Journal of Andrology, Vol. 23, No. 6, November/December 2002

Molecular Cytogenetic Detection of Meiotic Segregation Patterns in Sperm Nuclei of Carriers of 46,XY,t(15;17)(q21; q25)

TULIN ÇORA*, HASAN ACAR* AND MURAT KAYNAK†

*From the Departments of * Medical Genetics and† Biology, Medical Faculty, Selçuk University, Konya, Turkey.*

Correspondence to: Dr Tulin Cora, Tibbi Genetik Bilim Dali, Selçuk Universitesi, Tip Fakültesi, 42080, Konya, Turkey (e-mail: yaseminsena{at} hotmail.com).

Received for publication November 2, 2001; accepted for publication May 23, 2002.

Abstract

Structural chromosomal abnormalities in gonadal tissue represent an important category of parentally transmittable unbalanced chromosomal abnormalities to the offspring. A child with multiple anomalies was sent for cytogenetic analysis, and his karyotype was 46,XY,der(17)t(15;17)(q21; q25). This abnormality was transferred from his grandfather to his father and to the proband. In this family, 5 persons (1 female and 4 male) are the carriers of this abnormality. In this study, fluorescence in situ hybridization (FISH) on sperm nuclei of 4 male carriers was studied to determine the distribution of segregation patterns of the balanced translocation 15q;17q. The segregation results showed that the segregation products in the third carrier (the grandfather) were different, but they were not statistically significant. The segregation patterns in the other carriers were similar. Overall, 50.3% of the sperm nuclei (mean value for 4 carriers) analyzed were the result of alternate segregation; 36.9%, of adjacent I segregation; 9.0%, of adjacent II segregation; and 2.4%, of 3:1 segregation; the remaining 1.3% could be diploid sperm nuclei or of 4:0 segregation. Multicolor FISH analysis appears to be a rapid and reliable method for the direct analysis of segregation patterns in sperm nuclei of carriers of balanced reciprocal translocation, and it also provides interesting information for determining the possible risks for the offspring.

Key words: Fluorescence in situ hybridization, balanced translocation, carrier

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Balanced reciprocal translocations result from an exchange of fragments between 2 chromosomes,

without any gain or loss of genetic material. Although balanced reciprocal translocation carriers usually do not exhibit any particular phenotypes, they are responsible for a large proportion of infertility, pregnancy loss, mental retardation, behavioral abnormalities, and morbidity and mortality. Carriers of reciprocal translocations have reduced fertility and thus form an increased risk of having a spontaneous abortion or an unbalanced karyotype in their offspring (De Braekeleer and Dao, 1990). During meiosis, reciprocal translocation can produce unbalanced gametes through 3 principal modes of segregation: adjacent I, adjacent II, and 3:1. Many male carriers with a balanced translocation have a decreased number of gametes, and these gametes may have an unbalanced chromosomal constitution because of this translocation (Pellestor et al, 1997). Studies on live-born offspring or fetuses do not provide accurate information about meiotic segregation, since lethal segregations are lost through spontaneous abortions. Thus, the true mechanisms responsible for structural rearrangement at segregation remain unknown. Nevertheless, several methods for predicting the risk of imbalance at term have been proposed that are essentially based on the analysis of the chromosomal pachytene quadrivalent (Daniel, 1979; Jalbert et al, 1980; De Arce et al, 1986).

Data obtained directly by cytogenetic analysis of sperm from men having reciprocal translocations should lead to a better understanding of abnormal segregation products and mechanisms. The direct karyotyping of human sperm has been carried out using the human sperm-hamster egg technique to study segregation products (Rudak et al, 1978). However, this strategy is time-consuming and labor-intensive and does not permit the chromosome analysis of large numbers of sperm nuclei. The development of chromosome-specific probes now permits the use of fluorescence in situ hybridization (FISH) to study cells at interphase. The application of FISH using different probe combinations appears to be a reliable, sensitive, and rapid method of establishing the segregation patterns for specific chromosomes in sperm.

In this study, we analyzed sperm nuclei of 4 carriers of a balanced reciprocal translocation between the long arms of chromosomes 15 and 17 in the same family by means of the FISH technique.

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Donors

This study consists of 4 carriers of balanced translocation 15q; 17q in the same family. The proband was a boy (3 months old) whose cytogenetic analysis was determined to be of the 46,XY,der(17)t(15;17)(q21; q25) karyotype. This translocation was inherited from his father by an adjacent I orientation in 2: 2 segregation, although his 2½-year-old sister's karyotype, 46,XY,t(15;17)(15pter→15q21::17q25→17qter;17pter→17q25::15q21→15qter, was produced by an alternate orientation in 2:2 segregation, as was observed in the father and uncles and was probably inherited from the grandfather. The proband's mother had a normal karyotype. Her first pregnancy was aborted at the second month. Clinical, conventional, and molecular cytogenetic results of the proband have been published elsewhere (Çora et al, 2000). Briefly, the proband had a long upper lip, low-set ears, a prominent nose, a short neck, micrognathia, a prominent occiput, an arched palate, strabismus, cardiopathy, skeletal malformations, convulsions, and finger flexion deformities.

