

## Cytogenetic, Molecular and FISH Analysis of an Isodicentric Chromosome 21 idic(21)(q22.3) in a Mildly-Affected Patient with Down Syndrome

Frenny J Sheth<sup>1</sup>, Uppala Radhakrishna<sup>2</sup>, Michael A Morris<sup>3</sup>, Jean-Louis Blouin<sup>3</sup>, Jayesh J Sheth<sup>1</sup>, Asha Multani<sup>4</sup>, Stylianos E Antonarakis<sup>2,3</sup>

<sup>1</sup>*FRIGE HOUSE (Foundation for Research in Genetics and Endocrinology), Genetic Centre, 15, Kapidway, Jodhpur Gam Road, Satellite, Ahmedabad 380 015. Gujarat, India*

<sup>2</sup>*Division of Medical Genetics, University of Geneva Medical School and*

<sup>3</sup>*Cantonal Hospital, Geneva, Switzerland*

<sup>4</sup>*Department of Cell Biology, University of Texas, Houston, Texas, USA*

**KEYWORDS** Down syndrome; FISH; isodicentric; mental retardation; trisomy 21; YAC

**ABSTRACT** Most cases of Down syndrome (DS) result from a supernumerary marker chromosome 21; however there are rare cases in which DS is due to partial trisomy of chromosome 21, involving various segments of the chromosome. The characterization of DS that are due to partial trisomy 21 allows the phenotype to be correlated with the genotype. We present a case of a five-year-old male referred for cytogenetic analysis because of mild mental retardation and facial features typical of Down syndrome for whom karyotypic analysis showed a “mirror” reverse tandem duplication of chromosome 21. Fluorescence In-Situ Hybridization (FISH) using a half YAC containing the telomere of chromosome 21q and the adjacent marker D21S1575 revealed the deletion of both copies of this region on the translocated chromosome. Microsatellite analysis further delineated the breakpoint to be between CD18 and D21S1446, excluding the possibility of uniparental disomy and demonstrated that the rearranged chromosome was of paternal origin.

### INTRODUCTION

Down syndrome (DS) are usually caused by trisomy 21 and is the most common and best-known chromosome disorder with the birth incidence of 1:700 to 1:1000 (Hook 1982; Krivchenia et al. 1993). Clinically, it is characterized by a cohort of variable phenotypic features including morphological, anatomical and physiological. In addition, a constant but with varying severity of mental retardation is observed. In 4.8% of the cases, 21 trisomy is due to translocation (Hook 1982, Thuline and Pueschel 1982). These translocations are essentially centric fusions or Robertsonian translocations, which occur between chromosome 21 and D-group chromosomes or between chromosome 21 and chromosomes of the same group G. Isochromosome or tandem duplication of an acrocentric chromosome (partial trisomy 21) is rare (fewer than 1%) in children with clinical diagnosis of DS and was published as early as 1963 (Warkany and Soukup 1963; Zellweger et al. 1963) and in addition quite a few cases have been reported in the literature (Antonarakis et al. 1990; Blouin et al., 1991; Pangalos et al. 1992; Shaffer et al.

1992). A minimum Down syndrome critical region (DCR) in 21q22.2 between marker D21S17 and gene ETS2 has been suggested by molecular studies of patients with partial trisomy 21 (Neilson and Gibbs 2004; Vesa et al. 2005). Korenberg et al. 1991 challenged single DCR region by reporting some rare patients with several phenotypic elements of DS and proximal trisomy of HC21 (outside of the D21S17-ETS2 region).

In this study, we report a DS child with the karyotype 46,XY,idic(21)(q22.3). The break points were localized by using Fluorescence in situ hybridization (FISH) and microsatellite analysis.

### Case Report

A five-year-old male was referred for cytogenetic analysis because of mild mental retardation and facial features typical of DS. He was the second child born preterm (8 months) to non-consanguineous parents after normal delivery; his older sister and younger brother were normal. Father was 25 and mother was 24 years old at the time of his birth. Many of the typical features of DS were present in the proband, including brachycephaly, oblique eye

fissures, flat nasal bridge, macroglossia, neonatal hypotonia, high arched and narrow palate, short neck, short and incurved fifth fingers, transverse Palmer crease (right hand only), joint hyper flexibility and cardiac murmur which is associated with endocardial cushion defect.

