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A Retrospective Survey of Perinatal Risk Factors of 104 Living Children With Holoprosencephaly

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Holoprosencephaly (HPE) is a brain malformation resulting from a primary defect in development of the basal forebrain during early gestation. Prenatal genetic and environmental factors and birth outcomes were described in a population of 104 children with holoprosencephaly referred to three clinical centers from 1998 through 2002. The mean child age was 4 years. Of cases karyotyped, 9% presented with a chromosomal abnormality. This study of living children with holoprosencephaly, the majority of whom are cytogenetically normal, provides new information on the subsample of children with a less severe phenotype. Most children were born at term; about 51% were microcephalic at birth. Consistent with previous research, the association between HPE and maternal history of diabetes merits further investigation. Several findings have important implications for future research. Only 22% of the children in this study sample were diagnosed with holoprosencephaly prenatally. The vast majority of children (72%) were diagnosed with HPE between birth and 1 year of age. Also, 19% of the cases referred to the Carter Centers with HPE were not confirmed on scan review. When possible, future population-based epidemiological studies should emphasize mechanisms that identify children with HPE outside of the newborn period and confirm the diagnosis by review of MRI or high quality CT brain scan.

KEY WORDS: holoprosencephaly; risk factors; prenatal diagnosis

INTRODUCTION

Congenital malformations are a significant public health concern affecting 3–4% of all live births and many pregnancy terminations and miscarriages [Hobbs et al., 2002]. Holoprosencephaly (HPE) is a brain malformation that results from a primary defect in induction and patterning of the basal forebrain during the first 4 weeks of embryogenesis [Golden, 1999]. This defect results in incomplete separation of the cerebral hemispheres. Although the incidence of this relatively rare disorder ranges from 0.5 to 1.2 per 10,000 births [Croen et al., 1996; Rasmussen et al., 1996; Forrester and Merz, 2000; Bullen et al., 2001], it results in substantial morbidity and mortality throughout childhood.

Based on the degree of hemispheric non-separation, HPE has traditionally been classified into three types: alobar, semilobar, and lobar, with alobar being the most severe type [DeMyer et al., 1964]. A fourth type—middle interhemispheric variant of holoprosencephaly (MIH)—was recently described [Barkovich and Quint, 1993; Simon et al., 2002]. In the MIH variant of HPE, the cerebral hemispheres fail to divide in the posterior frontal and parietal regions, while the interhemispheric separation of the basal forebrain, anterior frontal lobes, and occipital regions are preserved. While classic HPE is likely due to a defect in basal forebrain induction and patterning, MIH is thought to be caused by a defect in roofplate induction and differentiation. Most children with all types of HPE experience neurological problems including cognitive and developmental delays, motor impairments, seizure disorders, and endocrinologic dysfunction. The severity of neurological problems and delay generally correlates with the degree of hemispheric non-separation (type of HPE) [Barr and Cohen, 1999; Plawner et al., 2002].

In general, about 30% of birth defects are thought to result from single-gene mendelian mutations, chromosomal abnormalities, non-mendelian mutations, and known teratogens. The majority of congenital malformations are attributed to multifactorial etiologies resulting from interactions between genes, environment, and possible socio-cultural factors [Hobbs et al., 2002]. Ming and Muenke [2002] have identified eight candidate genes associated with HPE: *SHH*, *SIX3*, *ZIC2*, *TGIF*, *PATCHED1*, *GL12*, *TDGF1*, and *FAST1*. However, mutations in these genes were identified in only 15–20% of their HPE cases. Thus, a number of other genes, yet to be discovered, are likely to be involved in HPE.

Maternal periconceptual exposures associated with holoprosencephaly in humans include maternal diabetes, ethyl alcohol, cigarette smoking, and retinoic acid [Croen et al., 2000; Cohen and Shiota, 2002]. Ethyl alcohol and retinoic acid have also produced HPE in experimental animals [Sulik and Johnston, 1982; Sulik et al., 1995]. While anecdotal reports also suggest maternal virus, low-calorie diet, hypocholesterolemia, salicylates, and contraceptives as potential teratogenic factors for HPE, none of these anecdotal reports have been validated as a cause of HPE in either humans or experimental animals [Cohen and Shiota, 2002]. The wide variability in phenotypic expression of HPE noted in previous papers is consistent with a model of multiple causes [Ming and Muenke, 2002; Plawner et al., 2002].

