Chromosomal Structural Abnormalities among Filipino Couples with Recurrent Pregnancy Losses

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ABSTRACT

Introduction. Recurrent pregnancy loss is a devastating reproductive problem that affects 5% of couples trying to conceive. Majority of the cases are due to cytogenetic errors. This study determines the prevalence of chromosomal structural abnormalities in Filipino couples who presented with 2 or more pregnancy losses.

Methods. Results from chromosomal analysis of couples referred for 2 or more miscarriages done at the Institute of Human Genetics-National Institutes of Health-University of the Philippines, Manila on peripheral blood samples from 1991 to 2010 were retrospectively reviewed.

Results. There were 356 couples with a history of 2 or more miscarriages sent for chromosomal analysis from 1991-2010 included in this study. Among these 356 couples, 17 couples (4.8%) were found to be carriers of different chromosomal abnormalities, 1 of whom had both of them affected with chromosomal abnormalities. From a total of 18 cases, there were 13(3.6%) translocations, 1(0.3%) insertion, 2(0.6%) with marker chromosomes, 1(0.3%) pericentric inversion and 1(0.3%) deletion.

Conclusion. The overall frequency of chromosomal structural abnormalities among patients with RPL in this study is 4.8% with translocations being the most common type detected. The results of this study are similar to that of previous large-scale studies which have demonstrated that parental chromosomal abnormalities are associated with RPL.

Key Words: recurrent pregnancy loss, chromosomal structural abnormalities

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Introduction

Recurrent pregnancy loss (RPL) is a devastating reproductive problem that affects approximately 0.5% to 1.0% of women attempting pregnancy and 5% of couples trying to conceive.[1] It is classically defined as the occurrence of three or more consecutive pregnancy losses. However, several clinicians start evaluation with two or more because of the recent increase in childless miscarriages.[2] The American Society for Reproductive Medicine currently defines RPL as the occurrence of two or more failed pregnancies.[3] Its etiology can be either maternal or embryonic. Maternal factors include chromosome abnormalities, antiphospholipid antibodies, uterine anomalies, endocrine abnormalities, thrombophilic disorders, immune dysfunction, infection and psychological distress. Embryonic factors include abnormal embryonic karyotype, genetic abnormalities, such as mutations in genes coding for inflammatory cytokines and coagulation factors, and epigenetic changes like reversible modifications of DNA and chromatin structure resulting from inappropriate methylation and expression patterns of imprinted genes.[2,4]

Embryonic aneuploidy is the most important cause of miscarriage before 10 weeks of gestation. A previous study showed that 70% of sporadic spontaneous abortions were caused by an abnormal embryonic karyotype. More recent molecular techniques such as the microarray comparative genomic hybridization showed that about 80% of sporadic spontaneous abortions were caused by abnormal embryonic karyotype. [5]

It is estimated that 30 to 50% of all pregnancies are lost prior to 6 weeks of gestation, of these, 70% are due to numeric cytogenetic errors. Pregnancy loss between 6 and 10 weeks of gestation occurs in approximately 15% of clinical pregnancies, of which 50% are due to numeric cytogenetic errors. After 10 weeks of gestation, pregnancy loss is dramatically reduced to 2 to 3%, of which only 5 to 6% is due to numeric cytogenetic errors.[6] Though majority of RPL are due to numeric cytogenetic errors, some may be due to unbalanced structural chromosome rearrangements.[6] Approximately 2% to 4% of RPL is associated with a parental balanced structural chromosome rearrangement, of

which balanced reciprocal or Robertsonian translocation are the most common. Additional structural abnormalities associated with RPL include chromosomal inversions, insertions, and mosaicism.[7]

In a prospective cohort study of 1,284 couples with recurrent pregnancy loss, it was shown that carriers of a reciprocal translocation had a higher miscarriage rate compared with non-carrier couples.[8] A study done in Quebec, Canada, where computerized database on 22,199 couples who experienced repeated pregnancy losses was analyzed, showed a rate of 4.7% for chromosomal structural rearrangements in couples suffering from two or more abortions. It also appeared that only translocations (both reciprocal and Robertsonian) and inversions were associated with a higher risk of pregnancy wastage.[9]

The present study aims to determine the prevalence of chromosomal structural abnormalities in couples who presented with RPL and were referred for chromosomal studies to the Cytogenetics Laboratory of the Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila.

