

Brains and faces in holoprosencephaly: pre- and postnatal description of 30 cases

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ABSTRACT

Objective To investigate the prenatal appearance of the holoprosencephaly spectrum.

Methods A database of 1750 fetuses with congenital anomalies identified by ultrasound was prospectively collected from 1987 to 2000. Among them, 30 cases (1.7%) with holoprosencephaly were prenatally identified and described.

Results The prevalence of holoprosencephaly in the Health Region of the National Center for Fetal Medicine in Norway was 1.26 : 10 000; the sex distribution (male : female) was 1.4 : 1. Holoprosencephaly was found in one dichorionic twin pregnancy and one pair of conjoined twins. Among the 30 cases of holoprosencephaly, 18 were alobar, five were semilobar, two were lobar, two were lobar variants, and three were anencephalic. The facial features varied considerably. Sixty-seven per cent (20/30) had associated structural anomalies that were not related to the cerebral and facial holoprosencephaly condition. Thirty-seven per cent (11/30) had detectable chromosome aberrations and 23% (7/30) had nonchromosomal syndromal origin. The size or shape of the head was abnormal in 83% (25/30) of holoprosencephaly cases.

Conclusion This study indicates that holoprosencephaly represents a heterogeneous entity with different etiologies and clinical appearances. The fact that holoprosencephaly features are found associated with particular conditions such as fronto-nasal dysplasia (2/30; 6.7%), agnathia-otocephaly (3/30; 10%), and anencephaly (3/30; 10%), suggests that these may be underreported conditions in other large holoprosencephaly series.

INTRODUCTION

Holoprosencephaly (HPE) is a heterogeneous entity of central nervous system anomalies caused by the impaired midline cleavage of the embryonic forebrain. In most cases it is associated with facial anomalies, with cyclopia representing an extreme variant.

Today, the categorization introduced by DeMyer and Zeman in 1963¹ and modified by DeMyer in 1977² is generally accepted. Holoprosencephaly is graded according to the severity of the brain anomaly as alobar, semilobar or lobar³. In the alobar form, only one single ventricle (holosphere) of variable size is present, lacking interhemispheric diversion. At the back, a membrane usually closes the holosphere. This membrane may bulge dorsally and form a fluid-filled 'dorsal sac'. The thalami and corpora striata are undivided across the midline; the olfactory tracts and bulbs and the corpus callosum are always absent. In semilobar HPE, rudimentary cerebral lobes with an interhemispheric posterior fissure may be present. The olfactory tracts and bulbs are absent or hypoplastic and the corpus callosum is rudimentary. In lobar HPE, some midline continuity may be present in spite of a distinct interhemispheric fissure. The olfactory tracts and bulbs may be absent, hypoplastic or normal. Midline cleavage of the thalami and the corpora striata may be incomplete.

The facial anomalies are usually categorized into four main types⁴:

- 1 cyclopia with a single eye or various degrees of doubling of the eye anlage, with or without proboscis;
- 2 ethmocephaly with ocular hypotelorism and proboscis located between the eyes;
- 3 cebocephaly with ocular hypotelorism and single-nostril nose;
- 4 median cleft lip and palate (premaxillary agenesis) and ocular hypotelorism.

Holoprosencephaly has been known since antiquity through the figure of the cyclopean shepherd Polyphemos in Homer's *Odyssey* (c. 800 BC). Throughout antiquity until the 17th century, the occurrence of a cyclopic newborn, whether it was a human or an animal, was associated with mystic and fabulous narrations^{5,6}; not until the 18th century were HPE and other anomalies recognized to be congenital conditions, and HPE cases were collected and described scientifically^{6,7}.

The prenatal recognition of HPE by ultrasound was first described by Kurtz *et al.* in 1980⁸, and has since been documented in many case reports and studies. Studies on postnatal HPE have shown a large variety of the clinical expressions of this condition⁵. There is reason to believe that the full range of this variety of expression of HPE and associated anomalies has not yet been described in prenatal studies.

We describe the sonographic appearance and the pre- and postnatal clinical data of HPE in a study group of 30 fetuses. We compare our findings with results from prenatal and postnatal studies and reviews, and discuss epidemiological aspects and etiological alternatives responsible for the HPE spectrum.

MATERIALS AND METHODS

During the period January 1987–December 2000, all sonographically detected cases of fetal anomalies were prospectively registered at the National Center for Fetal Medicine in Trondheim, Norway. The registrations included data about blood sampling, karyotype, ultrasound and X-ray examination, delivery, outcome and surgical treatment or autopsy reports. Norway is divided into five health regions. The National Center for Fetal Medicine is the referral center for prenatal diagnosis and therapy of all fetal anomalies detected in Health Region IV, and the tertiary referral center for the rest of Norway.

Of a total of 1750 cases with fetal anomalies detected prenatally by ultrasound, 360 had anomalies of the central nervous system. Thirty fetuses (1.7% of the total and 8.3% of those with central nervous system anomalies) were included in the study group, presenting facial anomalies such as cyclopia, proboscis/probosces, single-nostril nose, median cleft lip and palate, and/or alobar, semilobar or lobar HPE. Cases with isolated arrhinencephaly or septo-optical dysplasia were not considered to be part of the HPE spectrum and were therefore not included. For the calculation of the prevalence of HPE in Health Region IV, all local hospitals were contacted for information about prenatally missed cases.

All statements of gestational age including the age of embryos are expressed in completed weeks plus days based on the last menstrual period.

RESULTS

Pregnancies and outcome

The mean age of the mothers at the time of detection was 27 (range, 18–43) years. The mean gestational age at detection at the National Center for Fetal Medicine was 21⁺³ (range, 9⁺²–37⁺²) weeks, and at delivery it was 23⁺² (range, 12⁺¹–

39⁺³) weeks. Twenty-six of 30 (87%) fetuses/infants were delivered/terminated in Trondheim, while four were delivered at other hospitals.

Termination of the pregnancy was performed in 23 of the 30 cases (73%). One fetus died *in utero* and four died during or shortly after delivery. One child died at the age of 1 year 6 months. One child with frontonasal dysplasia was born in 1999, and was still alive at the age of 24 months. Twenty-seven autopsies were performed. In three cases, when the parents refused autopsy, information was based on prenatal ultrasound observations, laboratory tests and postnatal inspection.

Central nervous system and facial anomalies

The cerebral and facial features of the fetuses are described in Tables 1 and 2, and Figures 1–8. There were 18 alobar (60%), five semilobar (17%) and two lobar (6.7%) cases of HPE. In alobar HPE, the relative size of the holosphere and of the dorsal sac varied considerably. Two fetuses (Cases 15 and 16; Figures 2 and 3) presented agnathia-otocephaly and alobar HPE. A further two cases with fronto-nasal dysplasia (Figures 4 and 5) showed a variant of lobar HPE (6.7%), and three fetuses presented anencephaly (10%) (Table 1; Figures 6–8).

