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Chromosomal Origin of Small Ring Marker Chromosomes in Man: Characterization by Molecular Genetics

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Summary

Ten cases of small ring chromosomes which did not stain with distamycinA/DAPI and did not possess satellite regions associated with nucleolus-organizing regions are described. In situ hybridization with a battery of biotinylated pericentric repeat probes specific either for individual chromosomes or for groups of chromosomes allowed the identification of the chromosomal origin of these marker chromosomes. There was one example of a marker derived from each of chromosomes 1, 3, 6, 14, 16, 18, 20, 13 or 21, and the X, and there were two examples of markers derived from chromosome 12. One case possessed two markers, one derived from chromosome 6, and one derived from the X. The mechanism of generation of ring marker chromosomes is discussed. Five of seven cases who could be phenotypically assessed were abnormal. Three of these—the first with a ring chromosome derived from chromosome 1; the second with two markers, one derived from chromosome 6 and the other from the X chromosome; and the third with a ring chromosome derived from chromosome 20—each possessed distinctive facies. Additional cases with identified rings may allow the delineation of new chromosomal syndromes.

Introduction

Marker chromosomes occur in humans with a frequency of approximately 1.5/1,000, with some 40% being familial (Sachs et al. 1987). When encountered at prenatal diagnosis or in the young child, the presence of a de novo marker chromosome presents a difficult problem for the genetic counselor. It is usually not possible to give parents precise information regarding the long-term prognosis for the child.

Classical cytogenetic procedures (Rooney and Czepulkowski 1986) can provide information regarding the structure of a marker. The size and shape is often more clearly observed in solid-stained preparations, since G-banding is often uninformative, especially for the smaller-sized markers. Chromosomal satellites (nucleolus-organizing regions [NORs]) may be present at one or both ends of the marker and can be demonstrated by either silver staining or observation of satellite association between the marker and other acrocentric chromosomes. Centromeric heterochromatin can be demonstrated by C-banding. If a marker chromosome has two centromeres, one may be suppressed, either in all or in a proportion of cells. DistamycinA/DAPI staining identifies the heterochromatin of chromosomes 1, 9, 15, and 16 and of the Y chromosome. If a marker has chromosomal satellites and, in addition, a distamycinA/DAPI-staining region, then an origin from chromosome 15 is likely, and such cases have been published (Wisniewski et al. 1979). This is the only example where classical cytogenetic procedures allow the determination of the chromosomal origin of a marker.

In situ hybridization provides a means of identifying the origin of marker chromosomes. Mattei et al. (1985) demonstrated that a small marker chromosome was an i(18p), by using a unique single-copy DNA probe to a locus located on 18p. Callen et al. (1990*a*) have confirmed the presence of an i(18p) in nine patients by a combination of in situ hybridization with both a pericentric alphoid repeat probe specific

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for chromosome 18 and a single-copy unique DNA probe. The development of probes which detect pericentric repeats specific for either a particular chromosome or subset of chromosomes provides the opportunity to determine the chromosomal origin of any marker chromosome. Using such biotinylated probes, Callen et al. (1990*b*) have determined the chromosomal origin of three distamycinA/DAPI-positive markers. These small ring chromosomes were found to be derived from chromosomes 1, 9, and 16, respectively.

In the present study we report the chromosomal origin and clinical details of 10 cases of small ring marker chromosomes which were distamycinA/DAPI negative and did not contain NOR-staining material.

Material, Methods, and Cases

Cytogenetics

Metaphase spreads were prepared either from cell suspensions of short-term phytohemagglutinin-stimulated lymphocyte cultures or from fibroblast cultures harvested by standard methods. These cell suspensions were stored in methanol:glacial acetic acid fixative (3:1) at −20°C until required. G-banding, NOR-banding, and distamycinA/DAPI staining were by standard procedures (Rooney and Czepulkowski 1986).

Probes

All the probes used, except pHOM48, were derived from pericentric repeats which were classified as either alphoid, satellite II, or satellite III. Details of probes and their origins are given in table 1. The probe pHOM48 identifies a novel pericentromeric repeat which is specific to chromosome 22 (Metzdorf et al. 1988).

In Situ Hybridization

The probes for *D1Z5, D3Z1, D11Z1, D12Z3, D16Z2,* and *D20Z1* were purchased as biotin-labeled DNA from ONCOR (Gaithersburg, MD). All other probes were labeled, by nick-translation, with either

Table 1

Summary of Results of In Situ Hybridization with Biotinylated-Specific Repeat Probes to Patients with Marker Chromosomes

Chromosome Detected (locus), Probe	Repeat Type	Source or Reference	Hybridization Status[a] of Case									
			1	2	3	4	5	6	7	8	9	10
1 (. . .), RR6	Satellite II	Fowler et al., submitted	−		−	−			−		−	−
1 (*D1Z5*), p1.79	Alphoid	Oncor	+							−		
3 (*D3Z1*), palpha3-5	Alphoid	Oncor		+		−	−					−
6 (*D6Z1*), p308	Alphoid	Jabs and Persico 1987	−		+[b]	−	−	−	−		−	−
7 (. . .), RR38	Alphoid	Fowler et al., submitted	−	−	−	−	−	−	−		−	−
9 (. . .), 228S	Satellite II	Fowler et al. 1989	−	−	−	−	−	−	−		−	−
9 (. . .), RR17	Alphoid	Fowler et al., submitted						−			−	
10 (*D10Z1*), α10RP8	Alphoid	Devilee et al. 1988	−	−	−	−	−	−	−	−	−	−
11 (*D11Z1*), pLC11A	Alphoid	Oncor		−		−	−					−
12 (*D12Z3*), . . .	Alphoid	Oncor				+	+					
16 (*D16Z3*), pHUR195	Satellite III	Moyzis et al. 1987	−	−	−	−	−	−	−		−	−
16 (*D16Z2*), pSE16	Alphoid	Oncor								+		
17 (*D17Z1*), TR17	Alphoid	Choo et al. 1987	−	−	−	−	−	−	−	−	−	−
18 (. . .), L1.84	Alphoid	Devilee et al. 1986	−	−	−	−	−	−	−/+[c]	−	+	−
20 (*D20Z1*), 3-4	Alphoid	Oncor		−		−	−		−			+
22 (. . .), pHOM48	Other	Metzdorf et al. 1988							−			
X (. . .), TRX	Alphoid	Yang et al. 1982		−	+[b]	−	−	−			−	−
Y (*DYZ3*), pDP97	Alphoid	ATCC		−	−	−	−	−			−	−
1,5,16,19 (. . .), pGF2	Alphoid	Hulsebos et al. 1988	+		−	−	−	−		+	−	
5,19 (. . .), pGA16	Alphoid	Hulsebos et al. 1988	−	−					−	−		−
13,21 (. . .), L1.26	Alphoid	Devilee et al. 1988	−	−	−	−	−	+	−	−	−	−
14,22 (. . .), alphaXT	Alphoid	Jorgensen et al. 1987	−	−		−	−	−	+	−	−	−

[a] A plus sign (+) indicates that probe hybridized to marker chromosome; a minus sign (−) indicates that probe did not hybridize to marker chromosome; a blank indicates that probe was not tested.

