Am J Hum Genet 36:1092–1102, 1984

Incidence and Significance of Supernumerary Marker Chromosomes in Prenatal Diagnosis

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SUMMARY

The finding of a supernumerary marker chromosome in amniotic fluid cells poses a considerable counseling dilemma. In 6,500 cases referred to our laboratory over a 4½-year period, eight such cases were identified (0.123% of all cases). In five of the eight cases, a diagnosis of true mosaicism between cells with 46 and 47 chromosomes was made. In the remaining three cases, the marker was present in 100% of the cells. In three cases, the marker was determined to be familial in nature with mosaicism present in the parents of two of these cases. Detailed cytogenetic findings for each case are provided. In no cases were abnormalities noted in either abortuses or live borns.

The high incidence of mosaicism in these cases seems to indicate a propensity for supernumerary chromosomes to be lost. Familial markers may not be passed on for many generations, and they may arise as new mutations relatively frequently.

There is an urgent need for more information on the risks associated with the prenatal detection of supernumerary chromosomes. We recommend that in considering the implications of the prenatal detection of marker chromosomes cases be considered in at least four distinct groups: type 1—familial and nonmosaic; type 2—familial with mosaicism in either the amniotic fluid cells, a parent, or both; type 3—de novo markers and nonmosaic; and type 4—de novo with mosaicism present in the amniotic fluid cells.

Received January 13, 1984; revised April 27, 1984.

This paper was supported by the New York State Department of Health, contract C-113258, and was presented in part at 34th annual meeting of the American Society of Human Genetics, Norfolk, Virginia, October, 1983.

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 0002-9297/84/3605-0014$02.00

INTRODUCTION

There have been numerous reports of supernumerary chromosomes associated with both normal and abnormal phenotypes [1]. In a few cases, the specific identification of the origin of these accessory chromosomes can be established [2], but in most cases, classification of such "marker" chromosomes is not possible. Attempts to find correlations between cytological appearance and phenotypic effect of supernumerary marker chromosomes have been based on relatively few cases [3, 4]. Many cases where the marker has been established as being familial appear to be associated with normal phenotypes, but even among this group, there are an appreciable number of reports, possibly coincidental, of mental or physical abnormalities (14 cases reviewed Bernstein et al. [5]; and also [1, 6–11]). A further complication in the interpretation of cells with supernumerary chromosomes is the fact that they are frequently found in a mosaicism with normal cells [12].

Thus, the finding of a supernumerary chromosome in prenatal diagnosis poses a considerable counseling dilemma [5, 13–22]. We report here our experience of the incidence and outcome of such pregnancies and suggest that in considering the implications of the prenatal detection of marker chromosomes a distinction be made between cases where mosaicism exists as well as whether or not the marker was familial in origin.

MATERIALS AND METHODS

Prenatal diagnosis of cases with supernumerary chromosomes were recorded over a 4½-year period among 6,500 referrals to our laboratory, 90% of which were referred because of advanced maternal age (≥ 35). Details of cell-culture techniques and routine case work-up have been provided [23, 24]. Chromosomes were identified by a modified trypsin-Giemsa-staining technique (G-banding) [25], quinacrine dihydrochloride staining (Q-banding) [26], constitutive heterochromatin staining (C-banding) [27], and silver staining of nucleolar organizer regions (NOR-banding) [28].

CASES STUDIED

For all cases in which supernumerary marker chromosomes were detected, the reason for referral to the laboratory was advanced maternal age (see table 1). None of the patients or their partners had known histories of exposure to radiation or chemicals with the exception of case no. 4421 in which the father had been working in the furniture manufacturing industry and may have been exposed to clastogenic chemicals.

Cytogenetic findings of the amniotic fluid cells are given in table 2. Of special interest are the cells with multiple abnormalities found in one culture from case no. 4421. The culture was not contaminated with mycoplasma, and there was no obvious evidence of other chromosome instability or damage. In the 6,500 cases studied in this laboratory, we have had no other examples of clonal evolution in an amniotic fluid cell culture. Unfortunately, a third culture was not available in this case to determine whether any of the secondary abnormalities might have been present in vivo.