In this study, we analyzed segregation patterns of the translocation 15;17 in sperm nuclei of the 4 carriers (the proband's father, 2 uncles, and 1 grandfather).

Semen Samples

Semen samples were obtained from 3 carriers for both morphological and FISH analysis. All donors gave their informed consent prior to participation in the study. However, the fourth carrier (the grandfather, 58 years old) donated at home; therefore, his semen sample was directly processed for FISH analysis without morphologic sperm analyses. For the other 3 carriers, a morphological sperm examination was carried out using Kruger strict criteria on a sperm smear (Kruger et al, 1986).

Preparation of Sperm Nuclei for FISH Analysis

This part of the study was carried out in the Kombassan Experimental Medical Research and Application Center of Selçuk University.

Semen samples were processed for FISH analysis. After being washed in phosphate-buffered saline and fixed in methanol : acetic acid (3:1), the sperm nuclei were decondensed by incubation in 0.01 M dithiothreitol/2x sodium chloride-sodium citrate solution.

FISH Study

The probes used consisted of a mixture of a Prader-Willi/Angelman region probe (SNRPN) labeled with a red fluorophore and a chromosome 15q telomere-specific probe labeled with a green fluorophore (Cytocell, Adderbury, Banbury, United Kingdom), as well as a chromosome 17 centromere-specific alpha satellite probe (Vysis, Downers Grove, Ill) labeled with spectrum green and spectrum red mixture in a 1:1 ratio, giving a pink color. Standard routine chromosome preparations of the carriers were used for FISH analysis to examine the probe localization on the chromosomes. For sperm samples, the hybridization procedure and analysis were carried out as previously described (Acar et al, 2000). The slides were stained with a counterstain medium containing DAPI (4',6-diamidino-2-phenyl-indole).

Scoring Criteria

Slides were examined with an epifluorescence microscope (Optiphot, Nikon, Melville, NY) equipped with a set of DAPI, fluorescein isothiocyanate (FITC), rhodamine, and dual-band pass filters. A total of 8790 sperm nuclei were scored in 4 carriers. Certain populations of sperm nuclei were eliminated from scoring: 1) overlapping nuclei when it was impossible to assign a signal to a given nucleus, 2) a disrupted nucleus with indistinguishable margins, and 3) the nuclei with diffuse and indistinguishable signals.

The probe cocktail consisted of an SNRPN (red), a chromosome 15q telomere-specific probe (green) (Cytocell), and a chromosome 17 centromere-specific alpha satellite probe (Vysis) (a green and red mixture in a 1:1 ratio, giving a pink color). To facilitate the identification of the different combinations, we assigned a letter to each color as follows: r was red, an SNRPN; g was green, a chromosome 15 telomere-specific probe; and p was pink, a chromosome 17 centromere-specific alpha satellite probe. With this probe combination, the normal and balanced products correspond with the combination 1r-1g-1p (Table; Figure). The following abnormalities were scored for adjacents I and II as well as other possible segregation products, such as 1) a nucleus with 1 red signal and 1 pink signal, which corresponds with sperm nuclei bearing normal chromosome 17 and der(15), or a nucleus with 2 green, 1 red, and 1 pink signal, which corresponds with sperm nuclei bearing normal chromosome 15 and der(17), representing the adjacent I product; 2) a nucleus with 1 green and 2 red signals, which corresponds with sperm nuclei bearing normal chromosome 15 and der(15), or a nucleus with 1 green and 2 pink signals, which corresponds with sperm nuclei bearing normal chromosome 17 and der(17), representing the adjacent II product; 3) 3:1 segregation products representing a) a nucleus with 1 green, 2 red, and 1 pink signal, which corresponds with sperm nuclei bearing normal chromosomes 15 and 17 and der(15), b) a nucleus with 2 green, 2 red, and 1 pink signal, which corresponds with sperm nuclei bearing normal chromosome 15 and der(15) and 17, c) a nucleus with 2

green, 1 red, and 2 pink signals, which corresponds with sperm nuclei bearing normal chromosome 17 and der(15) and der(17), and d) a nucleus with 1 green, 1 red, and 2 pink signals, which corresponds with sperm nuclei bearing normal chromosome 17 and der(15) and der(17); and 4) others such as 4:0 segregation or diploid sperm nuclei.