[b] mar1 hybridized to p308; mar2 hybridized to TRX.

[c] Hybridized to L1.26 at low stringency. Under these conditions signal is found on chromosomes 13, 14, 18, and 21.

biotin-11-dUTP or biotin-14-dATP. The conditions for in situ hybridization and detection of the resultant signal by an antibody/peroxidase system with gold/silver amplification were as described elsewhere (Callen et al. 1990*b*). For each probe, initial experiments were necessary to determine the optimal probe concentration in the hybridization mix, which would restrict the signal either to a single chromosome or to a small subset of the chromosomes. High-stringency posthybridization wash conditions used were 0.1 × SSC (300 mmol/liter NaCl, 30 mmol/liter sodium citrate) at 65°C. Specific hybridization for each probe had occurred if there were a clear signal on the expected normal pair(s) of homologues and absence of signal on other chromosomes. The marker was considered negative if there was no signal in 10 such metaphases. For the probe L1.26 low-stringency posthybridization wash conditions were 0.1 × SSC at 42°C. When wash conditions are at high stringency, a specific signal on chromosomes 13 and 21 is present, and when wash conditions are at low stringency, the signal is present on chromosomes 13, 14, 18, and 21 (Devilee et al. 1986).

Case Descriptions

Case 1.—This case was a male born at 42 wk gestation after normal pregnancy, labor and delivery. Birth weight was 3,717 g (50th percentile), and length was 51 cm (50th–90th percentile). Chromosome studies were requested at 2½ years of age because of delayed speech development and dysmorphic features. At 4 years of age the Merrill Palmer scale of mental tests showed a mental age of 5 years 2 mo, and the Reynell developmental language scale showed receptive language abilities to be at a level of 2 years 11 mo. Bat ears were corrected at 5 years of age. When seen at 8 years 3 mo of age, he was functioning satisfactorily in a language-disorder unit of a normal school. Height was 1.35 m (90th percentile), weight was 25 kg (50th percentile), and head circumference was 50.3 cm (10th percentile). He had a lean build with narrow shoulders, bifrontal narrowing, a long face, ear-lobule creases, slightly up-slanting palpebral fissures, a long nose with broad nasal bridge, featureless philtrum, upturned corners to the mouth, mild micrognathia, bilateral clinodactyly of the little fingers, and inverted nipples. The facies are shown in figure 1.

Case 2.—This case was a female born at 40 wk gestation after normal pregnancy, labor and delivery. Birth weight was 2,980 grams (10th percentile), length was 47 cm (10th percentile), and head circumference was 31.5 cm (<10th percentile). The case was ascertained during a neonatal chromosome-screening program (Sutherland 1985). Gastroesophageal reflux was present to 16 mo of age, and the child suffered from mild asthma. Development was delayed and at a chronological age of 37 mo, the Merrill Palmer test showed a mental age of 32 mo, and the Reynell developmental language scale showed comprehension to be at a 30-mo level and expressive language to be at a 16-mo level. When seen at 6 years 11 mo, she was in a special education class at a normal school. Height was 1.1 m (3d percentile), weight was 22.5 kg (50th–75th percentile), and head circumference was 46 cm (<2d percentile). Microcephaly, a short neck, and a low anterior hairline were present, but the facies was not dysmorphic.

Case 3.—This case was a male who was noted to be small on ultrasound from 18 wk of pregnancy. Labor and delivery were normal at 39 wk gestation. Birth weight was 2,440 g (< 10th percentile), length was 44 cm (<10th percentile), and head circumference was 31.5 cm (<10th percentile). At 5 wk of age he had gastroesophageal reflux associated with poor weight gain; this resolved rapidly with treatment. At 4½ mo of age, in association with a viral respiratory-tract infection, seizures occurred, and these continued following recovery from the infection. Epilepsy was diagnosed and required continuing treatment with antiepileptic medication. Chromosome studies were undertaken at this time because of developmental delay. When seen at 6 mo of age, the child was functioning at a 3-mo level. Head circumference 40.8 cm (<2d percentile). The child was dysmorphic with telecanthus (90th percentile), widely set eyes (75th percentile), a right epicanthic fold, short nose, broad nasal bridge, broad nasal tip, long philtrum, thin and down-turned upper lip, widely spaced nipples, limited abduction of the left thumb, soft tissue syndactyly of toes two through four bilaterally, and a deep furrow extending from the base of the first interdigital cleft of the toes onto the sole. The facies are shown in figure 1.

Case 4.—The pregnancy of this female fetus was complicated by spontaneous rupture of the membranes at 16 wk gestation. An amniocentesis was performed at 21 wk because the pregnancy seemed to be ongoing and because it was felt prudent to determine the fetal karyotype. The pregnancy was terminated following discovery of a marker chromosome. Autopsy was not performed.

Case 5.—Birth of this male baby was at 41 wk gestation after a normal labor and forceps delivery,

