A subtelomeric translocation apparently implied in multiple abortions

Sandra Monfort,¹ Francisco Martínez,¹ Mónica Roselló,¹ Lourdes Badia,¹ Félix Prieto,¹ and Carmen Orellana^{1,2}

Submitted May 5, 2005; accepted February 3, 2006; published online April 5, 2006

Purpose: Chromosomal alterations are a major cause of clinically recognized abortions. So cryptic chromosomal rearrangements could be responsible for recurrent miscarriages.

Methods: The study was performed on 18 clinically normal couples who had four or more spontaneous abortions and whose karyotypes were found to be normal. Fluorescence in situ hybridization with specific probes of subtelomeric regions was used after optimizing the amount of probe.

Results: Only one subtelomeric translocation was detected between 2p and 3p chromosomes, in a female with seven miscarriages.

Conclusion: Subtelomeric rearrangements are infrequently related to multiple miscarriages. This kind of studies can be considered specially worthy when a couple have had not only abortions but also offspring with mental and physical anomalies. Thus, possible initial studies should focus on affected children or fetus.

KEY WORDS: FISH; rearrangement; recurrent miscarriage; subtelomeric translocation; telomeric probe.

INTRODUCTION

A major cause of clinically recognized abortions is genetic. The main chromosomal abnormalities include autosomal trisomies, polyploidy, monosomy X, and unbalanced structural rearrangements. In addition, chromosomal translocation is the most common structural rearrangement involved in recurrent miscarriage (1). Unbalanced chromosomal rearrangements involving telomeres are emerging as an important cause of mental retardation and congenital malformations in humans. As suggested by several authors (2,3), these kinds of rearrangements

could also be responsible for recurrent miscarriages, being, in these cases, one of the parents a carrier of the balanced translocation.

Cytogenetic analysis at a 400–550-band resolution is the standard methodology employed when a chromosomal rearrangement is suspected. However, rearrangements smaller than five megabases cannot be detected. So, it is necessary to employ specific techniques to detect these kind of small translocations. Fluorescence in situ hybridization (FISH) with specific probes can detect chromosomal rearrangements affecting the subtelomeric regions.

The aim of this study was to investigate the presence of subtelomeric translocations in couples with recurrent miscarriages. A cryptic translocation between chromosomes 2 and 3 was detected in a woman with normal gynaecologic and hormonal examinations, who had seven miscarriages and no viable offspring.

¹ Unidad de Genética y Diagnóstico Prenatal, Hospital Universitario La Fe, Valencia, Spain.

² To whom correspondence should be addressed at Unidad de Genética y Diagnóstico Prenatal, Hospital Universitario La Fe, Avd. Campanar 21, Valencia 46009, Spain; e-mail: orellana.car@gva.es.

Fig. 1. (a) FISH with 2p (green), 2q (red), X/Yq (orange), and X centromeric (blue) probe mixture. A signal of 2p is localized on a chromosome 3p arm. (b) FISH with 3p (green), 3q (red), 22q (orange), 22q11 (blue) probe mixture. A signal of 3p is localized on a chromosome 2p arm.

MATERIALS AND METHODS

This study was performed on 18 clinically normal couples who have had at least four spontaneous

abortions, or more than two spontaneous abortions together with infants with congenital anomalies. The more frequent causes of abortions were previously excluded in these patients and conventional

Fig. 2. Genealogical tree of the patient carrier of the balanced translocation (II-13). The arrow points to the consultant in this case.

cytogenetic analysis in peripheral blood, at a 400–550-band resolution, resulted normal in all of them.