This paper describes prenatal genetic and environmental factors, diagnosis, and birth outcomes in a population of

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children referred to three clinical centers focused on holoprosencephaly.

MATERIALS AND METHODS

Patient Selection

The target population consisted of children (birth to 18 years) with a diagnosis of holoprosencephaly that were referred to one of the three Carter Centers for Brain Research in Holoprosencephaly and Related Brain Malformations (a national consortium funded by a non-profit private foundation) from 1998 through 2002. The patients were referred to the Centers through various sources, including pediatricians, neonatologists, geneticists, and neuroradiologists. Some patients were self-referred via the Carter Center web site [http://hpe.stanford.edu/].

Inclusion criteria for this database study included: (1) diagnosis of holoprosencephaly confirmed by neuroimaging scan review, (2) completion of a database tool through multiple sources—parent report, medical record review, and clinical evaluation, and (3) parental consent. Neuroimaging studies (MRI or high-quality CT) of 257 patients were evaluated by two pediatric neuroradiologists who were unaware of the patient's clinical status. To be included in the study, the CT scans had to have adequate image quality to allow assessment of key structures (basal ganglia, thalami, and interhemispheric fissure). Two of the scans were excluded because of poor image quality or insufficient images. The type of HPE (alobar, semilobar, lobar, or MIH) was determined based on the extent of the interhemispheric separation and ventricular morphology [Barkovich and Quint, 1993; Lewis et al., 2002; Plawner et al., 2002]. Of note is that of the 255 patient scans reviewed with a previous diagnosis of holoprosencephaly, only two hundred and six children met the HPE neuroimaging criteria.

From this sample of children with confirmed HPE, 107 children received a clinical evaluation at one of the three Carter Centers and were invited to participate in the database study. The final study sample consisted of 104 children and their parents enrolled prospectively from 1998 through 2002. Forty-two patients were evaluated at the Kennedy Krieger Institute, 38 at Texas Scottish Rite Hospital, and 24 at Stanford University Medical Center. The study was approved by the Institutional Review Board at each of the Centers. Consent was obtained from the parents before enrollment.

Since this study was descriptive and lacked a control group, many of the findings are compared to equivalent values in the normal population. The majority of children in this study were born in the 1990s; therefore, normative data taken from US census statistics for the years 1990–2000 were used for comparison. These general comparisons are for clinical reference only and no statistical inferences should be drawn.

RESULTS

Patient Demographics

An important finding is that 19.2% (49/255) of referred patients failed to have their diagnosis of HPE confirmed upon neuroimaging scan review. Demographics by type of holoprosencephaly for the 104 study children are summarized in Table I. The diagnoses of the child participants were: 18 (17%) alobar type, 55 (53%) semilobar, 15 (14%) lobar, and 16 (15%) MIH. There were 45 males and 59 females. The mean age of child participants at the time of the study was 49.8 months (4 years and 2 months) with a range from 3 days to 18 years of age. The mean age of study children by type of HPE was 24.3 months for alobar, 50.4 months for semilobar, 79.2 months for lobar, and 48.7 months for MIH. Seventy-three children were Caucasian (70%), ten African-American (10 %), 18 Hispanic (17%), and three Asian/other (3%). The children came from a wide geographic area including 30 USA states (99), Puerto Rico (1), England (1), Ireland (1), and Mexico (2).

Prenatal and Birth Data

Prenatal ultrasounds were performed in 93% (95/104) of mothers. Yet only 21 of 95 children in this sample (22%) were diagnosed with holoprosencephaly prenatally. Although those children with the more severe types of HPE were likely to have been diagnosed prenatally, still 50% of children with alobar HPE and 83% with semilobar HPE were not diagnosed until after birth (Table I). The vast majority of children (72%) were diagnosed with HPE between birth and 1 year of age.

