

# Different proximal and distal rearrangements of chromosome 7q associated with holoprosencephaly

Brigitte Benzacken, Jean Pierre Siffroi, Corine Le Bourhis, Kader Krabchi, Nicole Joyé, Fadela Maschino, Franck Viguié, Joelle Soulié, Marie Gonzales, Gisèle Migné, Martine Bucourt, Ferechte Encha-Razavi, Lionel Carbillon, Jean Louis Taillemite

Laboratoire d'Histologie, Embryologie, Cytogénétique et Biologie de la Reproduction, Hôpital Jean Verdier, 93 Bondy, France  
B Benzacken

Laboratoire d'Histologie, Biologie de la Reproduction et Cytogénétique, Hôpital Tenon, 4 Rue de la Chine, 75020 Paris, France  
J P Siffroi  
C Le Bourhis  
K Krabchi  
F Maschino  
F Viguié

Laboratoire d'Embryologie Pathologique et Cytogénétique, Hôpital Saint Antoine, 75012 Paris, France  
N Joyé  
M Gonzales  
G Migné  
J L Taillemite

Service de Biologie, Hôpital Notre Dame de Bon Secours, 75674 Paris, France  
J Soulié

Service d'Anatomopathologie, Hôpital Jean Verdier, 93 Bondy, France  
M Bucourt

Service d'Histologie Embryologie, Hôpital Henri Mondor, 94 Creteil, France  
F Encha-Razavi

Service de Gynécologie Obstétrique, Hôpital Jean Verdier, 93 Bondy, France  
L Carbillon

Correspondence to: Dr Siffroi.

Received 4 March 1997  
Revised version accepted for publication 2 July 1997

## Abstract

**Four new cases of holoprosencephaly are described in fetuses exhibiting abnormal karyotypes with different distal and proximal rearrangements of the long arm of chromosome 7. Three of them showed terminal deletions of chromosome 7q, confirming the importance of the 7q36 region in holoprosencephaly. The karyotype of the fourth fetus showed an apparently balanced de novo translocation, t(7;13)(q21.2;q33), without any visible loss of the distal part of chromosome 7q. The involvement of new genes, different from the human Sonic Hedgehog gene (hShh) responsible for holoprosencephaly, or a positional effect are discussed.**

(*J Med Genet* 1997;34:899-903)

Keywords: holoprosencephaly; chromosome 7q; reciprocal translocation; Sonic Hedgehog gene

Holoprosencephaly (HPE) is a common and complex malformation affecting the cleavage of the developing forebrain and is associated with defects of the mid face. HPE occurs in about 1 in 16 000 live births but is more frequently encountered in spontaneous abortions with an incidence of 1 in 250.<sup>1,2</sup> Teratogenic factors, like maternal diabetes which increases the risk of fetal HPE to 1-2%, but also genetic causes, such as mendelian autosomal inheritance or recurrent cytogenetic abnormalities, show causal heterogeneity in HPE. Among chromosomal aberrations, trisomies 13 and 18 and triploidies are commonly associated with HPE. Non-random chromosome anomalies include deletions of chromosomal regions which are known to contain genes involved in the normal development of the brain and the face. Four of these genes were designated at the Human Gene Mapping 11 Conference<sup>3</sup>: HPE1 on chromosome 21q22.3, HPE2 on 2p21, HPE3 on 7q36, and HPE4 on 18p.

The purpose of this work is to present three new cases of terminal monosomy of chromosome 7q associated with holoprosencephaly and the case of an apparently balanced de novo translocation t(7;13)(q21.2;q33) in a fetus with semilobar holoprosencephaly, which suggests that a new region of the long arm of chromosome 7 may be involved in this kind of cerebral malformation.

## Case reports

### CASE 1

This was the second pregnancy in a 30 year old woman who had already had a normal daughter. The 31 year old father was unrelated and there was no history of congenital malformations or hereditary diseases in either family. At 21 weeks of gestation, a first morphological ultrasound examination of the fetus showed an excess of amniotic fluid and bilateral renal dilatation without any other visible anomaly. Amniocentesis was performed at 22 weeks of gestation. Fetal R banded karyotype showed a terminal deletion of the long arm of one chromosome 7 (fig 1A). Parental karyotypes were normal. A submicroscopic reciprocal translocation was eliminated by fluorescence in situ hybridisation (FISH) using a chromosome 7 paint (Biosys Cambio) which did not show any signal other than the two chromosomes 7 (fig 1B). Before medical termination of the pregnancy at 25 weeks of gestation, a second ultrasound examination showed a biparietal diameter of 59 mm, absent corpus callosum, no visible cerebral convolutions, and an abnormal sacrum.

