

Marker Chromosomes in Korean Patients: Incidence, Identification and Diagnostic Approach

The identification of marker chromosomes is important for genetic counseling. However, the origin or composition can rarely be defined with conventional cytogenetic technique alone. In this study, we investigated the incidences and types of marker chromosomes in Korean patients and attempted to establish a cost-effective diagnostic approach for marker chromosomes. We reviewed the karyotypes of 2,984 patients that were requested for the cytogenetic analysis between 1997 and 2003 at the Samsung Medical Center. Ten marker chromosomes were found and identified using fluorescent in situ hybridization (FISH). Among the ten marker chromosomes, six were supernumerary marker chromosomes (SMCs) and the rest were marker chromosomes in Turner syndrome (TS). The incidence of SMCs was 2.01/1,000, slightly higher than that previously reported. Five of six SMCs were satellited marker chromosomes. Three bisatellited marker chromosomes originated from chromosome 15 and two from chromosome 22. The origin of one SMC could not be identified. All marker chromosomes in TS originated from X- or Y chromosome. The application of FISH is indispensable to identify marker chromosomes, and the appropriate selection of probes is necessary for cost-effective analysis. For analyzing satellited marker chromosomes, application of probes for chromosome 15 followed by those for chromosome 22 is recommended and in cases of TS, probes for sex chromosomes should take precedence.

Key Words : Genetic Markers; Chromosome Markers; In situ Hybridization, Fluorescence; Incidence

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INTRODUCTION

Marker chromosomes are defined as unidentified structurally abnormal chromosomes and their consequences may range from being harmless to detrimental. Most of the marker chromosomes are supernumerary marker chromosomes (SMCs) that occur in addition to the 46 normal chromosomes and sometimes as constituents of the 46 chromosomes by replacing normal chromosomes. The latter is frequently found in patients with Turner syndrome (TS). About 3% of TS patients have a marker chromosome in addition to monosomy X (1). Meanwhile, prevalence of SMCs at birth is estimated at 0.6-1.5/1,000 (2, 3). About 80% of SMCs arise de novo and occur more frequently with advanced maternal age (4). However, there has been no study estimating the incidence of SMCs in Korea.

Despite the importance of identifying the origin or composition of the marker chromosomes in genetic counseling, they can rarely be defined with conventional cytogenetic techniques alone. In recent years, the ascertainment of chromosome origin has been made possible by the use of fluorescent in situ hybridization (FISH) (5, 6). The use of FISH has rendered possible the delineation of genotype/phenotype correlations in

some cases. However, the application of all kinds of probes is practically impossible due to high costs and limited specimens.

We conducted this study to investigate the incidences and types of marker chromosomes in Korea and to establish a cost-effective approach for identifying unknown marker chromosomes by FISH analysis.

MATERIALS AND METHODS

Chromosome analysis for constitutional abnormality was performed on 2,984 patients (1,541 amniotic fluid specimens, 1,443 peripheral blood specimens) between 1997 and 2003 at the Samsung Medical Center. Ten patients with marker chromosomes were found and studied.

Metaphase chromosome analysis of cultured amniocytes or peripheral blood lymphocytes and GTG-staining were performed using standard methods. Karyotypes were interpreted according to the International System for Cytogenetic Nomenclature, 1995 (7).

FISH studies using probes *D15Z1*, *D15S10*, *SNRPN*, *TUPLE1*, *ARSA*, *SRY*, and whole chromosome paint probes (WCP) or chromosome enumeration probes (CEP) for X- and

Y chromosomes (Vysis Inc., Downers Grove, IL, U.S.A.) were performed according to the manufacturer's instructions (Table 1). Additional studies for sex-determining region Y (SRY) sequences were done using polymerase chain reaction (PCR) in one case of TS.

The probes were hybridized to metaphase cells and the images were captured with the Cytovision Image Analyzer (Applied Imaging International Ltd., Newcastle, England). In each analysis, the signals from the normal homologous chromosomes served as internal controls and the marker was scored positive only if the normal homologues and the marker were labeled in at least five metaphases.

Table 1. The panel of FISH probes* used to identify marker chromosomes

Chromosome	Region	Locus
15	Centromere	<i>D15Z1</i>
15	15q11.2	<i>SNRPN</i>
15	15q11.2	<i>D15S10</i>
22	22q11.2	<i>TUPLE1</i>
22	22q13.3	<i>ARSA</i>
X	Centromere	<i>DXZ1</i>
X	Whole chromosome	-
Y	Centromere	<i>DYZ1</i>
Y	Whole chromosome	-
Y	Yp11.3	<i>SRY</i>

*All probes are made by VYSIS (Vysis Inc., Downers Grove, IL, USA).

