Genetic Epidemiology of Birth Defects: Nonsyndromic Cleft Lip and Neural Tube Defects

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INTRODUCTION

Birth defects have been defined by the March of Dimes as any abnormality of structure or function, whether inherited or acquired during the prenatal or perinatal period, and whether it presents itself in utero, at birth, or later in life (1). This broad definition includes inherited diseases with adult onset that are not generally considered to be birth defects. However, even when limited to abnormalities that are present at or shortly after birth, the definition of a birth defect encompasses a staggering number of conditions and a multitude of etiologic mechanisms.

Birth defects are currently the leading cause of infant mortality and represent a significant source of childhood disability in the United States and other developed countries (2). Known causes of birth defects include single gene disorders (e.g., van der Woude syndrome) chromosome abnormalities (e.g., trisomy 21) and environmental exposures (e.g., alcohol). However, causative factors have not been identified for as many as two-thirds of all birth defects (3). The subgroup of birth defects with largely unknown etiologies includes many structural malformations, such as orofacial clefts, neural tube defects, and cardiac malformations. These malformations are generally referred to as being nonsyndromic in order to differentiate them from phenotypically identical conditions which occur as part of recognized malformation syndromes (e.g., trisomy 13, Van der Woude syndrome, velocardiofacial syndrome). Although they are clearly not inherited in a simple Mendelian fashion, many of the nonsyndromic structural malformations tend to aggregate within families. Hence, genetic factors are thought to be involved in their etiology.

This review focuses on the genetic epidemiology of nonsyndromic structural malformations. Individually, many of these malformations are relatively common, and collectively, they represent a significant proportion of all birth defects. Identification of the specific factors which predispose an individual to the development of these malformations would, therefore, represent an important step towards reducing infant mortality and childhood disability.

BACKGROUND

The tendency for specific structural malformations to aggregate within families has long been recognized, and by the beginning of the twentieth century, hereditary factors were widely accepted as a cause of congenital malformations (4). However, it is only relatively recently that the familial aggregation patterns for various structural malformations have been well documented (e.g., 5–10).

Family studies have identified several characteristics that are common to the various structural malformations and inconsistent with simple Mendelian inheritance. These characteristics are well illustrated by the familial aggregation pattern exhibited by cleft lip with or without cleft palate. Briefly, cleft lip with or without cleft palate is a common, serious malformation of human structure that affects approximately 1 in 1,000 live-born Caucasians. Affected individuals have either unilateral or bilateral malformations of the upper lip that may or may not be associated with clefting of the palate. The familial aggregation pattern exhibited by cleft lip with or without cleft palate is characterized by risk ratios (i.e., the risks to relatives of affected individuals compared with the population prevalence) that are low compared with those observed for single gene disorders (table 1). This reflects the fact that, compared with single gene disorders, cleft lip with or without cleft palate tends to be more common in the general population, while the absolute risks to relatives of affected individuals tend to be lower. Additional characteristics of cleft lip with or without cleft palate that are inconsistent with simple Mendelian inheritance include a concordance rate for monozygotic twins that is markedly less than 100
percent, a nonlinear decline in the risks to relatives with decreasing degree of genetic relationship to the proband, and recurrence risks that are dependent on the severity of the proband's defect, as well as the number of affected family members (11, 12).

Numerous theories have been proposed to explain the nontraditional patterns of inheritance that characterize cleft lip with or without cleft palate and other common structural malformations. The majority of these theories have invoked modifications of simple Mendelian inheritance and are largely unsatisfactory explanations for the observed familial patterns. However, during the 1960s, it was recognized that multifactorial threshold models of inheritance could account for many of the familial patterns that characterize the structural malformations (13).

