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## Further evidence for a maternal genetic effect and a sex-influenced effect contributing to risk for human neural tube defects

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### Abstract

**Background**—Neural tube defects (NTDs), including spina bifida and anencephaly, are the second most common birth defect with an incidence of 1/1000. Genetic factors are believed to contribute to NTD risk and family-based studies can be useful for identifying such risk factors.

**Methods**—We ascertained 1066 NTD families (1467 affected patients), including 307 multiplex NTD families. We performed pedigree analysis to describe the inheritance patterns, pregnancy outcomes and recurrence risks to relatives of various types.

**Results**—Myelomeningocele or spina bifida (66.9%) and cranial defects (17.7%) were the most common NTD subtypes observed. The overall male:female ratio for affected individuals was 0.82, and there were even fewer males among individuals with an upper level NTD (0.62). Among twins, two of the five monozygotic twins and only three of 35 dizygotic twins were concordant, while 27% of the same sex twins were concordant, but none of the different sex twins. The estimated 6.3% recurrence risk to siblings (CI 0.04–0.08) is consistent with previous reports. Families with two or more affected individuals show a higher proportion of female transmitters ( $P = 0.0002$ ). Additionally, the number of affected relatives in maternal compared to paternal lineages was more than double ( $P = 0.006$ ). There were significantly more miscarriages, infant deaths, and stillborn pregnancies in the maternal aunts and uncles ( $P < 0.0001$ ) and of first cousins ( $P = 0.04$ ).

**Conclusions**—Our data provide several lines of evidence consistent with a maternal effect, as well as a sex-influenced effect, in the etiology of NTDs.

### Keywords

neural tube defects; spina bifida; anencephaly; maternal effect; recurrence risk

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## Introduction

Neural tube defects (NTDs) are one of the most common birth defects, occurring in approximately 1 in 1000 live births worldwide. NTDs are likely caused by a complex interaction of multiple genetic loci and environmental components. Early family-based research and segregation analysis found that NTDs were more likely to be caused by an autosomal dominant gene with reduced penetrance than by sporadic causes<sup>1,2</sup>, although no major gene has been identified. A role for imprinting or a parent of origin effect has also been suggested in the etiology of NTDs and other multifactorial disorders.<sup>3</sup>

The recurrence risk in full siblings of an NTD affected child is approximately 2–5%, representing up to a fifty-fold increase over the general population risk.<sup>2,4</sup> The recurrence rate for half siblings is estimated to be around 0.8%, and has been found to be higher in maternal versus paternal half sibs<sup>5</sup>, providing support for a maternal effect. After the birth of two children with an NTD, the risk to a third child is as high as 11%.<sup>2</sup> Offspring of NTD patients have an estimated recurrence risk of 4%, although few studies are available since survival into childbearing years has only recently become common. For second degree relatives the recurrence risk declines to 0.5% and for third degree relatives and beyond approaches that of the general population.<sup>6</sup> Twin studies, although generally based on very small data sets, showed the concordance rate to be 7.7% for monozygotic twins compared to 4.0% for dizygotic twins<sup>2</sup>, a trend consistent with genetic contribution to risk.

In addition, there is increasing evidence that gender of the child influences risk for developing an NTD. Overall, NTDs are more common in females than in males. However, this excess of females is greater among anencephaly and other upper level defects compared to spina bifida patients.<sup>7,8,9</sup> Other studies have confirmed this trend, but extended the phenotypic presentation of the female excess to include craniorachischisis.<sup>10,11</sup> Moreover, these studies have also shown a male excess in cases with lower spinal lesions.<sup>10,11</sup>

