Karyotype 69,XXX/47,XX,+15 in a 2½ year old child

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Abstract

We present the clinical findings in a 2½ year old girl with an unusual mosaic karyotype. Amniocentesis was performed at 35 weeks because of intrauterine growth retardation. The in situ cultures showed 47,XX,+15 in seven colonies, 69,XXX in four colonies, and in two colonies 46,XX was detected. Subcultures showed 69,XXX/47,XX,+15 with no normal cells. A small dysmorphic baby was born at term. Cytogenetic studies were performed on cord blood, amnion, and placental tissue immediately after birth and further studies on peripheral blood, bone marrow, muscle biopsy, and skin cultures at 1½ years of age. FISH with two autosomal centromeric probes was performed on the peripheral blood sample. A normal cell line could not be seen in any postnatal tissue by either technique. The predominant cell line postnatally was 69,XXX. There were no cytogenetic polymorphisms and the parental origin of the different cell lines was not determined. Marked red cell macrocytosis of peripheral blood was noted on routine blood count. Bone marrow aspiration showed megaloblastic haemopoiesis without evidence of vitamin B12 or folate deficiency. At 2½ years, the patient has significant developmental problems.

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People with both an abnormal cell line and a normal cell line are well known, the commonest comprising mosaic Down syndrome and sex chromosome aneuploidies.[1] Subjects with two abnormal cell lines and no normal cell line are also known but are much rarer. Cases include Turner syndrome and Down syndrome together, 45,X/47,XX,+21,[2] Turner syndrome and trisomy 18 mosaicism 45,X/47,XY,+18,[3] and mosaic trisomy 8, 45,X/47,XY,+8.[4] In one case, trisomy 15 was present together with 47,XXX mosaicism.[5] These cases could be attributed to multiple non-disjunction events which are increasingly being detected by studies for uniparental disomy.[1] However, unusual mosaicisms with separate cell lines also suggest the possibility of chimaerism. Interpretation of the phenotype is hindered by the difficulty of examining a large number of cells from a variety of different tissues. We present a 2½ year old girl with marked developmental delay who had an unusual mosaicism involving triploid and trisomy 15 cell lines. The phenotype in this case was compatible with triploidy and marked akinesia.[5]

Case report

The patient first came for our investigation before surgery at the age of 1½ years. She was the third pregnancy to healthy unrelated parents, both aged 33 years at her birth. There are two healthy older children and no miscarriages. During the pregnancy, there was a small amount of bleeding at nine weeks of pregnancy but nothing else unusual until 30 to 32 weeks when the mother considered that growth had stopped and movements were very weak. An ultrasound at 35 weeks showed intrauterine growth retardation (IUGR). A late amniocentesis was performed, mainly to aid further management. The baby was born at term by normal vaginal delivery with birth weight 2100 g, head circumference 33 cm, and length 46 cm (all below the 10th centile).

The patient had multiple congenital anomalies, noted from birth. The hands were small with a single palmar crease. There was syndactyly involving the middle and ring fingers bilaterally with some nail share and diminished flexibility in the terminal joints. The thumbs were hypoplastic with small thenar musculature and unstable metacarpal phalangeal joints. She had small palpebral fissures, a small mouth, high bossed forehead, fine scant hair (fig 1), and partial syndactyly of the second and third toes. She had complex congenital heart disease, consisting of mild pulmonary artery branch stenosis, a bicuspid aortic valve, and a large patent foramen ovale. Urine metabolic screen was normal.