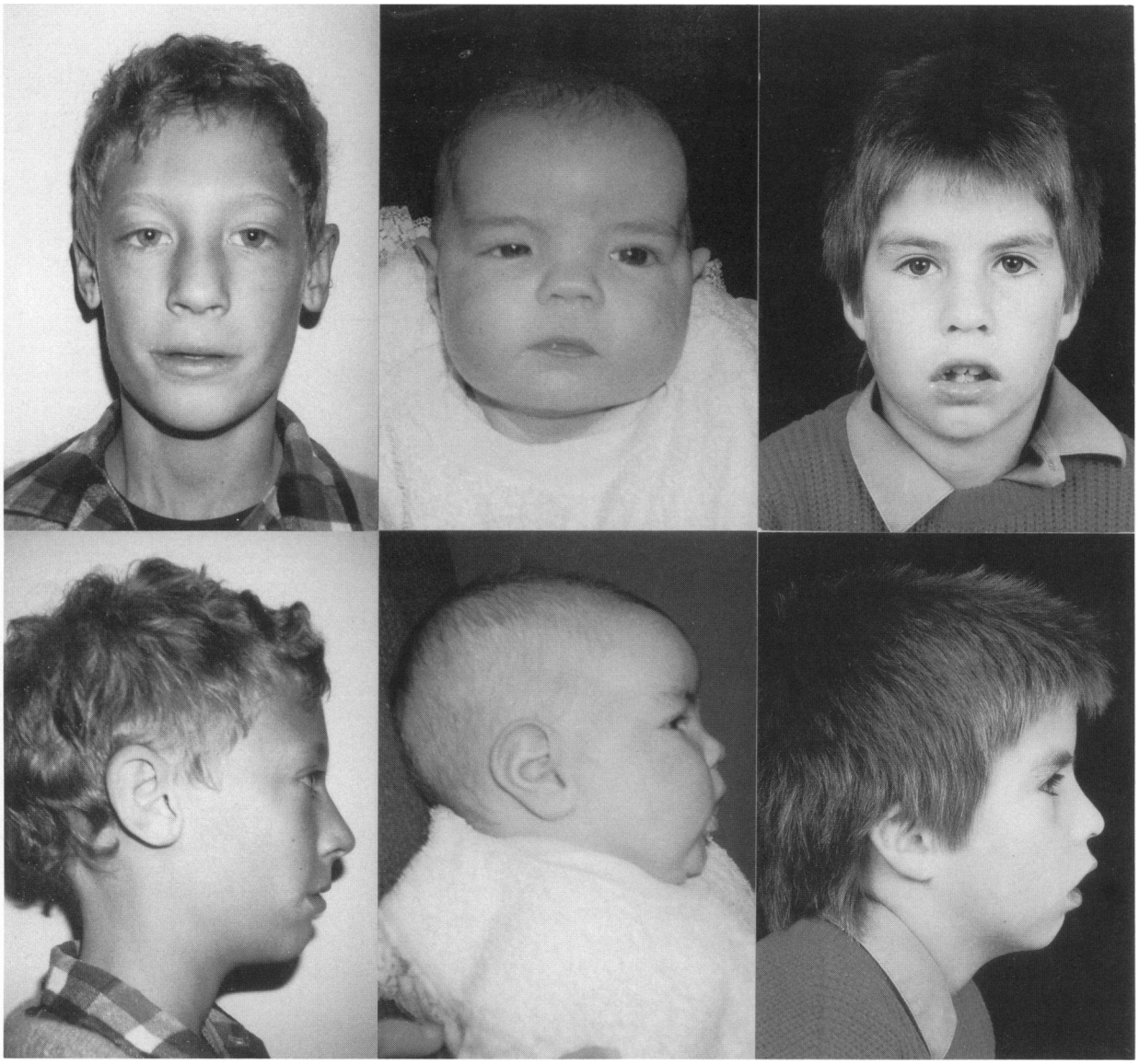


Figure 1 Photographs of dysmorphic patients with marker chromosomes. From left to right they are as follows: case 1, possessing a marker derived from chromosome 1; case 3, possessing two markers, one derived from chromosome 6 and the other from the X chromosome; and case 10, possessing a marker derived from chromosome 20.

although the pregnancy was complicated by hypertension. Birth weight was 4,320 g (>90th percentile), length was 54 cm (>90th percentile), and head circumference was 36.8 cm (90th percentile). An umbilical hernia was noted at birth and resolved over the first year of life. A chromosome study was performed at 21 mo of age because of delayed development and dysmorphic features. At that time the child's mental age was determined to be 12 mo. Bilateral vesico-ureteric reflux was detected at this time and was treated by ureteric reimplantation. Congenital strabismus was corrected at 4 years of age. When seen at 14 years of age, the boy was functioning at a 7-year-old level and attended a special school. Height was 1.7 m (75th percentile), weight was 85 kg (>97th percentile), and head circumference was 58.5 cm (>98th percentile). There were a prominent forehead, up-slanting palpebral fissures, and almond-shaped eyes. The latter two characteristics were thought not to represent dysmorphic features, because the boy's mother was of Filipino

extraction. No other abnormalities were noted on clinical examination.

Case 6.—Amniocentesis because of advanced maternal age showed a male fetus with a marker chromosome. The pregnancy was continued and was complicated by gestational diabetes and hypertension. Birth was by vaginal delivery at 38 wk gestation after an induced labor. Birth weight was 4,335 g (>90th percentile), length was 53 cm (>90th percentile), and head circumference was 36 cm (90th percentile). A left torticollis was present at birth and was treated successfully by physiotherapy. When the infant was seen at 4 mo of age, growth and development were normal, and there were no significant dysmorphic features.

Case 7.—This case was a female born at 40 wk gestation after normal pregnancy, labor, and delivery. Birth weight was 4,350 g (>90th percentile). The clinical features of Down syndrome were noted at birth, and chromosome studies were initiated. When seen at 18 mo of age, the child was functioning at an 11–12-mo developmental level and had a weight of 1.09 kg (50th percentile), a length of 79.3 cm (25th percentile), and a head circumference of 46.5 cm (25th percentile). The clinical features were those of Down syndrome, and there were no extraclinical findings which could be attributed to the marker chromosome.

Case 8.—Amniocentesis because of advanced maternal age showed a male fetus with a marker chromosome. The pregnancy was terminated. Autopsy was not performed.

Case 9.—Delivery of this female was at 40 wk gestation, by Caesarean section performed because of cephalopelvic disproportion. Birth weight was 2,500 gm (<10th percentile). At 11 years of age she was investigated for short stature, with a view to treatment with growth hormone, and chromosome studies were performed. When seen at 11 years 8 mo of age, she had a height of 1.33 m (<3d percentile), a weight of 38.5 kg (50th percentile), and a head circumference of 54 cm (50th–98th percentile). Intelligence was normal, and there were no dysmorphic features. The short stature was ultimately thought to represent idiopathic familial short stature.

Case 10.—This case was a male born at 42 wk gestation after normal pregnancy, labor, and delivery. Birth weight was 2,530 g (<10th percentile). At 14 mo of age he was considered to be dysmorphic, and there was concern about his development. A chromosome study was performed at 2 years of age. When seen at 7 years 10 mo of age, he was attending a normal school and was considered to have normal intelligence. Psychological assessment by the Wechsler scale showed that performance IQ was in the average range, that verbal IQ was in the high-average range, and that full-scale IQ was in the high-average range. Height was 1.12 m (<3d percentile), weight was 18.7 kg (<3d percentile), and head circumference was 52.5 cm (50th percentile). He had scaphocephaly, a high-pitched voice, low anterior hairline, abnormally folded low-set ears, synophrys with bushy eyebrows, a featureless philtrum, high palate, open bite and dental crowding, micrognathia, narrow shoulders, hyperextensible elbows and fingers, clinodactyly of fingers 2, 4, and 5 toward the third finger, transverse palmar creases, and partial soft-tissue syndactyly of the fingers 2–5. The right lower limb was 1 cm shorter than the left, resulting in a mild compensatory scoliosis. The facies are shown in figure 1.