The characteristics of the markers identified are summarized in table 3. Figures 1–4 show the supernumerary marker chromosomes identified by the various staining techniques. The marker for case no. 2594 strongly resembled that described by Nielson et al. [29] in five mentally retarded individuals and tentatively suggested as being an isochromosome 18p.

TABLE 1

PATIENT INFORMATION FOR CASES WITH SUPERNUMERARY MARKER CHROMOSOMES

CASE NO.	MATERNAL AGE	PATERNAL AGE	RACIAL BACKGROUND	REPRODUCTIVE HISTORY			
				Previous normal pregnancy	Spontaneous abortions	Induced abortions	Father's other children
0018	37	28	East European	0	0	0	0
0860	37	35	Hispanic	3	0	0	3
1860	34	30	Hispanic	1	0	0	0
2594	40	51	Black	3	1	3	1
3607	36	36	East European	2	2*	0	0
4421	36	33	Hispanic	2†	0	2†	3
4693	34	38	Black	1‡	1§	0	1
6183	40	35	Hispanic	3‖	0	1‖	2

* Both spontaneous abortions at approximately 4 months.
† All with a previous partner.
‡ With a previous partner.
§ Ectopic pregnancy.
‖ All with a previous partner.

In each case, patients received detailed genetic counseling following the completion of laboratory studies. Two patients with de novo marker chromosomes (cases nos. 2594 and 4421) elected to terminate their pregnancies. In no case was any abnormality noted in abortuses or live borns. No long-term follow-up information was available for these cases; case no. 0860 was reported by the mother to be developmentally normal at 2½ years, and case no. 3607 was reported by the father to be developmentally normal after 1 year.

TABLE 2

CHROMOSOME COUNTS IN AMNIOTIC FLUID CELLS

Case no.	Culture	46 (%)	47, + mar (%)
0018	A	0 (0)	18 (100)
	C	0 (0)	20 (100)
0860	A	13 (72)	5 (28)
	B	12 (100)	0 (0)
	C	6 (56)	5 (45)
1876	A	5 (45)	6 (56)
	B	5 (28)	13 (72)
2594	A	8 (73)	3 (27)
	D	4 (36)	7 (64)
3607	A	0 (0)	18 (100)
	B	0 (0)	10 (100)
4421	A	2 (10)	18*(90)
	B	15 (75)	5 (25)
4693	A	5 (14)	31 (86)
	B	0 (0)	15 (100)
6183	A	0 (0)	10 (100)
	B	0 (0)	10 (100)

* Includes: one cell 48,XX, + mar, + mar; one cell 47,XX, + mar, + mar, −20,t(2;3)(p13;p23); four cells 48,XX, + mar, + ring; four cells 48,XX, + mar, + ring,t(2;3)(p13;p23); one cell 47,XX, + mar + ring, −8; one cell 46,XO, + ring.

TABLE 3

CHARACTERISTICS OF SUPERNUMERARY MARKER CHROMOSOMES

Case no.	G-banding	Q-banding	C-banding	NOR-banding	Origin
0018	Metacentric	Satellites, both arms	Predominantly dark	Positive, both arms	Paternal
0860	Submetacentric	No satellites*	...	...	De Novo
1876	Metacentric	No satellites	Uniformly dark	Positive near centromere	Maternal†
2594	Metacentric	No satellites	...	Negative, both arms	De Novo
3607	Fragment-like	No satellites	Uniformly dark	Negative	Paternal‡
4421	Fragment-like	No satellites (4)	Pale and dark bands§	Positive, one arm (4)	De Novo
4693	Metacentric	Satellites, both arms	Pale and dark bands	Positive, both arms	De Novo
6183	Metacentric	No satellites	Pale and dark bands	Positive, both arms	De Novo

* By Q-banding, this marker had the appearance of a "Y" chromosome but smaller.
† Mother carried this marker in nine out of 30 lymphocytes.
‡ Father carried this marker in three out of 50 lymphoctyes.
§ The ring chromosome seen in one culture from this case had similar staining properties.

Successful confirmatory studies were carried out in cases nos. 0860 and 6183 (both studied by Dr. K. David at the Brooklyn Hospital, New York), 4693 (by Dr. R. Verma at the Interfaith Hospital, New York), and 2594 and 3607. For case no. 4693, in one cell in 30 from the blood sample and in one cell in 20 from a skin fibroblast culture, two marker chromosomes were found.

