

See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/236839300>

Fluorescence-in situ-hybridization”: Technology and its illustrated application in molecular medicine

ARTICLE · DECEMBER 2012

DOWNLOADS
90

VIEWS
86

2 AUTHORS:



Vijaya Kumar Pidugu

Academia Sinica

14 PUBLICATIONS 20 CITATIONS

SEE PROFILE



Usha Dutta

The Centre for DNA Fingerprinting and Diagnostics

24 PUBLICATIONS 25 CITATIONS

SEE PROFILE

VOLUME 13 (NS)

NUMBERS 1 & 2

JANUARY - DECEMBER 2012

THE JOURNAL OF CYTOLOGY & GENETICS



ISSN 0253-7605

FLUORESCENCE IN-SITU HYBRIDIZATION : TECHNOLOGY AND ITS APPLICATION IN MOLECULAR MEDICINE

VIJAYA KUMAR PIDUGU AND USHA R. DUTTA*

Diagnostics Division, Center for DNA Fingerprinting and Diagnostics, Tuljaguda, Nampally,

Hyderabad 500 001, India

*ushadutta@hotmail.com

(Received 27 January 2012, accepted 5 November 2012)

SUMMARY

The combination of molecular and cytogenetic techniques in the early 1980s have led to the field of molecular cytogenetics, which now plays a significant role in both clinical diagnostics and clinical research. Molecular cytogenetic techniques are based mainly on fluorescence *in situ* hybridization (FISH) which has become an invaluable tool in the field of diagnostics. FISH involves a fluorescently labeled DNA probe being hybridized to genomic DNA sequences used to study a specific site on a chromosome. Application of this modern cytogenetic technique has improved the detection of chromosomal aberrations. Here, we describe the technology and also illustrate a human case with a chromosomal abnormality and apparently characterize the abnormality by this molecular cytogenetic technique. We describe here 2 types of FISH one Direct and the other Indirect; we also describe an 18 months - old male child with delayed milestones. The cytogenetic analysis revealed a karyotype of 45,X,22p+. After ruling out the 22p+ region as extra chromosomal material, we could identify the p+ region with whole chromosome paint probe. The origin of the dicentric nature was confirmed by satellite enumeration probe of the derivative chromosome. Fine mapping with bacterial artificial chromosomes helped us to find the extent of deletion. Precise identification of such rearrangements helps in proper diagnosis and genetic counselling.

Key Words: FISH, breakpoint, derivative chromosome, probe, bacterial artificial chromosomes.

INTRODUCTION

Chromosome analysis relied on the study of chromosomal bands until the advent of molecular cytogenetic techniques. Application of these modern cytogenetic techniques have improved the diagnosis of chromosomal aberrations. Molecular cytogenetic techniques are based on fluorescence *in situ* hybridization (FISH). FISH can be used to identify chromosomal rearrangements by detecting specific DNA sequences with fluorescently labelled DNA probes.

This FISH technique and its application in the analysis of chromosomal aberrations is discussed in more detail below exemplifying how a chromosomal rearrangement was delineated in a case using this modern molecular cytogenetic technique.

FISH involves a fluorescently labelled DNA probe being hybridized to genomic DNA sequences which can be used to study a specific site on a chromosome. The first step in FISH is the availability of DNA probes. These probes are made by labelling the required DNA by Nick translation, Random priming and by PCR methods. Generally, probes less than 40 bases are synthetically manufactured and probes that range from 80 kb to 1 Mb are manufactured with molecular biology methods using cosmids, yeast artificial chromosomes (YACs), P1-derived artificial chromosomes (PACs) or bacterial artificial chromosomes (BACs) (Dutta et al. 2011). FISH probes are highly specific for their target DNA and divided into 4 main types. They are, gene-specific probes, centromere probes, telomeric probes and painting probes. However, there are 2 types of FISH techniques based on labelling patterns.

i) **Direct FISH:** In this technique the required DNA is isolated; fluorochromes of interest, for example, Fluorescein isothiocyanate (FITC) or Tetramethylrhodamine-5-(and 6) isothiocyanate (TRITC) are directly incorporated by labelling. All the commercially available probes fall into this type.

ii) **Indirect FISH:** The indirect FISH labelling is done by enzymatic methods such as nick translation. Here, the hapten or reporter molecules such as, biotin or digoxigenin are incorporated into the DNA. Such indirect labels require a detection step in which the reporter molecule is labelled with an agent such as avidin or antidigoxigenin that is conjugated to a fluorochrome. The detection methods for biotinylated probes employ avidin-fluorochrome conjugates, whereas those for digoxigenin-labelled probes employ anti-digoxigenin-fluorochrome conjugates.

