Significance of detection of extra metacentric microchromosome in amniotic cell culture

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SUMMARY A metacentric bisatellited microchromosome was detected in all metaphases from an amniotic culture performed because of maternal age. A wide-ranging survey of the literature failed to disclose any consistent anomaly associated with such a marker, but did reveal that the clinical picture of patients manifesting it could range from complete normality through mental retardation to a variety of deformities. The parents elected for termination, and the only deformity detected in the abortus was fixed talipes equinovarus. The implications of the finding of this marker chromosome on amniocentesis, believed to be reported for the first time here, are discussed particularly in the context of genetic counselling.

Numerous accounts of the finding of an extra metacentric microchromosome have appeared since the anomaly was first reported by Ilberry *et al.* (1961). In some of these reports the microchromosome has been found incidentally in a phenotypically normal person during surveys, or as a family trait; in others, it has been associated with congenital abnormalities.

We wish to report what we believe to be the first detection of such a marker in an amniotic cell culture, and to discuss the prenatal implications of this finding in the context of genetic counselling.

Case report

A 40-year-old Caucasoid woman was referred rather late (at the 18th week of pregnancy) for amniocentesis on account of advanced maternal age. She had produced 2 normal liveborn sons, now aged 8 and 5 years, and had aborted spontaneously three times, always during the first trimester. She is Rh-negative, and her husband Rh-positive; the first abortion took place after the birth of her first son, when she did not receive anti-D serum, and the second and third after the birth of her second son, who is Rh-negative. No investigations had been carried out on either abortus. There was no paternal or maternal family history of inherited disease, and the present pregnancy was proceeding normally. Questioning about drug ingestion, infection, or exposure to radiation elicited nothing of relevance. Transabdominal amniocen-

Received for publication 24 January 1977

tesis was performed after prior ultrasonic localisation of the placenta; a sample of clear fluid was obtained, and cells from it were cultured for karyotyping. Routine α -feto-protein estimation of amniotic fluid showed normal levels for the period of gestation ($<16\mu\text{g/ml}$).

CYTOGENETIC FINDINGS

Analysis of 45 Giemsa-stained amniotic fibroblast metaphases disclosed the karyotype 47,XY+mar (Fig. 1). There was random technical loss of chromosomes from 9 cells, but the extra chromosome was present in all of them. It was small and metacentric, and without obvious satellites in unbanded metaphases, though in 10 of the 45 cells association with acrocentric chromosomes was obvious. The regular chromosome complement showed a normal banding pattern on trypsin-Giemsa and quinacrine mustard treatment. Giemsa banding did not assist in identifying the nature of the micro-marker (Fig. 2). The marker chromosome fluoresced clearly in all metaphases and in some cells it had a distinctly bisatellited appearance (Fig. 3a).

Karyotyping of both parents and both children disclosed no evidence of any chromosomal anomaly, and screening of 100 cells failed to detect any sign of mosaicism for the marker in either parent. The mother had prominent satellites of one chromosome 21 and one 22 (Fig. 3c), and one paternal chromosome 22 had very elongated stalks terminating in prominent satellites (Fig. 3b). The latter had been inherited

Fig. 1 Unbanded karyotype of amniotic cells showing the presence of the extra metacentric microchromosome.

Fig. 2 GTG-banded karyotype of amniotic cells showing non-distinctive banding of the extra metacentric microchromosome.

Fig. 3 Partial Q-M-banded karyotypes showing (a) in the fetus, the bisatellited microchromosome and segregation of the maternal chromosome 21 lacking prominent satellites and chromosome 22 possessing them; (b) in the father, a prominent satellite of chromosome 22 having an elongated stalk and hence easily differentiable from the chromosome 22, ps^+ of the mother; and (c) in the mother, prominent satellites of one chromosome 21 and one chromosome 22.

by the younger son, and the elder son had inherited the maternal 21 satellite variant as well as the paternal one.

OUTCOME OF THE PREGNANCY

After extensive discussion of the literature and weighing up the evidence for the possibility of congenital defect (see below), the parents elected for termination of the pregnancy. Labour was induced with prostaglandins, and a male fetus, phenotypically normal except for left-sided fixed talipes equinovarus, was delivered vaginally. Necropsy did not disclose any internal abnormalities in the fetus.

