

CONSIDERATIONS REGARDING THE IMPLICATION OF POLYMORPHIC VARIANTS AND CHROMOSOMAL INVERSIONS IN RECURRENT MISCARRIAGE

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Abstract

Recurrent miscarriage becomes a problem that affect an increasing number of couples, almost 1% of the people who want to conceive. The aim of this study is to present the role of heterochromatic regions heteromorphism and chromosomal inversions in occurrence of recurrent miscarriage. In this study were included 354 couples with recurrent miscarriage, which presented at Medical Genetic Department of University of Medicine and Pharmacy Timisoara between octomber 2003 and november 2007. The most common findings were pericentric inversion of chromosome 9, heteromorphism of heterochromatic regions of chromosomes 1 and 16. Our date suggests that cytogeneticists should not ignore these variants that play an important role in reproduction failure.

Key words: heteromorphism, heterochromatin, recurrent miscarriage

Introduction

Recurrent miscarriage becomes a problem that affect an increasing number of couples, almost 1% of the people who want to conceive. Causes of recurrent abortions are: chromosomal abnormalities of genitors, gynecological anomalies, antiphospholipid syndrome, polycystic ovary syndrome and different kind of infections.

In approximated 50% of cases, the cause of reproduction failure remains unknown. In a small number of cases, the abortions arise from transmission of structurally aberrant chromosomes from the parents.

Chromosome variants or polymorphisms are microscopically visible regions that vary in size, morphology and staining properties and have no apparent effect on the phenotype. They are inherited in a Mendelian fashion and are mostly found in the highly variable regions of chromosomes 1, 9, 16, the distal two thirds of the long arm of the Y chromosome and the short arms and satellites of the acrocentric chromosomes.

A number of findings revealed that chromosome inversions are more frequent than deduced from classical cytogenetic studies. Inversions in which a breakpoint is in heterochromatic regions (1qh, 9qh, 16qh, and Yq) are relatively frequent. We like to mention also the other

chromones inversions who are responsabile during the gametogenesis for aberant recombinations and in the same time will be the cause of aneuploid gametes. The role of constitutive heterochromatin is still unknown and the heteromorphism of constitutive heterochromatin was thought to cause no phenotypic alterations.

The aim of this study is to present the role of heterochromatic regions heteromorphism and inversions of chromosomes in occurrence of recurrent miscarriage. The reports regarding the heterochromatic regions found in literature are controversial; there were studies that suggest no correlation between this chromosomal rearrangements and abortions, but in the last years many scientists paid a special attention to this heteromorphism and chromosomal inversions and their implications in reproduction failure. In 2005 Madon reported a study including 842 individuals with primary infertility or repeated miscarriages and showed that polymorphic variants are involved in reproduction failure.

Methods

In this study were included 354 couples with recurrent miscarriage, which presented at Genetic Department of University of Medicine and Pharmacy Timisoara between octomber 2003 and november 2007. The selection of the couples included in this study: one or more consecutive spontaneous abortions; both genitors with normal genitalia. The chromosomal preparations were analyzed after applying a trypsin G-band method. C banding was also used. For each individual, a minimum of 30 metaphase plates was counted and at least five cells were karyotyped.

Results

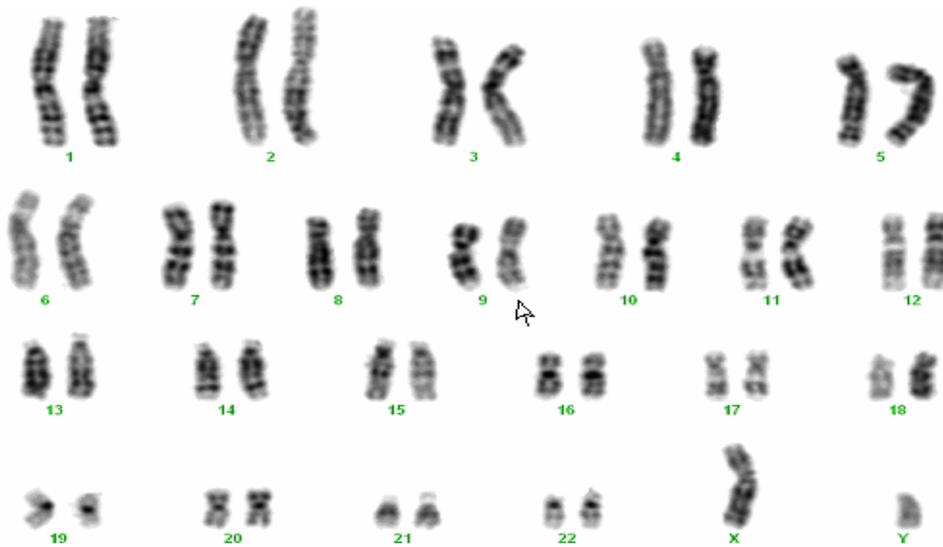
The chromosomal polymorphisms were classified as follows: obvious pericentric inversion of the constitutive heterochromatin; significantly enlarged heterochromatic region of the long arm; small Y (less than size of a G-group chromosome).