In this paper we describe the FISH technique and its application in the analysis of chromosomal aberrations in detail by illustrating a case of Y-autosomal translocation. Identification of chromosomal rearrangements are very important as it leads to the identification of new genes. Many different types of chromosomal rearrangements are reported involving autosomes and also sex chromosomes which led to the identification of new genes.

Chromosomal rearrangements involving both chromosome Y and 22 are rare, and may result in a number of different phenotypes (Borie et al. 2004). Translocations between sex chromosome Y and an autosome have a reported frequency of 1/2000 in the general population (Alves et al. 2002). The most frequent Y-autosomal translocations involve an acrocentric autosome and are frequently of familial origin. There are rare forms of Y-autosomal translocations resulting in an unbalanced 45-chromosome karyotype that includes a dicentric chromosome product of a Y+autosome fusion (Gardner & Sutherland 2004). These individuals are phenotypically males have SRY, the testis-determining gene located at Yp. Most of the Y-autosomal translocations have been reported in association with male infertility (Brisset et al. 2005), but not in a child with delayed milestones.

Here we report a child with delayed milestones and Y-autosomal translocation involving chromosome Y and 22. This derivative chromosome is dicentric with a deletion at the distal end of chromosome Yq. We showed the derivative chromosome to contain Yp, Y centromere and a small proximal part of the Yq chromosome along with the long arm of chromosome 22. We confirmed this derivative chromosome by the use of molecular cytogenetic techniques.

MATERIAL AND METHODS

The patient was referred to our centre for chromosomal analysis due to delayed milestones. He was 18 months - old and was the second child of non-consanguineous parents. The family history was unremarkable.

Metaphase chromosome preparations from the peripheral blood cultures were made according to the standard cytogenetic protocols. Cytogenetic analysis was performed by G-banding using Trypsin and Giemsa at approximately 400–450 band levels. Fifty metaphases were analyzed and reported according to the International System for Human Cytogenetic Nomenclature (ISCN). Silver staining for the nucleolar organizing regions was also performed.

FISH technique was performed on lymphocyte metaphase spreads of the patient using standard protocols. Chromosome denaturation, hybridization and signal detection were done according to the published protocols (Licher et al. 1988). The following DNA probes were used for FISH analysis: satellite enumeration probes or centromeric specific probes of X chromosome (DXZ1), Y chromosome (DYZ3) and chromosome 14/22 (D14Z/ D22Z1), DiGeorge probe specific for TUPLE1 and ARSA on chromosome 22 (Poseidon probes from Kreatech, Netherlands) and whole chromosome painting probes for chromosome 22 and Y (Applied Spectral Imaging, Israel).

To find the extent of deletion further, fine mapping was performed by selecting 4 BAC clones RP11-400O10 on Yp11.31 (SRY region), RP11-375P13 on Yp11.2 region, RP11-71M14 on Yq11.221, and RP11-20H21 on Yq11.222 region according to the current human NCBI Reference Sequence (Pruitt et al. 2007) utilizing the UCSC genome browser (Kent et al. 2002) and obtained from imaGenes, Berlin, Germany. The international standard nomenclature was used for clone names. BAC DNA was isolated using NucleoBond Plasmid Midi kit (Macherey-Nagel, Dueren, Germany) according to manufacturer's instructions. The isolated BAC DNA was labelled with biotin-16-dUTP (Roche Diagnostics, Mannheim, Germany) by nick translation (Langer et al. 1981) and FISH analysis was done on the metaphase slides as described by the standard protocols.

OBSERVATIONS

The cytogenetic analysis was initially interpreted as a 45, X, 22p+ karyotype (Fig.1). Silver staining revealed no active nucleolar organizing regions on the 22p+ region, thus confirming the presence of euchromatic material. The karyotypes of both the father and sib showed normal 46, XY.

Two-colour FISH with whole chromosome paint probes for Y chromosome (red) and chromosome 22 (green) showed that the normal chromosome 22 was in green whereas the derivative chromosome showed both red and green colours confirming the translocation of Y chromosome on chromosome 22 (Fig. 2).