External examination of the non-macerated female fetus (fig 1C) showed growth retardation (-2 SD) and facial dysmorphism including hypotelorism with oedematous eyelids, upward slanting palpebral fissures, a short and bulbous nose, retrognathia, a small mouth, large and misshapen ears, and a short neck. Hirsutism of the face was also noted (fig 1C). Visceral dissection showed bilateral hydronephrosis and abnormal pulmonary lobulation with a single lobed left lung. Radiological examination showed sacral S1 and S2 hemivertebrae. The diagnosis of semilobar holoprosencephaly was made because of the basal fusion of the frontal hemispheres, the absence of olfactory tracts, and the existence of only one optic nerve. In frontal section, the brain showed a single dilated ventricle and partial fusion of the thalami. The corpus callosum was present.

Further analysis of the fetal chromosomes by FISH, using a distal 7q probe (ONCOR, p5224 tel.7q/D7Z1 alpha sat) confirmed the deletion of the 7q36-qter chromosomal region (fig 2C). Therefore, the proband's karyotype was 46,XX.ish del(7)(q36)(wcp7+, tel7q-). The integrity of the telomeric sequences of the deleted 7q chromosome was verified by PRINS using a specific telomeric oligonucleotide



Figure 1 Case 1. (A) Partial karyotype of the proband showing the terminal deletion (R banding). (B) Chromosome 7 painting (arrows) failed to detect any fluorescent signal from another chromosome. (C) Face of the proband at 25 weeks of gestation.

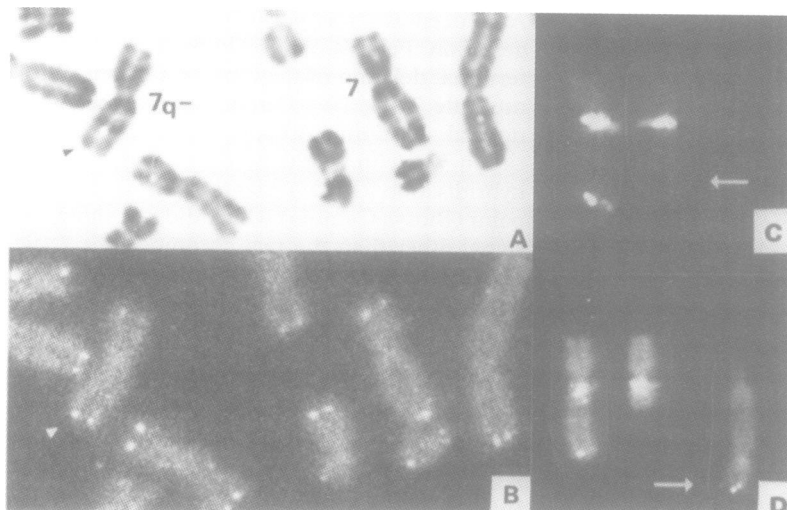


Figure 2 Molecular study of cases 1 and 2. (A, B) Case 1: PRINS with telomeric oligonucleotides after R banding showing the preservation of the telomeric sequences on the deleted chromosome 7 (arrowhead). (C) Case 1: FISH of a distal 7q probe confirming the deletion of the 7q36-qter region (arrow). (D) Case 2: analysis by FISH of the  $t(7;13)$  using a distal 7q probe and showing the presence of the 7q36-qter region (arrow).

(TAACCC  $\times$  5n) elongated in situ with digoxigenin 11dUTP (fig 2A, B).

#### CASE 2

This was the first pregnancy in unrelated parents, aged 28 and 29 years respectively. There was no history of malformations in either family. At 20 weeks of gestation, ultrasound examination of the fetus showed holoprosencephaly associated with agenesis of the corpus callosum. A medical abortion was performed at 22.5 weeks of gestation. The fetus showed mild craniofacial dysmorphism with hypertelorism, a flat occiput (fig 3C), and abnormally positioned thumbs. The semilobar holoprosencephaly and agenesis of the corpus callosum were confirmed by brain examination. There were no associated visceral malformations.

