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Adverse Reproductive Outcomes Among Pregnancies of Aunts and (Spouses of) Uncles in Irish Families With Neural Tube Defects

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Adverse pregnancy outcomes may be more frequent among sibs of individuals with neural tube defects (NTDs), and transmission of risk in families with an NTD may be more frequent among maternal relatives. In a study designed to evaluate matrilineal risk for NTDs, we compared adverse pregnancy outcomes among maternal and paternal first cousin pregnancies. Pregnancy histories were obtained by interview with 288 uncles and aunts (parents of the first cousin pregnancies) in 48 Irish NTD families. We analyzed pregnancy outcomes (preterm deliveries, stillbirths, and miscarriages) among 1,033 singleton first cousin pregnancies and compared risk among maternal versus paternal relatives. Maternal first cousin pregnancies were more likely to end adversely when compared to paternal first cousin pregnancies (17.4% vs. 11.7%, $P=0.01$). In a logistic regression analysis of pregnancies unaffected by birth defects, maternal line remained independently associated with adverse outcomes (odds ratio (OR) = 1.55, 95% confidence interval (CI) 1.06, 2.27) after controlling for NTD type, maternal age, maternal smoking during pregnancy, first cousin pregnancy's year of birth. The excess risk with maternal line related mainly to spina bifida occulta families (OR = 42.4; CI 2.64, 681; $P=0.008$); risk in open spina bifida families was 1.24 (CI 0.82, 1.87; $P=0.3$). These results support the hypothesis of excess risk for adverse pregnancy outcomes among maternal relatives in NTD families. Further work is needed, epidemiological as well as clinical and molecular, not only to confirm these findings, but also to define the underlying biological mechanisms linking adverse reproductive outcomes, excess maternal risk and occurrence of NTDs. © 2005 Wiley-Liss, Inc.

Key words: neural tube defects; pregnancy outcomes; miscarriages; preterm births; Ireland; family study; uncles and aunts

INTRODUCTION

Neural tube defects (NTDs) are among the most common and most serious of all birth defects worldwide. Their causes include environmental factors, as well as an underlying genetic susceptibility. Rates of NTDs vary greatly over both time and place. Epidemics have been reported, and in recent decades rates in many locations have declined significantly [Elwood et al., 1992]. In Ireland, about 50 years ago, rates at birth were around 8 per 1,000 [Coffey, 1983]. Since then NTD rates have dropped precipitously, reaching between 0.5 and 1 per 1,000 births in the year 2004 [Botto et al., 2005]. It is not known why rates have dropped so sharply in Ireland, improved nutrition with incorporation of more folate-containing foods is a possibility. Although it is widely accepted that folic acid prevents about 70% of new NTD cases from occurring, its method of action remains largely unknown [Botto et al., 1999].

While most NTDs may be environmental in origin, the excess risks for NTDs of 15- to 30-fold among close family members strongly suggest that genetic factors are also at work [Elwood et al., 1992; Byrne et al., 1996]. However, concordance for type of NTD is low, with different types of NTDs occurring within sibships [Hall et al., 1988]. Current molecular efforts are focussed on evaluating genes in the folic acid metabolism pathways. Studies in the Irish population suggest a role for specific genetic variants of the *MTHFR* gene among the Irish [Kirke et al., 2004].

There is considerable epidemiological evidence from earlier studies suggesting that genetic factors

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related to NTDs may be transmitted preferentially from the mother's side of the family. The evidence for this hypothesis includes excess rates of NTDs among mothers' relatives, whether full blood relatives or half-sibs [Carter and Evans, 1973; Nevin and Johnston, 1980; McManus, 1987; Chatkupt et al., 1992]. Among families with distant affected relatives the linking parent is more often female than male [Chatkupt et al., 1992; Mariman and Hamel, 1992].

The observation that miscarriages may occur more frequently in NTD sibships has led some to speculate that these may represent severe forms of NTDs that are incompatible with life [Elwood et al., 1992], though no studies known to us have evaluated this idea directly. Excess miscarriages may represent another form or expression of the underlying genetic susceptibility to NTDs.

The overall hypotheses for this study of relatives in Irish NTD families is that underlying hereditary factors exist in NTD families and can be detected in distant relatives using epidemiological methods. In this report, we evaluate the hypothesis that maternal relatives are more likely than paternal relatives to exhibit evidence for the hereditary basis for NTDs. We use occurrence of adverse reproductive outcomes as the outcome variable.

MATERIALS AND METHODS

This study reports results from Phase II of studies into Irish NTD families by the Boyne Research Institute (BRI). Phase I evaluated pregnancy histories and the health of siblings in the original nuclear families. Phase II subjects were identified by their siblings during Phase I. Therefore, subject participation in Phase I appears first below. Phase I families are called "nuclear families." References to 'pregnancies of uncles' are understood to be pregnancies *fathered* by uncles.

