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Genetics, Gene Carriers, and Environment

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Among different parts of the human body, the orofacial region is one of the most often affected by birth defects. One reason for this fact could be that it consists of many structures developing from all three embryonic germ layers. If we look to the Wilson scheme[1] of human prenatal development (Figure 1), on which critical periods of embryonic and fetal development can be seen, we readily notice that environmental factors acting as potential teratogens in the embryonic period frequently could disturb structures of the orofacial region.

On the other hand, intrinsic (genetic) factors cause a wide spectrum of anomalies, either single malformations or syndromes or multiple malformations that are not recognizable syndromes. It is characteristic of clefts that the structural or numerical aberrations of any human autosome can yield a cleft lip and/or palate anomaly among other phenotypic abnormalities.

Considering the development of birth defects as deviations from a point of so-called "normality" in human populations, it has become quite obvious that it is insufficient to analyze only genetic and environmental factors acting separately. If, in addition, their interactions are considered, not only genetic principles, but also the impact of environmental factors, mechanisms of development, and principles of etiology can be recognized.

We have to consider a balance of intrinsic (genetic) and extrinsic (environmental) factors. They exert their influences from very early stages—starting in developing germ cells, and continuing through fertilization, embryonic, fetal, perinatal, and postnatal periods. During this rather long time, the intrinsic and extrinsic factors are perpetually in interaction. The balance between them is dynamic—at one time genetic, and at other times environmental factors may

Figure 1. Critical Periods in Human Prenatal Development According to Wilson (1973).

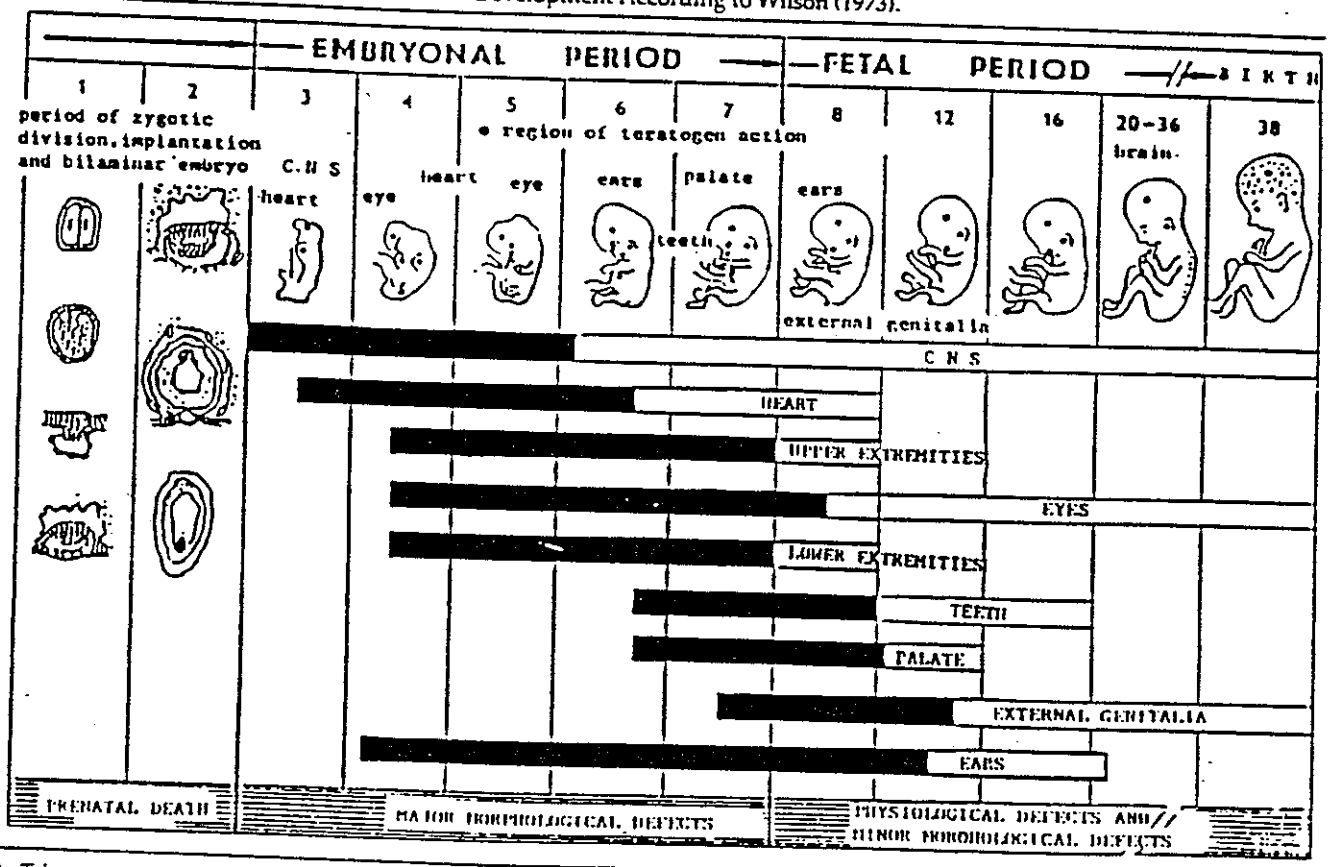

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Figure 2. Proportions of Genetic and Nongenetic (Environmental) Factors in Etiology of Human Malformations

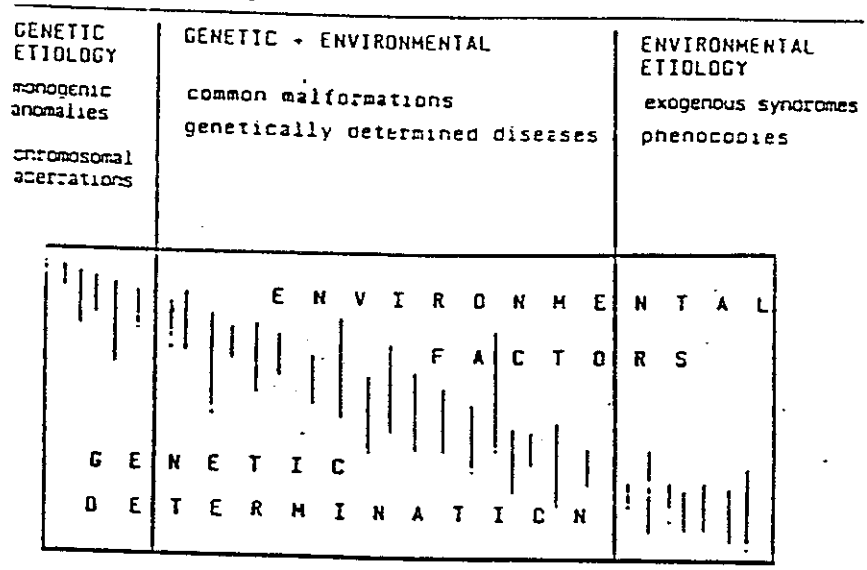


Figure 3. Pedigree of the Family with Treacher Collins Syndrome (The individual III/16 is clinically unaffected and has the son with typical manifestation.)

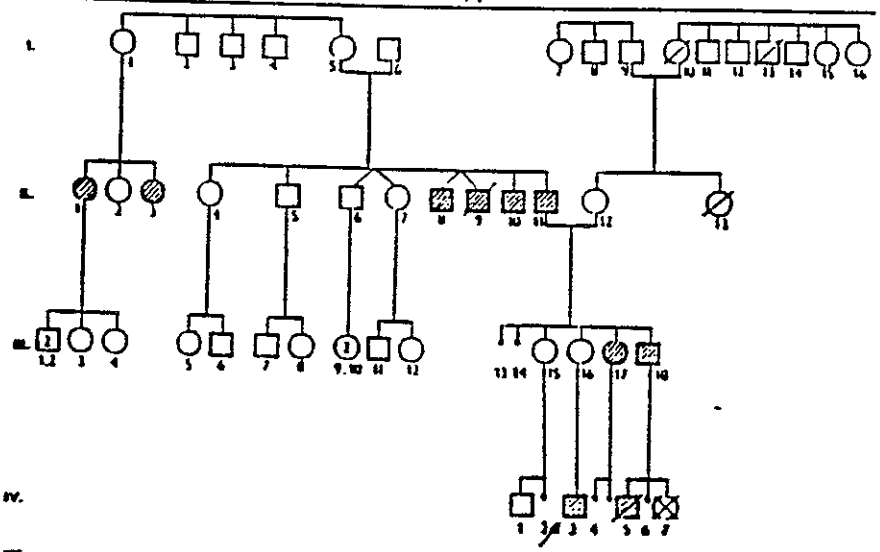
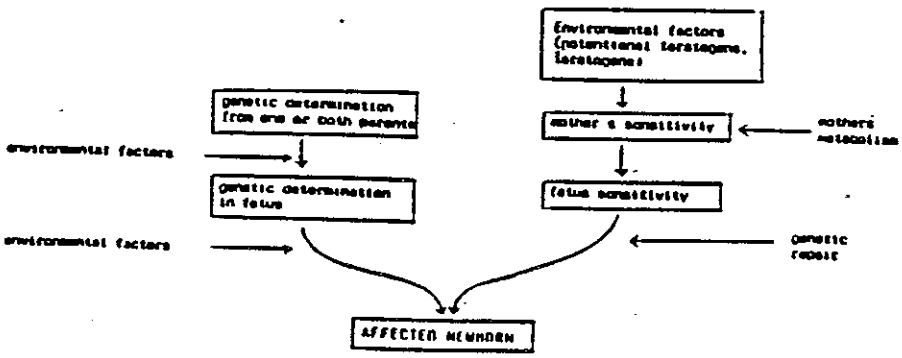


Figure 4. Model of the Multifactorial Etiology Consisting of Genetic Determination and Genetically Determined Susceptibility to Environmental Factors in Mother and Fetus (In this situation, both mother and fetus are sensitive to the potential teratogens.)



prevail. A large capacity for genetic and cellular repair mechanisms enables correcting of many "mistakes" in development. In the end, a baby is born, either normal, by our rules, or abnormal.