Some reports have suggested a maternal effect, or the preferential transmission of NTD-related genes through the maternal side of the family. In families with more than one case of non-syndromic NTD, unaffected individuals who may harbor and pass on a genetic predisposition are more likely to be female than male.<sup>12,11</sup> There also appears to be an excess of affected family members in mothers' relatives, with the highest rate among mothers' sisters' children.<sup>13,14,15,16</sup> Additionally, the recurrence rate to half sibs is higher than expected and greatest when the mother is the common parent<sup>13,5</sup>, with one study suggesting that the recurrence rate for maternal half sibs is equal that of full sibs.<sup>17</sup> Lastly, the number of adverse pregnancy outcomes (stillbirth, spontaneous abortion, or preterm delivery) is also associated with the risk for a neural tube defect. In Italian NTD families, there were 14% fewer siblings of case mothers versus case fathers, possibly because of excess miscarriages<sup>16</sup> and in Irish families with NTDs, the maternal first cousin pregnancies were more likely to end adversely than those on the paternal side.<sup>18</sup> Together, these findings support the hypothesis that imprinting or maternal effects play a role in the development of NTDs.

We analyzed our large collection of NTD singleton and multiplex (multiple case) pedigrees for evidence of sex-influenced and maternal (imprinting) effects. To investigate this hypothesis, we examined the sex ratios in NTD phenotypes and in putatively trait-transmitting individuals and investigated the relationship of additional affected individuals to the proband.

## Materials and Methods

### Ascertainment of families

Ascertainment of these families was carried out during the years 1993 through 2007 from a variety of sources, including myelodysplasia clinics, spina bifida and other NTD support groups, collaborating neurosurgeons, and our study website. Families contacted the study coordinator to express interest in participating in the study. Eligibility of the families was determined during a brief phone interview to establish that at least one individual in the family had a diagnosis of the following types of NTD's: acrania, anencephaly, unspecified caudal defect, craniorachischisis, encephalocele, lipoma of the spinal cord, lipomyelomeningocele, meningocele, myelocystocele, total myelomeningocele, unspecified spina bifida, myeloschisis, rachischisis, sacral agenesis, split cord malformation, tethered cord (cause unknown) or other (thickened filum terminale, fatty filum/spina bifida occulta (SBO), dermal sinus tract, dermoid, sacral teratoma, spina bifida occulta of unknown type). All participating individuals were consented using a protocol approved by the Institutional Review Board at Duke University Medical Center.

Upon enrollment in the study, a standardized, three generation pedigree was obtained.<sup>19</sup> The proband, or index case, in each family was the first sampled affected individual irrespective of diagnosis listed above. Additionally, a sequential sampling strategy was employed such that lines of descent from all affected relatives were pursued. In other words, we sampled all available affected individuals, their siblings and parents and any additional family members needed to connect those individuals (ie grandparents of first cousins). The mother of the proband or index case was the primary informant in all families. Fathers of the proband were also interviewed as a standard practice and if the proband was an adult, that individual was also interviewed.

In conjunction with the federal mandate for folic acid supplementation, in 1998, a detailed pregnancy risk factor questionnaire was developed and administered to the families. Questions on this questionnaire included information about medication usage, vitamin (folic acid) supplements, chemical exposures (including possible agent orange exposure), thyroid function, and other concurrent medical conditions (such as diabetes). Since this questionnaire was developed after we had begun enrolling families, information on this questionnaire was only available for a subset of families (n=697).

We attempted to obtain medical records to confirm the NTD diagnosis for all affected individuals enrolled in the study. When affected individuals had multiple neural tube lesions, we categorized based on the most severe diagnosis, or the highest level lesion. For example, a myelomeningocele starting at T10 extending through the sacrum would be categorized as a thoracic level myelomeningocele. A neurosurgeon evaluated medical records (operative report if applicable and available), fetal ultrasound images or radiographic images to further classify the NTD type in a subset of 146 cases. This subset of cases was prioritized based on whether or not the affected individual was a prenatal case or the family was currently being utilized for genetic analysis (see Rampersaud et al., 2005; Stamm et al., 2008). Individuals with a suspected or unknown type of defect were not included as affected. Singleton families with spina bifida occulta alone were excluded from enrollment. However, families were included in the study if an individual with spina bifida occulta occurred in conjunction with a second individual with another NTD. To the extent to which we could identify individuals with syndromic NTD, such as chromosome abnormalities or syndromes, we removed those families from analysis. However, due to cost constraints chromosomal studies were not routinely performed on all cases.