Methods

Results from chromosomal analysis done on peripheral blood samples from 1991 to 2010 were retrospectively reviewed. Samples were from couples referred for 2 or more miscarriages from different government and private hospitals, and private Obstetrics and Gynecology practitioners all over the country.

Submitted blood samples were processed at the Institute of Human Genetics-National Institutes of Health-University of the Philippines Manila according to established routine protocols for peripheral blood. The International System for Human Cytogenetics Nomenclature (ISCN 2009) was used in the reporting of results.

Results

A total of 356 couples with a history of 2 or more miscarriages were sent for chromosomal analysis from 1991-2010. Among these 356 couples, 17 couples (4.8%) were found to be carriers of different chromosomal abnormalities, 16 of whom had one of the partners presenting with chromosomal abnormalities and 1 couple had both of them affected with chromosomal abnormalities (Table 1 and Table 2). There were 12 females and 6 males affected. Of the 18 cases, there were 13 (3.6%) cases of translocation composed of 7 cases of two-break reciprocal and 6 cases of Robertsonian translocation, 1 (0.3%) case of insertion, 2 (0.6%) cases of marker chromosome, 1 (0.3%) case of pericentric inversion, and 1 (0.3%) case of deletion. Figures 1 to 5 show the different representative karyotypes of couples from this study. The abnormalities found in the couple who were both affected were a marker chromosome and reciprocal translocation in the female and male, respectively.

Table 1. Institute of Human Genetics- Cytogenetics Laboratory Data on Referral for Miscarriages (1991-2010), National Institutes of Health, UP-Manila

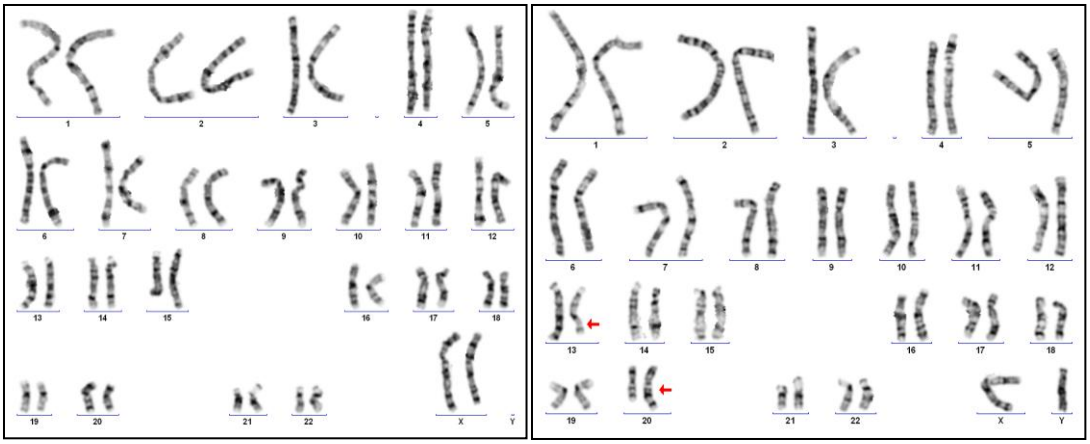
KARYOTYPE	NUMBER OF COUPLES	(%)
Normal	338	94.9%
Structural Abnormality	17	4.8%
Total	356	100%

