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A recent aetiological study on facial clefting in Hungary

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A case-control epidemiological and family study was organized of 2024 index patients born between 1970 and 1976 affected by isolated cleft lip ± cleft palate, isolated posterior cleft palate and multiple congenital abnormalities including facial clefting and of their matched control cases. The specific rate of affected parents and sibs was 2.4% and 4.2% in the isolated cleft lip ± cleft palate sample, while 2.2% and 3.2% in the isolated posterior cleft palate group. The proportion of polygenic liability was about 77 ± 8% in isolated cleft lip±cleft palate cases. Among teratogens, the triggering impact of certain anticonvulsants was confirmed.

Previously we have published the data of epidemiological [2], genetic [3] and teratological [4, 5] studies on isolated cleft lip with or without cleft palate, and isolated posterior cleft palate of approximately all index patients born in Budapest, 1962–1967. These index patients were, however, analysed without matched control cases, therefore the environmental, maternal and obstetrical factors could not be evaluated adequately. This drawback, some new aetiological ideas and a concrete request have prompted us to conduct a new complex aetiological study (1977–1981) on Hungarian index patients with facial clefting born in 1970–1976. Some data of this second study were published elsewhere [8], here the aetiological factors will be discussed.

Materials and Methods

The material of the Hungarian Congenital Malformation Registry [5, 6] involved 2024 index patients with facial clefting born between 1970 and 1976. The completeness of notification was 92–98% in these types of congenital abnormalities (CAs). First, 8 groups of facial cleftings were separated as follows:

(i) Isolated cleft lip (CL) and cleft lip with cleft palate (CLP); the sum of these two subgroups CL(P) was N = 1086.

(ii) Specified CA-syndromes including CL(P) (N = 26).

(iii) Unspecified multiple CAs including CL(P) (N = 295).

(iv) Isolated posterior cleft palate (CP) (N = 365).

(v) Specified CA-syndromes including CP (N = 15).

(vi) Unspecified multiple CAs including CP (N = 143).

(vii) Robin sequence (N = 65).

(viii) Other facial cleftings (e.g., holoprosencephaly, median and oblique facial clefting) (N = 29).

The specified CA-syndromes and CA-entities with CL(P) and CP were trisomy 13 (Patau) 7, trisomy 18 (Edwards) 3, trisomy 21 (Down) 6, ADAM-sequence 6, Meckel-Gruber 3, fetal hydantoin 3, Apert 2, SCE 2, congenital rubella 1, OFD-I 1, thoracopagus conjoined twins 1 (2), Goldenhar 1, Marfan 1, diastrophic dysplasia 1, Larsen 1, popliteal web 1, Roberts 1. These CA-syndromes were not evaluated in this study. Owing to the low number of index patients with Robin sequence and the group entitled "Other facial cleftings" were also excluded. The distribution of CAs within unspecified multiple CAs including CL(P) and CP was published previously [8]. These two groups were combined under the abbreviation MCA. Eventually the aetiological factors were planned to be analysed in three groups of facial clefting: CL(P), CP and MCA involving 1,954 recorded cases.

A questionnaire was sent to the parents of all index patients studied. Lists of drugs and diseases were enclosed and parents were asked to have a look at them before filling in the questionnaire in order to standardize and refresh their memories. The prenatal logbook of pregnancies studied, furthermore photos and medical documentation of index patients and affected first degree relatives were requested from the parents.

A similar method was used in control cases matched with birth place, week of birth, sex and outcome (still- or livebirth, infant death) of index patients. Three matched control cases were ascertained in records of obstetrical institutions where index patients were born. As it appeared, 63 control cases also had CAs: liability for dislocation of the hip 21, congenital inguinal hernia 15, congenital cardiovascular malformations 8, eye anomalies 5, undescended testicles 4, pyloric stenosis 2, congenital clubfoot 1, polydactyly 1, omphalocele 1, dermoid cyst 1, anal atresia 1, choanal atresia 1, obstructive urological anomaly 1, auricular anomaly 1. The affected matched control cases represent a 5.2% total birth prevalence of CAs.

Response rates were significantly different within the groups of facial clefting (Table I). Only those cases were evaluated where all important questions were answered unequivocally. In order to prevent the further case loss, unpaired index patients were matched with the second or, if it was necessary, with the third control case. CA of first degree relatives was confirmed by the help of medical records or personal checks.

Biomathematical analysis was performed in two approaches: (i) index patients and their matched control cases were compared by the McNemar test, while (ii) all control cases were considered to be a total control group and this was compared with groups of index patients (χ^2 test). In general the latter is shown in the Tables.