## Adverse birth outcomes

We recorded the total number offspring and pregnancy outcomes for case mothers, resulting in the siblings of the probands. Additionally, we asked the interviewee (proband's mother) about the pregnancies of both maternal and paternal grandparents and aunts and (spouses of) uncles of her children. No formal verification of spontaneous abortions (SAB), stillbirths (SB), therapeutic abortions (TAB) or other pregnancy outcomes were done in either the NTD mothers or other relatives. Statistical analysis was performed using 2 x 2 contingency tables for proportions.

## Recurrence risk

All data for recurrence risks were calculated using data collected from interviewing the mothers of NTD cases. Full sibling recurrence risk figures were calculated based on all siblings born subsequent to the proband. The overall occurrence risk was calculated by including all full siblings of the proband, regardless of the birth order. When the proband or affected subject was part of a twin pair, the co-twin (N=45) was removed from subsequent analyses. Pregnancies that ended in spontaneous abortion (SAB), stillbirth (SB), or therapeutic abortion (TAB) were included as affected if the status was known.

## Twin studies

Twin data included all pregnancies resulting in a twin birth. Members of triplet pregnancies were excluded from the analysis regardless of outcome. Recurrence rates for NTD were calculated in the same way as for the full siblings. Twin zygosity was based on that reported by the mother and no attempt was made to verify the type unless it was reported in the medical records. The zygosity was not known for seven of the twin sets and the sex of both members of one affected twin pair were not known since the pregnancy was terminated by therapeutic abortion.

## Sex ratio of normal and affected transmitters of NTD risk

In the multiplex families, the sex of the connecting relatives, or normal transmitters of NTD risk, was scored under the assumption that these cases shared a common genetic susceptibility. This analysis was performed using all affected individuals from the families, as well with only those affected individuals within three degrees of relationship from the proband, similar to a previous study.<sup>11</sup> In a small subset of the families, the transmission of NTD appeared to occur in an autosomal dominant manner and the sex of the affected transmitter was scored. Statistical analysis was performed using a chi-square test with one degree of freedom, under the assumption of an equal sex ratio.

## Results

### Description of study population

This study included 1066 families, including 1467 affected individuals. Among the 297 multiplex families, 194 had at least two affected members where the degree of relationship between them was not more distant than third degree ( $\leq 3^\circ$ ) (Table 1). The majority of the families were Caucasian (95.2%), 23 (2.2%) of the families were African American and 28 (2.2%) were of another ethnic background.

The majority (66.9%; n=982) of affected individuals had an open neural tube defect, defined as spina bifida or myelomeningocele. The next largest group of patients (17.7%; n=259) had cranial defects, defined as having either anencephaly, acrania, craniorachischisis, encephalocele, or an unspecified cranial defect. Detailed breakdown of the NTD phenotypes can be found in Table 2.

A detailed pregnancy history questionnaire, including extensive environmental exposures such as folate and multi-vitamin usage, was obtained for 697 families. A total of 290 (41.6%) of the interviewed mothers in this study reported having folate supplementation in the three months prior to conception of the proband, either in the form of multivitamins, prenatal vitamins, or folic acid tablets.

We sought to determine what percentage of our probands were born subsequent to the federal mandate of folic acid supplementation in food. Among all our probands, 114 were missing date of birth, either because there was no date of birth due to terminations ( $n=90$ ) or because the information simply could not be obtained ( $n=24$ ). For those probands whom we obtained the date of birth ( $n=952$ ), the range of birth dates is from 03/01/1926 to 05/04/2007. Among those 952 probands with known dates of birth, 160 probands (17%) were conceived post January 1998.