DISCUSSION

A total of eight cases of supernumerary marker chromosomes were identified out of a total of 6,500 cases, giving an incidence of approximately 0.123% or one in 812 cases. In five of the eight cases, the marker appeared to be de novo in origin, giving a crude estimate for incidence of 0.077% for prenatally diagnosed de novo markers, and 0.046% for familial markers.

Little data are available for comparison from other laboratories. Combining data from several larger studies in which full details of all abnormalities have been reported in detail, only three instances of supernumerary chromosomes were reported in 7,536 cases (0.04%) [30–32]. Of these three cases, two at least were familial in origin. In a large survey on prenatally detected de novo chromosome rearrangements, Warburton [33] collected 22 cases of supernumerary marker chromosomes out of a total of 98,745 amniocenteses (0.022%). The rate of de novo supernumerary marker cases reported to the New York State Chromosome Registry and United States Interregional Chromosome Registry System was 0.026%–0.070%, incidence showing an association with increased maternal age [34]. Soudek et al. [7] reviewed the incidence of supernumerary chromosomes in newborns and reported a frequency of 0.017% or one in 5,604 cases. By contrast, the frequency in mentally subnormal and/or socially deviant individuals was reported to be 0.287% or one in 348 cases.

All estimates on the incidence of supernumerary marker chromosomes are subject to methodological bias. Many of the consecutive live-born surveys are