Except for two of the anencephalic fetuses and one growth-restricted hydrocephalic fetus (Case 25), HPE was assumed prenatally in all cases. The two anencephalic fetuses had rudimentary orbits, which were very close together (Figure 6). In a third case of anencephaly, which was detected at 13⁺³ weeks, the finding of proboscis-like facial features raised

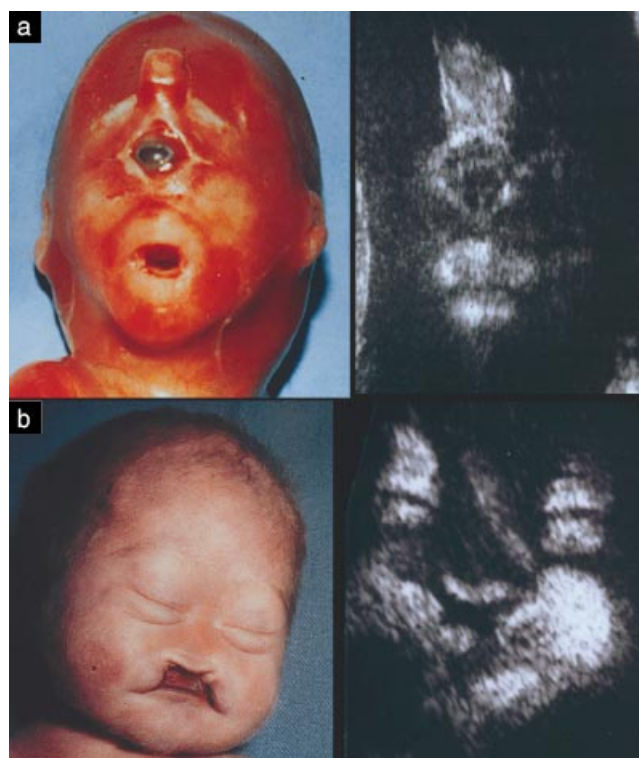


Figure 1 Postabortem photographs and ultrasound images of faces of holoprosencephalic fetuses: cyclopia and proboscis in a fetus with alobar holoprosencephaly (a, Case 9) and premaxillary agenesis, absent nose, and hypotelorism in a fetus with semilobar HPE (b, Case 19).

Table 1 The cerebral features in 30 cases with holoprosencephaly

Case	HPE type	Hemispheres	Dorsal sac	Corpus callosum	Olfactory tract
1	Alobar	Small holosphere	–	–	–
2	Alobar	Small holosphere	–	–	–
3	Alobar	Holosphere	–	–	–
4	Alobar	Small holosphere	–	–	–
5	Alobar	Holosphere	ND	–	–
6	Alobar	Holosphere	ND	–	ND
7	Alobar	Holosphere	–	–	–
8	Alobar	Holosphere	Large	–	ND
9	Alobar	Holosphere	–	–	–
10	Alobar	Small holosphere	–	–	–
11	Alobar	Small holosphere	Large	–	–
12	Alobar	Hydrocephaly	Large	–	–
13	Alobar	Small holosphere	Large	–	–
14	Alobar	Small holosphere	+	–	–
15	Alobar	Holosphere	+	–	–
16	Alobar	Holosphere	Small	ND	ND
17	Alobar	Holosphere	+	–	ND
18	Alobar	Holosphere	+	–	–
19	Semilobar	Fused anteriorly	–	–	–
20	Semilobar	Fused anteriorly	–	–	+
21	Semilobar	Fused anteriorly	–	–	–
22	Semilobar	Fused anteriorly	–	–	–
23	Semilobar	Fused anteriorly	–	–	–
24	Lobar	+	–	Hypoplastic, CSP –	–
25	Lobar	+	–	Hypoplastic	–
		Hydrocephaly, asymmetric hemispheres, fronto-nasal dysplasia			
26	Lobar variant	Hydrocephaly, common ventricle, fronto-nasal dysplasia	–	–, CSP –	ND
27	Lobar variant	fronto-nasal dysplasia	–	–, CSP –	
28	Anencephalic	–			–
29	Anencephalic	–			–
30	Anencephalic	–			–

HPE, holoprosencephaly; –, absent; +, present; CSP, cavum septi pellucidi; ND, not described.

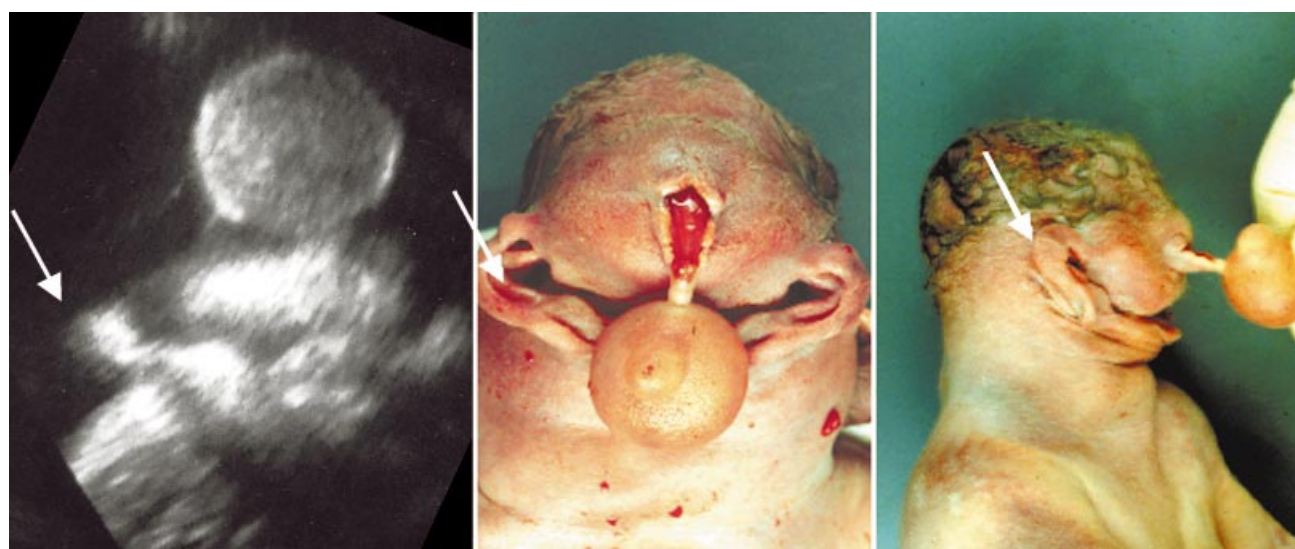


Figure 2 Ultrasound image and postabortem specimen of agnathia-otocephaly and the tumor-like proboscis in Case 15; the arrows point to the ear anlagen.

the suspicion of HPE in combination with anencephaly. This fetus had agnathia-otocephaly with cyclopia (Figures 7 and 8).

Cyclopia was present in eight cases; five of these had a single eye anlage (Figure 1a), while three had double eye anlagen (Table 2). Hypotelorism was found in 14 cases. Case 17 was



Figure 3 Dicephalus dipus tribrachius with agnathia-otocephaly of the right head (Case 16); this head had alobar holoprosencephaly.

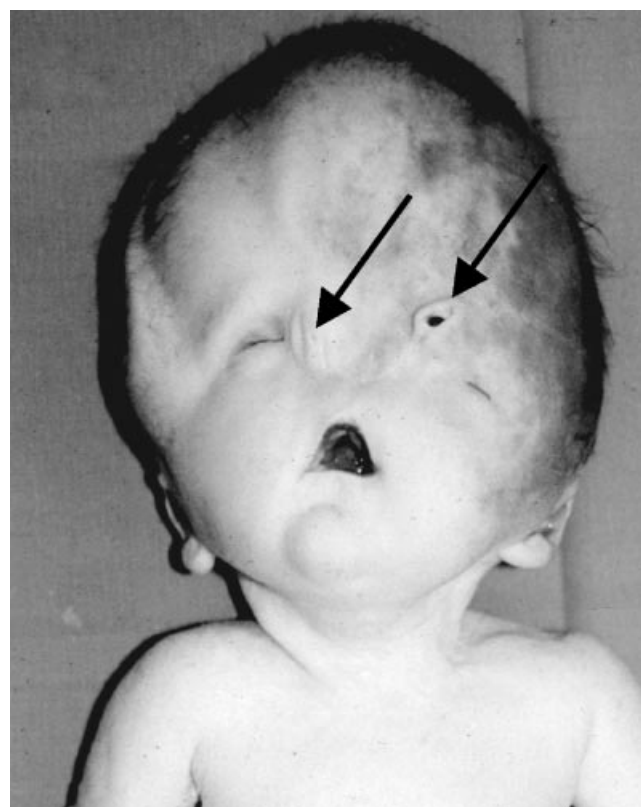


Figure 4 Face of Case 26 with fronto-nasal dysplasia; the arrows point to the nostrils. Hypertelorism is present.

