Am. J. Hum. Genet. 47:493–498, 1990

The Isochromosome 18p Syndrome: Confirmation of Cytogenetic Diagnosis in Nine Cases by In Situ Hybridization

D. F. Callen,* C. J. Freemantle,† M. L. Ringenbergs,* E. Baker,* H. J. Eyre,* D. Romain,‡ and E. A. Haan†

Departments of *Cytogenetics and Molecular Genetics and †Medical Genetics, Adelaide Children's Hospital, North Adelaide, South Australia; and ‡Cytogenetics Laboratory, Wellington Hospital, Wellington, New Zealand

Summary

Nine cases are described of tetrasomy 18p resulting from the presence of an isochromosome 18p [i(18p)]. The initial diagnosis of i(18p) was by standard cytogenetic techniques and was confirmed by in situ hybridization with a biotinylated alphoid probe (L1.84) specific for the pericentric region of chromosome 18 and with a tritium-labeled chromosome 18 probe (B74) which hybridizes to the *D18S3* locus situated at 18p11.3. The clinical features of the cases are summarized and shown to constitute a distinct and recognizable syndrome. Common features were low birth weight, a characteristic facies, neonatal hypotonia with subsequent limb spasticity, short stature, microcephaly, mental retardation, and seizure disorders. On the basis of size and cytogenetic banding a marker chromosome can be suspected to be an i(18p). In situ hybridization with the alphoid probe L1.84 provides confirmation of chromosome 18 origin. This more precise diagnosis will be an advantage in situations of pre- and postnatal diagnosis, since parents can be provided with a more confident prognosis for their child.

Introduction

A small metacentric marker chromosome described in a number of patients was first suggested to be an isochromosome 18p [i(18p)] by Froland et al. (1963). Since then, banding studies have confirmed that such metacentric marker chromosomes are consistent with the appearance of an i(18p), and various authors have suggested that a distinctive syndrome is associated with an additional i(18p), i.e., tetrasomy 18p (Batista et al. 1983; Rivera et al. 1984; Fryns et al. 1985). However, it remained to be confirmed whether these small metacentric chromosomes are all i(18p)s. Chromosomes with a large deletion of 18q or translocation products of 18 with another chromosome may appear cytogenetically indistinguishable from an i(18p).

In the present report nine patients with a small metacentric marker thought cytogenetically to be an i(18p) were evaluated by in situ hybridization with the biotinylated alphoid probe L1.84, which is specific for the pericentric region of chromosome 18, and with the tritium-labeled chromosome 18 probe B74. Detailed clinical evaluation of these patients, and comparison with published cases, documents the clinical syndrome associated with tetrasomy 18p.

Received March 12, 1990; revision received May 8, 1990.

Address for correspondence and reprints: Dr. David F. Callen, Department of Cytogenetics and Molecular Genetics, Adelaide Children's Hospital, North Adelaide, South Australia 5006, Australia.

0002-9297/90/4703-0014$02.00

Material and Methods

Cytogenetics

PHA-stimulated lymphocyte cultures were harvested, and metaphase spreads prepared, by standard procedures. G-banding was by a hydrogen peroxide pretreatment trypsin procedure, and C-banding was by treatment with barium hydroxide (Rooney and Czepulkowski 1986).

Probes

Probe L1.84 is specific for the pericentric alphoid repeat of chromosome 18 when hybridized and then washed under conditions of high stringency (Devilee

et al. 1986). Probe B74 is a 6-kb unique probe which is at the *D18S3* locus situated at 18p11.3 (Mattei et al. 1985).