The most widely accepted multifactorial threshold model of inheritance was proposed by Falconer (14). This model assumes that 1) the risk of developing a discrete condition, such as a structural malformation, is determined by an underlying, continuously distributed attribute which is referred to as liability, 2) liability is determined by the equal, additive, and relatively small effects of numerous genetic and environmental factors, and is normally distributed, and 3) the observed dichotomy in phenotypic expression is determined by a threshold beyond which individuals are affected. This model was particularly attractive because it allowed for the estimation of the heritability of liability (i.e., the relative proportion of the variation in liability that is determined by genes) for discrete traits (14).

Estimates of heritability obtained from Falconer's multifactorial threshold model provided the first clues regarding the relative importance of genetic factors in the etiology of structural malformations, and indicated that genes play an important role in the development of many of these conditions. For example, the heritability of liability to cleft lip with or without cleft palate is estimated to be approximately 80 percent (11). The assumptions of this model have, however, been criticized as being unrealistic, and it is generally accepted that the true mode of inheritance for many structural malformations is likely to lie between the extremes of single locus and multifactorial threshold inheritance. Recent progress in molecular and statistical genetics is helping to bridge the gap between these two extremes.

**SPECIAL CONSIDERATIONS**

Defining the nature of the genetic contribution (i.e., the number of genes involved and the magnitude of their effect) to the nonsyndromic structural malformations is not a straightforward task. Many of the methods which have served well in the identification of Mendelian disease genes are ill-suited for traits that are, genetically, more complex. Issues related to the dissection of genetically complex traits have been reviewed in detail by others (15–17). Although many of these issues relate equally to structural malformations and complex genetic diseases of adulthood (e.g., cardiovascular disease, cancer, psychiatric disorders), several are largely specific to structural malformations and deserve special consideration.

Family studies of many structural malformations are hampered by the problem of underreporting of affected relatives due to high rates of spontaneous abortion and, in some cases, elective termination of affected fetuses. Malformations that are associated with high neonatal mortality rates are also subject to underreporting which can distort the true pattern of familial aggregation and, therefore, bias conclusions regarding mode of inheritance. Familial aggregation patterns may also be distorted by reduced reproductive fitness among affected individuals (18). Although reduced reproductive fitness is not unique to structural malformations, its impact on familial aggregation is likely to be higher for these conditions than for diseases that develop during or after the reproductive years. In addition, genetic epidemiologic studies of structural malformations may be subject to complications arising from the involvement of maternal genes, or interactions between maternal and fetal genes, in disease etiology.

Compared with many of the complex diseases of adulthood, there are also several features of structural malformations that render them more amenable to genetic dissection. One such feature is the relative ease of diagnosis. Structural malformations tend to be well defined and easily recognized at or shortly after birth. Concerns regarding diagnostic heterogeneity and issues relating to age-at-onset are, therefore, dramati-

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**TABLE 1. Characteristics of the familial aggregation pattern for cleft lip with or without cleft palate**

<table>
<thead>
<tr>
<th>Risk</th>
<th>Prevalence</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Monozygotic cotwins</td>
<td>0.4040</td>
<td>404.0</td>
</tr>
<tr>
<td>First-degree relative</td>
<td>0.0322</td>
<td>32.2</td>
</tr>
<tr>
<td>Second-degree relative</td>
<td>0.0056</td>
<td>5.6</td>
</tr>
<tr>
<td>Third-degree relative</td>
<td>0.0035</td>
<td>3.5</td>
</tr>
<tr>
<td>Risk to first-degree relative if proband has:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral cleft lip with or without cleft palate</td>
<td>0.0225</td>
<td>2.25</td>
</tr>
<tr>
<td>Bilateral cleft lip with or without cleft palate</td>
<td>0.0479</td>
<td>4.79</td>
</tr>
<tr>
<td>An affected sibling</td>
<td>0.1558</td>
<td>15.58</td>
</tr>
<tr>
<td>An affected parent</td>
<td>0.1576</td>
<td>15.78</td>
</tr>
</tbody>
</table>

* Data are from Mitchell and Risch (11).
ally reduced. The period during which relevant environmental exposures may occur is also relatively narrow and well defined. Hence, nondifferential misclassification of exposure status is likely to be reduced, particularly when exposure data are collected shortly after the diagnosis of a malformed child. This should reduce heterogeneity by allowing for the exclusion of cases with completely environmental etiologies and increase the likelihood of detecting true gene-by-environment interactions. Finally, the existence of numerous population-based congenital malformation registries provides the opportunity to conduct large-scale studies and to compare results across racial and ethnic boundaries.