From experimental studies, as well as from studies on human fetuses, it is presumed that many times during prenatal life a human embryo undergoes such a disturbance of development, but thanks to a genetic repair or regaining of balance between genetic and exogenous (environmental) influences, the baby is born without any abnormalities. (Many irreparably damaged embryos are aborted.) Also, an opposite situation is possible: more or less genetically normal development may be influenced by exogenous factors, the threshold of reparatory capacity is overcome, and the newborn is abnormal.

Such mechanisms seem to work in a majority of common anomalies and also in genetically determined diseases, such as cardiovascular diseases, duodenal ulcers, diabetes, or allergies. Close interactions of genetic factors, represented by a polygenic background, and of environmental factors are characteristic. In common anomalies such as cleft lip and/or palate, pyloric stenosis, and congenital heart defects, environmental factors obviously act only in early stages of embryonic development during critical periods for respective organs. In genetically determined diseases, exogenous factors act most often during the postnatal period of life.

As has been said, this concept is valid probably for the major part of the human population (Figure 2). If we add together common birth defects, their micromanifestations, and genetically determined diseases of adult life and their mild forms, only quite a small number of individuals remain.

Even the extreme situations are never "pure." On one side, only genetically determined conditions such as monogenic anomalies (e.g., most types of syndactyly) or chromosomal aberrations (e.g., trisomy 21) are modified in their phenotypic expression by either environmental factors or polygenic background (or other intergenic interactions). This extreme situation could be documented by the pedigree of autosomal dominant Treacher Collins syndrome (Figure 3), in which clinically unaffected individuals had offspring with typical manifestation of this syndrome. I would think that in these situations, which we call incomplete

Figure 5. Model of the Multifactorial Etiology Consisting of Genetic Determination and Genetically Determined Susceptibility to Environmental Factors in Mother and Fetus (In this situation, mother is sensitive and fetus is resistant to the potential teratogens.)

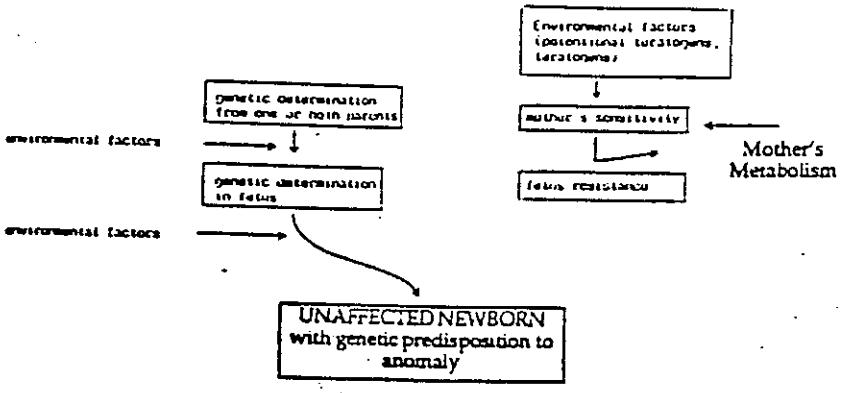
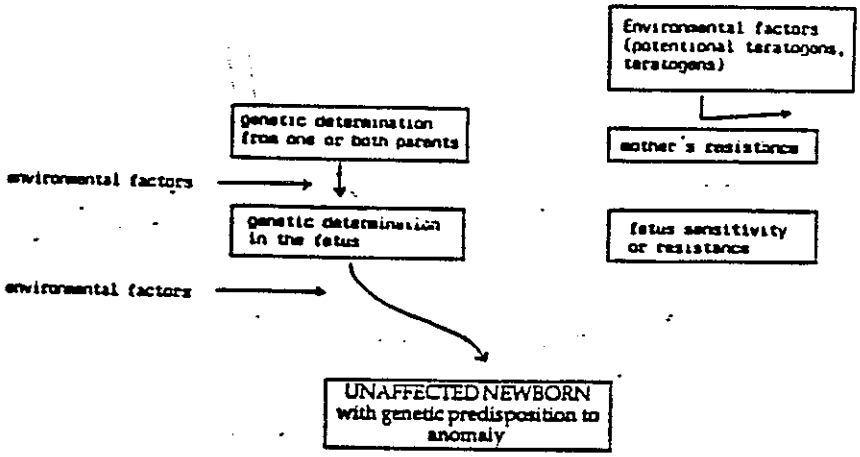


Figure 6. Model of the Multifactorial Etiology Consisting of Genetic Determination and Genetically Determined Susceptibility to Environmental Factors in Mother and Fetus (In this situation, mother is resistant and fetus is sensitive to the potential teratogens.)



penetrance and incomplete or varied expression in genetic terminology, we should take into account either environmental or genetic background influences acting in the periconceptional and early embryonic periods.

The opposite situation occurs in the typically environmental syndromes, like congenital rubella embryopathy and fetopathy. Here, the genetic background determines susceptibility to the noxious agent—the extent to which the embryo or fetus will be damaged, if at all. We know that not all mothers infected with rubella even, during the most critical period of their pregnancy, give birth to an affected baby. Also included in this classification are the so-called "predispositions" to different diseases, in which a pathogenic agent is well known, but whose clinical course and healing is modified by a genetic background.

To sum up, it is clear that to understand the principles of etiology of any malformation or disease, we should search for both intrinsic and extrinsic mechanisms, as well as for factors that could modify their balance. Some of these mechanisms could be demonstrated through differences of genetically determined susceptibility, either resistance or sensitivity, to environmental factors (potential teratogens) in mother and embryo (or fetus). If genetically determined sensitivity exists in both mother and fetus, the exogenous factor can influence development (Figures 4–6). It seems obvious that understanding interactions between intrinsic and extrinsic factors is a necessary base for assessing risk factors. If the risk factors are known, then the most appropriate strategy and tactics of prevention can be chosen.

For this largest subgroup of anomalies, in which both genetic and environmental factors participate in etiology, the orofacial clefts are still one of the best models for study. These malformations are heterogeneous in etiology. They could be caused by a wide scale of interactions between genetic and environmental factors (as is shown in Figure 2). A diagram representing three main etiological groups of clefts is shown in Figure 7. On the left side is situated a subgroup of clefts in which a genetic determination prevails. They are represented mainly by monogenic syndromes (in which cleft lip, cleft lip and palate, or cleft palate the more or less constant features), or by chromosomal syndromes. The right subgroup is represented by cases in which disturbance of normal genetically predetermined development of the embryo is caused by teratogens or other environmental factors (teratogens). This subgroup includes both single cleft malformations (mainly cleft lip) and multiple malformations, including clefts. In the majority of cases, the etiology is multifactorial—i.e., both genetic and nongenetic (exogenous) factors participate (the middle part of the illustration). A mechanism of development of the cleft malformation (Figure 8) could be derived from basic principles shown in Figures 4–6.

Before I describe in detail the results of our analysis of cleft lip and palate families, which support a multifactorial threshold model of inheritance at least for the majority of cases, and on which a method of primary prevention of orofacial clefts has been based, let me cite several authors whose work in genetics of cleft lip and palate represents important steps to a better understanding of the etiology of these anomalies.

