Efficiency of parental chromosome analysis in couples with recurrent miscarriage

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Efficiency of parental chromosome analysis in couples with recurrent miscarriage

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“En toute chose il faut considérer la fin”

Van alle dingen moet men het einde in het oog houden

Jean de La Fontaine, uit Fables (1668)

Aan mijn ouders

Contents

1

Introduction and outline of the thesis

In couples trying to conceive recurrent miscarriage causes tremendous grief, feelings of insecurity and ambivalence about each subsequent pregnancy. As a consequence, these couples often desperately seek help to carry a next pregnancy to term. To identify or exclude an underlying cause these couples are offered an extensive diagnostic work-up, but only few aetiological factors have been identified so far.[1,2]

One of the most evident aetiological factors in recurrent miscarriage is either of the partners being a carrier of a structural chromosome abnormality. Structural chromosome abnormalities involve the rearrangement of chromosome segments. They can be subdivided into translocations, inversions, deletions and duplications. Only translocations and inversions are known to be associated with recurrent miscarriage due to their ability to subsist in a balanced form, where there is no overall gain or loss of genetic material, enabling phenotypically normal carrier parents.

In 1962, when chromosome banding was not yet available, the first report of a cytogenetic analysis in a couple with an obstetric history of two miscarriages was published.[3] The male partner proved to have a chromosome of what at that time was known as the '21 to 22 group' which had an extra chromatin piece, of undetermined origin, translocated to its short arm. The same abnormality was found in his father. In the nineteen seventies, more reports appeared confirming a causal relationship between structural chromosome abnormalities and recurrent miscarriage.[4,5] In the following decades larger studies appeared establishing the increased incidence of parental structural chromosome abnormalities among couples with recurrent miscarriage.[6,8] It was demonstrated that the incidence of either of the partners being a carrier of a structural chromosome abnormality was approximately 0.7% in the general population, 2.2% after one miscarriage, 4.8% after two miscarriages, and 5.2% after three miscarriages.[9,10]

In couples carrying a structural chromosome abnormality the karyotype of their products of conception can be normal, balanced, or unbalanced. Depending on the particular combination of abnormal gametes an unbalanced karyotype can lead to miscarriage, stillbirth, or the birth of a child with major congenital impairments. In carrier couples, invasive prenatal diagnosis is offered in

subsequent pregnancies, so that parents may decide to terminate the pregnancy in case of an unbalanced foetal karyotype.

Background of the research described in this thesis

In 2000, when we started the studies that led to this thesis, it had been good clinical practice for many years to offer parental chromosome analysis to couples with recurrent miscarriage in view of their increased risk of carrying a structural chromosome abnormality and thus the potential birth of a child with an unbalanced karyotype. However, guidelines on recurrent miscarriage did not agree on whether parental chromosome analysis should be performed after two or three miscarriages, it was unknown whether other factors, such as maternal age, would influence a couples' probability of carrying a structural chromosome abnormality, it was unknown how many pregnancies were terminated as the result of this screening strategy and how many children with an unbalanced karyotype were born.[11-13]

In 1995 the value of parental chromosome analysis in couples with recurrent miscarriage to prevent viable unbalanced offspring had already been discussed by geneticists and providers of healthcare insurance.[14] In 2000 the databases of postnatal cytogenetics in the Netherlands demonstrated that the annual number of parental chromosome analyses in couples with two or more miscarriages had nearly doubled, from 1298 couples in 1992 to 2362 couples in 2000. At the same time the incidence of carrier couples decreased from 6.8% to 3.8%.[15] Thus, around the turn of the century, the increase in chromosome analyses had not resulted in identifying more carrier couples. Since this time-consuming and expensive procedure with a low detection rate of carriers is a burden to the healthcare system, its efficiency needed to be explored.

Little was known on the actual diagnostic and therapeutic management of recurrent miscarriage and the impact of introducing guidelines on clinical practice. Therefore, we first evaluated the implementation of the Dutch guideline which was introduced in 1999.[11]

The primary aim of our research was to investigate whether the efficiency of parental chromosome analysis in couples with recurrent miscarriage could be improved. If couples at high probability of carrying a structural chromosome abnormality could be distinguished from couples at low probability, it might be possible to withhold a substantial number of couples with recurrent miscarriage from the screening procedure, saving costs to the healthcare system.

Whereas the incidence of structural chromosome abnormalities among couples with recurrent miscarriage was established at the time we started our research, large studies on the reproductive outcome of couples with recurrent miscarriage and carrying a structural chromosome abnormality were lacking. Risk estimates for viable unbalanced offspring in these couples were mainly derived from data on prenatal diagnoses. Some of these studies indicated that the risk of viable unbalanced offspring in carrier couples depended on the mode of ascertainment of the structural chromosome abnormality; carrier couples ascertained through recurrent miscarriage appeared to be at much lower risk of viable unbalanced offspring (1.5 to 5%) compared to couples ascertained through the previous birth of a child with an unbalanced karyotype (20 to 25%), but other studies could not confirm this.[16-18] To safely decrease the number of parental chromosome analyses in couples with recurrent miscarriage we needed to be better informed on their actual reproductive outcome, in particular on the incidence of viable unbalanced offspring. Finally, in the past decade preimplantation genetic diagnosis (PGD) has become available as an alternative for invasive prenatal diagnosis to avoid termination of pregnancy in couples with structural chromosome abnormalities.[19-21] PGD has also been suggested to improve live birth rates in couples with recurrent miscarriage carrying a structural chromosome abnormality.[22,23] It was unclear to what extent this claim was substantiated by evidence.

Outline of the thesis

Chapter 2 evaluates the changes in the management of recurrent miscarriage among Dutch gynaecologists after the introduction of the guideline on recurrent miscarriage from the Dutch Society of Obstetrics and Gynaecology in 1999, as well as the adherence to the guideline. By means of a questionnaire returned by 84 of the 101 practices for obstetrics and gynaecology in the Netherlands, data concerning the use of definition, diagnosis and treatment of recurrent miscarriage were obtained. Results were compared with a similar study conducted before the introduction of the guideline and with the recommendations in the guideline.

Chapter 3 reports on factors influencing the probability of either of the partners being a carrier of a structural chromosome abnormality in couples with recurrent miscarriage. Among 279 carrier couples and 428 non-carrier couples referred for parental chromosome analysis after two or more miscarriages, factors influencing the probability of carrier status were identified. A model was developed to distinguish couples at high probability of either of the partners being a carrier from couples at low probability of being so.

Chapter 4 describes the reproductive outcome after parental chromosome analysis in couples with recurrent miscarriage. During a mean follow-up period of 5.8 years after parental chromosome analysis the reproductive outcomes of 278 couples carrying a structural chromosome abnormality and of 427 couples with both normal parental karyotypes were compared.

Chapter 5 reports on the mode of ascertainment of 56 inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis. All inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis in three centres for clinical genetics in the Netherlands in a nine years period were recorded. It was investigated whether these abnormalities were ascertained through recurrent miscarriage in the obstetric or family history, or through other factors.

Chapter 6 reviews the effect of preimplantation genetic diagnosis (PGD) on the chance of having a healthy child and the chances of a subsequent miscarriage in couples with recurrent miscarriage carrying a structural chromosome abnormality. Results of 21 studies reporting on the reproductive outcome of carrier couples with recurrent miscarriage after PGD and 4 studies reporting on the reproductive outcome of these couples after attempting natural conception are presented.

Chapter 7 provides a general discussion of the results presented in this thesis, outlines their clinical implications and provides suggestions for future research.

Chapter 8 presents the summary of this thesis.

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2

Management of recurrent miscarriage: evaluating the impact of a guideline

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Abstract

Background Little is known on the actual diagnostic and therapeutic management of recurrent miscarriage and the impact of introducing guidelines on this topic. The objective of this study was to evaluate any changes in the management of recurrent miscarriage among Dutch gynaecologists after the introduction of the Dutch guideline 'Recurrent Miscarriage' in 1999.

Methods Questionnaires were sent to all practices for obstetrics and gynaecology in the Netherlands. Data concerned definition, diagnosis and treatment of recurrent miscarriage. Results were compared with a similar study conducted before the introduction of the guideline and with the recommendations in the guideline.

Results The response rate was 83%. Regarding gestational age, only 3% of the respondents used the definition as advised in the guideline. After the introduction of the guideline, thrombophilia factors were tested more frequently, anticoagulants were prescribed more frequently and more respondents reported to correct uterine malformations. Therapies not described in the guideline, e.g. donor insemination and oocyte donation, were still applied.

Conclusions The adherence to the Dutch guideline 'Recurrent Miscarriage' was rather poor, presumably due to guideline-related as well as physician-related barriers. Too many diagnostic tests and ineffective therapeutic interventions were performed. This study demonstrates the importance of appropriate implementation and revision.

Introduction

The incidence of recurrent miscarriage among couples trying to conceive is 1-3%. A causal factor can be identified in only half of these couples.[1,2] Primary antiphospholipid syndrome and structural chromosomal abnormalities are factors in which the causal relationship with recurrent miscarriage is the most evident.[3] Other risk factors are endocrine factors, uterine anomalies, thrombophilia and smoking. However, a proven effective therapy exists only in the case of antiphospholipid syndrome.[3]

The Dutch guideline 'Recurrent Miscarriage' was introduced in 1999.[4] Before the introduction of this guideline, a survey among Dutch gynaecologists demonstrated that no consensus existed on the definition and management of recurrent miscarriage and that diagnostic testing for factors without therapeutic consequences was performed frequently.[5]

Recently, many reports have been published, discussing the importance of evidence-based medicine in relation to recurrent miscarriage.[3-7] However, little is known on the actual diagnostic and therapeutic management of recurrent miscarriage and the impact of introducing guidelines on this topic.

To evaluate any changes in the management of recurrent miscarriage after the introduction of the guideline, we conducted a second survey among Dutch gynaecologists.

Materials and methods

A questionnaire was sent to all 101 practices for obstetrics and gynaecology in The Netherlands in July 2003. Eight of these practices were located in university hospitals, 36 practices in non-university teaching hospitals and 57 practices in non-teaching hospitals. The questionnaire consisted mainly of multiple choice questions, with the possibility to elucidate the answers. The questions dealt with the definition of recurrent miscarriage, risk factors and diagnostic testing and therapy in couples with recurrent miscarriage. Gynaecologists were asked

to indicate which factors they considered as risk factors, which diagnostic tests they ordered on a routine basis and which on a specific indication and which interventions were applied to prevent future miscarriages.
The data were compared with the results of the first survey conducted before the introduction of the guideline and with the recommendations made in the guideline 'Recurrent Miscarriage'.[4] The percentages in the tables are based on the total number of respondents who answered the questions.

Results

Completed questionnaires were returned by 84/101 (83%) of the practices, among which 7/8 (88%) were from university hospitals, 31/36 (86%) from non-university teaching hospitals and 46/57 (81%) from non-teaching hospitals. Two practices refused to participate and 15 practices did not respond.
In total, 74/77 (96%) of the respondents answered that a protocol for the management of recurrent miscarriage existed in their clinic, compared with 42% before the introduction of the guideline.

Definition

In the Dutch guideline, recurrent miscarriage is defined as three or more consecutive pregnancy losses, with a gestational age up to 22 weeks. This cut-off value for gestational age was applied by only 2/76 (3%) respondents, whereas 78% of the respondents used a maximal gestational age of 15–16 weeks, 12% a gestational age of 9 weeks and 1% used a different value. Before the introduction of the guideline, the cut-off value was similar: 71% of the respondents used a gestational age of 16–17 weeks, whereas the other respondents did not clarify this item at that time. 'Recurrent miscarriage' was defined as 3 or more miscarriages by 60/77 (78%) respondents and 2 or more by 22% of the respondents. Before the introduction of the guideline, this was similar (71% and 29%, respectively). For 50% of the respondents, it was essential that the recurrent miscarriage had occurred in consecutive pregnancies. For the other

respondents, this was irrespective of the outcome of interspersing pregnancies. The recurrent miscarriage had to occur with the same partner for 29/73 (40%) of the respondents, and for 60% of the respondents this was irrespective of the partner(s). Before the introduction of the guideline, these percentages were 34% and 46%, respectively. The remaining respondents had no opinion regarding this specific item.

Risk factors

Risk factors for recurrent miscarriage according to the respondents as well as the guidelines point of view are listed in Table I. In the previous survey, before the introduction of the guideline, this topic was not investigated. Although thyroid gland dysfunction and infections are not risk factors according to the guideline, 42% and 27%, respectively, of the respondents regarded them as such. On the other hand, a high LH level and/ or polycystic ovary disease are regarded as risk factors in the guideline, whereas they were not by 52% and 36%, respectively, of the respondents.