Table 2. Distribution of Karyotypic Findings from Individual Patients

KARYOTYPE	N
Normal	Total = 694
46,XX	344
46,XY	350
Structural Abnormalities (12 females and 6 males)	Total = 18 (4.8%)
Translocation	13 (3.6%)
Two-break reciprocal	
46,XX,t(3;7)(qter;q21)	1
46,XX,t(7;15)(q31;q21)	1
46,XY,t(10;20)(q23;q13)	1
46,XX,t(4;6)(q31;qter)	1
46,XY,t(13;20)(q22;q13.1)	1
46,XY,t(15;17)(q11;q12)	1
46,XY,t(3;10)(p22;q26)	1
Robertsonian	
45,XX,rob(13;14)(q10;q10)	3
45,XY,rob(13;14)(q10;q10)	1
45,XX,rob(15;21)(q10;q10)	1
45,XX,rob(13;14)(q10;q10)	1
Insertion	1 (0.3%)
46,XX,ins(9;?)(q13;?)	1
Marker chromosome	2 (0.6%)
47,XX,+mar/46,XX	2
Pericentric Inversion	1 (0.3%)
46,XX,inv(6)(p25q23)	1
Deletion	1 (0.3%)
46,XY ,del(6)(q25)	1
Total	712

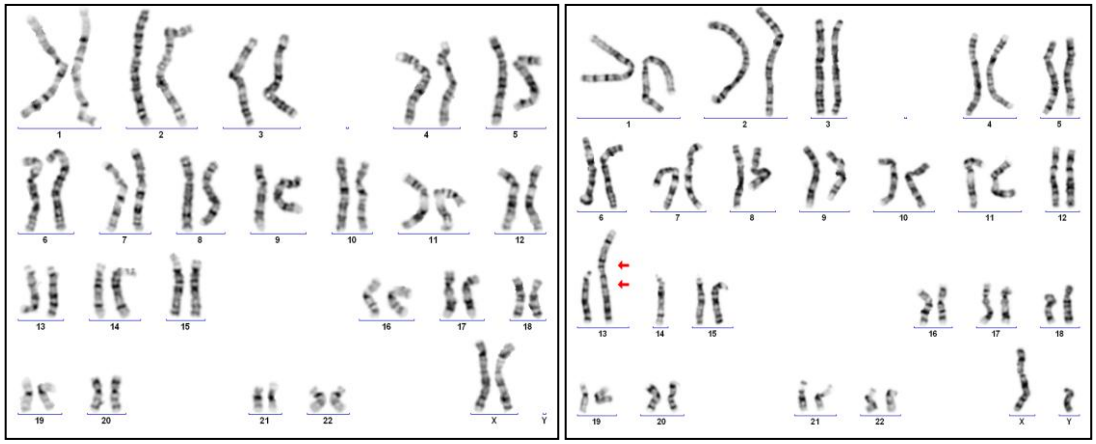
Discussion

Parental chromosomal abnormalities have long been recognized as a major cause of RPL.[10] The prognosis of subsequent pregnancies in couples with abnormal embryonic karyotype is poorer than in couples with normal karyotypes. Translocation in either partner is one of the most important causes.[2] The overall frequency of chromosomal structural abnormalities in the couples included in this study is 4.8% (17/356). This is similar to data from published studies that ranges from 4.7% to 12%.[9,11-14] The most common chromosomal abnormalities were translocations (7 two-break reciprocal and 6 Robertsonian) with an overall



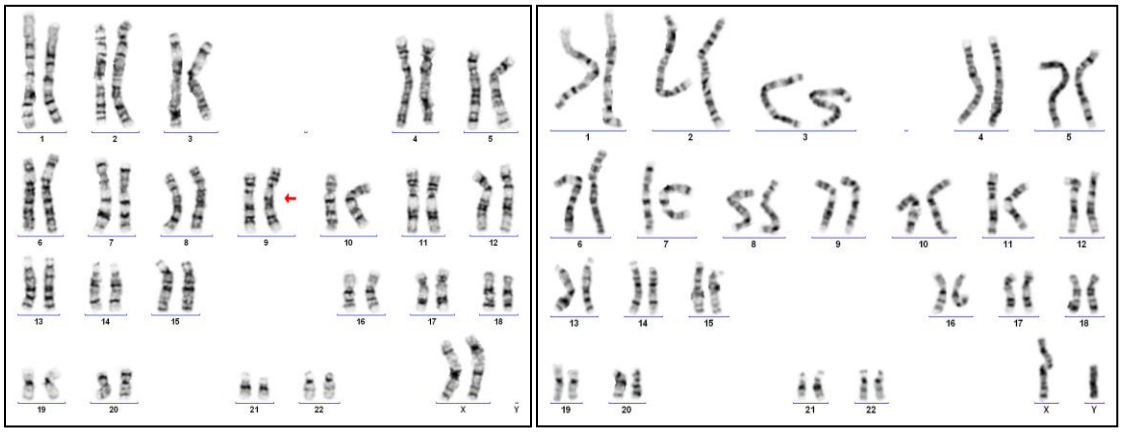
46,XX 46,XY,t(13;20)(q22;q13.1)