Open neural tube defects and cleft lip with or without cleft palate are two of the most easily recognized classes of structural malformations. The diagnosis of these conditions is straightforward and immediately apparent at the time of birth. It is likely that the ease of diagnosis, combined with the severity of these conditions, has contributed to the extensive research into their etiology. Although both conditions are generally believed to be inherited in a multifactorial fashion, epidemiologic and family data suggest that the underlying models of inheritance may be markedly different. Therefore, it is not surprising that recent progress in our understanding of the etiology of these conditions has followed from markedly different approaches.

CLEFT LIP WITH OR WITHOUT CLEFT PALATE

Several characteristics of cleft lip with or without cleft palate make it an ideal candidate for genetic epidemiologic studies: 1) The overt nature of the malformation and low perinatal mortality aid in the collection of reliable family history data; 2) affected relatives can be easily documented, since virtually all cases require surgical intervention; 3) the prevalence and familial aggregation pattern are unlikely to be dramatically influenced by elective termination or spontaneous abortion of affected fetuses; and 4) relative to other serious structural malformations (e.g., neural tube defects and congenital heart defects), reduced reproductive fitness is likely to have only a modest influence on the observed pattern of familial aggregation. In addition, genetic epidemiologic studies of cleft lip with or without cleft palate are relatively uncomplicated by the effects of environmental covariates. The prevalence and familial aggregation pattern in Caucasian populations of European descent are remarkably consistent across geographic location and time periods. Long-term secular trends have not been convincingly demonstrated (19) and no striking association with environmental variables, such as social class or season of conception, or with parental characteristics, such as age, occupation, or parity, have been identified (19, 20).

Race is the only demographic variable that has been consistently associated with the prevalence of cleft lip with or without cleft palate. Compared with Caucasians, the prevalence is higher in Asians (particularly Japanese) and lower in individuals of African descent (21–23). These differences appear to persist after migration, suggesting that they are genetically, rather than environmentally, mediated (23). Hence, based on both family and epidemiologic data, the evidence for a genetic component is the best etiologic clue that exists for cleft lip with or without cleft palate.

It is generally accepted that cleft lip with or without cleft palate is inherited as a multifactorial trait. However, this view has been challenged (24), and the specific nature of the genetic contribution continues to be debated (11, 25). Based on complex segregation analyses, which explicitly evaluate single major locus versus multifactorial threshold models of inheritance, it has been suggested that the familial aggregation pattern for cleft lip with or without cleft palate is more consistent with a model in which liability is determined by a single major locus (which may or may not act against a background of multifactorial inheritance) than it is with simple multifactorial threshold inheritance. The results of such analyses have been taken as strong evidence in favor of a major gene for this condition. However, a review of the literature indicates that, as a whole, the results of such analyses are far from conclusive.

Several segregation analyses of cleft lip with or without cleft palate family data have been unable to discriminate between alternative genetic models of inheritance (26–28) (table 2). Moreover, among the studies that have been able to discriminate between alternative models, there is no consensus regarding the most appropriate model of inheritance for this condition (29–34). For example, models which included a single major locus and a multifactorial threshold component were found to provide the best fit to family data in an English (29) and a Danish (30) data set, but different modes of inheritance were ascribed to the major locus in the two samples (table 2). These results are inconsistent with the similarity of the familial aggregation patterns in the English and Danish data (11). Moreover, there is compelling evidence against autosomal recessive inheritance of cleft lip with or without cleft palate: the risk to sibs does not exceed the risk to offspring (11, 35).