Phase I Study

Family identification. Nuclear families were identified from various sources, as follows: by their membership in the Irish Association for Spina Bifida and Hydrocephalus (Louth-Meath branch); by their membership in the Association for Spina Bifida and Hydrocephalus (of the United Kingdom, Ballymena, Northern Ireland, branch), or by word of mouth, including newspapers and local radio announcements, from among the general public living along the East Coast of Ireland. The nuclear families contained at least one child or one pregnancy that had been affected with any one of four types of NTD—anencephaly, open spina bifida, spina bifida occulta, or encephalocele.

Contact procedures. Families were contacted either in person or during meetings of the relevant associations, and a suitable date arranged for the interview. All interviews were done face-to-face, either in the subject's home, or at a hotel, or in the offices of the Boyne Research Institute. The original nuclear families were interviewed between 1995 and 1998. The respondent was the mother or father usually; in some cases the proband was the respondent.

Interview. The interview covered the proband's diagnosis and place of birth and treatment, date and place of birth for each parent, together with parents' educational level and their height and weight, mother's reproductive history, illnesses during pregnancy, and exposures before and during early pregnancy (drugs, medications, smoking, folic acid intake). In terms of family members, the interview identified individually each grandparent and each sibling of each parent. For each person, the interview covered their date of birth, date and cause of death and their health (birth defects and cancer).

Phase II Study

Subject participation. At the time that this Phase II study was started, 77 families had participated in Phase I. From among these families 48 agreed to participate in this Phase II study and they supplied the contact information for their brothers and sisters. Reasons for refusal by the original respondents included: family moved and not located, family contact deceased and no proxy, relationships within the family had deteriorated, family contact unwilling to provide contact information for their own siblings and/or those of the original spouse.

Contact procedures. Respondents from Phase I were recontacted by letter followed by a telephone call, to obtain their cooperation in this new study. The interviewer requested the name, address, and telephone number of each eligible sibling (uncles and aunts of the proband). In some cases, families preferred to notify each uncle or aunt themselves and obtain their cooperation personally.

Once the contact information had been obtained the interviewer sent a letter to each uncle or aunt explaining the aims and methods of the study; the letter was followed up with a phone call. Arrangements were made for a face-to-face interview for subjects living within 40 miles of the Boyne Research Institute. For subjects living further away, or overseas, a telephone or mailed interview was obtained.

Eligibility. To be eligible for the Phase II study, uncles and aunts had to be 18 years of age or older by January 3, 2000. If the uncle or aunt had died after reaching age 18 or became incompetent after age 18 a proxy respondent was sought from the next of kin, usually a spouse or a child. Ineligible subjects were uncles and aunts who were adopted, half-sibs, relatives who were younger than 18 years of age, or were mentally retarded or mentally ill, or confined to an institution since before age 18.

background rate of pregnancy loss in the community. The sample was constructed by a structured sampling procedure. Every tenth file was opened seeking an eligible female patient. For eligibility, women had to have had at least one pregnancy. If the patient was not eligible, the next file in the sequence was opened. This process continued until 100 women were identified. Of the 100, 8 could not be located; of the remaining 92, 70 agreed, giving a response rate of 76%. All interviews were done in person, either in the woman's home or in a neutral location. The interview covered details of their pregnancy histories, and was substantially similar to that for the nuclear families. Seventy women had 268 pregnancies with known outcomes, ending between 1948 and 1993. The outcomes consisted of 30 miscarriages, 3 stillbirths, 13 preterm livebirths and 222 pregnancies that ended in a full-term liveborn child. Mothers were born between 1925 and 1970. Gravidity ranged from 1 to 11, with a mean of 2.9. Results from this study provided comparison data for the NTD pregnancies.

Consent. The Ethics Board of the Boyne Research Institute approved all three studies separately, that is, Phase I, Phase II, and NEPHS. A consent form was completed by subject or proxy describing the objectives of the study and assuring them of procedures in place to secure their anonymity.

