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Invited Editorial: Mapping the Cleft-Lip Genes: The First Fix?

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Ever since Poul Fogh-Andersen's (1942) monumental report on the inheritance of harelip and cleft palate provided enough numbers for serious statistical analysis, geneticists have been speculating on the inheritance of cleft lip, with or without cleft palate (CL(P)) and isolated cleft palate (CP). Indeed, Fogh-Andersen, in his review of literature, lists claims for just about every imaginable mode of inheritance, including dominance with reduced penetrance, partial dominance (with homozygotes being severely affected and heterozygotes mildly affected or normal), double or "polyhybrid" recessivity, partial sex linkage (plus an autosomal recessive gene), and—most imaginative of all—"polymeric" recessivity, with two factors for the upper lip (right and left), two for the hard palate, and one for the soft palate. Fogh-Andersen's interpretation of his own data was that CL(P) showed conditioned dominance with sex limitation to males, meaning that the gene usually expressed itself in the homozygote but occasionally in the heterozygote. He also made the important observation that CL(P) was, in most cases, genetically different from CP, a distinction for which the embryological basis was subsequently demonstrated in the mouse (Trasler and Fraser 1963).

In the 1950s the idea was developed that cleft palate, induced by maternal treatment with cortisone in the mouse, was a multifactorial, threshold trait, and a biological basis was postulated for liability (the stage at which the palate shelves normally moved from vertical to horizontal, preliminary to fusion) and for a developmental threshold (the maximum amount of delay compatible with closure) (Fraser et al. 1957). Various factors involved in shelf movement to the horizontal and resistance of the intervening tongue—and how each of these factors could be influenced by genes and environmental factors—were identified, illustrating the multifactorial nature of the system (Trasler and Fraser 1977). Specific genes for differences in sensitivity to glucocorticoid-induced cleft palate were identified and even assigned to specific chromosomes (Bonner and Slavkin 1975; Vekemans et al. 1981; Liu and Erickson 1986). Usually two or three genes (maternal and/or fetal) accounted for the difference between any two pairs of strains, but the specific genes involved differed between different pairs of strains. Thus, on a population basis, the number of genes influencing liability to cortisone-induced cleft palate must be quite large. Furthermore, their effects are small enough that it takes extensive backcrosses, or development of congenic lines, or of recombinant inbred strains, to demonstrate their existence—experimental approaches not applicable to human studies. And they only reflect *differences* in response to cortisone; they do not tell us anything about the causes of spontaneous clefts.

Susceptibility to spontaneous cleft lip, as manifest in the A/Jax and related strains, also involves a "major" (i.e., detectable) genetic difference, although it has a penetrance of only about 5% on the A/Jax background and is modified by other, particularly maternal, genes (Biddle and Fraser 1986; Juriloff 1986). In this case the shape of the embryonic facial prominences was the indicator of cleft-lip liability (Trasler 1968). The map location of the gene remains stubbornly elusive.

Parallel to and independently of these experimental studies, statistical geneticists were developing a multifactorial threshold model to account for liability to the common, familial, human disorders, such as diabetes mellitus (Falconer 1965). Cedric Carter (1961) showed how such a model could account for the seemingly paradoxical fact that, in the case of "congenital" hypertrophic pyloric stenosis, the recurrence risk was higher in the near relatives of probands of the least-often-affected sex (female). This and other predictions of the model were shown to apply also to cleft lip (Carter

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1969; Fraser 1970, 1980*a*), though there were dissenting views (Melnick et al. 1980), and it was recognized that the predictions of the multifactorial threshold model stemmed from the presence of a threshold more than from the shape of the liability distribution.

Carter assumed that the genetic component of the model was polygenic, but he used the term loosely, to mean simply "more than a few" genes. It was pointed out that the data did not quite fit the expectation for a polygenic (strict sense) model (Melnick et al. 1980), which is not surprising, since the polygenic assumption (of equal, additive, small effects of many genes) is an idealistic simplification for purposes of statistical treatment. Real genes are not like that.