The following table presents the heteromorphism of heterochromatic regions and the chromosomal inversions in our study group.

Chromosome	Females	Males
9	46,XX, inv(9)(p12;q22.3)	46,XY, inv(9)(p24.3;q31.1)
	46,XX, inv(9)(p24.1;q31.1)	46,XY, inv(9)(p24.1;q31.1)
	46,XX, inv(9)(p24.3;q31.1)	46,XY, inv(9)(p21.1;q31.1)
	46,XX, inv(9)(p13.2;q21.11)	46,XY, inv(9)(p24.1;q31.1)
	46,XX,9qh+	46,XY, inv(9)(p24.3;q31.1)
	46,XX, inv(9)(p11;q13)	46,XY, inv(9)(p13;q13)
	46,XX, inv(9)(p22;q21.2)	
1	46,XX,1qh+, 16qh+	46,XY,1qh+
	46,XX,1qh+,	
	46,XX,dir dup (1)(q12)	
	46,XX,1qh+,	
16	46,XX,dir dup(16)(q11.2)	46,XY,16qh+
	46,XX,16 inv dup(q11.1;q11.2)	
8	46,XX,inv(8)(p23.1;q21.2)	
10	46,XX,inv(10)(p11.2q21)	
15	46,XX,inv(15)(p12q12)	
Y		46,XY, del(y)(p11.2-p11.3)

The most frequent polymorphism found in this study lot was pericentric inversion of chromosome 9. The incidence of this variant is ranging from 1% to 1.65% in the general population. DNA sequence analysis of human

chromosome 9 has shown that it is highly structurally polymorphic, with much intrachromosomal and interchromosomal duplication, and contains the largest autosomal block of heterochromatin.



B. A. Iama 4, poz. 9,5/163
Cariotip: 46,XY,inv(9)(p24.1;q31.10)

Figure 1 showing pericentric inversion of chromosome 9.

Heteromorphism of chromosomes 1 and 16 were also found in these patients, with prevalence in feminine subjects. This is according with the dates from literature where the incidence of chromosomal abnormalities is higher in females than that in males. A case of 46,XX,dir

dup(16)(q11.2) was diagnosed using prenatal diagnosis due to the suspicion based on echographic indicators of chromosomal aneuploidy and revealed the same chromosomal anomaly as the one found at her mother.