Also two-colour FISH with the X chromosome centromeric (green) and the Y chromosome centromeric (red) specific probes showed the presence of both centromeres. But the Y centromeric specific probe showed that the hybridization signal was located on derivative

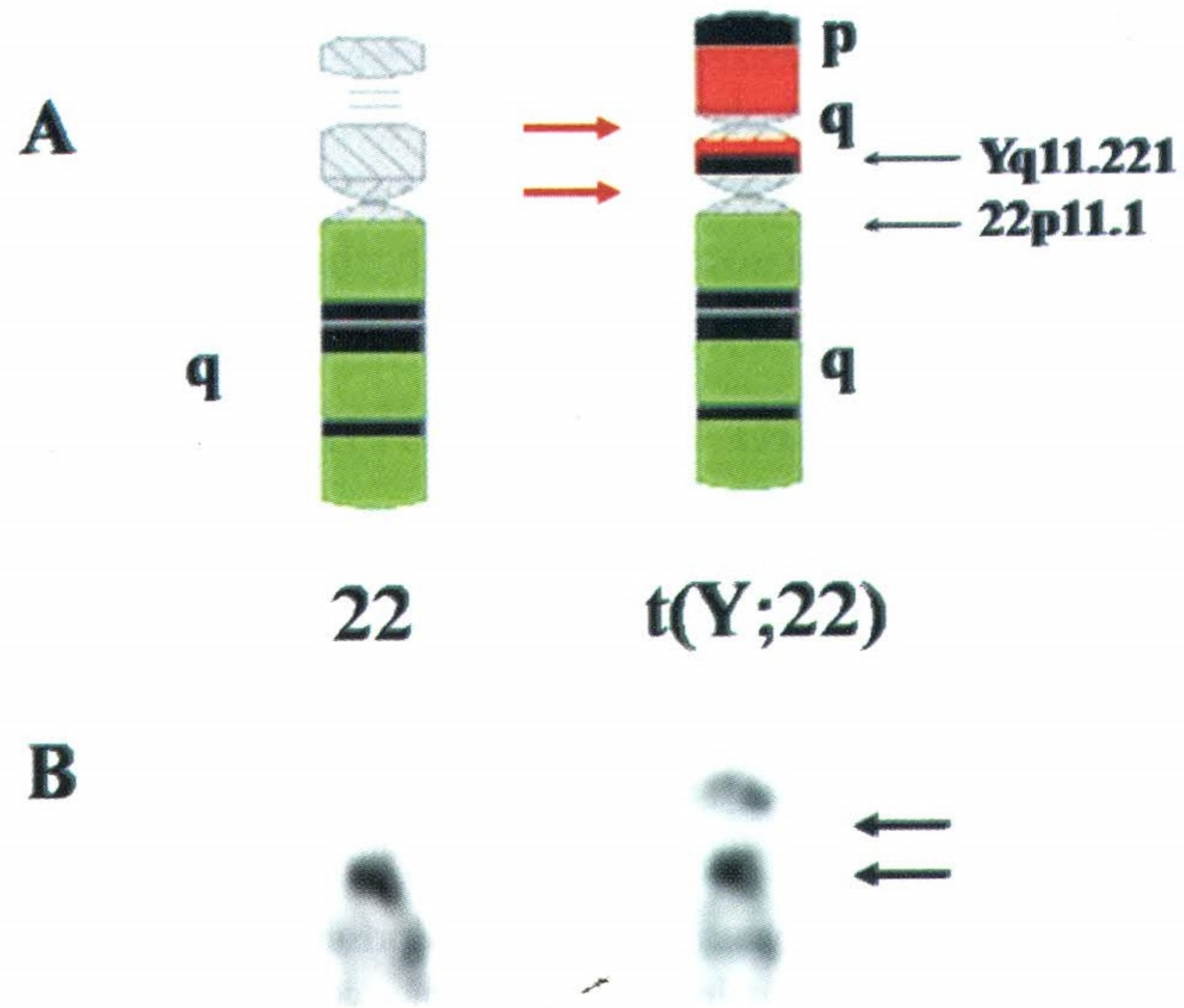


Fig.1: Idiogram and partial karyotype of the derivative chromosome in the proband. **A.** Idiogram showing normal 22 chromosome and derivative chromosome t(Y; 22) showing the breakpoint regions. **B.** The GTG-banded partial karyogram showing the translocation breakpoints.