Table 1 Risk factors for recurrent miscarriage

	Dutch guideline risk factor yes/ no	Respondents risk factor (yes) (%)
Maternal age (years)	Yes	83
≥ *35*	*	31
≥ *36*	*	31
≥ *40*	*	25
Number of miscarriages	Yes	98
Structural chromosome abnormalities	Yes	98
Endocrine factors		
Increased LH/ polycystic Ovary disease	Yes	48
Thyroid dysfunction	No	42
Diabetes	Yes, if poorly regulated	65
Uterine anomalies	Yes	76
Infections	No	27
Coagulation		
Antiphospholipid syndrome	Yes	98
Thrombophilia	Yes	93
Hyperhomocysteinemia	Yes	95
Lifestyle		
Smoking	Yes	64
Alcohol	Questionable	45

Interview among 84 practices for obstetrics and gynaecology in the Netherlands, 2003
*not asked/ not described

Diagnostic testing

Tests applied in the case of recurrent miscarriage are listed in Table II. In the Dutch guideline, it is recommended that parental chromosome analysis be performed after two (or more) miscarriages and other tests only after three (or more). Almost all respondents (96–98%) claimed to have performed parental chromosome analysis in couples with recurrent miscarriage, compared with 78% before the introduction of the guideline. Parental chromosome analysis was offered after 2 miscarriages by 63/77 (82%) respondents and after 3 by 18% of the respondents. Other diagnostic tests were applied after 2 miscarriages by 24/76 (32%) respondents and after 3 by 52/76 (68%) respondents. In the

Dutch guideline, hysterosalpingography or hysteroscopy is advised on a specific indication. However, to examine the uterine cavity, 4% of the respondents reported to have performed saline-infusion sonography on a routine basis and 32% only on a specific indication. Using this technique, the uterine cavity is examined sonographically while saline is infused in the uterus. In the Dutch guideline, this rather new technique is not yet mentioned.

Tests for endocrine abnormalities were claimed to be performed frequently before as well as after the introduction of the guideline. Besides examining LH/FSH and plasma glucose on indication, this is not recommended in the Dutch guideline.
Many of the respondents routinely screened for antiphospholipid syndrome. Testing for other thrombophilia factors is also performed more frequently after the introduction of the guideline. In particular, protein C (75% versus 31%), protein S (74% versus 30%), antithrombin III (62% versus 23%) and factor V Leiden (65% versus 12%) are investigated on a routine basis. In the Dutch guideline, this is recommended only on a specific indication. Activated protein C resistance was performed as a routine test by 73% of the respondents after the introduction of the guideline. This item was not included in the questionnaire in 1996. Other tests were also ordered on a routine basis, whereas in the guideline, they are advised only on a specific indication; for example, APTT, DRVVT and prenatal diagnosis in the case of structural chromosome abnormalities.
Testing for infections was still applied, although by less respondents, whereas in the guideline, it is stated that this is not indicated. Immunological testing was no longer performed after the introduction of the guideline. Ultrasound examination in early pregnancy was performed frequently before as well as after the introduction of the guideline (85% and 83%, respectively), even though it is not mentioned in the guideline.

Table 2 Diagnostic testing in case of recurrent miscarriage

	Goddijn *et al.*, 1999	Dutch guideline	Respondents	
	Routine test (%)		Routine test (%)	On indication (%)
Genetic factors				
Maternal karyotype	78	After 2 miscarriages	96	4
Paternal karyotype	78		98	2
Uterine factors				
Ultrasound	79	Yes	93	2
Hysterosalpingography	56	On indication	17	51
Hysteroscopy	41	On indication	8	67
Saline infusion sonography	*	*	4	32
MRI	10	*	0	11
Endocrine factors				
Endometrial biopsy	20	*	0	7
Serum progesterone	52	*	22	7
Serum LH/ FSH	46	Optional	35	6
TSH	72	*	52	19
T4/ T3	40	*	25	23
FT4	46	*	31	23
Plasma glucose	65	On indication	55	12
Antiphospholipid syndrome				
Platelet count	43	*	35	8
Lupus anticoagulans	69	On indication	94	6
Anticardiolipin (IgG, IgM)	56	Yes	89	7
ANA	27	No	40	11
APTT	29	Yes	53	8
DRVVT	7	On indication	13	6
Trombophilia				
PTT	19	*	27	11
Protein C	31	On indication	75	18
Protein S	30	On indication	74	18
Factor II	*	*	32	18
Factor VIII	*	*	30	15
Factor XII	17	On indication	28	17
Plasminogen	13	*	8	11
AT III	23	On indication	62	18
APC-resistence	*	On indication	73	14
Factor V Leiden mutation	12	On indication	65	21

Infections				
Ureaplasma urealyticum	20	No	1	7
Mycoplasma hominis	41	No	1	8
Chlamydia trachomatis	29	No	25	13
CMV	17	No	10	11
Other infections	20	No	6	7
Immunologic factors				
Natural killer cell activity	2	No	0	5
HLA-sharing	3	No	0	5
Blocking antibodies	2	No	0	5
CL-precursors	2	No	0	5
Miscarriage product				
Karyotype	17	*	8	8
Chorionic villus biopsy	2	*	0	6
Virology	6	*	1	4
Ongoing pregnancy				
HCG	21	*	6	4
Progesterone	6	*	5	5
Ultrasound < 8 weeks	85	*	83	5
HbA1c	16	*	11	18
Serum glucose	33	No	30	13
PND	7	On indication	5	35

Interview among 84 practices for obstetrics and gynaecology in the Netherlands, 2003
*not asked/ not described; MRI, magnetic resonance imaging; TSH, thyroid-stimulating hormone; T3, tri-iodothyronine, T4, thyroxine; FT4, free thyroxine; ANA, anti nuclear antibodies; APTT, activated partial thromboplastin time; DRVVT, dilute Russel's viper venom time; PTT, partial thromboplastin time; AT III, antithrombin III, APC, activated protein C; CMV, cytomegalovirus; HLA, human leucocyte antigen; CL precursor, cytotoxic T-lymphocyte precursor; HbA1c, haemoglobin A1c glycolysated; PND, prenatal diagnosis.

Therapy

Therapeutic interventions performed to prevent further miscarriage are listed in Table III. After the introduction of the guideline, more respondents advised to stop smoking (80% compared with 46%) and prescribed folic acid (85% compared with 53%). Furthermore, more respondents claimed to correct uterine anomalies (uterine septum resection 74% compared with 23%, myomectomy 79% compared with 39%, cerclage 43% compared with 18%), even though this is not recommended in the guideline. Fewer respondents prescribed progesterone (13% compared with 28%) and HCG (7% compared with 21%). After the introduction of the guideline, more respondents prescribed anticoagulants; aspirin 51% compared with 27% and heparin 45% compared with 9%. In

total, 57% of the respondents reported to prescribe aspirin in combination with heparin to prevent further recurrent miscarriage. In 1996, this item was not mentioned in the questionnaire.
Donor insemination and oocyte donation are not mentioned in the guideline, whereas 37% and 35%, respectively, of the respondents reported to apply these interventions.

Table 3 Therapeutic interventions in case of recurrent miscarriage

	Goddijn *et al.*, 1999 (%)	Dutch guideline	Respondents (%)
General			
Discourage smoking	46	Yes	80
Discourage coffee consumption	4	*	29
Folic acid	53	Yes, if hyperhomocysteinemia	85
Genetic factors			
Donor insemination	7	*	37
Oocyte donation	2	*	35
Uterine factors			
Septum resection	23	Not proven	74
Myomectomy	39	*	79
Cerclage	18	*	43
Endocrine factors			
Progestagens	28	No	13
Clomiphene citrate	15	*	11
HCG	21	No	7
T4	3	*	20
Corticosteroids	1	*	5
Tocolysis	2	*	4
Coagulation			
Aspirin	27	No	51
Heparin	9	No	45
Combination aspirin/ heparin	*	Yes, if antiphospholipid syndrome	57
Infections			
Antibiotics	0	No	11
Immunologic factors			
Immunisation IgG	0	*	11
Immunisation leukocytes	0	No	8
Immunisation other	0	*	10

Interview among 84 practices for obstetrics and gynaecology in the Netherlands, 2003
*not asked/ not described

Discussion

The existence of a previous survey on the definition and management of recurrent miscarriage enabled us to compare its management before and after the introduction of the Dutch guideline in 1999 and thereby to evaluate its impact. We demonstrated that the adherence to the guideline is rather poor. Even though the introduction of the guideline 'Recurrent Miscarriage' resulted in more structural testing for antiphospholipid syndrome, and aspirin and heparin were prescribed more frequently, many tests not recommended in the guideline were still applied and ineffective therapy was offered frequently. The high response rate (83%) suggests that the results of this study are representative for the management of recurrent miscarriage in The Netherlands.

Since 2004, the Dutch Society of Obstetrics and Gynaecology has explicit instructions for developing guidelines on the basis of the criteria of the Appraisal of Guidelines, Research and Evaluation in Europe (AGREE) instrument.[8] However, in 1999, at the time the guideline 'Recurrent Miscarriage' was developed, these instructions were not as detailed. The guideline was developed by experts in the field. The concept was discussed by a guideline committee, after which it was put to the vote and approved by the members of the Dutch Society of Obstetrics and Gynaecology. After approval, it was published on the Society's website and a paper version was sent to the members. The validity of the guidelines expires after 5 years, and the present guideline is under revision.

The reasons for not following a guideline can be diverse. Barriers to guideline adherence can be guideline related (the guideline can be outdated, difficult to use or items can be controversial), physician related (for instance, lack of awareness or agreement), patient related (resistance to guideline recommendations) or related to environmental factors (for instance, lack of a reminder system or counselling materials).[9] In The Netherlands, in the field of Reproductive Medicine, it has been reported that adherence to the guideline on intrauterine insemination was mainly impeded by the physician's lack of self-efficacy and low-outcome expectancy.[10]

Reasons for not following the Dutch guideline 'Recurrent Miscarriage' may be guideline related. To make good guidelines on the topic of recurrent miscarriage

is extremely difficult. Many aetiological factors, tests and treatments for recurrent miscarriage are still controversial.[3,6] In 2003, at the time this survey was conducted, new data had become available, requiring a different approach. For instance, in the guideline, alcohol consumption is considered a questionable risk for recurrent miscarriage, but a later study documented that alcohol consumption during pregnancy strongly increases its risk.[11] Elevated LH levels are considered risk factors for recurrent miscarriage in the guideline, but later studies could not confirm this.[12,13] In the guideline, testing for the antiphospholipid syndrome is recommended on a specific indication, whereas after the development of the guideline, international consensus was reached on the usefulness of routine testing.[14] In the guideline, ultrasound examination is recommended to detect intracavitary uterine anomalies, whereas later it was demonstrated that saline-infusion sonography is a much more sensitive and specific investigation for this purpose.[15] The guideline may also be confusing or difficult to use. The definition of recurrent miscarriage according to the Dutch guideline is seldom followed. Lack of international consensus on a definition may be a contributing factor in this respect.[3,16] In the Dutch guideline, it is recommended that parental chromosome analysis be performed after two miscarriages and the remaining tests after three. In The Netherlands, for convenience, this might result in performing the complete workups after two miscarriages. In most other countries, the complete workup is recommended after three miscarriages.

Other reasons for not following the guideline seem to be physician related rather than guideline related. For example, testing serum glucose without clinical manifestation of diabetes is not effective.[17] Many tests were performed on a routine basis, even though it is advised to perform these tests only in the presence of a specific indication, for example, testing for thrombophilia. After the introduction of the guideline, more gynaecologists reported to correct uterine anomalies to prevent recurrent miscarriage, even though no convincing evidence exists on its effectiveness.[18] This is also the case for artificial insemination with donor semen and for oocyte donation. Ultrasound examination in early pregnancy was frequently performed even without clinical implications. Justification for doing so may, however, be to reassure the patient in the case of an ongoing pregnancy.[19]

The data of this study indicate that physicians and/or patients apparently wish to initiate treatment for recurrent miscarriage, even if the effectiveness has not been established or has proved to be ineffective. On the other hand, if there is a physicians' lack of awareness of the guideline, this may be due to poor advertisement and dissemination to interested parties. Successful implementation of the guideline requires more interventions than distribution or (electronic) publication, such as educational meetings, local consensus processes, the employment of local opinion leaders and audit and feedback.[20] In the UK and Scandinavia, early pregnancy units exist with main focus on early pregnancy loss. In The Netherlands and other European countries, these specialized units are not established so far. It could be expected that centralized care also leads to more consensus in management and better adherence to guidelines.

In conclusion, the adherence to the Dutch guideline 'Recurrent Miscarriage' is rather poor, presumably due to guideline-related as well as physician-related barriers. Too many diagnostic tests and ineffective therapeutic interventions are performed. This study demonstrates the importance of appropriate implementation and revision.

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3

Selective chromosome analysis in couples with two or more miscarriages: case-control study

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Abstract

Objective To identify additional factors, such as maternal age or factors related to previous reproductive outcome or family history, and the corresponding probability of carrying a chromosome abnormality in couples with two or more miscarriages.

Design Nested case-control study.

Setting Six centres for clinical genetics in the Netherlands.

Participants Couples referred for chromosome analysis after two or more miscarriages in 1992-2000; 279 carrier couples were marked as cases, and 428 non-carrier couples served as controls.

Main outcome measures Independent factors influencing the probability of carrier status and the corresponding probability of carrier status.

Results Four factors influencing the probability of carrier status could be identified: maternal age at second miscarriage, a history of three or more miscarriages, a history of two or more miscarriages in a brother or sister of either partner, and a history of two or more miscarriages in the parents of either partner. The calculated probability of carrier status in couples referred for chromosome analysis after two or more miscarriages varied between 0.5% and 10.2%.

Conclusions The probability of carrier status in couples with two or more miscarriages is modified by additional factors. Selective chromosome analysis would result in a more appropriate referral policy, could decrease the annual number of chromosome analyses, and could therefore lower the costs.