In Situ Hybridization

Probe L1.84 was biotinylated and hybridized to metaphase chromosomes, and the signal was detected by using an antibody detection system with a gold/silver amplification of the signal according to a method described elsewhere (Callen et al. 1990). Probe B74 was tritium labeled to a specific activity of 8×10^7 cpm/μg and was hybridized to metaphase chromosomes, and the signal was detected by autoradiography according to a method described elsewhere (Callen et al. 1988). For each patient, silver grains positioned over or touching the following regions were scored in 50 metaphases: the short arm of chromosome 18, the long arm of chromosome 18 in a region extending distally from the centromere to a region equivalent in size to the short arm, the marker chromosome, and the remainder of the chromosome complement. The number of background grains expected on the region of the long arm of chromosome 18 scored is 0.91% of the total number of grains scored on the remainder of the chromosome complement. The value of 0.91% is derived from the percentage of the

Table 1

Clinical Features of Nine Patients with i(18p)

	Patient								
	1	2	3	4	5	6	7	8	9
Age at review	7 wk	16 mo	21 mo	7 years	11 years	13 years	23 years	30 years	36 years
Sex	F	F	M	F	F	F	F	F	F
Parental age (mother:father)	36:22	31:34	37:43	34:30	29:28	26:26	35:35	39:41	36:46
Physical characteristics:									
Growth:									
Birth weight (g)	2,500	2,250	2,580	2,860	3,260	2,130	2,355	2,950	3,630
Height (percentile)	3	10	3–10	3	3	10	<3	10	<3
Head circumference (percentile)	10	<3	50	<3	3	<3	<3	<3	3
Neurological:									
Feeding difficulty	+	+	+	–	NK	+	+	–	NK
Developmental delay	Moderate to severe	Moderate	Moderate	Severe	Moderate	Mild to moderate	Moderate	Mild to moderate	Severe
Spasticity	+	+	+	+	+	+	+	+	+
Seizure disorder	+	–	–	+	+	+	+	–	–
Craniofacial:									
Low-set/malformed ears	–	+	+	+	–	+	+	–	+
High eyebrows	+	+	+	+	–	+	+	–	–
Strabismus	–	–	+	+	+	+	+	+	–
Short palpebral fissures	+	+	+	+	–	+	+	–	–
Up-slanting palpebral fissures	–	–	+	+	–	–	+	+	–
High nasal bridge	–	+	–	–	–	–	+	+	+
Small nose	+	+	+	+	–	+	–	–	–
High arched palate	+	–	–	–	+	–	–	+	–
Philtrum	Long	Long	Long	Long	NK	Long	Short	N	N
Prominent upper lip	+	–	+	–	–	+	–	–	+
Small mouth	+	+	+	+	–	+	+	–	–
Prognathism	–	–	–	+	–	+	+	+	+
Limbs:									
Clenched hand/ camptodactyly	+	+	–	–	–	–	–	–	+
Adducted thumbs	+	+	–	–	+	–	–	–	–
Tranverse palmar creases	–	+	+	+	–	–	–	–	+
Trunk:									
Scoliosis	–	–	–	–	–	+	+	–	+

Note.—NK = not known; N = normal.

haploid autosomal length represented by the chromosome 18 short arm as determined from the relative chromosome lengths as given by ISCN (Harnden and Klinger 1985).

Since the arms of the putative i(18p) cannot be cytogenetically distinguished, the following strategy was used to confirm its structure: From the 50 metaphases scored for each patient the expected ratio of number of grains on the short arm of chromosome 18 to number of grains on the marker chromosome would be 1:1 if the marker is an i(18p). If the marker is a deleted 18q, then this ratio is 2:(1 plus the expected number of background grains on this region of 18q). The expected number of background grains can be estimated from 0.91% of the total number of grains on the remainder of the chromosome complement.

Results

The nine patients were assessed at ages between 7 wk and 36 years. Seven of the nine patients were referred for chromosome studies within the postnatal period because of dysmorphic facies and/or developmental delay. Patients 8 and 9 were aged 11 years and 21 years, respectively, when referred for chromosome studies because of mental retardation. The clinical features are shown in table 1, and photographs are shown in figure 1. In most cases, pregnancy, labor, and delivery were normal. One child was premature, and another was delivered by Caesarean section. Six of the nine cases had birth weights at or below the tenth percentile. Feeding difficulties in infancy occurred in five of seven cases for whom a feeding history could be obtained.

