Birth Defects in Uncles and Aunts from Irish Families with Neural Tube Defects

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BACKGROUND: Previous studies suggested an excess of matrilineal cases of neural tube defects among distant relatives in NTD families. There is little information on patterns of heredity of other birth defects among distant relatives. **METHODS:** Between 1995 and 2003, 78 nuclear families and 373 uncles and aunts were interviewed about birth defects among uncles and aunts in Irish families with an NTD. **RESULTS:** Among 783 total uncles and aunts, those related through the mother had more birth defects overall than those related through the father (8.4 vs. 4.0%, $p = 0.01$). The excess persisted after controlling with logistic regression models for maternal and paternal age, gender of uncle/aunt, proband's NTD diagnosis, and year of birth (OR 2.52; 95% CI: 1.29, 4.91; $p = 0.007$). Among individual birth defects, significant excesses over expected rates were seen for spina bifida, congenital heart defects, and syndactyly. **CONCLUSIONS:** This study of reported birth defects suggests that maternal uncles and aunts in Irish families have significantly more birth defects than paternal uncles and aunts. These results, if confirmed, support the hypothesis that NTD relatives carry a susceptibility to other birth defects, preferentially on the mother's side of the family, suggesting opportunities for prevention. *Birth Defects Research (Part A) 82:8–15, 2008.* © 2007 Wiley-Liss, Inc.

Key words: neural tube defects; congenital heart defects; birth defects; family studies; Ireland

INTRODUCTION

Birth defects are common; 1 in every 33 babies in the United States is born with a birth defect (http://www.cdc.gov/ncbddd/bd/faq1.htm). Birth defects are among the leading causes of death in developing countries, and among birth defects, NTDs are second in frequency following congenital heart defects (http://www.cdc.gov/ncbddd/bd/faq1.htm#CommonBD). NTDs are major congenital abnormalities of the CNS. The defect is called anencephaly when the brain tissue is exposed to the surface through a lesion in the scalp and skull (Lemire, 1988). Defects of the spine are commonly called spina bifida and involve the spinal cord, vertebrae, and skin (Mitchell et al., 2004). Spina bifida occulta (SBO) comprises lesions of the spine that are skin-covered; many may be asymptomatic and clinically silent. However, some percentage of SBO is associated with intraspinal lipoma, tethered cord syndrome, genitourinary dysfunction, increased incidence of disk pathology, lumbar spondylolysis, foot deformities, and syringomyelia (Gregerson, 1997).

The East Coast of Ireland has had rates of NTDs that are among the highest recorded and has seen the steepest drop in recent years. Only 50 years ago, NTD rates in Dublin were about 8 per 1,000 births (Coffey, 1983), while in 2000, NTDs affected approximately 1 in every 1,000 births (Dublin EUROCAT Working Group, 2001). The reasons for the steep fall in NTD incidence, which is unique in Europe (Botto et al., 2005), are unknown, though improved diet may be a factor.

The causes of most birth defects are unknown; both genetic and environmental factors are implicated. Folic acid prevents a large fraction of NTDs from occurring (MRC, 1991). Thus, the environment in the form of diet plays a major role in the origins of NTDs; other environmental associations of NTDs include maternal diabetes, maternal obesity, and certain exposures, including living near a hazardous waste site or in a lower socioeconomic status neighborhood (Lynberg et al., 1994; Ray et al., 2005; Dolk et al., 1998; Wasserman et al., 1998).

The role of heredity in the etiology of NTDs is supported by excess rates of NTDs in close relatives (Elwood

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et al., 1992) and occurrence of NTDs as components of syndromes, including Meckel-Gruber syndrome (Rampersaud et al., 2005). Studies of families with NTDs in the mid-twentieth century were concerned with understanding familial trends in a condition that was presumed to have a large genetic component. Investigators in many countries, evaluating many hundreds of family histories, found that increased NTD recurrence risks in siblings were common (Elwood et al., 1992). Maternal uncles and aunts were at increased risk of having an NTD compared to paternal uncles and aunts in most, but not all, studies (Williamson, 1965; Hunter, 1984; McManus, 1987; Chatkupt et al., 1994; Toriello and Higgins, 1983; Lippman-Hand et al., 1978). NTDs were also more common than expected in sibs of children with other malformations (Fraser et al., 1982).