Statistical analyses. For all studies, the data were entered and preliminary analyses done in EPI-INFO, a software package for epidemiologic studies, available free from the Centers for Disease Control (www.cdc.gov). For this report, statistical analyses were done with SAS (Statistical Analysis System, Cary, North Carolina). Simple comparisons were tested for statistical significance with chi-square tests or t-tests, as appropriate, with alpha = 0.05. Logistic regression models controlled for potential confounding. Before constructing the multivariate models, all variables of interest were first tested for their association with the dependent variable. Those associations that failed to reach at least $P = 0.2$ were not tested further. Variables that did not meet this standard were- gender of proband, gender of uncle/aunt relative, folic acid taken either before or during pregnancy, alcohol or illicit drug use before or during pregnancy, iron pills before or during pregnancy, measles, flu herpes, cold, diabetes or toxoplasmosis during pregnancy, and birth order. Of the three date of birth variables—that for proband, for uncle/aunt and for first cousin pregnancy—only the date of birth for first cousin pregnancy was tested in logistic regression models, since it was considered that there was substantial overlap between the three variables. When dichotomized, each of the three showed the same effect, all suggesting that adverse outcomes were more common in more recent years. Only the most relevant of the three variables—year of birth of the first cousin pregnancy- was used in logistic regression models. We considered models containing terms for folic acid use before and during pregnancy, maternal smoking before pregnancy, sex of proband, sex of parent, year of birth of uncle/aunt or of proband, as well as line, smoking during pregnancy, proband's diagnosis, maternal age and year of birth of first cousin pregnancy. Non-significant terms were dropped from the final model; we also dropped smoking before pregnancy, although significantly associated, since it did not improve the fit of the final model significantly. Conditional logistic regression models were used to obtain measures of association while stratifying on nuclear family in order to control for clustering of adverse outcomes within families. Potentially confounding variables were dichotomized for modeling purposes as follows: maternal smoking before or during the first trimester of pregnancy (ever/never), mother's age at birth of first cousin pregnancy ($\leq$35, 36+), line (maternal vs. paternal), type of NTD (spina bifida occulta vs. other), year of birth of first cousin pregnancy (<1987, 1987+).

Description of the families. The flow chart in Figure 1 shows the various family generations and participants in this study. The parents in the original nuclear families were born between 1916 and 1971, with a median of 1948; probands were born between 1957 and 1995 with a median year of birth of 1977, and first cousin pregnancies occurred between 1948 and 2000, with a median of 1978.

RESULTS

The 1,033 pregnancy outcomes consisted of 883 full-term pregnancies, 25 premature births, 12 stillbirths, and 113 miscarriages. Overall, the rate of adverse pregnancy outcomes (premature children, stillbirths, and miscarriages) was 14.5%. When examined according to maternal or paternal relationship, 11.7% of pregnancies to paternal relatives ended adversely compared to 17.4% for maternal relatives, a statistically significant difference ($P = 0.01$). The percentage of pregnancies ending adversely among maternal relatives was not significantly in excess of the percentage of pregnancies ending adversely reported by women participating in the NEPHS ($P > 0.05$). All components of the adverse pregnancy outcome (premature deliveries, stillbirths, and miscarriages) were elevated among maternal relatives compared to paternal relatives (Table I). For miscarriages, the difference failed to reach statistical significance (9.4% vs. 12.5%, $P = 0.13$); the difference in the percentages of premature children did achieve statistical significance (1.3% vs. 3.5%, $P = 0.04$). The duration of miscarried pregnancies was from 3 to 24 weeks, with the majority (64%) falling between 8 and 12 weeks. There was no difference in the distribution of gesta-

Enrollment. The 48 participating nuclear families yielded 532 uncles and aunts. Of these, 355 participated and were interviewed; 51 refused, giving a participation rate of 87.4%. Refusals were more likely paternal than maternal (65%), and more likely male than female (62%). The remainder (N = 126) were either not located, or were deceased, underage or incompetent (Fig. 1).

Interviews were carried out between April 2000 and February 2001 with the uncles and aunts. Of 355 uncles and aunts, 288 had 1,033 pregnancies included in this report, after eliminating twin pregnancies (N = 9), because of their excess risk for poor pregnancy outcomes, unknown pregnancy outcomes, including current pregnancies, ectopic pregnancies (N = 23) and uncles and aunts without pregnancies. These are called "first cousin pregnancies." Gravidity of uncles/aunts ranged from one to ten, with 30% of uncles/aunts reporting more than three pregnancies.

Medical records. We attempted to retrieve medical records for all reported NTDs in either uncles, aunts, or first cousins, but were not successful in instance. The reasons for failure to obtain documentation included records destroyed or missing from GPs offices, hospitals closed, and records unlocatable. We made no attempt to verify miscarriages or other pregnancy outcomes.

Reference group: North-eastern pregnancy and health study (NEPHS). In 1993, a sample of 100 parous women was drawn from the records of a local general practitioner, in order to determine the