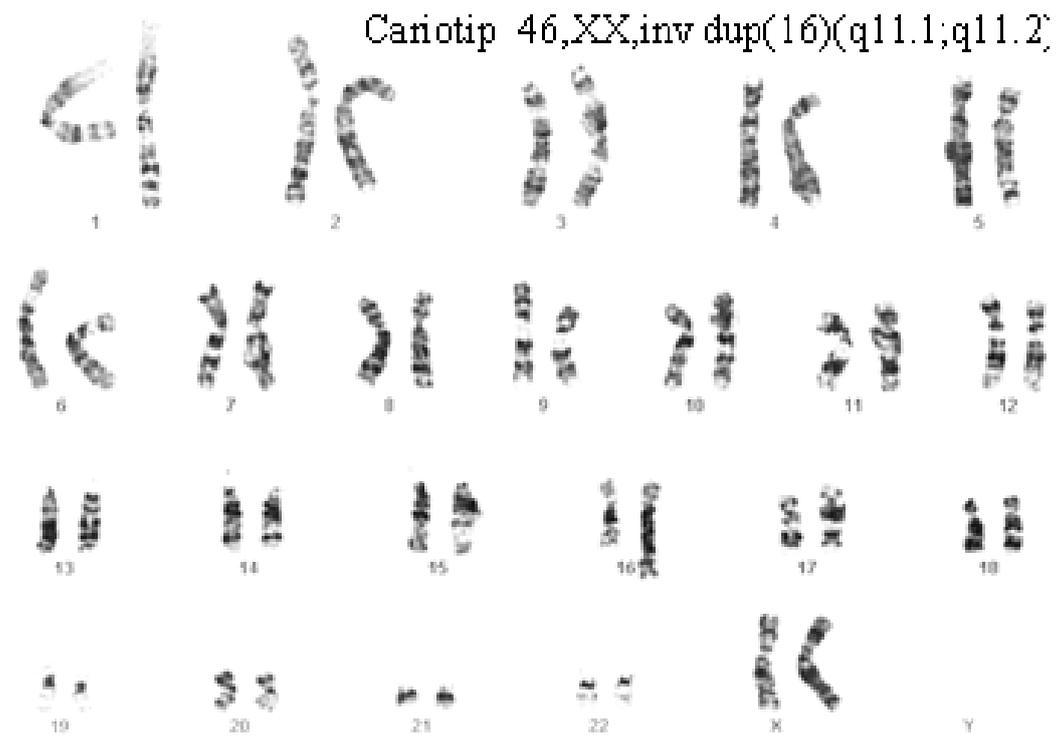


Figure 2 showing Karyotype 46,XX,inv dup(16)(q11.1;q11.2)

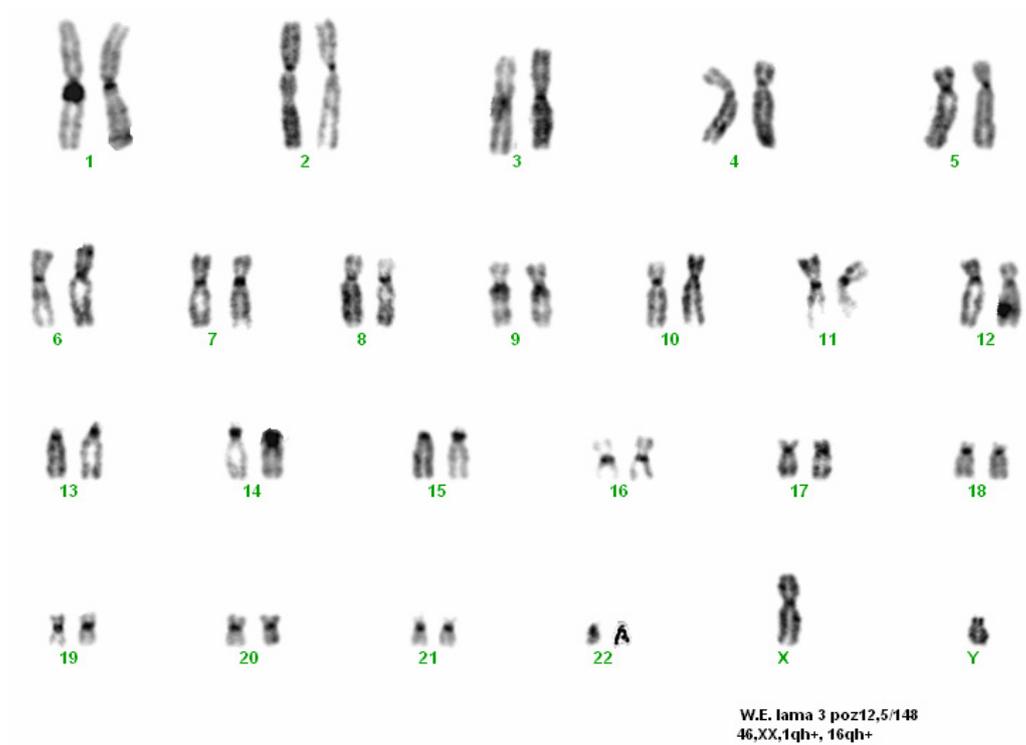
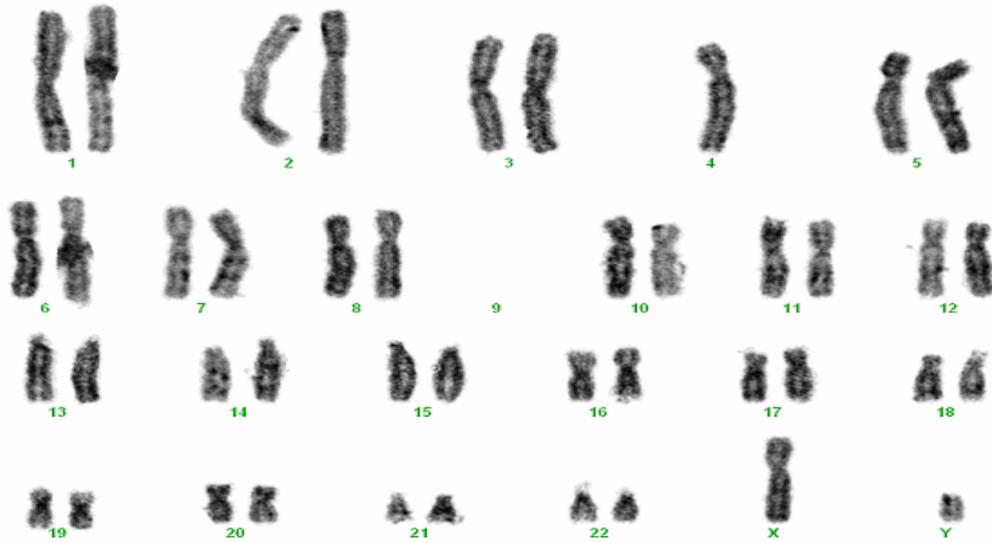