TABLE I

Response and evaluated rates in different groups

Group	Registered cases	Index patients				Matched control			
		Respondent		Evaluated		Respondent		Evaluated	
		No.	%	No.	%	No.	%	No.	%
CL(P)	1086	727	66.9	630	58.0	504	46.4	471	43.4
CP	365	218	59.7	179	49.0	182	49.9	151	41.4
MCA	503	429	85.3	392	77.9	219	43.5	202	40.2
Total	1954	1374	70.3	1200	61.4	905	46.3	824	42.4

Results and Discussion

Genetics

The results of the *family study* are summarized in Table II. The unreliable data of 49,981 second and 44,748 third degree relatives were excluded and only the confirmed CAs of parents and sibs were included in the study. The specific familial cluster (K) was obvious in the relatives of index patients with CL(P), CP and MCA, respectively, except in the sibs of index girls with CP. The rates of affected parents and sibs showed some differences as compared to our previous study [3] conducted essentially in the same populations 10 years earlier by the same method.

Table II

Data of family study

Group	Index patient			Father				Mother			
	Sex	p	N	m	M	q	K	m	M	q	K
CL(P)	B	0.133	402	395	6	1.5	11	398	8	2.0	26
	G	0.077	228	223	8	3.6	27	225	4	1.8	23
	Σ	0.103	630	618	14	2.3	17	623	12	1.9	25
CP	B	0.036	80	80	1	1.3	36	80	2	2.5	52
	G	0.048	99	98	3	3.1	86	99	2	2.0	42
	Σ	0.042	179	178	4	2.2	52	179	4	2.2	46
MCA	B	0.039	181	170	1	0.6	15	176	3	1.7	33
	G	0.051	211	210	3	1.4	36	211	3	1.4	27
	Σ	0.045	392	380	4	1.1	28	387	6	1.6	31
Control	B	—	454	453	0	—	—	453	0	—	—
	G	—	368	365	0	—	—	368	0	—	—
	Σ	—	824	819	0	—	—	823	0	—	—

Group	Brother				Sister			
	m	M	q	K	m	M	q	K
CL(P)	216	9	4.2	32	232	5	2.2	28
	146	8	5.5	41	129	4	3.1	40
	362	17	4.7	35	361	9	2.5	32
CP	43	3	7.0	194	40	3	7.5	156
	50	0	—	—	52	0	—	—
	93	3	3.2	89	92	3	3.3	69
MCA	131	5	3.8	97	102	1	1.0	20
	152	5	3.3	85	132	5	3.8	75
	283	10	3.5	90	234	6	2.6	51
Control	269	0	—	—	217	1	0.5	—
	217	0	—	—	211	1	0.5	—
	486	0	—	—	428	2	0.5	—

p = birth prevalence (per cent)
N = number of index patients
B = boy (male)
G = girl (female)
Σ = boy + girl

m = number of relatives studied
q = per cent of affected relatives $\left(\frac{M}{m} \cdot 100\right)$
M = number of affected relatives
K = q/p

In the seventies, the affected rate of index patients' parents was 2.1% (1.9%), while a 3.6% (4.9%) sib-occurrence was found in the group of *CL(P)*. The percentage rates of affected relatives in the previous study are shown in brackets. Additionally, three further sisters and one brother were mentioned by the affection of facial clefting, but they died and the necropsy report or adequate medical documents were not available. With the inclusion of these cases, the sib-occurrence would be 4.2%. Taking into consideration the retrospective approach and questionnaire method (owing to the incompleteness of ascertainment), our figures may be minimal. The h^2 was estimated as 0.77 ± 0.08 based on data of the first degree relatives in this study (Table III).

The affected rate of *CP* was 0.6% and 2.2% in parents while the sib-occurrence was 2.5% and 3.2%, respectively, in the sixties and seventies. Carter et al [1] found a $1.3 \pm 0.6\%$ sib-occurrence and a $2.9 \pm 0.9\%$ affection rate in children of probands. The estimate of h^2 was 0.82 ± 0.16.

The *MCA* group may involve cases of heterogeneous origin. The affected rate of parents was 1.3% while a 3.1% sib-occurrence was found. Out of 392 index patients, 26 had affected first degree relatives. It is worth evaluating these familial clusters separately.

TABLE III

Data of GAMT computer-program in CL(P) group
(Explanation of abbreviations in text)

