

Structural Chromosome Abnormality in Recurrent Pregnancy Loss in Gaza Strip: First Experience

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Short title: Chromosomal Abnormality in Recurrent Abortion.

Abstract: Objective This study was conducted in order to evaluate the type and frequency of structural chromosome abnormality in phenotypically normal couples suffering from recurrent pregnancy loss.

Subjects and Methods: Cytogenetic analysis was carried out for 50 Palestinian couples (100 cases), residing in Gaza Strip, who presented with repeated abortions at the Genetics Diagnosis Laboratory of the Islamic University in Gaza, Palestine.

Results: It was found that 5 (10.0%) couples showed a chromosomal abnormality in one of the partners. Two reciprocal translocations, one inversion, one deletion, and one derivative chromosome were identified. The reciprocal translocations and the inversion constitute novel structural abnormalities that have not reported before. The deletion case also was not recorded before in recurrent abortion. Additionally, nine (18.0%) chromosome variants were encountered in the study population.

Conclusion: These results confirm the notion that chromosomal abnormality is an important cause of recurrent abortion, and chromosomal analysis should be seriously considered by physicians working in Gaza strip as an etiological factor in couples suffering from recurrent abortions.

Key Words: Recurrent pregnancy loss, Cytogenetic, Chromosomal abnormality.

ملخص: تهدف هذه الدراسة إلى تحديد الخلل في بنية الكروموسومات (الصبغيات) وتقدير نسبته عند الأزواج الذين يعانون من مشكلة الإجهاض المتكرر. أجريت الدراسة في مختبر الفحوصات الوراثية في الجامعة الإسلامية بغزة حيث شملت 50 من الأزواج (أي 100 شخص) القاطنين في قطاع غزة وتم إجراء دراسة تحليلية لكروموسوماتهم. أظهرت نتائج الدراسة وجود خلل في كروموسومات خمسة (10.0%) من الأشخاص الذين شملتهم الدراسة، وتمثل الخلل في تبادل قطعة بين كروموسومين (Reciprocal translocation) في حالتين، والتفاف جزء في كروموسوم (Inversion) وهذه الحالات الثلاث لم يتم نشرها من قبل، وحذف في جزء من كروموسوم وهذا الخلل أيضا لم يتم تسجيله من قبل في حالات الإجهاض

وخلل في كروموسوم لم يتم التمكن من تحديده. وبالإضافة الى ماسبق تسع (18.0%) حالات
اظهرت اختلاف شكلي في كروموسوماتهم.
تؤكد هذه الدراسة وبشكل واضح على اهمية الخلل في بنية كروموسومات الانسان في حالات
الاجهاض وتوصي الاطباء المعنيين في قطاع غزة باخذ هذا الخلل بشكل جدي كاحد المسببات
الهامة للاجهاض المتكرر.

Introduction

Recurrent pregnancy loss (RPL) is a devastating problem, particularly to Palestinian families who are fond of having large families. RPL is usually defined as the loss of three or more consecutive pregnancies before 20 weeks of gestation. Around 1 to 2% of fertile women experience RPL [1].

RPL has many possible causes that can be categorized as genetic abnormalities, hormonal and metabolic disorders, uterine anatomic abnormalities, infectious causes, immune disorders and thrombophilic disorders [2]. No etiology, however, is found in at least 50% of RPL cases despite a thorough evaluation.

Results from numerous studies in different countries have shown that in about 2-8% of couples with RPL, at least one of the partners has chromosomal abnormality [3-8]. Higher percentages, reaching up to 80%, have been also reported [9].

Chromosomal content of couples with recurrent abortion is usually normal. The chromosomal abnormality, if present, is usually of structural nature. The most common of these abnormalities are balanced translocations, reciprocal and Robertsonian. A less common chromosomal abnormality that can cause RPL is a chromosome inversion [10].

Although the carrier of a balanced translocation or inversion is usually phenotypically normal, these structural abnormalities may cause pregnancy loss because unusual segregation of misaligned chromosomes during meiosis results in unbalanced gametes with consequent fetal loss.

The risk of miscarriage in couples with reciprocal translocations is approximately 25 to 70% and with Robertsonian translocations is approximately 25%. The risk for pregnancy loss with a chromosome inversion is not precisely known [2].

