Hemihypertrophy with hypomelanosis of Ito: A new syndrome combination

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A female hemihypertrophy patient with hypomelanosis of Ito is presented as a rare case combining classical features of both the syndrome. Chromosomal profile has been based on longitudinal study of repeated lymphocyte cultures during 1984-1992. The propositus has exhibited chromosomal mosaicism both hypoploid (42 ± 1) and hyperploid (48 ± 2 chromosome) counts, but the major stem line presented 46XX chromosomes. Ring chromosome with simple and complex translocations with marker dots appear to be the major cytogenetic assemblage of this child to posses unequal left and right halves of the body. Each and every organ from toe to the head has grown up unequally and lately the patient had been exhibiting different dark and light shapes of melanin on the skin. We believe that the patient had inherited, through her male parent, "a few" mutated loci on some chromosomes so as to generate different cell lines within the developing child. All sibs and the mother showed normal karyotype with no apparent aberration.

Keywords : Hemihypertrophy with melanosis of Ito, Chromosomal mosaicism, Ring chromosome, Marker dot, New syndrome combination

INTRODUCTION

The presence of chromosomal mosaicism in lymphocytes and/or in fibroblast cell cultures has been recognized with increasing frequency among phenotypically abnormal individuals. Such specific instances are often encountered in somatic cell cultures of patients with pigmentary changes in skin, areas of abnormal body growth, generalized undergrowth, asymmetry with unilateral hypoplasia, hypomelanosis of Ito and mental retardation (Glover *et al.*, 1989; Thomas *et al.*, 1989; Sybert *et al.*, 1990, Ritter *et al.*, 1990). Among above, chromosomal mosaicism has been a prominent feature in patients exhibiting pigmentary abnormalities often grouped under "Hypomelanosis of Ito" (Ritter *et al.*, 1990) sometimes associated with mental retardation. Though, the hypo and hyperpigmentation is known among patients of hemihypertrophy, the present report presents an observation on chromosomal mosaicism in a female patient assignable to a syndrome combining hemihypertrophy with hypomelanosis of Ito. This will clearly distinguish a classical case of hemihypertrophy where the hypo and hyperpigmentation may not have been distributed sporadically on some body parts.

MATERIALS AND METHODS

A female child born to non consanguineous parents was first examined at the age of 2 in 1979 for possessing unequal halves of the body at Bhopal, India. The left half of the body from "great toe to the head" was smaller than the corresponding right part. Longitudinal follow up studies over a period have been carried out during 1979-1992 and the mode of development of each part has been recorded. Lately in 1993 we had detected distinctly variable skin pigmentation on hands, calf muscles and shoulder area.

Peripheral blood samples have been obtained and cultured three times (RPMI and 199 media) during 1984-1992 at the Department of Genetics, Bhopal university.

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Fibroblast and lymphocyte cultures have been processed once at the Department of Paediatrics, Yamaguchi University in Ube and examined thoroughly. Chromosomes have been stained by usual Giemsa and also by Giemsa, and C banding methods. Both banded and nonbanded slides were scored blindly with a battery of about 12 kind of aberrations often encountered in most of chromosomal involvements (Goswami, 1986; Goswami *et al.*, 1992, 1997). These include hypoploid/hyperploid count, premature centromeric divisions (PCD), translocations (simple and complex), ring chromosome, deletions, inversions, chromosome breaks, presence of marker dot, double minutes; amitotically dividing lymphocytes, specific aberrations like acrocentric associations, telomeric adhesions or translocations, classical polyploid cells and several such configurations or structural variations which can never be missed by a keen eye.

RESULTS

Clinical observation

The propositus is exhibiting a classic picture of a hemihypertrophy patient (Fig. 1A). The frontal part of the head is more enlarged on the right side, ear temporal region nasal/nostrils size of the eyeballs, eyebrows, ears, jaws, all of them are larger in size than their corresponding part. Even the tongue was unequal on one side of the oral cavity (Fig. 1B). Right leg, hand, right part of the pelvic region, right knee, apparent size of the bones both in hands as well as in feet had a difference of 2 to 7cm in hands and 1 to 4cm in legs during the study of growth period in 1980-1992. Phalanges and metatarsals have been larger on the right side. Physicians and surgeons have had examined her many times and lately in 1990, she was confirmed to have Wilm's tumour. This further confirmed our patient to be a classical case of hemihypertrophy (Meadows *et al.*, 1974). The propositus died in 1994. Though the body was fragile (Fig. 1A, B, Fig. 2A and B), she was quite active, moved freely, talked, responded to phone calls and was a lively child in the family with normal intelligence but for her weak constitution. During common ailments (cough, cold and fever) she responded well to antibiotics and other medicines.