Postnatal development was delayed; she was hypotonic and unable to suck and was tube fed. She moved around very little and, as the hypotonia persisted, feeding was through a dropper. At surgery at 1½ years a gastrostomy tube was inserted and subsequent feeding has been through this tube. At 1 year 8 months her weight was 5.8 kg, length was 65 cm (below the 3rd centile), and head circumference was 43 cm (below the 2nd centile).
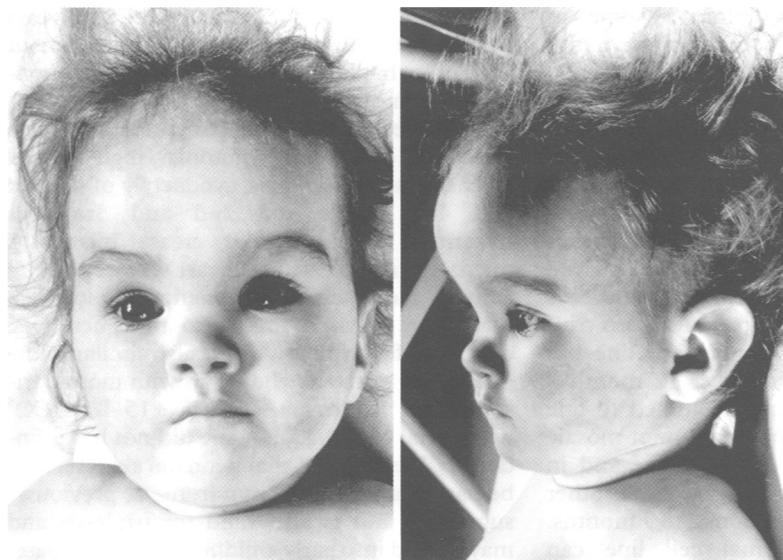
Figure 1   (Left) Anterior and (right) lateral view of the face.

Table 1   Cytogenetic analyses on different tissues from the patient

Tissue	Age	Total cells	Cells (%) with karyotype		
			46,XX	47,XX,+15	69,XXX
Amniotic fluid	35 wk				
In situ harvest		13	2 (15%)	7 (54%)	4 (31%)
			2 colonies	7 colonies	4 colonies
Subculture		36	0	14 (39%)	22 (61%)
Cord blood	Birth	85	0	5 (6%)	80 (94%)
Umbilical cord	Birth	25	0	5 (20%)	20 (80%)
Amnion	Birth	25	2 (8%)	3 (12%)	20 (80%)
Chorion	Birth	25	1 (4%)	4 (16%)	20 (80%)
Peripheral blood	1.5 y	360	0	2 (0.006%)	358 (99.996%)
Muscle biopsy	1.5 y	100	0	10 (10%)	90 (90%)
Skin biopsy	1.5 y	100	0	9 (9%)	91 (91%)
Bone marrow	1.5 y	163	0	0	163 (100%)

Review at 2½ years showed mid thoracic scoliosis with marked rotation and chest asymmetry. *X* ray, confirming the thoracic scoliosis, also showed segmentation anomalies at the level of the 6th to 7th thoracic vertebrae, a widening of the lower cervical and upper thoracic spinal canal, and marked osteoporosis,
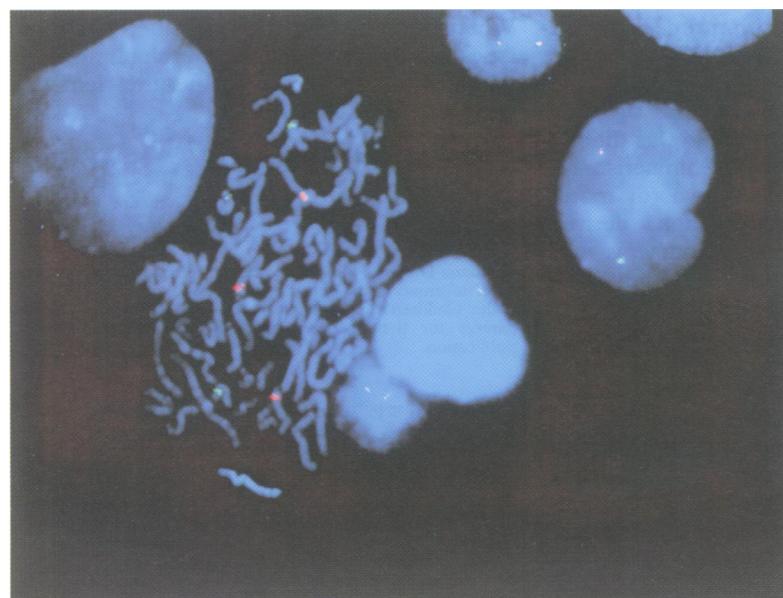