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Cytogenetic and Metaphase-FISH Analyses of Carriers

Conventional and molecular cytogenetic findings and clinical data of this family have been described in a previous report (Çora et al, 2000). In short, these carriers had 46,XY,t(15;17)(15pter→15q21::17q25→17qter;17pter →17q25::15q21→15qter karyotypes. All of these carriers are phenotypically normal, but according to strict Kruger criteria, they are subfertile. The first carrier had a boy with an unbalanced karyotype, which resulted from an adjacent I segregation product, and a girl with a balanced karyotype, the result of an alternate segregation product. In addition, the mother had a spontaneous abortion. The second and third carriers were unmarried.

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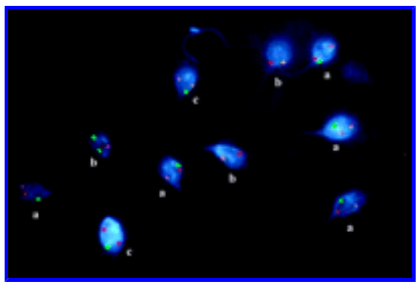
*Results of segregation products of 15;17 translocation by FISH**

The combination of an SNRPN (red), a chromosome 15q telomere-specific probe (green), and a chromosome 17 centromere-specific alpha satellite probe (green : red mixture; 1:1 ratio, giving a pink color) showed a normal localization of the FISH signal on the metaphase chromosome. In our previous study on the metaphase spread in this family, the chromosome 15-specific alpha satellite probe showed an extra signal on the centromeric region of chromosome 14, indicating the translocation of satellite DNA from chromosome 15 to the centromeric region of chromosome 14 (Çora et al, 2000).



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Sperm nuclei from the t(15;17) carrier (proband's father), hybridized with a Prader-Willi/Angelman region probe (SNRPN) (red), a chromosome 15q telomere-specific probe (green), and a chromosome 17 centromere-specific probe (red:green; 1:1 ratio, giving pink). **(a)** Nuclei bearing with the alternate segregation products (normal and balanced); **(b)** nuclei bearing the equipment with adjacent I products; and **(c)** nuclei bearing the adjacent II products.

Sperm-FISH Analysis

The sperm products resulting from alternate, adjacent I, and adjacent II segregations of the translocation 15;17 were determined by FISH analysis on sperm nuclei from the 4 carriers. These

segregation patterns were determined by the presence and/or absence of the fluorescent signals corresponding to chromosomes 15 and 17 (Figure).

In the 4 carriers, there were statistical differences for the distribution of segregation products (X^2 = 64.67, df: 12, P = .0001). This difference originated from a 2:2 adjacent I segregation product in the fourth carrier. For the other segregation products in the 4 carriers, the differences were insignificant (P > .05). In the 4 carriers, the incidence of sperm nuclei resulting from alternate, adjacent I, and adjacent II segregations was 50.3%, 36.9%, and 9.0%, respectively. A much smaller percentage of sperm nuclei were generated by a 3:1 segregation type (2.4%) than by any other segregation type. The remaining percentage of sperm nuclei was produced by 4:0 segregation or diploid sperm nuclei and aneuploidies (Table). Alternative segregants corresponding to normal and balanced gametes and to recombinant products were not distinguished by the application of this 3-probe combination.

Unbalanced adjacent I products are given in the Table. These products were observed in 36.9% of the analyzed sperm nuclei of the 4 carriers, with 18.9% bearing a normal chromosome 15 and der(17)t(15;17)(q21; q25) and 18% bearing a normal chromosome 17 and the der(15)t(15;17)(q21; q25) complement. Data analysis revealed no statistical differences between the incidence of the 2 products of adjacent I segregation, as expected (P > .05). The incidence of adjacent II products was 9.0% of the analyzed sperm nuclei of the 4 carriers, with 4.3% bearing a normal chromosome 15 and der(15)t(15; 17)(q21; q25) and 4.6% bearing a normal chromosome 17 and the der(17)t(15;17)(q21; q25) complement. Similarly, no statistical differences between the incidence of the 2 products of adjacent II segregation were found (P > .05).

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Initial studies of the meiotic segregation patterns of translocation carriers have been directly analyzed by human sperm karyotyping after the fertilization of hamster oocytes with human sperm nuclei (reviewed by Pellestor et al, 1997; Blanco et al, 1998). However, the incidence of segregation patterns of the balanced reciprocal translocation in sperm nuclei with the hamster oocyte-sperm fertilization technique is highly variable from one translocation to another and also from carrier to carrier (Pellestor et al, 1997; Blanco et al, 1998). Additionally, some investigators have shown evidence for an increase in the frequency of certain segregation products for some translocations (Pellestor et al, 1989; Martin et al, 1990; Estop et al, 1992). Furthermore, the 4:0 segregation in these studies was reported to be a more rare event.

