PRINT: ISSN 0972-3757 ONLINE: 2456-6360
Int J Hum Genet, 11(3): 177-182 (2011)
DOI: 10.31901/24566330.2011/11.03.05

Application of Fluorescence *in situ* Hybridization (FISH) Technique to Discern Complete/Partial Monosomy 21

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KEYWORDS Partial Monosomy. GTG. Interphase. Metaphase. FISH

ABSTRACT The present study pertains to a 7 month old female infant who showed dysmorphic features and developmental delay. Conventional cytogenetic analysis by GTG banding technique was carried out and this revealed monosomy 21. Interphase FISH and metaphase FISH were employed for better delineation of the observed results. FISH analysis confirmed unbalanced X;21 translocation. This study concludes that FISH technique along with conventional cytogenetic analysis serves for better understanding and interpretation of complete/ partial autosomal monosomies.

INTRODUCTION

Autosomal monosomies in humans are generally more unusual than the sex chromosomal monosomies and are suggested to be incompatible with life as such cases have been occasionally detected in spontaneous abortions (Riegel et al. 2005). The only monosomy potentially viable in humans seems to be that of the X-chromosome (Gravholt 2005). Non-mosaic monosomy 21 is listed as a "rare disease" by the National Institutes of Health (NIH) since the incidence is about 1 in 30,000 live births (Gupta et al. 2006) and is suggested to thrive only if it is present as a partial monosomy or mosaic (Cardoso et al. 2008; Lee et al. 2001; Schinzel 2001). The majority of monosomy 21 is the result of unbalanced translocations, where the pericentric region of chromosome 21 pairs with the X chromosome (Mattei MG et al. 1982). However, translocations involving X chromosome and an autosome are rare, since most of such individuals are often infertile (Layman 2002). So far, only about eight known cases of unbalanced X;21 translocations have been reported worldwide (Cardoso et al. 2008; Mattei MG et al. 1982). Currently available reports on monosomy 21 identified by conventional cytogenetics are actually cryptic reciprocal translocations resulting in partial monosomies or trisomies (Lee et al. 2001). With this background, the present study describes a female infant with multiple congenital malformations diagnosed with a non-mosaic monosomy of chromosome 21 after GTG-banding, which subsequently turned out to be a partial monosomy 21 caused by an unbalanced translocation of chromosomes X and 21 by FISH analysis.

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CLINICAL REPORT

A 7 month old female, born to 2° consanguineous parents was referred for dysmorphic features and developmental delay. The mother was aged 21 years and the father 32 years at the time of conception. After an uneventful pregnancy, the child was born full term by normal vaginal delivery (NVD) with a weight of 2 kg. There were no perinatal issues. There was a history of developmental delay with the social smile and head control appearing at 7 months of age. On examination she was found to have dysmorphic features like frontal bossing, low set large ears, a long philtrum, micrognathia, hemangioma of the alar nasi, long slender fingers with camptodactyly, a short neck, widely spaced nipples,

sacral dimple and café au lait spots (Fig.1). She had hypertonia and bilateral Congenital Talipes Equino Varus (CTEV) and bilateral Congenital Dislocation of Hips (CDH) and had surgical correction of the same. Ocular examination revealed bilateral iris coloboma. Audiometry evaluation was suggestive of bilateral moderate hearing loss. Magnetic Resonance Imaging (MRI) of the brain was normal. Echocardiogram revealed atrial septal defect. Ultrasonography of the abdomen was normal. Cytogenetics analysis for the proband and her family was performed.