### Sex ratios

Among the 1066 probands, 562 (54.04%) were female and 478 (45.96%) were male (F vs. M,  $P = 0.009$ ). For all 1467 affected individuals, the overall M/F ratio for all types of NTDs was 0.82. This represents a total of 767 (54.8%) females, 632 males (45.2%), and 68 individuals of unknown sex (Table 3). The overall observed difference in gender was also significant in the larger set of all affected individuals ( $P = 0.0003$ ).

When the sex ratios are analyzed according to the type of NTD and lesion level, those individuals with a cranial NTD had a greater female excess (Table 3). When the three broad categories of cranial, lower (spina bifida, myelomeningocele, meningocele, and myelocystocele) and closed defects (lipomylomeningocele and lipoma) are considered, the sex ratios within these categories are statistically different than that of the total sex distribution of all NTD affecteds ( $P < 0.05$ ). In individuals with cranial defects, including acrania, anencephaly, unspecified cranial defects, craniorachischisis, and encephalocele, the M/F sex ratio was 0.66. When anencephaly alone was considered the sex ratio is 0.76, although this is not statistically different than the 0.87 M/F ratio seen in spina bifida. An even greater female excess is seen in encephalocele, with an M/F ratio of 0.42. In individuals with spina bifida (myelomeningocele, meningocele, and myelocystocele) and closed NTD (lipomylomeningocele and lipoma), the female excess is either reduced or not seen, with sex ratios close to 1.0 (Table 3).

### Twins

A total of 47 twin pairs from the 1066 families were identified as having at least one affected sibling of the pair (Table 4). The majority, or 35 (75%), of the twins were reported to be dizygotic (DZ), five twin pairs were monozygotic (MZ) and the zygosity for seven sets of like-sex twins was unknown. Among the MZ twins, two of the five sets (40%) were concordant for the disorder, while only three of 35 (8.57%) DZ twin pairs were concordant. When concordance rates are considered based on like sex versus unlike sex twin pairs, a total of 27% of like sex twins and 0% of the unlike sex twins are concordant for the disease (Table 4). Among the twin pairs, both individuals were affected in eight families. Remarkably, all of these affected twin pairs were concordant for NTD type and were of the same sex regardless of zygosity.

In terms of the concordant twins, the phenotypes were almost exclusively myelomeningocele (lumbo/sacral and thoracic) or tethered cord. With the discordant twins, lumbo/sacral myelomeningocele represented over half of the diagnoses, followed by anencephaly, tethered cord, thoracic level myelomeningocele, lipomylomeningocele. Thus,

the lesions in the twins were generally similar in proportion to the frequency of the NTD subtypes seen in the overall data set.

### Recurrence risk

In the 1066 families, a total of 1514 live siblings of the proband were identified. Of these, 539 are younger. Among these at risk individuals, a total of 34 younger siblings also presented with some type of neural tube defect. Therefore, the recurrence rate to siblings in our sample set was 6.3%, with (95% CI 4.5 – 8.8). Thus, if we assume that the risk of NTD in the general population is 1/1000, the recurrence risk ratio to siblings in our data set is 63. Out of the total live siblings of the probands, ninety-four siblings were affected with a neural tube defect, therefore the overall occurrence rate of NTD in siblings is 6.2% (CI 0.051–0.076). When all pregnancies of NTD mothers are considered, under the conservative assumption that all SABs, TABs, and SBs of unknown phenotype are unaffected, the recurrence to siblings drops to 4.6%. Sibling recurrences also tended to be concordant, with greater than 60% of all affected sibling pairs being of the same sex and greater than 70% concordance for NTD type and level, in those pairs where the information was known (data not shown).

A larger proportion of affected half siblings was seen when the mother was the common parent. Of the 175 maternal half siblings, four (2.3%) were affected. On the paternal side, there was only one reported affected individual out of 115 half sibs (0.9%). Overall, five in 290 half siblings of the proband, or 1.7%, were affected (CI 0.006–0.042).

