Hum Genet (1988) 80:219–223

Human Genetics

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Original investigations

Molecular detection of a Yp/18 translocation in a 45,X holoprosencephalic male

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Summary. Prenatal diagnosis in a fetus with holoprosencephaly showed a 45,X karyotype and a suspected 18p abnormality. At birth, the fetus presented with normal male genitalia. Y chromatin was not cytogenetically detectable by Q-, G-, or G11-banding. Mosaicism for a cell line containing a Y chromosome was not observed in amniocytes, lymphocytes, or skin fibroblasts. Southern blot analysis for 11 different Y-DNA loci demonstrated the presence in the patient's genome of sequences derived from the short arm, centromeric region, and proximal long arm of the Y chromosome (intervals 1–5). The distal long arm of the Y (intervals 6 and 7) was absent. In situ hybridization with the Y-derived probe pDP105 showed silver grains over the short arm of the del(18) chromosome, suggesting a Y/18 translocation with loss of 18p and distal Yq material.

Introduction

The holoprosencephaly sequence is a midline field defect that involves the embryonic forebrain and the face and may result in complete failure of formation of the cerebral hemispheres and cyclopia. Holoprosencephaly is most often sporadic. However, familial occurrence has been observed (Dominok and Kirchmair 1961; DeMeyer et al. 1963; Hintz et al. 1968; Ardouin et al. 1968; James and Van Leeuwen 1970; Khan et al. 1970; Dallaire et al. 1971; Nivelon-Chevallier and Nivelon 1975) with autosomal dominant (Patel et al. 1972; Lowry 1974; Roach et al. 1975, pedigree 20; Gilbert and Opitz 1976; Petterson 1976; Cantú et al. 1978; Benke and Cohen 1983; Berry et al. 1984; Hattori et al. 1987), autosomal recessive (Klopstock 1921; Grebe 1954; Cohen and Gorlin 1969; Cohen et al. 1971), or possible X-linked inheritance (Begleiter and Harris 1980; Falk et al. 1982). In addition, it has been described in infants of diabetic mothers (Dekaban 1959; Barr et al. 1983), in congenital cytomegalovirus infection (Byrne et al. 1987), and in the following syndromes: DiGeorge syndrome (Conley et al. 1979); Meckel syndrome (Hsia et al. 1971); orofacial-digital syndrome (Váradi et al. 1980); velo-cardio-facial syndrome (Wraith et al. 1985); and the CHARGE association (Toriello 1986). Holoprosencephaly has also been associated with a variety of chromosome abnormalities (for review see Münke et al. 1988). To date, no sex chromosome anomaly has been associated with holoprosencephaly.

Here we report a male infant with alobar holoprosencephaly whose karyotype was initially interpreted as 45,X. Though no Y chromatin was detected cytogenetically, this infant's DNA was positive for several Y-specific sequences by Southern blot analysis. By chromosomal in situ hybridization we have demonstrated Y-specific material translocated to chromosome 18.

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Case report

The patient was born to a 22-year-old primigravida mother and a 27-year-old father. The parents were unrelated and healthy, with no evidence of any skeletal anomaly or midline defect. There was no history of maternal diabetes or known viral infections during the pregnancy. Ultrasound examination at 27 weeks demonstrated severe hypotelorism, hydrocephalus with a single anterior ventricle, and oligohydramnios. Analysis of metaphase chromosomes from amniocytes showed a 45,X karyotype and suggested an abnormality of 18p.

Birth was at 30 weeks of gestation. Both weight and length were at the 25th percentile (1200 g and 38 cm, respectively). In contrast, the infant had microcephaly (head circumference, 22 cm) below the 3rd percentile, a single fused eye, and a proboscis above this eye (Fig. 1). Despite the prenatal karyotype, there were no clinical signs of Turner syndrome. Instead the fetus presented with male genitalia, with normal penis and scrotum, but no palpable testes. The infant lived approximately 2 h.

Autopsy showed synophthalmia with a single midline orbit and globe with two irides and a midline hypoplastic optic nerve, alobar holoprosencephaly, and absent olfactory tracts and bulbs. The falx cerebri, crista galli, sella turcica, and cribriform plate were absent. Cranial nerves III to XII were present. There was pituitary agenesis with marked hypoplasia of adrenals, thyroid, and testes, which were in the pelvis adjacent to the gubernaculum. Histologically the testes had a decreased number of Leydig cells and germ cells.