Discussion

An extra metacentric chromosome has been reported in two large surveys of newborn populations. Walzer *et al.* (1969) found such a marker chromosome in 3 of 2400 consecutive phenotypically normal infants; in 2 the chromosome was bisatellited, and it was segregating in 2 of the 3 families. In their survey of 5049 consecutive newborns, Friedrich and Nielsen (1974) detected a bisatellited metacentric microchromosome in 1 abnormal and 2 phenotypically normal infants. The marker was found in normal members of the families of the abnormal and one of the normal neonates. In a survey of phenotypically normal adult females, Borgaonkar *et al.* (1971) found one subject with an extra metacentric chromosome.

It appears from these surveys that an extra microchromosome, usually bisatellited, can occur as an incidental variant marker in phenotypically normal individuals and their families. On the other hand, there are numerous accounts of association of this marker with phenotypic abnormalities. These accounts may be regarded as biased, since the proband would have been referred for cytogenetic assessment because of congenital malformation or mental retardation. In at least 14 such documented cases the marker has also been present in one parent and other phenotypically normal family members (Table 1); in 3 families the extra chromosome was bisatellited. There was great variation in the range of congenital abnormalities reported.

An even larger number of reports have appeared in the literature of cases in which an extra metacentric microchromosome in an abnormal proband was not present in normal family members (Table 2). Once again, the phenotypic abnormalities have spanned a wide range, from multiple gross congenital defects to mild mental retardation. Despite the proposal of a 'syndrome of the metacentric microchromosome' by Abbo and Zellweger (1970), it seems that though these chromosomes may look morphologically similar their origins may be various. Abbo and Zellweger's case, and those they review, are consistent with an isochromosome of the short arm of 18, and in the case of Taylor *et al.* (1975) banding techniques showed unbalanced segregation of 18 pi from a balanced translocation carrier. Investigation of the case of Borgaonkar *et al.* (1973), however, identified the origin of the extra chromosome as trisomy for a deletion of the long arm of 22. Similar derivations are suggested for marker chromosomes reported by Uchida *et al.* (1964) and Macintyre *et al.* (1971). Nevertheless, in the majority of instances the origins of the extra chromosomes could not be identified, though in some they could be inferred from the clinical picture.

Table 1 Cases referred for abnormality—familial marker present

Authors	Year	Phenotype of proband	Presumed chromosome identity
Abbo and Zellweger	1970	'Syndrome of the metacentric microchromosome'	? 18 p1†
Armendares <i>et al.</i>	1969	Multiple congenital abnormalities*	Bisatellited
Borgaonkar <i>et al.</i>	1971	Multiple congenital abnormalities*	Non-fluorescent†
Borgaonkar <i>et al.</i>	1971	Multiple congenital abnormalities*	Non-fluorescent†
Borges and Wald	1963	True hermaphrodite	Singly satellited
Frezza <i>et al.</i>	1966	Familial cerebropathy	?
Hoeft <i>et al.</i>	1970	Multiple congenital abnormalities*	?
Mukherjee and Burdette	1966	Features of partial Down's syndrome	? 21 p1
Nielsen and Hreidarsson	1973	Multiple congenital abnormalities*	Bisatellited
Nielsen <i>et al.</i>	1971	Oligospermia	? +Yq—
Pfeiffer <i>et al.</i>	1967	Multiple congenital abnormalities*	Bisatellited
Smith <i>et al.</i>	1965	Oligospermia	?
Soudek	1971	Multiple congenital abnormalities*	?
Ventruo <i>et al.</i>	1976	Kallman's syndrome	?

*No specific features related to any definite syndrome.

†Marker present in mosaic form in parent.