> ... further progress will probably depend less on the elaboration of better mathematical approaches than on identifying the biological attributes of the predisposing factors.[2]

> ... Thus, the etiology of cleft lip and/or cleft palate remains unclear at present. To be sure, our analyses do not rule out all possible multifactorial models; perhaps other models and other more discriminatory analytic methodologies would be more illuminating.[3]

> ... I do not think the MF/T (=multifactorial/threshold) model should be forsaken just yet. To expect human data to fit precisely the expectation of

Figure 7. Three Major Groups of Factors in Etiology of Orofacial Clefts

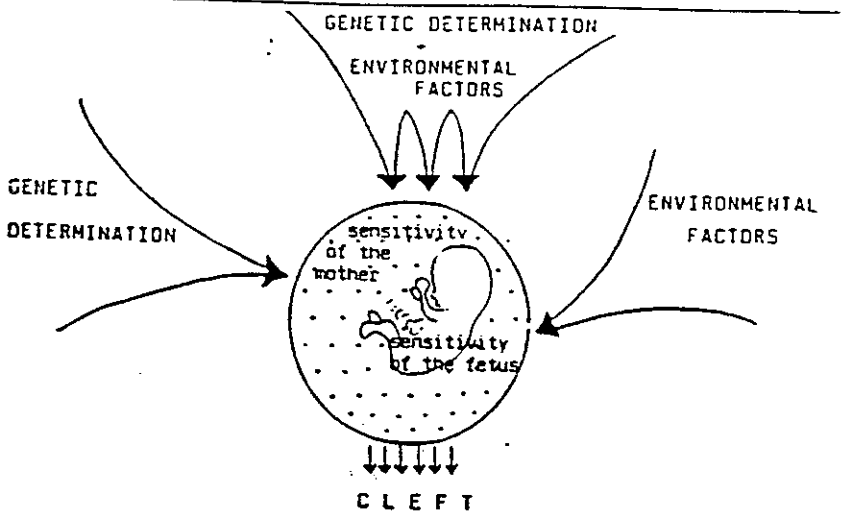


Figure 8. Possible Combinations and Results of Interactions Between Genetic Determination and Susceptibility to Environmental Factors in Etiology of Orofacial Clefts

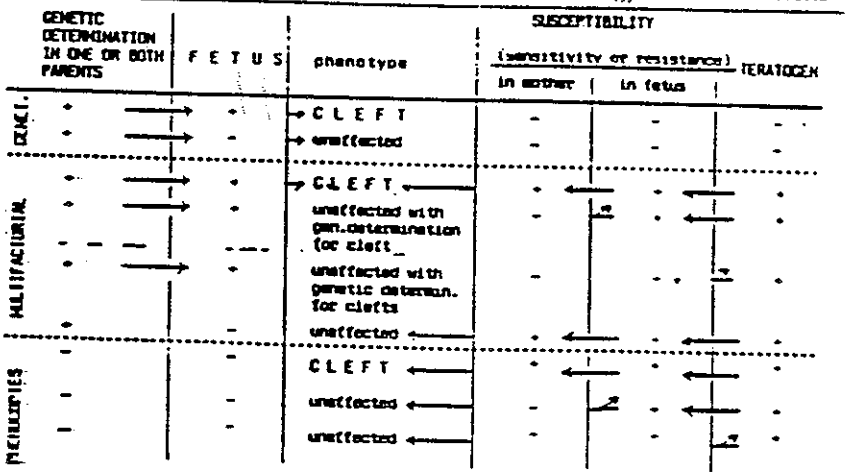


Table 1. Sample of 8,952 Probands with Orofacial Clefts Born between 1886-1986 in Bohemia by Type of Cleft and Period of Birth

	Year	Male	Female	Total	Cleft in Multiple* Malformations	Cleft in Syndromes	Total
CL	1886-1963	748	523	1271	**	**	1271
	1964-1986	589	371	960	17	5	982
	Total	1337	894	2231	17	5	2253
CLP	1886-1963	1258	586	1844	**	**	1844
	1964-1986	1095	552	1647	126	22	1795
	Total	2353	1138	3491	126	22	3639
CP	1886-1963	632	843	1475	**	**	1475
	1964-1986	547	758	1305	90	190	1585
	Total	1179	1601	2780	90	190	3060
Total		4869	3633	8502	233	217	8952

*Multiple malformations but no recognized syndromes.
**Not registered.

polygenic (in the strict sense) inheritance is unrealistic, since polygenes are a simplifying assumption made to reduce the complexity of real life to statistically manageable terms. To expect a fit to an SML (=single major locus) model is also unrealistic in view of the complexity of development and the variety of ways clefts can arise... different susceptible families will have different susceptible factors... If such factors are eventually identified, the MF/T model will begin to merge into the SML model, but by that time SML will mean several major factors, plus or minus. In the meantime, we should continue our efforts, at the morphological level, to enrich our biological understanding of distributions and thresholds.[4]

... It should be mentioned now that even though these analyses have not supported the MF/T model, it is probable that the etiology of cleft lip with and without cleft palate and isolated cleft palate is actually multifactorial—more clearly stated, that more than one factor is responsible for clefting, especially in isolated cases, for which there is some evidence of environmental causation.[5]

... The most economical hypothesis to explain the findings is the multifactorial threshold model. The birth frequency of the malformation and the family patterns found make it improbable that one single mutant gene makes a major contribution to the liability to develop the condition.[6]

Analysis of Clefts

Since 1965, we have collected 8,952 pedigrees of probands affected with orofacial clefts. The sample consists of 8,502 nonsyndromic cases, from which 2,231 are probands with cleft lip (CL) only, 3,491 are probands with cleft lip and palate (CLP), and 2,780 are probands with isolated cleft palate (CP) (Table 1). Dates of birth of our probands extend over one century: our oldest proband was born in 1886 and our youngest one in 1986.

One aspect of the study closely related to the quality of the data should be emphasized. Since 1965, all probands have been seen by me and my coworkers in our Clinical Genetics Laboratory. I can now see the second generation, children of our patients from the 1960s, or even the third generation, grandchildren of our oldest patients. We were also able to see personally the majority of the first-degree relatives of our probands. We looked carefully for minor manifestations, i.e.,

Table 2. The Incidence of Nonsyndromic Orofacial Clefts in Czech Population Born during 1964-86 (The total # of live births was 2,153,221.)

Cleft Type	Total	Incidence per 1,000	Proportion
CL	960	0.4458±0.0572	1:2243
CLP	1647	0.7607±0.0878	1:1307
CL=CLP	2607	1.2104±0.1094	1:826
CP	1305	0.6038±0.0787	1:1650

Figure 9. Incidence of Orofacial Clefts from 1964 to 1986 in Bohemia (Czechoslovakia)

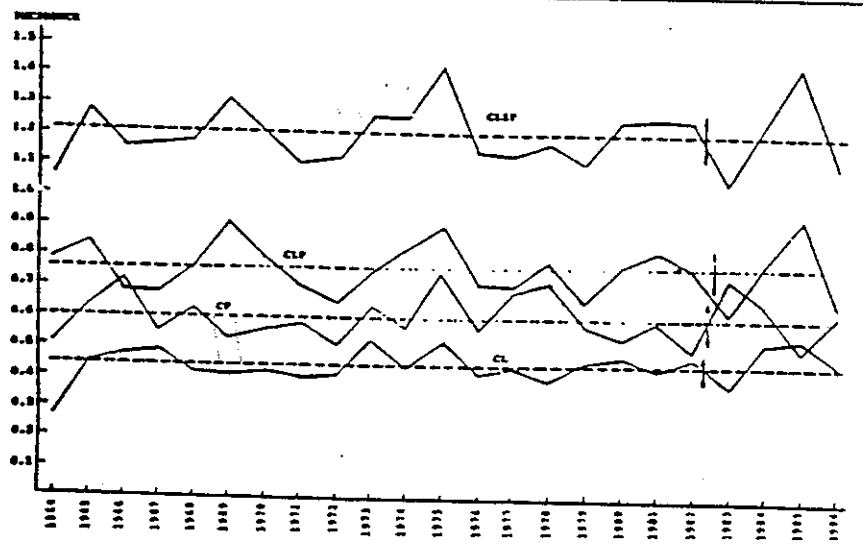
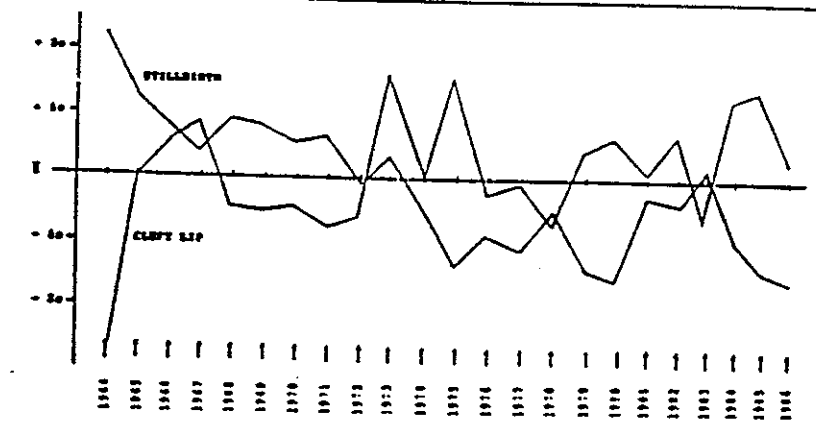


Figure 10. Reciprocity in the Incidence of Cleft Lip and in the Incidence of Stillbirth during the 23-year Period (1964-1986)



microforms. We examined all our probands and looked for associated malformations, and abnormalities of syndromes. This fact is crucial for both quality and completeness of the data, as well as for validity of our results. Many genetic and epidemiologic analyses of cleft data have been reported.[3,6-24] Unfortunately, a weak point of some of them is incomplete basic data.