Despite numerous investigations of excess NTD rates among relatives, excesses of other birth defects seem to have been little studied. If distant relatives had increased rates of other birth defects this could indicate the presence of a susceptibility factor, that in turn, could suggest opportunities for intervention and prevention. We set out to determine whether distant relatives—uncles and aunts—in NTD families had a raised risk of NTDs and other birth defects, and if this risk was higher among maternal relatives.

MATERIALS AND METHODS

This study describes results from Phase II (interviews with uncles and aunts) of the studies into Irish NTD families by the Boyne Research Institute. Phase I evaluated pregnancy histories and the health of siblings in the original ''nuclear families'' (results being prepared for publication). Phase II subjects were identified by the respondents in the nuclear families during Phase I.

Phase I Study

Family identification. Nuclear families were identified from various sources, as follows: by their membership in county branches of the Irish and Northern Ireland Association for Spina Bifida and Hydrocephalus, or by word of mouth, including newspapers and local radio announcements, and 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, spina bifida, SBO, or encephalocele.

Contact procedures and nuclear family interview. The nuclear families were interviewed between 1995 and 2002. The respondent was the mother (alone, 65%) or mother and father (21%); in some cases the proband was the respondent (6%). The balance (8%) comprised other family groupings. The interview with the nuclear family covered the mother's reproductive history, the proband's diagnosis, date and place of birth for each parent and grandparent individually, the health of each parent and grandparent, including birth defects and cancer. We asked individually about each parent's sibling (called ''uncles and aunts'' hereafter) and their health, including cancer and birth defects. For uncles and aunts we asked about their vital status, and if dead, date and cause of death. For grandparents, their years of birth and death, and occupation were asked. We did not ask about grandmothers' reproductive history, but only about liveborn children. We did not ask about grandmother's health, or smoking, alcohol, and medication use before or during her pregnancies.

Phase II

Participation, contact procedures, and eligibility. At the time that this Phase II study was started, 78 families had participated in Phase I. From among these families 50 agreed to participate in Phase II and they supplied the contact information for the uncles and aunts (details in Byrne and Carolan, 2006). Interviews were done face-to-face for the most part; relatives living at a distance or overseas were interviewed by telephone. To be eligible for Phase II, uncles and aunts had to be 18 years of age or older by January 3, 2000. If the uncle or aunt was deceased or incompetent 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 of the proband's parents, relatives who were younger than 18 years of age, or were mentally retarded or mentally ill, or confined to an institution.

Enrollment and interview. The 50 participating nuclear families yielded a total of 534 living uncles and aunts. Of these, 373 participated in Phase II and were interviewed between April 2000 and February 2001. Another 10 were eligible but not located, 55 were ineligible, 96 were located and refused. The participation rate was 79.5% (373/469). The Phase II interview asked about the health of the uncle or aunt, including birth defects and cancer, the pregnancy history and outcome of each pregnancy, and history of infertility and treatment sought, as well as health of spouse/partner. Respondents were asked about birth defects as follows: ''Did you have any of the birth defects or genetic syndromes listed?'' on a card that listed 27 of the most common birth defects. Other conditions mentioned by respondents were captured also. The Ethics Board of the Boyne Research Institute approved each Phase of the study.

Reference group. The Dublin component of the European Union's EUROCAT registry of congenital anomalies (www.eurocat.ulster.ac.uk) served as the comparison group for some individual birth defects that had reasonable comparability. Due to differences in ascertainment between the registry methods and reports by affected individuals and by relatives, we did not compare the overall rate of birth defects among uncles and aunts with the corresponding EUROCAT rate. EUROCAT is a European network of population-based registries for the epidemiologic surveillance of congenital anomalies, started in 1979. At present, 43 registries in 20 countries cover about 29% of births in Europe, with more than 1.5 million births evaluated each year. Among the objectives are provision of essential epidemiologic information on congenital anomalies in Europe, and early warning of new teratogenic exposures. The Dublin EUROCAT registry, started in 1980, is population-based, covering three counties, including Dublin. Termination of pregnancy for congenital anomaly is illegal in Ireland; the Dublin registry includes only fetuses greater than 23 weeks of gestation or weighing at least 500 g. Selected individual birth defects occurring from 1980 to 2003 were compared with birth defects from uncles and aunts, because the years of births of the uncles and aunts included those years also.