Table 2 Facial appearance in 30 cases with holoprosencephaly

Case	Eyes/orbits	Eye anlagen	Nose/proboscis	Mouth
1	Cyclopia	1	–	Small
2	Cyclopia	2	Proboscis	Small
3	Hypotelorism	2	Normal	Micrognathia
4	Hypotelorism	2, small orbits	–	Median cleft lip and palate
5	Hypotelorism	2	–	Median cleft lip and palate
6	Hypotelorism	2	–	Median cleft lip and palate
7	Hypotelorism	2	–	Small
8	Cyclopia	2	Proboscis	Small
9	Cyclopia	1	Proboscis	Small
10	Hypotelorism	2, large orbits	Normal	Small
11	Cyclopia	1	–	Median cleft lip and palate
12	Hypotelorism	2	Small	Normal
13	Cyclopia	2	Proboscis	Small
14	Cyclopia	1	Proboscis	Small
15	NI	NI	Proboscis, tumor-like	Agnathia-otocephaly
16	Hypotelorism	2	Proboscis	Agnathia-otocephaly
17	Hypotelorism	2	Normal	Normal
18	Hypertelorism, exophthalmus	2	–	Median cleft lip and palate
19	Hypotelorism	2	–	Median cleft lip and palate
20	Hypotelorism	2	Normal	Normal
21	Hypotelorism	2	–	Median cleft lip and palate
22	Hypertelorism	2	Small	Normal
23	Hypotelorism	2, small orbits	–	Median cleft lip and palate
24	Hypotelorism	2	Normal	Micrognathia
25	Normal	2	Small	Normal
26	Hypertelorism	2	Split nose/face	Triangular
27	Hypertelorism	1	Single nostril, split face	Triangular
28	Rudimentary orbits	NI	Single nostril	Small
29	Rudimentary orbits	NI	–	Median cleft lip and palate
30	Cyclopia	1	2 proboscides	Agnathia-otocephaly

NI, not identified; –, absent.

a boy with an alobar HPE; his mental development was seriously retarded. He died at the age of 1.5 years from complications after a shunt operation for hydrocephaly. Cases 26 and 27 had a variant of lobar HPE and showed hypertelorism as part of the split-face syndrome (fronto-nasal dysplasia; Figures 4 and 5). Case 26 presented no development of the corpus callosum and absent olfactory tracts. The fetus had extensive hydrocephaly with a large open communication between the hemispheres. The nose was split; one eye was only rudimentarily developed. Case 27 was still alive at the age of 24 months. The child had a common wide ventricular system without clear separation

into lateral ventricles/hemispheres. The thickness of the cortex varied and its contours were irregular. The thalami were fused. There was leakage of cerebrospinal fluid through a hole in the anterior calvarium at the forehead. The right orbit was atrophic and the eye was absent.

Proboscides were present in association with cyclopia in six cases. One of these had agnathia HPE with anencephaly (Figures 7 and 8), and one had it with hypotelorism. Median cleft lip and palate, also termed premaxillary agenesis, was seen together with hypotelorism (six cases; Figure 1b), hypertelorism (two cases), cyclopia (one case), and anencephaly (one case; Figure 6b).

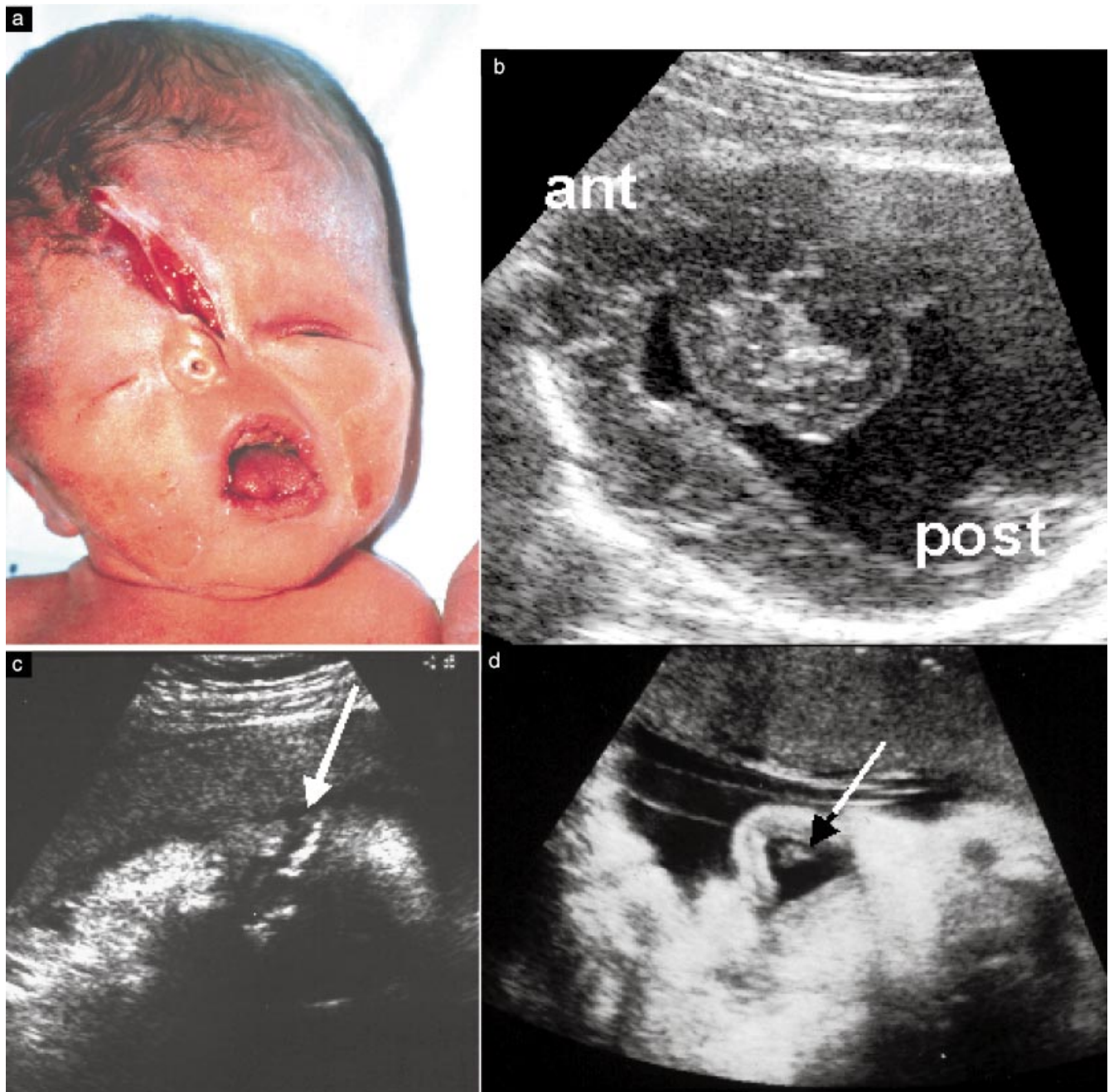


Figure 5 Fronto-nasal dysplasia (Case 27) showing one nostril, two eye anlagen, and a split forehead (a). In the horizontal ultrasound section through the brain (b), there is no falx, the brain seems disorganized, and there is one common ventricle. In the tangential ultrasound section through the fetal forehead (c), the arrow points to the split bony forehead. In the ultrasound image of the mouth (d): the upper lip is arched; the arrow points to the anterior palate.

