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Risk Factors for Nonsyndromic Holoprosencephaly: A Manitoba Case–Control Study

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Holoprosencephaly (HPE) is one of the most common developmental field defects, occurring in 1 in 250 conceptuses and in 1 in 10,000–20,000 live births. Nearly half of patients with HPE have a recognized syndrome or a single gene defect. However, little is known about the risk factors for the remainder with “nonsyndromic” HPE. In our case–control study, we examine factors associated with nonsyndromic HPE. We identified 47 patients with HPE from the genetics clinic database with an equal number of controls matched for gender and birthdate. Of the 47 patients, 23 were identified as nonsyndromic. No statistically significant differences were noted between the mean maternal and paternal ages of patients and controls. Factors associated with nonsyndromic HPE were: having an Aboriginal mother (unadjusted odds ratio [OR] 3.5, 95% confidence interval [CI] 1.1–11.1), an Aboriginal father (OR 12.8, 95% CI 3.0–55.1), at least one Aboriginal parent (OR 5.0, 95% CI 1.6–16.0), or two Aboriginal parents (OR 8.8, 95% CI 2.0–37.8), the presence of a family history of a midline facial defect (OR 8.2, 95% CI 1.5–45.2), and being of low socioeconomic status (OR 3.0, 95% CI 1.0–9.1). Having an Aboriginal background remained statistically significant after adjusting for low socioeconomic status. Other associations evaluated—history of prior spontaneous abortion, stillbirth, neonatal death, prepregnancy diabetes, infections during pregnancy, alcohol exposure, smoking, and substance abuse—were not significantly associated with nonsyndromic HPE. The use of periconceptional folic acid or vitamins was not associated with a lower risk of nonsyndromic HPE. © 2012 Wiley Periodicals, Inc.

Key words: holoprosencephaly; risk factors; epidemiology; Manitoba; Canada

INTRODUCTION

Holoprosencephaly (HPE) is one of the most common developmental field defects, occurring in approximately 1 in 250 conceptuses [Matsunaga and Shiota, 1977] and 1 in 10,000–20,000 live births [Croen et al., 1996; Rasmussen et al., 1996; Olsen et al., 1997; Forrester and Merz, 2000; Bullen et al., 2001; Ong et al., 2007]. HPE

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was initially thought to result from failure of cleavage of the embryonic forebrain, leading to lobar, semilobar, and lobar forms [DeMyer and Zeman, 1963]. However, it is now widely accepted that this malformation is caused by a defect in primary induction and patterning of the rostral neural tube [Golden, 1999]. HPE often occurs as part of a recognized syndrome or in association with a single gene defect. Several HPE genes have already been identified and other candidate genes proposed [Belloni et al., 1996; Roessler et al., 1996, 2003; Brown et al., 1998; Wallis et al., 1999; Gripp et al., 2000; Nanni et al., 2000; Ming et al., 2002; de la Cruz et al., 2002]. However, in 50% of patients, HPE is not associated with a specific cytogenetic abnormality or monogenic syndrome [Croen et al., 1996, 2000].

Little is known about the risk factors for the development of this form of HPE, termed “nonsyndromic” HPE. Some studies have suggested a causal link between maternal diabetes and HPE [Barr et al., 1983; Olsen et al., 1997]. This association has also been seen in other studies [Croen et al., 2000; Miller et al., 2010]. Other potential

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risk factors, largely based on case reports or small case series, include: female gender [Roach et al., 1975; Mastroiacovo et al., 1995; Rasmussen et al., 1996; Olsen et al., 1997; Croen et al., 2000]; early maternal menarche [Croen et al., 2000]; foreign maternal race [Croen et al., 1996, 2000; Rasmussen et al., 1996; Forrester and Merz, 2000; Ong et al., 2007]; young maternal age [Olsen et al., 1997]; young and older maternal age, with HPE occurring in a U-shaped distribution [Rasmussen et al., 1996]; poverty [Roach et al., 1975]; maternal lower education level [Croen et al., 2000; Miller et al., 2010]; previous pregnancy loss [Matsunaga and Shiota, 1977]; maternal anemia [Croen et al., 2000]; low-calorie weight reduction diets [Ronen, 1992]; maternal periconceptional exposures, including to alcohol [Jellinger et al., 1981; Bonnemann and Meinecke, 1990; Ronen and Andrews, 1991; Croen et al., 2000]; cigarettes or alcohol and cigarettes [Croen et al., 2000]; irradiation [Jellinger et al., 1981]; medications such as those for respiratory illness [Croen et al., 2000]; maternal flu [Orioli and Castilla, 2007]; salicylates [Khudr and Olding, 1973; Benawra et al., 1980; Agapitos et al., 1986; Croen et al., 2000; Miller et al., 2010]; retinoic acid [Lammer et al., 1985]; anticonvulsants [Kotzot et al., 1993; Holmes and Harvey, 1994; Rosa, 1995]; estrogen/progestin [Stabile et al., 1985; Orioli and Castilla, 2007]; TORCH infections [Byrne et al., 1987; Kilic and Yazici, 2005]; and use of assistive reproductive technology [Miller et al., 2010]. However, these variables are not consistently found to be risk factors for HPE across studies to date. Few population-based studies have rigorously examined potential etiologic agents, with only a few studies published to date that use a case-control design [Croen et al., 2000; Orioli and Castilla, 2007; Miller et al., 2010]. Furthermore, many earlier studies have included data from the 1970s to the 1980s, prior to significant improvements in prenatal ultrasound diagnosis. A review of proposed risk factors for nonsyndromic HPE based on four case-control studies has recently been published, highlighting the many uncertainties surrounding the etiology of nonsyndromic HPE [Johnson and Rasmussen, 2010].