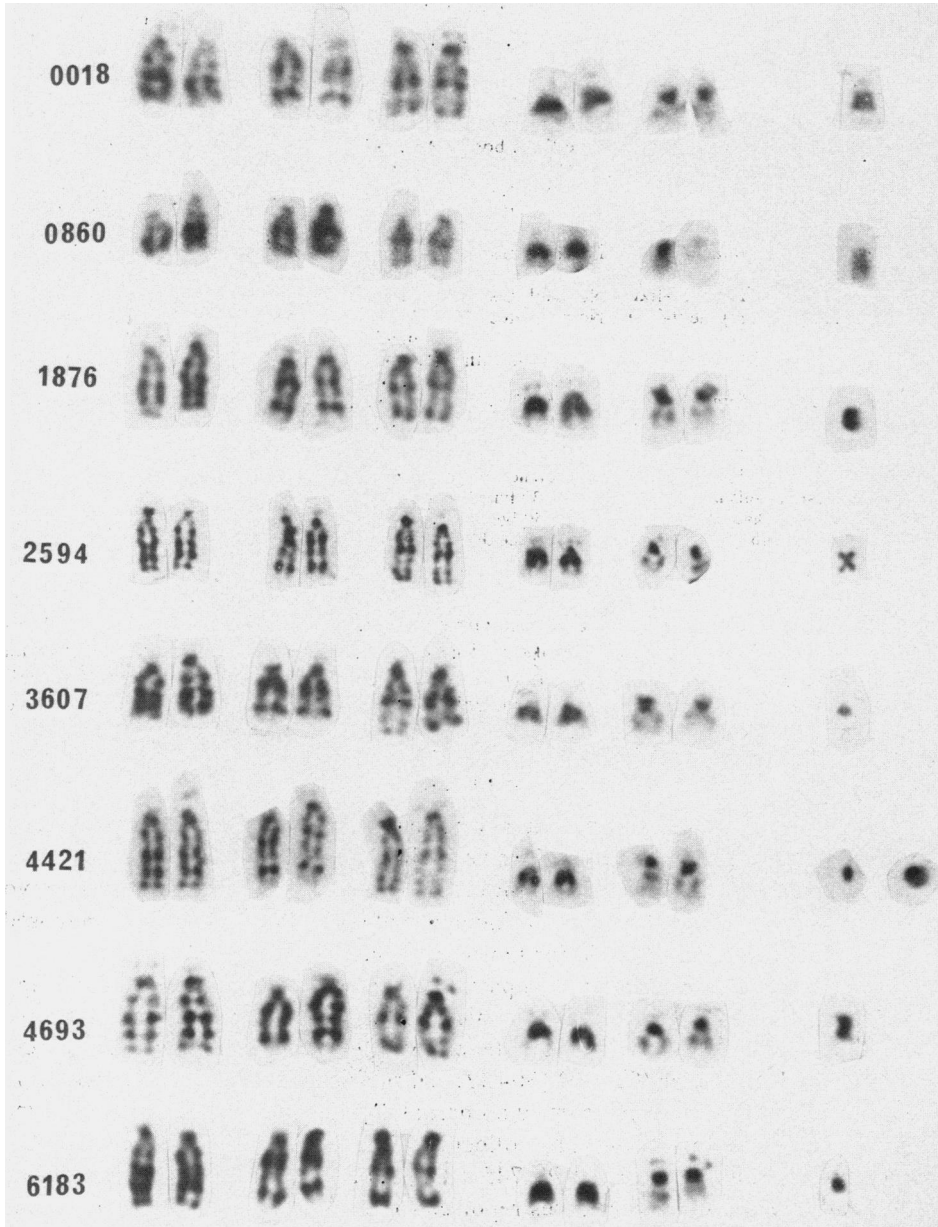

FIG. 1.—Supernumerary marker chromosomes from each case identified by G-banding. D- and G-group chromosomes are shown for comparison. In case no. 4421, the ring chromosome found in cells from one culture is also shown.