Our sample was evaluated with respect to incidence, sex ratio, severity and laterality of cleft, birth order, age of parents, seasonal incidence, associated malformations, twin analysis, proband's birth weight and length, proportion of familial and solitary cases, heritability, empiric risk figures, genetic counseling, syndromes and multiple malformations associated with the clefts, atypical clefts, type of inheritance, and effectiveness of primary prevention. There is not enough space to comment on all of the results obtained by analysis of our sample. Some of them were presented previously.[25,26] An analysis of others is still in progress and will be published soon; however, let me point out two of the most important results, which could influence further research in this field: (1) a hypothesis for a four-threshold model of liability to orofacial clefts, and (2) a hypothesis for and results from primary prevention of the cleft lip and palate. For both hypotheses, the crucial results were obtained from an analysis of incidence, sex ratio, severity of the clefts, and from a genealogical study.

Incidence. One of the most important basic characteristics for any kind of genetic and epidemiologic analysis is a precise population incidence. The incidence of orofacial clefts has been calculated from the most valuable part of our sample, which is represented by 4,362 probands born between 1964 and 1986 in Bohemia (Table 1). We registered and saw all children born with an orofacial cleft from 1964 until 1983, as well as the majority since 1983. Thus, we have almost a complete register.

Before calculation of the incidence, all syndromes and multiple malformations were excluded. Then, the incidence 0.4458 per 1,000 (1:2,243) for CL, 0.7607 (1:1,307) for CLP, and 0.6038 (1:1,650) for CP was found. For cleft lip with or without cleft palate (CL±P or CL+CLP) the incidence was 1.2104 (1:826) (Table 2).

Let me stress that for a precise estimate of incidence in a population, it is necessary to collect data from the same region for several years because the incidence could vary, probably due to differences in environmental factors. In our 23-year study, significant differences in some single year values of incidence have been found when these values were compared to the average value of the whole period (Figure 9).

Interesting results have been obtained when the relationship between stillbirth and incidence of each type of cleft has been evaluated. The results suggested that, in certain time periods, reciprocal

Figure 11. Sex Ratio Values in Orofacial Clefts during the 23-year Period (1964-1989)

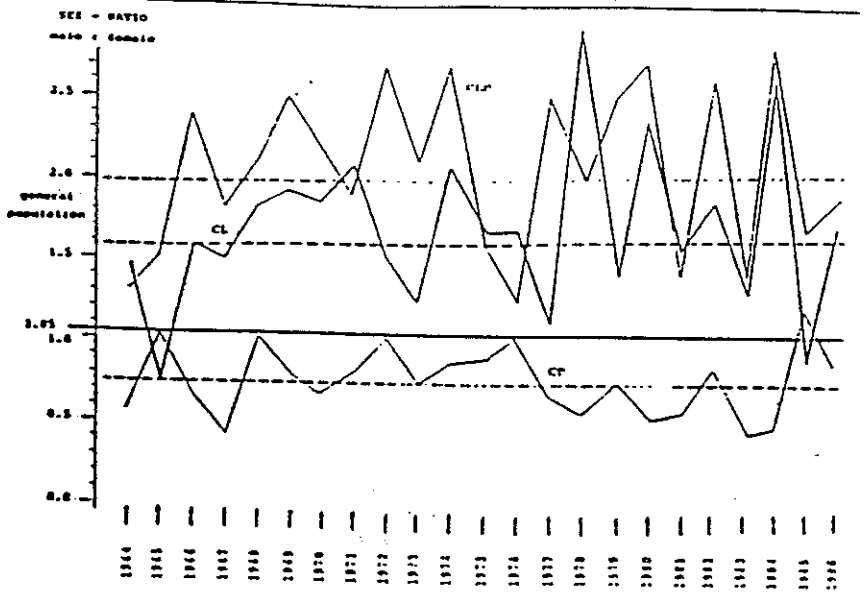


Table 3. Sex Ratios for Orofacial Clefts

	CL	CLP	CP
1886-1986	1.50	2.07	0.74
1964-1986	1.59	1.98	0.72
Isolated cases	1.68	2.07	0.74
Cleft (C) + 1 associated anomaly	0.93	1.53	0.67
C isolated and C + 1 associated anomaly	1.61	2.01	0.73
C + 2 or more associated anomalies	0.88	1.53	1.00
C in syndromes	1.00	0.54	0.81
C + 1 or more associated anomalies	1.91	1.53	0.76
C right	1.71	2.26	
C left	1.71	1.91	
C bilateral	1.00	2.14	

relations between the incidence of CL and stillbirth frequency might exist—i.e., a decreasing CL incidence occurred together with increasing stillbirth frequency. It was assumed that environmental factors in a certain combination or dose might cause one type of a birth defect (e.g., cleft lip) and in a different combination or in a lower or higher dose, another type of abnormality, which led to the death of a fetus before birth (Figure 10).

A higher incidence of CL and a lower incidence of CLP occurring in some periods (see, for example the period 1966-1970 in Figure 9) could be explained also by differences in combinations of environmental factors involved. The same explanations were also suggested in the previous literature.[27,28] When a hypothesis of independent fluctuations near a mean value was tested, no relation between incidence of the individual cleft types was proved.

However, if environmental factors are considered as a cause of differences between yearly incidences, it has to be pointed out that even such a small geographic region as Bohemia certainly includes smaller regions (districts) in which different sets of environmental factors may cause a variety of incidence values. Either an extreme local change in the incidence could influence the value for the whole region, or the environmental factor may be effective in the whole region (e.g., viral epidemic). In this way, the extremely high 1975 incidences of all types of clefts, but especially of CL, may be explained.

It has been found in our study that CL is the most variable type of cleft with respect to its incidence. This is in agreement with findings in other populations.[27,29-31] Therefore, in our opinion, CL seems to be the cleft type most sensitive to environmental factors.

The incidences of CL, CLP, and CP were compared and some interesting features were revealed. Districts with significantly (more than two standard deviations) higher incidence of CP did not have significantly different values of the incidence of CL or CLP. However, districts with a significantly lower incidence of CP had significantly higher incidence of CL. This can be explained by a finding of Leck,[29] who observed an increasing CL incidence and a decreasing incidence of spontaneous abortions following an influenza epidemic. It could also be considered that a more or less permanent environmental factor, probably geofactor, exists in this region. It may act as teratogen causing CL and CP. It may either increase resistance of CL embryos to spontaneous abortions, or decrease resistance to spontaneous abortions in CP embryos. These findings formed a base for further detailed analysis of these regions in respect to the incidence of clefts and also in respect to the incidence of other birth defects, and the incidence of spontaneous abortions.

The phenotypes of individuals with orofacial clefts were analyzed in respect to sex ratio, laterality, and severity of the CL and CLP, associated malformations, birth weight and length, occurrence of microforms, and season of birth.

Sex Ratio. The sex ratio is one of the main characteristics of a cleft population. It is well known that significant differences in the incidence of males and females are found both for CL±P and CP. Although a predominance of males over females is found in CL and CLP, the opposite situation, i.e., a significantly higher incidence of females compared to males, is found in CP. In our study, the following incidence rates were found: 0.5337/1,000 in males and 0.3534 in females with CL, 0.9923 in males and 0.5259 in females with CLP, 0.4957 in males and 0.7221 in females with CP.

The sex ratios 1.59 for CL, 1.98 for CLP, and 0.72 for CP were found for nonsyndromic cases in a subsample of cases born between 1964 and 1986. For the whole sample (1886-1986), sex ratios did

Figure 12. Comparison of Mean Roentgencephalograms in Fathers of Children with Isolated Cleft Palate and a Control Group of Males (Fathers—solid line)

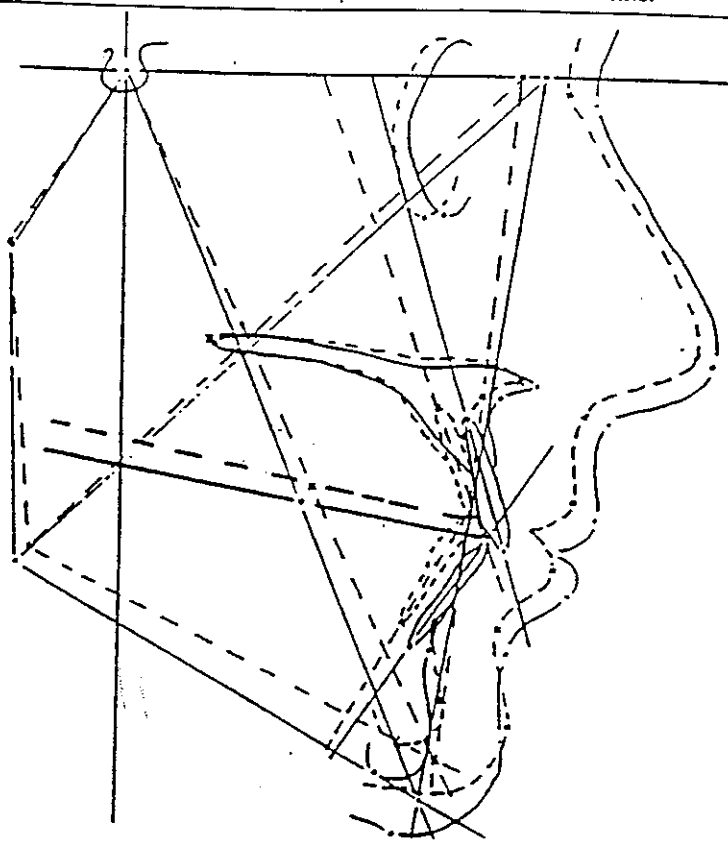
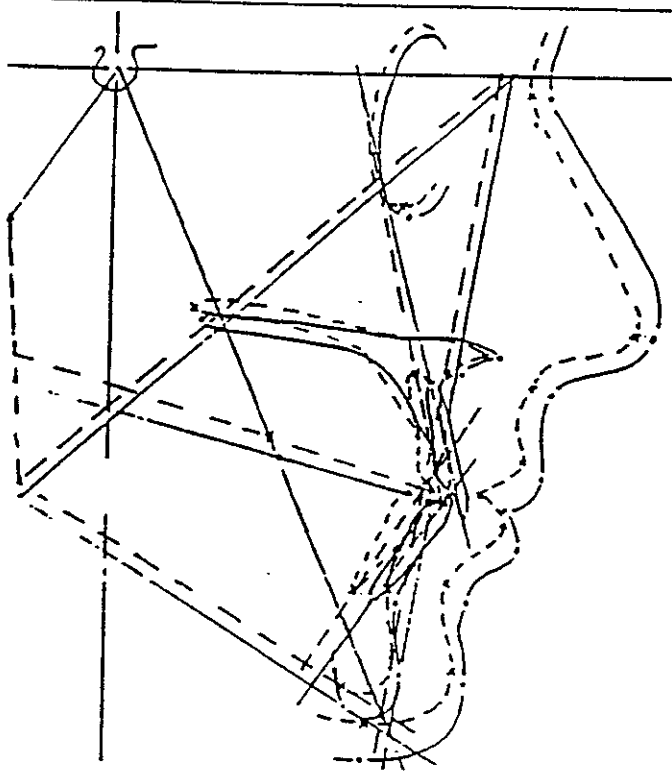