Chromosomal study

observations on chromosomes are grouped in two broad categories presented hereunder:

Numerical

As many as 50 slides to yield more than 500 metaphases were scored from three cultures until 1990 and we always

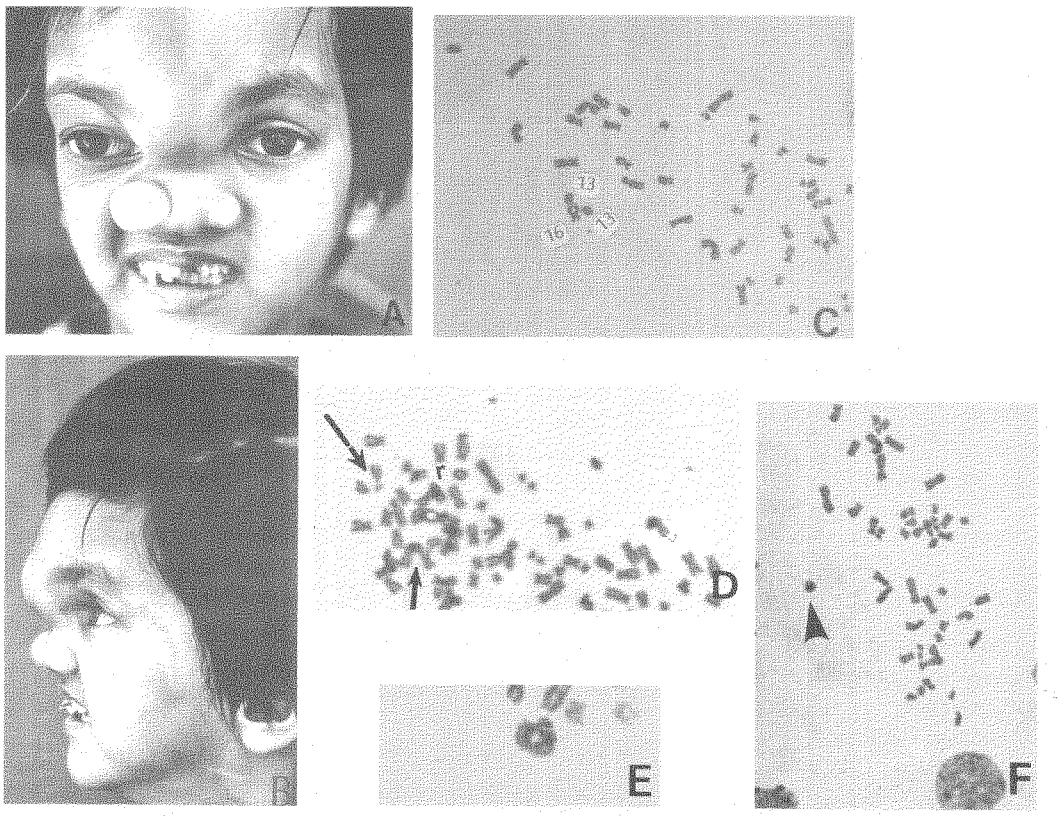


Fig. 1. A. A face of the propositus exhibiting right side enlargements on each corresponding organ (eye, nasal philtrum, teeth). Take note of a patch of darker skin on the forehead. **B.** Left side view of the face showing darker skin areas and smaller lower helix of the left ear. **C.** Giemsa stained metaphase chromosomes in cultured lymphocyte exhibitings makrer dots and irregular translocations involving three chromosomes (×1500). **D.** Spread metaphases showing ring (r) Levanian translocation (lower arrow) acrocentric association (broken arrow) and a marker dot (×2200). **E.** A part of metaphase showing a ring involving larger chromosome (×2500). **F.** A ring of smaller chromosome (×1500).