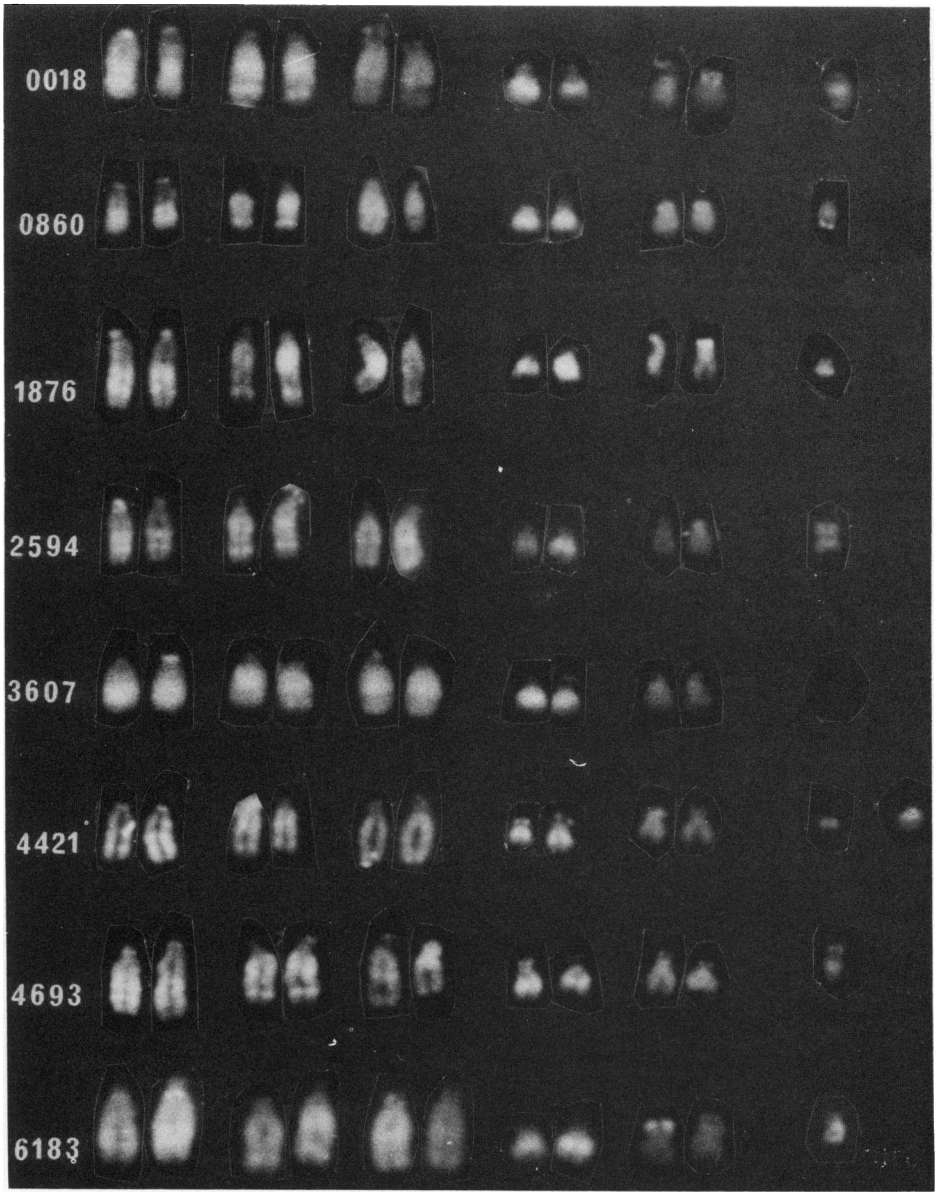

FIG. 2.—Q-banding of supernumerary marker chromosomes

based on the analysis of only a few cells and, consequently, some cases of mosaicism would be missed. Some markers are very small (e.g., case no. 3607) and can be easily missed even with good optics. Cytogenetic analysis based only on photographs of metaphase cells would result in misdiagnosis in many cases where supernumerary chromosomes are present. Thus, all reports on the incidence of such chromosomes should be considered as minimum frequencies.