Figure 13. Comparison of Mean Roentgencephalograms in Mothers of Children with Isolated Cleft Palate and a Control Group of Females (Mothers—solid line)



not differ from those found in the complete register: 1.50 (2,231 individuals for CL), 2.07 (3,491 individuals) for CLP and 0.74 (2,780 individuals) for CP.

Comparisons of yearly sex ratio values during the whole 23-year period confirmed that sex-ratio was a very sensitive parameter (Figure 11) that could be influenced by a variety of factors. It was clearly shown by results of our analysis that only a sample that is large enough (e.g., coming from a large geographic area over a long time period) could provide a precise overall value of the sex ratio. On the other hand, it should be pointed out that these average values conceal all yearly variations of the sex ratio values, which probably correspond to an altered spectrum of environmental factors.

The largest variations in sex ratio values were found again for CL, from a low of 0.73 in 1965 to a high of 2.91 in 1978. The values of sex ratio also varied for CP, but the differences were not so extreme. In 1965, 1973, and 1975, the values of sex ratio corresponded to the average. The most stable values were found for CLP. Significant differences existed between isolated cases and those that were associated with two or more other malformations, especially for CL and CP (counted in the 1964-1986 subsample of the complete register; see Table 3).

Laterality. The higher prevalence of left-sided clefting is the usual finding for CL as well as for CLP. The left side is affected twice as often as the right one. The same was observed in experimental animals. It was suggested as a possible explanation that major blood vessels supplying the right side of the head of the fetus leave the aortic arch closer to the heart and more in line with blood flow than those going to the left side.[32]

Table 4. Twins with Orofacial Clefts—Czechoslovakia (Bohemia Region): Year of Birth, 1918-82. Incidence of Twins in Subjects with Cleft is 1 in 67.7 (In the general population, it is 1 in 80 to 1 in 90.)

Type	# of Pairs	%
Discordant for cleft	64	73.6
Concordant for cleft	18	20.7
Syndromic clefts	2	2.3
Atypical clefts	3	3.4
Total	87	100.0

Table 5. Twins with Orofacial Clefts: Sex and Type of Cleft

Sex of Twins		Type of Cleft				
w/ Cleft	w/o Cleft	CL	CLP	CP	Atypical	Total
Male	Male	6	10	7	—	23
Female	Female	4	6	7	3	20
Male	Female	4	4	2	—	10
Female	Male	3	6	3	—	12
Total		17	26	19	3	65

Table 6. Twins with Orofacial Clefts: Zygosity

Zygosity	Number	%
MZ	16	19.0
DZ	40	47.6
Unknown	28	33.4
Total	84	100.0

Our analysis of laterality has been conducted from several points of view. Prevalence of left unilateral cases in ratio 1.3:1 (left to right) in CL and CLP was a very consistent finding, changing neither with the severity of the cleft, nor with the sex of the proband. The sex ratio (male/female) in individual subgroups divided according to laterality (Table 3) was highest for CLP-right side (2.26), lower for bilateral CLP (2.14), CLP-left side (1.91), and CL-left or CL-right (1.71). In a rather small group of bilateral CL subjects (although it was the largest one in the literature), the sex ratio was 1.00 (same number of males and females).

The ratio of unilateral to bilateral cases was almost the same in both the whole sample of 8,952 cases (born between 1886-1986) and the subsample of the complete register from Bohemia (4,362 cases born between 1964-1986). The ratio for CL was 11.1 and the ratio for CLP was 2.5. In the literature, this ratio of unilateral to bilateral cases varies and is probably also influenced by race—e.g., in blacks the bilateral form is described more frequently than in white populations.[17]

Severity. It is important to know for further analysis that the expression of uni- or bilaterality of the cleft is a significant indicator of degree of severity. This is much more significant and specific for evaluation of the degree of severity than occurrence of associated cleft of the secondary palate, i.e., the CL versus the CLP.

The ratio CL:CLP in different populations is probably influenced by a variety of different factors, even when we have to consider that the less severe forms of CL could be underrepresented in some studies. In our sample, the ratio of CL:CLP was 0.64 and 0.59 in the subsample of the complete register. Racial factors apparently also play a role—e.g., the highest proportion of CL was found

Table 7. Twins with Orofacial Clefts: Sex and Severity of Clefting

	Sex of Twins			
Type of Cleft	Male-Male	Female-Female	Male-Female	Total
CL and CL	—	1	—	1
CL and CLP	3	—	1	4
CLPuni and CLPuni	1	4	—	5
CLPuni and CLPbi	4	—	—	4
CLPbi and CLPbi	1	1	2	4
Total	9	6	3	18

Table 8. Proportion of Affected and Unaffected Individuals in Children of 578 Probands with CL±P and CP

Proband		Proportion of Aff:Total (empiric risk figures)		
Type of Cleft	Sex	in Sons	in Daughters	Total
CL±P unilat	Male	11:224	5:220	16:440
		4.91±1.48	2.27±1.02	3.60±0.9
	Female	6:132	4:131	10:236
		4.55±1.86	3.05±1.53	3.80±1.2
CL±P bilat	Male	6:52	2:41	8:93
		11.54±4.71	4.88±2.83	8.60±3.04
	Female	5:29	2:26	7:55
		17.24±7.71	7.69±5.54	12.73±4.54
Total CL±P		28:437	13:418	41:855
		6.41±1.21	3.11±0.86	4.80±0.75
CP	Male	2:68	6:70	8:138
		2.94±2.08	8.57±3.59	5.80±2.05
	Female	1:126	3:105	4:231
		0.79±0.79	2.86±1.65	1.73±0.87
Total		3:194	9:175	12:669
		1.55±0.89	5.14±1.71	3.25±0.94

Figure 14. Strong Influence of Sex of the Parent and Sex of the Affected Offspring on Empiric Risk Figures

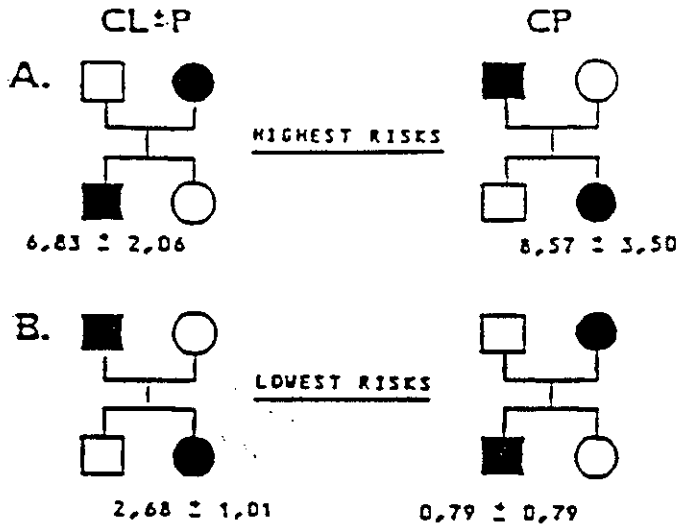


Figure 15. Two Threshold Model of Liability to Orofacial Clefts Taking into Account Sex of Proband