Construction of the analytic dataset. Because the interview did not include questions about grandparents' fetal losses, any volunteered information on miscarried pregnancies was not included, due to the potential for ascertainment bias. The analytic dataset was created by merging data from interviews with the nuclear families about the uncles and aunts (Phase I) with self-reported data obtained by interview data with the uncles and aunts (Phase II). This dataset included 783 liveborn children. Differences resulting from these reporting methods are evaluated in the analysis.

Birth defects removed from consideration. A number of birth defects was excluded from consideration for this report. These included heart murmur, hip click, mental retardation, isolated dislocated hip, and birth marks. Two unrelated individuals, both maternal relatives, with Down syndrome were also excluded from the analysis; two related maternal relatives with Kallmann syndrome were also excluded from the analysis.

Birth defects included in the analysis. Birth defects included in this report were any NTD, including those that are considered clinically related, such as dimple at base of spine, any defects of the eye present at birth, syndactyly, cleft lip and palate, and others. Two of three cases of heart defects were called ''hole in the heart'' and assumed to be ventricular septal defects; the third case was called ''congenital heart disease'' and is included with the other two as ventricular septal defects. Eye defects, where specified, included ''blocked tear duct—operated at birth'' ($n = 1$), ''squint from birth'' ($n = 3$), ''cast, requiring operation'' ($n = 2$), ''lazy eye'' ($n = 2$), ''shortsighted'' ($n = 2$), ''detached retina'' ($n = 1$), ''weak eye'' ($n = 1$).

Statistical Analyses

For all studies, the data were entered and preliminary analyses done in EPI-INFO. For this report, statistical analyses were carried out with SAS (Statistical Analysis System, Cary, NC). Simple comparisons were tested for statistical significance with chi-square tests with $\alpha = .05$, and two-tailed tests. Unconditional logistic regression models controlled for potential confounding. Variables were dichotomized for modeling purposes as follows: sibship size was split at 3 (1–3, 4–14), dichotomies based on median splits were applied to grandmother's age at birth of uncle or aunt (<32, 32+), grandfather's age (<35, 35+), grandmother's year of birth (1865–1917, 1918–1952), grandfather's year of birth (1860–1918, 1919–1952), uncle/aunt year of birth (1901–1949, 1950–1989, respectively), and proband year of birth (1934–78, 1979–1995). Factors considered as covariates were included in the multivariate models if they reached a value of $p = .2$ or less in univariate tests. Backward and forward selection procedures were evaluated. The final model was based on achievement of a c statistic greater than 0.70.

RESULTS

There were 783 uncles and aunts with known gender reported by the nuclear families; the characteristics of the families—probands, grandparents, and uncles and aunts—are set out in Table 1, along with the proportion with birth defects for each level of each characteristic. Among the 783 uncles and aunts, 48, or 6.1%, had a birth

Table 1
Irish Families with Neural Tube Defects: Distribution of Uncles and Aunts and the Proportion of Uncles and Aunts with Birth Defects According to Family Characteristics