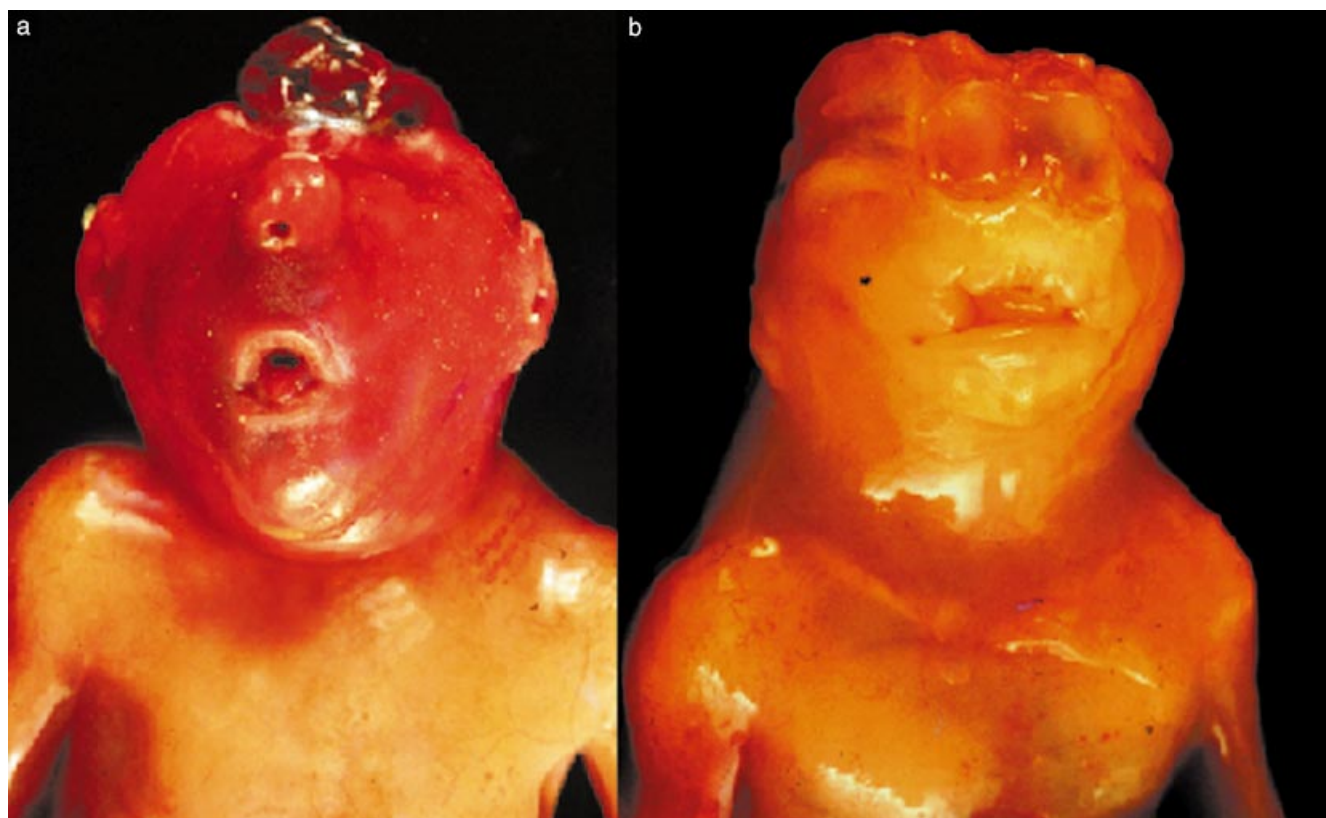


Figure 6 Postabortem images of holoprosencephalic fetuses with anencephaly: single nostril in Case 28 (a) and the premaxillary agenesis, absent nose, and rudimentary orbits lying close together in Case 29 (b)⁵⁸.

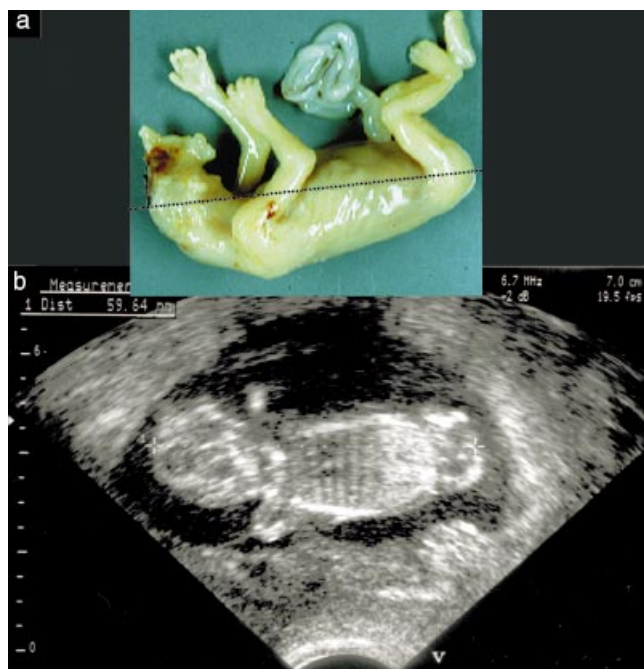


Figure 7 Anencephalic fetus (Case 30) at 13 weeks (a); it had a crown-rump length of 60 mm; the broken line indicates the coronal section of the ultrasound image in (b).

Head size in holoprosencephaly

Except for three fetuses with anencephaly (10%), measurements of the size of the head were made using biparietal

diameter (BPD) (Table 3). Five fetuses (17%) had a BPD ± 4 mm corresponding to the mean BPD of the relevant last menstrual period-based pregnancy length. In 17 cases (57%) the BPD values were 5–37 mm less than the corresponding mean. Eight of them had abnormal chromosomes and the other nine proved to have normal chromosomes. The head was ≥ 5 mm larger than the normal mean in five fetuses (17%): Case 12 with alobar HPE and a large dorsal sac and Cases 26 and 27 with fronto-nasal dysplasia, had increased cerebrospinal fluid, which gave the impression of hydrocephaly. Case 14, with alobar HPE, and Case 24, with lobar HPE, showed moderately increased BPD (+5 mm).

Karyotype and sex

A karyotype was obtained for 29 (97%) fetuses; 11 had abnormal chromosomes (Table 4). The male : female sex ratio for all cases was 1.4 : 1.0. Among the cases with detectable chromosome aberrations the male preponderance was 1.2 : 1.0.

Associated anomalies and syndromes

Table 5 shows the variety of associated structural anomalies found together with HPE. Twenty-one fetuses (70%) had associated structural anomalies, which were found in addition to cerebral and facial HPE features. Most associated anomalies were found in fetuses with abnormal chromosomes. Apart from anomalies of the head including brain

and face, the distribution of structural anomalies was (abnormal chromosomes in brackets): two vertebral (1), nine cardiovascular (3), three pulmonary (0), five body wall and diaphragm (2), one gastrointestinal (1), nine genitourinary (4), three hydrops or hygroma (0), five hypoplastic

adrenal glands (4) and three single umbilical artery (2). Anomalies of the limbs were found in 12 fetuses: five polydactyly (1), two reduction/aplasia of fingers (1), two syndactyly (0), one pes equinovarus (0), one abnormally positioned foot (1) and one rockerbottom feet (1). Structural