RESULTS

Incidence and identification of marker chromosomes

Marker chromosomes were observed in ten patients. Among the ten marker chromosomes, six were SMCs and the rest were marker chromosomes in TS. The incidence of SMCs in this study was 2.01/1,000. The origins of the marker chromosomes were identified in nine cases. In the remaining one case, no signal was observed on FISH with the aforementioned battery of probes. The markers were first categorized into two groups according to the presence or absence of chromosomal satellites, and later into three groups according to the FISH results. Five of six SMCs were satellited marker chromosomes. Three bisatellited marker chromosomes were found to be idic(15q) while two satellited markers originated from chromosome 22. Two of the three idic(15q) contained euchromatin including the region responsible for Prader-Willi/Angelman syndrome (PWS/AS) and both showed no deletion of the PWS/AS region in normal homologous chromosome 15. Analysis for PWS/AS region in the other idic(15q) case (case 1) could not be done due to the insufficient amount of specimen. All marker chromosomes in TS originated from the X- or Y chromosome: two originated from X chromosome and the other two from Y chromosome. One patient (case 7) with a marker originating from the Y chromosome showed SRY sequence while two patients with 45,X/46,X,mar(X) did not.

The inheritance status of the marker was ascertained in three

Table 2. Summary of ten cases showing marker chromosomes

Case No.	Karyotype	Age/Sex	Chr. Origin	Morphology	Size	Parental	Phenotype
A. Markers with satellites							
1	47,XX,+mar.ish idic(15)(q13)(D15Z1++)	6 yr/F	15	bi-satellited	≤22	NE	Developmental delay, tip-toeing gait, mental retardation, impaired speech, seizure
2	47,XX,+mar.ish idic(15)(q13)(D15Z1++, D15S10++,SNRPN++)	5 yr/F	15	bi-satellited	≤22	NE	Developmental delay, hyperactivity
3	47,XX,+mar.ish idic(15)(q13)(D15Z1++,D15S10+)	10 mo/F	15	bi-satellited	≤22	NE	Developmental delay, high-arched palate, microcephaly
4	47,XY,+mar.ish +del(22)(q13.1)(TUPLE1+,ARSA-)	3 days/M	22	satellited	<22	NE	Imperforated anus, iris coloboma, heart malformations
5	47,XX,+mar.ish +der(22)t(11;22)(q23.3;q11.2)mat (TUPLE1+,ARSA-)	15 days/F	22	satellited	<22	maternal	Intrauterine growth retardation, hypotonia, preauricular tag, ptosis of left eye
B. Markers accompanied with monosomy X							
6	45,X[3]/46,X,+mar.ish der(Y)(wcpY+)[7]	15 yr/F	Y	small	≤22	NE	Short stature, no secondary sex characteristics
7	45,X[94]/46,X,+mar.ish der(Y)(wcpY+,SRY+)[6]	18 yr/F	Y	small	≤22	NE	Short stature, primary amenorrhea
8	45,X[4]/46,X,+mar.ish der(X)(wcpX+,SRY-)[6]	26 yr/F	X	small	≤22	NE	Primary amenorrhea, poor secondary sex characteristics
9	45,X[10]/46,X,+mar.ish der(X)(wcpX+,SRY-)[10]	2 yr/F	X	small	<22	de novo	Developmental delay, poor feeding
C. Markers without satellites							
10	47,XY,+mar.ish mar(D15Z1-,TUPLE-,wcpX-,wcpY-) mat[21]/46,XY[9]	17 ⁺ GA/M	unidentified	small	<22	maternal	Follow-up loss

Chr, chromosomal; GA, gestational age; NE, not established.