In addition to classic chromosomal abnormalities, chromosome variants such as pericentric inversion of chromosome 9, duplication 9q13 and polymorphic variants in constitutive heterochromatin e.g., 1qh+ have been implicated by some authors as a cause of RPL [11,12].

The aim of this study was to determine the incidence of structural chromosome abnormalities in couples with RPL referred to Genetics Diagnosis Laboratory at the Islamic University of Gaza. Noteworthy, this is

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the first study to be done in our area. The results reported here should help physicians in Gaza strip realize the importance of cytogenetic investigation when evaluating cases with RPL.

Materials and Methods

Study subjects

A total of 50 couples experiencing RPL were included in the study. The age of the referred female subjects ranged from 19-40 years, while the age of male subjects ranged from 23-44 years. The couples were referred to our Genetics Diagnosis Laboratory, during the period December 2009 to April 2011, by local physicians from all over Gaza Strip. All couples had a history of three or more consecutive spontaneous abortions.

Cytogenetic study

Chromosomes were prepared from peripheral blood samples using standard methods [13,14]. Briefly, 0.5 ml heparinized blood was added to 10 ml complete RPMI-1640 in a sterile Falcon culture tube. For induction of lymphocyte proliferation, 2% (w/v) phytohemagglutinin-M was added to each culture tube. Tubes were incubated for 72 hours at 37°C under 5% CO₂. Lymphocytes were arrested at metaphase by addition of colchicine (20 µg/ml) 30 minutes before harvesting the cells. The cells were collected by centrifugation, resuspended in pre-warmed (37°C) hypotonic solution (0.075 M KCl), fixed in methanol: acetic acid (3:1), and spread on thoroughly cleaned microscope slides. The preparations were aged at 80 °C for 72 hours. Chromosome spreads were then banded by treatment with 0.25% trypsin for 3 to 10 seconds, and staining in 4% Giemsa solution (pH 6.8) for 3 minutes. In each case 15-20 complete metaphase plates were visually analyzed and karyotypes were prepared using a computerized Applied Spectral Imaging system. Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).

Results

Data on the number of abortions in the study population is presented in Table-1. The highest percentage (72%) of the couples had experienced 3 abortions.

Among the 50 couples (100 cases) investigated, structural chromosome abnormalities were encountered in 5 subjects distributed as 3 males and 2 females. All the abnormalities were autosomal, and no sex chromosome abnormalities, whatsoever, has been detected. Moreover, neither numerical chromosome nor mosaic abnormalities was observed in any of the couples. The observed structural abnormalities are presented in Table 2.

In addition, 9 individuals, 5 males and 4 females, were found to possess chromosome variants, as indicated in Table 3.

Three of the chromosomal abnormalities that are presented in Table 1, namely the inv(2) and the reciprocal translocations t(1;7) and t(2;5) constitute new structural abnormalities that have not been found in the literature, despite a thorough search. Karyotypes of the cases that showed structural chromosome abnormalities are illustrated in Figures 1 through 5.

Discussion

Naturally, pregnancy is a complicated process that ends up with an abortion in about 15 to 20% of married couples, and 1-2% of couples experience RPL. Existence of several etiological factors and the fact that in around 50% of the abortion cases the cause remains unknown add to the complexity of the problem.

This study constitutes the first published record on the type and frequency of structural chromosome abnormalities in cases of RPL in Gaza Strip. The study showed that 10.0% of the RPL couples harbored a structural chromosome abnormality in one partner. The percentage of chromosomal abnormalities associated with RPL reported by various investigators show wide variation ranging from few percentages to as high as 80% [7,9]. One explanation for these differences between the various studies is the sample size investigated. Studies with large number of samples, for instance, have shown a figure of about 5% [8,15,16]. Additional factors include nature of the cases studied, method and resolution of the banding technique used and the experience of the investigators. The relatively high percentage of cases with chromosomal abnormalities reported in this study could be due to both the small sample size and to selective nature of the cases since physicians in Gaza Strip request cytogenetic analysis after they exclude all the known causes and possible therapy options of abortion. This also explains why the cases are referred to our lab after they experience 3 or more consecutive abortions.