Introduction

Couples who have had two or more miscarriages are at increased risk of either of the partners carrying a structural chromosome abnormality. The incidence of carrier status increases from approximately 0.7% in the general population to 2.2% after one miscarriage, 4.8% after two miscarriages, and 5.2% after three miscarriages.[1,2] If one of the partners carries a structural chromosome abnormality, products of conception can have a normal karyotype, the same karyotype as the carrier parent, or an unbalanced karyotype. The last of these can lead to miscarriage, stillbirth, or the birth of a child with major congenital impairments. Prenatal diagnosis is therefore offered to carrier couples in subsequent pregnancies. No consensus exists between current guidelines for the management of recurrent miscarriage on whether chromosome analysis should be offered after two or three miscarriages. For example, the Royal College of Obstetricians and Gynaecologists recommends chromosome analysis after three miscarriages, whereas the American College of Obstetricians and Gynaecologists and the Dutch Society of Obstetrics and Gynaecology recommend chromosome analysis after two miscarriages.[3–5]

These guidelines are based on the fact that the probability of carrier status is increased after two or three miscarriages. Whether this probability is also modified by maternal age or by factors related to previous reproductive outcome or family history is not known. If it is, the possibility of withholding chromosome analysis from couples with a low probability of carrier status could be considered. We aimed to identify additional factors influencing the probability of carrier status in couples with two or more miscarriages and to calculate the associated probability of carrier status for every combination of these factors.

Methods

Patients

We used the databases of six centres for clinical genetics in the Netherlands to identify all couples referred for chromosome analysis after two or more miscarriages between 1 January 1992 and 1 January 2001.We marked as cases all couples in which one of the partners was found to be a carrier of a structural chromosome abnormality. As controls, we selected a random subset of two non-carrier couples for each carrier couple by identifying the last couple tested before the carrier couple and the first couple tested after the carrier couple in each centre. We recorded karyotypes according to the recommendations of the International Standing Committee on Human Cytogenetic Nomenclature.[6] We included only couples with at least two miscarriages with a gestational age up to 20 weeks and verified by a pregnancy test or ultrasonography. We excluded patients with other genetic diseases likely to cause fetal chromosome abnormalities and those with a language barrier.

Data collection

We contacted eligible couples by mail and invited them to participate in the study. After obtaining written informed consent, we examined the medical records of the relevant department of clinical genetics, and both partners filled out a questionnaire. We collected additional information by using telephone interviews and from medical records of the referring physician or midwife. The data collection was focused on the parental characteristics at the time of chromosome analysis, including general history, maternal age, obstetric history, and family history.

Statistical analysis

We used logistic regression analysis to identify factors influencing the probability of carrier status and to calculate the corresponding probability of carrier status. We divided variables into five subgroups: general history; maternal age at chromosome analysis, at first miscarriage, and at second miscarriage; number

of miscarriages; obstetric history; and family history. We used splines analysis to determine whether a linear relation existed between continuous variables and the probability of carrier status. In the case of a non-linear relation, we transformed continuous variables into categorical variables on the basis of the results of the splines analysis. We then did univariate logistic regression analysis with all variables. We retained variables with $P \leq 0.2$ in the univariate analysis for subsequent steps.

In the multivariate logistic regression analysis, we added variables to the model by subgroup. We retained only variables with $P \leq 0.1$ in the model. If two variables were highly correlated, we retained the one leading to the best improvement of the model. To determine whether the sequence of the subgroups influenced the final model, we repeated the analysis using different selection orders and comparing the results from each model.

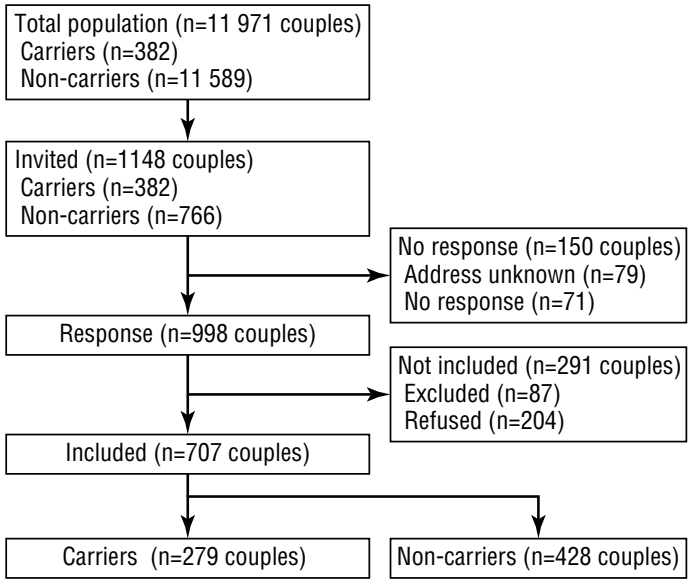
At selection, we matched the non-carrier couples to the carrier couples within each genetic centre and by time of chromosome analysis. To exclude a bias introduced by these potential confounders, we compared the results of logistic regression analysis with the results of conditional regression analysis.

As this was a nested case-control study, we had to adjust the model for the relative proportions of cases and controls in the total population of couples referred for chromosome analysis after two or more miscarriages.[7] We then calculated the probability of carrier status from the final model for every combination of variables. We used SPSS 11.5.1 for all analyses.

Results

Between 1 January 1992 and 1 January 2001, 11 971 couples had been referred to the participating centres for chromosome analysis after two or more miscarriages. We invited 1148 couples to participate in the study - all 382 carrier couples and 766 non-carrier couples. We included 62% of the invited couples - 279 (73%) carrier couples and 428 (56%) non-carrier couples (fig 1).

Figure 1 Flowchart of trial population and inclusion



Couples had been referred by gynaecologists from general hospitals (56%); gynaecologists from academic hospitals (29%); geneticists (11%); and general practitioners, midwifes, and paediatricians (4%). For 94% of couples the country of birth was the Netherlands.

At the time of chromosome analysis, differences existed between carrier couples and non-carrier couples (table 1). The mean maternal age was significantly lower and the mean number of miscarriages was significantly higher in carrier couples than in non-carrier couples.

The 279 structural chromosome abnormalities recorded consisted of 174 (62%) reciprocal translocations, 44 (16%) Robertsonian translocations, 3 (1%) (Y;22) translocations, 21 (8%) pericentric inversions, 21 (8%) paracentric inversions, 7 (3%) marker chromosomes, and 9 (3%) other structural chromosome abnormalities. Male and female carriers were not distributed equally: 177 (63%) carriers were women and 102 (37%) carriers were men.

Table 1 Baseline characteristics of couples at time of chromosome analysis
values are mean (range) unless stated otherwise

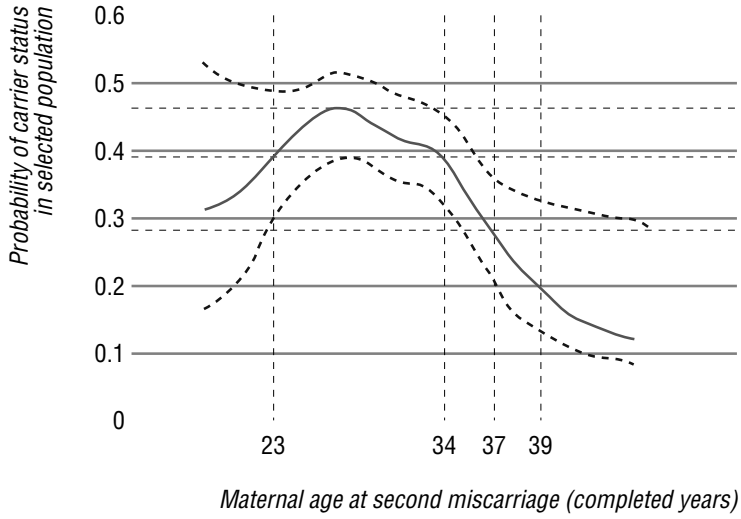
Characteristics	Carriers	Non-carriers	P value
No (%) of couples with parental chromosome analysis			0.010*
after 2 miscarriages	108 (39)	212 (50)	
after 3 miscarriages	112 (40)	153 (36)	
after ≥ 4 miscarriages	59 (21)	63 (14)	
Maternal age (years), at time of			
chromosome analysis	31.8 (20-43)	32.7 (19-47)	0.012†
first miscarriage	29.0 (17.3-41.3)	30.2 (16.0-47.7)	0.001†
second miscarriage	30.5 (19.0-41.5)	31.6 (17.7-48.1)	0.002†
Miscarriages before chromosome analysis			
no of miscarriages	3.0 (2-10)	2.8 (2-12)	0.002†
gestational age (weeks)	9.4 (5.2-15.3)	9.4 (4.8-15.0)	0.925†
No of children before parental chromosome analysis			
healthy	0.6 (0-6)	0.7 (0-5)	0.151*
stillborn	0.04 (0-1)	0.04 (0-1)	0.793*
diseased	0.01 (0-1)	0.02 (0-1)	0.404*
Ill or handicapped	0.05 (0-2)	0.04 (0-1)	0.462*

*χ^2 test
†Student's *t* test

A non-linear relation existed between maternal age and the log odds of carrier status. On the basis of the results of splines analysis, we decided to divide maternal age at second miscarriage into five categories: < 23 years, 23-33 years, 34-36 years, 37-38 years, and ≥ 39 years (fig 2). Figures for the other age variables were similar (data not shown). Variables with P ≤ 0.2 in univariate analysis were retained for multivariate analysis (table 2).

After multivariate logistic regression analysis, four factors influencing the probability of carrier status were retained in the final model: maternal age at second miscarriage, a history of three or more miscarriages, a history of two or more miscarriages in a brother or sister of either partner, and a history of two or more miscarriages in the parents of either partner (table 3). The sequence in which we added the subgroups did not influence the final model. Application of conditional regression analysis did not substantially alter the results.

Figure 2 Splines analysis: probability of carrier status of maternal age at second miscarriage, with 95% confidence intervals



Probability of carrier status is based on the selected population of included couples (279 carrier couples; 428 non-carrier couples); numbers of carrier couples and non-carrier couples need to be adjusted to determine the probability of carrier status in the total screening population

Table 2 Factors influencing the probability of carrier status after univariate logistic regression analysis (P≤0.20)

Risk factors	OR	95% CI	P value
Maternal age (years) at first miscarriage			0.001
<22	4.3	1.2 to 14.9	
22-31	4.7	1.6 to 13.8	
32-34	3.5	1.1 to 10.1	
35-37	1.7	0.5 to 5.8	
≥38	1.0	-	
Maternal age (years) at second miscarriage			0.006
<23	4.6	1.3 to 16.6	
23-33	4.0	1.4 to 12.0	
34-36	2.6	0.8 to 8.1	
37-38	1.8	0.5 to 6.2	
≥39	1.0	-	
Number of miscarriages			
3 and ≥4 compared to 2 miscarriages			0.010
2 miscarriages	1.0	-	
3 miscarriages	1.4	1.0 to 2.0	
≥4 miscarriages	1.8	1.2 to 2.8	
≥3 compared to 2 miscarriages	1.6	1.1 to 2.1	0.005
General history			
Exposure to radiation, either partner	0.3	0.1 to 1.4	0.140
Obstetric history			
≥1 ectopic pregnancies	0.5	0.2 to 1.2	0.117
≥1 healthy children	0.7	0.6 to 1.0	0.062
Family history			
≥2 miscarriages in a brother or sister	1.7	1.1 to 2.6	0.021
≥2 miscarriages in parents	1.5	0.1 to 2.2	0.055
Exposure to diethylstilbestrol	0.5	0.2 to 1.3	0.144

Table 3 Factors influencing the probability of carrier status after multivariate logistic regression analysis (P≤0.10)

Covariates	OR	95% CI	P value
Maternal age (years) at second miscarriage			
<23	6.2	1.1 to 34.3	0.04
23-33	6.1	1.3 to 27.7	0.02
34-36	3.3	0.7 to 16.1	0.13
37-38	2.3	0.4 to 12.0	0.33
≥39	1.0	-	-
3 vs. ≥2 miscarriages	1.4	1.0 to 2.1	0.05
≥2 miscarriages in a brother or sister	1.9	1.1 to 3.2	0.02
≥2 miscarriages in parents	1.4	0.9 to 2.2	0.10

Multivariate regression analysis was limited to 528 couples in whom the data collection was complete.

We calculated the probability of carrier status for every combination of variables in the final model (table 4). We found a probability of carrier status of 10.2% in couples with a maternal age < 23 years at the second miscarriage, referred after three or more miscarriages, and with a brother or sister as well as parents with a history of two or more miscarriages. At lowest risk (0.5%) were couples with a maternal age ≥ 39 years at the second miscarriage, referred after two miscarriages, and without a brother or sister or parents with a history of two or more miscarriages. Couples with a probability of carrier status below 2.2%, which is the reported incidence in couples with only one miscarriage, are noted in table 4.

As the multivariate model can be used only if all variables are known, which may not always be the case, we also built a model with maternal age at second miscarriage as the only variable (table 5). According to this model, couples with a maternal age of ≥ 37 years have a probability of carrier status below 2.2%.

If chromosome analysis had been withheld from couples with a probability of carrier status below 2.2%, the number of chromosome analyses would be reduced by 18% according to the multivariate model. If the model based on maternal age at the second miscarriage was applied, the reduction would be 7% (table 6).