Fig.2: WCP FISH on the lymphocyte metaphase spreads of the proband. The paint Y was labeled by TRITC (red signals) and paint 22 by FITC (green signals). The normal 22 is shown in green where as the t(Y; 22) is shown both in red and green colours.

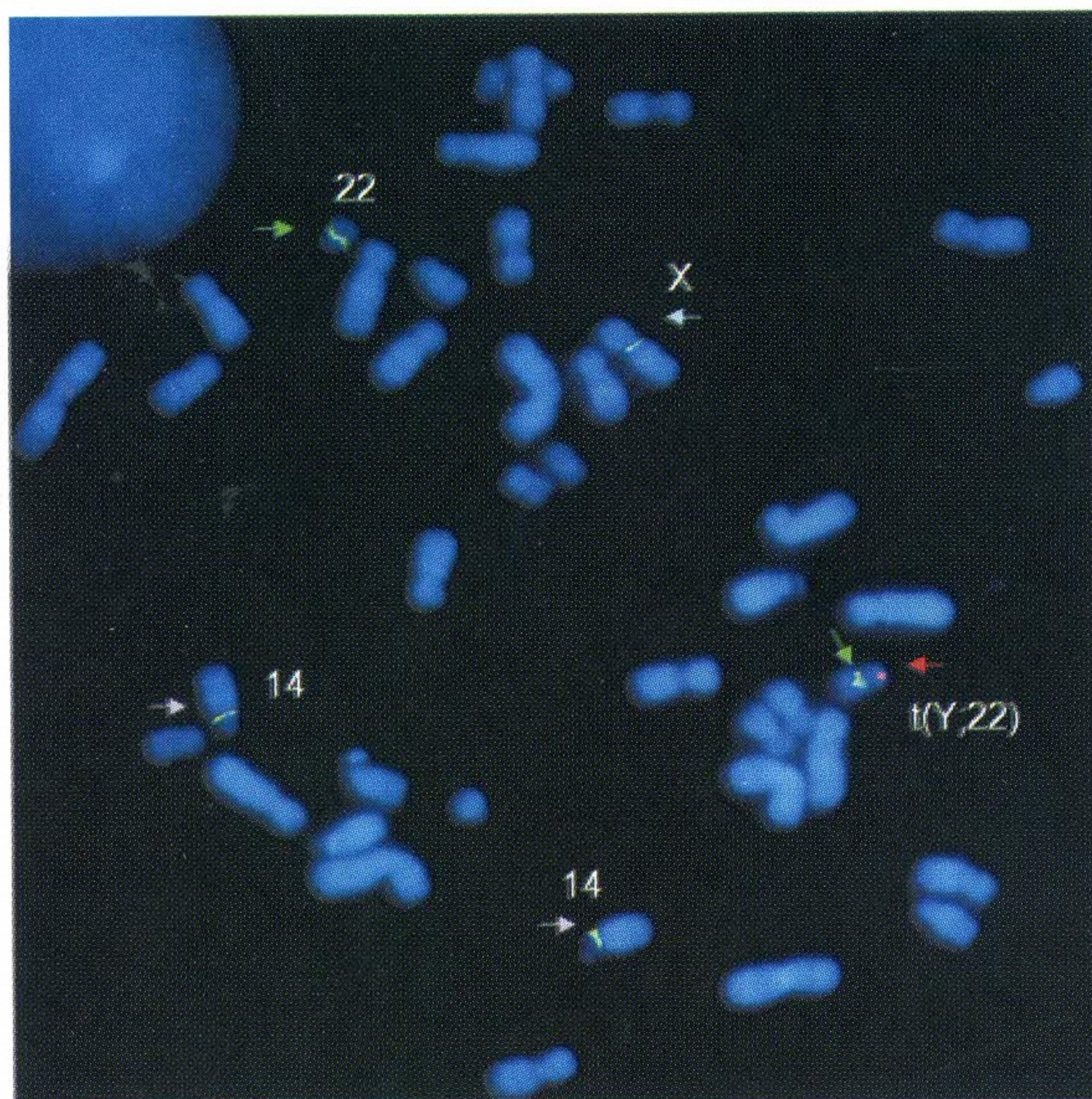


Fig.3: FISH with SE (Y/X) and SE (14/22) on the lymphocyte metaphase of the proband. The green arrows show the centromere 22, the violet arrows show both centromeres of chromosome 14, the blue arrow shows the X chromosome but the dicentric chromosome is shown in both green and red arrows.

