Determination of Centromere Number of Chromosomes Involved in Robertsonian Translocation by using Fluorescence in Situ Hybridization (FISH)

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ABSTRACT

Robertsonian translocation is one of the major chromosomal re-arrangements and constitutes 18% of all genetic abnormalities with an incidence of 1/1000 in the general population. Robertsonian translocations are characterized centric fusion of the long arms of the acrocentric chromosomes and generally occur between chromosome 13q, 14q and 14q,21q. In this study, the centromere numbers of 22 patients with Robertsonian translocations were evaluated by using fluorescence in situ hybridization with repetitive DNA probes localized on the centromeric region of acrocentric chromosomes. We detected that all Robertsonian translocation have a single cetromere. However some of case showed a distinct size of FISH signals on cetromere region, which may indicate the duplication or two different satellite DNA sequences. Therefore these cases should be clarified for the composition of monocenric centromere sequence.

Key Words: *Robertsonian translocation, centromere, dicentric chromosome, FISH*

Robertsonian Translokasyonlarda İşe Karışan Kromozomların Sentromer Sayısının Floresan in Situ Hibridizasyon (FISH) ile Belirlenmesi

ÖZET

Robertsonian translokasyon major kromozomal yeniden düzenlenmelerden biridir. Bu anomali genel populasyonda 1/1000 sıklık ile tüm genetik anomalilerin % 18'ini oluşturur. Robertsonian translokasyonlar, akrosentrik kromozomların uzun kollarının füzyonu ile karekterize ve genellikle kromozom 13q ve 14q, 14q ve 21q arasında meydana gelmektedir. Mevcut çalışmada 4 farklı tipde 22 Robertsonian translokasyon taşıyıcı ve hasta bireyin, akrosentrik kromozomlarındaki sentromer sayıları bu bölgelere spesifik repetetif DNA probları ile fulorescence in situ hybridization tekniği kullanılarak incelendi. Elde edilen FISH sonuçlarına göre Robertsonian translokasyonların tümünün monosentrik olduğu tesbit edildi. Bununla birlikte vakaların bazılarının setromer bölgesinde farklı büyüklükte FISH sinyalleri gözlendi. Bu farklı yapının sebebi sentromeri oluşturan dizilerin duplikasyonu veya iki farklı satellit DNA sekansı olabilir. Sonuç olarak, çalışmaya dahil olan tüm vakalar monosentrik olmakla birlikte, monosentrik yapıyı oluşturan satellit DNA dizilerinin karekterize edilmesi gerekmektedir.

Anahtar Kelimeler: *Robertsonian translokasyonlar, sentromer, disentrik kromozom, FISH*

INTRODUCTION

Robertsonian translocations are one of the most common structural chromosomal aberrations observed in humans and occur in approximately 1 in every 1000 newborns (1). This translocation can be either de novo or be transmitted by one of carrier parent. Robertsonian translocations are characterized centric fusion of the long arms of the acrocentric chromosomes 13, 14, 15, 21 and 22, with the translocated chromosome bearing either one or two centromeres and resulting in a 45 or 46 chromosome karyotype. The majority of Robertsonian translocations involve two nonhomologous acrocentric chromosomes. The most frequent Robertsonian translocations are der t(13q;14q) and t(14q;21q) have an estimated frequency of 0.97 and 0.20, respectively (2). The acrocentric chromosomes in man have been intensively investigated, both because of their frequent involvement in chromosomal aberrations and aneuploidy and because of their unique structural properties (3). Rearrangements of the acrocentric chromosomes are associated with an increased risk of aneuploidy. In generally, carriers of Robertsonian translocations are phenotypically normal but are at increased risk for infertility, spontaneous abortions or chromosomally unbalanced offspring. Malsegregation of Robertsonian translocation, results in trisomy or monosomy of complete chromosomes (4).