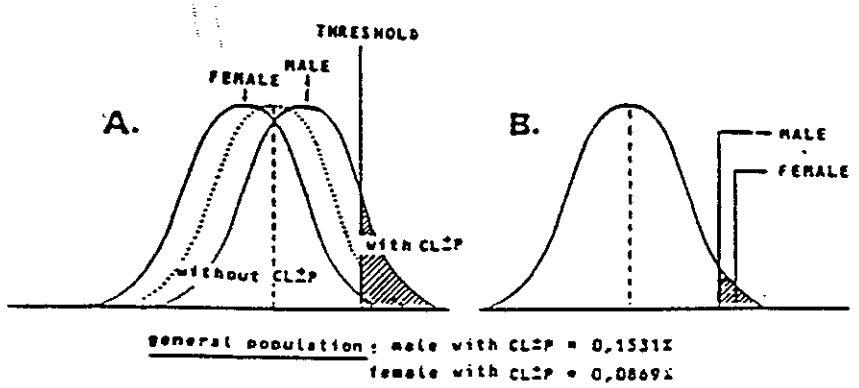
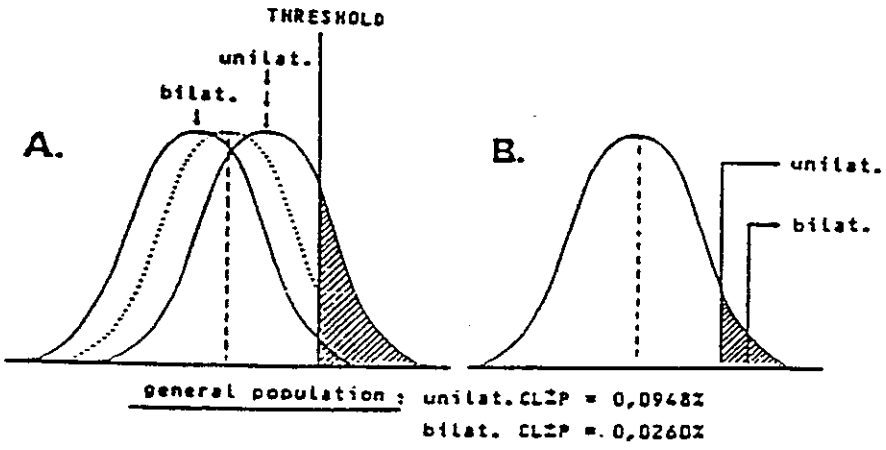


Figure 16. Two Threshold Model of Liability to Orofacial Clefts Taking into Account Severity of the Affection



in Japanese studies. There could ex etiologically different types of CL (tl would be supported by an experimen study of Trasler and Fraser[33]), or the d ferences may be connected with differ morphological shapes of the face det mined by racial factors.[11]

Morphological features of the hum skull and face could include such char teristics that are "predisposing" or se as "markers" (microforms) for orofa clefts in their offspring. This has been s gested also for CP.[35,36] In 1980, Fras suggested a hypothesis that a face sh could be related to manifestation of orofacial cleft.

A study of craniofacial morphology the parents of children with isolated c palate was conducted in our institut The aim of that study was to try to f possible markers of genetic predisp tion to cleft palate. The significant ferences in craniofacial morphol between the parents of cleft pa children and general population may nal the presence of a part of the polyg system for this defect, i.e., a poss predisposition.

Altogether, 53 fathers and 52 mot of children affected with cleft palate v examined. A control sample was for by university students from Prague Bratislava, 35 males and 40 fem Anthropometry of head and face, r gencephalometry (Figures 12 and 13). analysis of dental casts were perfor In these examinations, 94 param were analyzed. In the sample of par Angle class II dental relations were f more often compared to controls. brachycephalic type of head and the toprosop type of face were more com in both fathers and mothers. Statisti significant differences in certain p meters were found: the head was sh and wider in the parent sample: the i ocular distance was greater; and was increased height of the lower particularly the mandible. Also length of the anterior cranial base increased. The analysis of dental showed statistically significant diff ces only in the width of the palate.

It could be concluded on the ba these results that some morphol features in skeletal and soft tissues head and face may bear close relatio to the predisposition for isolated cle ate in humans. For this reason, hypo cal threshold values for the "pre palate type" of head and face were gested. Examination of craniofacial

To

phology using eight anthropometric and 14 cephalometric parameters was indicated. When five of eight anthropometric parameters and ten of 14 roentgencephalometric parameters were above the threshold, the unaffected individual could be considered to express a part of the polygenic background for isolated cleft palate. From the clinical point of view, it could mean a higher risk for transferring a genetic predisposition to the offspring and a higher risk of developing cleft palate.

Genealogical analysis. To evaluate the participation of genetic factors in the etiology of orofacial clefts, an evaluation of 87 cleft twin pairs (Tables 4-7) were carried out. These evaluations are contributing to the development of a model of liability to cleft lip with or without cleft palate.

The incidence of clefts among 21,147 relatives of the first, the second and the third degree was determined for two subsamples. The first one was formed by 789 probands born between 1970-1974. An assumption of closed sibship (very low chance of further pregnancy of the mother) served as a criterion for inclusion in this sample. In this group, the proportions of affected individuals in siblings, parents and second- and third-degree relatives were evaluated. The second sample was formed by our older 837 probands. The proportions of affected individuals among children, grandchildren, and siblings were evaluated.

From the results of this part of the analysis, empiric risk figures suitable for many kinds of counseling situations in respect to type of cleft, sex of proband, degree of relationship to proband with cleft, etc., were obtained (Table 8). In CL±P, a higher proportion of affected individuals among the first degree relatives was found for bilateral clefts (10.14±2.62 percent) standard error, compared to the unilateral clefts (3.68±0.72 percent). Important results were obtained when combinations of sex and clefts of parents and their children were studied. The risk was found to be higher for children of affected mothers (5.35±1.30 percent) than for children of affected fathers (4.47±0.91 percent). The risk for sons of affected mothers was highest (6.83±2.06 percent) and lowest (2.68±1.01 percent) for daughters of affected fathers. The reverse situation regarding sex was found for CP. The highest risk (8.57±3.50 percent) was obtained for daughters of fathers with cleft palate and lowest (0.79±0.79 percent) for sons of mothers with CP (Figure 14).

Figure 17. Four Threshold Model of Liability to Orofacial Clefts Taking into Account Sex of Proband and Severity of Affection

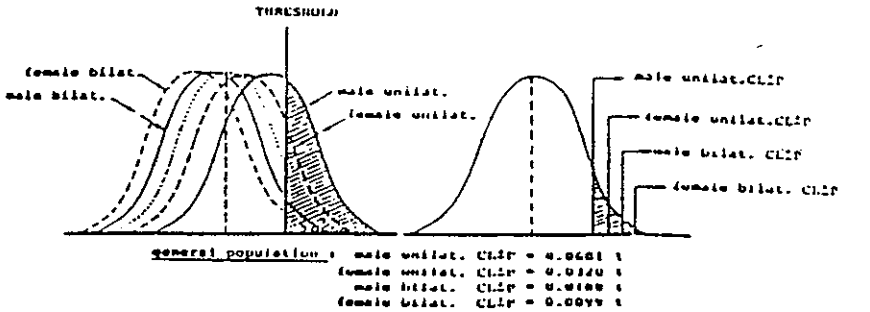
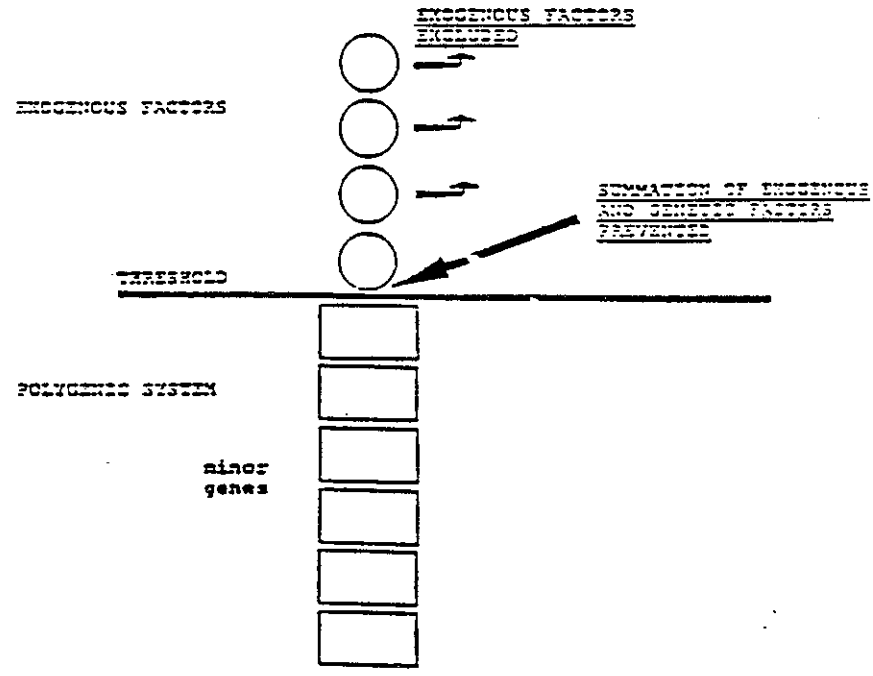


Table 9. Main Characteristics of Our Subgroups of CL±P

	Unilateral		Bilateral	
	Male	Female	Male	Female
Incidence in general population	0.0601	0.0320	0.0188	0.0099
Risk in siblings or children (%)	2.85	4.32	6.57	8.89
Heritability	0.6385	0.7682	0.8749	0.8779
Effectiveness of primary prevention*	−2.76	−2.09	−1.18	−0.04

*The difference between number of expected and observed cases in treated group.