The structural abnormalities reported here were observed in otherwise phenotypically normal individuals. This indicates that these abnormalities have no apparent effect on the phenotype of the carrier individual and the major concern is on his/her reproductive ability in terms of production of unbalanced gametes that may lead to abortion or the production of a malformed offspring. Unbalanced gametes are produced by those individuals because of the abnormal alignment and consecutive abnormal segregation of their chromosomes during meiotic division in the gametogenesis process. The risk for a pregnancy to end up with a miscarriage varies with the type of the structural abnormality and whether it is carried by the male or the female partner. For example, 50 to 70% of the gametes of reciprocal translocation carriers are unbalanced [17]. Most

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carriers of structural chromosome abnormalities, however, have a chance of producing also a phenotypically normal offspring.

Derivative chromosomes are known chromosomes that have an unidentified abnormality in their structure. In our study group, one case showed derivative chromosome 15, where an additional material was detected on the short arm of the chromosome. This abnormality has been reported before [18,19], and some investigators have shown, by molecular analyses, that the additional material on 15p is derived from chromosome Y [18]. The risk of recurrent abortion associated with der(15) is not documented but, Chen et al. have shown that high proportion of the gametes produced by a female carrier of such an abnormality were abnormal [19].

Few reports on interstitial deletions of the long arm of chromosome 1 have been published. The deletions, however, were larger than the one observed here, and they were associated with complex phenotypes including dysmorphism, growth and developmental delay, and organ abnormalities [20,21]. Melis et al., however, have shown in their reported case that interstitial deletion in 1q is associated with a mild phenotype [22]. Discrepancies in the associated phenotypes could be due to variation in the size of the deleted fragment in the different reports. Nonetheless, none of the previous reports has linked this abnormality with RPL. Assuming regular Mendelian segregation, the abortion recurrence risk for carriers of such an abnormality is expected to be at least 50%.

In addition to the structural chromosome abnormalities, polymorphic variants in the form of pericentric inversion in chromosome 9, duplication in 9q13 and increased constitutive heterochromatin of chromosome 1 (1qh+) were observed in 9 of the investigated couples. Polymorphic variants are microscopically visible regions that vary in size and position of heterochromatin. They are mostly found in the regions 1qh, 9qh, 16qh, and Yq. Some variants are sometimes referred to as inversions of heterochromatin. Most authors exclude these variants as a cause of abortion and consider them as harmless variants since they are also encountered in fertile normally reproducing individuals in comparable frequency [23]. Some other authors, however, consider them as a risk factor for male infertility [24] and abortion [25,26]. In our cases it is not possible to decide on whether these variants contribute to RPL since no data is available on their incidence in the reproductive individuals in our population. But their presence in a high percentage in the investigated group suggests that they have a role in abortion. This issue, however, needs further investigation since discrepancies in their role might be linked to the ethnicity of the research subjects.

This study constitutes the first published record on structural chromosome abnormality in RPL in Gaza Strip. Here, we also describe for the first time three unique cases of chromosome rearrangement namely, 46,XY,t(1;7)(q43;p15), 46,XY,t(2;5)(q37;q12), and 46,XX,inv(2)(p25q22) associated with RPL.

The results also showed that an appreciable percentage of the RPL couples had a structural chromosome abnormality. The importance of detecting chromosomal abnormality lies in providing the necessary information for genetic counseling, risk for having a consequent abortion, and discussion of the various reproductive options that are available to couples with RPL problem. Preimplantation genetic diagnosis (PGD), for instance, is one feasible option for producing a healthy offspring in couples with chromosomal abnormality [19,27].

In conclusion, chromosomal analysis is strongly recommended in evaluating couples with a history of abortion and concerned physicians in Gaza Strip should seriously consider chromosomal abnormalities as one cause of RPL.

Acknowledgements

The author is thankful to the technical staff at the Genetics Diagnosis Laboratory of the Islamic University of Gaza.