TABLE I. Demographics by Type of Holoprosencephaly (HPE) (n = 104)

	Alobar	Semilobar	Lobar	MIH	Total (%)
Diagnosis	18 (17.3%)	55 (52.9%)	15 (14.4%)	16 (15.4%)	104 (100)
Gender					
Male	5 (27.8%)	25 (45.5%)	8 (53.3%)	7 (43.8%)	45 (43.3)
Female	13 (72.2%)	30 (54.5%)	7 (46.7%)	9 (56.3%)	59 (56.7)
Race					
Caucasian	11 (61.1%)	38 (67.1%)	13 (86.7%)	11 (68.8%)	73 (70.2)
Hispanic	3 (16.7%)	9 (16.4%)	1 (6.7%)	5 (31.3%)	18 (17.3)
African-American	3 (16.7%)	6 (10.9%)	1 (6.7%)	0	10 (9.6)
Asian/other	1 (5.6%)	2 (3.6%)	0	0	3 (2.9)
Chromosome Analysis					(88/104 reporting)
Normal	13 (86.7%)	44 (89.8%)	11 (100%)	12 (92.3%)	80 (90.9)
Abnormal	2 (13.3%)	5 (10.2%)	0	1 (7.7%)	8 (9.1)
Time of Diagnosis					(95/104 reporting)
Prenatal	9 (50%)	9 (17.6%)	0	3 (21.4%)	21 (22)
Birth-6 months	9 (50%)	32 (62.7%)	10 (83.3%)	6 (42.9%)	57 (60)
6–12 months		6 (11.8%)	1 (8.3%)	4 (28.6%)	11 (11.6)
1–2 years		3 (5.9%)	1 (8.3%)	1 (7.1%)	5 (5.3)
>2 years		1 (2%)	0	0	1 (1.1)
Mean age in months at time of study (range)	24.3 months (11 days–10.6 years)	50.4 months (3 days–17.9 years)	79.2 months (2.4 months–18.8 years)	48.7 months (6.4 months –14.7 years)	49.8 months (3 days–18.8 years)

Eight of the 88 children with HPE (9%) in whom chromosomal analysis was performed had an abnormality (13q deletion, interstitial deletion of the long arm of chromosome 13, ring 13, mosaic 46,XO Turner, and 47,XX plus marker). Three were identified with a *ZIC2* mutation, one of the eight known genes associated with HPE. The majority of children with abnormal chromosomes (7/8) were diagnosed with a moderate or severe type of HPE (semilobar or alobar). Most of the children in this sample had sporadic HPE without a positive family history. There were two sibling pairs in this cohort: a male with lobar HPE and female with semilobar HPE, and a male and female sibling pair with semilobar HPE. There were no reported cases of consanguinity.

The mean gestational age at birth for children in this sample was 38.8 weeks (range 23 to 43 weeks; median 40 weeks). Approximately 21% of the study children were born prematurely (gestational age < 38 weeks). All children were singletons except for one dizygotic twin gestation (co-twin was unaffected). Birth weight ranged from 0.48 to 5.50 kg, with a mean birth weight of 3.2 kg (n = 101). Head circumference data at birth were only available on 37 children. The occipitofrontal head circumference (OFC) at birth of this subsample ranged from 27 to 51 cm, with a mean OFC of 33.9 cm. Ten children were macrocephalic at birth and diagnosed with hydrocephalus. The mean birth OFC of the subsample of children without hydrocephalus (n = 27) was 30.9 cm. Approximately 51% (19/37) of the children had birth head circumferences in the microcephalic range (OFC <10th percentile by gestational age). The Apgar scores and delivery data with norm comparisons are summarized in Table II.

Maternal Risk Factors

The mean maternal age at this child's birth was 28.5 years. Fifteen percent of the mothers (15/101) were 35 or more years of age. The mean paternal age was 32.2 years (n = 98). Twenty-seven percent of the mothers were college graduates (13% had graduate degrees); 62% of the mothers graduated from high school and/or had partial college education, and 12% had not completed high school.

The time of the first prenatal visit ranged from 1 to 28 weeks gestation, with a mean of 6.8 weeks. Thirty-five percent (30/85) of the mothers had a previous history of miscarriage. The majority (93%) of mothers reported taking prenatal vitamins during this pregnancy.