Results

Cytogenetic Studies

Results of cytogenetic studies on the 10 cases and their parents, as well as a summary of the clinical phenotype, are shown in table 2. The appearance of each marker is illustrated in figure 2. All markers were negative for distamycinA/DAPI banding, did not contain satellites associated with NORs, and had an appearance consistent with a small ring chromosome. One case was not mosaic, and five cases had a normal diploid cell line in addition to the line with 47 chromosomes. The other four cases had more complex karyotypes. In case 2, 22% of cells had the ring chromosome (mar1), while all cells possessed from one to five copies of a small fragment (mar2). Case 3 also had two markers with the larger ring chromosome (mar1) present in all cells, while mar2 was seen in 56% of cells. All cells of case 10 contained the marker, which was present in either one or two copies. Case 7 had Down syndrome with trisomy 21 present in all cells. In addition, 45% of the cells possessed a small marker chromosome.

The parental karyotypes of eight cases were normal and therefore established the markers to be de novo. In one instance, case 1, it was possible to study only one parent. The marker in case 8 was maternally derived. The mother was mosaic, with 15% of cultured lymphocytes showing the marker.

Molecular Studies

A variety of pericentric repeat probes which were

Table 2

Summary of Cytogenetic Results and Clinical Data

Case (sex) and Karyotype (% frequency[a])	Chromosomal Origin[b]	Parental Karyotype		Phenotype
		Mother	Father	
1 (M: 46 (30) 47, + mar (70)	1	Not determined	Normal	Specific language deficit, dysmorphic features
2 (F): 47, + mar2[c] (78) 48, + mar1, + mar2 (22)	3, unknown	Normal	Normal	Delayed development, short stature, microcephaly
3 (M): 47, + mar1 (44) 48, + mar1, + mar2 (56)	6,X	Normal	Normal	Delayed development, seizure disorder, microcephaly, dysmorphic features
4 (F): 46 (70) 47, + mar (30)	12	Normal	Normal	Not defined[d]
5 (M): 46 (80) 47, + mar (20)	12	Normal	Normal	Delayed development, vesico-ureteric reflux
6 (M): 47, + mar (100)	13 or 21	Normal	Normal	Normal
7 (F): 47, + 21 (55) 48, + 21, + mar (45)	14	Normal	Normal	Down syndrome
8 (M): 46 (50) 47, + mar (50)	16	Normal	46 47, + mar	Not defined[d]
9 (F): 46 (15) 47, + mar (85)	18	Normal	Normal	Short stature, probably familial
10 (F): 47, + mar1 (72) 48, + mar1, + mar1 (28)	20	Normal	Normal	Dysmorphic features, short stature

[a] For cases 4, 6, and 8 marker frequencies were from amniocyte cultures. All other cytogenetic results are from lymphocyte cultures.

[b] Details of determination are given in table 1.

[c] mar1 was a small ring chromosome which in 24% of cells was present as two copies; mar2 was very small and was present as 1 copy/cell (27%), 2 copies/cell (48%), 3 copies/cell (23%), and four or five copies (2%).

[d] Pregnancy was terminated, and no autopsy was performed.

specific for either particular chromosomes or subsets of chromosomes were hybridized to metaphase spreads from each of the 10 cases. The results obtained with this battery of probes are given in table 1 and are summarized in table 2. Photographs of representative positive results for each case are presented in figure 2.

For case 1 the marker chromosome was positive for two probes which detected pericentric alphoid sequences; these probes were pGF2, which hybridizes to sequences present on chromosomes 1, 5, 16, and 19, and p1.79, which is specific for chromosome 1. It should be noted that probe RR6, which hybridizes only to the satellite II sequences of chromosome 1, did not hybridize to this marker. Similarly, the marker of case 8 hybridized to the alphoid probes pSE16 and pGF2 and was therefore derived from chromosome 16. The probe pHUR195, which detects satellite III pericentric sequences on chromosome 16, was negative.

Cases 2, 4, 5, 9, and 10 possessed markers which were identified as originating from chromosomes 3, 12, 12, 18, and 20, respectively. The additional small marker (mar2) in case 2 could not be identified with any of the probes used. Case 3 had two markers; mar1 was derived from chromosome 6, and mar 2 was derived from the X chromosome. In case 6 the probe L1.26 hybridized to the marker. This indicates an origin from either chromosome 13 or chromosome 21. Probes which could differentiate between these two chromosomes were not available.

The marker in case 7 originated from chromosome 14. Initially, it was determined that the probe alphaXT, which detects both chromosome 14 and chromosome 22, hybridized to this marker. An origin from chromosome 14 was then indicated by both the lack of hybridization of the chromosome 22–specific probe pHOM48 and the positive signal found when probe L1.26 was used at low stringency. Under these conditions L1.26 detects chromosomes 13, 14, 18, and 21 but not chromosome 22.

The clinical data on the 10 cases are summarized in table 2. In three cases it was not possible to assess whether the marker chromosome had a phenotypic effect; the pregnancies of cases 4 and 8 were terminated, and case 7 had Down syndrome. A normal phenotype was present in cases 6 and 9. The phenotypes of the other five cases (1–3, 5, and 10) were abnormal. Three of these (cases 2, 3, and 5) were developmentally delayed, case 1 had a specific language deficit, and case 10 was of normal intelligence. Cases 1, 3, and 10 possess dysmorphic features, as shown in figure 1.