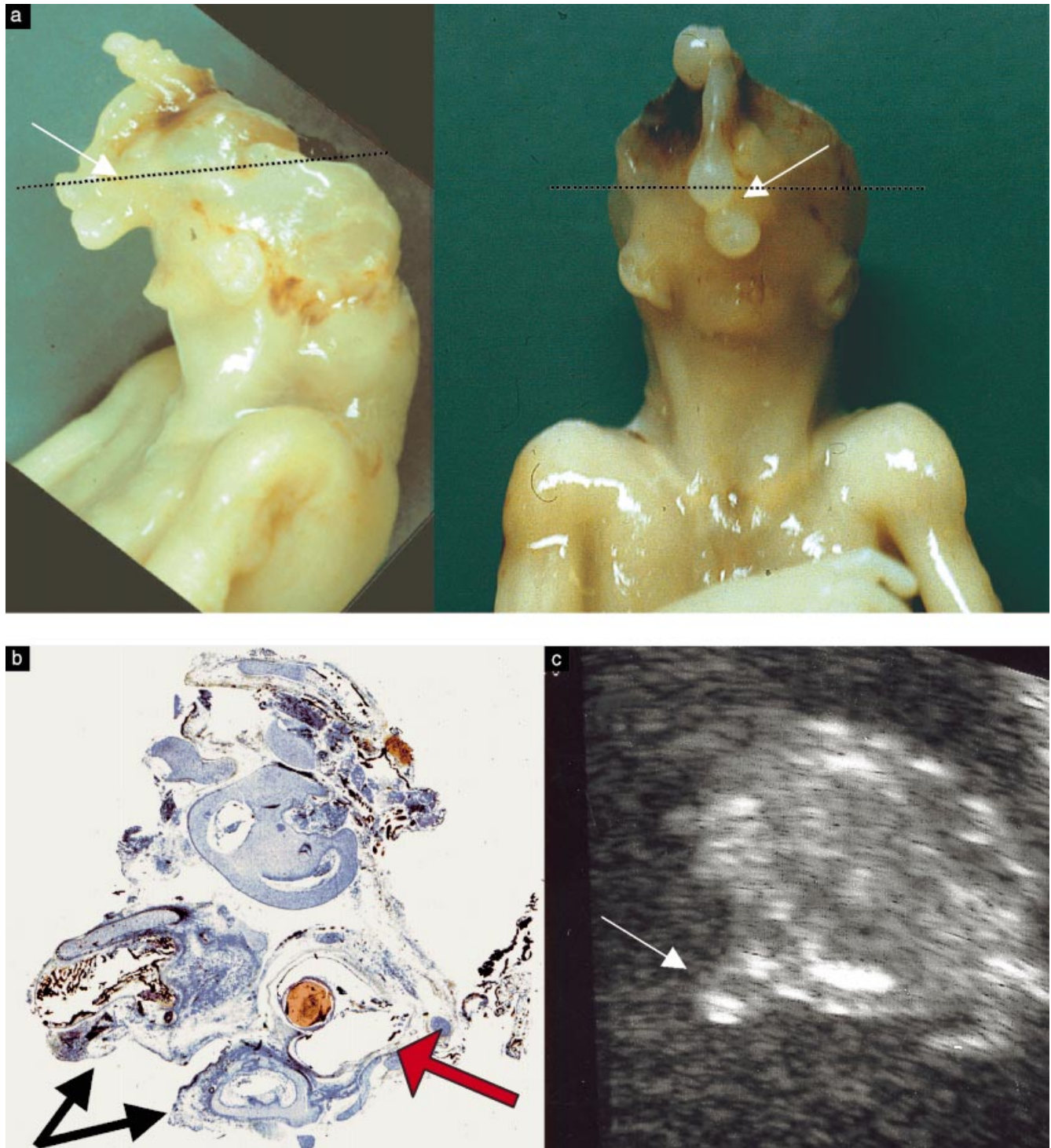


Figure 8 Photographs of the head of Case 30 (a), showing agnathia-otocephaly and two proboscides. The white arrows point out the area where the histological examination (b) revealed a single eye anlage. The broken lines indicate the horizontal section through the head for the ultrasound image (c). The histological slice (b) is of the proboscides (black arrows) and the single eye anlage (red arrow). The proboscides consist of cartilagenous cylinders covered with skin. The upper one is cut longitudinally, the lower one is cut obliquely through its base. The eye anlage contains the red shrunken corpus vitreum. In the horizontal ultrasound section (c) through the head (see broken line in a b), the arrow points to the proboscides.

anomalies associated with conjoined twins are not included in this overview.

Multiple pregnancies

There were two twin pregnancies. One twin in a dichorionic-diamniotic twin pregnancy had semilobar HPE with median cleft lip and palate (Case 19). The nose was absent. One pair of conjoined twins (dicephalus dipus tribrachius) presented one normal head and one head with agnathia-otocephaly HPE (Case 16). In addition to agnathia-otocephaly HPE, this conjoined twin had a cystic enlargement of the pharynx/larynx.

Prevalence of holoprosencephaly

Thirteen women from the study group came from Norwegian Health Region IV; 17 had been referred from institutions from all over Norway. Two additional HPE cases from Health Region IV were detected not prenatally, but shortly after birth. One had lobar HPE (normal chromosomes), and the other had semilobar HPE (trisomy 13). Both died postnatally. In the period from 1987 to 2000, the total number of deliveries (≥ 16 gestational weeks) in Health Region IV was 119 411 fetuses/infants. The prevalence of HPE was calculated to be 1.26 : 10 000 deliveries (15 cases in 119 411 births).

DISCUSSION

This study shows a large variety of prenatal clinical findings and etiological aspects of the HPE spectrum. In order to assess these different aspects and the particular varieties of HPE, it is necessary to refer to the many pre- and postnatal studies and case reports that have been published.

Epidemiology

The populations in studies on HPE differ in terms of the age of the specimens, the time of the detection of HPE, and the number of cases. The largest study is from Japan, where 150 cases with HPE were identified among 36 380 first-trimester induced abortions⁹. This study included embryos with cyclopia, ethmocephaly, cebocephaly and some minor degree of orbital hypotelorism with a flat nose. The histological diagnosis of HPE was made when the embryo was at Carnegie stage 13–23, which corresponds to approximately 6⁺⁰ weeks to 10⁺¹ weeks last menstrual period-based gestational age. The prevalence of HPE was 41 : 10 000. This Japanese study did not classify the types of HPE according to brain anomalies. The karyotype was not known. Associated anomalies were found in 46% (52/113) of the analysable cases, which is less than those found in the present study.

A perinatal study from California involved 121 HPE diagnoses in 1 035 386 live births/fetal death deliveries,

Table 3 Width of the head in 30 fetuses with holoprosencephaly

Case	GA at detection (weeks ^{+days})	Expected BPD (mm)	Measured BPD (mm)	Expected – measured BPD (mm)
1	23 ⁺⁶	63	34	29
2	9 ⁺²	10	10	0
3	19 ⁺⁴	48	41	7
4	15 ⁺¹	31	26	5
5	20 ⁺⁶	53	47	6
6	18 ⁺⁰	44	45	–1
7	21 ⁺³	55	32	23
8	20 ⁺⁴	52	47	5
9	20 ⁺¹	51	39	12
10	24 ⁺⁰	64	35	29
11	19 ⁺⁰	47	41	6
12	34 ⁺¹	91	103	–12
13	18 ⁺³	45	45	0
14	19 ⁺⁰	47	52	–5
15	30 ⁺⁶	84	47	37
16	20 ⁺⁵	53	41	12
17	37 ⁺¹	97	90	7
18	19 ⁺⁴	48	47	1
19	21 ⁺⁰	54	41	13
20	18 ⁺³	45	34	11
21	17 ⁺⁵	43	34	9
22	19 ⁺⁰	47	37	10
23	18 ⁺⁴	45	46	–1
24	33 ⁺⁰	87	92	–5
25	18 ⁺⁶	46	33	13
26	36 ⁺³	95	114	–19
27	37 ⁺²	96	102	–6
28	19 ⁺⁶		Anencephaly	
29	17 ⁺²		Anencephaly	
30	13 ⁺³		Anencephaly	

GA, gestational age; BPD, biparietal diameter.

registered from ≥ 20 weeks' gestational age to 1 year after delivery (prevalence, 1.2 : 10 000). It reported 56/121 (46%) alobar, 24/121 (20%) semilobar and 11/121 (9%) lobar HPE cases, whereas 30 specimens had an undetermined HPE type¹⁰.