Eighteen children (18/99, 18%) were born to diabetic mothers: gestational diabetes (9/99, 9%) or diabetes mellitus (9/99, 9%). Nine (9%) of the mothers reported a history of high blood pressure.

During a span of 6 months pre-conception to the first 3 months of this pregnancy, the majority of mothers reported taking medications (71/92, 77%). Figure 1 displays maternal medication use in order of frequency. On recall, 39% (35/91) of the mothers reported the presence of viral and/or bacterial infections during this time frame. Twenty-eight percent (27/98) of mothers reported drinking alcohol and 18 mothers (19%) reported smoking during this periconceptional period.

DISCUSSION

It is important to note that this is a convenience sample of living children with HPE and not a population-based study. Therefore, the study sample may be skewed toward more mildly affected cases. The majority of study children had isolated, non-syndromic holoprosencephaly. However, it is likely that other population-based studies have missed these mild HPE cases and included some cases that do not truly have HPE. Upon scan review, a significant proportion (19%) of the cases referred to the Carter Centers with HPE were found not to have HPE, but rather other types of brain malformations. This has important implications for future research. If possible, future population-based epidemiological studies should emphasize mechanisms that identify children with HPE outside of the newborn period and, when possible, confirm the diagnosis by review of MRI or high quality CT brain scan.

The proportion of patients with the various types of HPE in our study differs from what has been described in the literature. Previous epidemiological studies reported a majority of patients with alobar HPE: California, 46–67% [Croen

TABLE II. Delivery Data for HPE With Norm Comparisons (n = 104)

	HPE	Norm reference[a]
Mean maternal age (years)	28.5	27.2 (all births)
Range	15–42	
Mean paternal age (years)	32.2	29.7
Range	17–48	
Mean gestational age at birth	38.8 weeks (n = 87)	40
Range	23–43 weeks	
Percent delivered at		
<38 weeks	20.7 (18/87)	11.8
38–41 weeks	75.9 (66/87)	
≥42 weeks	3.4 (3/87)	
Percent born cesarean delivery	36.3 (33/91)	22.0
Mean birth weight (kg)	3.2 kg (n = 101)	
Median birth weight (kg)	3.07 kg	3.35 kg
Range	(0.50–5.50 kg)	
Percent birth weight, <2,500 g	11.9	7.6
Mean birth occipitofrontal head circumference (OFC, cm)	33.9 (n = 37)	
Percent OFC > 90th percentile	27.0 (10/37)	
Percent OFC < 10th percentile	51.4 (19/37)	
Apgar scores	1 min (n = 58); 5 min (n = 54)	5 min
7–10 (%)	50 (86.2%) 51 (94.4%)	98.6%
4–6 (%)	6 (10.4%) 3 (5.6%)	(<7); 1.4%
0–3 (%)	2 (3.4%) 0	

[a]National Center for Health Statistics: births: final data for 1999.