Table 2 Cases referred for abnormality—marker absent in parents

Authors	Year	Phenotype of proband	Presumed chromosome identity
Armendares <i>et al.</i>	1971	Microcephaly, facial asymmetry	?
Beyer <i>et al.</i>	1968	Multiple congenital abnormalities*	?
Borgaonkar <i>et al.</i>	1971	Multiple congenital abnormalities*	Non-fluorescent
Buhler <i>et al.</i>	1972	'Cat-eye' syndrome	? +22q—
Christensen and Nielsen	1971	Patient in psychiatric institute	? +Yq—
Crawford and Lele	1965	Multiple congenital abnormalities*	?
Dekaban and Zelson	1968	Features of partial Down's syndrome	Submetacentric ? +21q—
Den Dulk <i>et al.</i>	1966	Multiple congenital abnormalities*	?
Dent <i>et al.</i>	1963	Features of partial Down's syndrome	? +21q—
Dollmann and Jaeger	1968	Multiple congenital abnormalities*	?
Ferrante <i>et al.</i>	1968	Multiple congenital abnormalities*	?
Finley <i>et al.</i>	1971	Microcephaly—skull asymmetry	Non-satellited, early replicating
Frøland <i>et al.</i>	1963	Features of partial trisomy 18	? 18 der.
Gamstorp <i>et al.</i>	1966	Features of partial Down's syndrome	? +21q—
Gerald <i>et al.</i>	1968	'Cat-eye' syndrome	? +22q—
Ginsberg <i>et al.</i>	1968	'Cat-eye' syndrome	? +22q—
Gustavson <i>et al.</i>	1964	Features of partial trisomy 18	? 18 der.
Hultén <i>et al.</i>	1966	Oligospermia	?
Ilberry <i>et al.</i>	1961	Features of partial Down's syndrome	? +21q—
Insley <i>et al.</i>	1968	Intersex	?
Ishmael and Lawrence	1965	Features of partial trisomy D	D group der.
Ishmael and Lawrence	1968	Mental retard—no specific features	?
Jalbert <i>et al.</i>	1967	Multiple congenital abnormalities*	?
Mukherjee <i>et al.</i>	1968	Microcephaly, facial asymmetry	?
Mulcahy and Jenkyn	1972	Mental retard—no specific features	Non-satellited
Noel and Quack	1970	Multiple congenital abnormalities*	?
Schachenmann <i>et al.</i>	1965	'Cat-eye' syndrome	? +22q—
Stevenson <i>et al.</i>	1966	Features of partial trisomy D	? +D p1
Subrt and Prohliková	1970	Features of partial Down's syndrome	? +21q—
Taft <i>et al.</i>	1965	Features of partial trisomy 18	? 18 der.
Tamburro and Johnson	1966	Features of partial trisomy 18	? 18 der.
Tangheroni <i>et al.</i>	1973	Multiple congenital abnormalities*	?
Ventruo <i>et al.</i>	1973	Psoriasis—normal mentally	?
Warren <i>et al.</i>	1972	Features of partial trisomy 18	? +18 p1
Weber <i>et al.</i>	1970	'Cat-eye' syndrome	? +22q—

*No specific features related to any definite syndrome.

There have been reports of 6 patients in whom the clinical features suggested partial trisomy 21, and 2 in whom partial trisomy 13 was likely (see Tables 1 and 2). In the review by Freedom and Gerald (1973) of case reports of the 'cat-eye' syndrome first defined by Schachenmann *et al.* (1965), they point out that in 12 of the 13 patients an extra microchromosome, usually acrocentric rather than metacentric, was

present. Buhler *et al.* (1972) suggested that such cases represented partial trisomy 22 with deletion of the long arm.

In some individuals the extra chromosome may be a partially deleted Y (Christensen and Nielsen, 1971; Nielsen *et al.*, 1971). Price *et al.* (1976) and Borgaonkar *et al.* (1971) have reported extra metacentric marker chromosomes in 2 of 611 and 1 of 72 inmates

of maximum security hospitals, respectively. In the latter case, and one of the former, it was suggested that the marker could represent a deleted Y chromosome.