S	P	TYPE	D	S*	P*	m	M	2	H2	H2L	H2U	—ML	CHI2	DF
CL(P)														
B	1.33	PR	1	B	1.33	395	6	15.19	0.56	0.32	0.77	1.83		
B	1.33	PR	1	G	0.77	398	8	20.10	0.76	0.55	0.96	1.97		
G	0.77	PR	1	B	1.33	223	8	35.87	0.78	0.56	0.97	1.97		
G	0.77	PR	1	G	0.77	225	4	17.78	0.68	0.40	0.94	1.63		
		P TOTAL											2.45	3
B	1.33	SB	1	B	1.33	216	9	41.67	0.88	0.64	1.07	2.03		
B	1.33	SB	1	G	0.77	232	5	21.55	0.78	0.50	1.04	1.74		
G	0.77	SB	1	B	1.33	146	8	54.79	0.92	0.67	1.12	1.97		
G	0.77	SB	1	G	0.77	129	4	31.01	0.86	0.53	1.12	1.63		
		SB TOTAL							0.86	0.74	0.98	7.61	0.48	3
			1 TOTAL						0.77	0.69	0.84	18.05	3.62	1
					1 + 2 + 3 TOTAL				0.78	0.68	0.84	18.10	0.10	0
					PR + 2 + 3 TOTAL				0.78	0.55	0.83	9.71	2.17	0
					SB DIFFERENCE								1.55	1
						BB TOTAL			0.70	0.54	0.85	5.74	3.76	3
						BG TOTAL			0.77	0.60	0.93	3.72	0.02	3
						GB TOTAL			0.84	0.68	0.98	4.30	0.72	3
						GG TOTAL			0.76	0.55	0.94	3.57	0.61	3
						SEX DIFFERENCE							1.56	3

Data of second (2) and third (3) degree relatives were omittel.

The 2,556 first degree relatives of 824 matched control cases had 2 CL(P). Both CAs occurred in the sisters and these were CL and CLP. This 0.08% observed rate somewhat lower than the expected one (0.2%), i.e., the combined birth prevalence of isolated and multiple CL(P) and CP. The difference could, however, be explained by chance.

The most plausible hypothesis to explain the aetiology of CL(P) is the multifactorial-threshold model on the basis of a number of other studies (e.g. 1), including our previous survey. In this second study the family patterns of CL(P) were tested again by the *GAMT* (Gaussian-Additive-Multifactorial-Threshold) program [7] for the confirmation or exclusion of the role of the multifactorial threshold model.

The calculation is based on the sex (S), i.e., boy (B) and girl (G), specified birth prevalences (P), i.e., parents (PR) or sibs (SB) and the type (D) and sex (S^x) specified expected and observed rates of first degree relatives $\left(q = \frac{M}{m} \cdot 1000\right)$. The principle of the GAMT program is that the theoretically expected h^2 (H 2) values ($\pm$ confidence limit: H2L and H2U) are estimated by the maximum likelihood method (—ML) in the different segments of relatives (degree, type, sex). The comparison of estimated h^2 figures may be done by the use of appropriate statistics of an CHI2 type asymptotic distribution depending on the degree of freedom (DF). If there is no significant difference between the expected and observed h^2 figures, this proves that the familial pattern fits the GAMT program, i.e., the multifactorial threshold model.

Results of the GAMT program in the *CL(P)* group are shown in Table III. The familial pattern corresponded well to the GAMT program in the different sex-specified affected relative segments, too. The figures of h^2 seemed to indicate the multifactorial threshold model in the aetiology of CL(P) (Table III). However, according to our previous study the difference of h^2 values was significant in parents (0.68 ± 0.12) and sibs (0.95 ± 0.14). It was opposed to the classical multifactorial threshold model [10] and was explained by reduced fertility, i.e., selection in parents and the dominance variance. This recent study has confirmed the difference of h^2 values in parents and sibs, but the deviation did not reach the level of significance. Thus it is possible to state that the most plausible aetiological explanation for the origin of CL(P) is the multifactorial threshold model. Of course, when the observed figures fit the expected ones based on a model does not prove unequivocally the confirmation of the hypothesis unless alternative models can be excluded. Significant progress would be expected with a comparative analysis of different aetiological models [17, 22, 23, 24, 27] in the same materials.

In the case of *CP*, as a whole, the familial patterns corresponded to the GAMT-program. Some specific data were, however, opposed to the multifactorial threshold model (e.g., h^2 ex-

ceeded 1.0 twice in sibs, while h^2 was O in two other segments). The explanation may be the low number of relatives and the heterogeneous origin of the CP group.

The multifactorial threshold model was excluded in the MCA group; h^2 was obviously O in the matched control group.

The *main conclusions of the family study* were as follows:

(i) In the seventies the rates of affected relatives in CL(P) and CP groups were somewhat higher than in the sixties. [There was only one exception, the sib-occurrence of CL(P) cases.] A slight methodological progress may explain this.

(ii) As a rule, the sib-occurrence was higher than the rate of affected parents. It indicates the selection and/or the dominance variance.

(iii) Both the specific rates of affected parents (2.4% and 2.2%) and the sib-occurrences (4.2% and 3.2%) in the CL(P) and CP groups showed considerable similarities.

(iv) The origin of the CL(P) group is explained by the multifactorial threshold model (i.e., its polygenic liability is triggered or suppressed by environmental factors). The CP group showed a contraversial picture. The familial pattern, as a whole, fitted the GAMT-program, but some details were against it. This indicated a heterogeneous origin of the CP group. The familial cluster of MCAs did not fit the multifactorial threshold model; these groups involved different CA-entities of heterogeneous origin.