References:

1. Yoshida A., Miura K., Shirai M. Chromosome abnormalities and male infertility. *Assisted Reproduction* 1996; 6: 93-100.
2. Lee M. and Silver R.M. Recurrent pregnancy loss: summary and clinical recommendations. *Seminars in Reproductive Medicine* 2000; 18: 433-440.
3. Dubey S., Chowdhury M.R., Prahlad B., Kumar V., Mathur R., Hamilton S., Kabra M., Menon P.S.N., Verma I.C. Cytogenetic causes for recurrent spontaneous abortions-an experience of 742 couples (1484 cases). *Indian Journal of Human Genetics* 2005; 11: 94-98.
4. Goddijn M., Joosten J.H., Knegt A.C., van der Veen F., Franssen M.T., Bonsel G.J. Leschot N.J. Clinical relevance of diagnosing structural abnormalities in couples with repeated miscarriage. *Human Reproduction* 2004; 19: 1013-1017.
5. Stephenson M.D. and Sierra S. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. *Human Reproduction* 2006; 21: 1076-1082.

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6. Elghezal H., Hidar S., Mougou S., Khairi H., Saâd A. Prevalence of chromosomal abnormalities in couples with recurrent miscarriage. *Fertility and Sterility* 2007; 88: 721-723.
7. Al Hussain M., Al-Nuaim L., Abu Talib Z., Zaki O.K. Cytogenetic study in cases with recurrent abortion in Saudi Arabia. *Annals of Saudi Medicine* 2000; 20:233-236.
8. Fryns J.P., and Van Buggenhout G. Structural chromosome rearrangements in couples with recurrent fetal wastage. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 1998; 81:171-176.
9. Mustaqahamed S., Balachandar V., Mohanadevi S., Arun M., Manikantan P., Sasikala K., Karthickkumar A., Silambuselvi, Sureshkumar S., Balamuralikrishnan B. Identification of cytogenetic alterations in infertile couples experiencing repeated spontaneous abortions-using Giemsa Trypsin Giemsa banding (GTG). *Scientific Research and Essays* 2011; 6: 182-186.
10. Simpson J.L., Elias S., Martin A.O. Parental chromosome rearrangements associated with repetitive spontaneous abortion. *Fertility and Sterility* 1981;36:584-590.
11. Demirhan O., Pazarbasi A., Suleymanova-Karahan D., Tanriverdi N., Kilinc Y. Correlation of clinical phenotype with a pericentric inversion of chromosome 9 and genetic counseling. *Saudi Medical Journal* 2008; 29: 946-51.
12. Caglayan A.O., Ozyazgan I., Demiryilmaz F., Ozgun M.T. Are heterochromatin polymorphisms associated with recurrent miscarriage? *Journal of Obstetrics Gynaecology Research* 2010; 36: 774-776
13. Moorhead P.S., Nowell P.C., Mellman W.J., Battips D.M., Hungerford D.A.: Chromosome preparations of leukocytes cultured from peripheral blood. *Experimental Cell Research* 1960; 20:613-616.
14. Seabright M. A rapid banding technique for human chromosomes. *Lancet* 1971; 2:971-972.
15. Tharapel A.T., Tharapel S.A., Bannerman R.M. Recurrent pregnancy losses and parental chromosome abnormalities: a review. *British Journal of Obstetrics and Gynecology* 1985; 92:899-914.
16. Campana M., Serra A., Neri G. Role of chromosome aberrations in recurrent abortion: a study of 269 balanced translocations. *American Journal of Medical Genetics* 1986; 24:341-356.
17. Gaber K.R., El-Bassyouni H.T., El-Gerzawy A. Pericentric inversion of chromosome 1 and 9 in a case with recurrent miscarriage in Egypt. *Journal of American Science* 2010; 6:154-156.