Figure 3 showing C banding

The Y chromosome has an abundance of low copy repeats which render this chromosome susceptible to a multitude of rearrangements that, when involving the long arm, are often the cause of spermatogenic failure. Deletions of short arms of Y chromosome that include a part of pseudoautosomal region have no phenotypical

manifestations due to the compensation of the pseudoautosomal region from X but the deletion of a part of SRY gene, found in this region, could explain the reproductive dysfunction.

Rarer were cases involving other autosomal chromosomes: 8, 10, and 15.



G.R.lama 2, poz.11,5/140
Cariotip 46,XY,del(y)(11.2-11.3)

Figure 4 showing del(y)(p11.2-p11.3)

Cariotip: 46,XX,inv(10)(p11.2q21)

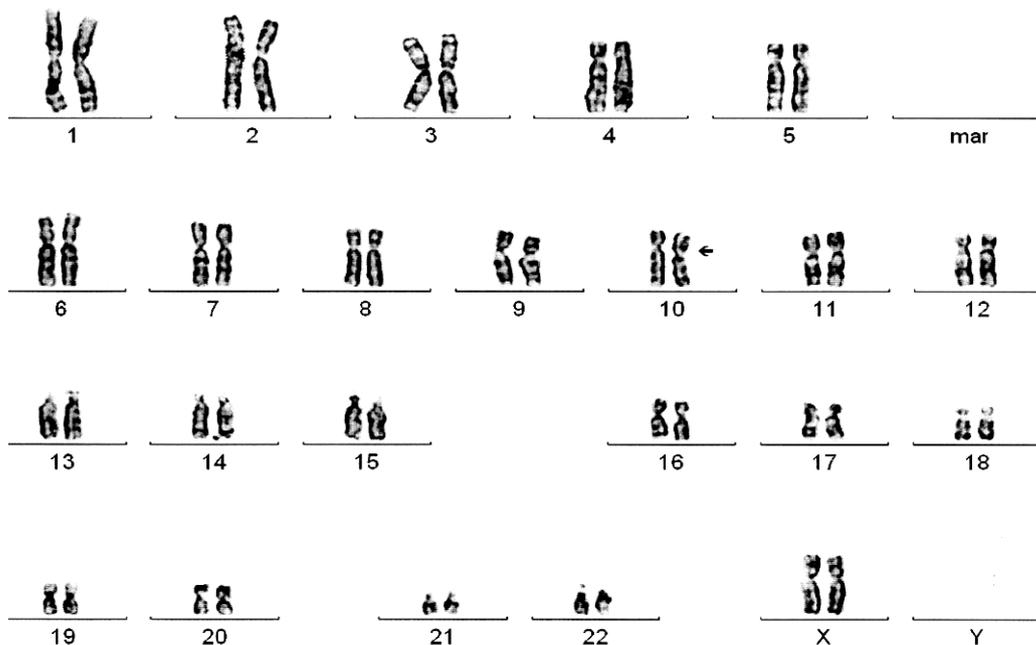


Figure 5 presenting 46,XX,inv(10)(p11.2q21).

