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18p⁻ Syndrome: an unusual case and diagnosis by in situ hybridization with chromosome 18-specific alphoid DNA sequence

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Summary. A patient with an atypical clinical picture of 18p⁻ syndrome is described. By the in situ hybridization technique we localized the chromosome 18-specific cloned repetitive sequence to metaphase chromosomes of the patient. The predominant hybridization of the probe was found in pericentromeric regions of homologous chromosomes 18. The amount of pericentromeric DNA measured by in situ hybridization differed between homologous chromosomes; and the number of radioactive grains was statistically greater in the normal chromosome 18 than in the aberrant chromosome 18p⁻. The results indicate that this probe may be useful in clinical cytogenetics for identification of aberrant chromosomes, localization of breakpoints, and studies of C-band DNA polymorphism of chromosome 18.

The proband, a boy, was born after the fifth pregnancy and second labour. The former pregnancies had been terminated by therapeutic abortions. The family history was unremarkable. The parents were healthy, the mother was 35 and the father was 39 years old. The patient was born by precipitated labour after a full-term normal pregnancy. Weight was 3400 g, length was 52 cm, circumferences of head and thorax were 34 cm. He cried immediately. Acrocyanosis, cyanosis of nasolabial triangle, and some congenital developmental defects were noted. Among them were microcephalia, short neck, cheilognathopalatoschisis, broad impressed ridge of the nose, deformed and low floor of the auricles, mongolian slant of the eyes, hypertelorism of ears and nipples, impressed thorax, hypoplastic penis, short trunk, and well defined network of venous vessels on the abdomen. Hepatosplenomegaly and the absence of the swallowing reflex were also marked. We failed to find any known teratogenic influence during the antenatal period.

The clinical picture of this case differed markedly from other cases of 18p⁻ syndrome. Such signs as birth weight of 3400 g, cheilognathopalatoschisis, hepatosplenomegaly, and mongolian slant of the eyes are not typical of 18p⁻ syndrome. Also alopecia (which supports the diagnosis of 18p⁻) was not observed in this case (de Grouchy and Turleau 1982).

The cytogenetic analysis was made on peripheral blood lymphocyte cultures. We found monosomy of the short arm of chromosome 18 in all cells leading to 46,XY,18p⁻ karyotype.

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In order to confirm the cytogenetic diagnosis and to localise the breakpoints we in situ hybridized the chromosome 18-specific cloned alphoid DNA fragment to chromosomal preparations of the patient. This probe was obtained from the collection of chromosome-specific cloned DNA sequences of the laboratory of Genetics, All-Union Research Center of Mental Health of AMS USSR. This fragment is about 1600 bp, cloned in pBR 322, inserted at the PstI site, and designated pBRHS 13 (Alexandrov et al. 1986). The in situ hybridization was performed as described previously (Yurov 1984). The cloned sequence pBRHS 13 hybridised predominantly to pericentromeric regions of chromosomes 18 and allowed one to identify chromosomes 18 not only in metaphase but also in interphase cells (Figs. 1, 2). The statistical analysis of radioactive grain number over homologous chromosomes 18 revealed that hybridization was more intense to normal chromosome 18, and the number of grains on the aberrant chromosome 18p⁻ was statistically less (Table 1).

We failed to find any other rearrangements in prometaphase and metaphase chromosomes of the proband using differential staining techniques (Q, G, C-banding). The karyotypes of the parents were normal. Therefore, we conclude that the aberrant chromosome 18p⁻ had arisen de novo. The unusual phenotype of the proband apparently is not due to the complex rearrangement with imbalance of a chromosome segment different from 18p⁻ and may result from clinical polymorphism of 18p⁻ syndrome.

Molecular cytogenetic analysis of the probands chromosomes by the in situ hybridization technique using chromosome 18-specific cloned DNA fragment has shown that chromosome 18p⁻ contains smaller amounts of centromeric heterochromatin than the normal chromosome 18 (25% and 36.4% of all radioactive silver grains, respectively, Table 1, Fig. 3). The detailed description of chromosome 18-specific repeated alphoid DNA will be presented in separate publications (Yurov et al. 1985; Alexandrov et al. 1986). This cloned DNA fragment is very specific to the centromeric region of chromosome 18 (more than 60% of all silver grains in metaphases, Table 1). Does the difference in C-heterochromatin content between normal and aberrant chromosomes 18 play an important role in the atypical clinical picture of 18p⁻ syndrome or is this phenomenum connected with homologous heteromorphism of the C-band DNA amount of chromosome 18? Theoretically, the possibility is not excluded that the amount of repetitive DNA in the C-band of chromosome 18 varies in the human population, for example, as described for chromosomes 1, 9, 16, and Y with large blocks of C-hetero-