An alternative approach to the evaluation of competing models of inheritance was recently suggested by Risch (36). Analyses using this approach have
TABLE 2. Summary of results from complex segregation analyses of cleft lip with or without cleft palate

<table>
<thead>
<tr>
<th>Population</th>
<th>Hypothesis</th>
<th>Conclusions†</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MFT*</td>
<td>SML*</td>
</tr>
<tr>
<td></td>
<td>(Inheritance, d, q)</td>
<td></td>
</tr>
<tr>
<td><strong>Caucasian populations (European descent)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>France (26)</td>
<td>Rejected</td>
<td>Not rejected</td>
</tr>
<tr>
<td>England (29)</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>Denmark (30)</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>Minnesota (27)</td>
<td>Not rejected</td>
<td>Not rejected</td>
</tr>
<tr>
<td>Italy (28)</td>
<td>Not rejected</td>
<td>Not rejected</td>
</tr>
<tr>
<td><strong>Non-European and racially mixed populations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan (30)</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>Hawaii (31)</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>China (32)</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>Madras, India (33)</td>
<td>Not rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>West Bengal, India (34)</td>
<td>Rejected</td>
<td>Not rejected</td>
</tr>
</tbody>
</table>

* Abbreviations: AD, autosomal dominant; AR, autosomal recessive; MFT, multifactorial threshold; SML, single major locus.
† Conclusions = best fitting Mendelian model (mode of inheritance at SML, d = degree of dominance at SML, q = frequency of the high risk allele, H = heritability associated with MFT component).

unequivocally rejected single major locus inheritance of cleft lip with or without cleft palate, and indicate that this condition is most likely determined by a multiplicative model of inheritance, under which a few genes (i.e., \( \leq 3 \) genes) of moderate effect act against a multifactorial background (11, 35, 37). Hence, it seems likely that the mixed results obtained in segregation analyses reflect the fact that neither the single major locus nor the multifactorial threshold model adequately describe the inheritance of cleft lip with or without cleft palate.

Statistical models, no matter how sophisticated or elegant, can offer only circumstantial evidence about mode of inheritance. Hence, it is not surprising that the quest for genes which are involved in the etiology of cleft lip with or without cleft palate has begun, despite an imperfect understanding of the genetic contribution to this condition. Efforts to identify genes for this condition have largely employed either population association (i.e., case-control) studies or parametric linkage analyses, using high density families (i.e., families with multiple affected individuals in at least two generations). Both approaches have limitations and have yielded conflicting results, making it difficult to draw firm conclusions regarding the role of putative susceptibility loci (38).

Population associations have been identified for cleft lip with or without cleft palate and the transforming growth factor alpha locus (39), the retinoic acid receptor alpha locus (40), and an anonymous marker (D4S192) on chromosome 4q (41). Although the existence of such population associations is taken as tentative evidence that the locus (or a gene in linkage disequilibrium with the locus) is related to disease etiology, this inference must be confirmed by a test of linkage (42). The power to detect linkage of cleft lip with or without cleft palate to transforming growth factor alpha, retinoic acid receptor alpha, or D4S192 is, however, likely to be quite low, since these loci do not appear to increase the risk to first degree relatives of affected individuals by more than 1.2-fold (11, 40, 41)—substantially less than the overall risk ratio of 32-fold for first-degree relatives.

Parametric linkage analyses offer one approach to the identification of cleft lip with or without cleft palate susceptibility loci. The application of this approach to complex traits is, however, limited by the need to specify a model that adequately explains the inheritance pattern. To overcome this limitation, most studies have been restricted to high-density families, the rationale being that such families are the most likely to segregate a rare, highly penetrant dominant disease allele. However, the ability to find a few high-density families does not prove that a major disease locus is segregating in these families; similar types of families will also be found for traits with more complex modes of inheritance (15). Since use of the wrong model can lead to both false-negative and false-positive linkage results (16), it is not surprising that parametric linkage analyses of cleft lip with or without cleft palate have been largely negative (43–52), and the few positive results have been weak and difficult to replicate. Such analyses have failed to detect linkage between this condition and either transforming growth factor alpha (44, 47, 49) or retinoic acid receptor alpha (48). However, loci on chromosomes 4q (53), 6p (54,
55), and 19q (56) have been implicated in the etiology of cleft lip with or without cleft palate on the basis of parametric linkage analyses.