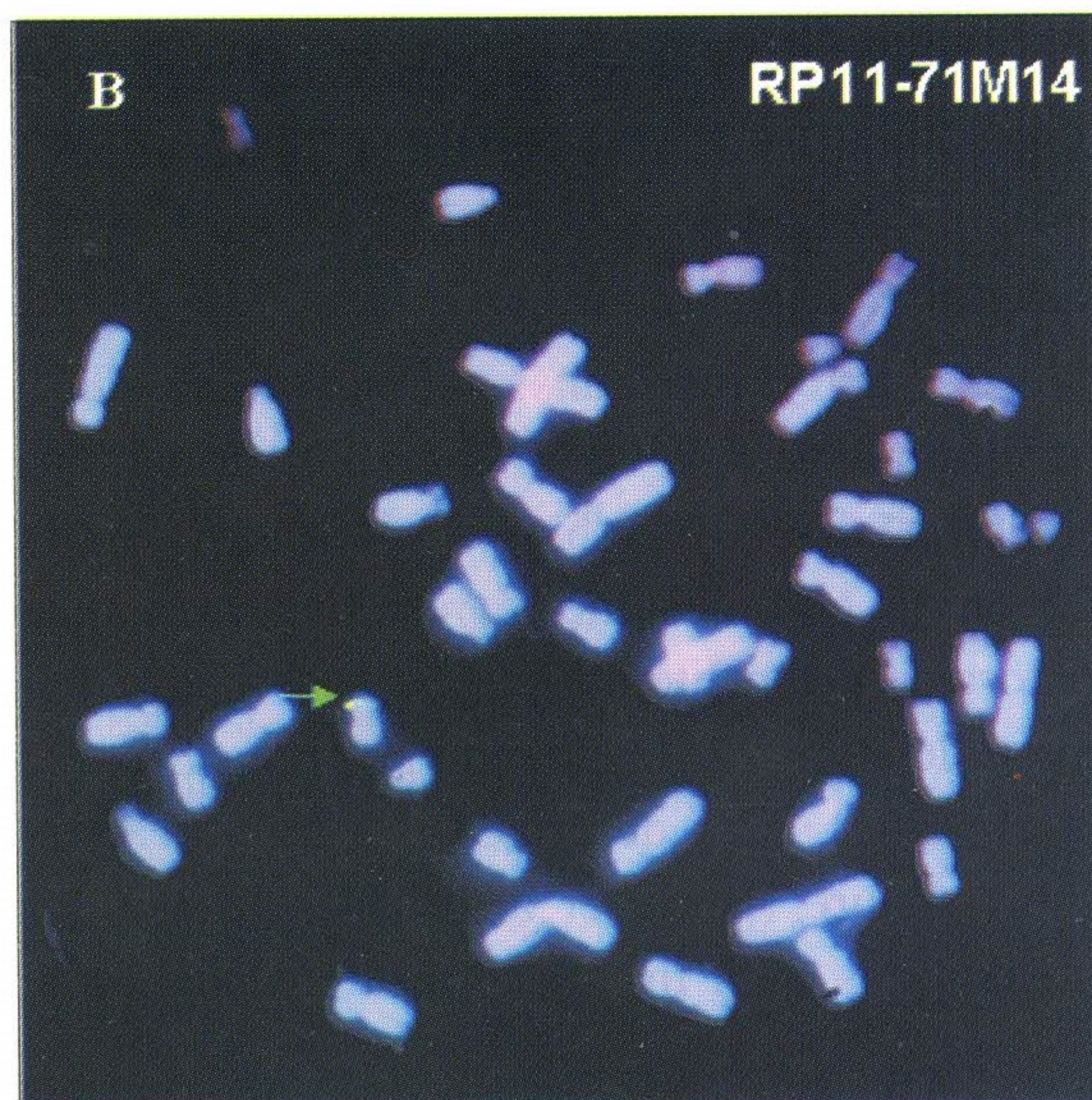


Fig.4: FISH with BAC clones on lymphocyte metaphase spreads of the proband. **A.** FISH with RP11-400O10 showing signals on Yp11.31 on the derivative chromosome. **B.** FISH with RP11-71M14 showing signals on Yq11.221 region.

chromosome (Fig. 3). The 14/22 α -satellite centromeric probe showed signals on both centromeres of chromosome 14 and with the 22 specific probe one signal was found on normal 22 chromosome and the other signal on derivative chromosome. This suggested that the derivative chromosome comprised both 22 and Y centromeric regions and is of dicentric origin (Fig. 3). Also FISH with DiGeorge probe showed signals on the TUPLE1 and ARSA regions on the normal chromosome 22 and also on the q arm of the derivative chromosome.