The occurrence of *non-specific CAs* was not higher in the first degree relatives of index patients than in those of the control cases (Table IV). The affected rate of relatives did not exceed the exceed the expected total prevalence of CAs (i.e., 6%), based on Hungarian experience. There was only one exception: the nearly 10% sister-occurrence in the CP group.

Particular stress was laid on the evaluation of other, so-called non-specific CA types in sibs of index patients with CL(P) and CP (Table V). (Data of parents were excluded owing to incompleteness caused by the selection and different levels of medical care.) The expected figures (E) were estimated on the basis of true birth prevalences (p) of CAs and the number of sibs (m). For evaluation of the comparison between the expected and observed figures (O) the χ^2 test was used. Only three significant differences were found in groups CL(P) and CP. Out of three, two were the *specific* familial cluster. The third one was a significantly lower figure in the group of congenital dislocation of the hip. The explanation may be an under-ascertainment. Five neural tube defects in sibs of CL(P) cases did not exceed the 0.05 level of significance in this study (Figs 1–5), however, their combination with some other materials of CL(P) indicated a higher sib-occurrence [14]. The relationship of these schisis-type CAs was published earlier [9]. According to the expectation, the other types of facial clefting did not occur more frequently in sibs of specified facial clefting groups, proving their independence

TABLE IV

Occurrence of non-specific (NS) congenital anomalies in the first degree relatives of index patients studied. (Facial cleftings and minor anomalies were excluded)

Group	Father			Mother			Brother			Sister		
	m	M_{NS}	q	m	M_{NS}	q	m	M_{NS}	q	m	M_{NS}	q
CL(P) (N = 630)	618	9*	1.5	623	8*	1.3	362	15*	4.1	361	16*	4.4
CP (N = 179)	178	2**	1.1	179	4**	2.2	93	4**	4.3	92	6**	6.5
MCA (N = 392)	380	3°	0.8	387	7°	1.8	283	10°	3.5	234	10°	4.3
Control (N = 824)	819	7°°	0.9	823	5°°	0.6	486	11°°	2.3	428	13°°	3.0

* dislocation of hip 1
cong. clubfoot 4
heart defect 1
cong. inguinal hernia 1
syndactyly 1
cong. myopia 1

** dislocation of hip 1
renal agenesis, unilat. 1

° polydactyly 2
spina bifida occulta 1

°° dislocation of hip 1
cong. clubfoot 1
syndactyly 1
limb reduction 1
cong. inguinal hernia 1
renal agenesis, unilat. 1
undescended testis, unilat. 1

* dislocation of hip 3
heart defect 2
polydactyly-syndactyly 1
spina bifida occulta 1
tongue defect 1

** dislocation of hip 2
cong. scoliosis 1
cong. myopia 1

° dislocation of hip 4
cong. clubfoot 1
heart defect 1
polydactyly 1

°° dislocation of hip 3
scoliosis 2

* dislocation of hip 1
cong. clubfoot 3
heart defect 1
pyloric stenosis 1
polydactyly 1
undescended testes 1
auricular CA 1
pectus excavatum 1
MCA (heart defect + renal agenesis, unilat.) 1
cong. inguinal hernia 4

** heart defect 1
pyloric stenosis 1
cong. clubfoot 1
cong. inguinal hernia 1

° heart defect 3
polydactyly 1
undescended testis 1
pyloric stenosis 1
spina bifida cystica 1
biliar atresia 1
multiple CA 2

°° dislocation of hip 1
cong. clubfoot 1
spina bifida cystica 2
cong. inguinal hernia 2
undescended testis 2
Down 1
heart defect 1
hypospadias 1

* dislocation of hip 4
cong. clubfoot 4
heart defect 3
anencephaly 3
spina bifida cystica 2

** dislocation of hip 3
heart defect 2
microphthalmia 1

° dislocation of hip 5
anencephaly + spina bifida 1
spina bifida cystica 1
haemangioma 3

°° dislocation of hip 3
scoliosis 1
heart defect 1
cong. inguinal hernia 3
pyloric stenosis 1
renal dysplasia 1
syndactyly 1
cong. clubfoot 2