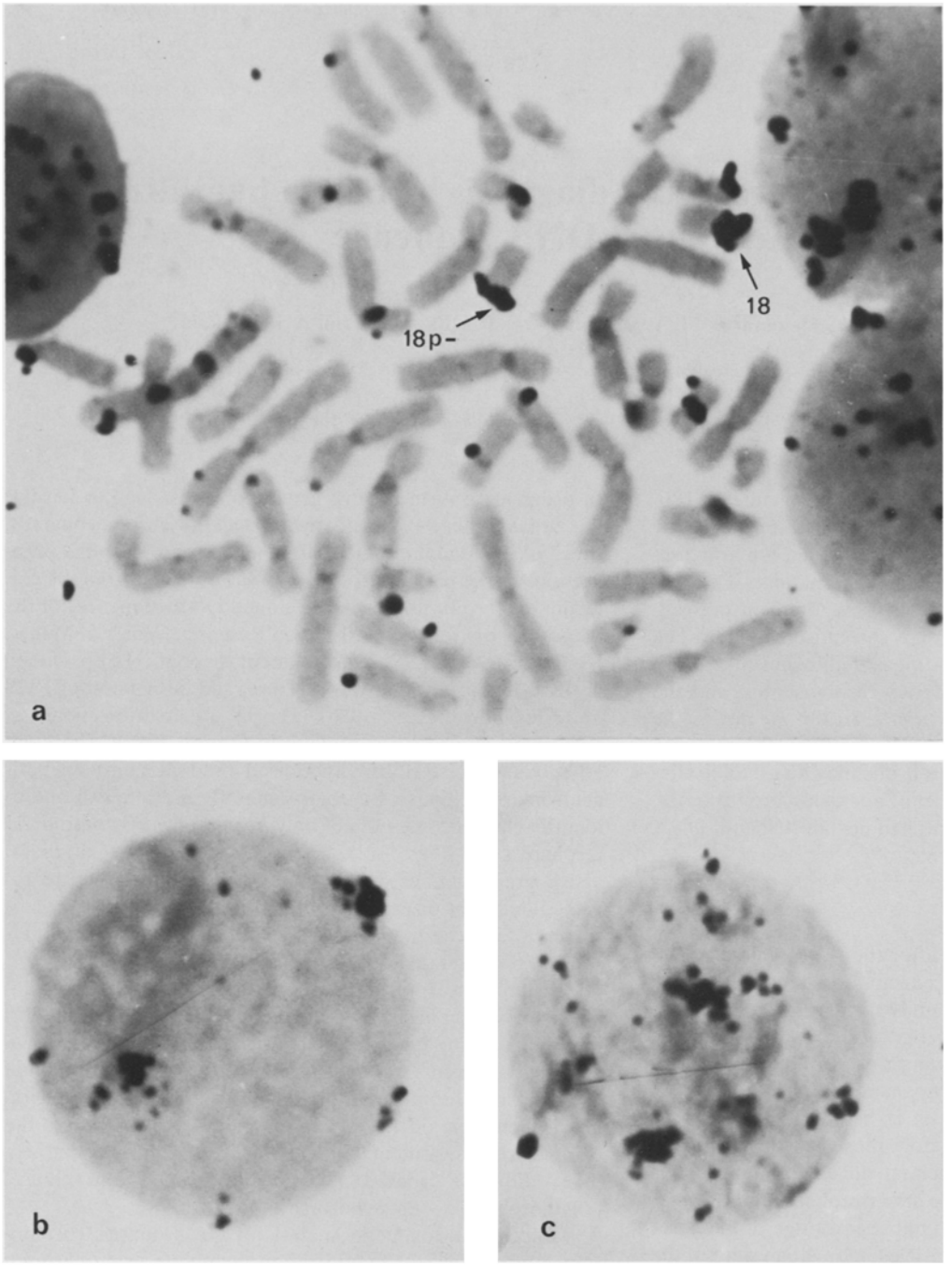

Fig. 1a, b. The probe pBRHS 13 was hybridized to chromosomal preparations from patient with $18p^-$ syndrome. Intense label was observed over centromeric regions of homologous chromosomes 18 (**a**). Minor hybridization sites were detected on chromosomes 20, 9, and 2. Intense sites of hybridization to centromeric condensed regions of chromosomes 18 were observed within most interphase cells (**b, c**). The DNA probe was nick-translated using dTTP to obtain specific activity of about 0.5×10^7 dpm/μg. Hybridization was carried out at 37°C for 18h in hybridization solution containing 50% formamide, 2 SSC, 10% dextran sulfate, and 0.5×10^6 dpm of the probe in 20μl. The detailed protocol of in situ hybridization was described previously (Yurov 1984)