Figure 2   Metaphase spread with FISH showing triploid cell with three signals for chromosome 18 (green) and three signals for chromosome 15 (red). Nearby interphase nuclei here are not informative.

attributed to the lack of movement. She had some hearing impairment, confirmed with brain stem auditory evoked responses. In addition, bilateral grade 2 vesicoureteric reflux was present. She had severe developmental delay, no speech, lack of movement, and inability to perform any activities.

Haematology tests at 1½ years showed macrocytic red blood cell changes. Haemoglobin was 13.9, white cell count 8.1, platelets 288 (all within the normal range). The mean corpuscular volume (MCV) was raised at 130 (normal range 80-100). The changes suggested megaloblastic haemopoiesis. This was confirmed by bone marrow examination which showed giant metamyelocytes, occasional hypersegmented neutrophils, and mild megaloblastic erythropoiesis at all stages of maturation. The bone marrow was moderately hypocellular. Serum vitamin B12 and folate studies were normal.

CYTOGENETICS

Prenatal and postnatal cytogenetic analyses were performed with standard techniques. All preparations were GTG banded. Table 1 shows the prenatal and postnatal test results. The triploid cell line predominated throughout all tissues except for the amniotic fluid in situ harvest where the trisomy 15 cell line predominated. The trisomy 15 cell line could be shown postnatally. It was present in low percentages in all tissues examined except the bone marrow which showed only 69,XXX. In the amniotic fluid, two colonies showed cells with 46,XX; these were derived from two different dishes (A and B) which had been set up separately from the two different tubes of fluid received. In the amnion culture, the two cells with 46,XX were from the same culture dish. The chorion culture showed one cell with 46,XX. These were the only normal cells seen and their interpretation is uncertain. They could represent a true very low grade mosaicism for a normal cell line, or result from loss of a chromosome 15 from the trisomy 15 cell line (by non-disjunction or anaphase lag) or indicate maternal contamination. The parents' karyotypes were both normal and there were no informative cytogenetic polymorphisms. Owing to the low numbers of 46,XX cells and the lack of informative cytogenetic polymorphisms, we could not establish whether the 46,XX cells were maternal in origin or fetal.

A further attempt to find normal cells in peripheral blood was undertaken with interphase FISH. Two probes were used simultaneously, the alpha satellite chromosome 18 (D18Z1, Oncor) and the chromosome 15 centromeric probe (pTRA25, donated by Dr A Choo, Murdoch Institute, Melbourne). The former was biotinylated and detected with avidin FITC and the latter was digoxigenin labelled and detected with rhodamine according to standard techniques. Hybridisation signals were visualised on a fluorescence Zeiss Axioskop 20 and captured on Cytovision image analysis system (Applied Imaging). A total of 75 interphase nuclei were scored. There were three nuclei with two signals for both chromosome 15 and chromosome 18 but this was too

low to establish a valid diploid cell line, as 42/75 (56%) of nuclei were uninformative (fig 2).