There is anecdotal evidence suggesting that Manitoba has a high occurrence of HPE and related malformations. This population, therefore, provides a unique opportunity to examine various factors that may influence the development of HPE. The objective of this case-control study was to elucidate potential risk factors for the occurrence of nonsyndromic HPE using data from the Winnipeg Children's Hospital Section of Genetics and Metabolism database.

METHODS

The study was approved by the Health Research Ethics Board, Faculty of Medicine, University of Manitoba.

Study Subjects

Patients. Patients (cases) were primarily ascertained from the database of the Winnipeg Children's Hospital Section of Genetics and Metabolism. Ascertainment is high as the Genetics section at our center is the only one in Manitoba and serves the entire province of approximately 1.3 million people. The database includes all of the cytogenetic studies done in Manitoba, both prenatally and postnatally. The section also has close ties to the provincial maternal

serum screening program, and serves as the consultant to both the single major fetal assessment unit and to the only place where terminations are done for congenital anomalies. To maximize case ascertainment, a search for patients with "HPE" as the primary diagnosis was also conducted on Pediatric Neurology clinic and Health Sciences Centre hospital databases.

The following were considered for potential inclusion: live births, terminations, and stillbirths between January 1990 and September 2001 encoded by the term "HPE," terms synonymous or often used interchangeably with HPE, facial features, or cranial anomalies consistent with HPE, and syndromes associated with HPE, including trisomies 13 and 18 (see Appendix I). HPE patients were ultimately selected for inclusion based on either of the following: radiologic or autopsy confirmation; or a strongly suggestive clinical sequence (including hypotelorism, cleft lip/palate, microcephaly, cyclopia, proboscis, ethmocephaly, cebocephaly, or a single central incisor in a patient with a family history of HPE) even if radiologic or autopsy confirmation was not available. Hypotelorism, cleft lip/palate or microcephaly, in isolation, were not used to define patients with HPE, given their common presentation in other conditions. Patients were excluded if enough data were missing without which a "tentative" diagnosis of HPE, could not be confirmed. Patients were also excluded if neuroimaging or autopsy indicated another diagnosis despite compatible facial features. In patients for whom the diagnosis was not clear, a medical geneticist and/or pediatric neurologist (A.C. and A.P.) determined eligibility for inclusion in the study.

Controls. For each patient (case), a control matched on gender who had the closest birthdate to the patient was selected from the Genetics database. To avoid both overlap between patients and controls and under-ascertainment of risk factors, potential controls were excluded if they had a midline craniofacial or a midline structural CNS anomaly or if they had multiple congenital anomalies with a strongly suspected structural CNS abnormality. If a potential control was excluded, the next eligible person entered in the database, based on the above criteria, was selected as the control.

Data Collection

Using a standardized form created for this study (See Supporting Information online eAppendix II), information from the Genetics records of patients and controls was abstracted on the following: parental characteristics; prenatal factors, including maternal illness, medications, and possible exposure to teratogens; family history and history of consanguinity; prenatal testing; birth data, including need for resuscitation; patient physical anomalies; results of investigations, including neuroimaging, cytogenetic studies, and autopsy, where applicable; and sequelae, including morbidity and mortality.

Data were abstracted solely from the Genetics records unless determination of eligibility for the study was in question. In this case, information from the hospital chart was sought strictly to clarify the patient's eligibility for study inclusion. All of the information collected was then recorded in an electronic database created for this study.

In construction of the database, a distinction was made among Aborigines—those who were Native, Inuit, Metis, and those with

more distant Aboriginal ancestry. However, the odds ratio (OR) for association of ethnic background with HPE was calculated after excluding those with mixed ancestry. Although an attempt was made to collect information on diabetes control, as well as the amount of alcohol consumption or smoking, the inconsistent and qualitative nature of the data made comparisons between patients difficult. Therefore, only the presence of prepregnancy diabetes and the use or non-use of alcohol and/or cigarettes during the pregnancy were used in the calculation of ORs. Similarly, although information on the various types of infections and substances used was collected, ORs were calculated for infections or substance use as a whole. We also attempted to collect information on the timing in gestation of certain exposures, but this information was not always available and was not used in the calculation of ORs. If “no teratogens” was documented in the chart, this was recorded as lack of alcohol or illicit drug use in the pregnancy, with no assumption made about maternal diabetes or smoking. Similarly, if the pregnancy was documented as “unremarkable,” absence of teratogens or significant maternal history was presumed, rather than classifying the data as unavailable. No distinction was made between unilateral, bilateral, and midline cleft lip/palate in family members.