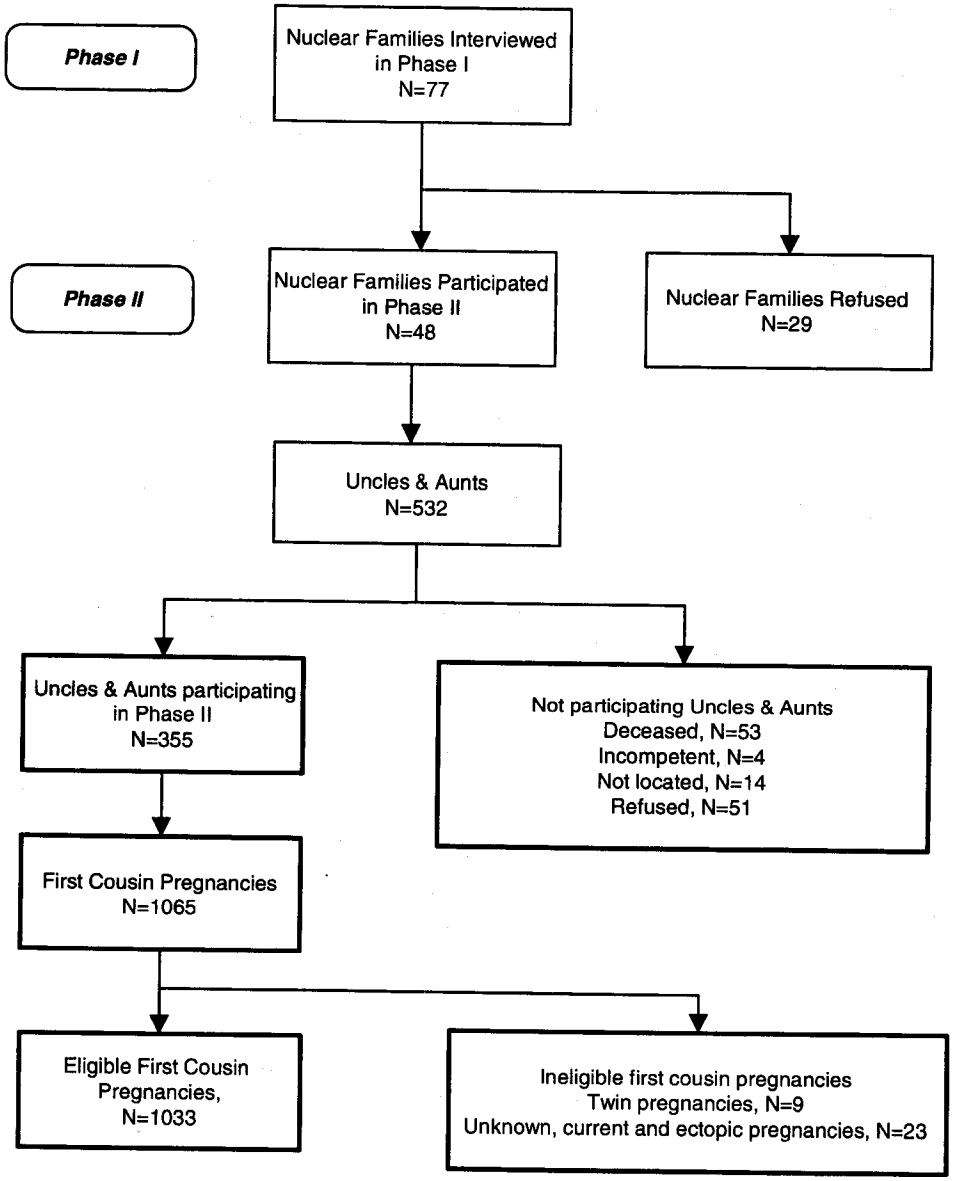

FIG. 1. Irish families with neural tube defects. Participation rates in Phase I and Phase II.

TABLE I. Irish Families With Neural Tube Defects. Adverse Outcomes To First Cousin Pregnancies According To Relationship (Paternal and Maternal) to Proband

	NEPHS reference group[a]		Paternal first cousin pregnancy outcomes		Maternal first cousin pregnancy outcomes		
Pregnancy outcome	N = 270	%	N = 522	%	N = 511	%	*P*
Full term liveborn child	233	86.3	461	88.3	422	82.6	
Premature child	4	1.5	7	1.3	18	3.5	
Stillborn child	3	1.1	5	1.0	7	1.4	
Miscarriage	30	11.1	49	9.4	64	12.5	0.03*
Any adverse pregnancy outcome	37	13.7	61	11.7	89	17.4	0.01**

[a]Pregnancy outcomes to NEPHS participants not statistically significantly different from either paternal or maternal outcomes among NTD first cousin pregnancies.
*Compares all pregnancy outcomes among paternal first cousin pregnancies with maternal first cousin pregnancies.
**Compares any adverse pregnancy outcome among paternal versus maternal first cousin pregnancies.

tional ages for miscarriage to paternal versus maternal relatives ($P > 0.05$).

Table II shows the distribution of first cousin pregnancies according to family characteristics. Thus, families where the proband was male had about the same numbers of first cousin pregnancies as families where the proband was female (50.2% vs. 49.8%); families with open spina bifida were most numerous, and contributed 83.7% of first cousin pregnancies; about equal numbers of first cousin pregnancies occurred in families where probands were born before 1978 as after. Among characteristics of uncles or aunts, somewhat more first cousin pregnancies were contributed by aunts, and 17.2% of first cousin pregnancies occurred to parents of ages over 35. About one-quarter of first cousin pregnancies were to mothers who smoked before getting pregnant, and slightly less to mothers who smoked during pregnancy. Folic acid was taken by only 5.9% of mothers before the first cousin pregnancy and by 13.2% during pregnancy. About half of first cousin pregnancies were at lower birth orders and half had occurred before 1979. A significant proportion of first cousin pregnancies (8.6%) ended in birth defects.