18. Rajcan-Separovic E, Robinson WP, Stephenson M. Recurrent trisomy 15 in a female carrier of der(15)t(Y;15)(q12;p13). *American Journal of Medical Genetics* 2001;99:320–324.
19. Chen Y., Chen G., Lian Y., Gao X., Huang J., Qiao J. A normal birth following preimplantation genetic diagnosis by FISH determination in the carriers of der(15)t(Y;15) (Yq12;15p11) translocations: two case reports. *Journal of Assisted Reproduction and Genetics* (2007); 24:483–488.
20. Cirillo S.M., Davi G.F., Bianco R. Interstitial deletion of chromosome 1 (q23-q25). Report of a case. *Clinical Genetics* 1984;25:549-552.
21. Takako T., Yamanouchi Y., Mori Y. Interstitial deletion of chromosome 1q [del (1)(q24q25.3)] identified by fluorescence in situ hybridization and gene dosage analysis of apolipoprotein A-II, coagulation factor V and antithrombin III. *American Medical Genetics* 1997;68:207-210.
22. Melis D., Perone L., Sperandio M.P., Sabbatino M.S., Tuzzi M.R., Romano A., Parenti G., Andria G. Mild phenotype associated with an interstitial deletion of the long arm of chromosome 1. *Journal of Medical Genetics* 1998;35:1047-1049.
23. Sayee Rajangam S. Tilak P., Aruna N., Devi R. Karyotyping and counseling in bad obstetric history and infertility. *Iranian Journal of Reproductive Medicine* 2007; 5: 7-12.
24. Sahin F.I., Yilmaz Z., Yuregir O.O., Bulakbasi T., Ozer O., Zeyneloglu H.B. Chromosome heteromorphisms: An impact on infertility. *J Assisted Reproduction and Genetics* 2008; 25: 191–195.
25. Caglayan A.O., Ozyazgan I., Demiryilmaz F., Ozgun M.T. Are heterochromatin polymorphisms associated with recurrent miscarriage? *Journal of Obstetrics and Gynaecology Research* 2010; 36: 774–776.
26. Fuente-Cortes B.E., Cerda-Flores R.M, Davila-Rodriguez M.I., Garcia-Vielma C., Alvarado R.M., Cortes-Gutierrez E. Chromosomal abnormalities and polymorphic variants in couples with repeated miscarriage in Mexico. *Reproductive BioMedicine Online* 2009; 18: 543-548.
27. Kyu Lim C., Hyun Jun J., Mi Min D., Lee H.S., Young Kim J., Koong M.K. Efficacy and clinical outcome of preimplantation genetic diagnosis using FISH for couples of reciprocal and Robertsonian translocations: the Korean experience. *Prenatal Diagnosis* 2004; 24:556-561.

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Table 1. Number of abortions in the study couples

Number of abortions	Number of couples	Percentage
3	36	72
4	7	14
5	3	6
6	2	4
12	2	4

Table 2. Structural chromosome abnormalities recorded in the study cases.

Case Number	Sex/age	Number of abortions	Type of structural abnormality	Karyotype result
15	Male/40 yrs	3	Reciprocal translocation	46,XY,t(1;7)(q43;p15)
18	Male/25 yrs	3	Derivative chr.	46,XY,der(15)
27	Female/25 yrs	3	Deletion	46,XX,del(1)(q21-q23)
31	Female/22 yrs	4	Inversion	46,XX,inv(2)(p25q22)
37	Male/34yrs	3	Reciprocal translocation	46,XY,t(2;5)(q37;q12)

Table 3. Variant chromosomes observe in the study cases.

Case Number	Sex/age	Number of abortions	Karyotype result
3	Female/20 yrs	4	46,XX,inv(9)(p12;q13)
8	Male/23 yrs	3	46,XY,1qh+
11	Male/37 yrs	3	46,XY,dup(9q13)
17	Female/29 yrs	3	46,XX,1qh+
33	Male/32 yrs	4	46,XY,inv(9)(p11;q13)
36	Female/32 yrs	3	46,XX,inv(9)(p12;q13)
42	Male/32 yrs	3	46,XY,1qh+
47	Female/30 yrs	4	46,XX,qh+
49	Male/29 yrs	3	46,XY,inv(9)(p12;q13)