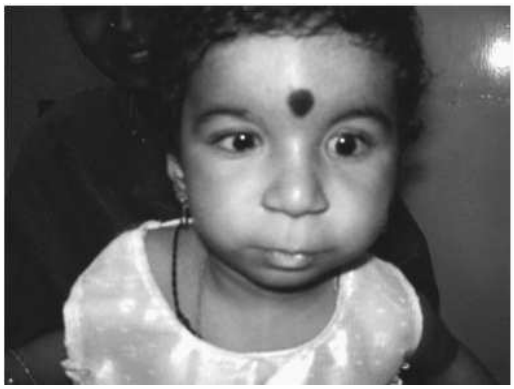

Fig. 1. Clinical photograph of the proband

METHODS

Conventional Cytogenetic Technique

The present study employed human leukocyte culture for metaphase slide preparation followed by GTG (G bands by Trypsin using Geimsa) banding to observe numerical and structural chromosome aberrations. Human leukocyte culture was established by adding 0.5 ml of peripheral venous blood obtained from the proband in a Sodium Heparin tube (BD Vacutainer, USA) into a 30 ml culture vial containing 5 ml of RPMI 1640 medium, 2 ml of FBS (Foetal Bovine Serum) and 0.2 ml of PHA (Phytohemagglutinin). The culture was incubated at 37 ºC and for 69 h of incubation, 0.1 ml of colchicine was added and the vials were re-incubated at 37 ºC for 12 min to arrest the cells in metaphase. The cell suspension was centrifuged at 1000 rpm for 10 min for the cells to settle. After removing the supernatant, 10 ml of 0.075 M pre-warmed hypotonic solution (KCl) was added and the vials were incubated at 37 ºC for 15 min. Further, the cell suspension was repeatedly centrifuged with the addition of 0.5 ml of fresh Carnoy's fixative containing methanol:glacial acetic acid in 3:1 ratio with gentle mixing until clear white cell pellet was obtained. Ice cold methanol slides were used for the metaphase preparation. Prepared slides were baked at 58 ºC for one week.

GTG Banding

The prepared metaphase slides were suspended in 0.005% trypsin for 2 sec followed by treatment in Sorenson's buffer for 5 sec. The slides were stained with Giemsa for 15 min and metaphase spreads were observed using a light microscope (100X magnification) (BH-2, Olympus, Japan) after a wash with distilled water. Cytovision software 3.1 version (Applied Imaging, US) was used to capture the metaphase spreads.

FISH Analysis

FISH analysis was performed for chromosome 21 to confirm the results of conventional analysis of metaphase spreads.

Interphase and Metaphase FISH

Interphase FISH was performed by using slides from 24 h culture. 10 µl probe (Aneu Vysion Multicolor DNA Probe Kit, Vysis CEP X - alpha satellite / LSI 13/21) was added to the slide, and sealed with the rubber solution. The slides were then denaturated for 5 min at 73 °C, followed by hybridization at 37 °C for 16-24 h. Further the slides were washed with 0.4 X SSC/ 0.3% NP 40 at 73 °C for 2 min and 2 X SSC/ 0.1% NP 40 at 37 °C for 2 min. The slides were counter stained with 10 µl of DAPI (Abbott Molecular Inc.) and viewed under fluorescence light microscope (BX-60, Olympus) using appropriate filters (DAPI, FITC, TRITC, Dual band pass filter, Multiband pass filter). The images were captured using the Cytovision FISH software (Version 3.1). The metaphase FISH analysis was performed using the metaphase slides prepared from 72 h cultures with similar FISH procedure.

RESULTS

Conventional Cytogenetic Analysis

Metaphase spreads of conventional GTG banding of the proband revealed an abnormal