## MATERIALS AND METHODS

**Cytogenetic Analysis:** Cytogenetic analysis of the proband and of family members was performed on peripheral blood lymphocytes. Giemsa (GTG), centromeric (CGB) and NOR banding techniques were performed by using standard techniques to identify the chromosomes.

**Fluorescence in situ Hybridization:** Fluorescence *in situ* hybridization (FISH) was performed with a pool of cosmids from the LL21NC02 library selected by hybridization with the human 21qter half-YAC C9. Hybridization was performed as described (Blouin et al. 1995) and images were captured and processed with Cytovision Probe workstation (Applied Imaging).

**DNA Polymorphism Analysis:** Genomic DNA was purified from peripheral blood lymphocytes from the proband, his parents, brother and sister by standard SDS-proteinase-K and phenol/chloroform extraction method. DNA polymorphisms were analyzed by polymerase chain reaction (PCR) amplification of short sequence repeat markers of chromosome 21. These markers, distributed throughout the long arm of chromosome 21 (table 1), were selected from the published linkage maps and from the Génethon and CHLC collections (Blouin et al 1995; Buetow et al. 1994; Dip et al. 1996; Gyapay et al. 1994, NIH/CEPH collaborative mapping group, 1992). Marker information can be obtained from <http://gdbwww.gdb.org/gdb>. One oligonucleotide primer of each marker was labeled with  $\gamma^{32}\text{P}$ -ATP using T4 polynucleotide kinase and PCR was performed by standard techniques. PCR products were separated by electrophoresis in a 6% denaturing urea/polyacrylamide gel and genotypes were scored after autoradiography.

## RESULTS

Cytogenetic analysis of the proband revealed constitutive karyotype 46,XY,idic(21)(q22.3) (Fig. 1A). However, the size of the chromosome and its banding pattern could not reveal loss of

material from either long arm. Over 400 metaphase cells were analyzed and no mosaicism was detected. Other family members, including the parents, did not show any chromosomal anomaly.

FISH using the telomeric half-YAC C9 and the adjacent marker D21S1575 showed signals only on structurally unaltered normal chromosome 21. Absence of same signals on the distal region of the structurally rearranged isodicentric chromosome revealed deletion of this region (Fig. 1B). To delineate the boundaries of the deletions more precisely, we performed a genotype analysis of over 20 polymorphic loci located from centromere to HC21q22.3 on blood DNA from proband and his parents. The results are shown in table 1. All the markers analyzed from D21S258 to CD18 showed duplication of a paternal chromosome 21 allele in the patient. Genotypes of the distal markers D21S1446 and D21S1575 show the absence of paternal alleles (Fig. 1C), confirming the existence of the distal deletion revealed by FISH mapping.

**Table 1: Genotypes of DNA polymorphism of proband's family {Markers on chromosome 21 are written according to their map location}**

<i>Chromosome 21 Markers</i>	<i>Genotypes Fa.Mo.FI</i>
D21S369	11.11.111
D21S215	11.12.111
D21S258	12.11.122
D21S120	24.13.144
D21S236	11.23.113
D21S192	12.00.112
D21S172	23.14.133
D21S1256	12.22.112
D21S406	12.12.111
D21S364	12.12.122
D21S366	22.00.122
D21S11	12.33.113
D21S145	24.13.344
D21S232	12.23.113
D21S210	33.12.233
D21S1244	22.12.222
D21S217	12.34.224
D21S167	23.12.122
D21S267	11.23.112
PFKL	23.11.133
D21S171	23.11.122
CD18/AcI	11.22.112
D21S1446	11.23.2
D21S1575	22.13.3

## DISCUSSION

Present case of DS with karyotype showing isodicentric chromosome 21(q22.3) that results in a nearly full trisomy 21. FISH analysis using