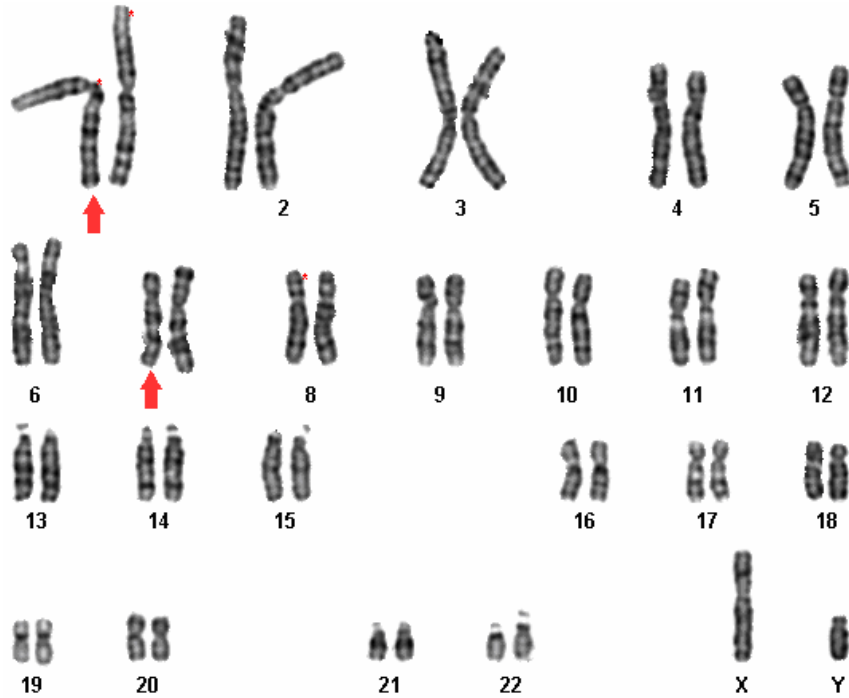


Figure 1. Karyotype of case number 15 showing the reciprocal translocation: $t(1;7)(q43;p15)$ in a male subject. Chromosomes 1 and 7 involved in the translocation are indicated by solid arrows.

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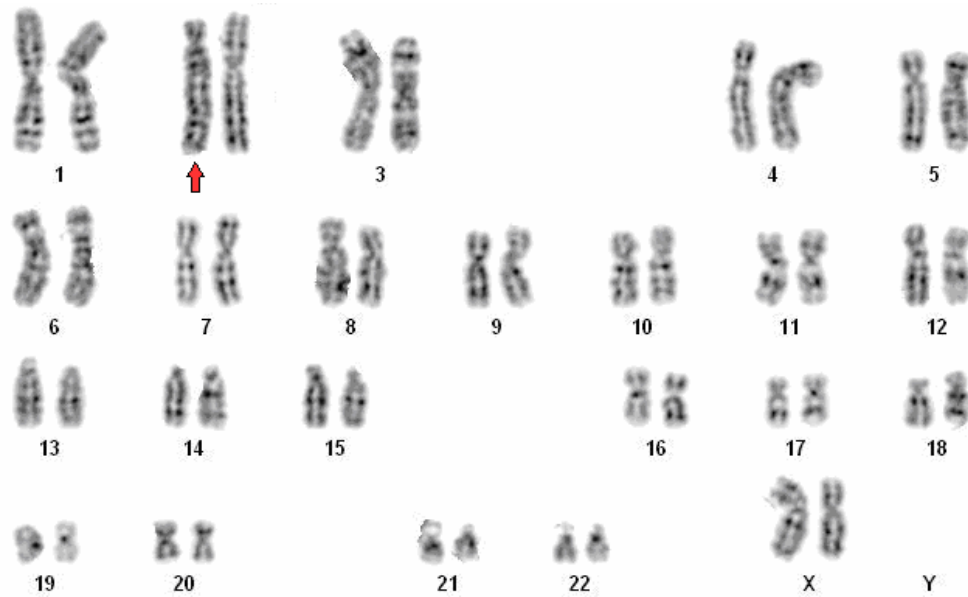


Figure 2. Karyotype of case number 31 showing the inversion in chromosome 2: $inv(2)(p25q22)$ in a female subject. Abnormal chromosome is indicated by a solid arrow.