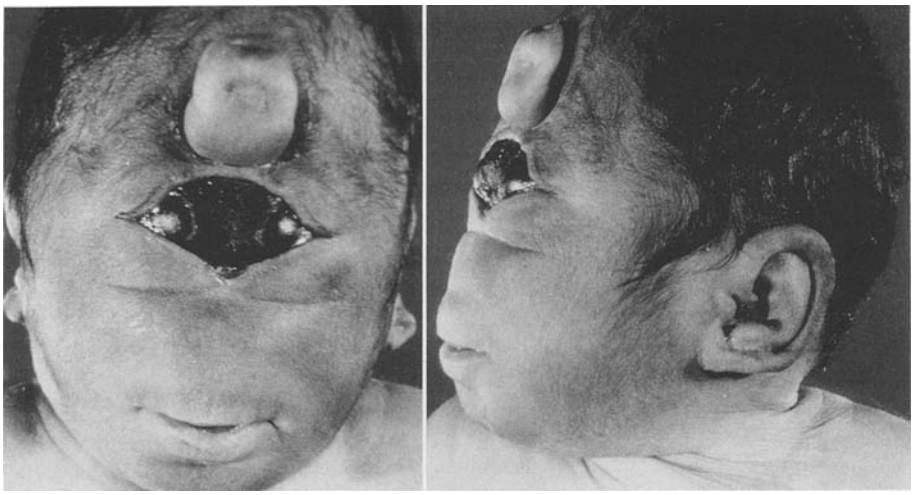

Fig. 1. Facial features of synophthalmia and proboscis in a male infant with alobar holoprosencephaly

Table 1. Southern hybridization of Y-DNA probes to genomic DNAs from the 45,X,18p− male infant and normal males and females

Interval	Probe/locus	Enzyme	Stringency[a]	Presence (+) or absence (−) of Y-specific restriction fragment		
				Proband	Normal males	Normal females
1	pDP 132	*Taq*I	H	+	+	−
2	pDP61	*Taq*I	H	+	+	−
3	50f2/A,B	*Eco*RI	M	+	+	−
3	pDP105/A	*Taq*I	M	+	+	−
4A	pDP34	*Taq*I	H	+	+	−
4B	pDP97	*Eco*RI	H	+	+	−
4B	50f2/D	*Eco*RI	M	+	+	−
5	12f	*Taq*I	H	−	+	−
6	50f2/C,E	*Eco*RI	M	−	+	−
6	pDP105/B	*Taq*I	M	−	+	−
7	pY431-HinfA	*Taq*I	M	−	+	−

[a] H, High stringency (hybridization at 47°C and washes at 65°C); M, medium stringency (hybridization at 42°C and washes at 55°C)

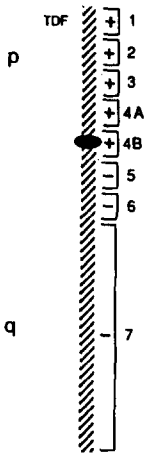

Fig. 2

Fig. 2. Deletion intervals of the Y chromosome (modified from Page 1986) indicating the presence (+) or absence (−) of Y-specific DNA sequences in the proband. *TDF* Testis-determining factor

Materials and methods

Chromosome analyses were performed on cultured lymphocytes of the proband and both parents with GTG-banding. Chromosomes from the proband's amniocytes, lymphocytes, transformed lymphoblastoid cells, and fibroblasts were also G-11 and/or QFQ-banded.

Hybridization probes

We used 11 Y-chromosome probes for filter hybridization and one of them (pDP105) for in situ hybridization. These probes have been derived from different regions or deletion intervals on the Y chromosome (Page 1986; Vergnaud et al. 1986; Fig. 2, Table 1): pDP132 (D.C. Page, unpublished work), pDP61 (D.C. Page, unpublished work, derived from plasmid 115; Geldwerth et al. 1985), 50f2 (Guellaën et al. 1984), pDP105 (D.C. Page, unpublished work), pDP34 (Page et al. 1984), pDP97 (D.C. Page, unpublished work, derived from cosmid Y97; Wolfe et al. 1985), 12f (Bishop et al. 1984), and pY431-HinfA (K. Smith, personal communication).

Filter hybridization

Genomic DNAs prepared from cultured amniocytes, blood, or lymphoblastoid cell lines were digested with restriction endonucleases, *Eco*RI or *Taq*I (Table 1), subjected to electrophoresis on 0.7% agarose gels, transferred to nylon membranes, and hybridized with ^{32}P-labeled DNA probes as de-

scribed elsewhere (Page et al. 1987). DNA from the infant's parents was not available for analysis.

In situ hybridization

Probe pDP105 was labeled by nick-translation with four tritium-labeled nucleotides to a specific activity of 1.5×10^7 cpm/μg DNA. This probe was hybridized to metaphase chromosomes from the patient and a normal male control as described elsewhere (Münke et al. 1984, 1985).