Figure 18. Hypothetical Mechanism of Primary Prevention of Orofacial Clefts Using Periconceptional Supplementation by Vitamins and Folic Acid



The fact that risk figures were higher in subgroups that were more rare in the population suggests a higher proportion of genetic factors. It was confirmed by an estimation of heritability. For the first-degree relatives of CL±P subjects, the

Figure 19. Ten-step Schedule of Primary Prevention of Orofacial Clefts and Dosage of Vitamins, Which Has Been Used

1. GENETIC EXAMINATION
2. estimation of the RISK FIGURE
3. MEDICAL EXAMINATION of the parents
4. GYNECOLOGICAL EXAMINATION of the mother
5. TREATMENT of patnological findings
6. suggestion of CONCEPTIONAL TIME
7. optimalization of LIVING CONDITIONS of parents before conception
8. VITAMIN AND FOLIC ACID supplementation of the mother minimaly two month before conception and lasting at least the first trimester of pregnancy
9. CONTINUAL CONTROL of pregnant mother
10. examination of the NEWBORN CHILD

"S P O F A V I T"

Vitamin A	2000 IU
Vitamin B_1	1 mg
Vitamin B_2	1 mg
Vitamin B_6	1 mg
Vitamin C	50 mg
Vitamin D_3	1000 IU
Nicotinamid(PP)	10 mg
Calcium panth.	1 mg

) this tablets daily

FOLIC ACID 10 mg daily

Table 10. Structure of Supplemented Group

Relatives with Cleft			Outcomes of Pregnancies (Newborns with Clefts in Parentheses)			
Siblings	CL	Unilateral	Male	16	34	35
			Female	18		
		Bilateral	Male	1	1	
			Female			
	CL±P	Unilateral	Male	55	68	94 (1)
			Female	13		
		Bilateral	Male	16	26 (1)	
			Female	10 (1)		
Parents	CL	Unilateral	Male	6	22	24
			Female	16		
		Bilateral	Male	2	2	
			Female			
	CL±P	Unilateral	Male	19	40 (1)	85 (2)
			Female	21 (1)		
		Bilateral	Male	15 (1)	21 (1)	61 (2)
			Female	6		
Two or more 1st-degree relatives						214 (3)
Other relatives						9 (2)
Total					275 (5)	52

heritability was 0.7302±0.0242. It was a little higher for children (0.8267±0.0442) than that for siblings (0.7302±0.0306). A higher value for heritability was found for bilateral cases compared to unilateral cases, and a higher heritability for females than for males. For CP subjects, the heritability for the first-degree relatives was 0.7482±0.0376. It was higher for children (0.7990±0.0726) than for siblings (0.6872±0.0606). We used the classical method of Falconer[37] as modified by Czeizel and Tusnady.[9]

Multifactorial Model of Liability With Four Different Thresholds

The results presented above revealed significant differences among the four basic subgroups. These findings led us to suggest a multifactorial model for liability with four different thresholds related to sex of the proband and severity of the anomaly.[23,26]

According to Carter[6,8] although opposed by Melnick et al.[3,20] who reanalyzed the classical Danish data, the hypothesis best explaining etiology of the majority of CL±P cases is the multifactorial threshold model. The liability is dependent on the sum of genetic and nongenetic (environmental) factors and is represented by a curve of normal distribution and a threshold, beyond which individuals are affected with CL±P.

Different values for population incidences in males and females subdivide the population above the threshold into two subgroups (Figure 15). A similar situation is found when a division is made with respect to severity of clefts, bilateral clefts are more severe and have higher heritability than unilateral ones (Figure 16). Therefore, the risk of transmitting the cleft to offspring is higher for females and bilateral cases than for males and unilateral clefts. Combination of both characteristics results in the four-threshold model of liability (Figure 17) with different thresholds for individual subgroups: (1) male with unilateral CL±P, (2) female with unilateral CL±P, (3) male with bilateral CL±P, (4) female with bilateral CL±P.

The main characteristics distinguishing these four subgroups are presented in Table 9. From the first (male with unilateral CL±P) to the fourth group (female with bilateral CL±P), the incidence in general population decreases and the risk of recurrence and the value

of heritability increase. Also, the results of our method of primary prevention of orofacial clefts (below) seem to support our hypothesis of the four-threshold model of liability.

Prevention

The problem of prevention of congenital malformations seems to be one of the most important branches of contemporary genetics. Although the scan technique (ultrasound) enables us to recognize individual structures in the craniofacial region of the fetus in utero, and although this method is developing rapidly, at present the only method of prenatal diagnosis by which the orofacial clefts can be diagnosed is visualization fetoscopy. We recommend this procedure for high-risk families with clefts (when the risk is above 10 percent), as well as for autosomal dominant and autosomal recessive syndromes of morphological malformations. This preventive method (terminating pregnancy) should be called a secondary prevention and is dependent on prenatal diagnosis.

More eificient, because it could affect a larger group of individuals at risk, seems to be primary prevention, which acts on the embryo before cleft development. The possibility of influencing the genetic background purposefully is far in the future. The only recent possibility of prevention is to prevent summation of genetic and environmental factors, or to exclude at least some of the environmental factors (see Figure 18).

It has been known for quite a long time that a connection exists between the composition of a mother's diet in pregnancy and the health status of her offspring. An association between nutritional deficiency in pregnant animals and birth defects in their offspring was probably first recorded in 1940 by Warkany and Nelson[38] and by Warkany and Schraffenberger in 1943.[31] Since that time, several attempts at dietary prevention of birth defects in humans have been carried out. Vitamins, particularly folic acid, were used to prevent orofacial clefts by Peer and coworkers,[39] later also by Douglas,[40] Conway,[41] Briggs,[42] Gabka,[43] and von Kreybig,[44] and more recently in prevention of neural tube defects (NTD) by Lawrence[45] and Smithells.[46]

Our method of primary prevention corresponds to our hypothesis of different thresholds based on different proportions of genetic and environmental factors in four individual subgroups of clefts. It is based also on another hypothesis: that environmental triggers, which probably play an important role in etiology of clefts, may be eliminated or decreased by this means.

We have used periconceptional supplementation with the multivitamin preparation SPOFAVIT (three times daily) and folic acid (10 mg daily) (Figure 19). Our present results, based on evaluation of 275 pregnancies (Table 10) in which primary prevention was applied, have reconfirmed our positive results published in 1982.[47] The primary prevention was applied in different counseling situations with different risks of recurrence. But only a homogeneous group with the same counseling situation and risks of recurrence was used for statistical evaluation. When the sample was evaluated as a whole, the effectiveness of prevention was expressed as differences in the numbers of expected and the numbers of observed affected cases (Table 11). The expected number was derived from control pregnancies that preceded initiation of a prevention program.

Following subdivision of the whole sample with respect to sex of probands, better results for male probands com-

Table 11. Prevention of CL±P by Periconceptional Vitamins (particularly high folic acid supplementation): Results for the Whole Group

	Prevention with Cleft			Control with Cleft			Results		
Proband	Total	#	%	Total	#	%	Expec. Cases	Observ. Cases	Difference
CL±P	214	3	1.40±0.81	1,901	77	4.05±0.46	8.67	3	-5.67

Table 12. Prevention of CL±P by Periconceptional Vitamins (particularly high folic acid supplementation): Results for Subgroups of Males and Females Separately

	Prevention with Cleft			Control with Cleft			Results		
Proband	Total	#	%	Total	#	%	Expec. Cases	Observ. Cases	Difference
Male	130	1	1.77±0.77	1,191	42	3.53±0.54	4.58	1	-3.58
Female	84	2	2.38±1.68	710	35	4.93±0.83	4.14	2	-2.14

Table 13. Prevention of CL±P by Periconceptional Vitamins (particularly high folic acid supplementation): Results for Subgroups of Unilateral and Bilateral Cases Separately

	Prevention with Cleft			Control with Cleft			Results		
Proband	Total	#	%	Total	#	%	Expec. Cases	Observ. Cases	Difference
Unilateral	164	1	0.61±0.61	1,566	55	3.51±0.47	5.76	1	-4.76
Bilateral	50	2	4.00±2.83	335	22	6.57±1.40	3.29	2	-1.29

Table 13. Prevention of CL±P by Periconceptional Vitamins (particularly high folic acid supplementation): Results for 4 Subgroups Divided by Sex and Severity of Cleft

		Prevention with Cleft			Control with Cleft			Results		
Proband	Sex	Total	#	%	Total	#	%	Expec. Cases	Observ. Cases	Difference
Unilateral	Male	96			973	28	2.88±0.54	2.76		-2.76*
	Female	68	1	1.47±1.47	593	27	4.55±0.88	3.09	1	-2.09**
Bilateral	Male	34	1	2.94±2.94	218	14	6.42±1.72	2.18	1	-1.18†
	Female	16	1	6.25±6.25	117	8	6.48±2.42	1.04	1	+0.04
Total		214	3	1.40±0.81	1,901	77	4.05±0.46	8.167	3	-5.67‡
2 or more 1-degree relatives		9		22.22±15.71			Not evaluated statistically			
Other relatives		52	2				Not evaluated statistically			

*p=0.1006. Chi: p=0.05975.
**p=0.2897. Chi: p=0.1678.
†p=0.4361. Chi=p=0.2529.
‡p=0.0659. Chi: p=0.0514.

pared to female probands were found (Table 12). This was expected by our hypothesis dealing with different thresholds for males and females. In the case of the male subgroup, we assumed that the value of liability contained more environmental factors in comparison with the female subgroup. As is shown in Figure 15, the threshold for males is nearer to the population average than the threshold for females.