Table 4 Probability of carrier status in couples with two or more miscarriages, according to multivariate regression model*

Maternal age (years) at second miscarriage	(RM_{bs})	($RM_{parents}$) +		($RM_{parents}$) -	
		≥3 misc	2 misc	≥3 misc	2 misc
<23	+	10.2%	7.3%	7.3%	5.2%
	–	5.7%	4.0%	4.1%	2.8%
23-33	+	10.0%	7.2%	7.2%	5.1%
	–	5.7%	4.0%	4.0%	2.8%
34-36	+	5.8%	4.1%	4.1%	2.9%
	–	3.2%	2.2%	2.2%	1.6%
37-38	+	4.0%	2.8%	2.8%	2.0%
	–	2.2%	1.5%	1.5%	1.1%
≥39	+	1.8%	1.2%	1.3%	0.9%
	–	1.0%	0.7%	0.7%	0.5%

RM_{bs-}=history of ≥miscarriages in a brother or a sister of either partner; $RM_{parents}$= history of ≥miscarriages in parents of either partner; ≥3 misc=history of ≥3 miscarriages in couple; ≥2 misc=history of ≥2 miscarriages in couple
*Limited to 528 couples with complete data
Grey area: couples with probability of carrier status <2.2%
Intercept based on the total population=-5,388

Table 5 Probability of carrier status in couples with two or more miscarriages, according to maternal age at second miscarriage

Maternal age (years) at second miscarriage	Probability of carrier status (%)
<23	4.2
23-33	3.7
34-36	2.4
37-38	1.7
≥39	0.9

Grey area: couples with probability of carrier status <2.2%
Logistic regression analysis limited to 669 couples with complete data
Intercept based on the total population = -4.648

Table 6 Couples with chromosome analysis, and percentage reduction compared with current policy in period 1992-2001

Screening strategy	Couples analysed*		Reduction†		
	Carriers	Non-carriers	Carriers (%, *95% CI*)	Non-carriers (%, *95% CI*)	Total reduction (%, 95% CI)†
Current policy	382	11 589	-	-	-
Restricted policy based on four predictive factors‡	351	9 503	31 (8, 6 to 11)	2086 (18, 17 to 19)	2117 (18, 17 to 18)
Restricted policy based on maternal age at second miscarriage	359	10 812	23 (6, 4 to 9)	777 (7, 7 to 8)	800 (7, 7 to 8)

*Numbers of analysed couples adjusted to numbers of carrier couples and non-carrier couples in total population.
†Reduction if chromosome analysis withheld from couples with probability of carrier status <2.2%.
‡Maternal age at second miscarriage; ≥3 miscarriages; history of ≥2 miscarriages in a brother or sister of either partner; history of ≥2 miscarriages in parents of either partner

Discussion

The results of this study show that in couples with two or more miscarriages, more factors than just the number of miscarriages influence the probability of carrier status. Low maternal age at second miscarriage, a history of three or more miscarriages, a history of two or more miscarriages in a brother or sister of either partner, and a history of two or more miscarriages in the parents of either partner all increase the probability of carrier status. We have shown that the efficiency of parental chromosome analysis could be increased by withholding the test from couples with a low probability of carrier status.

Possible limitations

The response rate among carrier couples was higher than that among non-carrier couples. This might be explained by a better understanding of the condition among carrier couples. A difference may also exist in the accuracy of data

obtained by questionnaires between carrier couples and non-carrier couples. For example, carrier couples might have a better knowledge of their family history. Even though many answers were confirmed by information from medical records, the existence of such a "recall bias" cannot be ruled out entirely.
The multivariate analysis included only couples in whom all risk factors were known; 528 of the 707 couples remained for multivariate analysis. Reduction of the sample size did not, however, change the proportions of carrier and non-carrier couples.

Comparison with literature

The reported incidence of carrier status in couples with recurrent miscarriage varies between 3.6% and 5.8%.[2,8,9] In this study, the incidence of carrier status was relatively low at 3.2%.
This lower incidence might be explained by our use of more restrictive selection criteria for structural chromosome abnormalities. We recorded structural chromosome abnormalities according to the recommendations of the International Standing Committee on Human Cytogenetic Nomenclature, and we did not mark people with a sex chromosome aneuploidy, a chromosome polymorphism, or a low level mosaicism as carriers.[6]
Identifying factors that influence the probability of carrier status and calculating the probability of carrier status by using a multivariate model has not been described previously. We found that maternal age at second miscarriage was the most influential factor and that the probability of carrier status decreased at advanced maternal age. Sporadic miscarriage rates increase steeply in women in their late 30s or older.[8] The recurrence of miscarriage in this group is probably more often due to age related chromosome abnormalities, mainly trisomies, than to structural chromosome abnormalities.[10–14]
The couples that had chromosome analysis in the Academic Medical Hospital have been presented elsewhere.[16] In this much smaller cohort, we found no significant difference in the incidence of carrier status between couples with maternal age below 36 years and couples with maternal age of 36 years and older. In the study reported here, we have clearly shown the influence of maternal

age on the probability of carrier status. This can probably be explained by the larger sample size in this study.

The available literature is divided as to whether the incidence of carrier status is higher after three miscarriages than after two miscarriages. Some studies have reported no significant difference, whereas others have reported a significant increase in the incidence of carrier status after three miscarriages.[17–19] Unlike our study, these studies all described series of patients without controls. We have shown an independent influence of a history of three or more miscarriages, compared with two miscarriages, on the probability of carrier status. This influence was less evident in the multivariate analysis than in the univariate analysis, because the number of miscarriages was, to some extent, correlated with the maternal age at the time of the miscarriages.

We have shown that a history of two or more miscarriages in a brother or sister of either partner or a history of two or more miscarriages in the parents of either partner influences the probability of carrier status in couples with two or more miscarriages. This finding is supported by the fact that structural chromosome abnormalities can exist within families.[20,21]

Clinical implications

Given the results of this study, the efficiency of chromosome analysis in couples with recurrent miscarriage needs to be reconsidered. We question whether offering chromosome analysis for all couples after two or three miscarriages can still be justified. After one miscarriage, in which the reported incidence of carrier status is 2.2%, chromosome analysis is not recommended. As a probability of 2.2% is apparently considered acceptable, it would seem reasonable to withhold chromosome analysis from couples with an even lower probability as well. However, 8% of the carrier couples would have remained undetected if selective chromosome analysis had been applied. The consequences of undetected carrier status is an important topic for future research.

We cannot exclude the possibility that in another clinical setting the savings might not be the same as in our study population. The referral practice might be different in other countries. Nevertheless, the results of this study are of great

interest in all countries, as we have shown that the number of miscarriages is not the only factor that should be taken into account. If couples are analysed after two miscarriages, many low risk couples will be analysed as well, such as couples with maternal age at second miscarriage between 34 and 36 years, without brothers or sisters with two or more miscarriages, and without parents with two or more miscarriages. On the other hand, if couples are analysed only after three miscarriages, high risk couples will not be detected until they have a third miscarriage - for example, couples with maternal age at second miscarriage between 23 and 33 years and with brothers or sisters as well as parents with two or more miscarriages.

Conclusions

Selective chromosome analysis in couples with two or more miscarriages - that is, withholding chromosome analysis from couples with a low probability of carrier status - would result in a more appropriate referral policy, could decrease the annual number of chromosome analyses, and could therefore reduce the costs to the healthcare system.

What is already known on this topic
The incidence of structural chromosome abnormalities is increased in couples with recurrent miscarriage.
Currently, chromosome analysis is offered to both partners after two or three miscarriages.
What this study adds
Low maternal age at second miscarriage, a history of three or more miscarriages, a history of two or more miscarriages in a brother or sister, and a history of two or more miscarriages in parents of either partner all increase the probability of carrier status.
Selective chromosome analysis could reduce the number of chromosome analyses by 18%.

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4

Reproductive outcome after chromosome analysis in couples with two or more miscarriages: index-control study

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Abstract

Objective To compare reproductive outcomes in couples carrying a structural chromosome abnormality and non-carrier couples referred for chromosome analysis after two or more miscarriages.

Design Index-control study.

Setting Six centres for clinical genetics in the Netherlands.

Participants 278 carrier couples and 427 non-carrier couples referred for chromosome analysis between 1992 and 2000 after two or more miscarriages before 20 weeks of gestation. Couples were followed up for at least 24 months after chromosome analysis.

Main outcome measures The birth of at least one healthy child, at least one more miscarriage, and viable offspring with unbalanced chromosomal abnormalities after parental chromosome analysis.

Results Mean follow-up after chromosome analysis was 5.8 years. 120 of 247 (49%) carrier couples had one or more miscarriage after chromosome analysis compared with 122 of 409 (30%) non-carrier couples (difference 19%, 95% confidence interval 11% to 26%; $P < 0.01$). The percentage of couples with at least one healthy child was not significantly different in carrier couples (83%) and non-carrier couples (84%) (difference – 1%, – 7% to 5%). Among 550 pregnancies in carrier couples, two viable unbalanced chromosome abnormalities were detected at prenatal diagnosis (0.4%) and the fetuses aborted and two children with an unbalanced karyotype were born (0.4%).

Conclusions Couples whose carrier status was ascertained after two or more miscarriages have a low risk of viable offspring with unbalanced chromosomal abnormalities. Their chances of having a healthy child are as high as non-carrier couples, despite a higher risk of miscarriage.

Introduction

Balanced structural chromosome abnormalities (abnormalities that involve the rearrangement of genetic material but no overall gain or loss, such as inversions and translocations) in parents can cause recurrent miscarriage. In couples with two or more miscarriages the incidence of these abnormalities varies between 3% and 6%.[1-4] In carrier couples the products of conception can have a normal karyotype, the same balanced structural chromosome abnormality as the carrier, or an unbalanced structural chromosome abnormality. The last scenario can lead to the fetus being miscarried, a stillborn child, or a child born with major congenital defects and severe mental handicap. Current guidelines for the management of recurrent miscarriage recommend chromosome analysis in both partners.[5-7] Once a structural chromosome abnormality has been detected, prenatal diagnosis in subsequent pregnancies and termination of pregnancy in the case of an unbalanced fetal karyotype is available.

To counsel carrier couples about their risk of viable offspring with unbalanced chromosomal abnormalities and their chances of having a healthy child or miscarriage we need to know the outcome in a population with similar abnormalities. Reports of reproductive outcome in carrier couples whose carrier status was ascertained after recurrent miscarriage provide information on only the first pregnancy after chromosome analysis or on the results of prenatal diagnosis in subsequent pregnancies, or they lack detailed information on reproductive outcome.[8-13] In most studies a control group was not investigated, and they all studied small numbers of carrier couples.[8-13]

We aimed to investigate the long term reproductive outcome in carrier couples whose carrier status was ascertained after two or more miscarriages and to compare this outcome with that in non-carrier couples with two or more miscarriages.

Methods

Study design

We used the databases of six centres for clinical genetics in the Netherlands to identify all couples presenting for parental chromosome analysis between January 1992 and January 2001, after two or more miscarriages. Couples referred to the Academic Medical Centre, Amsterdam, have been presented elsewhere.[14] When one partner was found to carry a structural chromosome abnormality we identified the couple as a carrier couple. We selected a random subset of two non-carrier couples per carrier couple by identifying the non-carrier couples tested immediately before and after the carrier couple. This matching was performed to obtain a sample balanced over time. We selected couples with at least two verified miscarriages before 20 weeks of gestation. Exclusion criteria were fewer than two miscarriages verified by a pregnancy test or ultrasonography, or if the couple did not speak Dutch, and the presence of genetic diseases in the couple that might cause chromosomal abnormalities in the fetus.

We contacted eligible couples by mail and invited them to participate in our study. After written informed consent had been obtained, we examined the medical records of the relevant department of clinical genetics and asked both partners to complete a questionnaire. Non-responders received reminders. We collected additional information from telephone interviews and from the medical records of the referring doctor or midwife. Data collection focused on the reproductive outcome of both categories of couples, which was recorded for at least 24 months after chromosome analysis. The main outcome measure was a successful reproductive outcome, defined as the birth of one or more healthy (phenotypically normal) children. Other outcome measures were miscarriages and other adverse reproductive outcomes, including stillbirths, viable offspring with unbalanced chromosomal abnormalities, and viable offspring with other chromosomal or congenital abnormalities, detected either prenatally or after birth.

Cytogenetic analysis

We obtained chromosome preparations from routine peripheral blood lymphocyte cultures. At least five GTG banded metaphases (minimal 500 band level) were evaluated for each person. Karyotypes were recorded according to the recommendations of the international standing committee on human cytogenetic nomenclature 1995.[15] We did not classify individuals with sex chromosome aneuploidy, chromosomal polymorphism, or low level mosaicism as carriers.

Statistical analysis

We tested differences between carrier couples and non-carrier couples with the Student's *t* test for normally distributed continuous variables, the Mann-Whitney U test for nonparametric continuous variables, and the χ^2 test for categorical variables. P values < 0.05 were considered significant. All statistical analyses were performed using SPSS version 11.5.1.

Results

Baseline characteristics

Between January 1992 and January 2001, 11 971 couples were referred for parental chromosome analysis to the six participating centres after two or more miscarriages. A structural chromosome abnormality was found in 382 couples (3.2%). We invited 1148 couples to participate in our study: all 382 carrier couples and 766 non-carrier couples. Of those invited, 61% were eligible for inclusion: 278 couples with a balanced structural chromosome abnormality (73%) and 427 couples with normal parental karyotypes (56%). Reasons for non-participation were exclusion, refusal to participate, non-response, and unknown address.

