for parental karyotyping by our Prenatal Diagnostic Center, after emergence of the history of two or more miscarriages. No unbalanced structural chromosome abnormalities were found. In nine cases, a balanced karyotype related to the parental carrier was found, including one marker chromosome. In another nine cases, a normal karyotype was obtained.

After the results of karyotyping were available a total number of 43 pregnancies was established in 25 carrier couples with childwish. Of these 43 pregnancies, 30 resulted in a live born healthy child (70%), 11 resulted in a miscarriage (26%), one pregnancy was ongoing (2%), and one abnormal pregnancy was reported (2%). Prenatal diagnosis was performed in 26 pregnancies. In 15 pregnancies (58%) a balanced karyotype, related to the parental carrier was found, in the resulting 11 pregnancies (42%), a normal karyotype was obtained. Mean maternal age at time of prenatal diagnosis was 33.0 years (range 22 – 44). The abnormal pregnancy occurred in a couple in which the mother was a carrier of a paracentrical inversion of chromosome 8 (karyotype 46, XX ish inv(8)(p21p23)). The couple initially decided against prenatal diagnosis. During routine ultrasound examination a Dandy-Walker malformation was detected. Before the chromosome result of a secondarily performed amniocentesis was available, the pregnancy was terminated at 24 weeks. The Dandy-Walker malformation diagnosis was postnatally confirmed by histology. The chromosome diagnosis was a balanced paracentrical inversion of chromosome 8 (karyotype: 46, XY,inv(8)(p21p23)) just like the mother, and seemed unrelated to the Dandy-Walker malformation.

Discussion

In couples with recurrent miscarriage, we compared frequencies of carriership of a structural chromosome abnormality in women with a maternal age ≥36 years and women with a maternal age <36 years at time of karyotyping, at second and at third miscarriage. Less carriers were found in women with a maternal age ≥36 years, indicating a lower risk of carriership in these women. The difference was not statistically significant (Odds Ratio 2.7; 95% CI: 0.9 – 7.8). This trend reflects the biological phenomenon of a higher miscarriage rate at older maternal age, due to a higher number of numerical chromosome abnormalities[1-8]. To our knowledge, defining frequencies of carriership of a structural chromosome abnormality in different maternal age groups has not been reported previously in couples with recurrent miscarriage. When the frequency of carriership in our study population was multiplied to the level of the original screening population, we found a low projected prevalence (1.9%) in the group with a maternal age ≥36 years, when compared to the group with a maternal age of <36 years at second miscarriage (4.6%).

After one single miscarriage, couples will not be karyotyped. The risk of a structural chromosome abnormality after one single miscarriage has been found to be 1.16 % in individuals, and approximately 2.3% in couples[9]. It seems therefore reasonable to withhold karyotyping when risks are below these percentages. The projected prevalence we calculated in the higher maternal age group at second miscarriage was slightly lower, i.e. 1.9% in couples. So it seems justified to withhold karyotyping, when maternal age at second miscarriage is 36 years or higher. In this group, possible structural chromosome abnormalities of the fetus, can be detected by prenatal diagnosis. Even more important is the fact that no unbalanced reproductive outcome was found in our study population, which stresses the low risk for such an serious adverse event in women with recurrent miscarriage. The mean duration of follow-up for carrier couples was six years. Karyotyping of 1324 couples ascertained for recurrent miscarriage did not prevent the birth of a single child with congenital abnormalities. In one case, the structural chromosome abnormality in the father, a 46,XY,inv(5)(p15.3q35) karyotype, was only detected at second instance after the birth of a child with microcephaly, an atrial septal defect and a ventricular septal defect. Many studies which describe frequencies of carriership of structural chromosome abnormalities in couples with recurrent miscarriage, do report obstetrical history, but data about obstetric follow-up is limited. We found three relatively small studies in which registration of subsequent pregnancies in case of carriership and recurrent miscarriage took place.

No unbalanced reproductive outcome was reported in respectively 7, 35 and 17 carrier couples

with recurrent miscarriage[10-12]. These studies favour a normal pregnancy outcome in carriers who were traced after the occurrence of miscarriages. The results of our study, with an even higher number of carriers, are in concordance with their findings.

The risk of having a handicapped child with an unbalanced karyotype depends on the type of translocation, and on the sex of the transmitting parent[13-15]. Generally a 5-10% risk on a live born child with multiple congenital abnormalities is mentioned in case of carriership[16]. When the 5-10% risk figure of a live born child with multiple congenital handicaps is used, then, in our study population three live born children with multiple congenital handicaps were to be expected (43 pregnancies in carrier couples multiplied by 0.075). But, beside a case with Dandy Walker malformation, most probably unrelated to the parental carrier, in our study population no children with handicaps were born out of a total number of 43 pregnancies.

The risk that parents will bear offspring with an unbalanced chromosome complement depends, besides the sex of the carrier and type of rearrangement as stated above, also on the method of ascertainment[17]. The frequencies of unbalanced reciprocal translocations at prenatal diagnosis in subsequent pregnancies are much higher when a family is ascertained through prior full-term unbalanced progeny than when they are ascertained through recurrent miscarriage (19.8% versus 4.8% for maternal carriers; 22.2% versus 1.4% for paternal carriers)[18].

Whether our study population is a selection rather than a reflection of the whole population of couples with recurrent miscarriage is difficult to determine. It is unlikely that couples with recurrent miscarriage who are not referred for karyotyping have a worse obstetric history, thus reflecting a subgroup with translocations leading more often to unbalanced offspring. In conclusion, a relatively low frequency of carriership is found in women with recurrent miscarriage who are ≥36 years, without any unbalanced offspring after the detection of carriership. Withholding karyotyping in the older maternal age groups seems justified, but this needs to be confirmed with more data. A nation wide study is currently underway in the Netherlands.

Diagnosis Study Courses, the abnormalities in Table, after recurrent miscarriage, the relevance of maternal age

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