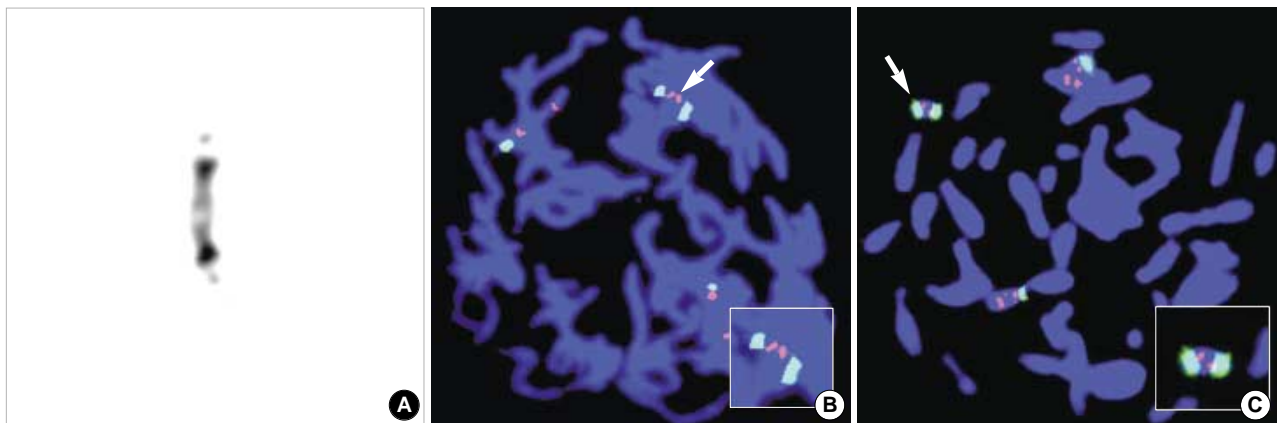


Fig. 1. Routine and metaphase FISH analysis of the bisatellited SMC, idic(15)(q13). (A) GTG-banding of the SMC. (B) FISH analysis with the *D15S10* (red), *D15Z1* (green), and control *PML* (red) probes. Normal chromosome 15 homologues showed a signal for the *D15Z1*, *D15S10*, and *PML* probes. SMC has two copies of *D15S10* and *D15Z1*. (C) FISH analysis with the *SNRPN* (red), *D15Z1* (green), and control *PML* (red) probes. SMC has also two copies of *SNRPN* and *D15Z1*. Arrows indicate SMCs. Images in the lower right box showed the magnified marker chromosomes.

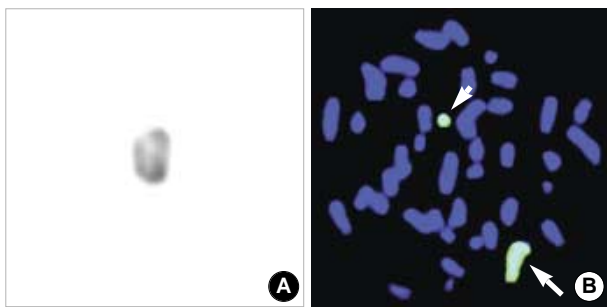


Fig. 2. Routine and metaphase FISH analysis of the marker chromosome accompanied with Turner syndrome. (A) GTG-banding of the SMC. (B) Identification of the X chromosomal material using metaphase FISH analysis with the whole chromosome paint probe (wcp) X (green). Big arrow indicates the normal X chromosome and small arrow indicates the marker chromosome.

cases. Two marker chromosomes were of maternal origin and one was de novo. In case 5, the mother of the patient was a carrier of the unique, non-Robertsonian, reciprocal balanced translocation $t(11;22)(q23.3;q11.2)$. In case 10, the same abnormal marker chromosome was found in a phenotypically normal mother. Table 2 shows the summary of the present series and Fig. 1-3 reveals the morphology and FISH results of the marker chromosomes.

Diagnostic approach to the marker chromosomes

The selection of probes was made on a case-by-case basis. The first criterion of selection was the morphology of the marker chromosomes observed on G-banding analysis. If there were probable chromosomes derived from, the probes for those chromosomes were applied first. The presence of satellites or accom-