Overall proportion with birth defects	All uncles and aunts $n = 783$	With birth defects $n = 48$	% = 6.1	p*
Characteristics of proband				
Proband gender				
Male	345	20	5.8	0.73
Female	438	28	6.4	
Proband's NTD diagnosis				
Anencephaly	56	0	0	0.24
Spina bifida occulta	113	7	6.2	
Open spina bifida	585	41	7.1	
Encephalocele	29	0	0	
Proband's year of birth				
1934–1978	352	17	4.8	0.17
1979–1995	431	31	7.2	
Who was interviewed in the nuclear family?				
Proband	53	6	11.3	0.86
Mother	483	26	5.4	
Father	16	1	6.3	
Mother & father	192	13	6.8	
Proband & mother or sister & mother	27	0	–	
Mother & grandmother	12	2	16.7	
Did the family participate in Phase II?				
Yes	575	45	7.8	0.001
No	208	3	6.3	
Characteristics of grandparents				
Grandmothers' age at birth of uncle/aunt				
15–31	350	17	4.9	0.13
32–57	382	29	7.6	
Grandfathers' age at birth of uncle/aunt				
17–34	319	17	5.3	0.30
35–63	401	29	7.2	
Grandmothers' year of birth				
1865–1917	364	23	6.3	1.00
1918–1952	368	23	6.3	
Grandfathers' year of birth				
1860–1915	374	22	5.9	0.57
1916–1952	347	24	6.9	
Characteristics of uncle/aunt				
Sex of related uncle or aunt				
Male	395	27	6.8	0.41
Female	388	21	5.4	
Uncle/aunt year of birth				
1901–1949	369	19	5.2	0.21
1950–1989	393	29	7.4	
Family size				
1–3	353	21	6.0	0.85
4–14	430	27	6.3	
Line				
Paternal	400	16	4.0	0.01
Maternal	383	32	8.4	
Was uncle/aunt living at time of interview?				
Yes	690	40	5.8	0.30
No	93	8	8.6	
Did uncle/aunt do Phase II interview?				
Yes	378	36	9.5	0.0001
No	405	12	3.0	

*p evaluates the differences in percentages of uncles and aunts with birth defects according to levels of each characteristic.

Table 2
Irish Families with Neural Tube Defects: Proportion of Uncles and Aunts with Birth Defects by Line and by Gender

	Total number	With birth defects	
		n	%
All uncles and aunts	783	48	6.1
Paternal line, total*	400	16	4.0
Paternal uncles	205	10	4.9
Paternal aunts	195	6	3.1
Maternal line, total*	383	32	8.4
Maternal uncles	190	17	9.0
Maternal aunts	193	15	7.8

*Comparing all maternal with paternal relatives, $p = 0.01$.

defect. There were no statistically significant differences in the proportion of uncles and aunts with birth defects according to levels of these variables: gender of proband, proband's NTD diagnosis and year of birth, the interviewee in the nuclear family, grandmother's and grandfather's age at birth of uncle/aunt, grandmother's or grandfather's year of birth, gender and year of birth of related uncle or aunt, the size (number of pregnancies, including livebirths) of the family of birth of the uncle/aunt, or whether the uncle/aunt was living when the data were collected. Some differences were observed within levels of certain characteristics. Thus, families who elected to participate in the Phase II interview study had more uncles and aunts with birth defects than those who did not participate ($p = 0.001$). Uncles/aunts related via the mother were more likely to have birth defects than those related via the father ($p = 0.01$), with an unadjusted OR (uOR) of 2.19 (CI: 1.14, 4.25). Within paternal or maternal line, male relatives had a similar rate of birth defects to female relatives. The difference in the proportion with birth defects was apparent in both genders within line. There were approximately equal numbers of male and female uncles and aunts, both overall and within line (Table 2).

Table 3 presents the results obtained from a logistic regression analysis that included all 783 uncles and aunts. The effect of maternal line remained statistically significantly elevated with an adjusted OR of 2.52 and a 95% CI from 1.29 to 4.91. The model also contained terms for maternal age, family's participation in Phase II, open spina bifida, year of birth of uncle/aunt, year of birth of proband, father's age, grandmother's and grandfather's year of birth. Despite a strong independent contribution from the term for participation, the risk of having a birth defect among maternal uncles and aunts remained significant. The adjusted rate was similar to the unadjusted rate, 2.52 and 2.19, respectively.

Table 4 lists the individual birth defects included in this study, and compares the percentages of each birth defect among maternal versus paternal relatives. Eye defects of all types were the most frequently reported type of defect ($n = 16$), followed by spina bifida, webbed toes, inguinal hernias and scoliosis (five cases each). None of the different types of birth defects were statistically significantly more likely to occur among maternal uncles and aunts compared to paternal relatives. However, 11 types of birth defects were reported more frequently among maternal than paternal relatives, in contrast to five types occurring more often to paternal relatives. When the percentages of individual birth defects in uncles and aunts were compared with those reported by EUROCAT, three entities occurred significantly more frequently (Table 5). Thus, uncles and aunts were 6.37 times more likely to have open spina bifida than the Irish population evaluated in EUROCAT, 7.76 times more likely to have ventricular septal defects, and 14.77 times more likely to have syndactyly. Down syndrome was not more frequent among uncles and aunts.