Discussions

The present study in which were included 708 individuals with primary infertility or repeated miscarriages, showed polymorphic variants in 3 males and 6 females. Inversions of chromosomes were observed in 10 females and 6 males.

The banding techniques and the high resolution banding permit the evidencing of more discrete chromosomal anomalies and revealed a great variety of heteromorphisms. In the cases of polymorphic inversions the different orientation of chromosomal segments may lead to misalignment between non-allelic segmental duplications.

The carriers of the inversion may have a risk of *de novo* deletion or other chromosomal rearrangement during meiosis. It is important to know if these variants are “normal” or may be “disease-causing” and it is now known that the contribution of structural variation to the overall heterogeneity of the human genome is considerable.

Due to the fact that the heterochromatin has no coding potential and contain genes for rARN, polymorphic variants on chromosomes were considered “normal”. Despite being categorized as a minor chromosomal rearrangement that does not correlate with abnormal phenotypes, many reports in the literature raised conflicting views regarding the association with recurrent abortions and abnormal clinical conditions. The associations of this “variants” and cases with infertility or recurrent abortions have been reported. Using refined molecular techniques, it is

now thought that genes for fertility and viability are resided in heterochromatin.

Conclusions

For the carriers there is a risk of formation of a recombinant aneusomy and later the transformation of the inverted chromosome during gametogenesis. The chromosomal unbalance of gametes may produce spontaneous fetal death and malformed offspring. This suggests that cytogeneticists should not ignore these variants and that these play an important role in reproduction failure. Prenatal examination is also indicated. No treatment is available for patients diagnosed as carriers of an abnormal karyotype, and they should be thoroughly counseled to avoid unnecessary reproductive wastage.

The characterization of polymorphisms at the molecular level is not as yet systematic. Due to the fact that banding techniques increased the number of polymorphisms that could be detected microscopically, it is clear that molecular cytogenetics may increase this number even further, leading to the detection of new forms of polymorphisms in the human genome not detectable by previous methods. As we gain more insight into the human genome, the identification and eventual understanding of chromosome variation such as common population inversions and acrocentric short arm variants will probably receive new connotations.

References

1. E. D. S. Borgaonkar, Chromosomal variation in man. A catalog of chromosomal variants and anomalies Wiley US, 1998.
2. H.E. Wyandt, V.S. Tonk, Kluwer Atlas of Human Chromosome Heteromorphisms. Ed. Acad. Press, Netherlands, 2004.
3. Feuk, L. *et al.* Discovery of human inversion polymorphisms by comparative analysis of human and chimpanzee DNA sequence assemblies. *PLoS Genet.* 1, e56 2005.
4. Iafrate, A. J. *et al.* Detection of large-scale variation in the human genome. *Nature Genet.* 36, 949–951 2004.
5. JIANG Jing, FU Manfen, WANG Defen. Cytogenetic analysis in 61 couples with spontaneous abortions. *Chinese Medical Journal*, 2001, vol. 114 No.2: 200-201.
6. Madon PF, Athalye AS, Parikh FR. Polymorphic variants on chromosomes probably play a significant role in infertility. *Reprod Biomed Online.* 2005. Dec; 11(6): 726-32. Review. PMID: 16417737 [PubMed - indexed for MEDLINE]
7. Samonte, R.V., Conte, R.A., Ramesh, K.H., Verma, R.S.: Molecular cytogenetic characterization of breakpoints involving pericentric inversions of human chromosome 9. *Human Genet.* 98: 576-580, 1996.
8. Sharp, A. J. *et al.* Segmental duplications and copy number variation in the human genome. *Am. J. Hum. Genet.* 77, 78–88 2005.
9. Tabet, A.C., Dupont, A., Lebbar, Couturier-Turpin M.H., Feldemann, D., Rabineau, D. Heteromorphism 18qh+: with or without reproductive consequences? *Ann. Genet.* 44, 139-142, 2001.
10. Teo SH, Tan M, Knight L, Yeo SH, Ng I. Pericentric inversion 9--incidence and clinical significance. *Ann Acad Med Singapore.* 1995 Mar; 24(2): 302-4

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