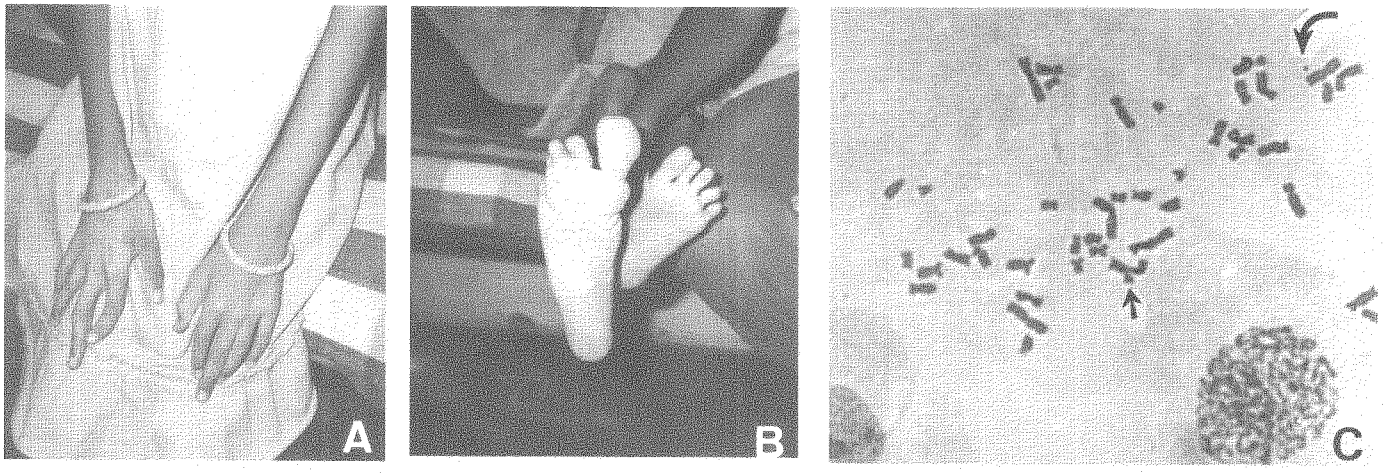


Fig. 2. A. Close up picture of hands of the propositus exhibiting darker skin on right thumb and similar patches on palm. Left hand had uniform colour of the skin. **B.** Differences in the right and left soles: note wrinkles on the sole due to hypertrophied skin. **C.** Metaphase plate from lymphocyte cultures in 1991 showing a marker dot (litted) and complex Levanian translocation (×2200).

found some cells with 42 ± 1 chromosomes many with 48 ± 2 chromosomes and, of course, the major stem line belonged to possess 46 chromosomes with two X chromosomes. In none of the hypoploid or hyperploid counts either of the X chromosomes was affected, but among the hypoploid counts we often found one chromosome 13 and both chromosome 17 missing in several cells. This was repeatedly proved by karyotyping G banded plates. For further confirmation attempts were made to use NaCl instead of Kcl for hypotonic treatment which yielded many intact cells with hypoploid counts (Goswami and Goswami, 1993).

Structural aberrations

Table 1 presents those features whose frequency estimates exceed 3% in peripheral blood cultures and this have been consistently observed in all three longitudinal studies. Chromosomally mosaic cells with ring chromosome and with both simple and complex translocations (involving more than two chromosomes: chromatid as well as chromatid types) appear to be a major cytogenetic assemblage of this hemihypertrophy patient with features of hypomelanosis of Ito (Fig. 1C-E).

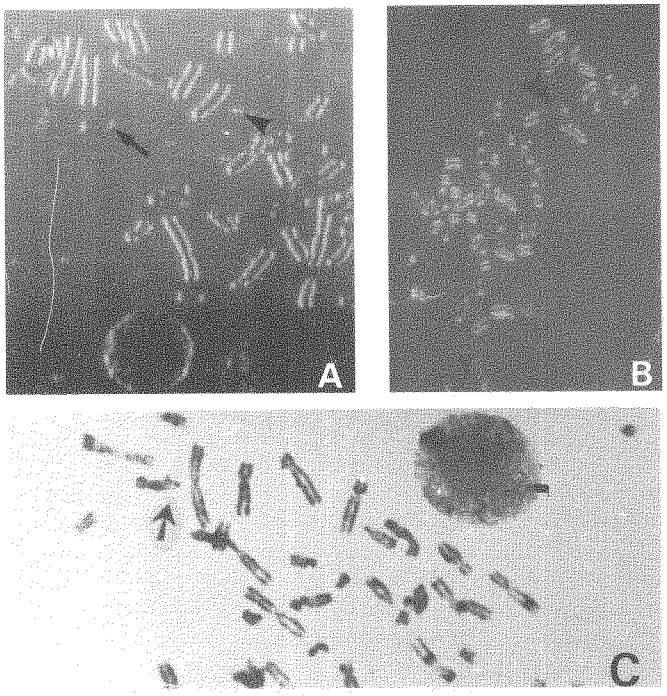
The propositus can not be mistaken for pigmentary dysplasia (Flannery, 1990) because of the presence of features of hemihypertrophy with lately detected Wilms tumour (in fact, observation of marker dots, Fig. 1C enabled search for any malignant feature). This was further verified by fluorescence microscopy (Fig. 2C). after acridine orange staining using yellow-red filters.

All the above observations on aberrations have been found to be negative except 7 to 8 % metaphase plates in father's lymphocytes with PCD (premature centromeric divisions) and about 3.5-4% lymphocytes with hypoploid counts (rarely, one hyperploid !!). Thus table 1 presents an overall chromosomal constitution of the female patient (46 XX) mixed with a few chromosomally mutated cell lines.