Identification of the marker as a derivative of one of the acrocentric chromosomes is facilitated by the presence of satellites on one or both arms. Where the marker is bisatellited it could be an isochromosome of the short arm of an acrocentric chromosome, or have been formed by centric fusion of the short arms of two acrocentric chromosomes during acrocentric association at the time of nucleolar organisation. Court Brown *et al.* (1966) and Lubs and Ruddle (1971) have shown that variation in length of the short arms and satellite regions of the acrocentric chromosomes are simple human polymorphisms without phenotypic effects. Others, however, have suggested that satellite association may predispose to breakage and translocation, possibly increased where the short arm is enlarged (Ohno *et al.*, 1961; Ferguson-Smith and Handmaker, 1963; Kong-oo Goh, 1968; Bauchinger and Schmid, 1970; Hamerton, 1971). A bisatellited chromosome in a phenotypically normal parent may consequently predispose to non-disjunction at meiosis. In fact, two families in which infants with trisomy 21 have had a phenotypically normal parent with an extra metacentric microchromosome have been reported (Ricci *et al.*, 1968; Mulcahy and Jenkyn, 1972). In the latter family the marker chromosome was bisatellited.

Lubs (personal communication to Friedrich and Nielsen, 1974) found a significantly higher incidence of congenital malformations in children with enlarged G-group satellites, and suggested (Lubs and Ruddle, 1971) that changes in the amount or distribution of heterochromatin may affect the expression of genes and particularly nucleolar organization. Freidrich and Nielsen (1974) have postulated that a bisatellited chromosome by association with acrocentric chromosomes may interfere with mitosis at a critical stage of fetal development.

The prominent satellites and short arms of G-group chromosomes in both parents of our case are of particular interest. It is conceivable that the increase of satellite material, and the unusual length of the short arm of chromosome 22 in the father, may have predisposed to centric fusion of the satellited regions and so to formation of the marker chromosome in the fetus.

The presence of a marker microchromosome in mosaic form in a phenotypically normal parent has been reported in 3 cases (Abbo and Zellweger, 1970; Borgaonkar *et al.*, 1971). It is possible that the marker may be selectively lost in successive cell divisions, and disappear gradually with increasing age. A prospective study of phenotypically normal

newborns with the marker would be of great interest. It is also possible that gonadal mosaicism may be present in a parent and not be detectable on lymphocyte culture, and thus be responsible for fetal abnormalities. One hundred lymphocyte metaphases of either parent of the present case were examined and revealed no indication of mosaicism.

Evaluation of the significance of the detection of this extra chromosome in amniotic cells presented great difficulty. On the one hand were the findings reported in surveys of phenotypically normal individuals, and on the other were the numerous accounts of variously severe congenital anomalies with extra metacentric microchromosomes. We were fortunate inasmuch as the father is a biologist, able to weigh up the conflicting published accounts for himself, once his attention had been drawn to them. Genetic counselling of a parent without such appropriate training would have been very much more burdensome, since the counsellors could never have been completely certain that the alternative possibilities had been grasped fully. As it happened, the decision of the parents was painfully made, but we believe that it was reached in full understanding of what was involved.

Assessment of the normality of the fetus was not easy. A fixed talipes equinovarus was the only morphological deformity detected. This deformity has been found in association with other congenital abnormalities in a long arm deletion of 13 (Allerdice *et al.*, 1969), occasional cases of trisomy 13, and fairly commonly in trisomy 18 (Warkany *et al.*, 1966). This case was patently none of these. Even where a well-known chromosome anomaly is detected in amniotic culture and subsequently confirmed on fetal karyotyping, the abortus may show no phenotypically recognisable stigmata of the corresponding syndrome. In 5 out of 6 cases of antenatal diagnosis of Down's syndrome, Laurence and Gregory (1976) failed to identify the characteristic Down's features in the fetus. Milunsky (1976) states that 'prenatal genetic studies are unique in that the crucial result depends upon a single laboratory test performed without prior examination of the patient'.

It would be helpful to see in the literature further reports of the antenatal detection of extra metacentric marker chromosomes and of the management of such cases.

We wish to thank Dr A. Porter for referring the patient for genetic counselling; and Drs E. Goudie and T. Kopenhager for performing the ultrasound examination and amniocentesis, respectively. We also thank Professor Trefor Jenkins for reading and commenting on this paper.

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Addendum

When this case was investigated we had not yet established recently developed silver staining techniques for the differential staining of satellite regions of acrocentric chromosomes. Such techniques should prove informative in the investigation of future similar cases.

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