Diplospermy II indicated as the origin of a liveborn human triploid (69,XXX)

SUMMARY A 69,XXX liveborn baby was shown to have the Rh genotype *CDe/cDE/cde* which suggested that 46 of her chromosomes were of paternal origin. Studies on C band polymorphisms and other genetic markers indicated that the most likely origin of this triploid was a failure of male meiosis II.

Triploidy is not uncommon in spontaneous abortuses but is a rare condition in term liveborn babies.^{1,2} After the birth of such an infant, the opportunity was taken to attempt to determine the origin of the triploidy by blood grouping and cytogenetic studies.

Case report

The 31-year-old mother had a history of three normal pregnancies in 1967, 1969, and 1970 and her children remain well. Her fourth baby was female and delivered by caesarian section in 1975 at 35 weeks because of suspected haemolytic disease of the newborn, as well as poor maternal weight gain. The infant was growth retarded and weighed 920 g. Her face was rather triangular in shape with a flattened nose and low set ears. She had syndactyly of hands and feet, there was mild jaundice, petechial haemorrhages were seen, and slight hepatomegaly was detected (fig 1). The infant survived for 17 hours, death being attributed to early bronchopneumonia. At necropsy the gall bladder was absent but the heart, diaphragm, ureters, bladder, uterus, fallopian tubes, and ovaries

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FIG 1 (a) Foot of triploid showing syndactyly.

(b) Complete triploid baby.

all appeared normal. Permission was not granted for dissection of the head and neck.

The placenta weighed 120 g and there was extensive squamous metaplasia at the cord insertion, with keratinisation on the surface of the amnion and dense fibrosis underneath.

Materials and methods

GENETIC MARKERS

Red cells from the patient and her parents were tested at the MRC Blood Group Unit using standard blood grouping methods. Enzyme types and plasma protein types were determined on blood samples, extracts of placenta and liver, and cultured fibroblasts using standard methods employed by the MRC Human Biochemical Genetics Unit.³

CYTOGENETICS

A bone marrow aspiration was taken for chromosome analysis. This test is recommended for speedy diagnosis (2 hours) in cases of poorly thriving or deformed babies, since the results can help in clinical management, for example, to determine whether surgery should be performed.⁴

The C banding method of Chandley and Fletcher was used. As this method commonly produces residual G bands, chromosome identification is aided by this accidental hybrid technique. The inheritance of each chromosome variant was studied by five independent observers without the serological information that two paternal contributions might be present. Each observer was given

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five C banded metaphase prints from mother, father, and triploid child. Karyotyping variation and inability to distinguish between parental homologues eliminated chromosomes 2,3,4,5,7,8,10,12,14, 19,20, and X from the exercise immediately. Concentrating on the remaining 11 chromosomes, each observer was asked to comment on the pattern in each chromosome triplet and on the possible parental origin of each member of the trio.

Results

GENETIC MARKERS

The blood groups of the family are given in table 1. The Rh groups are informative and since they reflect the triploidy the reactions of the various Rh antisera are given in the table. From the reactions of the first five antisera, those routinely used in Rh grouping, the Rh genotypes of the father and baby could be interpreted as *CDE/cde* and of the mother as *cde/cde*. However, the use of antisera to the compound Rh antigens ce, CE, and Ce combine to show the true genotypes to be mother *cde/cde*, father *CDe/cDE*, and the triploid baby *CDe/cDE/cde*. Thus the Rh groups confirm the baby's triploid state, with two contributions from the father.

The Kell, Duffy, Kidd, and Xg results for the parents are not recorded since no results for the baby were achieved. These groups require an anti-globulin test which could not be done since the baby's cells were sensitised with her mother's anti-D.

The phosphoglucomutase (PGM₁) phenotype listed in table 2 as 2-1-1 had PGM₁¹ isozymes which were approximately twice as strong as the PGM₁² isozymes, a pattern which we have observed in two other triploids. Given that there were two paternal contributions, the red cell acid phosphatase (ACP₁) phenotype suggested that two different paternal genes had been inherited, while the PGD and GPT phenotypes suggested that two identical paternal genes had been inherited. The phenotypes are listed in table 2.

CYTOGENETICS

The baby had a 69,XXX karyotype with no evidence of diploid mosaicism. Mild random hypotriploidy was apparent in 45% of metaphases. This was considered to be an artefact, principally because increased hypotonic pretreatment was used to

TABLE 2 Enzyme types and plasma protein types of family MRC 3462

Marker	Mother	Father	Baby
AK ₁	1	1	1
ADA	1	1	1
PEPA	1	1	1
PEPB	1	1	1
PEPC	1	1	1
GPT	2-1	2-1	2 (confirmed on tissues)
Pi	M	M	M
Gc	2-1	1	1
EsD	1	1	1
GOT ₈	1	1	1
ME ₁			1 (tissues)
ACON ₈			1 (tissues)
PGM ₁	2-1	2-1	2-1-1
PGM ₂	1	1	1
PGM ₃			1 (tissues)
PGD	A	AB	A
ACP ₁	A	BA	?BAA

facilitate adequate spreading of cells with such a large chromosome complement.

Table 3 illustrates the inheritance pattern of cytogenetic polymorphisms on the 11 chromosomes under consideration. Unfortunately, there were no outstanding heteromorphisms, so assessment was made on small relative differences in size, shape, and position of C bands or satellites⁶ (fig 2).