A characteristic and pleasant facies was seen in young children, the most consistent features being an oval facial shape, low-set and/or malformed ears, high eyebrows, short and slightly up-slanting palpebral fissures, strabismus, small nose, long philtrum, the center of the upper lip overlapping the lower lip and small mouth. With increasing age, prognathism developed, the nose and mouth became more normal in size, and the nasal tip became pointed.

Short stature and microcephaly were frequent with height or length at or below the third percentile in five patients and at or below the 10th percentile in all of them, and head circumference was at or below the third percentile in seven. Both delayed development of at least moderate severity and limb spasticity were present in all individuals, and a seizure disorder was a feature of five.

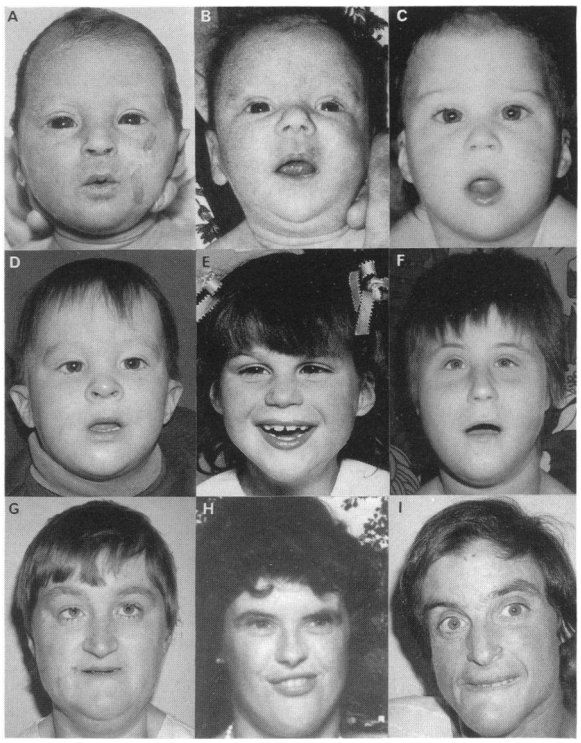

Figure 1 Photographs of individuals with i(18p). *A*, Patient 2 as a neonate. *B*, Patient 1 at 2 mo. *C*, Patient 4 at 5 mo. *D*, Patient 3 at 21 mo. *E*, Patient 4 at 6 years. *F*, Patient 5 at 13 years. *G*, Patient 7 at 23 years. *H*, Patient 8 at 30 years. *I*, Patient 9 at 36 years. Patient numbers refer to the patient numbers in table 1.

Two children at birth had adducted thumbs, camptodactyly, and overlapping fingers as in trisomy 18, one had adducted thumbs alone, and one had camptodactyly alone. Three adults had mild scoliosis not requiring treatment, and one had facial asymmetry. None of the cases had malformations of the brain, heart, kidney, or gastrointestinal tract, but more minor malformations did occur: dislocated hips (two cases), talipes equinovarus (one case), preauricular pit and glandular hypospadias and undescended testes (one case), and unilateral choanal atresia (one case).

Parental age appeared advanced, with mean ± SD maternal age being 33.7 ± 3.9 years and mean ± SD paternal age being 33.9 ± 7.7 years. Appropriate mean parental age figures for the communities from which the patients were drawn and for the long time period over which they were born are not available for statistical comparison. Parental chromosomes were normal in all cases.

The markers in all nine patients showed a similar G-banding and C-banding appearance. A representa-