Table 1 lists the baseline characteristics of the couples. A total of 320 couples (45%) had undergone chromosome analysis after two miscarriages, 263 couples (37%) after three miscarriages, and 122 couples (17%) after four or more miscarriages. The mean duration of follow-up was 5.8 (range 2.0-11.4) years.

We found significant differences between carrier couples and non-carrier couples. Women in carrier couples were younger at the time of chromosome analysis (mean 31.8 vs. 32.7 years, difference – 0.9 years, 95% confidence interval – 1.6 to – 0.2 years), had experienced more miscarriages (3.0 vs. 2.8), and had a lower mean number of healthy children (0.6 vs. 0.7).

The 278 recorded structural chromosome abnormalities consisted of 177 reciprocal translocations (64%), 43 Robertsonian translocations (15%), 21 pericentric inversions (8%), 21 paracentric inversions (8%), and 16 other structural chromosome abnormalities (6%). The sex distribution of carriers was unequal: 176 (63%) carriers were women.

Table 1 Baseline characteristics of couples carrying a structural chromosome abnormality and non-carrier couples referred for parental chromosome analysis after two or more miscarriages *values are numbers (percentages) of couples unless otherwise indicated*

	Carrier couples *(n=278)*	Non-carrier couples *(n=427)*	*P value*
Maternal age (years)			
Mean (SD)	31.8 (± 4.3)	32.7 (± 5.0)	
Median (inter quartile range)	32 (29 to 35)	32 (29 to 37)	0.02
Pregnant	73 (26.3%)	111 (25.9%)	0.56
Number of previous miscarriages			0.01
2	108 (38.8%)	212 (49.6%)	
3	111 (39.6%)	152 (35.6%)	
≥ 4	59 (21.2%)	63 (14.8%)	
Mean	3.0	2.8	<0.01
Number of healthy children			0.05
0	154 (55.4%)	207 (48.5%)	
1	98 (35.3%)	156 (36.5%)	
≥ 2	26 (9.4%)	64 (15.0%)	
Mean	0.6	0.7	0.04
Number of handicapped, stillborn or diseased children			0.75
No previous abnormal offspring	252 (90.6%)	384 (90.0%)	
≥ 1 abnormal offspring	26 (9.4%)	43 (10.0%)	

Follow-up

Figure 1 shows the follow-up of all couples entered in our study. After the results of chromosome analysis became available, 49 couples decided not to conceive (31 carrier couples (15%) and 18 non-carrier couples (6%)). In carrier couples the main reasons were the risk of having a child with congenital abnormalities (n = 17) and not wanting to have more miscarriages (n = 11). In non-carrier couples the main reasons were advanced maternal age (n = 10), fear of further miscarriages (n = 5), and other (n = 7).

Pregnancy occurred at least once after chromosome analysis in 239 carrier couples and 390 non-carrier couples. Table 2 shows the outcome of these pregnancies. A significantly greater proportion of carrier couples than non-carrier couples had one or more miscarriages after the analysis (120 of 249, 49% vs. 122 of 409, 30%; difference 19%, 95% confidence interval 11% to 26%).

Figure 1 Follow-up after parental chromosome analysis of 705 couples with two or more miscarriages

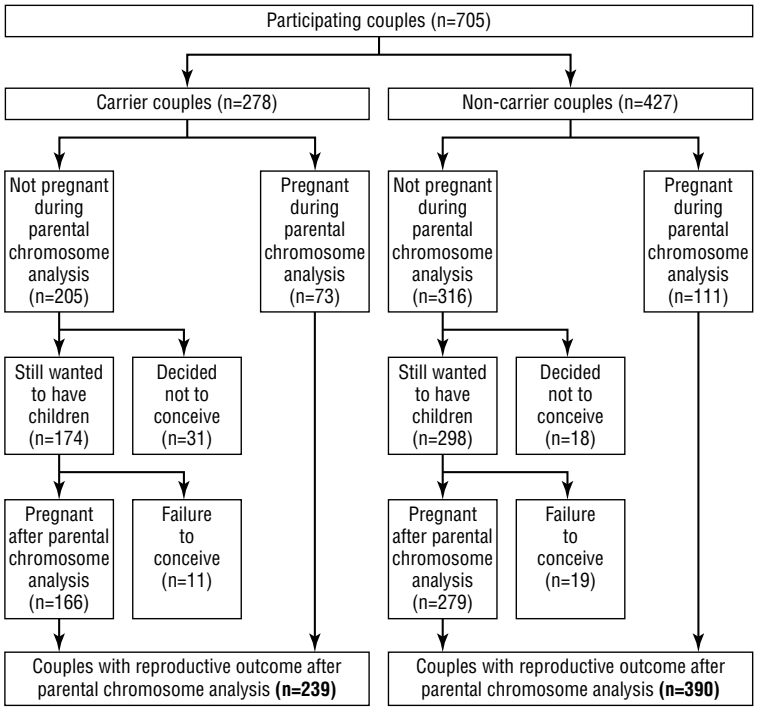

Table 2 Reproductive outcome after parental chromosome analysis in couples with recurrent miscarriage *values are numbers (percentages) unless otherwise indicated*

Reproductive outcome	Carrier couples *(n = 247)*	Non-carrier couples *(n =409)*	Difference in % (95% CI)§	P value
Failure to conceive	8 (3.2)	19 (4.6)	-1.4 (-4.4 to 2.0)	0.38
One or more miscarriages	120 (48.6)	122 (29.8)	18.8 (11.1 to 26.3)	<0.01
One or more terminated pregnancies	6 (2.4)	8 (2.0)	0.5 (-1.8 to 3.4)	0.69
One or more ectopic pregnancies	3 (1.2)	13 (3.2)	-2.0 (-4.3 to 0.7)	0.11
One or more stillbirths	3 (1.2)	6 (1.5)	-0.3% (-2.1 to 2.2)	0.79
One or more children who died postpartum	1 (0.4)	4 (1.0)†	-0.6 (-2.1 to 1.4)	0.41
One or more ill or handicapped children	2 (0.8)	11(2.7)‡	-1.9 (-4.0 to 0.5)	0.09
One or more healthy children	205 (83.0)	344 (84.1)	-1.1 (-7.2 to 4.6)	0.71

*Limited to couples who still wanted to conceive after chromosome analysis and those pregnant at the time of chromosome analysis
†One couple with children who died after birth
‡One couple with two ill or handicapped children
§Calculated difference might be different from the crude percentages owing to rounding off of numbers

The success rate -defined as the birth of a healthy child- was lower in carrier couples than in non-carrier couples for both the first pregnancy and second pregnancy after parental chromosome analysis (table 3). After the second pregnancy the rate of successful pregnancies was not significantly different in the two groups. In the total follow-up, at least one healthy child was born to 83% of the carrier couples and 84% of the non-carrier couples (difference – 1%, – 7% to 5%; P = 0.047%), and adverse pregnancy outcomes were similar in the two sets of couples.

Among the carrier couples, 85 of 157 (54%) with reciprocal translocations had one or more miscarriages compared with 18 of 37 (49%) with inversions, 13 of 38 (34%) with Robertsonian translocations, and 4 of 15 (27%) with other types of abnormality.

Proportions of couples giving birth to one or more healthy children during the follow-up period were similar in the various types of structural chromosome abnormality: 83% (131 of 157) for reciprocal translocations, 82% (31 of 38) for Robertsonian translocations, 78% (29 of 37) for inversions, and 93% (14 of 15) for other abnormalities.

Six pregnancies were terminated in carrier couples: three for social reasons; one because of trisomy 21, not related to the parental structural chromosome abnormality; and two because of an unbalanced karyotype resulting from a structural chromosome abnormality in the carrier. In these last two cases the parental structural chromosome abnormality had been ascertained after two or more miscarriages. In the first of these cases the fetal karyotype was 46,XY,der(18)t(3;18)(q27;p11.1) and the parental karyotype was 46,XY,t(3;18)(q27;p11.1). In the second case the fetal karyotype was 46,XY,der(9)t(3;9)(q25.3;p24) and the parental karyotype was 46,XX,t(3;9)(q25.3;p24).

Three stillbirths occurred in carrier couples after carrier status had been established. In all three cases the couple had not had prenatal diagnosis. In one case the karyotype of the child was not determined after birth. In another case the karyotype was uncertain owing to culture failure. In a third case culture also failed, but comparative genomic hybridisation showed no signs of an unbalanced karyotype. Congenital abnormalities were not found in any of the cases.

Two children with an unbalanced karyotype were born to carrier couples after parental chromosome analysis. In the first case the parental karyotype was 46,XX,t(16;22)(p13;q11.2). The unbalanced chromosome abnormality 46,XY,der(22)t(16;22)(p13;q11.2) was detected when amniocentesis was performed at 19 weeks of gestation. A severely handicapped child with Potter's syndrome and weighing 1500 g was born at 43 weeks; the child died immediately after birth. In the second case, the parents decided to refrain from prenatal diagnosis after the ultrasound scan in the second trimester was normal. The parental karyotype was 46,XX,t(6;8)(q26;q24.1). A child weighing 3900 g who had multiple congenital abnormalities and a 46,XY,der(6)t(6;8)(q26;q24.1) karyotype was born at 38 weeks of gestation.

One child with oesophageal atresia was born to a carrier couple. The karyotype of this child was not established because the abnormality was thought not to be related to the parent's chromosome abnormality.
In total, we found four unbalanced karyotypes: two were detected at prenatal diagnosis and followed by induced abortion, one was detected at prenatal diagnosis but not followed by pregnancy termination, and one was found in a severely handicapped child. All four unbalanced karyotypes resulted from a reciprocal translocation in one of the parents: three resulted from a translocation in the mother and one in the father.

Table 3 Successful reproductive outcome after parental chromosome analysis in couples with two or more miscarriages* *values are numbers (percentages) of couples unless otherwise indicated*

	Success rate per pregnancy†				Cumulative success rate‡			
	Carriers (n=239)	Non-carriers (n=390)	Difference in % (95% CI)	P value	Carriers (n=247)	Non-carriers (n=409)	Difference in % (95% CI)§	P value
Pregnancy after chromosome analysis								
1st	148/239 (62)	280/390 (72)	-10 (-18 to -2)	.01	148 (60)	280 (68)	-9 (-16 to -1)	<0.01
2nd	66/151 (44)	119/215 (55)	-12 (-22 to -1)	.03	173 (70)	324 (79)	-9 (-16 to -2)	<0.01
3rd	45/85 (53)	35/87 (40)	13 (-2 to 27)	.12	194 (79)	332 (81)	-3 (-9 to 4)	0.41
4th	14/40 (35)	18/48 (38)	-3 (-22 to 17)	.63	200 (81)	339 (83)	-2 (-8 to 4)	0.54
5th	10/23 (43)	6/23 (26)	17 (-10 to 41)	.22	205 (83)	342 (84)	-1 (-7 to 5)	0.84
6th	2/12 (17)	4/16 (25)	-8 (-36 to 24)	.60	205 (83)	342 (84)	-1 (-7 to 5)	0.84
Total follow-up	-	-		-	205 (83)	344 (84)	-1 (-7 to 5)	0.71

*Success rate defined as the birth of at least one healthy child.
†Limited to couples with at least one pregnancy after chromosome analysis.
‡Limited to couples pregnant during chromosome analysis or still wanted to conceive after the analysis, or both (including couples with failure to conceive after chromosome analysis).
§Calculated difference might be different from the crude percentages owing to rounding off of number

Discussion

The risk of viable offspring with unbalanced structural chromosomal abnormalities was low in carrier couples whose carrier status was ascertained after two or more miscarriages. Their chances of having a healthy child were as high as non-carrier couples, despite a higher risk of a subsequent miscarriage.

Comparison with related research

The incidence of structural chromosome abnormalities in our study (3.2%) was at the low end of the range of incidences found in previous studies (3-6%).[1–4] This might be because we used restrictive selection criteria for structural chromosome abnormalities, as recommended by the International Standing Committee on Human Cytogenetic Nomenclature.[15] We did not include individuals with a chromosomal polymorphism (such as inversion 9), low level mosaicism, or sex chromosome aneuploidy, abnormalities that are included in many other series describing the incidence of structural chromosome abnormalities in couples with recurrent miscarriage.

In agreement with two recent studies, we found that the birth of a healthy child at first pregnancy after chromosome analysis was lower in carrier couples (59%) than non-carrier couples (72%). Carp *et al*. found that a parental chromosomal abnormality decreased the chance of a live birth in the subsequent pregnancy: 45% of pregnancies in 73 carrier couples compared with 55% of pregnancies in 588 non-carrier couples, although this decrease was not significant.[8] Sugiura-Ogasawara *et al.* reported a significantly increased rate of miscarriage in the first pregnancy after chromosome analysis: 52% in 49 carrier couples compared with 28% in 1184 non-carrier couples.[9]

Limitations

Out of a total of 550 pregnancies after parental chromosome analysis in couples whose carrier status was ascertained after recurrent miscarriage, only two cases of viable offspring with chromosomal abnormalities were detected at prenatal diagnosis (0.4%) after which the pregnancies were terminated. In two other

cases severely handicapped children with an unbalanced structural chromosome abnormality were born (0.4%). Even though the response rate among carrier couples was good (73%), a selection bias could have occurred: couples with viable offspring with unbalanced chromosome abnormalities may have been more likely to refuse to participate in our study, thus leading to an under-representation of such abnormalities.