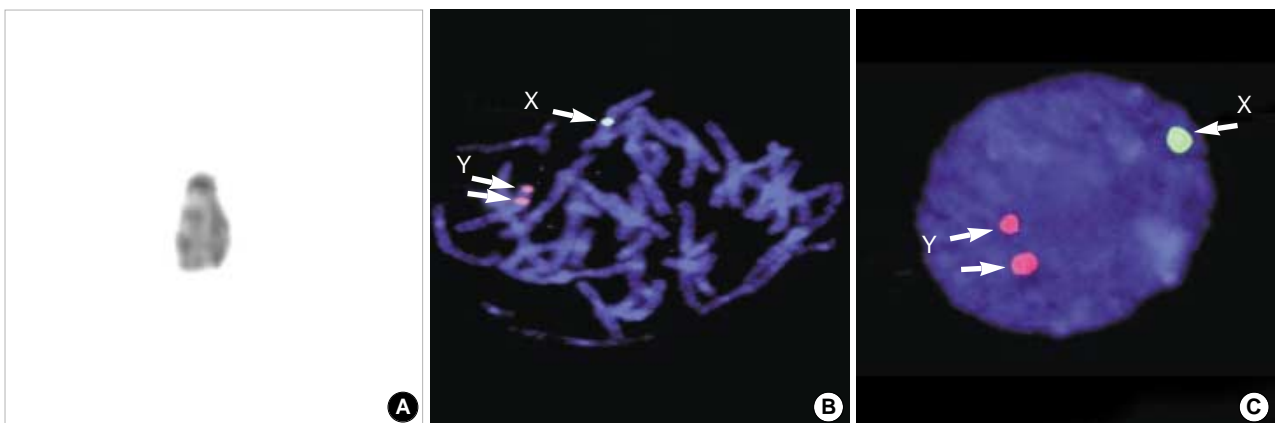


Fig. 3. Routine and FISH analysis of the marker chromosome accompanied with Turner syndrome. (A) GTG-banding of the SMC. (B) Identification of the Y chromosomal material using metaphase FISH analysis with the probes for Y centromere (red) and X centromere (green). Two red signals were observed. (C) Identification of the Y chromosomal material using interphase FISH analysis with the probe for Y centromere (red).

panied chromosome abnormalities was also important clues for approach.

According to this study, we would like to recommend the following. In patients with satellited marker chromosomes, probes for chromosome 15 can be applied first followed by probes for chromosome 22. If the origins of the markers could not be identified by these probes, probes for other satellited chromosomes could be considered in addition to testing the probes for X- and Y chromosome. In cases with marker chromosomes accompanied with monosomy X, probes for X- and Y chromosome should be applied simultaneously first. If possible, parental studies should be done to determine inheritance status.

DISCUSSION

The presence of a marker chromosome draws particular attention in genetic counseling because it may be associated with malformations and developmental abnormalities, although it is also found in phenotypically normal individuals. If marker chromosomes contain only the paracentromeric region and/or satellites, and thus little or no euchromatic material, they are considered to present lower risks of adverse clinical sequela (1, 8). In any case, a detailed analysis of marker chromosomes is mandatory for appropriate genetic counseling.

The incidence of SMCs in the present study was 2.01/1,000, higher than 0.6-1.5/1,000 previously reported (2, 3). We presume the reason to be the selection bias. That is, the subjects that were cytogenetically investigated here had been clinically suspected as having constitutional chromosome abnormalities.

According to a study by Buckton *et al.* on a large population, approximately 86% of the SMCs were derived from the acrocentric chromosomes (2). However, Blennow *et al.* (9) reported 5 of 37 SMCs of acrocentric origin containing no satellite region. This result could have been an underestimation considering only SMCs with satellites were recognized cytogenetically. This observation underlines the need for FISH in order to make a correct determination of the origin. In the present study, 5/6 (83.3%) SMCs, a similar proportion to that reported, were derived from the acrocentric chromosomes.

The most common SMCs are known to be SMCs originating from chromosome 15 (SMC(15)), in the form of inv dup (15), constituting approximately half of the cases in population studies (2). The present series also showed three (60%) SMC(15)s among five acrocentric SMCs. The effect of the SMC(15)s exerted upon the phenotype is determined by the extent of the duplicated region and the parental origin (10). SMC(15)s containing no euchromatin or only euchromatin proximal to the PWS/AS region have been generally associated with a normal phenotype (11, 12). In contrast, larger SMC(15)s containing the PWS/AS region have been associated with a wide spectrum of clinical features observed in PWS/AS, from normal to full PWS/AS phenotype (13). However, even if

SMC(15)s contained the PWS/AS region, paternally-derived SMC(15)s were associated with normal phenotype, while, in contrast, maternally-derived SMC(15)s showed an abnormal phenotype (13). All cases except case 1 showed larger SMC(15)s containing the PWS/AS region. Furthermore, all showed PWS/AS-like clinical features, but none met the diagnostic criteria of PWS or AS. Although SMC(15)s were noted to occur at a higher frequency in patients with PWS than in the normal population, they lacked the PWS/AS region and the symptoms were assumed to be caused by maternal uniparental disomy (UPD) or loss of the PWS/AS region (14). So molecular studies using methylation PCR to detect UPD or deletion of the PWS/AS region should be considered in all SMC(15) cases including familial cases inherited from a phenotypically normal carrier parent.