Figure 1. Wife with normal karyotype and husband with a reciprocal translocation



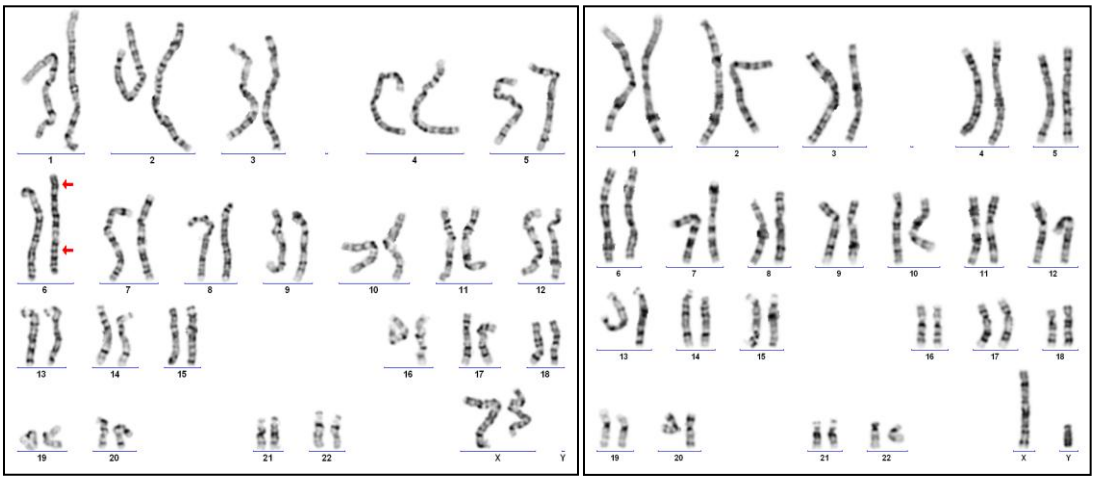
46,XX 45,XY,rob(13;14)(q10;q10)

Figure 2. Wife with normal karyotype and husband with a Robertsonian translocation