Another perinatal study from Scotland investigated 50 cases in 694 950 live births and still births at > 28 weeks' gestation (prevalence, 0.7 : 10 000). It described only 33 HPEs with normal chromosomes in detail, and found 21/33 (64%) alobar, 11/33 (33%) semilobar and 1/33 (3%) lobar subtypes of HPE¹¹.

Roach *et al.*¹² looked at 30 families to determine genetic and environmental factors involved in HPE, excluding cases with abnormal chromosomes. They described 25 (83%) alobar and 7 (23%) lobar HPE cases, and found a prevalence of only 0.63 : 10 000 live and still births.

The comparison of the distribution of HPE types in the present study with those described above is only partly possible, either because the study excluded chromosome aberrations^{11,12} or because it was not possible to determine the HPE type¹⁰. However, it is obvious from postnatal studies that the alobar form is the most common type, while the least severe lobar form is rare. None of the postnatal studies described anencephalic specimens. The California study reported 1/121 (0.8%) cases with agnathia, while we had 3/30 (10%) such cases, one of which was associated with anencephaly.

Many reports describe prenatal diagnosis of HPE (Table 6). The two largest series include 14 cases¹³ and 38 cases¹⁴. Berry *et al.*¹⁴ did not specify the type of HPE. A review of prenatally

diagnosed HPEs^{8,13,15–33} revealed the following subtypes: 61 alobar cases, seven semilobar cases, 12 lobar cases, and one exencephaly case. Since the introduction of transvaginal ultrasound, early diagnosis of HPE in the postembryonic and embryonic phases has become more common. The earliest diagnosis of alobar HPE was made at 9⁺2 weeks¹⁶, the earliest reported diagnosis of semilobar HPE was made at 13 weeks³¹, and the earliest description of lobar HPE was made at 21 weeks³³.

The discrepancy between the embryonic, fetal and postnatal populations indicates a high early first-trimester mortality rate of HPE. In addition, we believe that some HPE cases are not recognized perinatally and, thus, are not registered. Semilobar and lobar HPE cases in particular may be missed because facial signs can be absent. However, Pilu *et al.*^{33,34} have described the sonographic signs of fetal lobar HPE, and have shown that it is feasible to diagnose this condition prenatally. The inclusion of diagnoses until the age of 1 year in the postnatal study carried out in California probably explains the relatively high prevalence and the large share of mild HPE types.

The prevalence numbers cited in the postnatal studies are lower than the prevalence of 1.26 : 10 000 in Norwegian Health Region IV found in the present study. It might be that particular conditions with HPE, such as the two anencephalic cases and two cases with fronto-nasal dysplasia detected in the nonselected population of our health region, have been overlooked or ignored in other studies.

Table 4 The karyotype and sex in 30 cases with holoprosencephaly

Case	Karyotype	Comments
1	46,XY	
2	46,XX,t(8; 14)(p21.1; q24.1)	Apparently balanced translocation
3	45,XY,der(14)t(14; 20)(q13; p13)	Partial monosomy 14q
4	47,XY + 13t (13; 14)	Unbalanced translocation trisomy 13
5	47,XY + 13	Trisomy 13
6	47,XY + 13	Trisomy 13
7	69,XXX	Triploidy
8	47,XX, + 13	Trisomy 13
9	46,XY	
10	46,XY,del(13)(q22)	Monosomy (part 13q)
11	46,XY	
12	46,XX	
13	46,XX	
14	46,XY	
15	46,XY	
16	46,XX	
17	46,XY	
18	46,XY,der(11)t(11; 18)(p15?; q22?) inv(11)(q13?; q13?),der(18)t(11; 18)(p15?; q22?)	Partial monosomy 11?
19	46,XY	
20	46,XY	
21	Karyotyping failed	Male phenotype
22	46,XX,r(13)(p11.2; q21) ring chromosome	
23	46,XX	
24	46,XX	
25	47,XX + 13	
26	46,XY	
27	46,XY	
28	46,XX	
29	46,XX	
30	46,XY	

Environmental influences

The present study showed a remarkable variety of facial and cerebral defects. A common denominator for these anomalies may be defective induction of the prosencephalic neuroepithelium including the neural crest population during embryonic development, which may lead to HPE with its variable facial and cerebral appearance³⁵. Environmental or maternal factors such as diabetes have been associated with HPE³⁶. According to a study from 1983, the approximate risk to infants of diabetic mothers is 1%, a 200-fold greater risk than that of the general population³⁷. Today, the improved treatment of diabetes reduces congenital anomalies in diabetic pregnancies. None of the patients in the present study had diabetes.

Animal studies have revealed that teratogens such as ethanol⁵ and cyclopamine³⁵ may cause HPE. The latter alkaloid exerts its effect along the Sonic hedgehog pathway (see

below). Patient 30 (Figures 7 and 8) had taken drugs, namely lithium and mianserin hydrochloride (tricyclic antidepressant). An association of these drugs with human HPE and anencephaly is not known. However, Rogers³⁸ described cyclopia in chick embryos after exposure to lithium chloride. A study from 1922³⁹, in which leopard frogs were exposed to lithium chloride, resulted in ‘microcephalic to anencephalic embryos with “fused” suckers and olfactory pits’, and HPE in some cases (‘there are probably some cyclopic embryos among these’).

Abnormal karyotype

The present study confirmed the well-known association between HPE and individuals with cytogenetic abnormalities. Certain chromosomes, such as chromosome 13, display recurrent involvement in HPE, and vice versa; HPE may be present in as many as 70% of trisomy 13 cases⁴⁰.

Table 5 Associated structural anomalies and outcome in 30 cases with holoprosencephaly (see also Tables 1 and 2)

Case	Associated anomalies in addition to HPE features	Delivery, outcome
1	None, hypoplastic adrenal glands	TOP
2	None	TOP
3	Dysmorphic face, micrognathia, cleft posterior palate	TOP
4	Medial omphalocele, bilateral polydactyly (hands), hygroma, SUA	TOP
5	VSD, truncus communis, bilateral hydronephrosis, bilateral polydactyly (hands), hydrops, SUA	TOP
6	Diaphragmatic hernia, bilateral hydronephrosis	IUFD, no autopsy
7	Myelomeningocele, low-set ears, unilobar right lung, VSD, hydronephrotic dysplastic kidney, renal agenesis, omphalocele, syndactylies, hypoplastic adrenal glands, molar	TOP
8	Hypoplastic left heart, polydactyly	Spontaneous; died
9	Right hydronephrosis	TOP
10	Small cerebellum, bilateral absent lung lobes, ASD secundum, VSD, overriding aorta, right ventricular hypoplasia, hypospadias, absent scrotum, right displaced small thumb, left aplastic thumb, bilateral syndactylies 4–5	TOP
11	Hypoplastic right ventricle, tricuspid atresia, hypoplastic arteria pulmonalis, hypoplastic adrenal glands	TOP
12	Hydrocephalus, small cerebellum	Induced; died
13	None	TOP
14	Low-set dysmorphic ears, thoracic hemivertebrae, tetralogy of Fallot, rockerbottom feet	TOP
15	None	TOP
16	Dicephalus dipus tribrachius, right head microcephalic, astomia, cyst of larynx; additional defects related to conjoined twins	TOP
17	None	Spontaneous; died at 1 year 6 months
18	Low-set ears	TOP
19	Esophageal atresia, left ureteral stenosis, left hydronephrosis	Spontaneous; died
20	Urethral agenesis, PUV, left kidney dysplasia, right double kidney	TOP
21	Buphthalmia, urethral agenesis, recto-vesical fistula, cystic dysplastic kidneys, large placenta, anhydramnios	TOP
22	Low-set ears, VSD, no lobation of lungs, dysplastic kidneys, pes equinovarus	TOP
23	None	TOP
24	Dysmorphic ears, preauricular tags, micrognathia, coarctation, VSD	TOP
25	Hydrocephalus, dysmorphic face and ears, cleft palate, cystic nuchal hygroma, postaxial polydactyly (hands and feet), hypoplastic left heart/aorta	TOP
26	Hydrocephalus, reduction of fingers and toes, hypoplastic adrenal glands, hyperplastic thyroid gland, amniotic band	Induced; died
27	Hydrocephalus	cephalocentesis 450 mL Spontaneous; alive at 24 months
28	Low-set ears, medial omphalocele, bilateral ulnar deviation of hands, low-set large abducted toes, hypoplastic adrenal glands, SUA	TOP
29	Medial omphalocele, preaxial polydactyly feet, claw hands	TOP
30	None	TOP