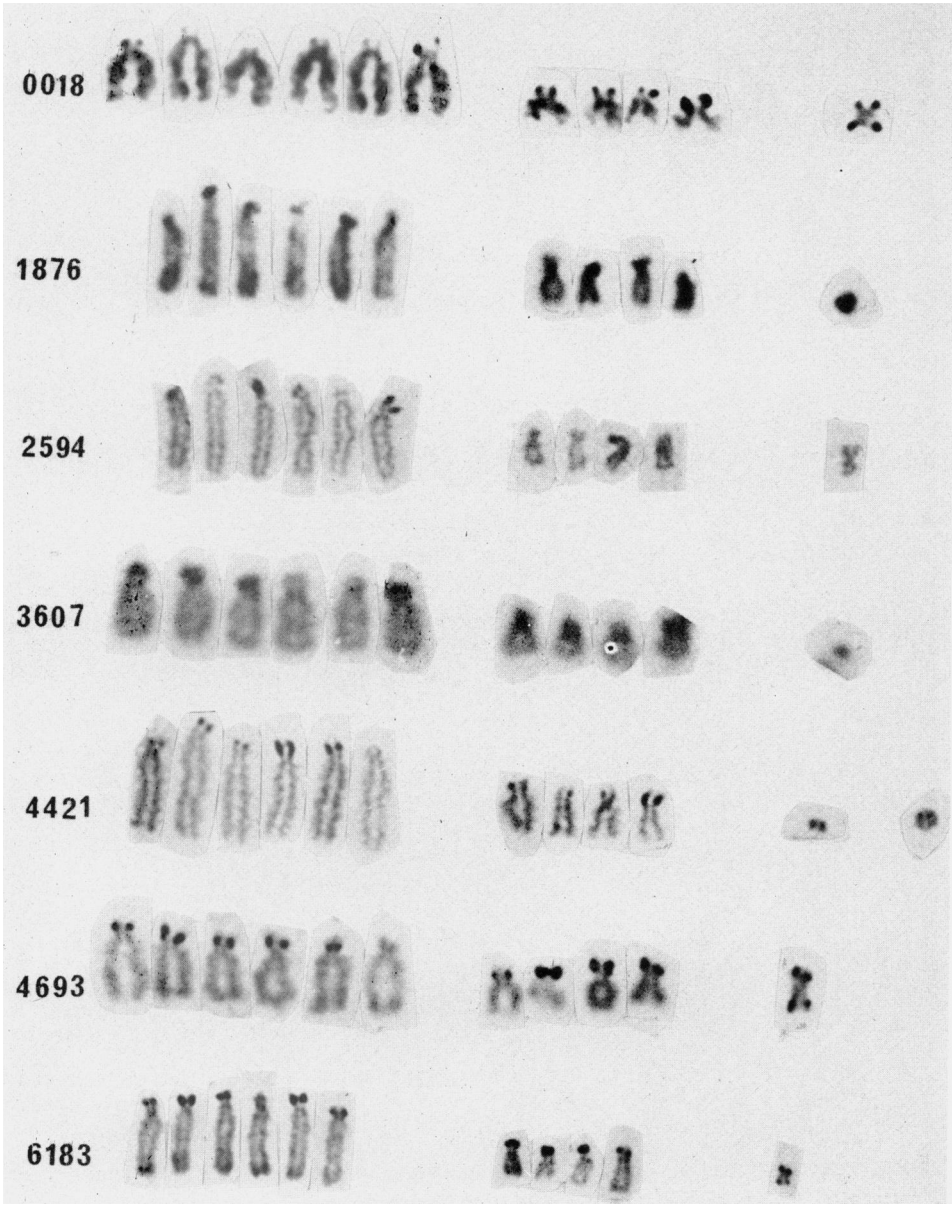

FIG. 3.—Silver staining (NOR-banding) of supernumerary marker chromosomes from seven of the cases.