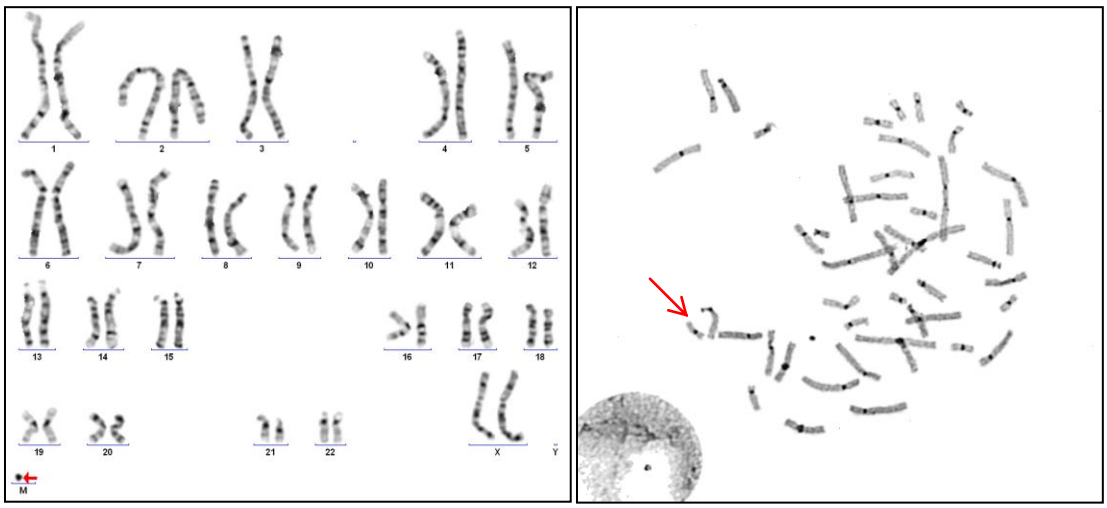
46,XX,ins(9;?)(q13;?) 46,XY

Figure 3. Wife with an insertion in chromosome 9 and husband with normal karyotype



46,XX,inv(6)(p25q23) 46,XY

Figure 4. Wife with pericentric inversion of large segment on chromosome 6 and husband with normal karyotype



47,XX,+mar[20]/46,XX[10] C-banding reveals dark chromatin material on marker chromosome probably from a centromere region

Figure 5. Woman with history of miscarriages showing a mosaic karyotype with an additional small marker chromosome

frequency of 3.6% which is similar to previous reports.[10] Couples with translocations are at risk for an unbalanced fetal karyotype.[15] There were 2 (0.6%) cases with marker chromosome and a single (0.3%) case of insertion suggesting that probably the inserted genes and genes in the marker chromosomes led to the production of unbalanced gametes causing pregnancy loss. The risk of a fetal abnormality depends on the origin of the marker chromosome and fluorescent-in-situ hybridization (FISH) using various probes is usually required for its precise identification. Chromosomal insertions are relatively rare because they require three chromosomal breaks. Carriers of insertions are at risk of producing gametes with duplication or deletion of the inserted segment.[16] In a study done by Stephenson and Sierra investigating the reproductive outcomes associated with a parental carrier of a structural chromosome rearrangement, three cases of insertion associated with RPL were reported, namely, a maternal carrier of a Y heterochromatin insertion on the distal end of chromosome 15, a maternal carrier with complex insertion involving chromosomes 2 and 16, and a paternal carrier with an insertion resulting from three break points on chromosome 17.[17] The types of translocations seen in this study are similar to those previously published.

In this study, there was 1 (0.3%) case of pericentric inversion in chromosome 6, specifically inv(6)(p25q23). A pericentric inversion can lead to the production of unbalanced gametes due to a duplication or deficiency of the material flanking the inverted segment.[16] As far as we know, we here add a single case to the only four previously published reports of pericentric inversion in chromosome 6 in couples who had RPL.[18]

There was a single case of deletion in chromosome 6 [46, XY ,del(6)(q25)] in this study. Unlike translocations and inversions, chromosomal deletions causing recurrent abortion is not commonly reported in the literature. Deletions in chromosome 16 and 19 had been associated with RPL.[12,19] This may be explained by the decreased fertility of individuals with chromosomal deletions. It is demonstrated in this study that there are more females who are carriers of autosomal anomalies as compared to males (2:1); similar trends of a preponderance of female carriers are seen in literature.[14] A possible explanation for this is that chromosomal aberrations in male carriers may cause severe meiotic disturbances and spermatogenic arrest leading to sterility.[14]

In recent years, specialized chromosomal studies such as comparative genomic hybridization (CGH) are being offered to couples with RPL. CGH uses differentially labeled fluorophore-tagged DNA from the patient and a normal control, applied on a metaphase slide which detects unbalanced chromosomal changes such as excesses or deficiencies.[20] However, the clinical utility of CGH in RPL has yet to be determined.[21]

Conclusion

The overall frequency of chromosomal structural abnormalities among patients with RPL in this study is 4.8%; with translocations being the most common type of chromosomal abnormality detected. The results of this study are similar to that of previous large-scale studies which have demonstrated that parental chromosomal abnormalities are associated with RPL. It underscores the recommendation that chromosomal analysis be included as an integral part in the evaluation of couples with 2 or more pregnancy losses because detection of chromosomal abnormalities is vital in genetic counseling and in the management of subsequent pregnancies.

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