TABLE V

Occurrence of non-specific type CAs in the sibs of index patients

Congenital abnormality (CA)	CL(P) (m = 723)		CP (m = 185)		Control (m = 914)	
	E	O	E	O	E	O
Anencephaly-spina bifida cystica (p = 2.6)	1.9	5	0.5	0°	2.4	2
Cleft lip ± palate (p = 1.0)	0.7	26□	1.2	1	0.9	2
Cleft palate (p = 0.4)	0.3	2	0.1	6□	0.3	0
Eye CAs (p = 0.5)	3.6	4	0.9	1	4.6	4
Ear CAs (p = 3.0)	2.2	4	0.6	0	2.7	1
Cardiovascular CAs (p = 10.7)	7.7	5°°	2.0	3	9.8	2*
Pyloric stenosis (p = 1.5)	1.1	1	0.3	1	1.4	1
Urogenital CAs (p = 3.0)	2.2	1°°	0.6	0	2.7	1
Hypospadias (p = 2.2)	1.6	0	0.4	0	2.0	1
Undescended testis (p = 7.8)	5.6	1	0.4	0	7.1	2
Cong. inguinal hernia (p = 11.4)	8.2	4	2.1	1	10.4	5
Cong. dislocation of hip (p = 28.0)	20.2	5x	5.2	3	25.6	4x
Clubfoot (p = 10.0)	7.2	7	1.9	1	9.1	3
Poly- and/or syndactyly (p = 0.5)	0.4	1	0.1	0	0.5	1
Other limb CAs (p = 0.6)	0.4	0	0.1	0	0.6	0
Vertebral and rib CAs (p = 0.5)	0.4	1	0.1	0	0.5	1
Down (p = 1.2)	0.9	0	0.2	0	1.1	0
Other CAs (~p = 10.0)	7.2	9*	1.9	4**	9.1	7***
Total	71.7	76	18.6	21	90.8	37*

□ = occurrence of specific type CA
x = $p < 0.05$
° = Anencephaly stated without medical record
°° = one component CA of multiple CA
* = haemangioma 5, hydrocele testis 3, micrognathia 1
** = haemangioma 4
*** = Down syndrome 1, tongue CA 1, haemangioma 3, torticollis 2