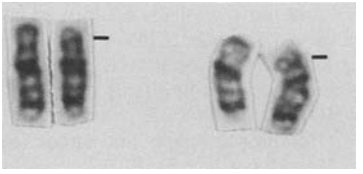

Fig. 3. GTG-banded partial karyotype of the proband with holoprosencephaly demonstrating two examples of chromosome 18 homologs. Normal chromosomes 18 are on the *left* of each pair; the t(Yp;18) translocation chromosomes are on the *right*

Results

The proband's karyotype was initially interpreted as 45,X. In addition to the single sex chromosome, a deletion of 18p was detected following GTG-banding (Fig. 3). There was no evidence of Y heterochromatin as judged by quinacrine mustard or G-11 staining. Analysis of over 100 cells, each from amniocytes, fibroblasts, and lymphocytes, demonstrated 45 chromosomes per cell with no cell carrying a Y chromosome. This excludes mosaicism for a Y-bearing cell line at a level of 3% with 95% confidence (Hook 1977). The parents had normal karyotypes.

As this patient was phenotypically male, we tested for the presence or absence of Y-specific sequences from eight previously characterized deletion intervals (Vergnaud et al. 1986; Page 1986). The results of these DNA hybridization studies are summarized in Fig. 1 and Table 1. We detected the presence of most if not all of the short arm (deletion intervals 1–4A) and the centromere (interval 4B) of the Y chromosome. Most of the long arm was found to be absent. Thus, the breakpoint appears to be in the proximal portion of the long arm of the Y chromosome.