HPE was classified as alobar, semilobar, lobar, or midline interhemispheric fissure variant. If this information had not been recorded in the chart, it was determined based on available information. HPE was also classified as syndromic or nonsyndromic. Syndromic patients included those who had abnormalities on conventional or molecular cytogenetic testing, as well as those with normal chromosomes but with a constellation of features suggestive of a known syndrome.

Statistical Analysis

Using Manitoba census data, the incidence of HPE among live born infants in the Manitoba population was calculated [CANSIM II SERIES V470802]. With information obtained from the 2001 Census profiles, median household incomes based on postal codes were used in the estimation of socioeconomic status. In this estimation process, the mean of all of the median household incomes for the study population was calculated. Patients and controls were then grouped into low and high socioeconomic status above and below this mean. The nonsyndromic patients were then compared to controls to identify potential risk factors for this form of HPE. Logistic regression was used to calculate unadjusted ORs and their 95% confidence intervals (CIs) for potential risk factors for HPE. Logistic regression was also used to calculate ORs for having an Aboriginal mother, an Aboriginal father, at least one Aboriginal parent or two Aboriginal parents, adjusted for low socioeconomic status. The independent-sample *t*-test was used to compare continuous variables between groups. A *P*-value <0.05 was considered statistically significant.

RESULTS

Case Ascertainment

Forty-five of 47 (96%) patients with HPE were identified from the Genetics database. Two additional patients were ascertained from

the Pediatric Neurology database. The information on these two patients was abstracted from the Genetics database, where the patients were subsequently found listed under an alternate diagnosis.

Incidence of HPE and Its Subtypes

There were 47 patients with HPE identified over our study period, of which 27 were live born. Of these patients, 25 were live born over the years for which we have complete data (excluding 2001). Using census data indicating 175,338 live births over these 11 years, the prevalence of HPE in Manitoba, therefore, approximates 1.4 in 10,000 (95% CI 0.9–1.9) live births per year. Of the 47 patients, 24 were syndromic and 23 were nonsyndromic forms of HPE. In the majority of patients with syndromic and nonsyndromic HPE, the diagnosis of HPE was confirmed postnatally by either neuroimaging (12/23 or 52.2% nonsyndromic) or autopsy (2/23 or 8.7% nonsyndromic), in approximately one-third of patients, by antenatal ultrasound (8/23 or 34.8% nonsyndromic), and in one case each of syndromic and nonsyndromic HPE, by examination only (4.3% nonsyndromic).

The results of chromosome testing, done either prenatally by amniocentesis, or postnatally, were available for 89% (42/47) of the patients with HPE. Results of molecular testing were available in only 28% (13/47) of the patients, and only among those who already had conventional cytogenetic studies where results did not already indicate trisomy 13. Most of the cytogenetic test results provided were for deletions of 7q, although in a few cases it was apparent that deletions of chromosome 7p, 18p/q, and 21q were also sought. In other cases, the specific molecular tests were not specified. In some cases, other molecular tests were done as considered appropriate (e.g., 22q11), and these are not included in the previous numbers, as they do not represent a genetic locus associated with HPE. Further genetic information may become available in the future as, in many cases, the DNA was banked.

Chromosomal and molecular abnormalities accounted for 32% (15/47) of HPE patients, or for 36% of those with available chromosome study results. Trisomy 13 was most frequent followed by deletions involving chromosome 7. Non-chromosomal syndromic forms accounted for 19% (9/47) of HPE patients. Nonsyndromic HPE was, therefore, present in the remaining 49% (23/47) of patients.

Identification of Potential Risk Factors for Nonsyndromic HPE

Initially, 47 controls matched for gender and birthdate were selected, 1 for each case of syndromic and nonsyndromic HPE. The 23 nonsyndromic forms of HPE were chosen for further study and elucidation of potential risk factors. Making use of all of the original controls, this provided two controls for each case of nonsyndromic HPE.

The ORs calculated for potential risk factors and their association with HPE is shown in Table I. The following factors were significantly associated with HPE in univariate analyses: having an Aboriginal mother, having an Aboriginal father, having at least one Aboriginal parent, having a family history of a midline facial defect, and being of low socioeconomic status. Although there was a trend in our study suggesting that prepregnancy diabetes