Table II also shows the proportion of first cousin pregnancies that ended adversely according to each of these characteristics, with the relevant *P*-value. There were no differences in the proportion of first cousin pregnancies ending adversely by gender of proband, nor by type of NTD. There was a significant excess risk for relatives of probands who were born more recently (>1977). In considering characteristics of uncles or aunts, there were no differences by gender of relative, nor did folic acid intake make a difference. However, first cousin pregnancies were more likely to end adversely if the uncle or aunt was born after 1945, and if the mother smoked before or during pregnancy. The birth order of the first cousin pregnancy and the year of birth, split at the median of the distribution, did not make a statistically significant difference in the proportion of pregnancies ending adversely. However, there was a suggestion that year of birth might be associated with excess risk. When year of birth was split into quartiles, the proportion of pregnancies ending adversely was similar for the first three quartiles, while births ending from 1987 to 2000 were more likely to end adversely—20.5% ($P < 0.04$). First cousin pregnancies were more likely to end adversely if they also had a birth defect ($P < 0.001$).

Table III evaluates the risk of adverse pregnancy outcome according to the type of NTD of the proband, by maternal or paternal line and by the gender of the related parent. For this analysis, we combined data from uncles reporting pregnancies of their spouses either separately, or with the spouse present also, and evaluated those outcomes compared to those of aunts reporting their own pregnancies. None of the differences between uncles and aunts within line were statistically significant ($P > 0.05$). In each comparison of paternal with maternal line, there were more adverse outcomes for the relatives on the maternal side. For the relatively small number of spina bifida occulta families, there was a considerable excess risk of a maternal first cousin pregnancy ending adversely ($P = 0.001$), and for the larger group of families with other types of NTDs the risk was smaller, but still statistically significant ($P = 0.048$).

These relationships were evaluated in a series of logistic regression models (Table IV). In this study, pregnancies affected by a birth defect were significantly more likely to end adversely (Table II). Therefore, the association of line with adverse pregnancy outcome was restricted to pregnancies that did not also end in a birth defect. The potential association of line with birth defects will be considered in a separate report. Model 1 evaluates all pregnancies. The independent effect of maternal line remained statistically significant in a model that contained a number of other significant terms. The odds ratio for maternal versus paternal line was significant at 1.55 ($P = 0.02$), mother's smoking during pregnancy was strongly associated with adverse pregnancy outcome (OR = 1.86, $P = 0.005$). Finally, recent year of birth was strongly significant with an odds ratio of 1.8 ($P = 0.005$). Because families where the proband had spina bifida occulta seemed more at risk for adverse

TABLE II. Irish Families With Neural Tube Defects. Distribution of First Cousin Pregnancies According to Relatives' Characteristics, and Percentage Ending Adversely According to Relatives' Characteristics (Unadjusted Stratified Analysis)

	Distribution of first cousin pregnancies		First cousin pregnancies ending adversely		
	N = 1033	%	N	%	P*
Characteristic of proband					
Proband gender					
Male	519	50.2	72/519	13.9	
Female	514	49.8	78/514	15.2	0.61
Proband's NTD diagnosis					
Anencephaly	55	5.3	3/55	5.5	
Spina bifida occulta	91	8.8	19/91	20.9	
Open spina bifida	865	83.7	123/865	14.2	
Encephalocele	22	2.2	5/22	22.7	0.13
Proband's year of birth					
1957–1977	496	48.0	58/496	11.7	
1978–1995	537	52.0	92/537	17.1	0.02
Characteristic of uncle or aunt					
Sex of related uncle or aunt					
Male	466	45.1	68/466	14.6	
Female	567	54.9	82/567	14.4	0.96
Age of uncle or aunt at interview					
<36	801	79.7	99/801	12.4	
36+	204	17.2	35/204	17.2	0.09
Uncle/aunt year of birth					
1916–1945	496	48.0	56/496	11.2	
1946–1971	537	52.0	94/537	17.5	0.006
Maternal smoking before each pregnancy					
Yes	260	25.4	49/260	18.9	
No	765	74.6	101/765	13.2	0.03
Maternal smoking during each pregnancy					
Yes	191	18.6	40/191	20.9	
No	834	81.4	110/834	13.2	0.009
Folic acid taken before each pregnancy					
Yes	60	5.9	8/60	13.3	
No	959	94.1	142/959	14.8	0.90
Folic acid taken during each pregnancy					
Yes	134	13.2	17/134	12.7	
No	881	86.8	131/881	14.9	0.59
Characteristic of first cousin pregnancies					
Birth Order					
1–2	541	52.4	83/541	15.3	
3+	492	47.6	67/492	13.6	0.49
First cousin pregnancy's year of birth					
1948–1969	266	25.8	35/266	13.2	
1970–1978	251	24.3	32/251	12.7	
1979–1986	248	24.0	28/248	11.3	
1987–2000	268	25.9	55/268	20.5	0.04
Birth defect present**					<0.001
Yes	78	8.6	10/78	12.8	
No	833	91.4	19/833	2.3	