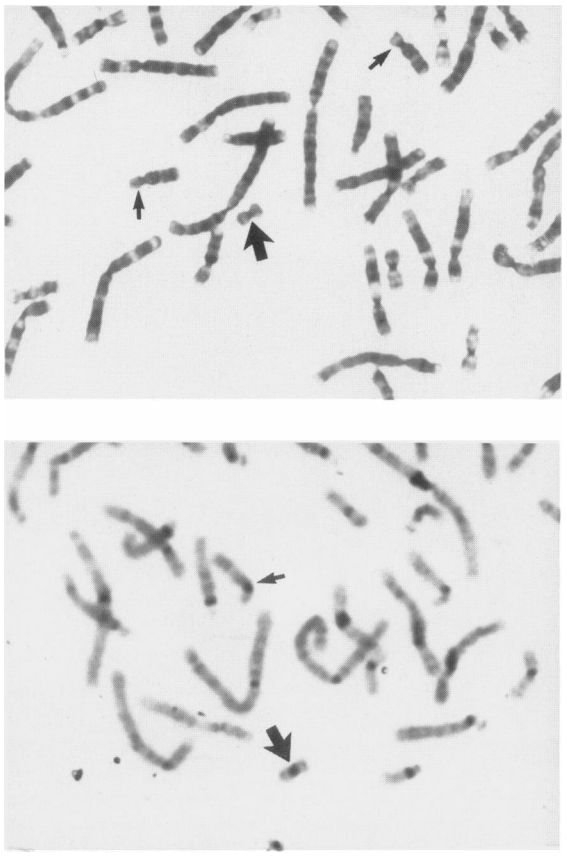

Figure 2 Partial metaphase spreads showing G-banding (*A*) and C-banding (*B*) of i(18p).

tive example is illustrated in figure 2. These metacentric markers showed a size and G-banding pattern consistent with an i(18p).

In situ hybridization with the biotinylated probe L1.84 demonstrated that the marker in all nine patients possessed the pericentric region of chromosome 18 (fig. 3). In each of the nine patients, the marker was confirmed to be an i(18p) by in situ hybridization of the tritiated probe B74. For each patient the number of grains scored over 50 metaphases is given in table 2. The data are homogenous ($\chi^2_{16} = 22.0$, $P > .05$), and thus the expected number of background grains on that region of the long arm of chromosome 18 which was scored can be calculated from the pooled totals. This expected number is 30 grains (0.91% of 3,286 grains), which is in agreement with the 31 grains actually scored. For each patient, the ratio of total grains scored on the normal chromosome 18 short arms to total grains scored on the marker was not significantly different from a 1:1 ratio ($\chi^2_1 < 3.84$ for each patient). However, for

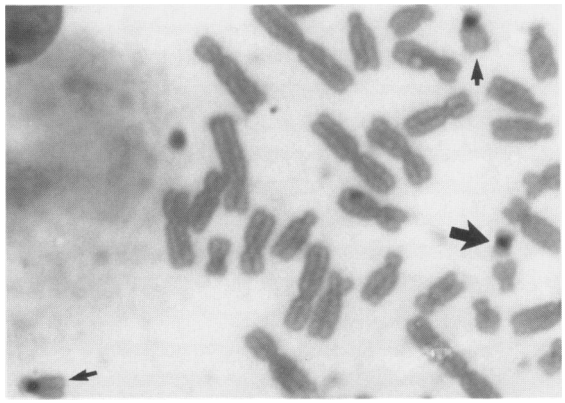

Figure 3 Partial metaphase spread showing in situ hybridization of biotinylated probe L1.84 to an i(18p). The two normal chromosome 18s are indicated by the small arrows, and the i(18p) is indicated by the larger arrow.

each patient this ratio was significantly different from a 2:1.2 ratio, which is the distribution of grains expected on the normal chromosome 18 short arms compared with those scored on the marker, if the marker is a del(18q) ($\chi^2_1 > 3.84$ for each patient). Therefore these data are consistent with the marker being an i(18p).

On this assumption there would be expected to be a proportion of markers with the label at both ends, since there are two sites for hybridization of the B74 probe. As can be seen from the pooled totals of table 2, there were 42 instances of the marker chromosome labeled at both ends, while the number of instances where two grains were observed on chromosome 18 was 13. The double-labeled chromosome 18s had a grain present on the short arm and also a grain present on that portion of the long arm extending from the centromere to a region equivalent in size to the short arm. The grains present on this latter portion of the chromosome can be attributed to background. Although for each patient there were a greater number of double-labeled marker chromosomes compared with double-labeled chromosome 18s, as would be expected if the marker were an i(18p), the numbers were too small for statistical analysis.