TABLE I. Proposed Risk Factors for the Development of Holoprosencephaly

Risk factor	Patients ^a (max n = 23)	Controls ^a (max n = 47)	Unadjusted odds ratio	95% CI
Aboriginal mother ^b	10/22	8/42	3.5	1.1–11.1
Aboriginal father ^b	11/21	3/38	12.8	3.0–55.1
At least one aboriginal parent ^b	12/21	8/38	5.0	1.6–16.0
Two aboriginal parents ^b	9/21	3/38	8.8	2.0–37.8
Family history midline facial defect	6/21	2/43	8.2	1.5–45.2
Low socioeconomic status ^c	16/22	22/47	3.0	1.0–9.1
Previous spontaneous abortion	7/22	12/44	1.2	0.4–3.8
Previous stillbirth	2/23	2/45	2.0	0.3–15.6
Previous neonatal death	1/23	0/45 ^d	4.1	0.1–126.7
Prepregnancy diabetes mellitus	3/23	1/47	6.9	0.7–70.4
Infections ^{e,f}	5/23	15/46	0.6	0.2–1.8
Alcohol exposure ^f	9/22	15/44	1.3	0.5–3.8
Smoking ^f	11/18	23/44	1.4	0.5–4.4
Substance abuse ^f	1/19	7/33	0.2	0.02–1.8
Folic acid/vitamins ^f	4/23	15/47	0.5	0.1–1.6

^aData not available for all patients and controls.

^bAboriginal does not include Metis, mixed race, etc.

^cBased on postal codes.

^dFor purpose of calculating OR, 0.5 rather than 0 is used.

^eThe five infections among cases include: Two upper respiratory tract infections, two urinary tract infections, and one genital infection (chlamydia). The 15 infections among controls include:

6 upper respiratory tract infections, 1 lower respiratory tract infection, 2 urinary tract infections, 5 genital infections (1 chlamydia, 1 chlamydia and warts, 1 herpes, 2 yeast), and 1 unknown.

^fIrrespective of timing in gestation.

may be associated with HPE, it did not attain statistical significance. Two of the three instances of prepregnancy diabetes occurred among Aboriginal mothers (with the third having an unknown racial background), as did the only instance of prepregnancy diabetes among controls. Numbers were, therefore, too low to allow for assessment of the risk associated with having an Aboriginal mother adjusting for pregestational diabetes.

Low socioeconomic status is likely correlated with having an Aboriginal parent (having an Aboriginal mother, $P=0.11$, having an Aboriginal father, $P=0.07$, having at least one Aboriginal parent, $P=0.08$, and having two Aboriginal parents, $P=0.05$), although these associations were generally not statistically significant. When adjusted for low socioeconomic status, having an Aboriginal mother (OR 3.3, 95% CI 1.0–10.8), having an Aboriginal father (OR 11.9, 95% CI 2.6–53.9), having at least one Aboriginal parent (OR 5.0, 95% CI 1.5–16.9), or having two Aboriginal parents (OR 7.5, 95% CI 1.6–34.2) remained significant predictors of HPE.

There was no association of HPE with previous spontaneous abortion, stillbirth, or neonatal death, or with infections, alcohol exposure, smoking, or substance abuse during the pregnancy. The use of periconceptional folic acid or vitamins did not appear to exert a protective influence. The mean maternal age (25.3 years vs. 27.6 years) and paternal age (28.8 years vs. 31.8 years) did not differ between nonsyndromic patients and controls ($P=0.10$ for each).

DISCUSSION

There have been very few case–control studies designed to look at risk factors for the development of nonsyndromic HPE. In our

case–control study, we found that having an Aboriginal parent, having a family history of a midline facial defect, and being of low socioeconomic status were associated with the development of nonsyndromic HPE. Our analysis of risk factors was based on live births, stillbirths, and terminations with nonsyndromic, non-chromosomal HPE. These comprised approximately half of our patients with HPE, consistent with the literature [Croen et al., 1996, 2000; Rasmussen et al., 1996].

The prevalence of HPE among live births of 1.4 in 10,000 found in our study is among the highest reported in the literature [Roach et al., 1975; Urioste et al., 1988; Mastroiacovo et al., 1992; Martínez-Frías et al., 1994; Croen et al., 1996; Rasmussen et al., 1996; Whiteford and Tolmie, 1996; Olsen et al., 1997; Forrester and Merz, 2000; Bullen et al., 2001; Ong et al., 2007; Orioli and Castilla, 2007]. A similar prevalence of 1.31 per 10,000 was found through the International Clearinghouse Birth Defects Surveillance Systems, based on over 7 million births from five continents; however, this figure included stillbirths and pregnancy terminations in addition to live births [Leoncini et al., 2008]. To the best of our knowledge, few populations report a higher prevalence. There was a reported prevalence of HPE of 1.7 per 10,000 in the West Midlands area of the United Kingdom, but again, this figure includes terminations, fetal losses, and stillbirths in addition to live births [Ong et al., 2007]. A much higher birth prevalence rate of 6.06 per 10,000 was found in Taipei, Taiwan [Chen et al., 2005].