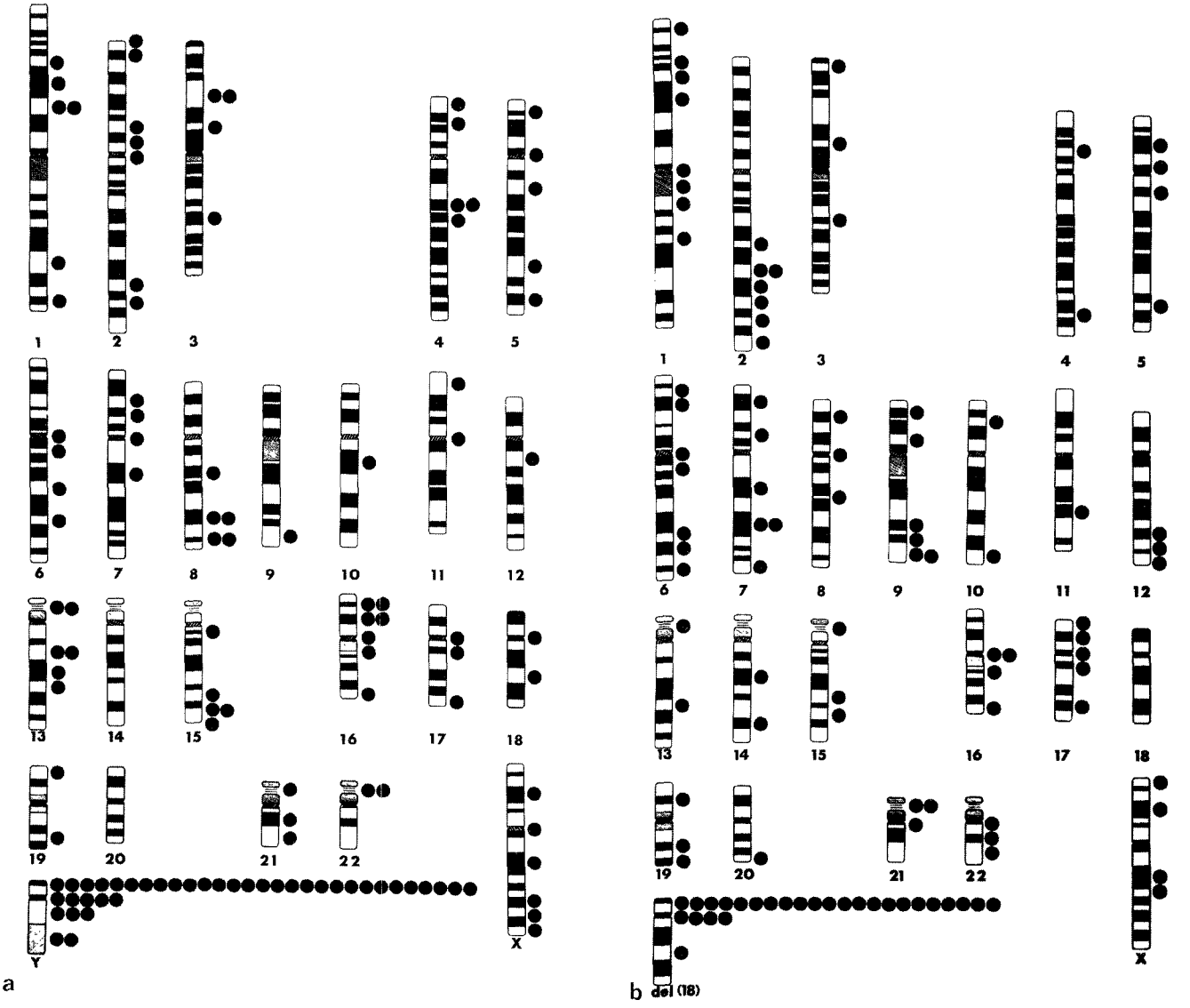

Fig. 4a, b. In situ hybridization of the Y-specific probe pDP105 to normal male chromosomes (**a**) and chromosomes of the male infant with the 45,X karyotype (**b**). Distribution of silver grains is recorded on ISCN (1985) ideograms at the 400-band stage

To determine the chromosomal location of the Y-specific DNA in the patient, we used in situ hybridization with a Y-specific repetitive probe (pDP105) shown to be present in the patient by Southern analysis. After in situ hybridization, the 60 cells analyzed had a total of 120 grains over chromosomes. Of the 120 grains, 26 (22%) were over 18p, and there was no significant accumulation of grains elsewhere (Fig. 4). In contrast, analysis of 60 cells of a male control after in situ hybridization with the same probe demonstrated that from a total of 130 grains over chromosomes, 39 grains (30% of total) were over the Y chromosome (Fig. 4). Although the del(18) and the normal chromosome 18 could not be distinguished in every cell, especially when covered by a silver grain, we concluded from these in situ data that Y-specific sequences are located on the short arm of the del(18) chromosome.

Discussion

We have described a male infant who was prenatally found to have holoprosencephaly and a 45,X karyotype. Review of the chromosomes, dictated by clinical findings, showed a deleted short arm of one of the chromosomes 18. Southern analysis revealed DNA sequences from the short arm (deletion intervals 1–4a) and centromeric region (interval 4B) of the Y chromosome to be present in the infant's DNA. The presence of interval 1, to which the testis-determining factor has been mapped (Page et al. 1987), accounts for the male phenotype. Using a probe from deletion interval 3 of Yp we were able to demonstrate by in situ hybridization that the Y material was translocated to the del(18) chromosome. Data from Southern analysis indicated that the breakpoint in the Y chromosome was in the proximal portion of the long arm with loss of Yq material distal to the break. The cytogenetic data do not unequivocally allow a breakpoint determination on chromosome 18. The alobar holoprosencephaly and cyclopia are most likely due to the deletion of 18p. Craniofacial anomalies occur in 16% of cases with del(18p) (de Grouchy and Turleau 1984). Holoprosencephaly has also been observed with translocations that result in a deletion of chromosome 18p material (Liberfarb et al. 1979; Buchinger et al. 1981).

To date, eight other 45,X males with Y; autosome translocations have been characterized with some of the same Y-DNA hybridization probes (Disteche et al. 1986; Maserati et al. 1986; Gal et al. 1987; Magenis et al. 1987; Weber et al. 1987; Andersson et al. 1988). Like the patient reported here, another 45,X male with a Y;18 translocation (Maserati et al. 1986), three 45,X males with Y;15 translocation (Disteche et al. 1986; Gal et al. 1987; Andersson et al. 1988), and two 45,X males with a Y;14 translocation (Andersson et al. 1988) also appear to carry the entire short arm and centromere of the Y chromosome. All seven individuals appear to have Yq breakpoints. Nonetheless, the Yq breakpoints are not identical in all these cases. The breakpoint in the present case is more proximal than those in most of the other 45,X males. However, one 45,X male with a Y;14 translocation (Andersson et al. 1988; case 3) was, like the present patient, missing Y-DNA from intervals 5–7. As the 45,X male with a Y;18 translocation (Maserati et al. 1986) was not tested for the presence of interval 5, we cannot establish whether its Yq breakpoint is distinct from that in the patient presented here.

Acknowledgements. We are grateful to Dr. A. Donnenfeld for transformation of lymphocytes of the patient presented, to Drs. J. Weissenbach and K. Smith for DNA probes, and to Drs. R. Lodato, D. Huff, and R. Iozzo for the autopsy results. This study was supported by funds from the National Institutes of Health (GM 32592 to B.S.E. and HD 22532 to D.C.P.).

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Received March 7, 1988 / Revised April 25, 1988