Jpn J Human Genet **41**, 215-219, 1996

Case Report

RETRIEVAL OF ANEUPLOIDY BY FISH-TECHNIQUE IN A CASE WITH 46,XX/47,XXX/47,XX,+8

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Summary We report on a 46 year old female with a new chromosomal finding [46,XX/47,XXX/47,XX,+8] who was referred for ovarian failure. The clinical presentation was highly unusual and the patient does not exhibit the characteristic phenotype of trisomy 8 syndrome. Interphase cytogenetics using FISH-technique revealed discrepancies with a different population of cells when compared with its metaphase index. Therefore, it is advised that patients with mosaic karyotypes should be evaluated by analyzing metaphase as well as interphase nuclei labeled with chromosome specific molecular tags, especially in the situations where the incidence of a mosaic cell line is very low. Nevertheless, in a cost-conscious environment, we must exercise caution prior to making universal recommendations concerning the usefulness of medical devices which are increasing at a logarithmic rate.

Key Words trisomy 8, trisomy X, ovarian failure, Turner syndrome, FISH-technique, interphase cytogenetics

INTRODUCTION

Trisomy 8 syndrome, which is relatively common, was first recognized over three decades ago (Stalder *et al.*, 1963). Although, the chromosomal modality is mosaicism, yet a syndrome can be clinically recognizable (de Grouchy and Turleau, 1984; Jones, 1988; Buyse, 1990). Nevertheless, phenotypic variability is quite profound (Riccardi, 1977) and cytogenetic analysis has been quite imperative. We report a new case of unusual mosaicism with rare clinical presentations.

Received October 6, 1995; Revised version accepted December 1, 1995.

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CLINICAL HISTORY

J.L. is a 46 year old female caucasian executive secretary who was referred for evaluation of osteoporosis. Her height was 5 feet, 7 inches. Bilateral clubbed feet were treated with triple arthrodesis in childhood. Her lower lip was reported normal prior to surgery at age 26 following trauma. Menarche occurred normally at age 11 or 12. As a teenager, she was treated with medications for oligomenorrhea which was followed by amenorrhea since age 22. Back pain began at adolescence and has increased in the past three years. Since age 41, she has been known to have kyphosis of the thoracic spine with wedging and narrowing of disc spaces as well as multiple level disc disease of the lumbar spine. Bone densitometry has revealed osteopenia of both hips with values 2.5 SD below age-and-gender-matched normal values. Her two sisters have normal menses.

Physical examination revealed normal development including normal female breast and pubic hair. Craniofacial dysmorphism, single transverse palmar creases were absent. Laboratory evaluation of amenorrhea revealed primary hypogonadism: FSH −35 MIU/ml, LH 32.3 MIU/ml with normal prolactin and thyroid function.

CYTOGENETIC AND MOLECULAR METHODS

Chromosomal study was performed on PHA-stimulated lymphocytes and harvested according to the routine procedure. Chromosome analysis was done by GTG and QFQ banding techniques (Verma and Babu, 1995). Patient refused skin biopsy for cytogenetic evaluation.

FISH-technique was performed according to the manufacturers (Oncor, MD or BDS, PA) instructions. For chromosome X, the biotin labeled alpha satellite centromeric probe (Oncor) and for chromosome #8 alpha satellite centromeric probe (BDS) were used. Chromosomal DNA was denatured in 70% formamide/2×SSC (pH 7.0) at 70°C. The probe was denatured at 70°C for 5 min. Post hybridization wash was in 50% formamide/2×SSC (for chromosome X); and 65% formamide/2×SSC (for chromosome #8) followed with a wash in 2×SSC for 8 min. The chromosomes were counter stained with propidium iodide/antifade and DAPI as required for the probe used. Hybridization signals were detected and photomicrographs were taken using a Zeiss axiophot microscope.

RESULTS AND DISCUSSION

Cytogenetic findings with G-banding from peripheral blood chromosomes provided the following impression:

2	cells:	46,XX	[1.6%]
5	cells:	47,XXX	[4.2%]
113	cells:	47,XX,+8	[94.2%]

Interphases were hybridized by FISH-technique using X-probe (Fig. 1). One hundred and twenty-five nuclei were observed. Thirty-one nuclei have three dots while 94 nuclei have only two hybridizing signals. Therefore, it was revealed that 25% cells have triple-X. In another experiment, slides were also hybridized using chromosome 8 specific centromeric probe again, 125 nuclei were observed. One