Implications

The two earlier studies had small numbers of carrier couples and limited their observations to the pregnancy immediately after parental chromosome analysis.[8,9] We recorded successive pregnancy outcomes during a long follow-up period (mean duration of 5.8 years) to obtain more accurate information on long term reproductive outcome. In our cohort, 83% of the carrier couples and 84% of the non-carrier couples gave birth to at least one healthy child after chromosome analysis; this finding could have implications for the counselling of couples with recurrent miscarriage due to chromosome abnormalities. However, a subgroup of women who repeatedly miscarry (four or more miscarriages) may have a worse prognosis because other factors might contribute to their miscarriages.

Currently, counselling couples about their risk of having a child with an unbalanced karyotype is based mainly on empirical risk estimates or databases that lack exact data on reproductive history or outcome, or both.[16-18] The risk of viable offspring with chromosomal abnormalities depends on the chromosome segment involved, the sex of the carrier parent, and the mode of ascertainment. In general, carrier couples ascertained after the birth of an affected child are at the highest risk of having viable offspring with chromosomal abnormalities (20-22%), whereas couples ascertained after recurrent miscarriage have an estimated risk of 2% to 5% (derived from data obtained by prenatal diagnosis after parental chromosome analysis).[19,20]

In our cohort, less than 2% of carrier couples had viable offspring with unbalanced chromosomal abnormalities: two cases were detected at prenatal diagnosis after which the pregnancies were terminated (0.4%) and two severely handicapped children (0.4%) were born. In the 278 carrier couples in our study, structural

chromosome abnormalities more commonly resulted in miscarriage rather than viable offspring with unbalanced chromosomal abnormalities. However, more than 10% of carrier couples decided not to conceive after parental chromosome analysis, so there may be a case for changing the guidance to these couples.
Preimplantation genetic diagnosis has been proposed as an option to reduce the occurrence of offspring with chromosomal abnormalities in carrier couples and further miscarriages in carrier couples with recurrent miscarriage, although its efficiency has not yet been established.[21,22] Our findings of the good reproductive outcome in these couples bring into question whether an assisted reproductive technique is desirable. Preimplantation genetic diagnosis is an expensive intervention, which requires an in vitro fertilisation procedure and therefore bears the risk of serious complications.

Conclusion

The risk of viable offspring with unbalanced chromosomal abnormalities is low in carrier couples whose carrier status was ascertained after two or more miscarriages. Their chances of having a healthy child are as high as non-carrier couples, despite a higher risk of a subsequent miscarriage. The more accurate risk information provided by our study should help carrier couples when deliberating between the risk of another miscarriage, a handicapped child, and the chance of a healthy child.

What is already known on this topic
Couples carrying a structural chromosome analysis whose carrier status is ascertained after recurrent miscarriage are at risk of having a child with severe congenital handicaps.
What this study adds
The risk of viable offspring with unbalanced structural chromosome abnormalities is low in couples whose carrier status is ascertained after recurrent miscarriage.
Their chances of having a healthy child are as high as for non-carrier couples (over 80%), but they have a higher risk of subsequent miscarriage.
The more accurate risk information provided by our study could help couples when deliberating between the risk of another miscarriage, a handicapped child, and the chance of a healthy child.

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5

Inherited unbalanced structural chromosome abnormalities at invasive prenatal diagnosis are rarely ascertained through recurrent miscarriage

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Abstract

Objective To determine the mode of ascertainment of inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis.

Methods From the databases of three centres for clinical genetics in the Netherlands, all cases of inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis in the period 1992-2000 were selected. The mode of ascertainment was identified by examining the reason for invasive prenatal diagnosis and the reason for parental chromosome analysis of the first structural chromosome abnormality detected within the family.

Results In total 56 cases of inherited unbalanced structural chromosome abnormalities were detected at invasive prenatal diagnosis. Only one case was ascertained through two previous miscarriages (2%). The main modes of ascertainment were a previous child with an unbalanced karyotype (48%), congenital abnormalities at ultrasound examination (20%), and advanced maternal age (9%). The remaining cases had a different mode of ascertainment.

Conclusion Inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis are rarely ascertained through two or more miscarriages.

Introduction

In couples with two or more miscarriages the incidence of either of the partners carrying a structural chromosome abnormality varies between 3% and 6%.[1-3] Of these carrier couples the products of conception can have a normal karyotype, the same balanced structural chromosome abnormality as the carrier, or an unbalanced structural chromosome abnormality. The last scenario can lead to miscarriage, a stillborn child, or a child born with major congenital defects and severe mental handicaps. Therefore, current guidelines for the management of recurrent miscarriage recommend chromosome analysis in both partners.[4-6] Once a structural chromosome abnormality has been detected, invasive prenatal diagnosis in subsequent pregnancies and termination of pregnancy in the case of an unbalanced foetal karyotype can be considered.

Our group conducted a large study among couples referred for parental chromosome analysis after two or more miscarriages.[7] We found that the risk of viable unbalanced offspring in carrier couples ascertained through recurrent miscarriage was very low; among 550 pregnancies of 278 carrier couples with a history of two or more miscarriages, only two pregnancies were terminated because of an unbalanced structural chromosome abnormality at invasive prenatal diagnosis (0.4%). Furthermore, two children with an unbalanced karyotype were born (0.4%). Even though carrier couples experienced more miscarriages after parental chromosome analysis than non-carrier couples, there was no difference in their chances of delivering a healthy child. These results demonstrate that carrier couples ascertained through recurrent miscarriage are mainly at risk for repeat miscarriage and only to a small degree for viable unbalanced offspring.

If this hypothesis holds, very few unbalanced structural chromosome abnormalities at invasive prenatal diagnosis will be detected in couples with recurrent miscarriage, and the majority of these abnormalities will be found in couples with a different mode of ascertainment.

To confirm this hypothesis we identified all inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis from the cytogenetic databases from three centres for clinical genetics in the Netherlands in the period 1992-2000, and established the mode of ascertainment.

Materials and Methods

From the cytogenetic databases of three centres for clinical genetics in the Netherlands all inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis in the period 1992-2000 were identified. Unbalanced structural chromosome abnormalities from couples with normal parental karyotypes (*de novo* abnormalities) were not included. Data were retrieved from the centres' patient and family files.

From all cases we identified the mode of ascertainment, defined as the primary reason for chromosome analysis, which could originate from the case pregnancy, from the obstetric history of the parents of the case, or from the family history. Therefore, a stepwise approach was used. We investigated the reason for invasive prenatal diagnosis, the reason for parental chromosome analysis, and the first balanced or unbalanced structural chromosome abnormality detected within a family. First, the reason for invasive prenatal diagnosis of the case was recorded. If the reason for invasive prenatal diagnosis was related to the case pregnancy, without prior knowledge of the parental karyotypes, for instance abnormalities at ultrasound examination or advanced maternal age, this reason was also considered the mode of ascertainment. If the reason for invasive prenatal diagnosis was a parental carrier, the reason for parental chromosome analysis was recorded. If the reason for parental chromosome analysis was a carrier in the family, the complete family files were studied to record the mode of ascertainment of the first structural chromosome abnormality within that family, which could be either balanced or unbalanced. If the reason for parental chromosome analysis was related to the obstetric history of the parents, for instance a previous child with an unbalanced karyotype, two or more miscarriages, or a previous unbalanced foetus ascertained trough advanced maternal age, this was considered to be the referral mechanism.

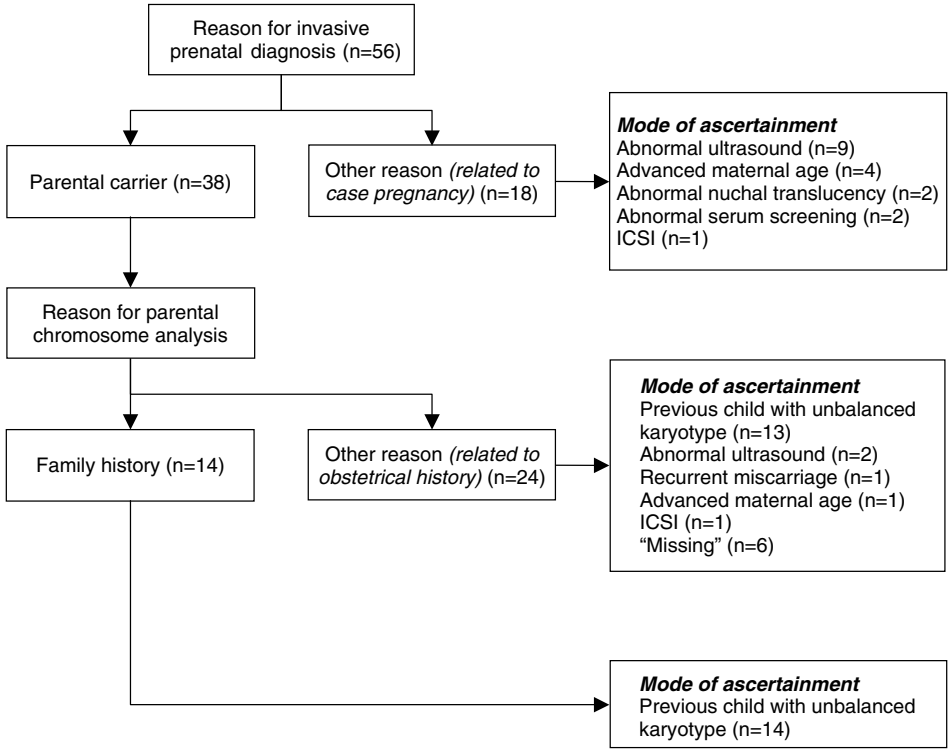
Data were analyzed using the Statistical Package for Social Sciences 11.5.1 (SPSS Inc. Chicago, IL) Given the small number of cases no attempts at statistical hypothesis testing were made.

Results

In total 56 inherited unbalanced foetal structural chromosome abnormalities were detected at invasive prenatal diagnosis in the period 1992 to 2000. These cases originated from 43 families; 33 families with 1 case, 7 families with 2 cases and 3 families with 3 cases. The structural chromosome abnormalities were 36 reciprocal translocations, 18 Robertsonian translocations, 1 insertion and 1 marker chromosome. Invasive prenatal diagnosis was performed by amniocentesis (n=31), chorionic villus sampling (n=24) and umbilical cord venepuncture (n=1). The mean gestational age at invasive prenatal diagnosis was 18 weeks (range 9 to 41). The mean maternal age at invasive prenatal diagnosis was 31.5 years (range 20 to 40).

The most frequent reasons for invasive prenatal diagnosis were a structural chromosome abnormality in either of the parents (n=38), congenital abnormalities at ultrasound examination (n=9) and maternal age (n=4) (Figure 1). In 38 cases in which the reason for invasive prenatal diagnosis was because of a carrier parent, the most frequent reasons for the initial parental chromosome analysis were a carrier in the family history (n=14) and the previous birth of a child with an unbalanced karyotype (n=13). In only one case, the reason for parental chromosome analysis was recurrent miscarriage. All 14 cases in which the parental carrier status had been established because of a structural chromosome abnormality within the family history, had been ascertained through the birth of a child with an unbalanced karyotype with no prior knowledge of the familial balanced chromosome abnormality'.

Figure 1 Flowchart of route to determine the mode of ascertainment of 56 inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis in three centres for clinical genetics in 1992-2000



The modes of ascertainment of the 56 cases of inherited unbalanced structural chromosome abnormalities at invasive prenatal diagnosis are summarized in Table 1. In total, the main modes of ascertainment were the birth of a child with an unbalanced karyotype in the obstetric history or in the family history (48%), congenital abnormalities at ultrasound examination (20%) and advanced maternal age (9%). Other modes of ascertainment were enlarged nuchal translucency (4%), abnormal maternal serum screening (4%), and ICSI (4%). In only one case the mode of ascertainment was two or more miscarriages (2%).

In six cases, originating from 4 case families, the mode of ascertainment could not be established due to missing patient files. In all of these cases the reason for invasive prenatal diagnosis was a parental carrier.

Table 1 Mode of ascertainment of 56 inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis in 1992-2000

Mode of ascertainment	N=56
Previous child with an unbalanced karyotype	27 (48%)
Suspected congenital abnormalities of the foetus	
Ultrasound abnormalities	11 (20%)
Abnormal nuchal translucency	2 (4%)
Abnormal serum screening	2 (4%)
Advanced maternal age	5 (9%)
ICSI	2 (4%)
Recurrent miscarriage	1 (2%)
'Missing'	6 (11%)

Discussion

This study demonstrates that inherited unbalanced structural chromosome abnormalities at invasive prenatal diagnosis are rarely ascertained through recurrent miscarriage. In 56 cases of inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis, in only one the mode of ascertainment was two or more previous miscarriages.

This study includes couples from three of the eight centres for clinical genetics in the Netherlands. The three centres are located in different parts of the country. Since national consensus exists on the reasons for prenatal diagnosis, it is unlikely that the rate of unbalanced progeny at invasive prenatal diagnosis differs for the various centres.[8] We therefore believe that the centres included in this study are representative for the Netherlands. However, between centres for clinical genetics differences in the approach of family members may exist once carrier status has been detected. Some centres actively approach family members while others have a less proactive policy.

In six cases, originating from four case families the mode of ascertainment could not be established. Given our findings in the other cases it seems highly unlikely that the mode of ascertainment in all of these cases has been through two or more miscarriages.