HPE, holoprosencephaly; SUA, single umbilical artery; PUV, posterior urethral valves; TOP, termination of pregnancy; IUFD, intrauterine fetal death; VSD, ventricular septal defect; ASD, atrial septal defect.

In the studies from California and Scotland on births with HPE, the frequency of cytogenetically verified chromosome abnormalities was reported to be 36% (43/121)¹⁰ and 34% (17/50)¹¹. In a series of cases of prenatally diagnosed HPE, 29% (11/38)¹⁴, 33% (4/12)²⁶ and 43% (6/14)¹³ had detectable chromosomal aberrations. These numbers agree with the 37% (11/30) of detected chromosomal aberrations in the present study. If we combined the results of all these studies, the proportion of chromosome aberrations would be 35% (92/265). Except for the study of Berry *et al.*¹⁴, the karyotype was not available in all the cases of the presented studies (i.e. in 41 of the total of 265 cases). Croen *et al.*¹⁰ classified seven additional cases based on clinical appearance to have trisomy 13, and they suggested that in their study, 41% (50/121) were chromosomally abnormal. Thus, the true proportion of detectable chromosomal aberrations among HPE cases may be higher than 35%.

In the present study, chromosome 13 was involved in eight cases, including one fetus with triploidy (Table 4). Except for Case 2, all cases with an abnormal karyotype had associated anomalies in addition to HPE features. Case 10 had the karyotype 46,XY,del(13)(q22), presenting a small cerebellum, abnormal lungs (two right lobes and one left lobe), ventricular septal defect, overriding aorta, right ventricular hypoplasia, secundum atrial septal defect, hypospadias, absent scrotum, right displaced small thumb, left aplastic thumb, and bilateral syndactylies of digits 4–5. A similar prenatally detected case of 13q-syndrome with HPE and agenesis of a thumb was recently reported⁴¹. Case 2 had an apparently balanced translocation 46,XX,t(8;14)(p21.1;q24.1) derived from the father. In a previous pregnancy with another partner 13 years previ-

ously, the mother had lost a child with hydrocephaly. The autopsy report, however, described the postmortem central nervous system findings insufficiently. The review of the prenatal ultrasound videotape in this hydrocephalic child was highly suspicious for HPE. Thus, it is possible that the paternal balanced translocation had nothing to do with the occurrence of HPE, and that this HPE was rather of maternal origin.

Based on nonrandom cytogenetic rearrangements, the research on the genetic etiology of HPE has revealed at least 12 chromosomal regions on 11 chromosomes, designated HPE 1–12, which may contain genes that play a role in the pathogenesis of HPE⁴². The Sonic hedgehog gene (SHH), which maps to the region 7q36⁴³, and ZIC2, which maps to the critical region in chromosome band 13q32, are genes in which mutations can cause HPE. While SHH functions in the ventral neural tube, the ZIC2 message is seen in the dorsal neural tube. In contrast to serious facial anomalies in SHH mutations (HPE 3), patients with ZIC2 mutations (HPE 5) do not usually present major malformations of the face⁴⁴. These two examples show how different signaling proteins affect neural development in different ways, and that defective signaling may result in quite different clinical appearances of HPE. In Case 3, the chromosome aberration 45,XY,der(14)t(14;20)(q13;p13) involved the long arm of chromosome 14 with partial loss of the distal end (14q13); therefore, the gene responsible for HPE 8⁴⁵ might have been defective in this fetus.

Gene mapping was not performed in any of our cases. In the future, laboratory techniques will improve and make gene mapping more accessible so that HPE variants can more easily be related to specific chromosome loci.

Table 6 Summary of published reports on prenatal ultrasound diagnosis of holoprosencephaly

Reference	Year	Case (n)	GA at detection (weeks)	Comments
Kurtz <i>et al.</i> ⁸	1980	1	Not mentioned	
Blackwell <i>et al.</i> ¹⁷	1982	1	Third trimester	
Filly <i>et al.</i> ²²	1983	5	23–30	
Cayea <i>et al.</i> ¹⁹	1984	1	30	
Chervenak <i>et al.</i> ²⁰	1985	7	27–37	
Tóth <i>et al.</i> ²⁹	1986	1	14	Exencephaly
Pilu <i>et al.</i> ²⁸	1987	8	21–32	
Nyberg <i>et al.</i> ¹³	1987	14	14–38	
Berry <i>et al.</i> ¹⁴	1990	38	16–36	
Bronshtein and Weiner ¹⁸	1991	1	14	TVS in early pregnancy
Gonzales-Gomez <i>et al.</i> ²⁴	1992	1	10	TVS in early pregnancy
Nelson and King ²⁵	1992	1	10	TVS in early pregnancy
Pilu <i>et al.</i> ³³	1992	12	21–35	Only lobar HPE
Achiron <i>et al.</i> ¹⁵	1994	1	10	TVS in early pregnancy
Gembruch <i>et al.</i> ²³	1995	1	13	TVS in early pregnancy
Zalen-Sprock <i>et al.</i> ³²	1995	1	12	TVS in early pregnancy
Pilu <i>et al.</i> ²⁷	1996	1	11	TVS in early pregnancy*
Chow <i>et al.</i> ²¹	1996	7	16–28	
Parant <i>et al.</i> ²⁶	1997	12	13–33	
Wong <i>et al.</i> ³¹	1999	3	10–13	TVS in early pregnancy
Turner <i>et al.</i> ³⁰	1999	1	10	TVS in early pregnancy
Blaas <i>et al.</i> ¹⁶	2000	1	9	TVS in early pregnancy
Present study		30	9–37	2 × fronto-nasal dysplasia 2 × anencephaly 2 × agnathia-otocephaly 1 × anencephaly + agnathia-otocephaly

*Case from Monteagudo. GA, gestational age; TVS, transvaginal sonography; HPE, holoprosencephaly.

Syndromes and associations

The occurrence of HPE in syndromes and associations is documented in many studies and reports^{5,10,46}. Examples of syndromes and associations in which HPE has been described are: Meckel syndrome⁴⁷, Martin syndrome⁴⁸, Fitch syndrome⁴⁹, Pallister–Hall syndrome⁵⁰, Steinfeld syndrome⁵¹, hypertelorism and ectrodactyly syndrome⁵², velocardiofacial syndrome⁵³, pseudotrisomy 13 syndrome⁵⁴, Lambotte syndrome⁵⁵, Genoa syndrome⁵⁶, Goldenhar syndrome⁵⁷, acrocallosal syndrome⁵⁸, and Smith–Lemli–Opitz syndrome⁵⁹. Interestingly, Smith–Lemli–Opitz syndrome is due to a deficiency in the final step in the cholesterol biosynthetic pathway at the level of 3 β -hydroxy-steroid- Δ^7 -reductase^{46,60}. Cholesterol is an essential part of the SHH pathway (discussed above).