Sperm-chromosome studies by the multicolor FISH technique appear to be of special value in determining the chromosomal constitution of sperm nuclei in translocation carriers. There have been only a limited number of cases reported using the FISH technique. In the literature, sperm FISH studies present shortcomings that need to be considered when applying them to the segregation analysis of reciprocal translocation in the sperm nuclei of carriers, since the probe combination is not able to distinguish between segregation products such as alternate and adjacent I products (Van Hummelen et al, 1997; Durak et al, 1999; Honda et al, 1999; Armstrong et al, 2000).

In the present study, we have been able to show the reciprocal translocation 15;17 in sperm nuclei of 4 carriers by the SNRPN and the chromosome 15q telomere-specific and chromosome 17 centromere-specific alpha satellite probes. Our results revealed a predominance of alternate segregation (50.3%), followed by adjacent I segregation (36.9%), adjacent II segregation (9.0%), 3:1 segregation

(2.4%), and other segregants (1.3%). The incidence of segregation patterns in our carriers is comparable to that found in previously studied reciprocal translocations with FISH (Goldman and Hulten, 1993). These data can be highly informative for the segregation behavior of reciprocal translocations and determination of the imbalance products in the progeny of the carriers of reciprocal translocation. Similar to the hamster technique, FISH data also showed that the incidence of segregation products has varied greatly from one translocation to another, ranging from 35.5% to 56.7%, with alternate segregation predominant as well as variance from carrier to carrier (Pellestor et al, 1997; Blanco et al, 1998; Estop et al, 1998; Martini et al, 1998; Mercier et al, 1998; Cifuentes et al, 1999; Giltay et al, 1999; Oliver-Bonet et al, 2001). In contrast, an earlier study reported that the predominant segregation product was adjacent II segregation (39.3%), followed by adjacent I (16.2%) and alternate (14.5%) products (Goldman and Hulten, 1993). Pellestor et al (1997) reported 2 cases. One of the cases carrying the 7; 18 translocation showed adjacent I (43.0%) as the predominant segregation, followed by alternate segregation (33.3%) and adjacent II segregation (19.4%); the other case carried the 7;9 translocation, and the predominant segregation product was alternate (44.4%), followed by adjacent I (37.0%) and adjacent II (12.9%). By contrast, Martini et al (1998) and Estop et al (1999) reported a high incidence of 3:1 segregation of 28.9% and 40.1%, respectively.

There are apparent discrepancies in data obtained by either the hamster oocyte-sperm or the FISH technique in the literature. One possible explanation of these differences is that the carriers studied might be heterogeneous because of conditions such as age, diseases of the reproductive organs, diet, and medical treatment, all of which may have an effect on the genetic constitution of sperm nuclei (Rubes et al, 1998). In fact, in this study, the fourth carrier had some differences in the segregation product when compared with the other 3 carriers. This may have been related to the carrier's age. Another possible reason is that there is a higher incidence of one segregation product type than another, depending on each individual chromosome involved in translocation. Jalbert et al (1980) hypothesized that these differences may be related to the unequal translocated segments, the position of break points, and the involvement of acrocentric chromosomes. In this study, although chromosome 15 is an acrocentric chromosome involved in translocation, the segregation patterns were different from those of t(11;22), as reported by Estop et al (1999). This may be attributable to differences in each individual acrocentric chromosome involved in the translocation. Another possible reason may be related to the technique and the criteria used to evaluate the results. In this study, in order to eliminate the probe localization factor, metaphase spreads obtained from the peripheral blood of each carrier were analyzed by an application of the same probe mixture, since there was an extra signal from the chromosome 15 centromere-specific alpha satellite DNA sequence on chromosome 14 (Çora et al, 2000). All carriers had the same intensity and specific signals for each probe. The hamster technique allows very few sperm nuclei to be analyzed, and it must be kept in mind that the relative ability of genetically unbalanced sperm to penetrate hamster eggs has not been extensively studied. In conclusion, the incidence of segregation products of reciprocal translocation in sperm nuclei of carriers can be evaluated by FISH, using the proper combination of probes, and will give patients more accurate genetic advice. In addition, further studies will enable us to explain the behavior of segregation patterns and the mechanism for each type of translocation from carrier to carrier and their effects on reproduction.

Acknowledgments

We thank Drs M. Aktan for his help in sperm analysis, F. Kara for his assistance in statistical analysis of the data, and M. S. Yildirim for technical assistance. We also express our appreciation to Drs Ahmet Arslan, Olivia Buchanan, Yususf Z. Igci, A. Kahan Karabulut, and Ercan Kurar for

reviewing and editing the manuscript.

Footnotes

Supported by grant TF 2001/00044 from the Selçuk University Research Fund.

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