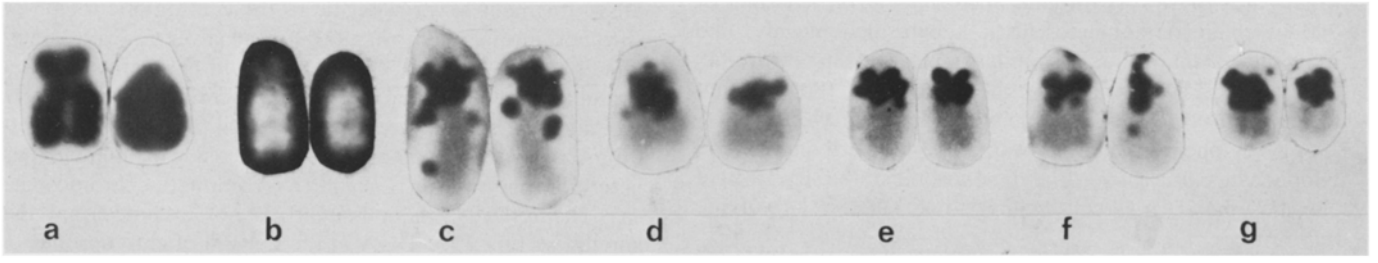

Fig. 2a–g. Examples of homologous chromosomes 18 from several metaphases after in situ hybridization (**c–g**), routine staining (**a**), and Q-banding (**b**)

Table 1. The quantitative analysis of radioactive grain numbers after in situ hybridization of cloned probe pBRHS13 to chromosomes 18 and 18p⁻

No. of metaphases	Number of radioactive grains			
	Normal chromosome 18	Aberrant chromosome 18p⁻	Both chromosomes 18	All chromosomes in metaphase
1	17	10		40
2	12	6		30
3	15	10		36
4	13	9		34
5	12	10		40
6	16	12		43
7	15	8		39
8	10	6		31
9	15	11		47
10	18	10		36
11	12	7		31
12	16	12		47
13	14	15		41
14	20	15		56
15	12	10		38
16	9	6		32
17	14	10		44
18	16	11		43
19	16	13		47
20	18	13		49
21	13	9		45
22	17	12		52
23	14	10		39
24	16	11		44
25	19	15		49
Total number of grains	369	261		1033
Mean value	14.76 (36.4%)	10.44 (25%)	25.2 (61.4%)	41.32 (100%)
Standard deviation	2.72	2.63		6.89
Standard error	0.54	0.52		1.37

Differences between degree of hybridization of probe pBRHS 13 in the normal and aberrant chromosomes 18 are highly significant (t_{st} = 5.69; $P < 0.001$)

chromatin (McKay et al. 1978; Gosden et al. 1975, 1981). The application of the C-banding technique to study a small region of centromeric heterochromatin is a very subjective approach with relatively low resolution. There are few reports about the G- and C-variants of chromosomes with small C-bands, for example, 18ph⁺ (Fordyce et al. 1983). In situ hybridization of chromosome-specific centromeric cloned DNA fragments is a new molecular-cytogenetic approach to study small C-band heterochromatin regions.

Syndrome 18p⁻ is very rare and for a really informative study collaborative efforts of researchers are necessary with material from rare cases of aberrant chromosomes 18. We believe that our results will encourage further detailed collaborative molecular-cytogenetic analyses of this syndrome. In summary, the data obtained indicate that the chromosome 18-specific probe may be useful in clinical cytogenetics for the diagnosis of unusual and structurally altered chromosomes, to study C-DNA heteromorphisms of chromosome 18, and for detailed mapping of chromosome 18.

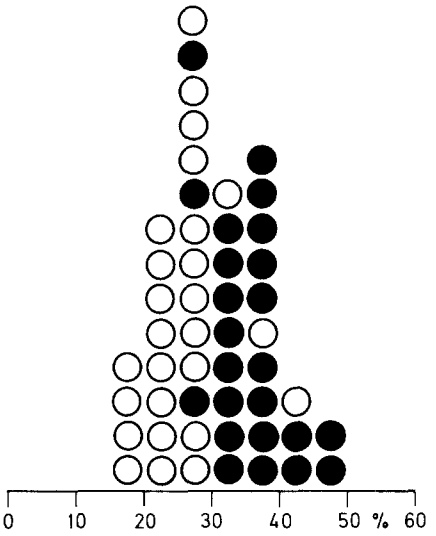

Fig. 3. The relative number of silver grains (in percent of all radioactive grains per metaphase) on normal chromosome 18 (●) and on aberrant chromosome 18p⁻ (○). The primary data on radioactive grain numbers are shown in Table 1

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