Specific probes for chromosomal telomeric regions (ToTelVysion™ Multi-Color, VYSIS, Downers Grove, IL) were used for FISH experiments following the recommended manufacturer's protocols with minor modifications. This specific set consists of 62 DNA FISH probes distributed in 15 mixtures, where all telomeres are represented except the acrocentric short arms [13, 14, 15, 21, and 22]. The hybridization volume could be reduced to 0.8 μ L by the use of immunofluorescence slides containing eight wells of 6 mm diameter. Approximately, 1.5 μ L of cellular suspension was dropped on each well. After examination with a phase-contrast microscope, 15 wells containing the metaphases of better quality were selected for posterior hybridization under standard conditions. After hybridization, every mix of probes was evaluated in a minimum of 10 metaphases by using a Nikon Eclipse E600 microscope (Tokyo, Japan) and the Quips R Imaging Software (VYSIS, Downers Grove, IL).

RESULTS

Among the 36 individuals studied, one subtelomeric translocation was detected. In a woman who had seven miscarriages and no viable offspring, the karyotype appeared normal at 550-band resolution. The analysis of the complete subtelomeric probe set allowed to detect a subtelomeric translocation between 2p and 3p chromosomes. The 2p probe signal was observed on one chromosome 2p and on the short arm of one chromosome 3. Whereas, as expected, the probe 3p was observed on one chromosome 3p and on the derivative chromosome 2p (Fig. 1).

No other member of her family has been studied so far, so we do not know whether this translocation was inherited or arose *de novo*. However, no remarkable familial antecedents were reported, except for sporadic abortions in two siblings and her mother (three abortions among a total of 18 pregnancies), which corresponds to the usual spontaneous abortion rate (Fig. 2).

The rest of the couples showed normal results for the complete subtelomeric probe set.

DISCUSSION

In a systematic screening of patients with recurrent miscarriages using subtelomeric probes, we have found one subtelomeric translocation among 18 couples. As far as we know, all the similar studies have reported only three cases with subtelomeric chromosomal rearrangements in a total of 175 couples (4–8). The relative incidence of these rearrangements described among couples with recurrent miscarriages usually ranges from 0 to 2%, close to the frequency found in the present study, 5% (Table I). Only one study, on a very short series, found an exceptionally high 20% positive findings (1/5 couples) (5).

The cryptic subtelomeric translocation found in the present study apparently is the cause of fetal loss of this woman (seven miscarriages and no viable offspring). In a search of the literature, we failed to find any case carrying the unbalanced del2pter/dup3pter or the dup2pter/del3pter translocations. So we do not know whether these chromosomal imbalances are viable or not. However, several living patients bearing longer 2p duplications or deletions, as well as longer 3p duplications or deletions have been reported previously, usually associated to a syndromic condition.

Table I. FISH Studies on Subtelomeric Translocations in Different Series of Patients with Recurring Miscarriages

Reference	Number of recurrent miscarriages	Number of couples studied	Cryptic subtelomeric translocation	Probe set
(5)	>4	5	2 [(3q;10p) male, (20p;Dq) female]	Multiprobe T system (cytowell)
(6)	>3	40	0	Multiprobe T system (cytowell)
(8)	Multiple	50	0	Multiprobe T system (cytowell)
(7)	Multiple	27	0	Multiprobe T system (cytowell)
(4)	>2	50	1 [(3q;10p) female]	Multiprobe T system (cytowell)
Present study	>3 or ≥ 2 + infant with congenital anomalies	18	1 [(2p;3p) female]	Totelvysion™ Multi-Color (VYSIS)

Therefore these rearrangements can be in principle viable. Furthermore, de Vries *et al.* (9) found a lower proportion of recurrent miscarriages among families of patients with known subtelomeric translocations than among families of patients with mental retardation without these rearrangements.

However, a question remains to be solved: the expected probability that all the seven gestations were generated from gametes bearing the corresponding unbalanced translocation is very low (0.5⁷). On one hand a possible explanation may be that this translocation predisposes to malsegregation of chromosomes during gametogenesis, and consequently to pregnancy losses. On the other hand, we cannot reject the possibility that an additional unknown cause as a gene truncated by the chromosomal translocation, is contributing to the reproductive failure of the carrier woman. These hypotheses deserve further consideration.