Discussion

A feature of our case is the long survival associated with two abnormal cell lines, both of which when present as the sole abnormality are non-viable. One case of liveborn trisomy 15 has been reported, in a female baby who survived for four days and was non-mosaic in 100 cells from peripheral blood culture.[6] Live born triploidy mosaicism with a normal cell line has been reported with survival over 12 months.[7] The mosaicism is frequently confined to one tissue such as skin. An apparently non-mosaic triploid girl survived to 10½ weeks,[8] and in their review these authors discuss seven other cases of long term survival, from 2 to 7 months. The presence of a normal cell line can significantly improve survival. Prenatally this has been shown in confined placental mosaicism involving trisomies of chromosome 7, 13, and 16, and in these non-viable aneuploidies the normal cell line in the placenta allows survival to term.[9] Postnatally, cases of trisomy 16 liveborns showed mosaicism; some showed mosaic trisomy 16 in skin fibroblasts but not in peripheral blood while one had trisomy 16 cells in blood but a normal skin culture,[10] [11] and long surviving people with trisomy 18 may all be mosaics.[12] In our patient we could not convincingly show a normal cell line either prenatally or postnatally even though many cells from different tissues were examined cytogenetically (table 1) and with FISH (fig 2). It remains unknown in our case whether normal cells, still present in a tissue not examined, are contributing to her survival.

Unusual mosaicisms with separate cell lines could be attributed to multiple non-disjunction events and in these mosaicisms the genetic material in the different cell lines is of the same origin. More than one population of cells within the body also suggests the possibility of chimaerism. One example was the 18 year old girl with features of Turner syndrome and 45,X in skin and other tissues, but 47,XY,+13 in lymphocytes.[13] There were no stigmata of trisomy 13 in this girl and no signs of masculinisation. Another example was the girl with 46,X,i(Xq)/47,XX,+13.[14] Chimaerism results when a person contains two (or more) genetically distinct cell populations derived from more than one zygote. Chimaeras may arise as "blood" chimaeras owing to mixing of blood from non-identical twins across a defective placenta or as the result of double fertilisation and fusion of the diploid nuclei. To establish the mechanism, markers, either informative cytogenetic heteromorphisms or DNA polymorphisms, from the proband and the parents need to be studied. In one case studied with DNA polymorphisms, a child with ambiguous genitalia and a 46,XX/46,XY karyotype was identified as the result of postzygotic fusion of two embryos.[15] Our case is unproven as to mechanism of origin, as we were unable to perform polymorphic studies but the mechanism of chimaerism with fusion of a trisomy 15 and triploid zygote involves a number of abnormal events, as does gain or loss of a haploid set accompanied by non-disjunction of chromosome 15.

The phenotype of our patient at 2½ years consisted of severe hypotonia, high bossed forehead, scant fine hair, syndactyly of the 3rd and 4th fingers and 2nd and 3rd toes bilaterally, single palmar crease bilaterally, microcephaly, congenital heart disease, lack of activity, and severe developmental and intellectual delay. This is similar to the phenotype described for triploidy.[7] [8] It is also similar to the phenotype of the newborn girl with mosaic trisomy 15 karyotype (47,XX,+15/47,XXX)[5] with "fetal akinesia", but she did not have syndactyly. We consider that neonatal akinesia may be part of a trisomy 15 syndrome as previously suggested[5] but is also valid for triploidy and may extend into early childhood, as in our case. Additional spinal and muscular abnormalities, including developmental delay and early scoliosis such as in our patient, are most likely to be secondary to the inactivity.

Our patient came to investigation at the Children's Hospital because of macrocytosis. Macrocytosis is well known in triploidy as detected prenatally, but few cases are reported past 1 year of age. The macrocytosis of triploidy is greater than for small for gestational age (SGA) fetuses with normal karyotype and also fetuses with other trisomies, such as trisomy 13. This was shown in a large study comprising 22 triploids, 31 SGA with other chromosomal defects, and 178 SGA with normal chromosomes.[16] Also, chromosomally abnormal fetuses have a significantly higher mean corpuscular volume than control fetuses.[17] [18] Interestingly, bone marrow aspiration in our patient showed morphologically megaloblastic haemopoiesis, which has not been previously documented in triploidy. We think that the megaloblastic changes are the result of the triploidy rather than the low grade persistence of trisomy 15 cells, as the latter were not detected in the bone marrow after extensive analysis. Whether malignancy is more common in those cases of triploidy with megaloblastosis is unknown, as is also the risk for our patient with two abnormal cell lines.