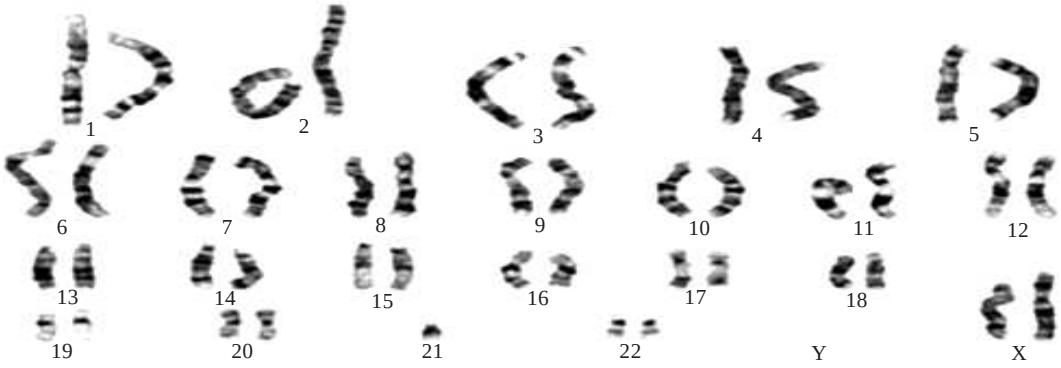

Fig. 2. Proband's karyotype showing monosomy of chromosome 21

female karyotype demonstrating full monosomy for chromosome 21 (45,XX,-21) in all the 45 metaphase spreads analysed with a 500 band resolution. Figure 2 represents the observed karyotype for monosomy 21. Parental chromosome analysis was found to be normal.

FISH Analysis

However, the possible changes in banding patterns within the proximal part of long arm of one of the homologous X chromosome lead to further analysis. To clarify whether the reported case was associated with X and 21 translocation, interphase and metaphase FISH analysis was performed.

The FISH analysis using LSI probe for chromosome 21 (Fig. 3A) revealed two signals representing disomy for that region of chromosome 21. Further analysis with 21 locus specific / X centromeric probe revealed an unbalanced translocation between chromosomes X and 21 (Fig. 3B) in all 100 metaphase spreads examined with one normal chromosome 21. The Karyotype was therefore revised as 45,X,der(X), t(X;21)(q25;q11.2),-21.

DISCUSSION

In spite of considerable advances in the field of clinical cytogenetics in the last three decades, the conventional GTG banding technique still faces cases with unforeseen findings (Iourov et al. 2008). The proband reported in this study exhibited many characteristic features of 'monosomy 21' reported by Schinzel (2001) in his catalogue. The comparison between the clinical features and the karyotype observed in the present study with that of the earlier reports have been illustrated in Table 1. The proband was initially diagnosed as a full monosomy 21. However, the characteristic fetal lethality of monosomy 21 prompted further molecular cytogenetic investigations using FISH analysis and this demonstrated the existence of X;autosome translocation leading to partial monosomy 21.

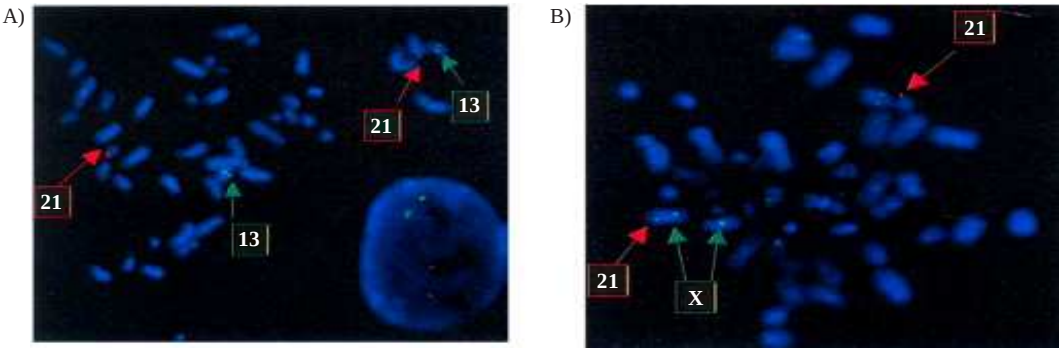

Fig. 3. A) Interphase and metaphase FISH with 13/21 probe; B) Metaphase FISH with 21/X probe

Table 1: Comparison of observations in the present study with earlier reported clinical features and karyotypes