Cytogenetic studies showed an apparently balanced translocation between the long arms of chromosomes 7 and 13 (fig 3A). Parental karyotypes were normal. Analysis by FISH of this translocation was performed by chromosome painting (Biosys Cambio) which confirmed the diagnosis (fig 3B) and by hybridisation of a telomeric 7q probe (ONCOR, p5224 tel.7q/D7Z1 alpha sat) which showed that there was no visible loss of the distal 7q region (fig 2D). The proband's karyotype was  $46,XX,ish\ t(7;13)(q21.2;q33)(wcp7+,tel7q-;wcp7+,tel7q+)$ .

#### CASE 3

During the first pregnancy of a young, unrelated couple, ultrasonography performed at 23 weeks showed a severe cystic brain malformation, a large cleft lip, and a left club foot. Termination of pregnancy was proposed and accepted at 23.5 weeks. The macerated female fetus showed growth retardation ( $-3$  SD), enlarged head and fontanelles, premaxillary agenesis, bilateral microphthalmia, and a left club foot (fig 4B). Internal examination showed bilateral renal hypoplasia, severe adrenal hypoplasia, and abnormal pulmonary lobulation. Neuropathological examination showed a deliquescent brain with alobar holoprosencephaly.

Cytogenetic study on amniotic fluid showed a deletion of the long arm of chromosome 7,  $46,XX,del(7)(q32)$  (fig 4A). The maternal karyotype was normal but paternal chromosome analysis was not performed.

#### CASE 4

This child, a girl, was born to 32 and 31 year old, non-consanguineous parents who already had a normal daughter and had had three spontaneous miscarriages. During the pregnancy, ultrasound examination at 14 weeks of gestation showed holoprosencephaly, a flat face, and a cardiac malformation (ventriculoatrial septal defect) in an otherwise normal fetus.

Fetal karyotyping was performed on an amniotic fluid cell culture and showed a female karyotype with a derivative chromosome 7. Parental karyotypes were then investigated. The father's karyotype was normal but the mother's chromosomes displayed two reciprocal translocations,  $46,XX,t(1;7)(q41;q35)t(8;9)(q24.23;q22.1)$  (fig 5A). Hence, the fetus was trisomic for the chromosome 1q41-qter region and monosomic for the distal portion of chromosome 7q. The fetal karyotype was  $46,XX,der(7)t(1;7)(q41;q35)$  (fig 5B). The parents decided to continue the pregnancy and the child died two hours after delivery.

External examination of the female fetus showed growth retardation ( $-4$  SD), craniofacial dysmorphism including microcephaly, cyclopia with proboscis, microstomia, low set, dysplastic ears, and a short neck, and bilateral camptodactyly (fig 5C).

Visceral dissection showed ambiguous thoracic and abdominal situs with a complex heart malformation, enlarged kidneys with hydronephrosis, and bilateral adrenal hypoplasia. Skeletal x ray examination showed 11 pairs of

ribs and partial sacral agenesis. Examination of the brain confirmed the alobar holoprosencephaly.

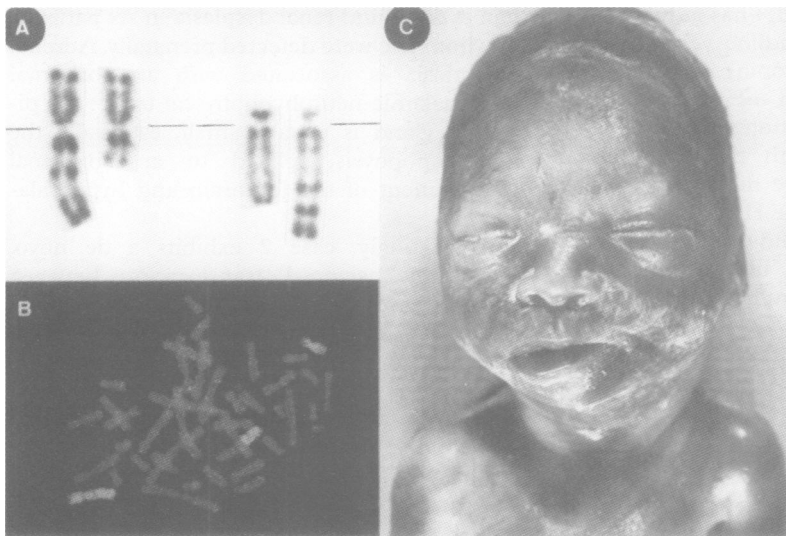


Figure 3 Case 2. (A) Partial R banded karyotype showing the apparently balanced de novo translocation  $t(7;13)(q21.2;q33)$ . (B) Chromosome 7 painting confirming the translocation. (C) Face of the proband at 22.5 weeks of gestation.