There were a total of 4 recurrences in the 122 live born children of the probands, making the recurrence risk to offspring 3.3% (CI 0.011–0.087). When all pregnancies of the index case are considered, there are an additional 21 SABs reported. Under this more conservative estimate, the recurrence risk to children of the proband would be 2.8%. Thus, the recurrence risk ratio to offspring in our data set is 28.

### Relationship of affected individuals to proband

When the number of affected individuals on the maternal vs. paternal side of the family is counted, there are more than twice as many affected maternal relatives ( $P = 0.0006$ ) (Table 5). The highest proportion of affected relatives is found in the maternal first cousins, or more specifically, in the children of mother's sisters. In total, there are 88 affected relatives within 3 degrees of relationship from the proband on the maternal side, but only 39 affected paternal family members. Of the affected relatives, there were 21 reported affected siblings of maternal grandparents and 8 affected siblings of paternal grandparents. These figures were not included in the overall calculation since the total number of grandparental siblings is not routinely collected and thus there would be no accurate measurement of the unaffected population.

### Sex ratio of normal and affected transmitters of NTD risk

All of the 297 multiplex families were considered for determining the number of transmitters of NTD risk (Table 6). For the overall analysis, 72 families were excluded because the affected individuals were siblings and the sex of the transmitting parent could not be determined. An additional 16 families were excluded because there were affected individuals on both the maternal and paternal side. For this conservative analysis, 194 pedigrees remained eligible because there were two or more affected relatives within three degrees. Of these, 84 were excluded since only affected sibling pairs were present and two were excluded because there were affected individuals on both sides of the family. In a total of 12 families, the inheritance pattern appeared to be consistent with autosomal dominant inheritance and the sex of the affected transmitting individual was counted.



The overall M:F sex distribution of unaffected carrier or transmitting individuals was 0.64, with a total of 224 male transmitters and 351 female transmitters. We observed an even greater excess of female gene carriers, with a M:F ratio of 0.52, when only closely related relatives are counted (Table 6). Among the affected individuals who also transmitted the predisposition to their children, a significant shift towards the female sex was also observed ( $P = 0.0002$ ). In the families with autosomal dominant inheritance pattern, a total of 9 affected transmitters were female and 5 were male (M:F = 0.56), but this difference is not statistically significant.

### Adverse outcomes

Pregnancy outcomes from both maternal and paternal grandparents resulting in aunts and uncles showed a significant increase in the adverse pregnancy outcomes on the maternal side of the family (Table 7). This was also true for pregnancy outcomes from maternal and paternal aunts and (spouses of) uncles resulting in first cousins. The difference between total adverse outcomes and miscarriages alone in the maternal versus paternal aunts and uncles (children of the proband's grandparents) is statistically significant ( $P < 0.0001$ ). When the pregnancies of first cousins are considered, the difference between the proportion of adverse outcomes between the maternal and paternal relatives is also statistically significant ( $P = 0.04$ ). There was no evidence for fewer children in any of the relative groups tested and the average sibship size did not appear to vary between either the maternal or paternal side of the family in both siblings of NTD mothers and fathers and in their offspring (data not shown).

The number of adverse outcomes or miscarriages is consistent with an increase in interbirth interval. The interbirth interval for paternal aunts and uncles is 3.8 years, but for the maternal aunts and uncles, it is 5.5 years. Although these results are suggestive for an increase in miscarriages in the maternal lineage, this difference did not reach statistical significance. For first cousins, there does not appear to be a difference in the interbirth interval. Paternal first cousins have an average interbirth interval of 3.9 years, whereas it is 3.3 years for maternal first cousins. It is important to note however that the dates of birth for first cousins are not routinely asked to the mothers of NTD probands, and these figures represent a small number of families.