Note added in proof

The patient died of respiratory failure following an upper respiratory tract infection at the age of 3 years 8 months.

1 Robinson WP, Blinkert F, Bernasconi F, Lorda-Sanchez I, Werder EA, Schinzel AA. Molecular studies of chromosomal mosaicism: relative frequency of chromosome gain or loss and possible role of cell selection. *Am J Hum Genet* 1995;56:444-51.
2 Jansen S, Kruger AJ, Liebenberg G. Turner/Downs mosaicism. A case report. *S Afr Med J* 1991;79:731-2.
3 Serville F, Fontan D, Laurent C, Cazauran JM, Verger P. Mosaic 45,X/47,XX,+18. *Hum Genet* 1977;36:351-3.
4 Schofield B, Babu A, Punales-Morejon D, et al. Double mosaic aneuploidy: 45,X/47,XX,+8 in a male infant. *Am J Med Genet* 1992;44:7-10.
5 Fryns JP, Kleczkowska A, Lagae L, Kenis H, van den Berghe H. A specific phenotype associated with trisomy 15 mosaicism. *Ann Genet (Paris)* 1993;36:129-31.

6 Coldwell S, Fitzgerald B, Semmens JM, Ede R, Batemen C. A case of trisomy of chromosome 15. *J Med Genet* 1981;**18**: 146-7.

7 Woods CG, Bankier A, Curry J, *et al.* Asymmetry and skin pigmentary anomalies in chromosome mosaicism. *J Med Genet* 1994;**31**:694-701.

8 Niemann-Seyde SC, Rehder H, Zoll B. A case of full triploidy (69,XXX) of paternal origin with unusually long survival time. *Clin Genet* 1993;**43**:79-82.

9 Kalousek DK, Dill FJ. Chromosomal mosaicism confined to the placenta in human conceptions. *Science* 1983;**221**:665-7.

10 Gilbertson NJ, Taylor JW, Kovar IZ. Mosaic trisomy 16 in a live born infant. *Arch Dis Child* 1990;**65**:388-9.

11 Lindor NM, Jalal SM, Thibodeau SN, Bonde D, Sauser KL, Karnes PS. Mosaic trisomy 16 in a thriving infant: maternal heterodisomy for chromosome 16. *Clin Genet* 1993;**44**: 185-9.

12 Smith A, den Dulk G. Follow up of case of advanced survival and trisomy 18. *J Ment Defic Res* 1980;**24**:157-8.

13 Eiben B, Hansen S, Goebel R, Hammans W. Tissue-specific 45,X0/47,XY,13 mosaicism in an 18-year-old woman. *Hum Genet* 1989;**82**:391-2.

14 Igarashi M, Tsukahara M, Sugio Y, Katayama K, Kajii T. 46,X,i(Xq)/47,XX,+13 mosaicism. *Ann Genet (Paris)* 1985;**28**:241-4.

15 Green AJ, Barton DE, Jenks P, Pearson J, Yates JRW. Chimaerism shown by cytogenetics and DNA polymorphism analysis. *J Med Genet* 1994;**31**:816-17.

16 Nicolaides KH, Snijders RJM, Thorpe-Beetson JG, van den Hof MC, Gosden CM, Bellingham AJ. Mean red cell volume in normal, anemic, small, trisomic and triploid fetuses. *Fetal Ther* 1989;**4**:1-13.

17 Fisk NM, Tannirandorn Y, Santolaya J, Nicolini U, Letsky EA, Rodeck CH. Fetal macrocytosis in association with chromosomal abnormalities. *Obstet Gynecol* 1989;**74**:611-16.

18 Sipes SL, Weiner CP, Wenstrom KD, Williamson RA, Grant SS. The association between fetal karyotype and mean corpuscular volume. *Am J Obstet Gynecol* 1991;**165**:1371-6.