All five cases of spina bifida in uncles and aunts were described as isolated, without other birth defects. Three of the cases were related to the proband through the maternal line; four were deceased, three in infancy and one at age 61. All were born into different families between 1936 and 1964. In four of the five families the proband had open spina bifida; in the fifth, the proband had SBO. The single aunt with SBO also had scoliosis; the proband in her family also had SBO. In terms of relationship to the proband, one case of spina bifida occurred in each of the four groups: paternal uncles, paternal aunts, maternal uncles, and maternal aunts. The fifth case of spina bifida was a child of unknown gender born to the maternal grandparents (Table 6).

Both cases of Down syndrome were born into separate families, both were in the maternal line, and in both families the proband had open spina bifida. One, a male, had died in infancy, the second, a female, was about 30 years of age at the time of the study. Maternal ages at birth were 35 and 42 respectively; paternal ages were 33 and 53 respectively.

One family with a child with encephalocele had two maternal uncles with Kallman syndrome (KAL1), an X-linked syndrome due to a defect of neuronal migration leading to hypopituitarism and hypogonadism (OMIM 308700). We tested for the association of Xp22.3 Kallmann syndrome locus with encephalocele within this family by studying polymorphic markers flanking the Kallmann locus (Meitinger et al., 1990). Haplotyping of the family showed that the two brothers received the same Xp22.3 region, which they shared with their mother; the opposite maternal haplotype was found in an unaffected brother. The proband received the grandpaternal haplo-

Table 3
Logistic Regression Model Based on all 783 Reported Uncles and Aunts with Birth Defects in Irish Families with Neural Tube Defects

	Odds ratio*	95% Confidence interval	*p*
Line, maternal vs. paternal	2.52	1.29, 4.91	0.007
Maternal age, 32+ vs. <32	1.79	0.75, 4.26	0.19
Family's participation in Phase II, Yes/No	5.77	1.73, 19.23	0.004
Open spina bifida vs. all other types	1.65	0.70, 3.87	0.25
Uncle/aunt year of birth, 1950+ vs <1950	1.22	0.45, 3.33	0.70

*Odds ratios derived from a model that also contained terms for proband's year of birth, father's age, grandfather's year of birth, grandmother's year of birth.

Table 4
Rates of Individual Birth Defects Overall and Among Maternal and Paternal Uncles and Aunts

	All uncles and aunts $n = 783$			Paternal uncles and aunts $n = 400$			Maternal uncles and aunts $n = 383$		
Birth defect	*n*	%	95% CI**	*n*	%	95% CI	*n*	%	95% CI**
Eye defects	16	2.04	1.17, 3.30	5	1.25	0.41, 2.89	11	2.87	0.44, 5.08
Spina bifida, open	5	0.64	0.21, 1.48	2	0.50	0.06, 1.79	3	0.78	0.16, 2.27
Webbed toes/feet	5	0.64	0.21, 1.48	1	0.25	0.00, 1.30	4	1.04	0.29, 2.65
Inguinal hernia	5	0.64	0.21, 1.48	3	0.75	0.16, 2.18	2	0.52	0.06, 1.87
Scoliosis	5	0.64	0.21, 1.48	–	–	–	5	1.31	0.43, 3.02
Cryptochidism*	4	1.01	0.28–2.57	3	1.46	0.30, 4.22	1	0.53	0.00, 2.90
Ventricular septal defect	3	0.38	0.08, 1.11	1	0.25	0.00, 1.30	2	0.52	0.06, 1.87
Limb defects	2	0.26	0.03–0.92	1	0.25	0.00, 1.30	1	0.36	0.00, 1.45
Clubfoot	2	0.26	0.03–0.92	1	0.25	0.00, 1.30	1	0.36	0.00, 1.45
Spina bifida occulta	1	0.13	0.00–0.71	–			1	0.36	0.00, 1.45
Dimple at base of spine	1	0.13	0.00–0.71	–	–	–	1	0.36	0.00, 1.45
Intestinal atresia	1	0.13	0.00–0.71	–	–	–	1	0.36	0.00, 1.45
Total	55	7.02	5.33, 9.05	20	5.00	3.08, 7.62	35	9.1	6.5, 12.5