With the aid of computers, the statistical geneticists were now developing and testing methods of complex segregational analysis of ever increasing complexity, involving various combinations of a "major" gene (with low penetrance), a polygenic contribution, sporadic (i.e., nonrecurrent) cases, and a familial environmental component (Morton et al. 1970; Elston and Stewart 1971; Morton and MacLean 1974; Lalouel and Morton 1981). "Major" seemed to mean "with a big enough effect to be detectable." The predicted recurrence risks were not very different from those of the multifactorial model (Melnick et al. 1980), except in situations where liability was high. The problem was the difficulty of trying to predict the shape of the liability distribution simply from the number of individuals that fell beyond the threshold (i.e., were affected) in various groups of relatives (Fraser 1980*b*).

In the meantime David Bixler, to his great credit, updated Fogh-Anderson's pedigrees, greatly enlarging the data base and providing a unique set of families for complex segregational analysis. Subsequent analyses of these data have led to the suggestions of (1) a dominant gene, with an ingenious hypothesis (allelic restriction) to account for the low penetrance (Melnick et al. 1977); (2) neither the multifactorial threshold nor the single-major-locus model (Melnick et al. 1980); and (3) either a major locus or the full mixed model plus a large admixture of sporadic cases caused by polygenic inheritance, phenocopies, or environmental agents (Marazita et al. 1986).

Most recently Chung et al. (1986) have, to some extent, reconciled the views of the multifactorial threshold and the major-gene supporters by showing that the Danish data are consistent with the presence of an autosomal recessive gene influencing liability in about one-third of the cases and also an important contribution from multifactorial inheritance, whereas in the Japanese population the data fit a multifactorial model with no major gene. Keep in mind that the postulated autosomal recessive gene in the Danish population has a penetrance of roughly 33%, so the recurrence risk in these families would be about 8%, not dramatically higher than that of the multifactorial group, which is about 4%.

The goal of the multifactorial supporter would be to identify some biological attribute of liability, such as face shape, that could be an indicator of increased risk (Trasler 1968; Fraser and Pashayan 1970; Coccaro et al. 1972; Erickson 1974). The goal of the major-gene supporters would be to identify a genetic marker that would indicate increased risk. The proof of the pudding is in the eating—if there is an epigenetic biological attribute of susceptibility, demonstrate it. If there is a major gene, find the marker. In either case, the difficulty of proving the pudding is compounded by its heterogeneity.

Studies of face shape in the unaffected cotwins of children with CL(P) have recently demonstrated heterogeneity (Johnston and Hunter 1989). About one-third had average to moderately increased nasal cavity widths, as if they had had small maxillary prominences. The other two-thirds had narrow nasal cavities, as if they had had small medial nasal prominences. The authors suggest that the latter (narrow) type may correspond to Chung's multifactorial group, and the former to the autosomal recessive group.

Now we have another breakthrough. In this issue Ardinger et al. (1989) have demonstrated an association of CL(P) with two RFLPs at the transforming growth factor alpha (TGFA) locus—a major advance. Ironically, the authors chose to look at the loci for TGFA and at several other growth factor and receptor loci because animal studies had suggested their possible involvement in secondary palate closure, whereas the human association is with cleft lip, an embryologically earlier and different process. But, in fact, TGFA may also be involved in fusion of the facial processes, so the association is still plausible (M. C. Johnston, personal communication). It will be exciting to see whether the relationship of CL(P) to TGFA is substantiated by linkage and whether classifying the CL(P) subjects by nasal cavity width clarifies the association by substantially reducing heterogeneity. No doubt this will not by the only marker associated with CL(P)—but at last the pieces of the puzzle are beginning to fit together.

It may not be long before prenatal diagnosis will be possible. And then we will have to start wrestling with the ethics of aborting a fetus with a low risk of having

a more or less correctable malformation. Or, to look on the brighter side, we may be able to test more critically the possible reduction in risk by prenatal vitamin supplementation (Tolarova 1987). And stop arguing about whether its a major-gene or multifactorial/threshold character!

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