	Couturier et al.(1979)	*Telvi et al.(1992)*	*Viljoen et al.(1992)*	*West and Allen (1998)*	*Cardoso LCA et al.(2008)*	*Our findings (2011)*
Monosomy 21 Karyotype	*45,X,-21, der(X) t(X;21)(q27; q11)*	*45,X,-21,der(X) t(X;21)(q28;q11)*	*45,X,-X,-21,+der(X) t(X;21)(Xqter→Xq 22.3::21q21.1→ qter)*	*45,X,-21,der(X) t(X;21)(q27;q21)*	*45,X,-21,+der(X) t(X;21)(q25;q21.1)*	*45,X,der(X), t(X;21)(q25; q11.2),-21*
Developmental delay	+	+	+	+	+	+
Hypertonia	+	+	NS	+	-	+
Downslanting small palperable fissures	-	+	-	+	+	-
Low hair line	+	NS	NS	+	+	-
Hypertelorism		+	-	+	+	-
Broad nasal base with a large tip	+	+	+	NS	+	+
Anteverted nostrils	NS	+	-	NS	-	-
Low set large ears	-	+	+	+	-	+
Prominent anthelix	-	+	NS	NS	+	-
Large lobes	-	+	NS	NS	-	-
Large carp mouth	+	-	-	+	+	-
Long Philtrum	NS	+	-	NS	+	+
Prominent occiput	NS	+	NS	NS	-	-
Short neck	+	+	NS	+	+	+
Micrognathia	NS	+	-	NS	-	+
Short thorax	NS	-	-	NS	+	-
Overlapping and/or flexed fingers/toes	-	+	+	NS	+	+
Kyphoscoliosis	NS	-	+	NS	+	-
Widely spaced nipples	NS	-	NS	NS	+	+

NS: Not Stated

The literature indicated that so far eight cases of unbalanced X;21 translocations have been reported worldwide as identified by FISH or molecular genetic studies of initially diagnosed 'full monosomy 21' (Cardoso et al. 2008; Mattei MG et al. 1982). The formation of such chromosome abnormalities are due to a reciprocal translocation involving chromosome 21 followed by the loss of one of the derivative chromosomes, regardless having an active centromere (Iourov et al. 2008). The majority of cases reported are de novo unbalanced translocations (Hertz et al. 1993; Lopez-Pajares et al. 1993; Flaherty et al. 1998; Phelan et al. 1988; Riegel et al. 2001). The proband in the present study has also demonstrated similar condition as the cytogenetic evaluation of the parents proved to be normal.

The phenotypic manifestations of monosomy 21 are most variable, depending upon the nature of the inactivated X chromosome or the deletion size and location. Several authors have suggested that the determining factor in the selection of the X to be inactivated would be the direction of the exchange of chromosomal material in the translocation process (Cohen et al. 1972; Crandall et al. 1974). Thus, in cases where X material would be translocated to an autosome, the normal X would be preferentially inactivated, and in cases of translocation from autosome to X, inactivation would be variable in nature. Hence, selective inactivation patterns are adopted to promote survival (Mattei MG et al. 1982). Inactivation of the translocated X would result in autosomal monosomy features, while inactivation of the normal X would maintain disomic condition of the autosome. Since the proband have showed features of a monosomy 21, it can be assumed that extreme skewed inactivation towards the unbalanced translocated X;21 has occurred in a larger group of cell lineages leading to the manifestations of the characteristic symptoms of a monosomy 21. For, if the translocated X which is a nullisomy for the deleted Xq23-Xq28 region and a monosomy for 21pter-21q11, were to be activated, the affected individual would probably exhibit short stature and streak gonads and not monosomy 21 features (Couturier et al. 1979; Schinzel 2001).

CONCLUSION

The present study signifies the importance of high resolution molecular cytogenetic analysis for the evaluation of cases that appear to be a full or partial monosomy of chromosome 21 due to unbalanced translocations. The conventional cytogenetic analysis could serve for initial diagnosis. However, in all cases of autosomal monosomies FISH technique along with conventional cytogenetic analysis could serve for better diagnosis and interpretation of results. Further studies establishing the X inactivation pattern are in prospect.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. S. Sridharan, Medical Director, KKCTH, Chennai, Tamil Nadu, India and Dr. Solomon F.D. Paul, Principal, Division of Human Genetics, Sri Ramachandra University, Chennai, Tamil Nadu, India for their support and help in the successful completion of the study.

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