*Denominator includes only males, $n = 395, 190, 205$ respectively.
**Exact binomial 95% confidence intervals.
Number of birth defects is greater than the number of individuals, since some subjects had more than one birth defect.

type, ruling out any association of the Xp22.3 locus with her NTD.

We considered the possibility that biases could account for the matrilineal excess seen here. The mother in the nuclear family was most often the respondent and might be expected to know more about her own relatives than those of the father. This did not seem to be the case. While the proportion of uncles and aunts with birth defects varied considerably according to who was interviewed in the nuclear family, the differences were not statistically significant (Table 1, $p = 0.48$). Families who participated in Phase II were more likely than those who chose not to participate to have had more uncles and aunts with birth defects; however, the multivariate analysis showed that the independent influence of maternal line remained significantly elevated. The reason for not participating was not significantly associated with birth defects in the maternal line ($p = 0.37$; data not shown). The association with maternal line remained when the data were divided according to individuals who self-reported birth defects, compared to those whose birth defects were reported by proxies (data not shown).

There was considerable lack of concordance in reporting of birth defects. Thus, of 48 individuals with birth defects, 10 were reported by both nuclear family and uncle/aunt, another 14 were reported only by the nuclear family, and 24 were reported by the uncle or aunt. Within each group more birth defects were reported in the maternal line, 6 of 10, 8 of 14, and 18 of 24, respectively. These analyses do not suggest that there was significant bias that could explain the matrilineal excess.

There is considerable support in the literature for the observation that maternal uncles and aunts are more likely to have a NTD than paternal uncles and aunts. In seven of eight studies (including the present study, Table 6) that enumerated NTDs in uncles and aunts, maternal uncles and aunts were more likely than paternal uncles and aunts to have an NTD (overall OR 1.86; 95% CI; 1.15, 3.03; $p = 0.01$). These data were further divided by gender for those studies that enumerated paternal and maternal uncles and aunts separately (data not shown). The resulting rate of NTDs among maternal aunts only was 1.22%, compared to 0.66, 0.74, and 0.75% for paternal uncles, paternal aunts, and maternal uncles, respectively. When the percentage of maternal aunts with NTDs was compared to the total of the other three groups of relatives combined (1.22 vs. 0.56%) the difference was statistically significant, with an OR of 2.13 (CI: 1.20, 3.75; $p = 0.008$).

Table 5
Comparison of Birth Defect Rates between Irish Uncles and Aunts and Dublin EUROCAT Registry

	EUROCAT Dublin Registry 1980–2003, $n = 510{,}328$*			All uncles and aunts $n = 783$			Unadjusted odds ratios		
Birth defect	*n*	%	95% CI	*n*	%	95% CI**	uOR	95% CI	*p*
Spina bifida	514	0.10	0.09–0.11	5	0.64	0.21, 1.48	6.37	2.33, 15.95	<0.0001
Ventricular septal defects**	252	0.05	0.04, 0.06	3	0.38	0.08, 1.11	7.76	1.99, 25.00	0.007
Webbed toes/feet	222	0.02	0.02–0.03	5	0.64	0.21–1.48	14.77	5.36, 37.18	<0.0001
Intestinal atresia	126	0.03	0.02–0.03	1	0.13	0.00–0.71	5.18	0.27, 34.08	0.18

*www.eurocat.ulster.ac.uk
**Assumes that entities called ''hole in the heart'' and ''congenital heart defect'' are equivalent to ventricular septal defect.