In the Californian study¹⁰, 11.6% (14/121) had a recognizable monogenic disorder. We suspected syndromal (not chromosomal) conditions or associations in 23% (7/30) of our cases (Cases 11, 14, 19, 20, 24, 28 and 29). Additionally, there were special conditions such as fronto-nasal dysplasia and agnathia-otocephaly. Most likely, these conditions represent defective development in only one developmental field, probably on the basis of one intrinsic defect. Therefore, we consider these conditions to be malformations and not syndromes.

The association of structural anomalies in the present study was consistent with the underlying condition (chromosome aberration, syndrome, association). Most anomalies were detected in limbs (12 cases), followed by the genitourinary system (nine cases) and cardiovascular system (nine cases) with a preponderance in chromosomally abnormal specimens.

Holoprosencephaly and anencephaly (Figures 6–8)

Three of the 30 (10%) HPE cases in the present study had anencephaly with facial anomalies typical for different HPE types, namely cebocephaly (single-nostril; Figure 1a), median cleft lip and palate (Figure 1b), and agnathia-otocephaly with cyclopia and two proboscides (Figures 7 and 8). The finding of anencephaly and HPE in the same family provides some genetic evidence that both central nervous system anomalies may be related in some instances^{7,61}. Roach *et al.*¹² found four cases of spina bifida/myelomeningocele in relatives of 30 HPE probands. Lemire *et al.*⁶¹ reviewed 19 published cases of anencephaly combined with facial features of HPE, including cyclopia, cebocephaly, and median cleft lip. Tóth *et al.*²⁹ were the first to report the prenatal diagnosis of the combination of HPE features with acrania/exencephaly. While the mechanism responsible for HPE associated with anencephaly is unknown, damage to the rostral end of the neural tube, with failure of its closure, is the commonly accepted explanation for this condition⁵. There were no anencephalic HPE fetuses/infants in the afore-mentioned large studies. We suggest that the combination of acrania/anencephaly and HPE represents an underdiagnosed association.

Holoprosencephaly in fronto-nasal dysplasia

Both fronto-nasal dysplasias in the present study (Cases 26 and 27; Figures 4 and 5) had cerebral HPE features. In 1981,

Roubicek *et al.*⁶² called attention to the brain anomaly found in a girl with frontonasal dysplasia, which was consistent with an ‘atypical example of the pseudohemispheric category of prosencephaly’ (i.e., HPE). As early as 1967, DeMyer⁶³, and later Sedano *et al.*⁶⁴, described this condition as ‘median cleft face syndrome’, and discussed its relation to HPE. The etiology of this condition is assumed to be the insufficient closure of the anterior neuropore.

Holoprosencephaly and agnathia-otocephaly (Figures 2, 3, 7 and 8)

Three cases with agnathia-otocephaly represent a relatively large share of our population (10%) and exemplify possible underdiagnosis of this particular condition in other HPE studies. It is known that features of the facial and cerebral dysmorphisms seen in the cyclopia/HPE spectrum may occur in association with agnathia-otocephaly^{5,65}. Cohen separated agnathia-otocephaly from other forms of HPE for two reasons. First, all of the 44 reported cases have been sporadic, except for the probably autosomal-recessive inheritance in two siblings, reported by Pauli *et al.*⁶⁶. Second, among causes of HPE with known etiology, such as trisomy 13, no cases of agnathia-otocephaly have occurred. None of our three agnathia-HPE cases (Cases 15, 16 (conjoined twin) and 30) had abnormal chromosomes. Case 30 was special, as it showed agnathia-otocephaly, combined with two proboscides, cyclopia rostral to the proboscides, and anencephaly (Figures 7 and 8).

Holoprosencephaly and multiple pregnancies

The two twin pregnancies in our selected population (one dizygotic twin pair, Case 19, and one conjoined twin, Case 16; Figure 3) represent a relatively high percentage. There is a higher incidence of anomalies in twins, especially in monozygotic twinning, which is assumed to occur on the basis of a teratogenic event⁶⁷. Holoprosencephaly in twins has been discussed in a review paper⁶⁸ and no significant conclusions were made except that the twinning rate in families with HPE probands is higher than the overall twinning rate¹² and that HPE is found in dizygotic as well as in monozygotic pregnancies. Roach *et al.*¹² ($n = 30$), Croen *et al.*¹⁰ ($n = 121$) and Whiteford and Tolmie¹¹ ($n = 50$) each reported only one twin pregnancy in their studies.

Head size in holoprosencephaly

Table 3 documents reduced head size (17/30; 57%), which is a common feature in HPE, both in fetuses with normal and in those with abnormal karyotype. In addition, there were three anencephalic fetuses. Recently, den Hollander *et al.*⁶⁹ presented a study group of 30 fetuses with microcephaly; nine of them had HPE (four with abnormal and five with normal karyotype). Increased size of the head can also be found, as shown in the present study (5/30; 17%). Increased BPD was mainly found in third-trimester pregnancies (4/5; 80%); this observation was also made by Berry *et al.*¹⁴. In conclusion, 83% of the fetuses in our study had an

abnormal shape and/or size of the head. The ultrasound findings of an abnormal size of the head, hydrocephaly, microcephaly, or even anencephaly, should trigger the sonographer to rule out the possibility of association with HPE.

Sex ratio

The sex distribution in large retrospective studies shows a female predominance. Whiteford and Tolmie¹¹ found a male : female ratio of 0.8 : 1 in 33 cases with normal chromosomes. Roach *et al.*¹² found a ratio of 0.3 : 1 in 32 cases, while Croen *et al.*¹⁰ calculated a ratio of 0.47 : 1 in 121 cases. This is in contrast to the present prenatal study with a male : female ratio of 1.4 : 1 in 30 cases. The composition of postnatal and prenatal study populations may be connected to this difference.

Inheritance and the risk of recurrence

The risk of recurrence depends on the basis for the actual condition, such as chromosome defect or syndrome. Both autosomal dominant and recessive conditions with HPE have been described⁵ but incomplete penetrance or an incomplete form or microform of HPE can make the interpretation of familial occurrence difficult. Patel *et al.*⁷⁰ described a family in which the mother had a single incisor as an abortive presentation of HPE, while her child showed alobar HPE with median cleft lip and palate, and ocular hypotelorism. In Case 2, an isolated form of alobar HPE, the mother had lost a child with hydrocephaly 13 years previously. This might have been a misdiagnosed HPE (see Case 2). Case 28 has recently been reported as a variant of acrocallosal syndrome associated with anencephaly⁵⁸. Here, the recurrence of the condition in a later pregnancy gave evidence for autosomal recessive inheritance.

CONCLUSION

This study illustrates the heterogeneity of HPE with different etiologies and different clinical appearances. Seventy percent of the cases had associated structural anomalies that were found in addition to cerebral and facial HPE features. Thirty-seven percent of the cases had an abnormal karyotype. Holoprosencephaly is commonly associated with an abnormal size of the head. The prevalence of HPE registered in Norwegian Health Region IV over a period of 14 years was 1.26 : 10 000.

We have described special conditions that were associated with HPE, such as fronto-nasal dysplasia, agnathia-otocephaly and anencephaly with HPE features. They are probably underreported conditions in other HPE studies.

The diagnosis of HPE by ultrasound has been made as early as 9⁺2 weeks' gestation¹⁶. The development of the fore-brain can be depicted in detail with ultrasound from 7 weeks on^{71,72}. Thus, alobar HPE may be detectable as early as the end of week 7. In the future, improvement of ultrasound technology and the widespread use of transvaginal ultrasound will lead to more early diagnoses of HPE. Systematic registration of early diagnoses may shed light on the discrepancy of HPE incidence found in embryonic vs. later studies.

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