### Discussion

The collection of pedigrees presented here represents one of the largest NTD family data sets reported to date, with greater than 1,000 families and nearly 1,500 affected individuals. From our dataset we found multiple lines of evidence for both sex-influenced and maternal (imprinting) effects in risk for human neural tube defects. Our results indicate an overall excess of affected females, a high recurrence risk for maternal half-siblings and increased frequency of affected relatives on the maternal side of the family. Additionally, there is an excess of females in unaffected individuals who appear to transmit NTD susceptibility and greater adverse pregnancy outcomes in maternal relatives.

The sex ratios suggest an excess of females in the overall NTD dataset, which becomes greater with increasing severity. We observed a nearly equal distribution of males and females in spina bifida and closed defects such as lipomyelomeningocele. For the most part, our results are in agreement with previous findings<sup>20</sup>, although the male excess in low level lesions is not as strong, and we did not replicate the strong female excess in thoracic level lesions. However, the previous study included only nine individuals with thoracic lesions. The female excess could result from a selection against males in utero, leading to an increase in the miscarriage rate or there could be some protective mechanisms in the male sex. Differences with previously published data<sup>20</sup>, which was based on postmortem

examination, may be accounted for by discrepancies in the survivability differences between the sexes. The female excess seen among anencephaly and encephalocele may be partially reflective of a greater proportion of spontaneous abortions occurring earlier on in male fetuses. Several theories have been proposed including differences in spontaneous abortion rates, teratogen susceptibility, and sex-linked genetic factors.

Our recurrence risk estimates may be slightly inflated due to an excess of multiplex pedigrees in our sample set. Our study actively recruits families with two or more affected individuals, although only one affected individual is required for enrollment. Additionally, our conservative assumption that all siblings with unknown birth order were unaffected and exclusion of non-live birth pregnancies may have affected the recurrence risk estimates. The recurrence risk estimates that we obtained for full siblings (6.3%) were slightly higher than previously reported.<sup>13,5,21,22,4</sup> Sibling recurrence risk figures were similar if younger sibs only were included or when all sibs regardless of birth order were counted. When all pregnancies of the mother are counted, and when all fetuses of unknown affection status are considered to be unaffected, the recurrence risk to siblings drops to 4.6%. This figure is perhaps a better estimate of the true risk and is more consistent with previous findings. The overall recurrence risk to half siblings (1.7%) was also found to be as great as the full sibling recurrence risk reported in one study.<sup>5</sup> Remarkably, the half sibling recurrences were greater when the relationship was through the mother, also suggesting a maternal effect. The recurrence risk of 3.3% to children of the NTD patients was also found to be similar to previous reported values, although there were only 4 recurrences seen in our sample set.

In our sample set, the 40% concordance rate for MZ twins is much higher than previously reported estimates of 7% for monozygotic twins and 4% for dizygotic twins<sup>2</sup>, although the percentages are based on a very small sample size in all reported series, including ours. The zygosity of the twin pair was assumed to be that reported by the mother, although there may have been some twin pairs incorrectly classified as identical or MZ. Often the proportion of monozygotic twins estimates are based on the number of like-sex twin pairs. Under this assumption, one study found that 52% of the twin pairs were concordant<sup>5</sup>, more closely matching our estimates. Additionally, our active recruitment of multiplex families may cause some inflation in these concordance rates. The trend that there is a higher concordance rate for both MZ and like sex twin pairs, however, is clearly seen in this study.

In the measurements of more distantly related relative pairs, such as the proportion of maternal and paternal relatives, sex of unaffected transmitters and our observed adverse outcomes, there is likely to be some effect due to bias. Some degree of reporting and recall bias will be inherent in the ascertainment and interview methods through the NTD mothers that we and others have used to collect data on birth defects. One way to test for the effect of differences in the recall bias is to interview the fathers separately to see if the effect is still present.<sup>12,18</sup> Another method for assessing the amount of underreporting is to measure the effect in a control population and then reduce the obtained results by this value.<sup>23</sup> Some studies have ruled out ascertainment bias by considering the same variables in other birth defects or in Mendelian diseases and by testing alternative ascertainment methods.<sup>11,12</sup> Although bias no doubt plays some role, the results in this study and many others have consistently provided evidence for a maternal effect.