Table 6
Neural Tube Defects in Paternal and Maternal Uncles and Aunts: Eight Studies

	Paternal uncles and aunts		Maternal uncles and aunts	
Author(s)	*n*	%	*n*	%
Williamson, (1965) Great Britain	0/285	0	3/311	0.97
Lippman-Hand et al. (1978) Canada	4/594	0.67	0/599	0
Nevin and Johnston (1980) Northern Ireland	2/333	0.60	4/342	1.17
Khoury et al. (1982) Atlanta	0/692	0	5/715	0.70
Hunter (1984) Canada	4/1,266	0.32	12/1,203	1.00
McManus (1987) Ireland	12/1,590	0.76	12/1,482	0.81
Chatkupt et al. (1994) United States	4/250	1.60	12/261	4.60
Present study, Ireland	2/400	0.50	3/383	0.78
Total	28/5,387	0.52	51/5,296	0.91
Combined odds ratio		1.86 (1.15, 3.03), $p = 0.01$		

DISCUSSION

In this study of Irish families with NTDs the risk of a birth defect was more than twofold higher among uncles and aunts of the proband who were related through the mother than through the father (aOR 2.52; CI: 1.29, 4.91). However, the excess risk for any individual birth defect among maternal relatives did not reach statistical significance. A number of birth defects occurred in the uncles and aunts significantly more often than expected compared to the population-based registry. These were spina bifida, with an uOR of 6.37, ventricular septal defects (uOR 7.76), and syndactyly (uOR 14.77).

This report is from Phase II of our studies of Irish families with NTDs. A previous report from this study described the reproductive experiences of these uncles and aunts and found that maternal aunts and (spouses of) uncles had excess rates of adverse reproductive outcomes, especially if the proband had SBO (Byrne and Carolan, 2006). The present study also finds evidence for maternal inheritance in the form of excess birth defects to uncles and aunts, but did not find clear evidence to implicate one particular type of NTD in the proband. Families where the proband had SBO showed the maternal line effect to the same degree as families with open spina bifida.

Results from combined studies of the occurrence of NTDs among maternal uncles and aunts provide strong evidence for an excess of NTDs among maternal aunts, with a combined OR of 2.13, comparing maternal aunts to all other groups of relatives. Thus, the combined studies from Europe and North America provide strong evidence that some factor or factors that is traveling along the maternal line confers an excess risk of NTDs to maternal aunts in families where an NTD has already occurred.

Another question of interest in these data is whether the magnitude of the maternal excess among just the three Irish studies might parallel the secular trend in NTD rates in Ireland. The ORs among the three Irish studies (Nevin and Johnston, 1980; McManus, 1987; and the present study) of 1.96, 1.07, and 1.57, respectively, do not suggest that the maternal excess of NTDs parallels the drop in new NTD cases documented by Botto et al. (2005).

One other study reported on other birth defects among parental siblings (uncles and aunts). Khoury et al. (1982) noted that there were more other birth defects among maternal siblings than among paternal siblings (2.5 vs. 1.3%) among pregnancies that occurred before the proband's birth. The present study found that ventricular septal defects and syndactyly occurred to excess among uncles and aunts compared to population expected rates. Hunter (1984) and Nevin and Johnston (1980) also reported occurrences of congenital heart defects in siblings. Hunter (1984) speculated that a defect in the midline field could explain at least some of the associated malformations. It is possible that this mechanism could account for other relatives having midline field defects, such as ventricular septal defects. However, the excess of syndactyly over expected is not easily explained. Syndactyly forms part of many syndromes, whose evaluation is beyond the scope of the present study. It is also possible that this excess could have arisen by chance.

A biological mechanism that could link family members with NTDs and other birth defects preferentially on the maternal side is not clear. X-linked inheritance may occur in some NTD families, as noted by Toriello et al. (1980). Mitochondrial transmission is another mechanism that could explain these results (Chatkupt et al., 1992). Interest in genes involved in the metabolic pathways leading from folic acid to NTDs is high. Available evidence suggests that maternal genes may be involved more often than embryonic genes in production of an NTD (Doolin et al., 2002). Future research that incorporates molecular studies with family data may provide clues to novel mechanisms of inheritance and help explain our observations.

The occurrence of Down syndrome among relatives in NTD families has generated some comment, with the suggestion that Down syndrome and NTDs may share a common etiology (Barkai et al., 2003). However, Olsen and Winther (2003) pointed out the potential for bias in ascertainment in a hospital-based series. Amorim et al. (2004) could not find evidence for an association in a large Latin American collaborative study of congenital malformations. In our community-based series of families, although two cases of Down syndrome occurred among mother's relatives, the overall rate of Down syndrome was not significantly higher than expected.