The results of this study demonstrate that the vast majority of viable unbalanced offspring detected at invasive prenatal diagnosis is ascertained through other factors than two or more previous miscarriages, even though the number of included cases is not large. This is in accordance with the results of our previous study, in which we observed a very low incidence of unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis in carrier couples ascertained through two or more miscarriages (0.4%).[7] More reports have been published on the reproductive outcome of carrier couples ascertained through recurrent miscarriage, describing the low incidence of viable unbalanced offspring among these couples.[9-15] In carrier couples the risk of viable unbalanced offspring varies markedly, depending on the type of chromosome abnormality, the chromosomes involved, the length of the translocated segment, the segregation mode and the sex of the parental carrier.[16-18] In one study the risk of unbalanced progeny at amniocentesis to carriers of chromosome rearrangements was reported to be between 1.5 and 5% in reciprocal translocation carriers ascertained through recurrent miscarriage, whereas couples ascertained through the birth of a previous unbalanced child had a risk between 20 and 25%.[17] In other studies the influence of the mode of ascertainment could not clearly be demonstrated.[19,20] Our results confirm the influence of the mode of ascertainment on the risk of viable unbalanced offspring.

By using a stepwise approach, we were able to determine the mode of ascertainment of unbalanced foetal structural chromosome abnormalities beyond the first degree relatives. In this way we demonstrated that, in this study group, no unbalanced structural chromosome abnormalities at invasive prenatal diagnosis were discovered through recurrent miscarriage in a family member. Since the vast majority of viable unbalanced offspring at prenatal chromosome analysis was ascertained through viable unbalanced offspring in the family, it seems that actively approaching family members can be considered in couples in

whom carrier status has been ascertained through viable unbalanced offspring. It is less obvious whether an active approach of family members is also required in carrier couples ascertained through two or more miscarriages.
It should be realized that unbalanced structural chromosome abnormalities at invasive prenatal diagnosis are frequently discovered without prior knowledge of the parental carrier status. In this study 22% of all cases was ascertained through maternal age, abnormalities at ultrasound or serumscreening, or ICSI. Furthermore, this study did not include *de novo* unbalanced structural chromosome abnormalities. It has been reported that 54% of all cases of chromosomal structural abnormalities at invasive prenatal diagnosis are not inherited from either of the parents.[21]

In conclusion, unbalanced structural chromosome abnormalities at invasive prenatal diagnosis are rarely ascertained through recurrent miscarriage. The most frequent mode of ascertainment is the birth of a child with an unbalanced karyotype in the obstetric history or family history, or through ultrasound abnormalities.

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7

General discussion

Couples with recurrent miscarriage are at increased risk of either of the partners being a carrier of a structural chromosome abnormality. The incidence of carrier status increases from approximately 0.7% in the general population to 2.2% after one miscarriage, 4.8% after two miscarriages, and 5.2% after three miscarriages.[1-3] Most recent studies report an incidence of approximately 3% among couples with a history of recurrent miscarriage.[4,5] Couples carrying a structural chromosome abnormality are at risk of unbalanced products of conception, leading to miscarriage, stillbirth or to the birth of children with severe congenital impairments. Therefore invasive prenatal diagnosis is offered to carrier couples in subsequent pregnancies, so that parents may decide to terminate the pregnancy in case of an unbalanced foetal karyotype.

Considering the increased risk of carrying a structural chromosome abnormality in couples with recurrent miscarriage, and thus the potential birth of a child with an unbalanced karyotype, it has been routine practice for many years to recommend parental chromosome analysis to all couples after two or three miscarriages.[6-8] However, evidence underpinning the efficiency of this screening policy was lacking. In the Netherlands the annual number of parental chromosome analyses in couples with recurrent miscarriage nearly doubled, from 1298 couples in 1992 to 2362 couples in 2000, while the incidence of identified carrier couples decreased from 6.8% to 3.8%.[9] Thus, the increase in chromosome analyses had not resulted in identifying more carrier couples. This raised the question whether performing such a time consuming and expensive procedure with a low detection rate of carriers in its present form was justified, and whether the efficiency of the screening policy could be improved.

In this thesis we tried to address these questions by performing a large nationwide study among couples referred for parental chromosome analysis after two or more miscarriages. We investigated factors influencing the probability of either of the partners being a carrier of a structural chromosome abnormality and recorded their actual reproductive outcome after parental chromosome analysis. The results of this research are discussed in the paragraphs below, together with their clinical implications. In addition, suggestions for future research are provided.

Our hypothesis was that the efficiency of screening for parental chromosome abnormalities in couples with recurrent miscarriage could be improved by reducing the annual number of chromosome analyses without loss of quality in healthcare. If couples at high probability of being a carrier could be differentiated from couples at low probability, the number of parental chromosome analyses could be reduced without substantially increasing the number of undetected carrier couples. To reach this goal, we developed a model based on four factors from the medical history that calculated the probability of being a carrier couple, maternal age at second miscarriage being the most influential factor.[10] We calculated that withholding parental chromosome analysis from couples with recurrent miscarriage at low probability of carrying a structural chromosome abnormality could reduce the annual number of chromosome analyses in these couples with at least 18%.

It was recognized that introducing this selective screening strategy would unavoidably lead to a small increase in undiagnosed carrier couples, which might result in the birth of more children with an unbalanced karyotype. Therefore, we next studied the reproductive outcome of carrier couples with a history of recurrent miscarriage. We found that extensive screening for parental structural chromosome abnormalities in couples with recurrent miscarriage had prevented the birth of only very few children with an unbalanced karyotype; in a period of ten years only two unbalanced foetal karyotypes at prenatal diagnosis (0.35%) and two live born children with an unbalanced karyotype (0.35%) were recorded.[11] The accuracy of this low incidence was confirmed by searching the prenatal cytogenetic databases of half of the participating centres for clinical genetics for viable unbalanced offspring in the same period.[12] At prenatal diagnosis, only one unbalanced foetal karyotype ascertained through recurrent miscarriage was detected and this case had been included in our previous study. Our results are in accordance with other, although smaller, cohort studies reporting on the reproductive outcome of this group of carrier couples.[13-15] Historical data from prenatal studies also report a low incidence of unbalanced products of conception in carrier couples with a history of recurrent miscarriage (between 1.5% and 5%). [16-18] It should be recognized that couples with recurrent

miscarriage carrying a structural chromosome abnormality represent a subgroup of carriers with much lower risk estimates of viable unbalanced offspring than couples with a previous child with an unbalanced karyotype. In couples in whom a structural chromosome abnormality has been ascertained through recurrent miscarriage unbalanced products of conception predominantly seem to lead to miscarriage rather than to viable unbalanced offspring. This might be explained by the extend of the imbalance: large imbalances most probably are not viable and therefore result in miscarriage, whereas small imbalances could lead to an ongoing pregnancy with viable unbalanced offspring. In addition, it should be realized that a proportion of all viable unbalanced offspring is not inherited from either of the parents. The incidence of spontaneous or *'de novo'* translocations may be up to half of the unbalanced cases detected at prenatal diagnosis or a fifth at birth, in which cases parental chromosome analysis will obviously not be of any value.[19,20]

Even though the risk of viable unbalanced offspring is very low in couples with recurrent miscarriage carrying a structural chromosome abnormality, knowledge of the parental karyotype might still be of substantial importance if it would determine a couple's prognosis in future pregnancies, but this is not the case. We demonstrated that couples with recurrent miscarriage carrying a structural chromosome abnormality have an excellent prognosis towards delivering a healthy child in future pregnancies. Although these couples are likely to experience more miscarriages than non-carrier couples with recurrent miscarriage, on the long term the chances of a healthy child are similar for carrier couples and non-carrier couples, increasing up to 83% and 84% respectively.[11] Other studies reporting a lower chance of a healthy child in future pregnancies generally only report on the outcome of the first pregnancy after parental chromosome analysis.[13,15]

Given the excellent prognosis towards delivering a healthy child by natural conception, we hypothesized that the live birth rate in couples with recurrent miscarriage and carrying a structural chromosome abnormality can not be improved by preimplantation genetic diagnosis (PGD). By reviewing the literature on PGD in carrier couples we demonstrated that there is insufficient data indicating that PGD improves the live birth rate in carrier couples with a

history of two or more miscarriages compared to natural conception.[21] Taking all this evidence together, we feel that recurrent miscarriage due to structural chromosome abnormalities is a natural selection mechanism which as to date can not be improved by clinical interventions.

Clinical implications

The selective screening strategy for parental chromosome analysis in couples with recurrent miscarriage, has been adopted in the ESHRE guideline and in the Guideline 'Recurrent Miscarriage' of the Dutch Society of Obstetrics and Gynaecology.[22,23] Our data on the reproductive outcome of couples with recurrent miscarriage carrying a structural chromosome abnormality demonstrate that the incidence of viable unbalanced offspring was extremely low (n=4, 0.7%). Extrapolating the number of unbalanced karyotypes to the total population of couples with recurrent miscarriage, this means that by screening 11 971 couples with recurrent miscarriage in a nine years period of time, only five cases with an unbalanced foetal karyotype would have occurred (0.02%) of which only half would have been detected at invasive prenatal diagnosis (table 1). This risk is not increased compared to the reported risk of approximately 0.06% viable unbalanced offspring in the in the general newborn population.[24,25] When applying our model to select couples at high probability of carrier status, even in couples at highest probability of carrier status (i.e. couples with maternal age <23 years at the time of the second miscarriage, ≥3 miscarriages and a family history of recurrent miscarriage) the risk of viable unbalanced offspring would be only 10.2% x 0.7% = 0.07%. Also in half of the cases with an unbalanced karyotype a handicapped child was born regardless of the screening strategy because parents decided to refrain from invasive prenatal diagnosis or because they decided not to terminate the pregnancy.

We can therefore now conclude that either with or without a selective screening strategy, parental chromosome analysis in couples with recurrent miscarriage is not efficient to prevent viable unbalanced offspring and does not provide additional information on a couples' long-term chance of a healthy child which is excellent anyhow. Therefore, in our opinion screening for parental structural chromosome

abnormalities in couples with recurrent miscarriage could be abandoned. For couples with recurrent miscarriage proper counselling is essential, but does not require knowledge of the parental karyotype. It is important that the low risk of viable unbalanced offspring and the good reproductive long-term chances for carrier couples are emphasized.

Recommendations for future research

We found that adherence to the guideline "Recurrent Miscarriage" of the Dutch Society of Obstetrics and Gynaecology was rather poor.[26] The results of our study demonstrate the importance of implementation if new guidelines on recurrent miscarriage are introduced, to prevent unnecessary tests and therapy. However, to persuade caretakers in the field of recurrent miscarriage to completely abandon a screening strategy that has been considered 'good clinical practice' for many years, might cause even more resistance. It is not clear which factors (barriers and facilitators) influence the implementation of new guidelines on recurrent miscarriage among gynaecologists, patients, general practitioners, midwives, clinical geneticists and clinical cytogeneticists. It is also unknown to what extent the outcome of parental chromosome analysis influences the choices on reproductive behaviour. We are unacquainted with the psychological pressure of known carrier status in couples with recurrent miscarriage. At present, research on this point is being conducted.[27]

As to day, in the Netherlands nuchal translucency measurement is offered to all pregnant women. Enlarged nuchal translucency has been reported in foetuses with an unbalanced structural chromosome abnormality.[28-30]. Even though viable unbalanced offspring is rare in couples with recurrent miscarriage carrying a structural chromosome abnormality, this non-invasive test might become a reassuring alternative to overcome the fear of undiagnosed viable unbalanced offspring. More research on this topic is needed.

The contribution of the birth of a child with an unbalanced karyotype to the total spectrum of children born with congenital abnormalities and handicaps seems relatively small. Comparing costs and effects of parental chromosome analysis for recurrent miscarriage and costs and effects of prenatal testing in Down's syndrome screening could generate additional worthwhile information.

Table 1 Calculated risk of viable unbalanced offspring in the total screening population of couples with recurrent miscarriage (nine years- period)

	Total screening population	Invited study population	Recruited study population	Reproductive outcome in study population	Pregnant after chromosome analysis	
Carriers	382	382	279/ 382 (73%)	278	239/ 278 (86%)	
No of pregnancies	756* *(382*86%*2.3)*				550 (2.3 per couple)	
No of viable unbalanced offspring	5* (0.7%)				4 (0.7%)	2 at birth 2 at PND
Non-carriers	11 589	766	428	427	390/ 427 (91%)	
No of pregnancies	24 256* *(11 589*91%*2.3)*				897* (2.3 per couple)	
Total	11 971	1148	707	705		
No of pregnancies	25 012*					

*calculated number of pregnancies after chromosome analysis resulting from couples with reproductive outcome after parental chromosome analysis
Risk of viable unbalanced offspring per pregnancy in the total screening population of 11 971 couples is 5/ 25012= 0.02%

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8

Summary en Nederlandse samenvatting

Summary

Chapter 1 outlines the relationship between recurrent miscarriage and structural chromosome abnormalities, and describes the objectives of this thesis. One of the most evident aetiological factors in recurrent miscarriage is either of the partners being a carrier of a structural chromosome abnormality. For many years it has been good clinical practice to offer parental chromosome analysis to all couples with recurrent miscarriage. Evidence to support the efficiency of this policy was lacking. Since it became clear that in the Netherlands a substantial increase in the annual number of chromosome analyses had not resulted in identifying more carrier couples, the efficiency of the screening procedure needed to be explored.