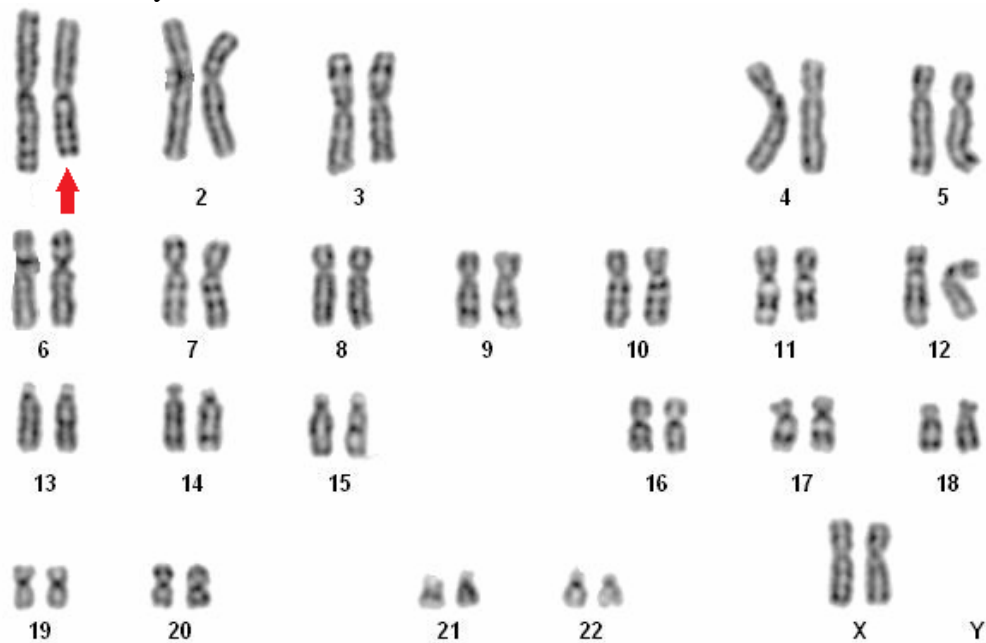


Figure 3. Karyotype of case number 27 showing the interstitial deletion in chromosome 1: del(1)(q21-q23) in a female subject. Abnormal chromosome is indicated by a solid arrow.

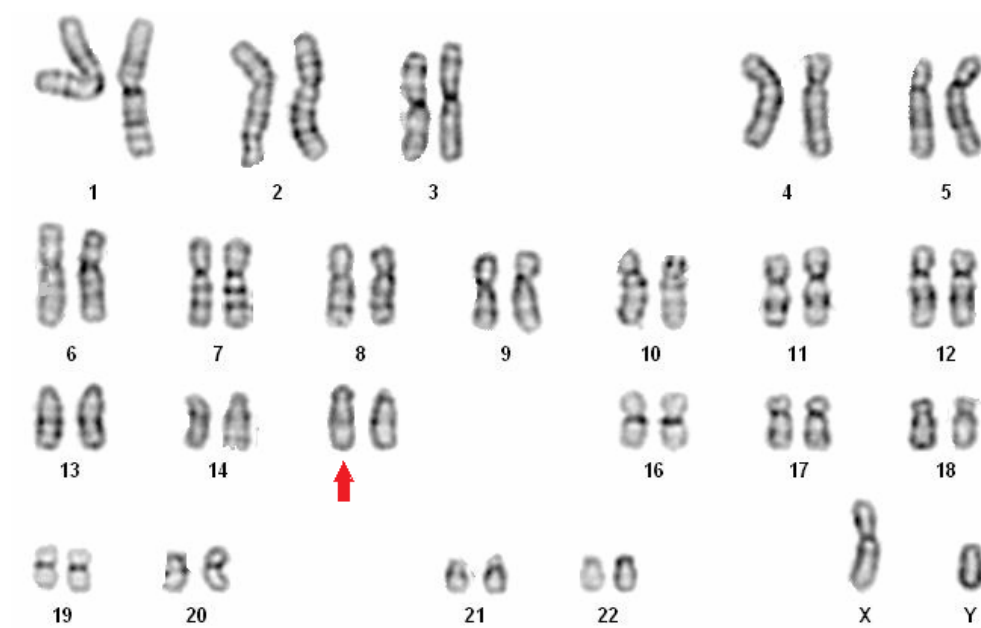
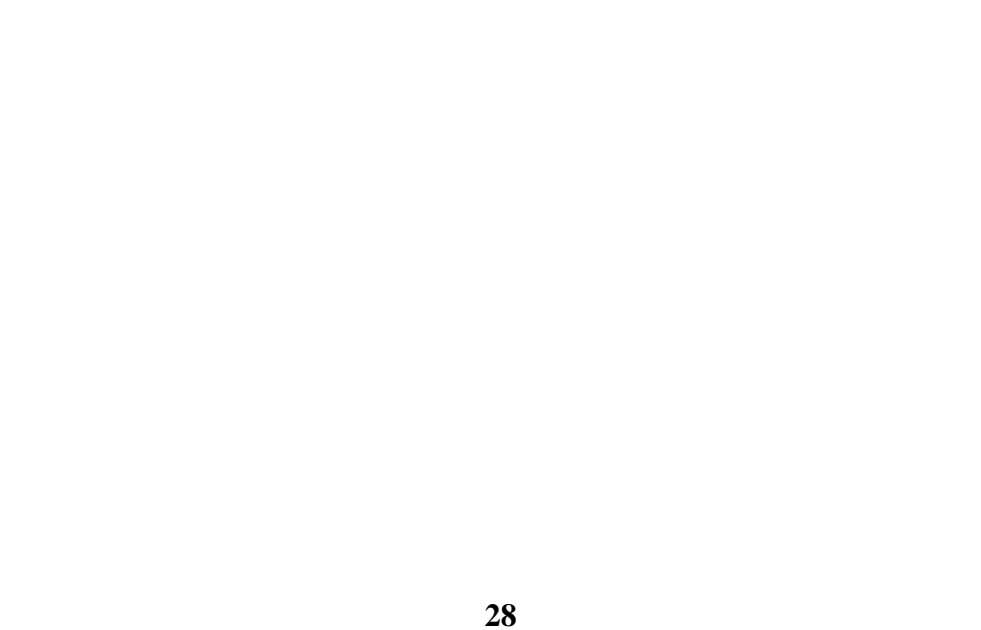