Although cross-study comparisons are challenging due to different combinations of live births, terminations, and/or stillbirths used in the numerator, we believe that the high incidence of HPE in our study is largely due to inherent characteristics of the Manitoba

population, rather than to different definitions for prevalence employed by different studies. As HPE is an abnormality where patients are detected prenatally and outcomes are poor [Ong et al., 2007], our calculation, which does not include terminations, is likely to be an underestimate of the true prevalence in Manitoba. This is especially true given that the prevalence of HPE appears to be 40 times higher among early spontaneous abortions (<10 weeks) than that of pregnancies that progress beyond 20 weeks [Matsunaga and Shiota, 1977]. In fact, one study found that the observed prevalence rate differences can largely be explained by the pregnancy outcome status of the studied cohort [Orioli and Castilla, 2010] with fewer than 1 in 10,000 patients with only live and stillbirths, more with terminations included, and between 40 and 50 per 10,000 in two classical Japanese studies on aborted embryos. Our prevalence of HPE among live births exceeds the expected rate based on this study's figures. This may be, at least in part, related to the large population of Aboriginals in Manitoba, as the Orioli and Castilla study found a trend toward higher birth prevalence rates in minorities, with likely a lower prenatal detection rate of HPE and consequently fewer terminations of pregnancy. Patients in our study were born between 1990 and 2001, during the same time period as some of the most recent studies. Therefore, it is unlikely that recent improvements in imaging techniques and diagnostic testing are important explanations for the higher incidence of HPE observed in our study.

Unique to the Manitoba population is the large population of Aboriginals, comprising 10.6% and 13.5% of the total population of Manitoba in 1991 and 2001, respectively [Statistics Canada], and our findings suggest that this racial background is associated with the development of HPE. In fact, the risk may be higher than we have estimated as we may have disproportionately missed babies born to Aboriginal parents living on remote reserves. The California Study [Croen et al., 1996] was the first to suggest racial background as a potential risk factor. In that study, among chromosomally normal patients, those with mothers who were Hispanic-White were at increased risk of HPE, as were those with mothers who were born in Mexico, although the former was not statistically significant. In a subsequent paper by the same authors, among non-syndromic patients, increased risks were observed for foreign-born versus U.S. or Mexico-born women [Croen et al., 2000]. Similarly, in a study performed in Atlanta, the risk for HPE and/or arhinencephaly was higher among Non-White than "White" infants [Rasmussen et al., 1996]. Among a Hawaiian population, having a mother who was Far East Asian increased the chance of HPE in the baby, compared to having a mother who was "White" [Forrester and Merz, 2000]. However, in this study, HPE patients had to fit into one of five racial categories, or they were excluded from the study. In the study done by Ong et al., there was again a greater risk of HPE in the Non-White population. This risk was statistically significant in the Pakistani and in the Black African populations, although with the latter there were wide CIs as numbers were small [Ong et al., 2007].

Given that minorities may be at increased risk of HPE, this suggests the possibility that lower socioeconomic status may play a role. Interestingly, Miller et al. [2010] found an association of lower education level with nonsyndromic HPE. Our study, in fact, found that being of lower socioeconomic status confers three

times the odds of having a baby with HPE, yet being of Aboriginal background also confers a risk of HPE independent of low socioeconomic status. Among the Aboriginal population, the following conditions suggest lower socioeconomic status: a higher unemployment rate and a lower income; a high rate of adolescent pregnancy and more single parent families; poor housing, inadequate water supplies and waste disposal in some communities; environmental contaminants; less access to health care; nutritional deficiencies; obesity and higher rates of type II diabetes; higher rates of smoking, caffeine intake and binge alcohol drinking among mothers; and higher rates of infectious diseases, including tuberculosis [MacMillan et al., 1996].

Any one or various combinations of these factors point to an inherent difference in the Aboriginal population that might predispose to the development of HPE. However, infections, smoking, alcohol use, and substance use were not associated with HPE in our study. Some studies have suggested a causal link between maternal diabetes and HPE [Barr et al., 1983; Olsen et al., 1997]. Our findings are consistent with these studies, with prepregnancy diabetes having an OR of 6.9, although not statistically significant, likely due to our small numbers. The few occurrences of prepregnancy diabetes in our study occurred primarily among Aboriginal mothers, although, again, numbers were small. Other studies [Croen et al., 2000; Orioli and Castilla, 2007] had found a statistically significant increased risk of having babies with HPE among women who took insulin for diabetes during the index pregnancy, but only two of our patients with HPE had mothers who used insulin, one of whom was known to be Aboriginal and the other whose racial background was unknown.

In addition to environmental factors, one cannot exclude the possibility that genetic factors may play a role in the development of nonsyndromic HPE. Aboriginal populations carry a founder effect and a relatively smaller gene pool. Similarly, in the study by Ong et al., the Pakistani population of the United Kingdom has a high prevalence for consanguineous unions. In this manner, some patients of apparently isolated HPE may represent recessively inherited HPE [Ong et al., 2007]. In our study, having a family history of a midline facial defect was found to be a risk factor. This may have a genetic basis. Consistent with this speculation is the finding that Sonic hedgehog, one of the genes implicated in the development of HPE, is essential for first pharyngeal arch development [Yamagishi et al., 2006]. Alternatively, shared risk factors may predispose one to the development of HPE as well as to other midline facial defects or other congenital anomalies. Interestingly, the risk of oral clefting is also increased among Aboriginals [Lowry et al., 1986; Bower et al., 1989; Tolarova and Cervenka, 1998].