We find a higher proportion of affected relatives on the maternal vs. paternal side of the family, but it is possible that this excess is due to some underreporting or recall bias on the paternal side. Our results remain significant ( $P < 0.05$ ) when the total of affected paternal relatives on the side are increased by a factor of 1.5 to account for underreporting. This would allow for a larger contribution of recall bias than the factor of 1.34 previously suggested.<sup>13,23</sup> When reporting the pregnancy outcomes, uncles interviewed alone on both



sides of the family reported about half the number of adverse pregnancy outcomes, suggesting a significant bias. There were, however, more adverse outcomes in the maternal side of the family, regardless of the interviewee.<sup>18</sup> In our sample set, the proportion of adverse outcomes on the maternal versus paternal side are consistent with the results of the interbirth interval data, which would not be expected to show this bias, and remains significant when there is underreporting of the paternal pregnancy outcomes by a factor of 1.3.

We find that the highest proportion of affected relatives other than siblings is with third-degree relatives, or mother's sister's children, as seen in several earlier reports.<sup>13,14</sup> One hypothesis for this finding is that the maternal grandparents pass on a sex-influenced trait to their daughters and that her children can develop NTDs, but because the trait is sex-influenced it appears that the grandparents do not pass on the trait to their sons.<sup>16</sup> The higher frequency of female transmitters would also be consistent with this hypothesis, as both the mother and maternal aunt would be unaffected transmitters of NTD risk. This hypothesis assumes that there is a single dominant gene with reduced penetrance segregating in these families and that all affected individuals presented with an NTD as a result of receiving the same genetic susceptibility. Additionally, unaffected transmitters may have spina bifida occulta or other type of undiagnosed neural tube defect. Results of segregation analysis and linkage studies suggest that this model is appropriate.<sup>24</sup> In the sample set as a whole, the possibility of X-linked or mitochondrial inheritance patterns can be excluded since there are several families with male-to-male and male-to-female transmission.

Although there is strong evidence for maternal and sex-influenced effects in neural tube defects, it is clear that environment factors also play an important role in the etiology of NTDs. As we continue to study the role of folate supplementation, additional studies are needed to investigate the importance of epigenetic factors and imprinting effects, as well as the gender of the child in altering NTD risk. Genetic association studies have suggested a role for several genes through a variety of analysis methods, but it is still unclear if the maternal or fetal genotype is most relevant. The incorporation of child's gender, genomic imprinting, methylation status, and folate supplementation into future genetic models is essential for accurate risk assessment and fully understanding the genetic influences of NTDs and other birth defects.

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**Table 1**

Description of NTD families

NTD family type	Total families	Total affected individuals
Simplex	769	769
Multiplex		
All families	297	689
Families with relatives $\leq 3^{\circ}$	194	434
Totals	1066	1467

**Table 2**

NTD diagnosis for probands and affected relatives

Diagnosis	Proband only	Other affected	GRAND TOTAL
Acrania	4	0	4
Anencephaly	127	84	211
Cranial defect - unspecified	5	5	10
Craniorachischisis	4	1	5
Encephalocele	22	7	29
Lipoma of the spinal cord	17	7	24
Lipomyelomeningocele	108	4	112
Meningocele	11	4	15
Myelocystocele	3	1	4
Myelomeningocele (total)	682	102	784
Cervical	0	3	3
Lumbo/Sacral	551	51	602
Thoracic	90	3	93
unknown level	41	45	86
Spina bifida, unspecified	38	160	198
Myeloschisis	1	0	1
Rachischisis	2	1	3
Sacral agenesis	3	2	5
Split Cord Malformation			
(type I, diastematomyelia (5), unknown type (3))	8	2	10
Tethered cord - cause unknown			
(fatty filum (3), thickened filum terminale (1), spina bifida occulta (3), lipoma (1), dermal sinus tract (1))	21	16	37
OTHER			
(thickened filum terminale (1, also syringomyelia), fatty filum/spina bifida occulta (3), dermal sinus tract (1), dermoid (1), sacral teratoma (1), spina bifida occulta (2), unknown (1))	10	5	15
TOTAL	1066	401	1467

**Table 3**

Sex ratios of NTD patients by type of defect.