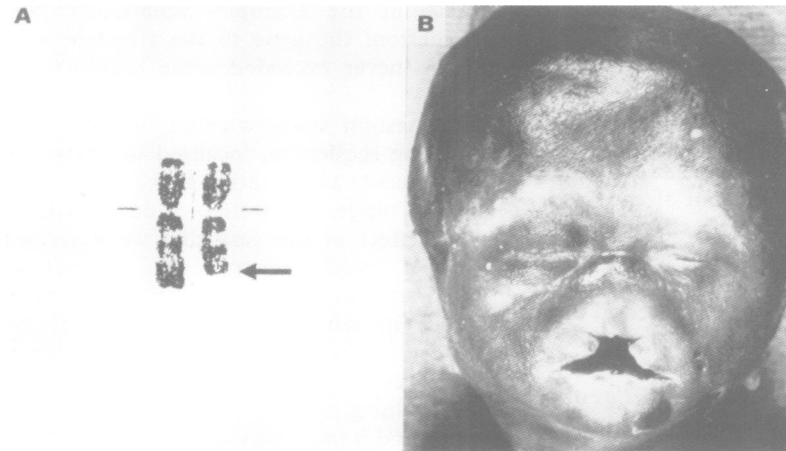


Figure 4 Case 3. (A) Partial R banded karyotype showing the abnormal chromosome 7 with deletion of 7q32-qter (arrow). (B) Face of the fetus at 23.5 weeks of gestation.

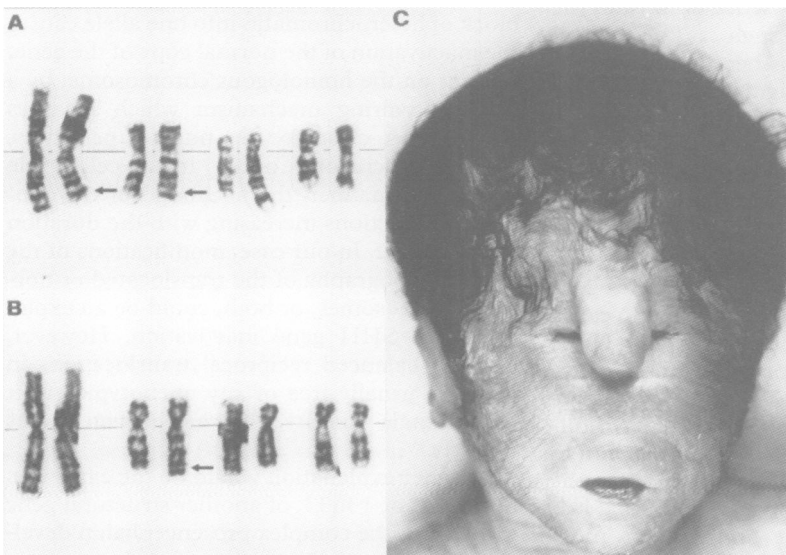


Figure 5 Case 4. (A) Maternal translocations  $t(1;7)(q41;q35)$  (arrows) and  $t(8;9)(q24.23;q22.1)$  (G banding). (B) Partial G banded fetal karyotype showing the unbalanced segregation of the  $t(1;7)$  translocation leading to monosomy of the 7q35-qter region (arrow) and trisomy of the 1q41-qter segment. (C) Face of the proband at birth.

## Discussion

Our report of three new cases of terminal deletion of the long arm of chromosome 7 (cases 1, 3, and 4) confirm the close association between the loss of this chromosomal region and the onset of developmental anomalies of the prosencephalon, leading to holoprosencephaly (HPE3), and of the notochord. This association has already been well documented by many observations made in newborn infants<sup>4-8</sup> or in fetuses,<sup>7-11</sup> including malsegregations of parental translocations or de novo deletions.