Our study has strengths and limitations. One of its major strengths lies in its case-control design, making it one of very few case-control studies in the literature that examines risk factors for the development of HPE, particularly nonsyndromic HPE. We believe that information on patients and controls was obtained consistently and reliably by specialist physicians in the Genetics department. While many patients with mild isolated anomalies or those with only a small genetic component would not be referred, the department would have been involved with the vast majority of other patients and would have a record of every patient for whom

chromosome studies were done. Our study also covers a time period in which we would have been able to take advantage of improvements in technology and prenatal ultrasound diagnosis.

Our study numbers are small and, therefore, did not permit a comprehensive multivariate analysis to identify independent risk factors for the development of HPE. Another important limitation to this study was the lack of molecular testing on all patients. While the karyotype was available for 87% of the patients, molecular cytogenetic and molecular tests were not performed consistently. At least 10% of HPE patients with normal chromosomes have microdeletions/duplications and remain undetected by usual karyotyping [Orioli and Castilla, 2010]. With the availability of current arrayCGH technology, the proportion of HPE patients with chromosomal deletions/duplication might have well been higher as has been demonstrated in other cohorts with HPE [Bendavid et al., 2009]. Therefore, patients with chromosomal or monogenic disorders may have mistakenly been classified as nonsyndromic, affecting the magnitude of the observed associations. This would be the case, for instance, if mutations in HPE-associated genes went undetected among Aboriginal parents.

Lacking a population-based registry of congenital anomalies in Manitoba, the identification of patients with HPE from the Genetics database represented the best alternative. However, this may have lead to the under-ascertainment of milder patients of HPE, those without an abnormal outward physical appearance. Although the retrospective design of this study represented the most efficient way to collect information on a large number of patients, information on a proposed risk factor was occasionally missing from the chart and the information was not collected in as standardized a manner as could have been achieved through direct study interviews administered to parents. Another limitation of the study arises from the use of the Genetics database for the selection of controls. However, by selecting patients and the controls from the same defined population, similar information was obtained and a comparable evaluation was performed for both patients and controls. There have been precedents to this kind of study design [de Vries et al., 2001].

In summary, we found that having an Aboriginal mother, an Aboriginal father, at least one Aboriginal parent, or two Aboriginal parents, having a family history of a midline facial defect and being of low socioeconomic status were associated with the development of nonsyndromic HPE. Future studies should be done in Manitoba and among other populations that incorporate more patients, with molecular testing performed routinely, to help to determine the significance of these potential risk factors among nonsyndromic patients with HPE. In particular, further research is needed to determine what it is about being Aboriginal that increases the risk of HPE.

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APPENDIX I—TERMS FOR IDENTIFYING PATIENTS WITH HOLOPROSENCEPHALY

We sought to maximize holoprosencephaly case ascertainment by searching the clinic databases using the following terms:

Holoprosencephaly
Anophthalmia
AR campomelic dysplasia
Arhinencephaly
Ceboccephaly
Cyclopia
Deletion 13q syndrome
Deletion 18p syndrome
Diabetes insipidus
Ethmocephaly
Hypotelorism
Infant of a diabetic mother (IDM)
Meckel syndrome
Microphthalmia
Pallister–Hall syndrome
Pseudotrisomy 13
Single central incisor
Smith–Lemli–Opitz syndrome
Trisomy 13
Trisomy 18.

REFERENCES

- Agapitos M, Georgiou-Theodoropoulou M, Koutselinis A, Papacharalamus N. 1986. Cyclopia and maternal ingestion of salicylates. *Pediatr Pathol* 6:309–310.
- Barr M Jr, Hanson JW, Currey K, Sharp S, Toriello H, Schmickel RD, Wilson GN. 1983. Holoprosencephaly in infants of diabetic mothers. *J Pediatr* 102:565–568.
- Belloni E, Muenke M, Roessler E, Traverso G, Siegel-Bartelt J, Frumkin A, Mitchell HF, Donis-Keller H, Helms C, Hing AV, Heng HH, Koop B, Martindale D, Rommens JM, Tsui LC, Scherer SW. 1996. Identification of Sonic hedgehog as a candidate gene responsible for holoprosencephaly. *Nat Genet* 14:353–356.
- Benawra R, Mangurten HH, Duffell DR. 1980. Cyclopia and other anomalies following maternal ingestion of salicylates. *J Pediatr* 96:1069–1071.
- Bendavid C, Rochard L, Dubourg C, Seguin J, Gicquel I, Pasquier L, Vigneron J, Laquerrière A, Marcorelles P, Jeanne-Pasquier C, Rouleau C, Jaillard S, Mosser J, Odent S, David V. 2009. Array-CGH analysis indicates a high prevalence of genomic rearrangements in holoprosencephaly: An updated map of candidate loci. *Hum Mutat* 30:1175–1182.
- Bonnemann C, Meinecke P. 1990. Holoprosencephaly as a possible embryonic alcohol effect: Another observation. *Am J Med Genet* 37: 431–432.
- Bower C, Forbes R, Seward M, Stanley F. 1989. Congenital malformations in aborigines and non-aborigines in Western Australia, 1980–1987. *Med J Aust* 151:245–248.
- Brown SA, Warburton D, Brown LY, Yu CY, Roeder ER, Stengel-Rutkowski S, Hennekam RC, Muenke M. 1998. Holoprosencephaly due to mutations in ZIC2, a homologue of *Drosophila* odd-paired. *Nat Genet* 20:180–183.