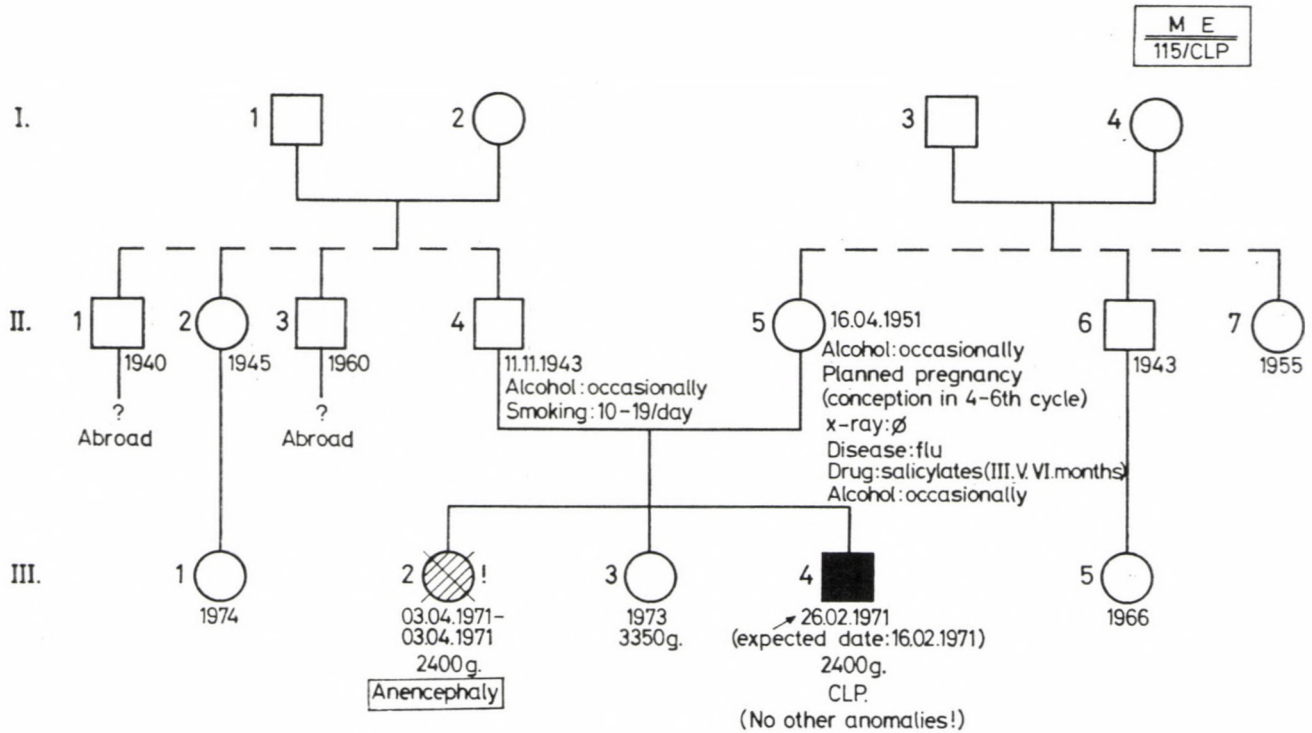

FIG. 1. Pedigree of Case 115

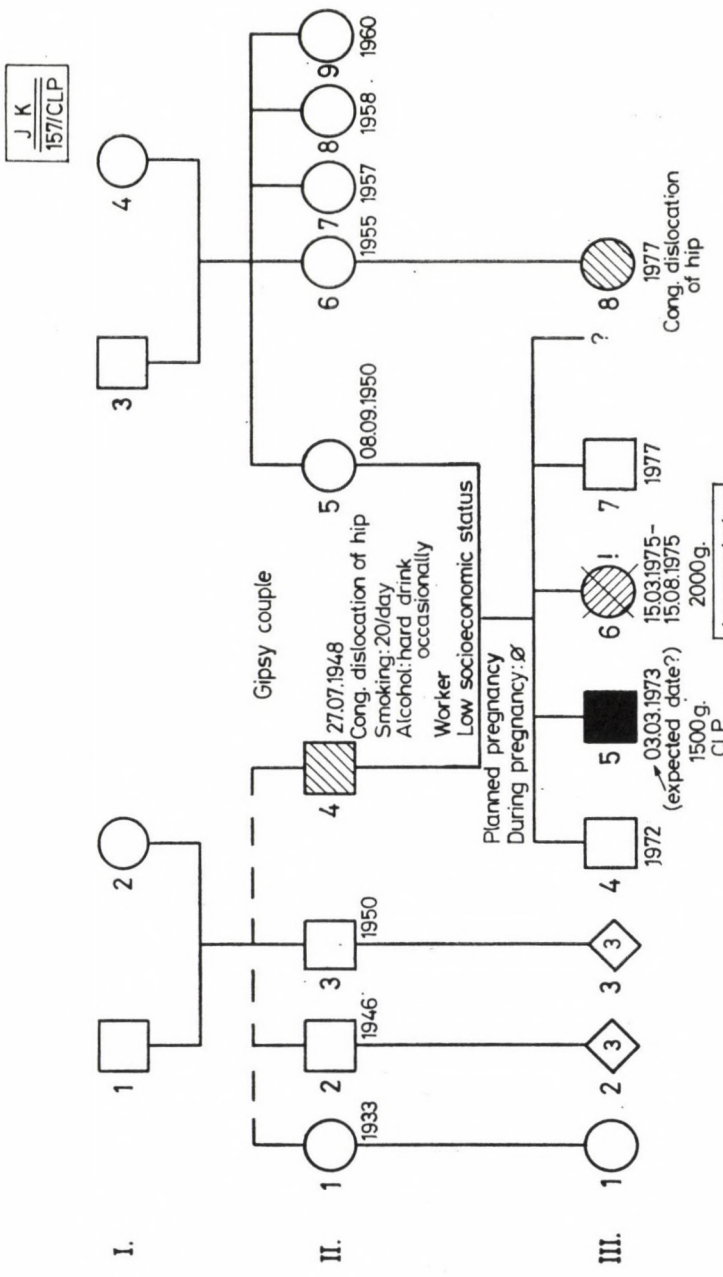

FIG. 2. Pedigree of Case 157

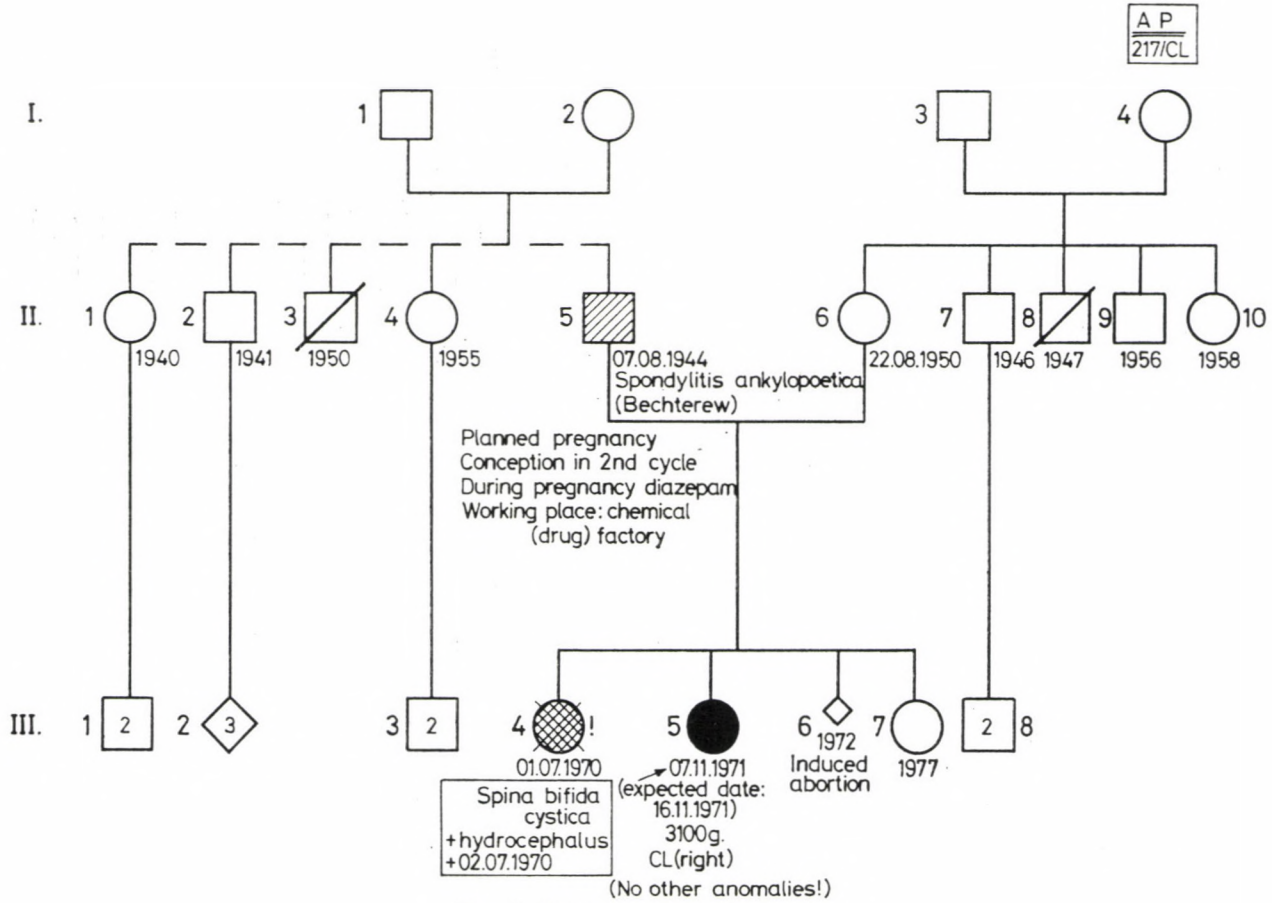

FIG. 3. Pedigree of Case 217

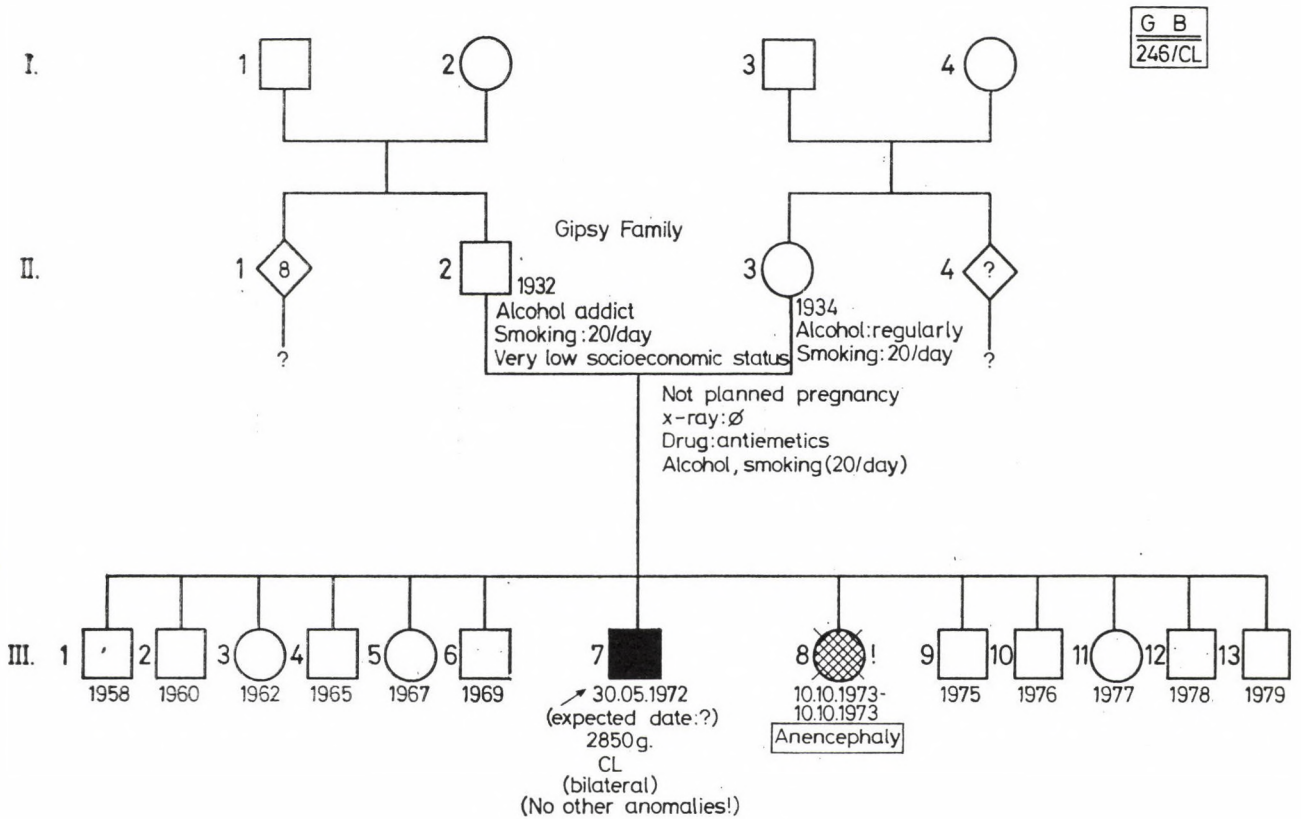

FIG. 4. Pedigree of Case 246

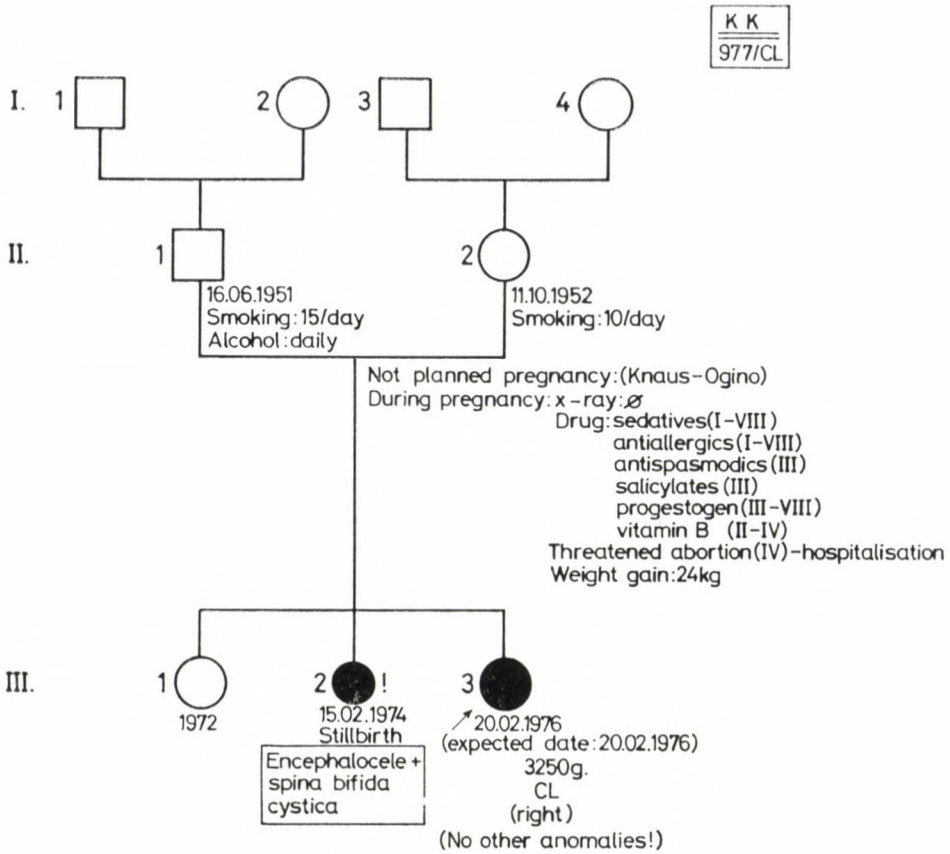

FIG. 5. Pedigree of Case 977

and different origin. In the control group the observed occurrence of liability for dislocation of the hip, congenital cardiovascular malformations and the total CAs were significantly lower, showing the ascertainment bias.