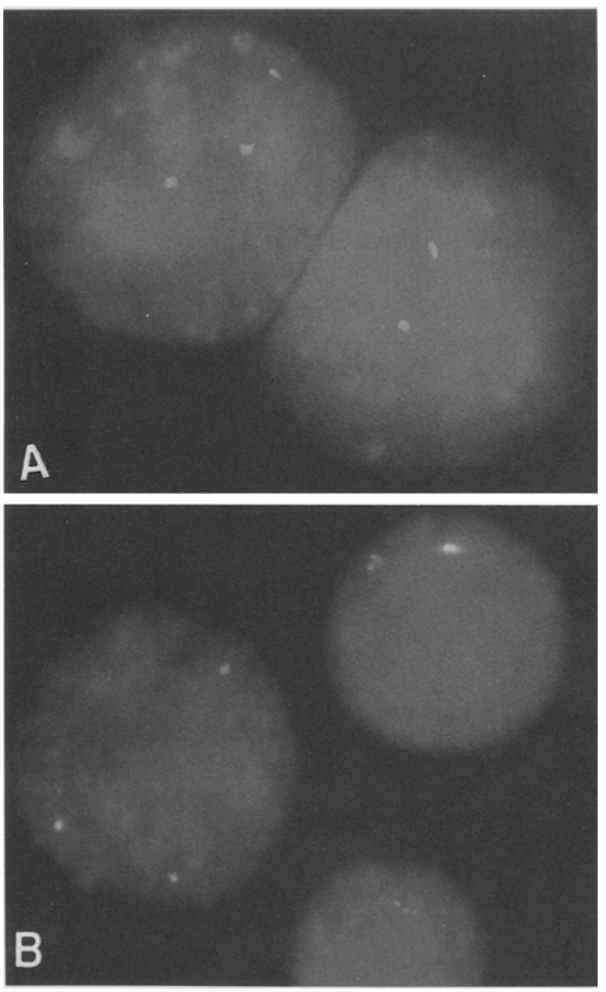

Fig. 1. FISH-technique using alpha satellite probe for chromosome 8 (A) showing normal interphase nuclei (2 dots) and trisomy for chromosome 8 (3 dots). Similarly, when alpha satellite probe for X-chromosome was used (B) normal interphase nuclei (2 dots) and triple-X (3 dots) were identified (see text).

hundred nuclei have three dots while 25 have only two dots. Therefore, 80% of the cells revealed trisomy for chromosome 8. There is no significant difference for trisomy 8 population, but a significant difference was noted for triple-X as findings are contrary to the metaphase analysis data.

It is a long held belief that aneuploid cells have a different cell cycle duration (Nevins, 1994) and the discrepancy can be interpreted here on a similar line of thought. For example, cells with triple-X may have been arrested at interphase stage giving rise to higher incidence, while cells with trisomy 8 may have similar cell division cycle. Consequently, the nuclei with the trisomy for different chromosomes have different duration of cell division.

Chromosomal mosaicism is one of the most common finding for a number of human chromosome disorders. Present findings clearly warrant that in cases of mosaicism, interphase cytogenetics should be supplemented before the final report is written to the physicians. This approach is especially useful in situations where cell line with mosaicism is quite low. Nevertheless, this pivotal approach will add cost. However, patients have become well educated consumers and demand state-of-the-art care. Usually, they do seek a second opinion. It is also well known that the proportion of abnormal cells in blood is not always representative of other tissue. Furthermore, blood mosaicism usually evolves with ageing. Our patient refused a skin biopsy. It would have been ideal to compare these findings by two methods. Undoubtedly, the differences observed between routine cytogenetic analysis and interphase by FISH-technique could drown a few cytogeneticists in the court where such a "discrepancy" may reflect as "negligence."

On a clinical note, the patient's features were not consistent with those recognized for the so called trisomy 8 syndrome. The major findings included: bilateral clubbed feet; ovarian failure at age 22; chronic back pain since teens; multiple level degenerative disc disease (early arthritis) and dorsal kyphosis.

Double aneuploidy involving a sex chromosome with autosomes is a rare occurrence, but cases with trisomy 8 syndrome (46,XX/48,XXY,+8) ambiguous genitalia (45X/48,XXY,+8) with numerous dysmorphic features and neurological anomalies (47,XY,+8/48,XXYY) have been reported (Casey *et al.*, 1981; Tegenkamp *et al.*, 1980; Hoovers *et al.*, 1989; Schofield *et al.*, 1992). Needless to say, when compared with other trisomy 8 syndrome, this individual had unusual clinical manifestation and was referred to us originally to rule out mosaic Turner syndrome.

REFERENCES

Buyse ML (1990): Birth defects encyclopedia. Center of Birth Defects Information Service, Dover, pp 351-352

Casey PA, Clark CE, Cowell HR (1981): 46,XY/48,XXY,+8 in a male with clinical and dermatoglyphic features of mosaic trisomy 8 syndrome. Clin Genet **20**: 60-63

de Grouchy J, Turleau C (1984): Clinical atlas of human chromosomes. John Wiley, New Yrok, pp 124-133

Hoovers JMN, Oothuys JWE, de Visser M (1989): Mosaic 47,XY+8/48,XXXY in a mentally non-retarded man with phenotypical and neurological abnormalities. Clin Genet **35**: 446-449

Jones KL (1988): Smith's recognizable patterns of human malformation. W.B. Saunders, Philadelphia, pp 26-29

Nevins JR (1994): Cell cycle targets of the DNA tumor viruses. Curr Opin Genet Dev **1**: 130-134

Riccardi VM (1977): Trisomy 8: An international study of 70 patients. Alan R. Liss, for the National Foundation-March of Dimes, New York, BD: DAS XXIII (3C), pp 171-184

Schofield B, Babu A, Punales-Morejon D, Popescu S, Leiter E, Franklin B, Penchaszedeh VB (1992): Double mosaic aneuploidy: 45,X/47,XY+8 in a male infant. Am J Med Genet **44**: 7-10

Stalder GR, Buhler EM, Weber JR (1963): Possible trisomy in chromosome group 6-12. Lancet **I**: 1379

Tegenkamp T, Gobrail M, Goodwin C, Swedlund S, Labidi F, Tegenkamp I (1980): Sex selection in a 45,X,−X/48,XY,+X,+8 mosaic pseudo true hermaphrodite with multiple anomalies. Am J Hum Genet **32**: 133A

Verma RS, Babu A (1995): Human chromosomes: principles and techniques. McGraw Hill, New York