Chapter 2 reports on the impact of the guideline on recurrent miscarriage from the Dutch Society of Obstetrics and Gynaecology, introduced in 1999, among gynaecologists as well as the adherence to this guideline. A survey was conducted among all 101 practices for obstetrics and gynaecology in the Netherlands. Data concerning definition, diagnosis and treatment of recurrent miscarriage were collected. Results were compared with a similar study conducted before the introduction of the guideline and with the recommendations in the guideline. It was demonstrated that adherence to the guideline was rather poor. Too many diagnostic tests and ineffective therapeutic interventions were performed. The results of this study demonstrate the importance of implementation once new guidelines are introduced, to prevent unnecessary tests and therapy.

Chapter 3 presents a model to distinguish couples with recurrent miscarriage at high probability of carrying a structural chromosome abnormality from couples at low probability. A nested case-control study was conducted among couples with two or more miscarriages referred for parental chromosome analysis between 1992-2000. In total 279 carrier couples and 428 non carrier couples were included. Data was obtained from questionnaires, medical records and telephone interviews. Using multivariate logistic regression analysis four factors associated with the probability of carrier status were identified: maternal age

at second miscarriage, a history of three or more miscarriages, a history of two in more miscarriages in parents of either partner and a history of two in more miscarriages in brothers or sisters of either partner. The calculated probability of carrier status varied between 0.5% and 10.2%. Selective chromosome analysis would result in a more appropriate referral policy, can decrease the annual number of chromosome analyses, and lower the associated costs.

Chapter 4 reports on the reproductive outcome after parental chromosome analysis of couples with two or more miscarriages carrying a structural chromosome abnormality compared to non-carrier couples with two or more miscarriages. An index-control study was conducted among 278 carrier couples and 427 non-carrier couples with two or more miscarriages prior to parental chromosome analysis. During a mean follow-up period of 5.8 years after parental chromosome analysis 49% of the carrier couples had one or more miscarriages compared with 30% of the non-carrier couples. The percentage of couples with at least one healthy child was not significantly different in carrier couples (83%) and non-carrier couples (84%). Among 550 pregnancies in carrier couples, two viable unbalanced chromosome abnormalities were detected at prenatal diagnosis and the pregnancies were subsequently terminated (0.4%) and two children with an unbalanced karyotype were born (0.4%). The risk of viable offspring with unbalanced chromosomal abnormalities is thus low in carrier couples whose carrier status was ascertained after two or more miscarriages. Their chances of having a healthy child are as high as non-carrier couples, despite a higher risk of a subsequent miscarriage.

Chapter 5 reports on the mode of ascertainment of inherited unbalanced structural chromosome abnormalities detected at prenatal diagnosis. From the databases of three centres for clinical genetics all inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis in the period 1992-2000 were selected. The mode of ascertainment was identified by examining the reason for invasive prenatal diagnosis and the reason for parental chromosome analysis of the first structural chromosome abnormality

detected within the family. In total 56 cases of inherited unbalanced structural chromosome abnormalities were detected at invasive prenatal diagnosis. Only one case was ascertained through recurrent miscarriage (2%). The main modes of ascertainment were a previous child with an unbalanced karyotype (48%) and congenital abnormalities at ultrasound examination (20%). We conclude that inherited unbalanced structural chromosome abnormalities detected at invasive prenatal diagnosis are rarely ascertained through recurrent miscarriage.

Chapter 6 reviews the reproductive outcome after preimplantation genetic diagnosis (PGD) in couples with recurrent miscarriage carrying a structural chromosome abnormality, as well as the reproductive outcome of these couples after attempting natural conception. MEDLINE, EMBASE and the Cochrane database were searched up to April 2009. Trials, patient series and case reports describing reproductive outcome in couples with recurrent miscarriage carrying a structural chromosome abnormality after attempting natural conception or after PGD were included. Primary outcome measure was the percentage of couples achieving a healthy child. Secondary outcome measure was the percentage of couples experiencing a subsequent miscarriage. Since no randomized controlled trials or non-randomized comparative studies were found, separate searches for both groups were conducted. Four observational studies reporting on the reproductive outcome of 468 couples after attempting natural conception and 21 studies reporting on the reproductive outcome of 126 couples after PGD were found. After attempting natural conception on average 53% of the couples achieved a healthy child in the first pregnancy after parental chromosome analysis; on average 35% miscarried. After PGD on average 35% of the couples achieved a healthy child, whereas on average 5% miscarried. Currently, there is insufficient evidence to recommend PGD as a method to increase the chance of a health child in couples with recurrent miscarriage carrying a structural chromosome abnormality.

Chapter 7 provides a general discussion of the results presented in this thesis and outlines their clinical implications. Our data have shown that parental chromosome analysis in couples with recurrent miscarriage is not efficient in preventing viable unbalanced offspring. Knowledge of the parental karyotype only provides additional information on a couples' short-term chance of a healthy child and as to date there is no evidence of an effective therapy to improve their chance of a healthy child. Therefore, in our opinion, screening for parental structural chromosome abnormalities in couples with recurrent miscarriage could be abandoned. In counselling these couples it is essential that the low risk of viable unbalanced offspring and the good reproductive chances on the long-term are emphasized.

Samenvatting

Hoofdstuk 1 beschrijft de relatie tussen herhaalde miskraam en structurele chromosoomafwijkingen en omschrijft de doestelling van dit proefschrift. Dragerschap van een structurele chromosoomafwijking bij één van de ouders is één van de meest evidente oorzakelijke factoren voor herhaalde miskraam. Sinds vele jaren is het gebruikelijk dat karyotypering van beide ouders wordt aangeboden aan alle paren met herhaalde miskraam. Onderzoek om de efficiëntie van een dergelijk beleid aan te tonen ontbrak echter. Omdat gebleken was dat een aanzienlijke toename van het jaarlijkse aantal karyotyperingen bij paren met herhaalde miskraam in Nederland niet had geresulteerd in een toename van het aantal opgespoorde dragerparen, was het noodzakelijk om de efficiëntie van deze screening te onderzoeken.

Hoofdstuk 2 beschrijft de invloed van richtlijn 'Habituele Abortus' van de Nederlandse Vereniging voor Obstetrie en Gynaecologie uit 1999 op het beleid van Nederlandse gynaecologen alsmede de mate waarin de richtlijn wordt gevolgd. Er werd een enquête gestuurd aan alle 101 gynaecologische maatschappen in Nederland. De vragen hadden betrekking op de definitie, diagnostiek en behandeling van herhaalde miskraam. De resultaten werden vergeleken met die van een zelfde onderzoek, verricht vóór de invoering van de richtlijn, en met de aanbevelingen die in de richtlijn gedaan worden. Er werd aangetoond dat de richtlijn 'Habituele Abortus' slechts matig werd gevolgd. Er werd te veel onnodig onderzoek gedaan en frequent ineffectieve therapie aangeboden. De resultaten van dit onderzoek laten zien dat bij de invoering van een richtlijn veel aandacht besteed moet worden aan de implementatie om onnodig onderzoek en overbehandeling te voorkomen.

Hoofdstuk 3 presenteert een model om onderscheid te kunnen maken tussen paren met herhaalde miskraam die een hoge kans hebben om drager te zijn van een structurele chromosoomafwijking en paren met een lage kans op dragerschap. Er werd een genest case-controle onderzoek verricht onder paren

die in de periode 1992 tot en met 2000 waren verwezen voor karyotypering van de ouders vanwege herhaalde miskraam. In totaal werden 279 dragerparen en 428 paren met een normaal karyotype geïncludeerd. Gegevens werden verkregen door middel van vragenlijsten, telefonische vraaggesprekken en uit medische dossiers. Door middel van multivariabele logistische regressieanalyse werden vier factoren gevonden die de kans om drager te zijn van een structurele chromosoomafwijking beïnvloeden: de maternale leeftijd ten tijde van de tweede miskraam, een voorgeschiedenis van drie of meer miskramen, ouders met twee of meer miskramen en een broer of zus met twee of meer miskramen. De berekende kans om drager te zijn van een structurele chromosoomafwijking varieerde van 0,5% tot 10,2%. Selectief karyotyperen, op geleide van deze kansen, kan resulteren in gerichter verwijsbeleid, waardoor het jaarlijkse aantal karyotyperingen kan afnemen en kosten worden bespaard.

Hoofdstuk 4 vergelijkt de reproductieve uitkomst na karyotypering van paren met herhaalde miskraam die drager zijn van een structurele chromosoomafwijking met de reproductieve uitkomst van paren met herhaalde miskraam met een normaal karyotype. Er werd een index-controle onderzoek verricht onder 278 dragerparen en 427 paren met een normaal karyotype met twee of meer miskramen voorafgaand aan de karyotypering. In een follow-up periode van gemiddeld 5,8 jaar na karyotypering van de ouders maakte 49% van de dragerparen en 30% van de paren met een normaal karyotype minstens één miskraam door. Het cumulatieve percentage paren met één of meer gezonde kinderen na de karyotypering verschilde niet tussen beide groepen (83% en 84% resp.). Onder 550 zwangerschappen van dragerparen na de karyotypering werden twee ongebalanceerde structurele chromosoomafwijkingen gevonden bij prenatale diagnostiek waarna de zwangerschappen werden afgebroken (0,4%) en werden twee kinderen geboren met een ongebalanceerd karyotype (0,4%). De kans op een kind met een ongebalanceerde structurele chromosoomafwijking is laag voor paren waarbij het dragerschap wordt ontdekt naar aanleiding van twee of meer miskramen. Hun kans op het krijgen van een gezond kind is even groot als voor paren met herhaalde miskraam en een normaal karyotype, ondanks een grotere kans op het opnieuw doormaken van een miskraam.

Hoofdstuk 5 laat zien wat de aanleiding was tot het opsporen van erfelijke ongebalanceerde structurele chromosoomafwijkingen bij invasieve prenatale diagnostiek. Uit de databases van drie centra voor klinische genetica werden alle overgeërfde ongebalanceerde structurele chromosoomafwijkingen geselecteerd die gevonden waren bij prenatale diagnostiek in de periode 1992-2000. De indicaties voor prenatale diagnostiek en voor karyotypering van de ouders van de eerste structurele chromosoomafwijking binnen een familie werden vastgesteld. In totaal werden 56 overgeërfde ongebalanceerde structurele chromosoomafwijkingen gevonden. Slechts één van deze afwijkingen was ontdekt naar aanleiding van herhaalde miskraam (2%). De meest voorkomende aanleidingen waren de eerdere geboorte van een kind met een ongebalanceerd karyotype (48%) en afwijkingen bij prenataal echoscopisch onderzoek (20%). Wij concludeerden dat overgeërfde ongebalanceerde structurele chromosoomafwijkingen, gevonden bij prenatale diagnostiek, zelden opgespoord worden naar aanleiding van herhaalde miskraam.

Hoofdstuk 6 laat aan de hand van een overzicht van de literatuur zien wat de reproductieve uitkomst is na preimplantatie genetische diagnostiek (PGD) bij paren met herhaalde miskraam en dragerschap van een structurele chromosoomafwijking, alsmede van paren die probeerden spontaan zwanger te worden. MEDLINE, EMBASE en de Cochrane database werden doorzocht tot april 2009. Trials, patiënten series en case reports die de reproductieve uitkomst beschreven na PGD en/ of na poging tot spontane zwangerschap bij paren met herhaalde miskraam en dragerschap van een structurele chromosoomafwijking werden geïncludeerd. Primaire uitkomstmaat was het percentage paren dat een gezond kind kreeg. Secundaire uitkomstmaat was het percentage paren dat een miskraam kreeg. Omdat er geen onderzoeken werden gevonden die de reproductieve uitkomst van beide groepen vergeleken, werd voor beide groepen een aparte zoekstrategie uitgevoerd. Vier onderzoeken die de reproductieve uitkomst beschreven van 368 paren die probeerden spontaan zwanger te worden werden geïncludeerd, alsmede 21 onderzoeken die de reproductieve uitkomst van 126 paren na PGD beschreven. Van de paren die spontaan probeerden zwanger

te worden kreeg 53% een gezond kind en 35% een miskraam in de eerste zwangerschap na karyotypering van de ouders. Na PGD kreeg 35% een gezond kind en 5% een miskraam. Momenteel is er onvoldoende bewijs om PGD aan te bevelen als een techniek om de kans op een gezond kind vergroten bij paren met herhaalde miskraam en dragerschap van een structurele chromosoomafwijking.

Hoofdstuk 7 bepreekt de resultaten die gepresenteerd worden in dit proefschrift en de klinische implicaties hiervan. Onze gegevens laten zien dat het karyotyperen van paren met herhaalde miskraam niet efficiënt is ten aanzien van het voorkómen van kinderen met een ongebalanceerde structurele chromosoomafwijking. Kennis van het karyotype van de ouders is alleen informatief ten aanzien van de kans op een gezond kind op de korte termijn. Tot op heden bestaat er geen bewezen effectieve interventie om de kans op een gezond kind te vergroten. Daarom zijn wij van mening dat het routinematig karyotyperen van paren met herhaalde miskraam afgeschaft zou kunnen worden. Bij de voorlichting van deze paren is het van essentieel belang dat het lage risico op een kind met een ongebalanceerd karyotype en de gunstige prognose ten aanzien van het krijgen van een gezond kind worden benadrukt.