Martinez-Frias (1994) reported a significantly elevated rate of hypospadias among cases of spina bifida in a Spanish birth defect registry. In our study, although there were no reports of hypospadias, reports of cryptorchidism were common, with a rate of 7% among uncles.

Cryptorchidism has been reported to occur more often than expected in children with other congenital anomalies, among them CNS anomalies (Biggs et al., 2002). The joint occurrence of GU anomalies and NTDs could suggest a midline fusion defect that might have a unique genetic mechanism, possibly X-linked (Toriello et al., 1980). There seem to be no reports of associations between cardiac anomalies and NTDs. Again, it is possible that a midline developmental problem could link the excess rate of cardiac defects seen in our study with NTDs.

The epidemiology of SBO has not been well studied, and its incidence is 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 SBO (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; Guggisburg et al., 2004). An earlier report from this study indicated that families where the proband had SBO had an excess rate of adverse reproductive outcomes, and an excess among maternal relatives in these families (Byrne and Carolan, 2006). In this analysis of birth defects, the maternal excess of birth defects applied equally to families where the proband had SBO and to families where the proband had open spina bifida. Inclusion of encephaloceles among the family of NTDs was explored recently; Rowland et al. (2006) concluded that the balance of the epidemiologic evidence favors retaining the NTD classification.

Factors that have previously been associated with NTD risk, maternal diabetes, obesity, or diet (Becerra et al., 1990; Ray et al., 2005; Suarez et al., 2003), could not be evaluated in this report, because we did not seek this information concerning grandparents. Hence, a number of potential confounders have gone unmeasured. It is not clear how these factors might explain the excess of birth defects among maternal relatives.

The possibility that certain biases might explain these results was evaluated in a number of different ways. Although mothers constituted the largest proportion of respondents in the nuclear families, the rates of birth defects among uncles and aunts were similar across all reporting relatives. In the same way, the excess of maternal uncles and aunts with birth defects was present among all types of nuclear family respondents. Thus, while failing to entirely rule out the possibility that mothers will preferentially report birth defects among their own relatives, these analyses do not provide support for this potential source of bias. Others (Elwood et al., 1992) have discouraged consideration of reports of excess risks among maternal relatives for this reason, but as far as we know the existence of this bias has not previously been evaluated. Participation bias, while present, did not change the influence of maternal line. Another analysis of these data showed that fathers reported about half as many adverse pregnancy outcomes as mothers (Byrne and Carolan, 2006); it is reassuring that mothers and fathers report similar percentages of birth defects (Table 1). Rasmussen et al. (1990) warn that information obtained from others concerning their children's birth defects should be used with caution, having found a positive predictive value of mother's reports of only 47% when compared with registry data. For this reason, the findings of the present study are strengthened by inclusion of self-reported birth defects. Proxy reporters were members of the nuclear family, mostly the parents of the proband, who were the siblings of these uncles and aunts. The accuracy of proxy reporting of birth defects by siblings has not been evaluated to our knowledge.

Ascertainment of birth defects in this study differs considerably from that of a population-based registry data; but it is not clear what biases might result. EUROCAT reports are based on medical examinations actively sought from hospitals and clinics; this study uses self-reports. Due to potential difficulties in comparing study data with registry data, the comparisons are limited to certain clinical entities. At least one study evaluated parental reporting of malformations with clinical evaluations and found a high degree of validity (Doyle et al., 2004). However, the comparison of maternal and paternal relatives is robust, because our study uses the same ascertainment methods for both.

The potential of folic acid to prevent birth defects other than NTDs is accumulating (Wilcox et al., 2007). This study provides evidence for excess rates of birth defects among maternal relatives. If there are common mechanisms that link the occurrence of NTDs with the occurrence of birth defects overall among other relatives in the same family, then possibly folic acid could prevent the occurrence of other birth defects, too. A broader preventive role for folic acid in preventing birth defects in distant relatives could be possible.

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