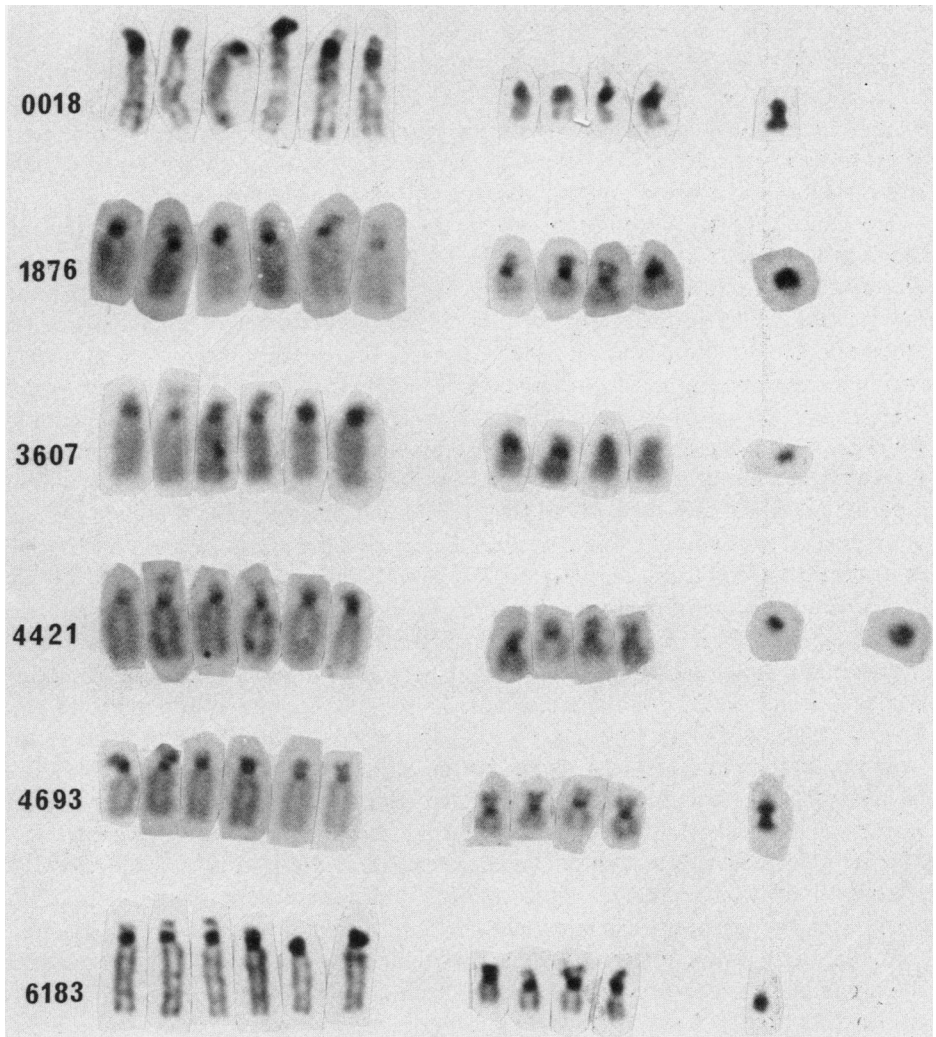

FIG. 4.—C-banding of supernumerary marker chromosomes from six of the cases

The term "supernumerary chromosome" has been used to refer to any unidentifiable marker chromosome and clearly covers a diverse range of cytogenetic abnormalities. At the present time, it is probably premature to attempt to assess the implications of such a prenatally detected marker only on the basis of its cytogenetic appearance. Many of the supernumerary markers appear to be satellited or bisatellited, and many appear to be composed of mostly darkly staining material by C-banding [3]. However, the fact that these markers can stain darkly with silver staining indicates that they are not composed entirely of constitutive heterochromatin. These chromosomes appear to have transcriptionally active ribosomal DNA sequences and may well contain other functionally active euchromatin. For

these reasons, these chromosomes should not be referred to as "genetically inactive" [3, 19].

Of the eight cases reported here, at least five were derived from acrocentrics as judged by Q- or N-banding. Neither of these two banding techniques is sufficient to conclude that a marker is not derived from an acrocentric. However, it is important to try to establish whether this is the case, since variability in acrocentric short arms seems to be a polymorphism that has no adverse phenotypic effect.

The cases reported here illustrate well the problems associated with assessing the significance of a marker chromosome. When a marker is identifiable in all amniotic fluid cells and in all cells from one of the phenotypically normal parents (as in case no. 0018), the interpretation of a normal pregnancy seems reasonable. However, the remote possibility that the effect of a supernumerary chromosome may vary in differing genotypes should not be overlooked [35]. Whether or not the presence of a supernumerary chromosome can increase the risk of nondisjunction for other chromosomes remains to be established [36]. In cases where a familial marker is present as a mosaicism in the amniotic fluid cells or as a mosaicism in a parent (as in cases nos. 1876 and 3607), the possibility exists that dosage may be important. A deficiency or excess in the relative proportions of each cell type in different tissues could lead to developmental abnormality.

A striking feature of the cases reported here is the high incidence of mosaicism. Of the eight cases reported, mosaicism existed in five. Of the three cases where the marker was familial, parental mosaicism existed in two of the cases. In cases nos. 4421 and 4693, nondisjunction of the marker may have given rise to cell lines with no marker and two markers. There may be, therefore, a propensity for supernumerary chromosomes to be lost at a rate much higher than that which occurs for other aneuploidies. There have been few cases of long-term serial studies of cases with supernumerary chromosomes. However, Fitzgerald and Mercer [37] describe a case where there appeared to be a progressive loss of aneuploid cells with the age of the carrier. Familial markers may not be passed on for many generations, and they may arise as new mutations relatively frequently.

We strongly endorse attempts to collect data on specific risks associated with the prenatal detection of supernumerary chromosomes ([12, 33] and E. B. Hook, communication to New York State Chromosome Registry participants, 1983). Thus far, emphasis has been placed on gathering information on de novo cases of supernumerary chromosomes, and even for these, long-term follow-up is scant [12]. While there does appear to be an increased risk of abnormality associated with de novo supernumerary markers [12], it needs to be firmly established that the reports of mental or physical abnormality in individuals with familial markers are simply due to ascertainment bias. We advocate dividing cases into four major groups: type 1—familial and nonmosaic; type 2—familial with mosaicism in either a parent or in the amniotic fluid cells or both; type 3—de novo markers and nonmosaic; and type 4—de novo with mosaicism present in the amniotic fluid cells. As data are accumulated within each group, risks based on the size, staining properties, presence of satellites, etc., can be established. Because of the rarity of these cases, careful documentation by all laboratories will be essential.

ACKNOWLEDGMENTS

We thank the entire technical and counseling staff of the Prenatal Diagnosis Laboratory for the careful involvement in all 6,500 cases. We also thank the following for referrals, counseling, and follow-up information (including laboratory data) on specific cases cited: P. Klass, New York Hospital; K. David and H. Schwartz, The Brooklyn Hospital; M. Wishnick and V. Jansen, Bellevue Hospital; W. Crombleholme and E. Beller, Downstate Medical Center; H. Mootabar, Roosevelt Hospital; A. Shanske and V. Diaz, Queens Hospital Center; K. Pilgrim, H. Dosik, and R. Verma, Interfaith Hospital; and K. Yeboa, Columbia Presbyterian Hospital.

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