Figure 4. Karyotype of case number 18 showing derivative chromosome 15 [der(15)] in a male subject. The derivative chromosome is indicated by a solid arrow.



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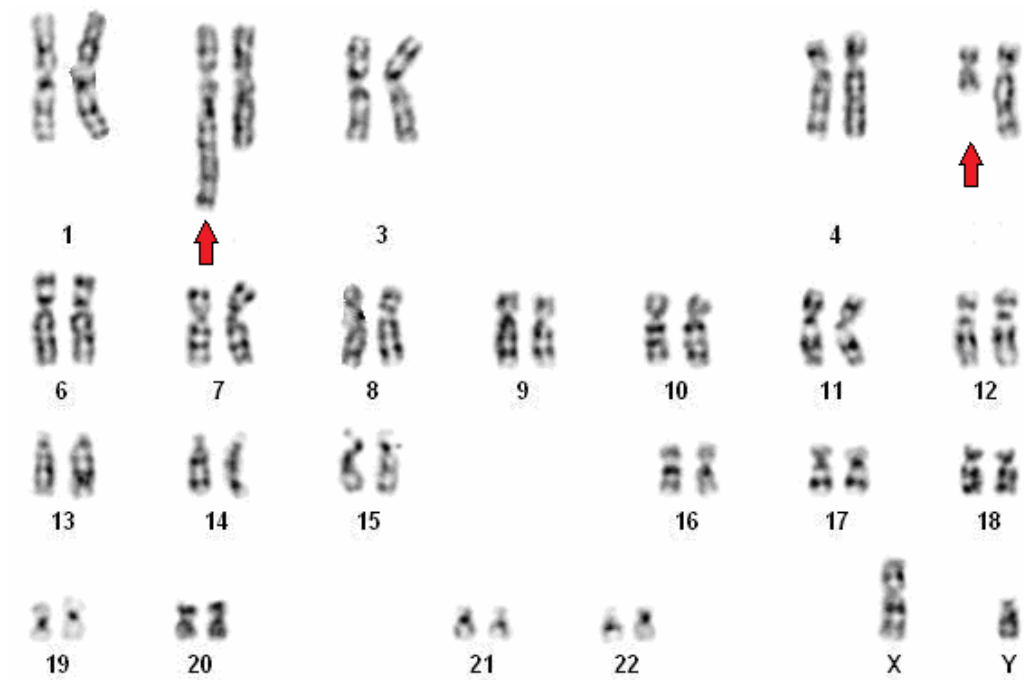


Figure 5. Karyotype of case number 37 showing the reciprocal translocation: $t(2;5)(q37;q12)$ in a male subject. Chromosomes 2 and 5 involved in the translocation are indicated by solid arrows.