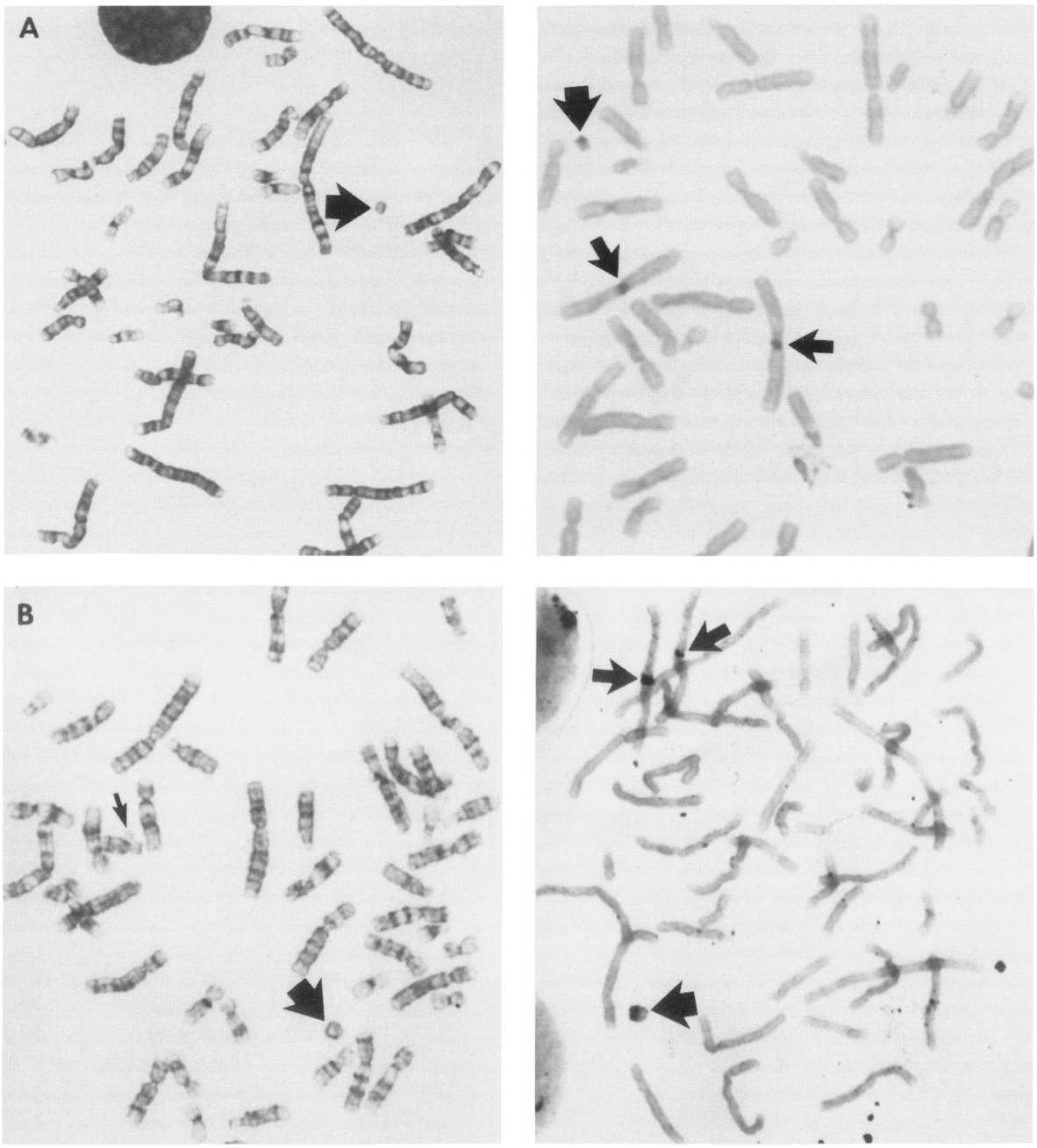
Discussion

The 10 cases of the present report all possessed small ring chromosomes which did not stain with distamycinA/DAPI and did not possess satellite regions associated with NORs. The results of in situ hybridization with a battery of biotinylated pericentric repeat probes specific either for individual chromosomes or groups of chromosomes allowed the identification of the chromosomal origin of the marker chromosomes in these 10 cases. There was one example of a marker derived from each of chromosomes 1, 3, 6, 14, 16, 18, 20, 13 or 21, and the X chromosome, and there were two examples of markers derived from chromosome 12 (tables 1 and 2). Case 3 possessed two markers, one derived from chromosome 6 and one derived from the X chromosome. Additional small markers seen in case 2 could not be identified with the probes used.

In cases 1 and 8 the markers originated from chromosomes 1 and 16, respectively. These markers were positive for the alphoid probes which specifically detect these chromosomes but were negative for probes which detect the adjacent satellite II and satellite III repeat regions. Callen et al. (1980*b*) have described three patients with small ring chromosomes—derived from chromosomes 1, 9, and 16, respectively—that were distamycinA/DAPI positive and also positive for the satellite II and satellite III pericentric probes specific for these chromosomes. It is also noteworthy that mosaicism of these marker chromosomes is a common feature both in the present report and in the study by Callen et al. (1990*b*).

Ring chromosomes are considered to arise from chromosome breaks occurring on either side of the centromere and subsequent rejoining of the broken ends of the segment containing the centromere (Hamerton 1971). To generate the small ring chromosomes reported here, one of these two breaks is either at the centromere or adjacent to the centromere in the region containing alphoid repeats. This would be consistent with the observation of ring chromosomes derived from chromosomes 1 and 16 which do not contain satellite II or satellite III sequences.

The presence of mosaicism in the majority of patients is consistent with either the postzygotic formation of marker chromosomes or a postzygotic instability resulting in loss during cell division. This contrasts



with markers which have been determined to be i(18p), which are not mosaic (Callen et al. 1990*a*) and probably arise by a different mechanism. Certain isochromosomes have been shown to be complex structures and to contain information from both sides of the centromere. For example, an i(Xq) has been shown to contain sequences present on the short arm of the X chromosome (Callen et al. 1987), and a chromosome in a mentally retarded boy was described as an iso(9) (pter→q12, q12→pter) (Wik Sjostedt et al. 1989). Such chromosomes can also be generated by a single "U-type" exchange event involving chromosome breakage and subsequent reunion between the sister chromatids. A U-type exchange can be generated by