*P evaluates the differences between levels of each characteristic in the percentage of first cousin pregnancies ending adversely.
**N = 122 first cousin pregnancies without birth defect information.

pregnancy outcomes compared to other families, we stratified the analysis by NTD type. Model 2 considers only first cousin pregnancies in open spina bifida families. In these families, the increased risk attributable to maternal line was reduced to 1.24, and was no longer statistically significant. Maternal smoking and age remained unchanged. First cousin pregnancies occurring in more recent years were significantly more likely to end adversely (OR = 1.76, $P = 0.01$). Model 3 evaluates only at spina bifida occulta (SBO) families and here the effect of maternal line is large (OR = 42) with a wide confidence interval. In these families, year of birth was not significant. In other models (not shown) that evaluated adverse pregnancy outcomes to first pregnancies of families where the proband had either anencephaly or encephalocele, with 74 pregnancies available for analysis, the odds ratio for maternal line was 5.1 with a confidence interval of 0.7–36.6 ($P = 0.1$). When all non-SBO families were combined, the OR for line

TABLE III. Irish Families With Neural Tube Defects. Percentage of First Cousin Pregnancies That Ended Adversely by Respondent Type, Proband Diagnosis, and Parental Line (Proxy Reporters Excluded)

	Paternal line		Maternal line		
Diagnosis and gender	N	%	N	%	*P*
Spina bifida occulta					
Uncles	1/11	9.1	5/10	50.0	
Aunts	0/14	0	13/53	24.5	
Total	1/25	4.0	18/63	28.6	0.001*
Other NTDs					
Uncles	25/191	13.1	28/176	15.9	
Aunts	28/260	10.8	37/219	16.9	
Total	53/451	11.8	65/395	16.5	0.048**

*Evaluates the difference in percentage of all first cousin pregnancies ending adversely in families where the proband had spina bifida occulta.
**Evaluates the difference in precentage of first cousin pregnancies ending adversely in families where the proband had all other types of NTDs.

was 1.32 (95% CI 0.89 to 1.97, $P = 0.17$). Conditional logistic regression models, stratified on nuclear family, gave similar results (not shown).

Table V evaluates the possible bias introduced by having fathers report pregnancy outcomes compared to mothers, and sets out the percent of first cousin pregnancies ending adversely according to who was interviewed and their relationship to the proband. In both lines, uncles interviewed alone reported about half the number of adverse pregnancy outcomes as uncles interviewed with aunts present, suggesting a significant bias in reporting. However, in the maternal line, there were significantly more adverse outcomes no matter which the reporter was, suggesting that the excess maternal risk can be detected even in the presence of a strong reporting bias.

Another bias concerned participation rates of nuclear families in Phase II. We evaluated the participation rates of nuclear families according to family characteristics collected during Phase I of these studies. There were no statistically significant differences in the average age of mothers at the time of interview of the nuclear family, nor in the number of pregnancies that they had, nor in the level of education of either the father or mother in the nuclear family. Likewise, there were no significant differences in the percentage of uncles and aunts with birth defects in either maternal or paternal line, though there were more uncles and aunts with birth defects reported by participating families, more so on the paternal side (20.8% vs. 6.9%; data not shown).

DISCUSSION

This exploration of pregnancy outcomes to uncles and aunts in Irish NTD families has confirmed the original hypothesis, that is, that pregnancies to maternal relatives were more likely to end adversely than pregnancies to paternal relatives. However, this effect was modified depending on the nature of the NTD in the proband. Families with spina bifida occulta had a strong maternal effect, though the numbers of pregnancies were small; all other families had a greatly weakened maternal effect. Although miscarriages constituted the largest component of the total adverse outcomes, the excess risk seemed to be located among preterm pregnancies (Table I). That is, miscarriages were slightly more common among maternal first cousin pregnancies, but preterm deliveries were significantly more common.

Our hypothesis was based on previous reports from studies that did not set out to directly measure a maternal effect. Earlier family studies showed that there were more NTDs among maternal relatives than among paternal relatives [Carter and Evans, 1973; Nevin and Johnston, 1980; McManus, 1987],

TABLE IV. Odds Ratios and 95% Confidence Intervals of First Cousin (FC) Pregnancy Ending Adversely According to Family Characteristics Derived From Logistic Regression Models. Only Pregnancies Not Affected by Birth Defects Included. Irish Families With Neural Tube Defects