22q11.2 has been known as a very unstable region of chromosome 22, which is involved in several syndromes related to constitutional anomalies such as Cat eye syndrome (CES, a trisomy or tetrasomy of 22q11), DiGeorge syndrome (a microdeletion), and supernumerary der(22)t(11;22) syndrome. Similar to SMC(15)s, SMC(22)s containing additional copies of proximal 22q euchromatin are associated with patients with one or more of the malformations related to CES (15). However, there was no straightforward correlation between the extent of additional copies found within the marker and the severity of the CES phenotype. Case 4 had an extra satellited monocentric chromosome 22 with terminal deletion at 22q13.1, trisomic of "critical region", 22pter-22q11. He showed an imperforated anus, coloboma of the iris and heart malformations, consistent with CES. Although colostomy had been performed immediately after birth, he died nine days thereafter. Case 5 had a SMC identified as a derivative chromosome 22 from the unbalanced translocation between chromosome 11 and 22 with breakpoints at 11q23.3 and 22q11.2, respectively. The patient showed intrauterine growth retardation, hypotonia, preauricular tag, and ptosis of the left eye, which were consistent with supernumerary der (22) syndrome. The clinical features of supernumerary der (22) syndrome are the combined effects of partial trisomies for both 11q and pericentromeric 22q (16).

Cases 6-9 were found to have mosaic 45,X/46,X,+mar. FISH with wcp X and wcp Y confirmed the Y origin of the marker chromosomes in cases 6 and 7 and the X origin in cases 8 and 9. Approximately 50% of patients with TS have either various structural sex chromosome rearrangements or mosaics with a variety of karyotypes and manifest the full or partial Turner phenotype (17). Meanwhile, a Y chromosome or derivative of Y is present in 6% of the TS patients, and an additional 3% have a marker chromosome that has been derived from either the Y chromosome or some other chromosome (18). The presence of Y chromosome sequences in TS patients merits special attention because it predisposes the patients to developing gonadoblastoma and virilization with an estimated risk of 15-25% (4, 19-21). Considering this potential risk, the

screening of all TS patients for the presence of Y sequences has been previously suggested (4). Two of our cases were also mosaic TS with Y chromosome sequences.

It has been reported that TS patients with 45,X/46,X,mar(X) karyotypes may have unusually severe phenotypes, which include mental retardation, soft tissue syndactyly, or abnormal faces (22-24). Lack of the X inactivation center (XIC) at Xq13.2 in these patients leads to functional disomy of the proximal part of the X chromosome, which may have contributed to the severe phenotypes observed in these patients (23). We could not determine whether or not case 9 had mental retardation due to her age. But case 8 with mar(X) did not have mental retardation, and we can assume that her mar(X) contained XIC, thus no functional disomy. Karyotyping for more than 30 metaphases is mandatory in TS patients with some degree of mental retardation to detect mar(X) chromosomes and, when detected, the parental origin and X inactivation status of the chromosome should be subsequently defined for accurate delineation of phenotype-genotype correlation.

In case 10, we could not determine the origin of the SMC. It may be assumed that the SMC ran in the patient's family without association to phenotypic abnormalities because her mother, who possessed the same marker chromosome, had a normal phenotype. Additional FISH studies for chromosomes other than chromosomes 15, 22, X, and Y would be needed to establish the make-up of the SMC.

In conclusion, we could estimate the rough incidence of SMCs in Korea. Satellited markers originating from chromosome 15 were most common. The molecular cytogenetic technique, FISH, accurately delineate the nature and origin of marker chromosomes, which is difficult by conventional cytogenetic procedures alone. In particular, the initial selection of appropriate probes according to the morphology on G-banding analysis is critical for cost-effective characterization. That is, in cases with satellited marker chromosomes, probes for chromosome 15 can be applied first followed by probes for chromosome 22. If the origins of the markers could not be identified by the above probes, the use of probes for other satellited chromosomes in addition to X- and Y chromosomes should be considered. For TS patients with marker chromosomes, beginning with probes for X- and Y chromosome is preferable. Besides the identification of marker chromosomes, clinical correlation, parental study, and consideration of other possibilities are important in genetic counseling for patients with marker chromosomes.

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