In conclusion, subtelomeric translocations may be the cause of multiple miscarriage although in a rather small percentage of cases. The high economical and labor costs of these studies make its clinical application impractical. However, given that subtle chromosomal anomalies are also estimated to be implied in 3–20% patients with idiopathic mental retardation (10), when a couple have had not only abortions but also offspring with mental and physical anomalies, this kind of studies can be considered worthy. Initial studies should focus on the affected child or abortion specimen, in order to detect unbalanced rearrangements. Currently there are alternative techniques, such as multiplex ligation-dependent probe amplification (MLPA) (11), that allow a mere rapid and cost-effective detection of this kind of imbalances, whose results would lead to posterior analysis of parents and other relatives.

CONCLUSIONS

Subtelomeric rearrangements are infrequently related with multiple miscarriages. This kind of studies can be considered specially worthy when a couple have had not only abortions but also offspring with mental and physical anomalies. A more practical approach would be to focus the initial studies on the affected child or fetus, when possible, in order to detect putative rearrangements in a cost-effective manner.

ACKNOWLEDGMENTS

This work was supported by Grant No. SAF2001-0404 from the Ministerio de Ciencia y Tecnología and FEDER (Fondos Europeo de Desarrollo Regional).

REFERENCES

1. Dhont M: Recurrent miscarriage. *Curr Women Health Rep* 2003;3(5):361–366. Review
2. Brackley KJ, Kilby MD, Morton J, Whittle MJ, Knight SJ, Flint J: A case of recurrent congenital fetal anomalies associated with a familial subtelomeric translocation. *Prenat Diagn* 1999;19(6):570–574
3. Shaffer LG, Spikes AS, Macha M, Dunn R: Identification of a subtle chromosomal translocation in a family with recurrent miscarriages and a child with multiple congenital anomalies. A case report. *J Reprod Med* 1996;41(5):367–371
4. Cockwell AE, Jacobs PA, Beal SJ, Crolla JA: A study of cryptic terminal chromosome rearrangements in recurrent miscarriage couples detects unsuspected acrocentric pericentromeric abnormalities. *Hum Genet* 2003;112(3):298–302.
5. Yakut S, Berker-Karauzum S, Simsek M, Zorlu G, Trak B, Luleci G: Telomere-specific fluorescence in situ hybridization analysis of couples with five or more recurrent miscarriages. *Clin Genet* 2002;61(1):26–31
6. Fan YS, Zhang Y: Subtelomeric translocations are not a frequent cause of recurrent miscarriages. *Am J Med Genet* 2002;109(2):154
7. Jalal SM, Harwood AR, Sekhon GS, Pham Lorentz C, Ketterling RP, Babovic-Vuksanovic D, Meyer RG, Ensenaue R, Anderson MH, Jr, Michels VV: Utility of subtelomeric fluorescent DNA probes for detection of chromosome anomalies in 425 patients. *Genet Med* 2003;5(1):28–34
8. Benzacken B, Carbillon L, Dupont C, Siffroi JP, Monier-Gavelle F, Bucourt M, Uzan M, Wolf JP: Lack of submicroscopic rearrangements involving telomeres in reproductive failures. *Hum Reprod* 2002;17(5):1154–1157
9. De Vries BB, White SM, Knight SJ, Regan R, Homfray T, Young ID, Super M, McKeown C, Splitt M, Quarrell OW, Trainer AH, Niermeijer MF, Malcolm S, Flint J, Hurst JA, Winter RM: Clinical studies on submicroscopic subtelomeric rearrangements: A checklist. *J Med Genet* 2001;38(3):145–150
10. De Vries BB, Winter R, Schinzel A, van Ravenswaaij-Arts C: Telomeres: A diagnosis at the end of the chromosomes. *J Med Genet* 2003;40(6):385–398
11. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G: Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* 2002;30(12):15, 30