	ODDS ratio*	95% CI		*P*
Model 1—all NTD cases, N = 1,000				
Line (paternal, maternal)	1.55	1.06	2.27	0.02
Smoking during pregnancy (no, yes)	1.86	1.21	2.85	0.005
Proband's diagnosis is spina bifida occulta (vs. other diagnoses)	1.65	0.95	2.88	0.08
Mother's age at birth of FC pregnancy (≤35, 36+)	1.41	0.91	2.18	0.13
Year of birth of FC pregnancy (≤1987, 1988+)	1.75	1.18	2.59	0.005
Model 2—open spina bifida only, N = 835				
Line (paternal, maternal)	1.24	0.82	1.87	0.31
Smoking during pregnancy (no, yes)	1.90	1.19	3.03	0.007
Mother's age at birth of FC pregnancy (≤35, 36+)	1.26	0.78	2.06	0.35
Year of birth of FC pregnancy (≤1987, 1988+)	1.76	1.14	2.71	0.01
Model 3– spina bifida occulta only, N = 91				
Line (paternal, maternal)	42.40	2.64	681.6	0.008
Smoking during pregnancy (no, yes)	19.85	2.14	184.06	0.009
Mother's age at birth of FC pregnancy (≤35, 36+)	1.80	0.50	7.02	0.40
Year of birth of FC pregnancy (≤1987, 1988+)	1.32	0.40	4.36	0.65

*Logistic regression models contained all terms shown.

TABLE V. Irish Families With Neural Tube Defects. Number and Percent of First Cousin Pregnancies Ending Adversely According to Who Was Interviewed and Their Relationship to the Proband

Line	Who was interviewed?	First cousin pregnancies ending adversely	
		N	%
Paternal line			
	Uncle interviewed alone	16/157	10.2
	Uncle interviewed with spouse	10/46	21.7
	Aunt interviewed alone	28/274	10.2
Maternal line			
	Uncle interviewed alone	24/150	16.0
	Uncle interviewed with spouse	9/35	25.7
	Aunt interviewed alone	50/272	18.4
Reference group NEPHS	Only mothers interviewed	37/268	13.8

even when the relationship was of half-siblings [Janerich and Piper, 1978]. In a study of families with more than one living case of isolated, nonsyndromic spina bifida, there were significantly more gene-carrier females than males [Chatkupt et al., 1992], and in another study of Dutch families with at least two affected members [Mariman and Hamel, 1992].

There have been no studies known to us that evaluated pregnancy outcomes to distant (third-degree) NTD relatives. Studies of relatives are generally limited to reporting on the occurrence of NTDs as outcomes, with some evaluations of miscarriages. We are not aware of reports of other adverse outcomes, such as stillbirth or premature births, occurring to excess among relatives in NTD families. In our study, we found a significant excess of premature births among pregnancies to maternal relatives. Miscarriages and preterm deliveries have many causes, including smoking. In our study, maternal smoking during pregnancy did indeed have a strong independent effect on adverse outcomes, but maternal line remained significant after taking smoking into account.

Miscarriages have been reported to occur to excess in NTD sibships [Elwood et al., 1992] but have not been evaluated among more distant relatives. An underlying assumption of this research project is that miscarriages and other adverse pregnancy outcomes are part of the variable expression of NTDs, in other words, they stem from the same cause or set of causes, but have a different manifestation. A corollary from this hypothesis is that if folic acid prevents the primary occurrence of NTDs, then it could also prevent that fraction of miscarriages that is linked causally to NTDs. There is some evidence that miscarriages are prevented among supplemented populations [George et al., 2002]. In this study, we found no suggestion that periconceptional folic acid was associated with lower rates of adverse reproductive outcomes, but folic acid use was low.

Preterm deliveries also arise for many reasons, both sociodemographic (education, income, marital status, maternal age, smoking) as well as medical (infection, fetal and uterine abnormalities, medical interventions), and recently, perhaps, a deletion allele of a gene in the folic acid pathway [Bibby and Stewart, 2004; Johnson et al., 2005]). Preterm deliveries share many risk factors in common with miscarriage, including smoking. We eliminated pregnancies ending in birth defects from consideration in the logistic regression, so the excess rates of adverse outcomes noted are likely independent of birth defects, though one cannot be sure of miscarriages in this regard. A biological mechanism that might plausibly link preterm births in maternal first cousin pregnancies with the occurrence of an NTD in a third-degree relative remains to be determined.

The observation of increased rates of adverse reproductive outcomes among pregnancies ending recently (1987–2000) was surprising. This observation was not present in the relatively few first cousin pregnancies among SBO relatives, but was noted among families with other neural tube defects, and was. It could be explained by improved recall of pregnancy outcome or better diagnoses or by chance, but was not seen among the NEPHS families, making these explanations unlikely.