Discussion

We studied nine individuals who appeared to have an i(18p) by standard cytogenetic methods. Their clinical features were documented, and in situ hybridization was performed with probes L1.84 and B74. Each marker was confirmed to be an i(18p), and a character-

Table 2

In Situ Hybridization of Probe B74 to Metaphases from Nine Patients with i(18p)

Patient	No. of Grains Scored				No. of Occurrences of Double Labeled Chromosomes	
	18p	18q[a]	Marker	Remainder	18	Marker
1	36	8	35	300	3	4
2	22	5	26	364	2	3
3	37	5	40	346	3	8
4	47	5	52	420	2	5
5	27	3	32	242	0	5
6	33	0	40	415	0	6
7	31	2	40	288	1	4
8	47	3	42	464	2	4
9	40	0	41	416	0	3
Total	320	31	348	3,255	13	42

[a] Number of grains given is for a region of 18q which extends from the centromere to a distance equal to the size of the short arm of chromosome 18.

istic phenotype was present, as suggested by other authors.

To our knowledge, there are only two reports of parental chromosome abnormality, in both cases 47,XX,del(18)(p11.21),+i(18p), leading to tetrasomy 18p (Taylor et al. 1975; Takeda et al. 1989). In our series and in previously published cases, parental chromosome studies have given normal results. Mosaicism of isochromosome 18p has been described once (Göcke et al. 1986) and was not present in our cases.

Mattei et al. (1985) used in situ hybridization of the 18p probe, B74, to demonstrate that the small metacentric marker chromosome in a mentally retarded boy was an i(18p). However, the detailed distribution of grains on the i(18p) as compared with the normal chromosome 18 was not given. The conclusion was based on the finding that, of a total of 53 metaphases in which there was label on the marker, there were eight instances to which the marker chromosome was labeled at both ends. It is possible that such double-labeled marker chromosomes could be due to a combination of signals derived from the hybridization of the probe and background grains.

In 1984 Rivera et al. reviewed the then reported cases of i(18p) and concluded that this chromosomal abnormality is associated with a distinct phenotype. Subsequently reported cases (Yoshihara et al. 1988; Takeda et al. 1989) and our own nine cases conform to this phenotype. It comprises, in the infant, low birth weight, microcephaly, hypotonia, feeding difficulties, camptodactyly or adducted thumbs, and a characteristic facial appearance, the main features of which are round or oval shape, low-set ears, high eyebrows, short palpebral fissures, small nose, long philtrum, and small mouth. Spasticity develops by the second year of life, mental retardation of at least moderate degree on all or some psychological tests is present in all, and seizure disorders are common.

Parental age appeared advanced in our series of nine patients, but appropriate control data were not available for statistical comparison. Batista et al. (1983), reviewing 11 cases, concluded that parental age did not appear to be increased. Cases published more recently by Rivera et al. (1984), Fryns et al. (1985), and Yoshihara et al. (1988) had maternal ages of 38, 30, and 38 years, and paternal ages of 40, 36, and 43 years, respectively. Parental age may be advanced in the isochromosome 18p syndrome, but further studies are needed to confirm this. Hook and Cross (1987) have documented advanced maternal age associated with prenatal detection of extra structurally abnormal chromosomes.

In all nine patients the small metacentric marker chromosome was consistent with an i(18p), on the basis of size and banding pattern. Two probes were used to confirm the structure of the marker. The probe L1.84 showed that the marker contained the centromere and pericentric alphoid repeats of chromosome 18, while the probe B74 was used to show that the marker contained two copies of the short arm of this chromosome.

In all nine patients the marker was confirmed as an i(18p), and in a further study of 36 patients with markers no other cases of small metacentric markers which hybridize to L1.84 have been found (unpublished data). On this basis it is considered that in situ hybridization with the alphoid probe L1.84 should be sufficient to confirm that a marker chromosome is an i(18p) when this is suspected on the basis of size and cytogenetic banding. In situ hybridization with biotinylated probes is a procedure which can be completed within 2 d and utilizes standard cytogenetic spreads. A metaphase containing a marker chromosome which has been probed using this procedure has two normal chromosome 18s, which provide a convenient positive control. This procedure is suitable for diagnosis of the i(18p) prenatally. The nine patients in the present report, together with those previously published, provide an accurate assessment of phenotype for counseling purposes.