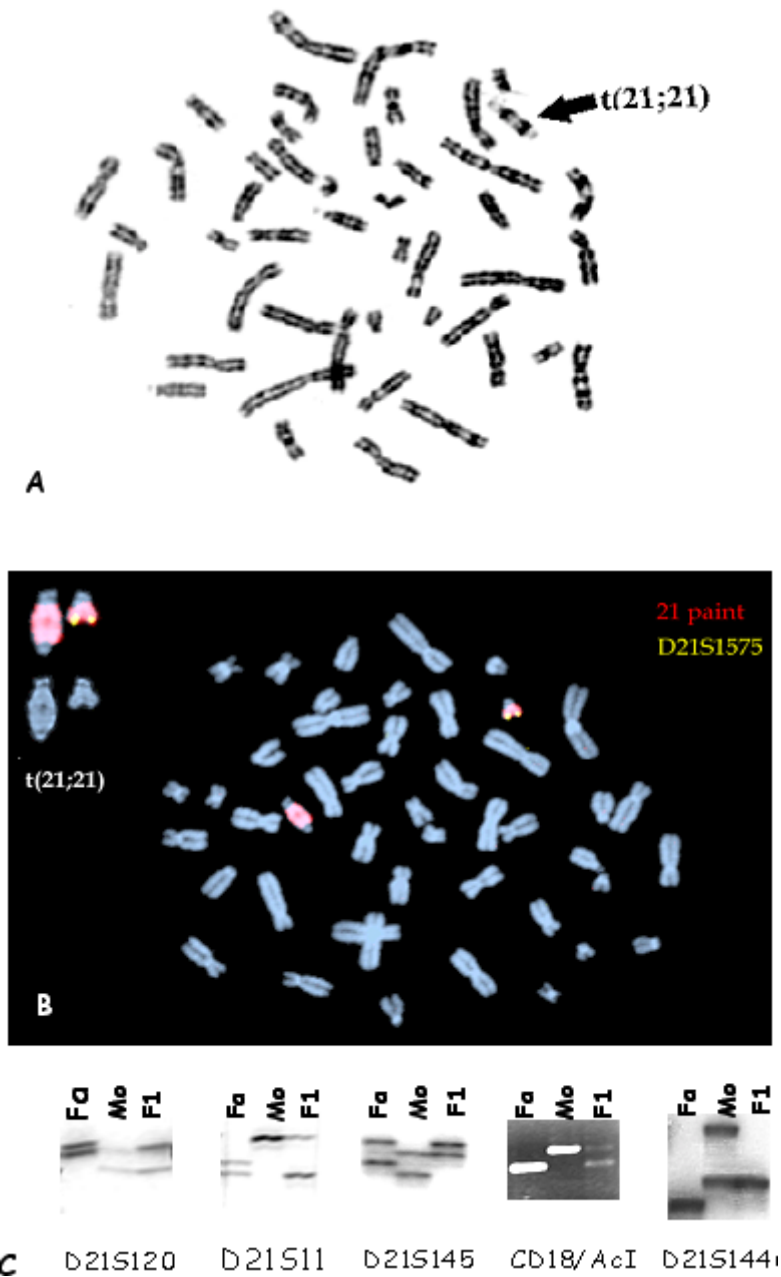


Fig. 1 A. Metaphase of the proband showing 46,XY,idelic(21)(q22.3)

B. Partial metaphase showing normal and translocated #21 with WCP #21 and C9 (D21S1575)

C. Autoradiograms of informative DNA polymorphism analyses: Fa: father; Mo: mother; F1: DNA from proband with idic(21;21).

➤ Microsatellite analysis showing biparental inheritance of alleles for the marker D21S120, D21S11, D21S145, CD18/AcI.

➤ Markers D21S1446 show the uniparental (maternal) contribution of most distal chromosome 21 segments.

HC21 telomeric probe indicates a deletion of at least 120-Kb most terminal region of chromosome 21 in both chromosome units involved in the isoformation. A genotype study using markers located in the long arm of HC21 had determined that the breakpoint on both arms of the aberrant chromosome was located between CD18 and D21S1446. Since CD18 and D21S1446 are separated by only 2.1 cM in the linkage map, we cannot exclude the possibility that the breakpoint occur at the exact same location on both chromosomal units. The HC21 linkage map indicates that the breakpoint should localize in the region, which span 2.1 cM and 120 KB from centromere. In addition to a partial trisomy 21, the patient shows a monosomy for the small region, which span the terminal part of HC21 containing markers D21S1446 and D21S1575.

The etiology of isodicentric chromosome 21 is uncertain, however, genotype analysis in table 1 revealed that aberrant chromosome has arisen during meiosis I when both the paternal chromatids 21 are still identical and no crossing over has occurred. This also excludes the possibility of uniparental disomy. Clinical examination of the patient indicates that most of the features of DS are present delineating a typical trisomy 21 phenotype. The phenotypic features in the present case are in the accordance with the triplication of the DCR region located on q22.2 and very proximal q22.3 both spanning a maximum of 6Mb as suggested by Delabar. No significant effect of monosomy for distal 21q22.3 has been seen in the present case as also observed by Pangolas .