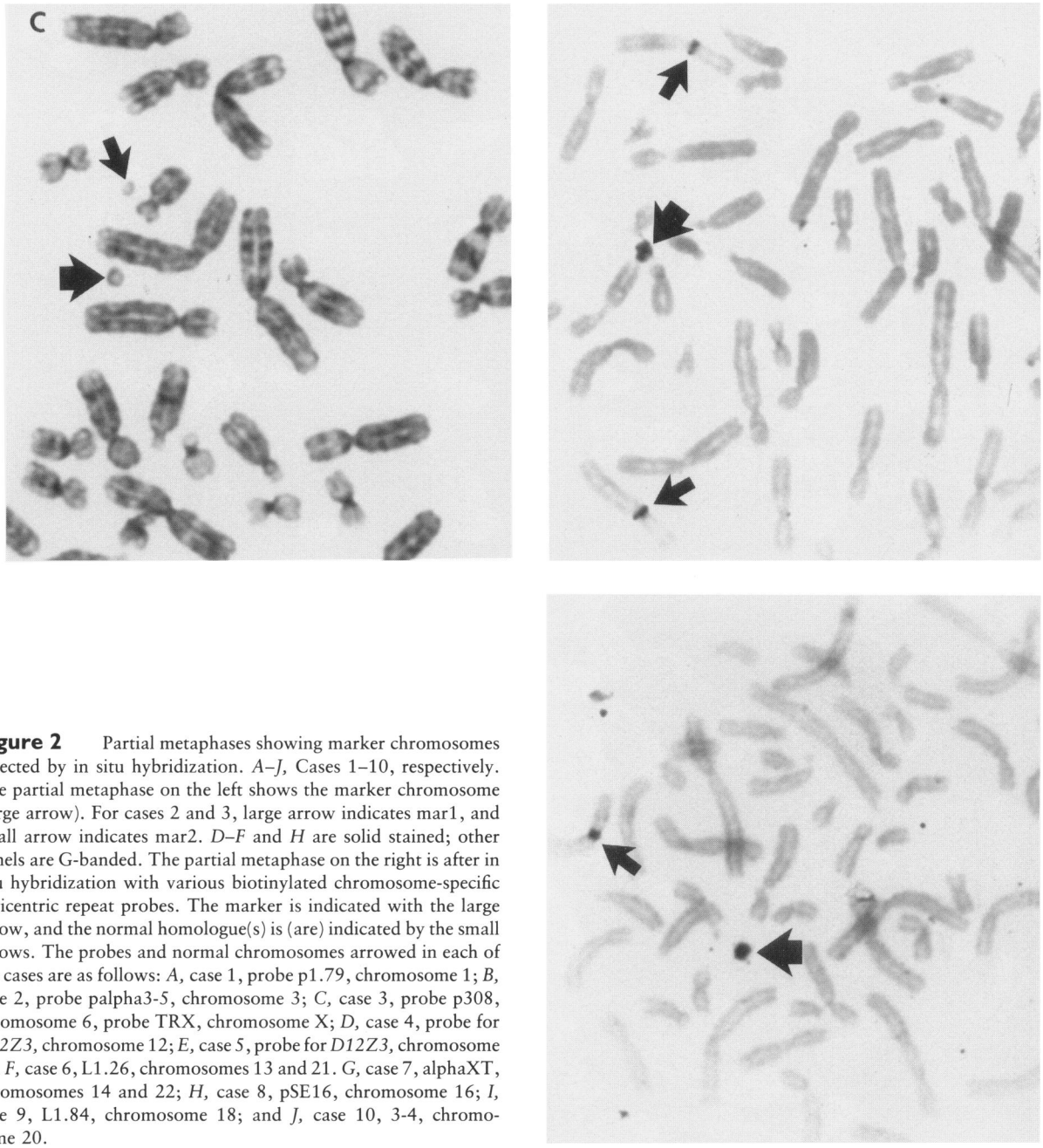
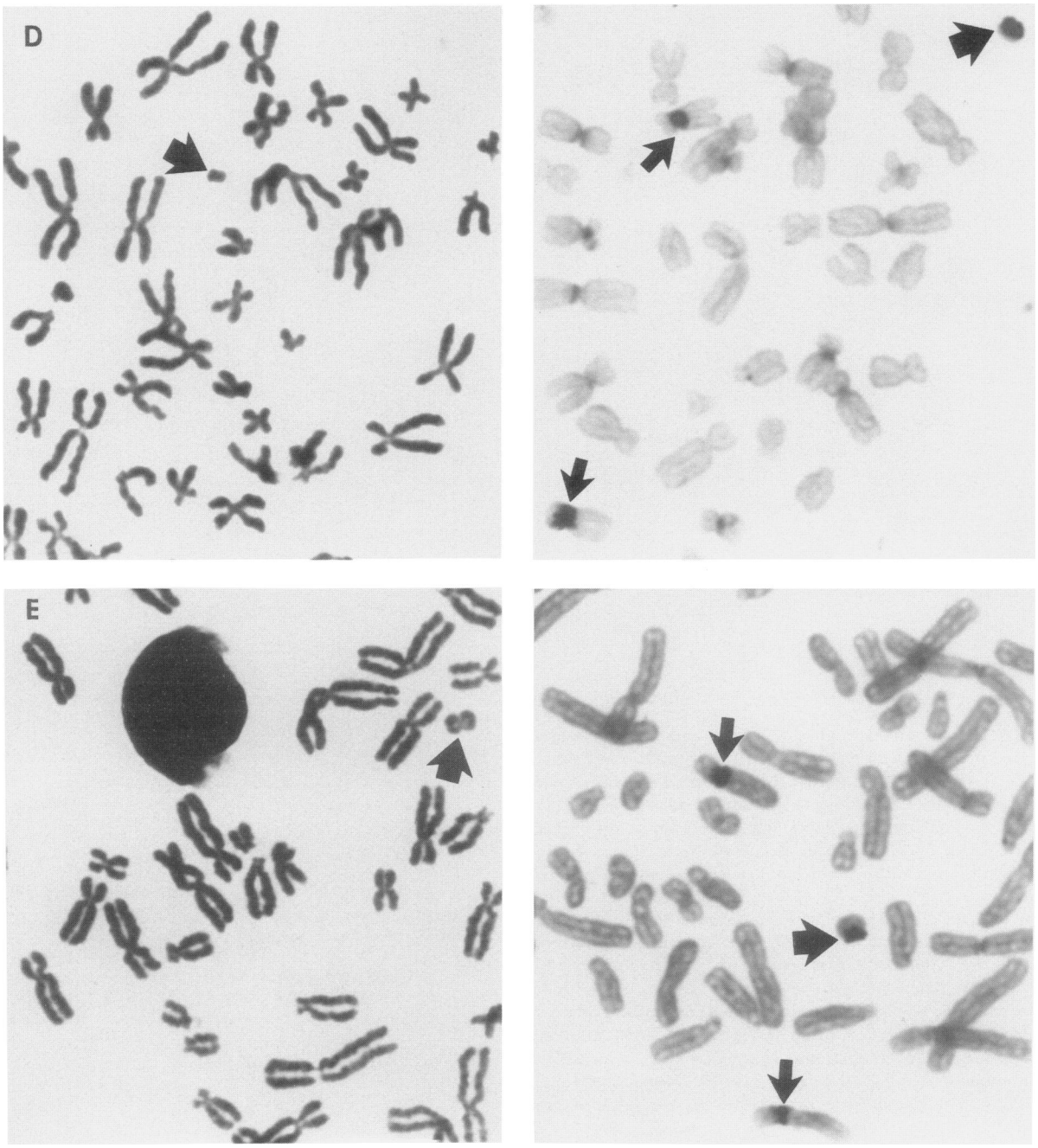