A similar situation was found when the effectiveness of prevention in the families with probands affected with unilateral or bilateral clefts was compared. Better results were obtained if the proband was affected with a unilateral cleft than if the proband was affected with a bilateral cleft (Table 13). If sex and severity were combined, the effectiveness of prevention could be evaluated in four subgroups with different thresholds of liability. In agreement with the four-threshold model, the best results were obtained in the most frequent subgroup, where the highest proportion of environmental factors occurs, i.e., in the subgroup of male probands with unilateral clefts (Table 14).

An interesting hypothesis was suggested by Nora in 1984 with respect to a molecular theory of this action.[48] A foreign chemical substance is subjected to a sequence of changes inside the cell. It binds to a receptor in cytoplasm and this complex moves to nucleus. There, a synthesis of oxidative enzymes is induced. Oxidation of the foreign chemical substance leads to formation of reactive intermediates that may act as mutagens or teratogens until they are conjugated and excreted from the cell. In humans, there exist two sorts of individuals: responders and nonresponders (capable or incapable of synthesizing oxidative enzymes in response to a foreign chemical). In relations of mother and fetus, four combinations are possible. The fetus can be damaged if both mother and fetus are responders. A similar hypothesis (see Figures 4–6) was tentatively suggested by us several years ago.[49] It is understandable that reactive intermediates and radicals are antagonized by antioxidants like vitamins C and E.

Still more data are required to conclusively confirm our hypothesis of primary prevention of orofacial clefts by multivitamin supplementation and folic acid.

Also, the population incidence cannot be decreased to a large amount by this method. However, if we consider the results of CL±P prevention by this method, the results of British studies on prevention of neural tube defects and experimental studies and human data in orofacial clefts, it seems to be a promising method, through which the frequency of cleft lip and palate can be decreased in families at risk, families where already either one of the parents suffered from cleft or where there has already been a baby born with cleft.

Periconceptional supplementation b vitamin preparations with large amount of folic acid as a method of primar prevention of orofacial clefts has man attractive features. There is no danger o negative influence either on fetal develop ment or on mother's health during th pregnancy. The medication with multi vitamins and folic acid is inexpensive And, as with other environmental factor such as is avoidance of smoking, its ap plication can have only favorable affect on both the fetus and mother.

If we exclude social and psychologica points of view and compare only th direct financial expenses for multidis ciplinary treatment of child with an orofa cial cleft, then it is clear that the sam amount of money can be used more effec tively for preventive activity in severa hundreds of families.

As Bruce Ross mentioned in this meet ing, in families in which we were helpin to counterbalance severe psychologica stress and other problems caused by th fact that the cleft baby was born, we d not want to see another affected baby an we do not want to go through everythin again with that family. Each baby bor after prevention without presenting a cleft is our little victory. If in this kind o primary prevention there seems to be a way to help, it is the duty of all of us wh are involved in research and treatment o cleft children to take advantage of all pos

sibilities for prevention.

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Reactor Paper: Cleft Lip and Cleft Palate—Whence and Whither?

Clarke Fraser, O.C., Ph.D. M.D.

This has been an illuminating session illustrating many of the problems—and some progress toward their resolution—of estimating risks relating to the occurrence of facial clefts both in relatives of those affected and in the general population.

Dr. Tolarova's family study is unique both in its size and in the long period of her observation. The fact that occurrence in siblings varies with both sex and severity (bilaterality) is consistent with expectation for a quasicontinuous variant (threshold trait). It is nice to see these relationships, which have been doubted by certain others, demonstrated so clearly. The four-threshold model (which is really a four-distribution model, but it is more convenient to deal with thresholds) is a logical extension of these relationships. Segregation analysis at an earlier stage did not distinguish between a single gene and the multifactorial threshold model,[1] but it would be interesting to apply some of the more recent and sophisticated models of complex segregation analysis (discussed by Dr. Elston) to this unique body of data to see if evidence for the presence of one or more genes of "major" (meaning detectable) effect could be teased out.

The sibling-occurrence figures will also be useful for counseling, as the differences between the highest (sons of bilateral female probands—17±7.7 percent) and lowest (daughters of unilateral male probands—2±1 percent) are appreciable. It is curious that the figures for children and siblings are quite similar except for the offspring of bilateral CL±P category, which is considerably higher than the sibling value[2] or the estimate (for siblings) from previous studies.[3] One wonders if this excess may represent sampling bias, since it has a large standard error. Perhaps the figure for siblings[2] would be a more reliable guide for counseling. This illustrates one difficulty of risk assessment in this area—causal heterogeneity. Splitting the data into more subgroups to achieve greater homogeneity reduces the sample size of each subgroup, resulting in large standard error, even with a very large study group such as this one. To get large subgroups may require collaborative projects, but that runs the risk of increasing heterogeneity again. A dilemma!

The most interesting of Dr. Tolarova's findings is the preventive effect of periconceptional multivitamins. It must have taken great courage to start such a trial knowing how long it would take to get an answer. I was very skeptical of the early American reports of an effect on facial clefts,[4] but the evidence suggesting that there might be an effect on neural tube defects was more convincing, and Dr. Tolarova has a much bigger sample of cleft families than the American studies. The epidemiologists will no doubt object that it is not a randomized trial, as they did for the neural tube defect study of Smithell's et al. The comparison group here is presumably all the families in the study group that were not in the treated group, most of whom were seen before the vitamin study began. Since there is much less association with environmental factors for facial clefts than for neural tube defects, these figures are probably quite stable, and it is difficult to see how treated families could have been selected from a group that had a lower recurrence risk than the rest of the sample, although it would be nice to know exactly how they were selected. These results need to be taken seriously, and perhaps we should be asking ourselves whether we should be recommending periconceptional vitamins for subsequent pregnancies of mothers who have had a child with cleft lip—particularly if the results of the British MRC randomized trial on neural tube defects turns out to be positive.

If maternal nutrition does indeed influence liability to both neural tube defects and cleft lip (and perhaps other malformations), this would provide a possible explanation for the fact that neural tube defects occur more often than expected in the siblings of probands with cleft lip, and of probands with several other types of malformations[5] and perhaps vice versa.[6] If so, one might expect to see social class differences (reflecting nutritional differences) in frequency of cleft lip. There is some evidence in support of this[7] and it would be useful to pursue this line of investigation.

As for Dr. Erickson's presentation, I have nothing but admiration for the dedication and determination of those who run birth defect surveillance programs, and sympathy for the frustration they must endure in dealing with largely negative findings and the lack of funds to follow up the positive ones that look promising. It is a largely unappreciated and thankless task. Yet even the negative findings may be useful in that they can reassure when the (usually false) alarm is rung for some suspected teratogen. And, of course, they can confirm the ones where the cause for alarm is real.

Dr. Erickson's thoughts on risk management are cogent. The discovery of a teratogen certainly raises the question of whether the benefits of the environmental agent justify the increased risk to the unborn of being born malformed. From a prenatal counseling viewpoint, a relative risk of even as much as 10 would mean, for cleft lip, an absolute risk of 1 in 100, which those weighing the risk and benefits might consider low, although those actually exposed might disagree. One gets the feeling that the benefit side of the question may well include economic benefit to those who market the agent, and one may wonder if this is appropriate, and what can be done to prevent financial profit from overbalancing the scales.

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Finally, there are the exciting findings in Dr. Johnston's twins, demonstrating genetic heterogeneity for cleft lip. As some of us have been trying to do for years, he has finally teased out a biological measure of liability. The next step will presumably be to see if some of the unaffected relatives (particularly siblings) show the same differences as the co-twins of the probands. If, as Dr. Johnston suggests, the "wide" group corresponds to the autosomal recessive type that, according to Chung et al.,[8] may account for about one-third of cleft lips in the Danish population, the search for a genetic marker would be greatly simplified. Remember that this gene would have a penetrance of roughly 33 percent, so the risk of recurrence in these families would be about 8 percent, not dramatically higher than that of the 4 percent risk predicted on the multifactorial model. But if a linkage could be found—e.g., with the TGFA marker shown to be associated with cleft lip in some families[9]—then the 8 percent risk would become close to either 0 or 33 percent and prenatal diagnosis could be done. Then we will have to wrestle with the ethical problem of doing prenatal diagnosis (presumably with the intention of aborting the fetus if affected) for a nonlethal condition that can be repaired with remarkable success. The search for improved risk assessment leads us down some unexpected pathways.

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