Physical mapping of the distal part of chromosome 7q (7q32-qter) has been performed, using 41 DNA markers previously mapped in this region, in 13 cases of 7q terminal or interstitial deletions<sup>12</sup>: the minimal critical region of deletion overlap in patients with HPE has been assigned to 7q36 between markers D7S292 and D7S392. Interestingly, three patients with deletions encompassing the critical region have shown mild midline structural defects, such as microcephaly, highly arched palate, or dysmorphic features, suggesting genetic heterogeneity or a complex regulation pattern of the gene(s) involved in HPE.

Familial HPE, with cytogenetically normal chromosomes, has also been reported in a number of families exhibiting autosomal dominant or apparently autosomal recessive modes of inheritance. A high degree of phenotypic variability has been observed in autosomal dominant forms of HPE with expression in obligatory gene carriers ranging from alobar HPE and cyclopia to features such as microcephaly, ocular hypotelorism, or single central upper incisor, or even to normal phenotypes.<sup>1,2</sup>

Linkage studies, performed in 125 subjects from nine families with autosomal dominant HPE, have shown a close association of the malformation with DNA marker D7S22, localised in 7q36, between markers D7S292 and D7S392, which define the minimal critical region.<sup>13</sup> This linkage was excluded in one family, confirming the genetic heterogeneity in HPE, although affected members or obligatory gene carriers in this family showed the same level of clinical variability as the eight other families.

The molecular analysis of the 7q36 chromosomal region has led to the isolation of a number of putative candidate genes responsible for the pathogenesis of HPE3. The human homologue of the rat RHEB gene (ras homologue enriched in brain) has been isolated and mapped to 7q36 by Mizuki *et al.*<sup>14</sup> Considering the chromosomal localisation of this gene as well as the potential function of the protein it encodes, a growth factor and synaptic action regulated protein, the authors have emphasised that hRHEB may be a strong candidate for the HPE3 gene.

More recently, the implication of a particular class of genes involved in normal embryonic development, hedgehog (HH), has been suggested: mice deficient in one of the three mammalian hedgehog homologues, Sonic Hedgehog (Shh), exhibit malformations of the frontal area of the brain and deformed skeletons and die around birth.<sup>15</sup> The study of 37 patients

with 7q36 chromosomal rearrangements led Belloni *et al*<sup>6</sup> to localise the human SHH gene within a 500 kb critical region, between D7S392 and D7S3029, which is free of any other candidate genes. SHH has inductive patterning effects on the midline ventral central nervous system, the developing limb buds, and the ventral specification of somites: these relevant biological functions and the close association of SHH with the translocation breakpoints analysed have made this gene an excellent candidate for the HPE3 locus.<sup>16</sup> This was confirmed by the study of 30 autosomal dominant HPE families, five of which were found to segregate different heterozygous SHH mutations.<sup>17</sup>

Cases 1, 3, and 4 of our report have deletions of one SHH allele which is consistent with the fact that alterations in the SHH gene lead to dominant effects on human development whereas, in the mouse, the consequences of *Shh* deletions have always been observed at the homozygous level.<sup>15</sup> In our patients, such deletions led to semilobar (case 1) or alobar (cases 3 and 4) holoprosencephaly, confirming the phenotypic variability in HPE. Moreover, fetuses 1 and 4 showed some form of partial sacral agenesis (SA), a rare disorder that is usually sporadic and commonly associated with maternal diabetes.<sup>18</sup> This malformation has already been described in several cases of chromosome 7q deletions including those where HPE was present. In 15 cases where a terminal deletion of the long arm of chromosome 7 was associated with HPE, signs of caudal deficiency were found in nine.<sup>10</sup> Haplotype analysis of this region in two families with an autosomal dominant form of SA has led to the localisation of one or more gene(s) in 7q36, between D7S396 and the telomere.<sup>19</sup>