- Bullen PJ, Rankin JM, Robson SC. 2001. Investigation of the epidemiology and prenatal diagnosis of holoprosencephaly in the North of England. *Am J Obstet Gynecol* 184:1256–1262.
- Byrne PJ, Silver MM, Gilbert JM, Cadera W, Tanswell AK. 1987. Cyclopia and congenital cytomegalovirus infection. *Am J Med Genet* 28:61–65.
- Chen CP, Chern SR, Lin CJ, Lee CC, Wang W, Tzen CY. 2005. A comparison of maternal age, sex ratio and associated anomalies among numerically aneuploid, structurally aneuploid and euploid holoprosencephaly. *Genet Couns* 16:49–57.
- Croen LA, Shaw GM, Lammer EJ. 1996. Holoprosencephaly: Epidemiologic and clinical characteristics of a California population. *Am J Med Genet* 64:465–472.
- Croen LA, Shaw GM, Lammer EJ. 2000. Risk factors for cytogenetically normal holoprosencephaly in California: A population-based case-control study. *Am J Med Genet* 90:320–325.
- de la Cruz JM, Bamford RN, Burdine RD, Roessler E, Barkovich AJ, Donnai D, Schier AF, Muenke M. 2002. A loss-of-function mutation in the CFC domain of TDGF1 is associated with human forebrain defects. *Hum Genet* 110:422–428.
- de Vries BB, White SM, Knight SJ, Regan R, Homfray T, Young ID, Super M, McKeown C, Splitt M, Quarrell OW, Trainer AH, Niermeijer MF, Malcolm S, Flint J, Hurst JA, Winter RM. 2001. Clinical studies on submicroscopic subtelomeric rearrangements: A checklist. *J Med Genet* 38:145–150.
- DeMyer W, Zeman W. 1963. Alobar holoprosencephaly (arhinencephaly) with median cleft lip and palate: Clinical, electroencephalographic, and nosologic considerations. *Confin Neurol* 23:1–36.
- Forrester MB, Merz RD. 2000. Epidemiology of holoprosencephaly in Hawaii, 1986–1997. *Paediatr Perinat Epidemiol* 14:61–63.
- Golden J. 1999. Towards a greater understanding of the pathogenesis of holoprosencephaly. *Brain Dev* 21:513–521.
- Gripp KW, Wotton D, Edwards MC, Roessler E, Ades L, Meinecke P, Richieri-Costa A, Zackai EH, Massagué J, Muenke M, Elledge SJ. 2000. Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. *Nat Genet* 25:205–208.
- Holmes LB, Harvey EA. 1994. Holoprosencephaly and the teratogenicity of anticonvulsants. *Teratology* 49:82.
- Jellinger K, Gross H, Kaltenback E, Grisold W. 1981. Holoprosencephaly and agenesis of the corpus callosum: Frequency of associated malformations. *Acta Neuropathol* 55:1–10.
- Johnson CY, Rasmussen SA. 2010. Non-genetic risk factors for holoprosencephaly. *Am J Med Genet Part C* 154C:73–85.
- Khudr G, Olding L. 1973. Cyclopia. *Am J Dis Child* 125:120–122.
- Kilic N, Yazici Z. 2005. A case of holoprosencephaly and cebocephaly associated to torch infection. *Int J Pediatr Otorhinolaryngol* 69:1275–1278.
- Kotzot D, Weigl J, Huk W, Rott HD. 1993. Hydantoin syndrome with holoprosencephaly: A possible rare teratogenic effect. *Teratology* 48:15–19.
- Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Grix AW Jr, Lott IT, Richard JM, Sun SC. 1985. Retinoic acid embryopathy. *N Engl J Med* 313:837–841.
- Leoncini E, Baranello G, Orioli IM, Anneren G, Bakker M, Bianchi F, Bower C, Canfield MA, Castilla EE, Cocchi G, Correa A, De Vigan C, Doray B, Feldkamp ML, Gatt M, Irgens LM, Lowry RB, Maraschini A, McDonnell R, Morgan M, Mutchinick O, Poetzsch S, Riley M, Ritvanen A, Gnansia ER, Scarano G, Sipek A, Tenconi R, Mastroiacovo P. 2008. Frequency of holoprosencephaly in the International Clearinghouse Birth Defects Surveillance Systems: Searching for population variations. *Birth Defects Res A Clin Mol Teratol* 82:585–591.
- Lowry RB, Thunem NY, Silver M. 1986. Congenital anomalies in American Indians of British Columbia. *Genet Epidemiol* 3:455–467.
- MacMillan HL, MacMillan AB, Offord DR, Dingle JL. 1996. Aboriginal health. *CMAJ* 155:1569–1578.
- Martínez-Frías ML, Bermejo E, García A, Galán E, Prieto L. 1994. Holoprosencephaly associated with caudal dysgenesis: A clinical-epidemiological analysis. *Am J Med Genet* 53:46–51.
- Mastroiacovo P, Cavalcanti DP, Zampino G, Serafini MA. 1992. Epidemiological and genetic study of holoprosencephaly in 106 cases observed in the Italian Multicentric Registry 1978–1989. In *Proceedings of the First International Meeting of the Genetic and Reproductive Epidemiology Research Society*. pp. 71–82.
- Matsunaga E, Shiota K. 1977. Holoprosencephaly in human embryos: Epidemiologic studies of 150 cases. *Teratology* 16:261–272.
- Miller E, Rasmussen S, Siega-Riz A, Frias J, Honein M. and the National Birth Defects Prevention Study. 2010. Risk factors for nonsyndromic holoprosencephaly in the National Birth Defects Prevention Study. *Am J Med Genet Part C* 154C:62–72.
- Ming JE, Kaupas ME, Roessler E, Brunner HG, Golabi M, Tekin M, Stratton RF, Sujansky E, Bale SJ, Muenke M. 2002. Mutations in PATCHED-1, the receptor for SONIC HEDGEHOG, are associated with holoprosencephaly. *Hum Genet* 110:297–301.
- Nanni L, Schelper RL, Muenke MT. 2000. Molecular genetics of holoprosencephaly. *Front Biosci* 5:D334–D342.
- Olsen CL, Hughes JP, Youngblood LG, Sharpe-Stimac M. 1997. Epidemiology of holoprosencephaly and phenotypic characteristics of affected children: New York State, 1984–1989. *Am J Med Genet* 73:217–226.
- Ong S, Tonks A, Woodward ER, Wyldes MP, Kilby MD. 2007. An epidemiological study of holoprosencephaly from a regional congenital anomaly register: 1995–2004. *Prenat Diagn* 27:340–347.
- Orioli IM, Castilla EE. 2007. Clinical epidemiologic study of holoprosencephaly in South America. *Am J Med Genet Part A* 143A:3088–3099.
- Orioli IM, Castilla EE. 2010. Epidemiology of holoprosencephaly: Prevalence and risk factors. *Am J Med Genet Part C* 154C:73–85.
- Rasmussen SA, Moore CA, Khoury MJ, Cordero JF. 1996. Descriptive epidemiology of holoprosencephaly and arhinencephaly in metropolitan Atlanta, 1968–1992. *Am J Med Genet* 66:320–333.
- Roach E, Demyer W, Conneally PM, Palmer C, Merritt AD. 1975. Holoprosencephaly: Birth data, genetic and demographic analyses of 30 families. *Birth Defects Orig Artic Ser* 11:294–313.
- Roessler E, Belloni E, Gaudenz K, Jay P, Berta P, Scherer SW, Tsui LC, Muenke M. 1996. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nat Genet* 14:357–360.
- Roessler E, Du YZ, Mullor JL, Casas E, Allen WP, Gillesen-Kaesbach G, Roeder ER, Ming JE, Ruiz i, Altaba A, Muenke M. 2003. Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proc Natl Acad Sci USA* 100:13424–13429.
- Ronen GM. 1992. Holoprosencephaly and maternal low-calorie weight-reducing diet. *Am J Med Genet* 42:139.
- Ronen GM, Andrews WL. 1991. Holoprosencephaly as a possible embryonic alcohol effect. *Am J Med Genet* 40:151–154.
- Rosa F. 1995. Holoprosencephaly and antiepileptic exposures. *Teratology* 51:230.