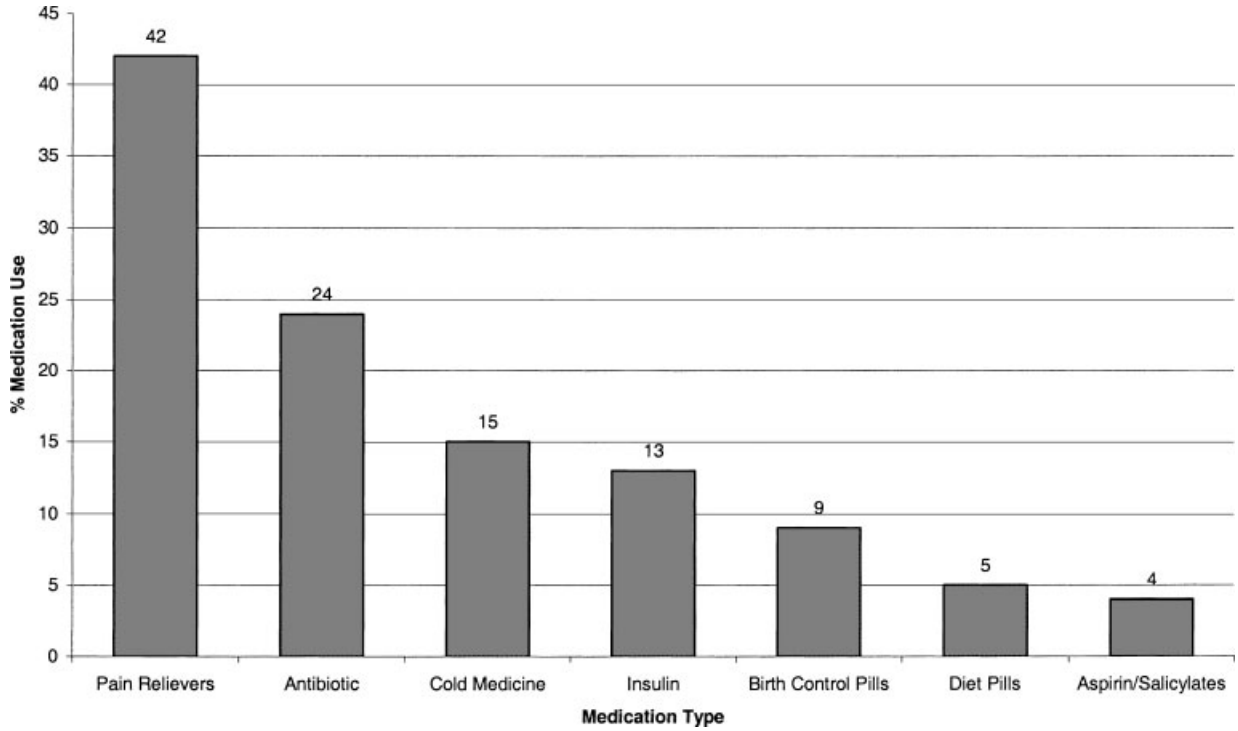

Fig. 1. Maternal periconceptional medication use: prevalence and type (n = 92).

et al., 1996, 2000] and New York State, 54% [Olsen et al., 1997]. In our centers, we evaluate a greater proportion of children with moderate and less severe HPE (semilobar, lobar HPE or MIH). This is likely due to the selection bias of patients clinically evaluated at the Carter Centers and the neuroimaging study inclusion criteria. The children with the less severe forms of HPE may also survive longer, allowing more opportunity to be evaluated at our Centers.

Only 22% of the children in this study sample were diagnosed with holoprosencephaly prenatally. In contrast, Bullen et al. [2001] found that prenatal diagnosis was achieved in 71% of cases, rising to 86% with a routine anomaly scanning program at 18 to 20 weeks gestation. Literature demonstrating prenatal diagnosis of alobar HPE as early as 9 weeks gestational age has been reported with visualization by two- and three-dimensional ultrasound [Blaas et al., 2000]. However, since the vast majority of our patients did not have alobar HPE, but rather less severe forms of HPE, we would expect that the rate of prenatal diagnosis would be lower. Lobar and MIH forms of HPE would be more difficult to detect by prenatal ultrasound, since in these forms the interhemispheric non-separation can be quite subtle and the characteristic dorsal cyst of HPE is often absent.

The higher occurrence of HPE among females reported in this research is consistent with previous published national and international studies [Roach et al., 1975; Rasmussen et al., 1996; Croen et al., 2000]. One possible explanation for the female predominance in HPE is that males are more likely to spontaneously abort [Rasmussen et al., 1996]. The higher number of males with HPE reported in Bullen et al.'s [2001] study population may reflect the inclusion of fetal deaths, stillbirths, and pregnancy terminations.

The 9% abnormal chromosomal analysis rate in this study is much lower than the rates reported in the literature. Estimates of holoprosencephaly associated with a karyotypic abnormality range from 38% [Bullen et al., 2001] to 55% of cases [Ming et al., 1976]. We can hypothesize several reasons for this significant difference in abnormal chromosome rate. Since most of these prior high estimates include fetal deaths and terminations, it may be that pregnancies of children with HPE and karyotypic abnormalities are more likely to be terminated or result in fetal death, and thus were excluded from our sample. Prior studies have found higher neonatal mortality among infants with syndromes, including chromosomal abnormalities and single-gene conditions, than infants with isolated non-syndromic holoprosencephaly [Croen et al., 1996; Rasmussen et al., 1996]. Our sampling bias of living children skewed toward mildly affected, clinically stable patients. Thus, severely affected babies that die shortly after birth or who are too unstable to travel for a clinical evaluation—high risk subgroups that may have a higher rate of karyotypic abnormality—were not included in our sample.