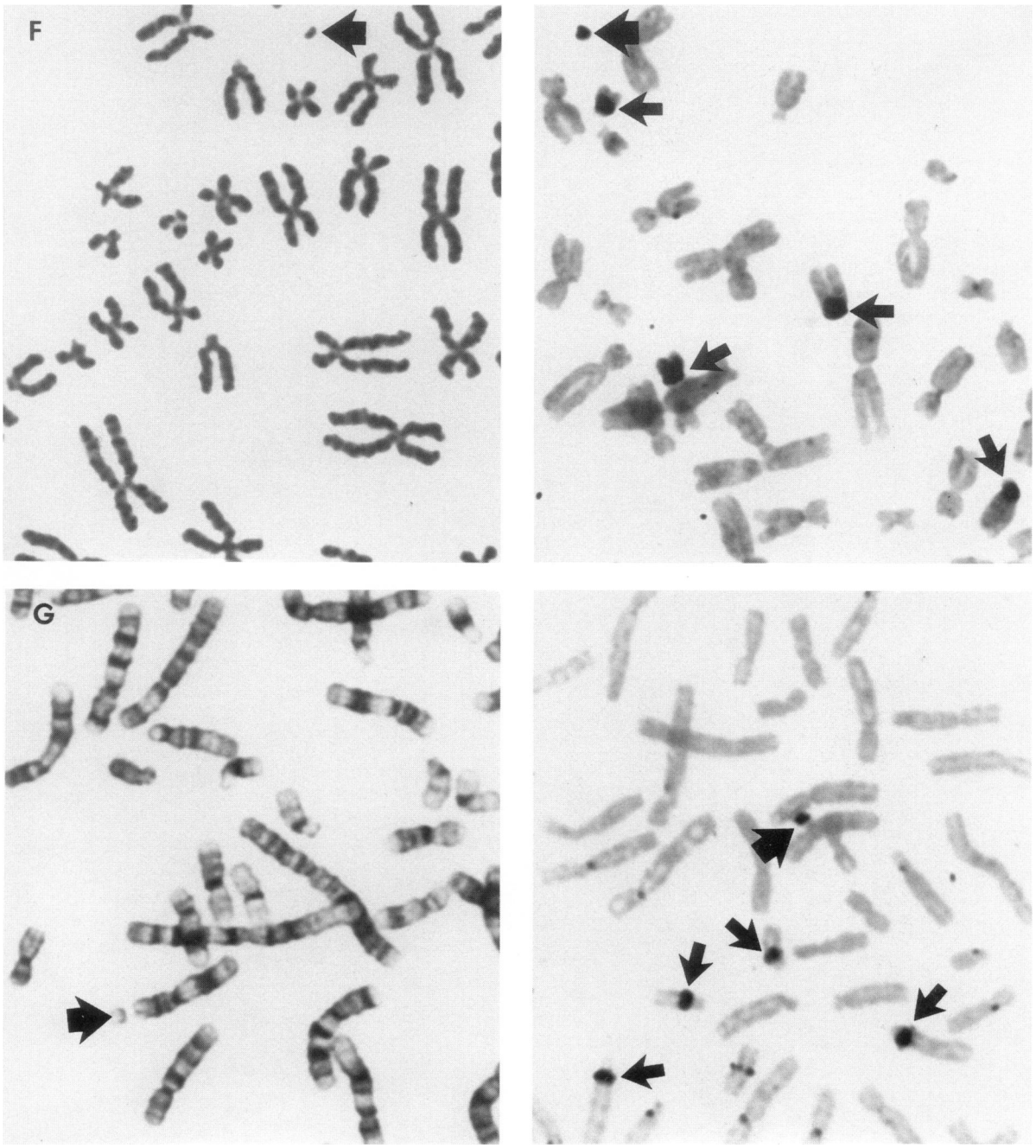
Figure 2 Partial metaphases showing marker chromosomes detected by in situ hybridization. *A–J,* Cases 1–10, respectively. The partial metaphase on the left shows the marker chromosome (large arrow). For cases 2 and 3, large arrow indicates mar1, and small arrow indicates mar2. *D–F* and *H* are solid stained; other panels are G-banded. The partial metaphase on the right is after in situ hybridization with various biotinylated chromosome-specific pericentric repeat probes. The marker is indicated with the large arrow, and the normal homologue(s) is (are) indicated by the small arrows. The probes and normal chromosomes arrowed in each of the cases are as follows: *A,* case 1, probe p1.79, chromosome 1; *B,* case 2, probe palpha3-5, chromosome 3; *C,* case 3, probe p308, chromosome 6, probe TRX, chromosome X; *D,* case 4, probe for *D12Z3,* chromosome 12; *E,* case 5, probe for *D12Z3,* chromosome 12. *F,* case 6, L1.26, chromosomes 13 and 21. *G,* case 7, alphaXT, chromosomes 14 and 22; *H,* case 8, pSE16, chromosome 16; *I,* case 9, L1.84, chromosome 18; and *J,* case 10, 3-4, chromosome 20.

a single abnormal event in DNA replication, as suggested by Wik Sjostedt et al. (1989). Possible mechanisms for the generation of small ring chromosomes are (*a*) two U-type exchanges on either side of the centromere or (*b*) one U-type exchange in combination with a transverse misdivision of the centromere. This latter possibility is consistent with the observation of two types of ring chromosomes from chromosomes 1 and 16: (1) those that contain satellite II or satellite III sequences and (2) those in which they are absent.

An aim of the present study was to document the clinical features present in individuals with marker chromosomes of defined chromosomal origin. In the