- Stabile M, Bianco A, Iannuzzi S, Buonocore MC, Ventruto V. 1985. A case of suspected teratogenic holoprosencephaly. *J Med Genet* 22:147–149.
- Tolarova MM, Cervenka J. 1998. Classification and birth prevalence of orofacial clefts. *Am J Med Genet* 75:126–137.
- Urioste M, Valcarcel E, Gomez MA, Pinel I, Garcia deLeón R, Diaz de Bustamante A, Tebar R, Martinez-Frias ML. 1988. Holoprosencephaly and trisomy 21 in a child born to a nondiabetic mother. *Am J Med Genet* 30:925–928.
- Wallis DE, Roessler E, Hehr U, Nanni L, Wiltshire T, Richieri-Costa A, Gillesen-Kaesbach G, Zackai EH, Rommens J, Muenke M. 1999. Mutations in the homeodomain of the human SIX3 gene cause holoprosencephaly. *Nat Genet* 22:196–198.
- Whiteford ML, Tolmie JL. 1996. Holoprosencephaly in the west of Scotland 1975–1994. *J Med Genet* 33:578–584.
- Yamagishi C, Yamagishi H, Maeda J, Tsuchihashi T, Ivey K, Hu T, Srivastava D. 2006. Sonic hedgehog is essential for first pharyngeal arch development. *Pediatr Res* 59:349–354.