Further fine mapping with the 4 BAC clones was performed. FISH with RP11-400O10 on Yp11.31 (SRY region) showed signals on the terminal end of the derivative chromosome. This showed the presence of SRY region (Fig. 4A). Subsequently, FISH with RP11-375P13 on Yp11.2 region also showed signals indicating that the Yp region was intact (data not shown). Apparently, FISH with RP11-71M14 on Yq11.221 showed signals on derivative chromosome (Fig. 4B) whereas FISH with RP11-20H21 on Yq11.222 region showed no signals. This indicated that the breakpoint is present in between these 2 clones. Thus we delineated the derivative chromosome and concluded that the breakpoint region is distal to the Yq11.221 region.

Finally, we could identify the p region of the derivative chromosome. This region anchored the Y chromosome specific sequences containing the short arm, centromere and a small portion of the long arm of chromosome Y. Hence, we characterized this breakpoint region and concluded that the breakpoint might have occurred distal to the Yq11.221 region. Further, we could assign the karyotype according to ISCN (2009) as 45,X,dic(Y;22)(q11.2;p11.1).ish dic(Y;22)(wcpY+,DYZ3+,wcp22+,D14Z1+,D22Z1+,DXZ1+,TUPLE1+,ARSA+)(RP11-400O16+,RP11-375P13+, RP11-71M14+, RP11-20H21-).

DISCUSSION

Y-autosomal translocations often result from the translocation of the heterochromatic region of chromosome Y on to the short arm of an acrocentric chromosome, mostly chromosome 15 (Kuhl et al. 2001, White et al. 1998). The translocation of an autosome, specially chromosome 22 on to chromosome Y resulting in a dicentric chromosome is a rare event. Few cases are reported in the literature, one case had a normal phenotype (Callen et al. 1987) and other had microdeletion 22q11 phenotype (Lupski et al. 1991) and another case with infertility (Arneemann et al. 1991). Also described is a translocation (Y; 22) resulting in the loss of SHOX and isolated short stature (Borie et al. 2004). To our knowledge there are no cases involving this unique breakpoints with the dicentric translocation and delayed milestones.

The proband described in the current work was showing initially only 45 X, 22p+. Generally, 45X is a Turner karyotype but in this case considering the phenotypic characters we could guess that 22p+ is a translocated chromosome comprising of 22 and Y. Hence, by conventional cytogenetics we could only describe this case to this extent. But further

characterization of this case was only possible by using the modern molecular cytogenetic technique i.e., FISH. In our case we had used both direct and indirect FISH techniques. In direct FISH technique we had used the commercially available probes (both paint probes and satellite enumeration probes). Hence, by WCP FISH we could confirm the translocation. And by satellite enumeration probes we could find the dicentric origin of the derivative chromosome. By subsequent characterization using the 4 BAC clones we could demonstrate the indirect FISH technique as well as delineated the breakpoint region.

An imbalance in the long arm of the derivative chromosome was unlikely, since the breakpoint occurred in the short arm of chromosome 22. Nonetheless we performed additional FISH with probes TUPLE1 and ARSA which allowed us to rule out an imbalance at these loci on chromosome 22. FISH using RP11-400O10 allowed us to confirm that the SRY was not deleted in this child. BAC FISH showed that the breakpoint occurred distal to Yq11.221 region.

The most distal region of the Yp chromosome contains PAR1 (primary pseudoautosomal region) that has a homologous region on the distal Xp region. Another segment, homologous between X and Y is the PAR2 region, located at the terminal region of Xq and Yq. In male meiosis the recombination between PAR1 is obligatory because it is necessary for pairing, chiasma formation and correct segregation during meiosis (Kuhl et al. 2001). However, recombination between the PAR2 region is less frequent (Gardner & Sutherland 2004) and it has been demonstrated that the loss of Y chromosome PAR2 does not disturb meiotic segregation and has no effect on the phenotype (Kuhl et al. 2001). In our case Yp terminal region is located at a free end of the dicentric chromosome, so PAR1 is accessible and could recombine with its homologue in Xp. On the other hand, PAR2 is virtually unavailable as it is located in the fusion region between chromosome 22 and Y, so recombination is disturbed, although it is not necessary for the progression of meiosis. Thus the phenotypic effect is not shown in this case too but the fertility status cannot be predicted now as he is a boy. In future, testing of AZF factors would help the patient in knowing the fertility status by checking whether any of those AZF factors are disturbed due to this chromosomal rearrangement.