Nonparametric linkage approaches, such as the transmission disequilibrium test (57) and the affected sib-pair approach (58), offer attractive alternatives to parametric linkage analyses, since they do not require specification of mode of inheritance. The transmission disequilibrium test is particularly attractive because it requires only one affected individual per family and is, therefore, applicable to the majority of families. Since this test can only detect linkage in the presence of linkage disequilibrium, it is, however, most useful when a population association between a disease and a marker has already been detected. The transmission disequilibrium test has been used to evaluate the relation between cleft lip with or without cleft palate and genetic variation at the transforming growth factor alpha locus (59) and a locus on 19q3.1 (56). In both cases, this approach has provided evidence in favor of linkage between cleft lip with or without cleft palate and loci in these regions of the human genome.

In contrast to the transmission disequilibrium test, the affected sib-pair approach can detect linkage in the absence of linkage disequilibrium. However, affected sib-pairs are relatively rare for cleft lip with or without cleft palate and several hundred would be required to detect linkage to loci with moderate effects (35). Not surprisingly, large scale affected sib-pair linkage analyses have not been reported for cleft lip with or without cleft palate. Collaborative efforts to accumulate a sample of affected sibling pairs with sufficient power to detect cleft lip with or without cleft palate suscept-ibility loci of moderate effect are, however, currently being organized by several groups (60).

Elucidation of the full complement of genetic risk factors for cleft lip with or without cleft palate is likely to represent a gradual, iterative process. Genetic and/or environmental risk factors that are individually weak and difficult (if not impossible) to detect, may interact to produce more substantial increases in risk. Therefore, as putative susceptibility loci are identified, the impact of other potential genetic and environmental risk factors should be reevaluated. Evidence for one gene-environment interaction has been identified in this manner; prenatal exposure to maternal cigarette smoke, which has been inconsistently associated with the risk of cleft lip with or without cleft palate, appears to be a more potent risk factor when present in conjunction with the transforming growth factor alpha C2 allele (61, 62).

**NEURAL TUBE DEFECTS**

As with cleft lip with or without cleft palate, neural tube defects (primarily anencephaly and spina bifida) are generally thought to be inherited in a multifactorial fashion. The evidence for a genetic component is, however, substantially weaker for neural tube defects than it is for cleft lip with or without cleft palate. Although this may reflect true differences in the genetic contribution to these two conditions, the role of genetic factors in the etiology of neural tube defects has been difficult to establish on the basis of existing family data. This is partially attributable to concerns regarding the validity of family data for neural tube defects—particularly for second- and third-degree relatives—due to the increased risk of spontaneous abortion (63), high perinatal mortality (64, 65), and, increasingly, elective termination of affected fetuses (66). Moreover, the risk to offspring has not been well established, due to the low reproductive fitness of affected individuals. Analysis of the familial aggregation patterns for neural tube defects have, therefore, been largely restricted to information on the siblings of affected individuals.

The risk of neural tube defects in the siblings of affected individuals (3–8 percent), is consistently increased compared with the risk in the general population (6, 7, 9, 10, 65). In addition, there appears to be a further increase in risk to individuals who have more than one affected sibling (6, 9, 10, 65). Increased risks of neural tube defects have also been reported for maternal half-siblings (9, 10, 67) and offspring of maternal aunts (9, 10, 68), but have not been convincingly demonstrated for other types of second- and third-degree relatives. This pattern of increased risk is consistent with the involvement of a maternal factor in the determination of neural tube defects. However, since differential reporting of maternal and paternal relatives cannot be excluded, the available family data are insufficient for the purposes of establishing mode of inheritance (65, 69, 70).