The mean maternal and paternal age at birth of index infant was slightly higher than the population mean. While 15% of the study mothers were $\geq$35 years at birth, the wide maternal age range is consistent with several previous reports of equal risks across the maternal age spectrum [Croen et al., 1996, 2000]. In a population-based study of HPE, Rasmussen et al. [1996] noted a U-shaped maternal age distribution for HPE with increased risk in women younger than 20 and 35 years and older when compared with women 25 to 29 years of age; however, when isolated HPE cases were evaluated separately, this association with maternal age was not observed.

Women who conceive holoprosencephalic embryos may be at greater risk of having miscarriages in other pregnancies [Cohen, 1989]. A significant percentage (35%) of mothers in this study and others report a history of previous pregnancy loss [Matsunaga and Shiota, 1977]. One report found the prevalence of holoprosencephaly among pregnancies terminated at less than 20 weeks gestation to be over 30 times higher than that of pregnancies that progress beyond 20 weeks [Matsunaga and Shiota, 1977]. In a recent study of 285 couples with recurrent miscarriage, 46% of the miscarriages were cytogenetically abnormal [Stephenson et al., 2002]. While

chromosome abnormalities occur in a high percentage of spontaneous abortions, other etiological factors may include maternal reproductive anatomic disease and systemic maternal diseases such as diabetes [Katz and Kuller, 1994].

With regard to teratogenic exposures, 18% of study children were born to diabetic mothers. Engelgau et al. [1995] estimated that maternal diabetes (gestational, non-insulin-dependent, or IDDM) is present in 4% of all US pregnancies. Comparatively, the US natality statistics for 1999 [National Center for Health Statistics, 2001] reported a diabetes rate of 27.3 per 1,000 live births (2.7%). While it is suspected that hyperglycemia is a major contributor to teratogenesis, hypoglycemia, hypoxia, maternal vasculopathy, ketones, amino acid abnormalities, glycosylation of proteins, hormonal imbalances, and somatomedin inhibitors may also play a pathogenetic role in diabetic embryopathy [Cohen and Shiota, 2002]. Previous studies have suggested that maternal diabetes may be associated with holoprosencephaly [Barr et al., 1983; Martinez-Frias et al., 1998; Croen et al., 2000; Cohen and Shiota, 2002]; additional research on this association is merited.

Maternal alcohol consumption during the periconceptional period has been associated with HPE in human [Ronen and Andrews, 1991; Croen et al., 2000; Cohen and Shiota, 2002] and experimental animal studies [Sulik and Johnston, 1982; Webster, 1983]. In a California population-based case-control study [Croen et al., 2000], increased risk for HPE was observed for women who drank any alcohol in early pregnancy (OR = 2.0, 95% CI 0.9–4.5); women who both drank and smoked in the periconceptional period were over five times as likely to have a baby with HPE. In our study, 28% of mothers reported drinking any alcohol and 19% reported smoking during the first 3 months of this pregnancy; however, it is difficult to draw conclusions without a control group. Further research with more precise measurements of maternal alcohol and smoking exposure is warranted.

Almost 39% of the mothers reported the presence of viral and/or bacterial infections during the periconceptional time frame. Pain relievers and antibiotics were used by over 24% of the mothers for treatment. A number of prior anecdotal case reports associate viremia with human holoprosencephaly [Khudr and Olding, 1973; Mollica et al., 1979] and a population-based case-control study found medications for respiratory illness and salicylate-containing medications increased the risk for non-syndromic HPE [Croen et al., 2000]. Future epidemiological studies in children with HPE will be helpful in determining the significance of first trimester viral and bacterial infection as a potential environmental teratogen.