The centromere regions are mystery for different scientists; for cytologists, it is the primary constriction of the mitotic chromosome, for geneticists it is the origin from which recombination distances are measured. Other equally valid definitions include the following: a specialized DNA sequence, a specialized type of heterochromatin, a structure that regulates sister-chromatid-pairing, a structure that attaches the chromosome to the ends of growing or shrinking microtubules, a marshalling region where chromosomal passenger proteins congregate prior to their function elsewhere during the closing stages of mitosis, and a signaling device that monitors chromosomal alignment and tells the cell when it is safe to segregate sister chromatids in mitosis and homologs in meiosis (5). Molecular studies have shown that the pericentromeric and short-arm regions of acrocentric chromosomes have extensive sequence homology, although some sequences are not common to all of the acrocentrics. Several distinct and tandemly repetitive sequences are localized to these regions. Alpha-satellite DNA sequence is a major class of repetitive DNA found at the centromeric region of each human chromosome (6). Centromers can be involved in chromosomal anomalies. One of those is Robertsonian translocations. Robertsonian translocations have either single or two centromeres. Therefore we aimed to evaluate the centromer numbers in the patients having Robertsonian translocation by using fluorescence in-situ hybridization (FISH) techniques with acrocentric centromere-specific probes.

MATERIAL AND METHODS

Patients

This study includes total 22 unrelated (13 female and 9 male) patients with Robertsonian translocation. Twelve of them were Robertsonian translocation carriers including 3 t(14;21) and 9 t(13;14) who have repeated miscarriages and infertility history. The other 10 patients were Down syndrome. Five of them were t(14;21), 3 t(21;21) and 2 t(15;21). The age of the patients ranged from one mounth to 28 years.

Fluorescence in Situ Hybridization (FISH)

In this study, patients consulting for different purposes such infertility, repeated miscarriages, and birth of a malformed child were included. Conventional cytogenetic analysis in lymphocyte chromosomes revealed a chromosomal translocation for each patient. Briefly, standard chromosome preparations from blood lymphocytes of the carrier and the control groups were used. In order to verify the number of centromere on Robertsonian translocation, FISH analyses were done by using D13Z1/D21Z1 and D14Z1/D22Z1 and D15Z1 probes (Cambio, UK). D13Z1/D21Z1 was labeled with Cy3 (fluoresces red), and D14Z1/D22Z1 was labeled with biotin and then was detected with avidin conjugated with fluorescein isothiocyanate (FITC; fluoresces green) according to previously published procedures (7). For the samples, the probe mixtures were denatured at 70ºC for 10 min, while DNA was denatured in 70%formamide/2 SSC at 70ºC for 2-3 min. The slides were then dehydrated through an ascending alcohol series (70, 85, and 100%), and airdried. The denatured probe mixture was applied onto the slides. The hybridization was performed in 40ºC moisture chamber overnight. The slides were washed twice for 5 min with 2 SSC, 50% formamide/2xSSC and 2xSSC at 42 ºC. They were then stained with a counterstain medium containing DAPI (40,6-diamidino-2-phenyl-indole).

***Table 1**. Karyotypes, circumstances of discovery of structural chromosomal abnormality and centromer constitution used to perform FISH of 22 patient or carriers of Robertsonian translocation.*

Patient	Karyotype	Discovery	FISH probe	Centromer constitution
1	46, XX, t (21;21)	Down Sendromu	D13Z1/D21Z1	Monocentric
2	46, XX, t (21;21)	Down Sendromu	D13Z1/D21Z1	Monocentric
3	46, XX, t (21;21)	Down Sendromu	D13Z1/D21Z1	Monocentric
4	46, XY, t (14;21)	Down Sendromu	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
5	46, XX, t (14;21)	Down Sendromu	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
6	46, XX, t (14;21)	Down Sendromu	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
7	46, XY, t (14;21)	Down Sendromu	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
8	46, XY, t (14;21)	Down Sendromu	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
9	46, XY, t (15;21)	Down Sendromu	D13Z1/D21Z1 and D15Z1	Monocentric
10	46, XY, t (15;21)	Down Sendromu	D13Z1/D21Z1 and D15Z1	Monocentric
11	45, XX, t (14;21)	Repeated miscarriages	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
12	45, XX, t (14;21)	Repeated miscarriages	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
13	45, XY, t (14;21)	Repeated miscarriages	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
14	45, XX ,t (13;14)	Repeated miscarriages	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
15	45, XX ,t (13;14)	Repeated miscarriages	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
16	45, XX, t (13;14)	Repeated miscarriages	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
17	45, XX ,t (13;14)	Repeated miscarriages	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
18	45, XX, t (13;14)	Repeated miscarriages	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
19	45, XY, t (13;14)	Primary infertility	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
20	45, XY, t (13;14)	Oligoasthenoteratozoo-spermia	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
21	45, XY, t (13;14)	Oligoasthenoteratozoo-spermia	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric
22	45, XY, t (13;14)	Oligoasthenoteratozoo-spermia	D13Z1/D21Z1andD14Z1/ D22Z1	Monocentric