Thus cytogenetic research and diagnostics have greatly benefited by employing molecular techniques. In particular the development of FISH technique had widened these applications. In summary our case shows the importance of documenting Y-autosomal translocations with molecular cytogenetic techniques. We could also identify the derivative chromosome and delineate it. Characterizing the breakpoint regions helps us in describing the phenotype and also explains the molecular mechanism responsible for the rearrangement.

ACKNOWLEDGEMENT

This study was supported by a core grant to the Centre for DNA Fingerprinting and Diagnostics, from the Department of Biotechnology, Government of India.

REFERENCES

- ALVES C, CARVALHO F, CREMADES N, SOUSA M & BARROS A 2002 Unique (Y; 13) translocation in a male with oligozoospermia: cytogenetic and molecular studies *Euro J Hum Genet* **10** 467-474
- ARNEMANN J, SCHNITTGER S, HINKEL G K, TOLKENDORF E, SCHMIDTKE J & HANSMANN I 1991 A sterile male with 45, X0 and a Y; 22 translocation *Hum Genet* **87** 134-138
- BORIE C, LE'GER J, DUPUY O, HASSAN M, LEDU N, LEBBAR A, CZERNICHOW P & EYDOUX P 2004 Translocation (Y; 22) Resulting in the loss of SHOX and Isolated short stature *A J M G* **125A** 186-190
- BRISSET S, IZARD V, MISRAH I, MABOURA A & MADOUX S 2005 Cytogenetic molecular and testicular tissue studies in an infertile 45,X male carrying an unbalanced (Y;22) translocation: case report *Hum Reprod* **20** 2168-2172
- CALLEN D F, SUTHERLAND G R & CARTER R F 1987 A fertile man with tdc(Y; 22): how a stable neo-X1X2Y sex-determining mechanism could evolve in man *Am J Med Genet Suppl* **3** 151-155
- DUTTA U R, PIDUGU V K & DALAL A 2011 Molecular cytogenetics illustrated: SKY and FISH *Newslet Genet Chapt Indian Acad Pediatrics* **4** 3-6
- GARDNER R J & SUTHERLAND G R 2004 *Chromosome abnormalities and genetic counselling* 3rd ed Oxford University Press New York
- KENT W J, SUGNET C W, FUREY T S, ROSKIN K M, PRINGLE T H, ZAHLER AM & HAUSSLER D 2002 The human genome browser at UCSC *Genome Res* **12** 996-1006
- KUHL H, ROTTGER S, HEILBRONNER H, ENDERS H & SCHEMPP W 2001 Loss of the Y chromosomal PAR2-region in four familial cases of satellited Y chromosomes (Yqs) *Chromosome Res* **9** 215-222
- LANGER P R, WALDROP A A & WARD D C 1981 Enzymatic synthesis of biotin-labeled polynucleotides: Novel nucleic acid affinity probes *Proc Natl Acad Sci* **78** 6633-6637
- LICHER P, CREMER T, BORDEN J, MANUELIDIS L & WARD D C 1988 Delineation of individual human chromosomes in metaphase and inerphase cells by in situ suppression hybridization using recombinant DNA libraries *Hum Genet* **80** 224-234
- LUPSKI J R, LANGSTON C, FRIEDMAN R, LEDBETTER D H & GREENBERG F 1991 Di George anomaly associated with a de novo Y: 22 translocation resulting in monosomy del (22) (q11.2) *A J Med Genet* **1** 196-198
- PRUITT K D, TATUSOVA T & MAGLOTT DR 2007 NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes transcripts and proteins *Nucl Acids Res* **35** D61-D65
- WHITE L M, TREAT K, LEFF A, STYERS D, MITCHELL M & KNOLL J H 1998 Exclusion of uniparental inheritance of chromosome 15 in a fetus with a familial dicentric (Y;15) translocation *Prenat Diagn* **18** 111-116