With the possible exception of families where the proband had anencephaly, families comprising the three other NTD groups had high rates of adverse pregnancy outcome (Table II). The high rate of adverse outcomes among families whose proband had spina bifida occulta compared to other types of NTDs was unexpected. The epidemiology of spina bifida occulta has not been well studied, and its incidence poorly understood. In this study, SBO is treated as part of the family of NTDs, or conditions arising from spinal dysraphism. Available evidence suggests that this is a reasonable approach: in a meta-analysis, parents of infants with spina bifida were more likely than controls to have relatives with spina bifida occulta [Elwood et al., 1992]. In addition to epidemiological associations, the clinical literature suggests the presence of associations between SBO and conditions such as enuresis, midline cutaneous

lesions, including dermal sinus [Ritchey et al., 1994; Kara, 2003; Guggisberg et al., 2004]. Since the association of adverse pregnancy outcomes was largely confined to SBO families and only weakly present in other types of NTDs, this argues further for the complexity of inheritance patterns within NTDs.

This project has a number of strengths and weaknesses. The main strength of the study lies in the direct reporting of pregnancy outcomes from the parents themselves, thus avoiding the inaccuracies and potential ethical difficulties inherent in proxy studies. The close ties within Irish families ensured a relatively high rate of participation and a low rate of non-location of participants. The relatively large numbers of pregnancies resulted in quite small confidence intervals, with point estimates with reasonable good precision. Our results are strengthened in light of the NEPHS results, which show lower, though not statistically significantly so, rates of adverse pregnancy outcomes, and fail to show the secular trends seen in these data. The NEPHS study is a reasonable comparison group: the range of years of birth for mothers and pregnancies and the family size is similar to the NTD families, as are the methods of data collection. In addition, none of the NEPHS children was born with an NTD.

However, there are a number of biases and other potential weaknesses in this study. Our study is a convenience sample, drawn from the community through a variety of methods, since we were interested in prevalent cases covering a wide range of years. While our series might differ from an incidence series covering the same range of years, it seems unlikely that participation in the sample could be influenced by knowledge of the hypothesis, since it did not have wide currency in the community. Another potential bias is reporting of adverse pregnancy outcomes by fathers, instead of mothers. When we evaluated adverse pregnancy outcomes reported by uncles alone versus those reported by uncles in the presence of their partners (Tables III and V), we found that on both sides of the family, the percentage was about half when reported by uncles alone, suggesting underreporting by fathers. As shown in both tables, and confirmed in the logistic regression, the effect of maternal line persists despite this apparent bias. This misclassification apparently occurred equally on both sides of the family. It seems unlikely, therefore, to have led to a major bias to the results. Further, we evaluated the gestational age distribution of miscarriages and found that overall, it conformed to expectation, based on earlier clinical miscarriage studies [Warburton et al., 1991]. Gestational age at miscarriage did not differ by line, suggesting that differences in recall by line was not a major issue. Bias in participation rates by the nuclear family was a concern. There seemed to be no differences by demographic characteristics between those families who agreed to give us access to their relatives and those who did not. While there were no significant differences in rates of reported birth defects, more families with birth defects among uncles and aunts participated. To attempt to deal with this we eliminated first cousin pregnancies that ended in birth defects from the logistic regression. Although the participation rate by uncles and aunts was high—87.4%, more direct refusals occurred among paternal relatives and among males. It is not clear how these small differences might bias the results. Recall of pregnancy outcome might be a problem, since we saw more adverse pregnancy outcomes more recently. However, the comparable results from NEPHS do not show any difference by year, suggesting that this result may be valid, though unanticipated.

One other limitation of this study lies in the lack of medical record backup. However, this is not unusual in studies of miscarriages at least, which are rarely documented medically. A further limitation may lie in the nature of the sample of the original nuclear families, that is, it is a convenience sample. At the outset the study aimed to enroll Association members, but the numbers of participants were not adequate. Since the birth dates of the probands cover about 40 years and much of the island of Ireland, it is not possible to estimate what fraction this sample represents of the total number of NTD births occurring during those years and in these locations. Non-paternity, which varies between 0.8% and 30% [Bellis et al., 2005], would result in misclassification and bias the results towards the null hypothesis of no association. We have no information on non-paternity. On the other hand, our study gains credibility from the finding of a statistically significant excess of adverse reproductive outcomes with cigarette smoking both before and during pregnancy, with odds ratios of 1.53 for smoking before pregnancy and 1.74 for smoking during pregnancy.

Taken as a whole, if these data can be confirmed, they suggest that there are underlying, presumably genetic, susceptibilities to adverse reproductive outcomes among NTD relatives that may be related to the occurrence of NTDs. It is possible that the expression of these susceptibilities is modified by the sex of the transmitting parent and by the transmitting line, whether maternal or paternal. This view would confirm the generally held view that NTDs are multifactorial in origin and probably polygenic as well. The mode of inheritance, if applicable, remains unclear, since in our data the excess risk in the maternal line applied equally to uncles as to aunts.

This report adds to the increasing body of knowledge that supports matrilineal inheritance in NTD families. However, it also provides a window on a biological system of considerable complexity. It seems likely that studies combining epidemiological with molecular approaches would be useful and should be undertaken.

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