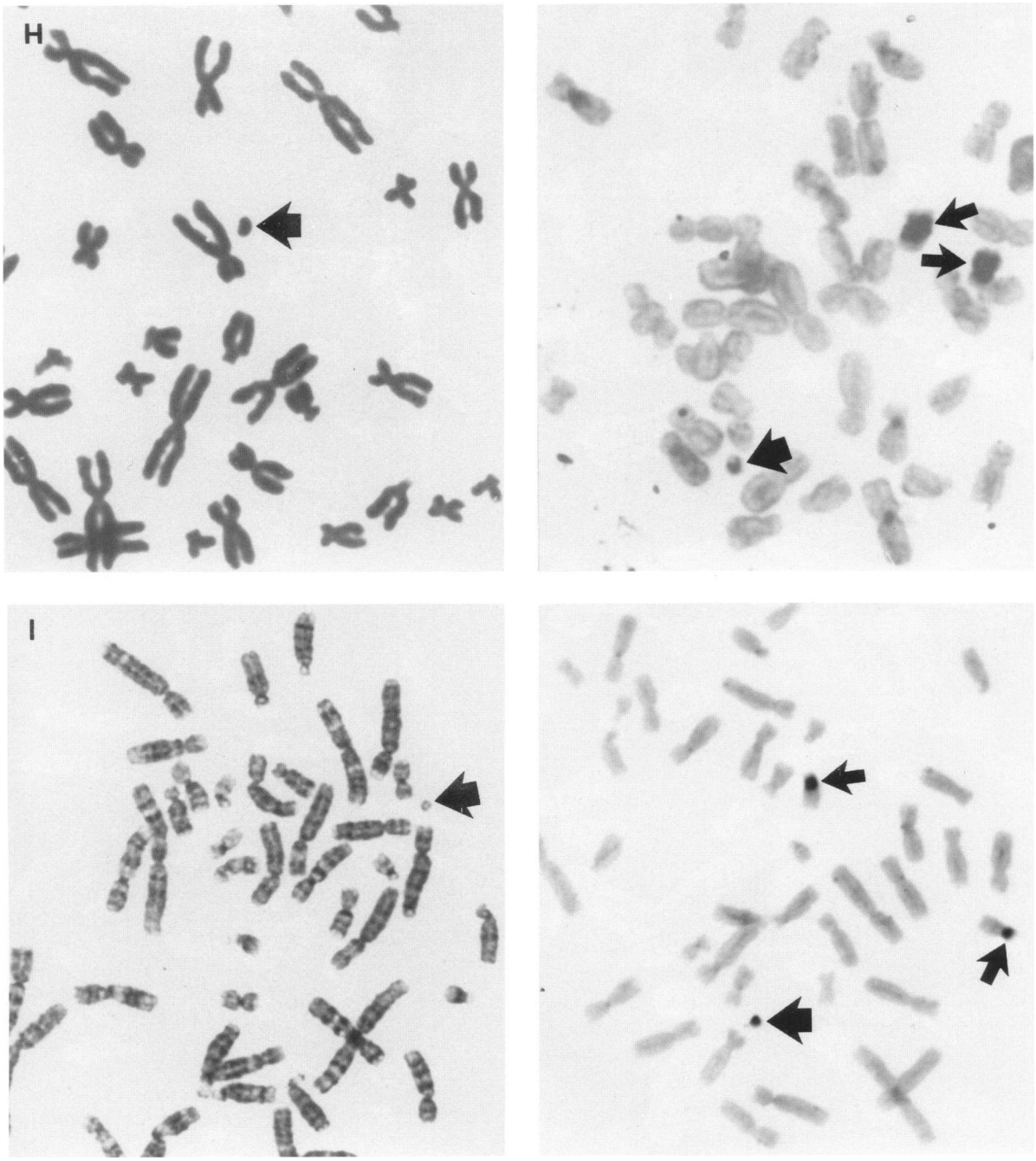


long-term future, as additional cases are ascertained, improved prognostic information will become available to the parents of children with marker chromosomes. Those markers without satellites associated with NORs usually consist of small ring chromosomes. These can be further subdivided by distamycinA/DAPI banding. A normal phenotype was associated with the only three patients who could be clinically assessed and who possessed a distamycinA/DAPI-positive ring marker chromosome (two cases in Callen et al. 1990*b* and one further, unpublished case). In the present report the phenotype of five of the seven cases who could be clinically assessed were abnormal. Our approach to classification of markers

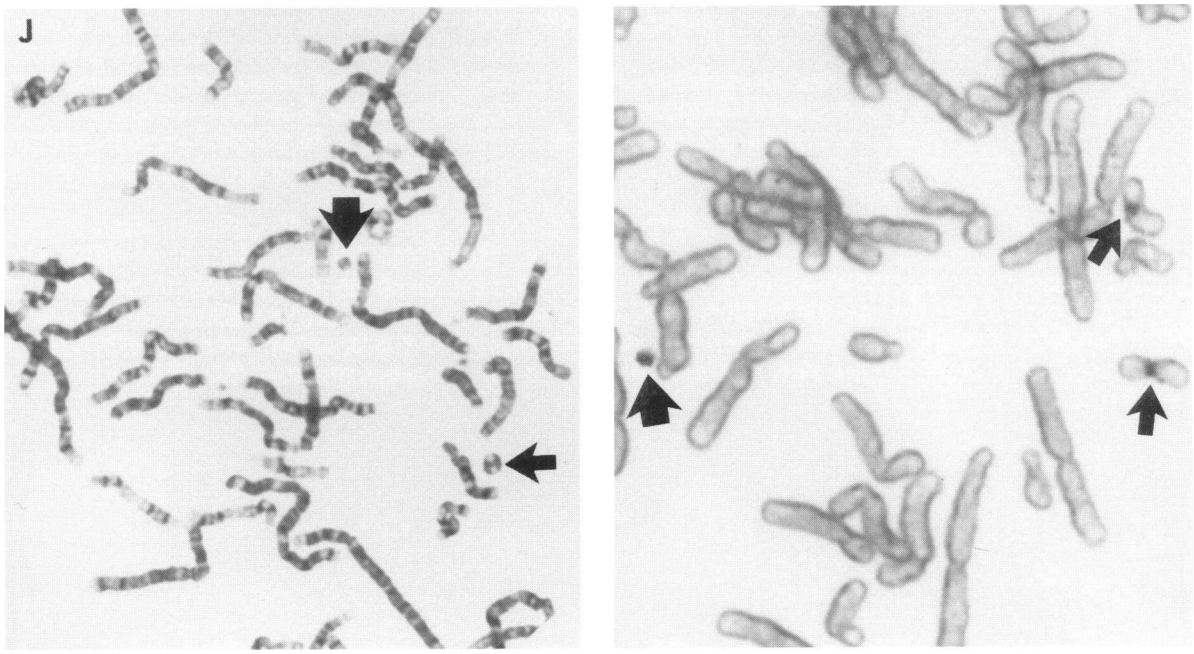


may therefore be useful in defining groups of patients in whom the prognosis is different. This classification is not unexpected, since small distamycinA/DAPI-positive ring chromosomes may consist solely of repetitive DNA sequences—alphoid sequences, and satellite II or satellite III sequences—and thus be devoid of euchromatin.

The present report demonstrates that small ring marker chromosomes originate from the centromere and from the adjacent pericentric regions of a wide variety of chromosomes. Will identification of a group of patients with markers originating from a particular chromosome allow the identification of new chromosomal syndromes? There will be variation in position



of breakpoints—and, accordingly, in the genetic content—of independently ascertained markers which have been identified as arising from the same chromosome. However, although there will be this variation, there may be a recognizable clinical syndrome due to the particular genetic content of the region adjacent to the centromere. The present report suggests that one of the two breakpoints which generate small ring chromosomes is at or adjacent to the centromere. Consequently, markers would contain genetic material located either distal or proximal to the centromere. Therefore, there may be two distinct syndromes associated with each group of centromere-derived markers. In many cases such syndromes will not be able to



be clearly defined—because of the nonspecific nature of the phenotype, as has been described in cases 5, 6, and 9. However, the distinctive facies and pattern of abnormalities present in case 1, where they are associated with a ring chromosome 1, and in case 10, where they are associated with a ring chromosome 20, may herald the identification of new chromosomal syndromes. Identification of further cases will allow both the clarification of these issues and the elimination of any biases due to ascertainment.

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