Relative to its effect on cleft lip with or without cleft palate, the environment appears to play a more important role in the etiology of neural tube defects. The occurrence of neural tube defects is associated with a number of environmental and demographic variables, including calendar year, season, geographic location, ethnicity, race, socioeconomic status, maternal age, and parity (71, 72). Moreover, several lines of evidence have strongly implicated maternal nutritional status as an important determinant of the risk (73) for neural tube defects. This view has recently been confirmed by several studies that have convincingly demonstrated a relation between folic acid and neural tube defects (74–76). Maternal periconceptional supple-
mentation with folic acid has been shown to significantly reduce the risk of this condition (74, 75), and a dose-response relation between maternal red blood cell folate levels and neural tube defects risk has been demonstrated (76). However, the mechanism by which folic acid exerts its protective effect remains unknown.

It has been suggested that folic acid supplementation may override an inherited disorder of maternal folate metabolism, and that such disorders could contribute to the familial aggregation of neural tube defects (77). From a genetic standpoint, if this hypothesis is correct, the phenotype of interest is not neural tube defects but having had a child with a neural tube defect. The observed increased risks to siblings, maternal half-siblings, and the offspring of maternal aunts are consistent with this latter possibility. However, formal evaluation is required to determine whether the familial aggregation of this maternal trait (i.e., having had an affected child/pregnancy) is consistent with a genetic mode of inheritance. Such analyses will be complicated by the sex-limited and parity-dependent expression of this trait. Moreover, it is possible that both the maternal and fetal genotypes contribute to the risk of neural tube defects.

The identification of the protective effect of folic acid has provided potentially important clues regarding specific genetic factors which may predispose to the development of neural tube defects. Genes that are involved in the metabolism and absorption of folic acid are now strong candidates for neural tube defect susceptibility loci. One such candidate is the gene for 5,10-methylenetetrahydrofolate reductase, an enzyme involved in folic acid metabolism. The frequency of the 677C—>T mutation in this gene, which results in a thermolabile variant of the enzyme, has been found to be significantly higher in patients with neural tube defects and in the parents of affected individuals compared with the general population (78–80).

A causal relation between methylenetetrahydrofolate reductase and neural tube defects remains to be confirmed by appropriately designed linkage analyses. Any such analyses of this gene, or other candidate loci for neural tube defects, will be complicated by the uncertain nature of the disease phenotype (i.e., neural tube defect versus having had a child/pregnancy with neural tube defects) as well as the relevant genotype (i.e., maternal versus fetal versus maternal plus fetal).

**SUMMARY**

This review has focused on only two common structural malformations. However, the difficulties and successes encountered while attempting to elucidate the genetic contribution to these two conditions are likely to be echoed in studies of other complex congenital anomalies. As our understanding of the mechanisms which give rise to a particular malformation gradually unfolds, the importance of a multidisciplinary approach to understanding the genetic contribution to these conditions becomes increasingly apparent. Experience indicates that both traditional and genetic epidemiologic approaches will be integral components of any such efforts, since the identification of environmental risk factors (e.g., folic acid) may provide clues regarding the nature of disease susceptibility loci, and the identification of susceptibility loci will provide new opportunities to explore potential environmental covariates of disease risk (e.g., maternal cigarette smoke).

Although we are not yet in a position to completely describe the genetic contribution to any single structural malformation, advances over the past decade have led to a rapid increase in our ability to elucidate the relevant genetic factors. Given the complex nature of the nonsyndromic structural malformations, there is undoubtedly much work to be done before we fully understand the genetic contribution to even a single malformation. However, for the first time, such an understanding and the accompanying potential for prevention seem within our reach.

**ACKNOWLEDGMENTS**

This work was supported by grant DE11388 from the National Institutes of Health.

**REFERENCES**