DISCUSSION

The propositus is a classical case of hemihypertrophy with hypomelanosis of Ito. Besides perfect unequal left and right halves of the body, she had shown signs of Wilms tumour and lately had exhibited darker and lighter areas of skin pigmentation on her head, shoulders and right upper arm. Chromosomally, she always exhibited both numerical as well as structurally mosaic cell lines. Theoretically, there were about 20% hypoploid, 10% hyperploid and 70%, normal diploid (2n-46XX) cells in the blood culture. This must be mentioned here that peripheral blood on keeping cool in TC 199 medium and sub culturing after 8 days did not yield cells with variant chromosome counts.

There appears a possibility that there have been more than one cell lines in the child which might have originated by clonal expansion of the mutated viable cells during prenatal life. The mosaicism can be caused either by chromosomal abnormalities or from single-gene mutations or possibly from the incorporation of extrachromosomal DNA. In our case, this appears possible that a mutant cell line with chromosomal aberration appeared in prenatal life and the propositus has developed with somaclonal mosaicism. Since the tissue on keeping (both skin as well as peripheral blood) for 8 days



does not yield cells with aberrations after subsequent cultures, we presume that aberrant cells must be dying out quickly while cells with normal cell line must sustain full life. Chromosomal mosaicism and aberrations are well documented in hypomelanosis of Ito (Sybert *et al*, 1990) though in some cases no chromosomal aberrations were observed either in peripheral blood or skin fibroblast cultures. Sometimes though, either aberrations or mosaicism or both may be either present in peripheral blood and absent in fibroblast cultures or vice versa, or may be present in both (Glover *et*

Fig. 3. Part of late metaphases under fluroscent light with yellow and green filters after staining with acridine orange; **A.** dark field enlargement showing marker dot (arrow) and DM (double minute, arrow head). There are a few chromatin breaks and a complex translocation (X 2500). **B.** Small arrow head shows two double minutes and larger shows detaching marker dot (X 2200). Assowed chromosome shows. **C.** Chromatin attenuation at the chromatid and telomeric end.

Table 1. Chromosomal profile of hemihypertrophy patient with hypomelanosis of Ito.

	Study period			Reamrk
	1984-87	1988-91	1992-94	
1. Number & metaphases scored	260	200	140	Other cells revealed
2. No. of metaphases 46, XX	172 (0.661)	128 (0.64)	86 (0.614)	variable counts (see text)
3. Chromosomal aberrations in metaphases				
(1) ring chromosomes	17 (0.065)	08 (0.04)	-	Fig. 2E, F
(II) Premature centromeric divisions	28 (0.107)	12 (0.06)	02 (0.014)	
(III) Somatic pairing	12 (0.046)	14 (0.07)	02 (0.014)	
(IV) Marker Dots	78 (0.3)	50 (0.25)	13 (0.092)	Fig. 2D, 3A, B
(V) Simple translocations	27 (0.103)	10 (0.05)	07 (0.05)	
(VI) Chromatin attenuation	14 (0.053)	07 (0.035)	05 (0.035)	Fig. 3C
(VII) Complex translocation	28 (0.107)	10 (0.05)	13 (0.092)	Fig. 2C, D, 3A
(VIII) Acrocentric associations	27 (0.103)	14 (0.07)	02 (0.014)	Fig. 2D
4. Nuclear aberration				
(1) fragmentation	12 (0.046)	12 (0.06)	05 (0.035)	
(2) amitotic division (chains of 3 or more nuclei)	14 (0.053)	14 (0.07)	05 (0.035)	

al., 1989; Ritter *et al.*, 1990)

Probably for the same reason hypolelanosis of Ito is considered to be a clinical marker for non-specific chromosome mosaicism (Happle, 1987; Ratter *et al.*, 1990), which was also reported as a rare feature in a hemihypertrophy patient (Jenkins *et al.*, 1971).

The present case is a unique one, probably, no single hemipypertrophy patient from "head to toe nail" might have exhibited so classic features, with hyponelanosis of Ito; chromosomal features showing mosaicism, rings, complex translocations and marker dots apart from Wilms tumour. The patient appears to have inherited through her male parent "a few" of mutated loci on some chromosomes causing PCD, an inherited chromosomal anomaly. This will be obviously resulting into nondisjunctions in a few hundred mitotic divisions among millions of cells during early developmental stages. Acrocentric associations, simple and complex translocations (Fig. 1C-E) and record of marker dots in nearly 10% lymphocyte cells are strongly suggestive of an early onset of mutagenic clone in the developing fetus. We tentatively assign this patient as a case of "Heminypertrophy with melanosis of Ito" a new syndrome combination. Since the mother and her two brothers are both chromosomally normal, we suspect the origin of mutant cell line through the paternal gamete.

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