The slides were examined with an epifluorescence microscope (Nikon, Optiphot) equipped with a set DAPI, fluorescein isothiocyanate (FITC) and rhodamine filters. These filters allow individual detection of red and green.

RESULTS

In this study, we analysed centromeric number in 22 unrelated (13 female and 9 male) Robertsonian translocation patients using FISH technique. FISH analysis on metaphase and interphase cells from the patient's lymphocyte using probes to the centromeres of chromosomes 13/21, 14/22 and 15 revealed that the translocation had a hybridization signal from each probe, indicating that these rearrangements are monocentric. The results obtained from both conventional cytogenetic and FISH analaysis with chromosome spesific probes are given in Table 1. FISH signals on some translocations chromosomes were appeared as in distinc size.

DISCUSSION

The centromere is an essential functional domain responsible for the correct inheritance of eukaryotic chromosomes during cell division. Molecular studies have shown that the pericentromeric and short-arm regions of these five pairs of acrocentric chromosomes have extensive sequence homology, although some sequences are not common to all of the acrocentrics. Several distinct and tandemly repetitive sequences are localized to these regions. Alpha-satellite is a major class of repetitive DNA found at the centromeric region of each human chromosome (6). Acrocentric chromosomes contain a number of satellite subfamilies, some of which are shared by different acrocentrics. These shared sequences have been postulated to be involved in the formation of Robertsonian translocations and in nonrandom participation of chromosomes 13, 14, and 21 in most Robertsonian translocations (1).

The short arms of acrocentric chromosomes can be divided into three distinct portions. The proximal short arm (pll) contains satellites I-IV (8, 9), beta-satellite (10) and the interspersed 724 repeated sequence (11). The stalk, or nucleolus organizer region (NOR) (p12), contains the 18S and 28S ribosomal genes (12). Finally, beta-satellite DNA and 724 DNA have been mapped to the distal cytological satellite (p13). In Robertsonian translocation, single or double centromeres may be formed depending on the breakpoint of chromosomal regions. These centromeric composition and centromer number have an effect on chromosomal segregation, resulting in the further abnormalities.

In the present study, we analysed centromeric number in 22 unrelated (13 female and 9 male) Robertsonian translocation patients or carriers who have four different type of this by using FISH technique. It has been determined that centromeric number is structurally monocentric in all of Robertsonian translocations. FISH analysis on metaphase cells from the patient's lymphocyte culture using probes to the centromeres of chromosomes 13, 14, 15, 21 and 22 revealed that Robertsonian translocations had a hybridization signal from each probe, indicating that this rearrangements is monocentric. However the structure at the centromere of the rearrangements could not be distinquish the origine of centromeric composition in single color FISH study.

In the literature, Lemyre E, et al (13) suggested that most Robertsonian translocations are dicentric and also the location of chromosomal breaks leading to their formation occur in the acrocentric short arm. Furthermore, number of authors also confirm that in situ hybridization methods were used to clarify that the majority of Robertsonian translocations retain the alpha satellite DNA from both chromosomes involved and thus are structurally dicentric, with the breakpoints usually located in the acrocentric short arms, proximal to the NOR (3,7,9,14,15,16). These kind of dicentric chromosomes are stable during the cell division due to one of centromer inactivation (17). However, this translocation may result in unbalanced ofspring in the next generation.

In our present study, some of case showed a distinct size of FISH signals, which may indicate the duplication or two different satellite DNA sequences in adjacent status. Therefore, these cases should be clarified for the satellite DNA composition of monocenric centromere sequence by using multicolor FISH study. These sequence composition may effect on the occurence of interchromosomal effect raising chromosome aneuploidy and/or the behavior of translocated chromosome segregation in next generation.

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