To the best of our knowledge, this may be the first report of FISH analysis of an isodicentric chromosome 21 idic(21)(q22.3) in patient with DS.

#### ACKNOWLEDGMENTS

We are grateful to members of the family for their co-operation and donation of blood samples. This study was supported with funds from the University & Cantonal Hospital of Geneva and FRIGE, Ahmedabad.

#### REFERENCES

- A Comprehensive Genetic Linkage Map of the Human Genome 1992. *NIH/CEPH Collaborative Mapping Group. Science*, **258**:148-162.
- Antonarakis SE, Adel Berger PA, Peterson MB, Binkert F, Schinzel AA 1990. Analysis of DNA polymorphism suggests that most de novo dup (21q) chromosomes in patients with DS are isochromosome and not translocations. *Am J Hum Genet*, **47**: 968-972.
- Blouin JL, Aurias A, Creau-Goldberg N, Apiou F, Alcaide-Loridan C, Bruel A, Prieur M, Kraus J, Delabar JM, Sinet PM 1991. Cytogenetic and molecular analysis of a de novo tandem duplication of chromosome 21. *Hum Genet*, **88**: 67-74.
- Blouin JL, Christie DH, Gos A, Lynn A, Morris MA, Ledbetter DH, Chakravarti A, Antonarakis SE 1995. A new dinucleotide repeat polymorphism at the telomere of chromosome 21q reveals a significant difference between male and female rates of recombination. *Am J Hum Genet*, **57**: 388-394.
- Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Duyk GM, Sheffield VC, Wang Z, Murray JC 1994. Integrated human genome-wide maps constructed using the CEPH reference panel. *Nat Genet*, **6**: 391-393.
- Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J 1996. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature*, **380**: 152-154.
- Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J 1994. The 1993-94 Genethon human genetic linkage map. *Nat Genet*, **7**: 246-339.
- Hook CG 1982. Epidemiology of Down syndrome. In: SM Poeschel, JE Rynders (Eds.): *Down Syndrome. Advances in Biomedicine and the Behavioral Sciences*. Cambridge: Ware Press, pp. 11-18.
- Korenberg JR, Kalousek DK, Anneren G, Pulst SM, Hall JG, Epstein CJ, Cox DR 1991. Deletion of chromosome 21 and normal intelligence: molecular definition of the lesion. *Hum Genet*, **87**: 112-118.
- Krivchenia E, Hether CA, Edmond LD, May DS 1993. Comparative epidemiology of Down syndrome in two United States populations. *Am J Epidemiol*, **137**: 815-825.
- Nelson DL, Gibbs RA 2004. Genetics. The critical region in trisomy 21. *Science*, **306**: 619-621.
- Pangalos C, Theophile D, Sinet PM, Marks A, Stamboulieh-Alazis D, Chettouh Z, Prieur M, Verellen C, Rethore MO, Lejeune J, Delabar JM 1992. No significant effect of monosomy for distal 21q22.3 on the Down Syndrome Phenotype in "Mirror" duplication of chromosome 21. *Am J Hum Genet*, **51**: 1240-1250.
- Shaffer LG, Jackson-Cook CK, Stasiowski BA, Spence JE, Brown JA 1992. Parental origin determination in 30 de novo Robertsonian translocations. *Am J Med Genet*, **43**: 957-963.
- Thuline HC, Poeschel SM 1982. Cytogenetics in Down syndrome. In: SM Poeschel, JE Rynders (Eds.): *Down Syndrome. Advances in Biomedicine and the Behavioral Sciences*. Cambridge: Ware Press, p. 133.
- Vesa J, Brown Y, Greenfield D, Korenberg JR 2005. Molecular and cellular characterization of the Down syndrome critical region protein 2. *Biochem Biophys Res Commun*, **328**: 235-242.
- Warkany J, Soukup SW 1963. A chromosomal abnormality in a girl with some features of Down's syndrome (mongolism). *J Pediatr*, **62**: 890-894. Website: <http://gdbwww.gdb.org/gdb/>
- Zellweger H, Mikamo K, Abbo G 1963. An unusual translocation in a case of mongolism. *J Pediatr*, **62**: 225-229.