Therefore, the question arises whether or not SA and HPE are both caused by mutations in the SHH gene. In addition to severe defects in the forebrain region, homozygous mutant *Shh*<sup>-/-</sup> mouse embryos exhibit anomalies of the axial skeleton including absence of most of the sclerotomal derivatives, such as the spinal column.<sup>15</sup> *Shh* protein has been implicated as the notochord derived signal responsible for induction of the sclerotome<sup>20</sup>; in mutant mice, absence of the sclerotome promoting effect of *Shh* would act directly on the modulation of other genes, such as the Pax family genes.<sup>15</sup> In man, mutation of one SHH allele leads to a mechanism of haploinsufficiency which is sufficient to disturb ventral midline neurogenesis and to cause HPE, but insufficient to cause ventralisation defects of sclerotome.<sup>17</sup> These differences between homozygous mutant mice and heterozygous mutant human patients might be explained by the requirement for different concentrations of SHH protein to produce various biological responses.<sup>17</sup> On the other hand, in chromosome 7q deletions, loss of both one SHH allele and one or more gene(s) responsible for SA and localised in the vicinity of SHH would lead to more important impairment of sclerotome induction and, consequently, to caudal regression syndrome.

Associated malformations include renal dysplasia (cases 1, 3, and 4) or adrenal hypoplasia (cases 3 and 4) which are common features in fetuses with HPE. In 27 cases of HPE, McGahan *et al*<sup>21</sup> found renal dysplasia in six patients, of whom five were detected prenatally. Adrenal hypoplasia is associated with an abnormal hypothalamic-neurohypophyseal unit<sup>22</sup>: the pituitary gland is usually small, especially the neurohypophysis, related to an abnormal development of the forebrain and hypothalamus.

Interestingly, case 2 exhibits a *de novo*, apparently balanced translocation between chromosomes 7 and 13, with a chromosome 7q breakpoint localised in 7q21.2, several million base pairs from the SHH locus. Although they are localised in the 500 kb critical region, none of the four translocation breakpoints described by Belloni *et al*<sup>6</sup> disrupts the SHH gene. A long range position effect has been suggested, decreasing SHH expression by separating *cis* acting regulatory elements or changing the gene neighbourhood by the juxtaposition of a silencing chromatin region. Such position effects have been recently reviewed by Bedell *et al*<sup>23</sup> but, in the examples mentioned, the distance from the gene to the chromosomal alteration never exceeded some hundreds of kilobases.

The question arises whether or not *cis* or *trans* acting sequences, localised in the same DNA molecule as the target gene, can exert a very long range effect that could explain a position effect in the case that we describe. This effect would implicate a particular action on chromatin structure as suggested by other examples in which deletions of regulatory sequences, mediating both chromatin structure and enhancer functions, can induce the silencing of distant genes.<sup>23</sup> Evidence for long range chromosome interactions and their impact on three dimensional nuclear architecture has been reported in *Drosophila*<sup>24</sup>: in brown dominant (*bw*<sup>d</sup>) mutants, the insertion of a large block of heterochromatin into one allele causes the inactivation of the normal copy of the gene, present on the homologous chromosome, by a physical pairing mechanism which localises both alleles close to the nuclear periphery. These associations occur in a cell cycle dependent manner, the likelihood of chromosome interactions increasing with the duration of interphase. In our case, modifications of the nuclear topography of the translocated or normal chromosomes, or both, could be an explanation of SHH gene inactivation. However, because balanced reciprocal translocations in man are usually free of any phenotypic effect except male sterility, this mechanism seems unlikely.

Another explanation would be the existence, in 7q21.2 or 13q33, of another structural gene involved in the complex prosencephalon development process. Disruption of such a gene or separation from its regulatory sequences by the translocation breakpoint would be responsible for the occurrence of holoprosencephaly.

The authors thank M Catala for brain examination of case 2, P Bitoun for useful paediatric advice concerning holoprosencephaly, and E Prieto for artwork.