Although the study sample is biased toward survivors, the birth data findings show that the majority of the study children were born at term. However, compared with the general population, a comparatively higher percentage of infants were born prematurely (Table II). Approximately 12% of the study infants were low birth weight (less than 2,500 g) compared to 7.6% in the general population. While approximately half of the children were born microcephalic, hydrocephalus was diagnosed in 27% of the children with recorded birth head circumferences.

STUDY STRENGTHS AND LIMITATIONS

Strengths of this study include: a large ethnically and geographically diverse population of children living with HPE and confirmed brain scan readings as the diagnostic outcome variable. In several previous studies of holoprosencephaly, ascertainment depended on recognition of characteristic facial features. However, most children with moderate and less severe forms of holoprosencephaly (semilobar, lobar, MIH) present with mild if any facial deformities. Lack of imaging confirmation of HPE in ascertainment biases the sample toward the severe spectrum.

Potential study limitations include: recall error on mother's retrospective report of prenatal exposures and selection bias. While 97% (104/107) of the children who received clinical evaluations at one of the three study centers participated in the study, only 51% (104/206) of children with confirmed HPE in the target population were enrolled. If the non-interviewed cases differed substantially from the interviewed families, our findings may be biased. Children who come to one of three national Carter Centers for evaluation and research participation are generally stable enough to travel, thus there may be a selection bias toward children with less severe malformations and better clinical outcomes. Also, the majority of mothers in this cohort were well-educated; 89% of study mothers had 12 or more years of schooling compared to 78% of all women who gave birth in 1999 [National Center for Health Statistics, 2001]. Most participating families have Internet access and the resources to travel to a center of excellence.

CONCLUSIONS

Human and animal studies suggest that prenatal exposure to multiple environmental teratogens, as well as genetics, influence development of the HPE phenotype. As Ming and Muenke [2002] hypothesize, "even relatively low doses of these teratogens, which by themselves may not be sufficient to cause HPE or any other clinical abnormality, may act in concert with other environmental or genetic variables to generate the HPE phenotype"(p 1026). While maternal diabetes and alcohol use have consistently been associated with HPE in both human and animal studies, the strength of exposure and interaction effects with genetics and other environmental teratogens remains unclear. As this is a relatively rare disorder, future studies might explore the use of non-traditional study designs that maximize the power of small sample sizes, such as family-based and countermatched studies, to test for gene-environment interactions [Hobbs et al., 2002].

A significant clinical finding of this study was that only 22% of the children with HPE were diagnosed prenatally on ultrasound. In order to improve parents' ability to make informed decisions regarding pregnancy and/or knowledgeable preparation for infant care, we need to increase prenatal detection of this severe brain malformation. Bullen et al. [2001] found that fetal anomaly screening by ultrasonography at 18 to 20 weeks gestation significantly improved prenatal diagnosis of holoprosencephaly. In mothers with high-risk pregnancies (i.e., family history of holoprosencephaly or a related neurodevelopmental disorder, history of multiple miscarriages, maternal diabetes), particularly with equivocal two-dimensional ultrasounds, the complementary use of 3D ultrasonography, color Doppler scanning and/or fetal MRI in improving HPE diagnostic accuracy should be explored.

Finally, upon diagnosis, families are frequently told that their child has no chance for survival or meaningful interaction. We note, however, that the mean age of the children participating in this study was 4 years. Fifteen percent of the children were between 10 and 19 years of age. The mean age of the 17 children with the most severe form of HPE, alobar HPE, was 2 years. While Barr and Cohen [1999] noted that half of the children with alobar HPE die within the first 5 months, in fact, many children with HPE live into adolescence and beyond. Children with moderate to severe holoprosencephaly generally develop minimal motor and language skills; however, children with less severe HPE (i.e., MIH) may walk with assistance, speak and function with mild cognitive impairment [Lewis et al., 2002; Plawner et al., 2002]. Therefore, when a fetus or child is diagnosed with

holoprosencephaly, it is important that pediatricians and obstetricians who help families make decisions involving pregnancy care, interruption, and/or birth adjustment provide accurate information about the spectrum of clinical outcomes.

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