	Totals	Females	Males	Unknown	Ratio (M:F)
All NTD	1467	767	632	68	0.82
All Cranial NTDs	259	134	89	36	0.66
Anencephaly only	211	102	78	31	0.76
Encephalocele only	29	19	8	2	0.42
All Spina bifida *	1001	519	453	29	0.87
All Myelomeningocele (MM)	784	403	371	10	0.92
Lumbrosacral MM only	602	319	279	4	0.87
Thoracic MM only	93	44	49	0	1.11
Closed defects (lipoMM, lipoma)	136	70	64	2	0.91

\* All spina bifida category includes the following NTD diagnoses from Table 2: meningocele, myelocystocele, myelomeningocele and spina bifida unspecified.

**Table 4**

## Description of Twins

	Concordant	Discordant	Total	%Concordance
Zygosity				
Monozygotic	2	3	5	40%
Dizyotic	3	32	35	8.6%
Unknown	3	4	7	
Sex				
Like sex	7	19	26	27%
Unlike sex	0	20	20	0%
Unknown	1	0	1	



**Table 5**

The total number of affected and unaffected individuals in all NTD pedigrees based on the relationship to the proband

Relationship to the proband	Total affected relatives (percent of affected relatives for type)	Total unaffected relatives
Paternal		
father	3 (0.003)	1063
half sibling	1 (0.009)	114
father's brother (uncle)	6 (0.005)	1178
father's sister (aunt)	6 (0.006)	1079
father's brother's child (first cousin)	9 (0.005)	1765
father's sister's child (first cousin)	5 (0.003)	1835
father's mother (grandmother)	1 (0.0009)	1065
father's father (grandfather)	0	1066
great grandparent	0	2132
Total	31	11297
Maternal		
mother	5 (0.005)	1061
half sibling	4 (0.02)	171
mother's brother (uncle)	10 (0.008)	1219
mother's sister (aunt)	17 (0.01)	1127
mother's brother's child (first cousin)	9 (0.005)	1853
mother's sister's child (first cousin)	17 (0.008)	2023
mother's mother (grandmother)	3 (0.003)	1063
mother's father (grandfather)	1 (0.0009)	1065
great grandparent	1 (0.0005)	2131
Total	67	11713

**Table 6**

Male to female ratios among affected and normal transmitters of multiple case NTD families

	Total families	Total individuals	Male	Female	M:F ratio
Normal transmitters	194	575	224	351	0.64
All affected relatives					
Affected relatives $\leq 3^{\circ}$	96	160	55	105	0.52
Affected transmitters	12	14	5	9	0.56
AD inheritance pattern					

Pregnancy outcomes resulting in first cousins, or aunts and uncles of the proband and broken down by either maternal or paternal relationship.

**Table 7**

Pregnancy outcome	Paternal first cousins		Maternal first cousins		Paternal aunt/uncle		Maternal aunts/uncles	
	N = 3699	%	N = 4031	%	N = 2396	%	N = 2589	%
Live births	3553	96.1	3830	95.0	2261	94.4	2354	90.9
Infant death (ID)	2	0.05	5	0.1	13	0.5	23	0.9
Stillborn (SB)	4	0.1	5	0.1	5	0.2	7	0.3
Miscarriage (SAB)	140	3.8	191	4.7	117	4.9	205	7.9
Any adverse outcome	146	4.0	201	5.0	135	5.6	235	9.1