- 1 Cohen MM. Perspectives on holoprosencephaly. Part I. Epidemiology, genetics and syndromology. *Teratology* 1989;40:211-35.
- 2 Cohen MM. Perspectives on holoprosencephaly. Part III. Spectra, distinctions, continuities and discontinuities. *Am J Med Genet* 1989;34:271-88.
- 3 Frezal J, Schinzel A. Report on the Committee on clinical disorders, chromosome aberrations and uniparental disomy. *Cytogenet Cell Genet* 1991;58:986-1052.
- 4 Schinzel A. Cyclopia and cebocephaly in two newborn infants with unbalanced segregation of a familial translocation rcp (1;7) (q32;q34). *Am J Med Genet* 1984;18:153-61.
- 5 Bürrig KF, Gebauer J, Terinde R, Pfitzer P. Case of cyclopia with an unbalanced karyotype attributable to a balanced 3/7 translocation. *Clin Genet* 1989;36:262-5.
- 6 Bogart MH, Cunniff C, Bradshaw C, Jones K, Jones OW. Terminal deletions of the long arm of chromosome 7: five new cases. *Am J Med Genet* 1990;36:53-5.
- 7 Lurie IW, Ilyina HG, Podlieschuk LV, Gorelik LB, Zaletajev DV. Chromosome 7 abnormalities in parents of children with holoprosencephaly and hydronephrosis. *Am J Med Genet* 1990;35:286-8.
- 8 Hatzioannou AG, Krauss CM, Lewis MB, Halazonetis TD. Familial holoprosencephaly associated with a translocation breakpoint at chromosomal position 7q36. *Am J Med Genet* 1991;40:201-5.
- 9 Kleczkowska A, Fryns JP, Moerman P, Vandenberghe K, Van Den Berghe H. Holoprosencephaly in a fetus with a 46,XX,der(7),t(7;8)(q36.1;p12)mat karyotype. *Ann Genet* 1990;33:111-12.
- 10 Morichon-Delvallez N, Delezoide AL, Vekemans M. Holoprosencephaly and sacral agenesis in a fetus with a terminal deletion 7q36-7qter. *J Med Genet* 1993;30:521-4.
- 11 Pluchon E, Giovangrandi Y, Labbe F, et al. Prenatal diagnosis of a fetus with partial monosomy 7 (q34-qter) and partial trisomy 18 (q21-qter). *Prenat Diagn* 1993;13:983-8.
- 12 Gurrieri F, Trask BJ, Van den Engh G, et al. Physical mapping of the holoprosencephaly critical region on chromosome 7q36. *Nat Genet* 1993;3:247-51.
- 13 Muenke M, Gurrieri F, Bay C, et al. Linkage of human brain malformation, familial holoprosencephaly, to chromosome 7 and evidence for genetic heterogeneity. *Proc Natl Acad Sci USA* 1994;91:8102-6.
- 14 Mizuki N, Kimura M, Ohno S, et al. Isolation of cDNA and genomic clones of a human Ras-related GTP-binding protein gene and its chromosomal localization to the long arm of chromosome 7, 7q36. *Genomics* 1996;34:114-18.
- 15 Chiang C, Litingtung Y, Lee E, et al. Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* 1996;383:407-13.
- 16 Belloni E, Muenke M, Roessler E, et al. Identification of *Sonic hedgehog* as a candidate gene responsible for holoprosencephaly. *Nat Genet* 1996;14:353-6.
- 17 Roessler E, Belloni E, Gaudenz K, et al. Mutations in the human *Sonic hedgehog* gene cause holoprosencephaly. *Nat Genet* 1996;14:357-60.
- 18 Harlow CL, Partington MD, Thieme GA. Lumbosacral agenesis: clinical characteristics, imaging and embryogenesis. *Pediatr Neurosurg* 1995;23:140-7.
- 19 Lynch SA, Bond PM, Copp AJ, et al. A gene for autosomal dominant sacral agenesis maps to the holoprosencephaly region at 7q36. *Nat Genet* 1995;11:93-5.
- 20 Fan CM, Porter JA, Chiang C, Chang DT, Beachy PA, Tessier-Lavigne M. Long range sclerotome induction by *Sonic Hedgehog*: direct role of the amino-terminal cleavage product and modulation by the cyclic AMP signaling pathway. *Cell* 1995;81:457-65.
- 21 McGahan JP, Nyberg DA, Mack LA. Sonography of facial features of alobar and semilobar holoprosencephaly. *AJR* 1990;154:143-8.
- 22 Verloes A, Ayme S, Gambarelli D, et al. Holoprosencephaly-polydactyly (pseudotrisonomy 13) syndrome: a syndrome with features of hydroletharus and Smith-Lemli-Opitz syndromes. A collaborative multicentre study. *J Med Genet* 1991;28:297-303.
- 23 Bedell MA, Jenkins NA, Copeland NG. Good genes in bad neighbourhoods. *Nat Genet* 1996;12:229-32.
- 24 Dernburg AF, Broman KW, Fung JC, et al. Perturbation of nuclear architecture by long-distance chromosome interactions. *Cell* 1996;85:745-59.