The conclusions arrived at independently by the five observers are summarised in table 4 which serves to illustrate how individual observations on polymorphisms can vary enormously. However, the results indicate that many of the sets of three display a pattern of 'one different and two alike' with no triplet consistently showing all three different (fig 3).

TABLE 3 Most likely inheritance pattern of chromosome variants

Chromosomes which could be unambiguously identified	Father	Mother	Baby
Chromosome 1	a b	a c	a a c
6	a b	a b	a a a
9	a b	a c	a a c
11	a b	a b	a a a
13	a a	a b	a a b
15	a a	a c	a a c
16	a a	a b	a a b
17	a b	a b	a a a
18	a b	a c	a a a
21	a b	a c	a a a
22	a b	c d	a a d

A change of symbol (a, b, c, or d) is used to represent chromosome variants as agreed by at least four of the five observers.

TABLE 1 The blood groups of the family

	Anti-							Rh groups		Other groups			
	C	c	D	E	e	ce	CE	Ce					
Mother	-	+	-	-	+	+	-	-	<i>cde/cde</i> (rr)	O	M	S-s+	P ₂ Lu(a-)
Father	+	+	+	+	+	-	-	+	<i>CDe/cDE</i> (R ₁ R ₂)	A ₁	M	S+s+	P ₁ Lu(a-)
Baby	+	+	+	+	+	+	-	+	<i>CDe/cDE/cde</i> (R ₁ R ₂ r)	A	M	S+	P ₂ Lu(a-)

FIG 2 Segregation of chromosomes 1, 9, 15, and 16 (C banded).

FIG 3 C banded karyotype (69,XXX) illustrating the residual G bands and the 'two alike and one different' pattern in many triplets. In particular note the small partial inversion of centromeric heterochromatin in the maternal chromosome 9 (RHS).

Therefore, taking cytogenetic analysis as a sole source of information, there is weak evidence of two paternal contributions, although this may be insufficient to eliminate digyny I as an alternative (it can be seen from table 3 that chromosome 22 is the only example where digyny is unlikely). However, combined with the serological information, the cytogenetic results are more useful. The 'two alike and one different' pattern was unanimously agreed by the five observers to exist in a minimum of five triplets (table 4) so that, given that only one chromosome came from the mother, it is clear that in each case the 'two alike' are identical to one of the father's relevant homologues (table 3).

Discussion

Table 5 lists the combined probability of each possible cause of triploidy. Digyny can be ruled out by the Rh blood groups and probably by the autosomal C band polymorphisms. Diplospermy I is excluded by the study of chromosome variants and the XXX sex chromosomes. The results from ACP₁, GPT, Rh, PGD, and PGM₁ were subjected to extensive and elaborate calculations which attempt to allow for the chromosomal location of these markers and the number of chiasmata expected between them and their centromeres. When completed these calculations did not distinguish between

TABLE 4 Summary of cytogenetic analysis by five independent observers

Observer	Number of triplets showing pattern (mean $\pm$ SE)			Comments on inheritance (E = to exclusion of all else)
	2 alike + 1 different	3 indistinguishable	3 different	
DJ	5 $\pm$ 0.28*	6 $\pm$ 0.28	0 $\pm$ 0	A mean of 3.4 $\pm$ 0.2 Triplets indicate diandry (E)
TB	7.2 $\pm$ 0.9	3.2 $\pm$ 0.81	0.6 $\pm$ 0.2	A mean of 1 $\pm$ 0.28 Triplets indicate diandry (E)
BP	6.8 $\pm$ 0.6	4.2 $\pm$ 0.6	0 $\pm$ 0	A mean of 3.6 $\pm$ 0.35 Triplets indicate diandry (E)
JW	6.4 $\pm$ 1.11	4.6 $\pm$ 1.11	0 $\pm$ 0	A mean of 1.6 $\pm$ 0.2 Triplets indicate diandry (E)
IB	7.4 $\pm$ 0.82	3.2 $\pm$ 0.8	0.4 $\pm$ 0.2	A mean of 0.2 $\pm$ 0.14 Triplets indicate diandry (E)

*Lowest mean used for probability estimation.

TABLE 5 Probability of observing triploid of given phenotype classified by mechanism of origin

Information from	Diandry		Digyny	
	Diplospermy I	Diplospermy II	Dispermy	
XXX chromosomes	0	0.5	0.25	0.5
Autosomal C band variants	0	about 1	< ($\frac{1}{2}$) ⁵	0
ACP ₁ BAA	0.73	0.54	0.5	0.5
GPT 222	0.0675	0.115	0.125	0.125
R ₁ R ₂ r and PGD AAA	0.038	0.0325	0.0325	0
Relative probability	0	63.87	1	0

diplospermy II and dispermy or significantly alter the odds in favour of either origin (table 5). The chance of two unrelated sperm having five identical autosomal G band polymorphisms and an X chromosome each is ($\frac{1}{2}$)⁶, so that the odds in favour of diplospermy II rather than dispermy are 64:1.

There are very few published cases of triploidy where diplospermy II origin was a possibility.⁷⁻⁹ We are not aware of any triploids where a diplospermy II origin is as likely as in our case. This triploid was also probably the first recorded case of 'double' haemolytic disease of the newborn having two D genes to stimulate maternal anti-D in addition to her other misfortunes.

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