Prognostication for the child found to have a marker chromosome can be difficult, especially when the marker is discovered unexpectedly at prenatal diagnosis. In situ hybridization with probes to defined chromosomal segments has the potential to determine the chromosomal origin of the marker, a finding which, if associated with a distinct phenotype, allows families to be given more precise information about the future of their fetus.

It can be anticipated that there will arise progressively more opportunities to combine the techniques of cytogenetics and molecular biology ("molecular cytogenetics") for diagnosis of chromosome abnormalities. Possible uses include definition of the nature of markers, deletions, duplications, insertions, and translocation breakpoints, thus providing improved diagnosis, prognosis, and chromosomal syndrome delineation.

Acknowledgments

We thank the parents of our nine patients for their cooperation and interest in the research, and we thank Professor J. G. Mortimer and Dr. A. A. Kerr for clinical details of cases 5 and 2, respectively. We acknowledge the technical assistance of P. Dagger. This work was supported by the National Health and Medical Research Council of Australia.

References

Batista DAS, Vianna-Morgante AM, Richieri-Costa A (1983) Tetrasomy 18p: tentative delineation of a syndrome. J Med Genet 20:144–147

Callen DF, Hyland VJ, Baker EG, Fratini A, Simmers RN, Mulley JC, Sutherland GR (1988) Fine mapping of gene probes and anonymous DNA fragments to the long arm of chromosome 16. Genomics 2:144–153

Callen DF, Ringenbergs ML, Fowler JCS, Freemantle CJ, Haan EA (1990) Small marker chromosomes in man: origin from pericentric heterochromatin of chromosomes 1, 9 and 16. J Med Genet 27:155–159

Devilee P, Cremer T, Slagboom P, Bakker E, Scholl HP, Hager HD, Stevenson AFG, et al (1986) Two subsets of human alphoid repetitive DNA show distinct preferential localization in the pericentric regions of chromosomes 13, 18 and 21. Cytogenet Cell Genet 41:193–201

Froland A, Holst G, Terslev E (1963) Multiple anomalies associated with an additional small metacentric chromosome. Cytogenetics 2:99–106

Fryns JP, Kleczkowska A, Marien P, Van den Berghe H (1985) 18p tetrasomy: further evidence for a distinctive clinical syndrome. Ann Genet 28:111–112

Göcke H, Muradow I, Zerres K, Hansmann M (1986) Mosaicism of isochromosome 18p: cytogenetic and morphological findings in a male fetus at 21 weeks. Prenatal Diagn 6:151–157

Harnden DG, Klinger HP (eds) (1985) ISCN: an international system for human cytogenetic nomenclature. Karger, Basel

Hook EB, Cross PK (1987) Extra structurally abnormal chromosomes (ESAC) detected at amniocentesis: frequency in approximately 75,000 prenatal cytogenetic diagnoses and associations with maternal and paternal age. Am J Hum Genet 40:83–101

Mattei MG, Philip N, Passage E, Moisan JP, Mandel JL, Mattei JF (1985) DNA probe localization at 18p113 by *in situ* hybridization and identification of a small supernumerary chromosome. Hum Genet 69:268–271

Rivera H, Moller M, Hernandez A, Enriquez-Guerra MA, Arreola R, Cantu JM (1984) Tetrasomy 18p: a distinctive syndrome. Ann Genet 27:187–189

Rooney DE, Czepulkowski BH (eds) (1986) Human cytogenetics: a practical approach. IRL, Oxford

Takeda K, Okamura T, Hasegawa T (1989) Sibs with tetrasomy 18p born to a mother with trisomy 18p. J Med Genet 26:195–197

Taylor KM, Wolfinger HL, Brown MG, Chadwick DL (1975) Origin of a small metacentric chromosome: familial and cytogenetic evidence. Clin Genet 8:364–369

Yoshihara S, Iinuma K, Nihei K, Naito H (1988) Case report of tetrasomy 18p in a girl. Acta Paediatr Jpn 30:635–637