Prenatal selection may modify the specific and non-specific occurrence of CAs in sibs, therefore the outcome of previous and subsequent pregnancies of the mothers of index patients were also evaluated (Table VI). The rates of spontaneous abortion were significantly higher in the group of MCAs before ($\chi^2 = 6.87$; $p < 0.05$) and after ($\chi^2 = 12.28$; $p < 0.001$) the birth of index patients. The high rates of spontaneous abortion and the subsequent stillbirth did not exceed the significance level in the CP group owing to the low number of cases. Furthermore a lower per cent of subsequent induced abortions is worth mentioning in the MCA group ($\chi^2 = 17.96$; $p < 0.001$).

The data presented indicate the effect of prenatal selection for the

TABLE VI

Previous and subsequent pregnancy outcomes

Group	Previous pregnancies								Total pregnancy°	
	Induced abortion		Spontaneous abortion*		Stillbirth**		Livebirth°			
	No.	%	No.	%	No.	%	No.	$\bar{x}$	No.	$\bar{x}$
CL(P) (N = 630)	179	22.7	119 (1)	19.5	19	3.7	473	0.75	790	1.25
CP (N = 179)	45	20.4	40	22.9	5	3.7	130	0.73	220	1.23
MCA (N = 392)	146	25.9	87 (5)	20.9	11	3.3	319	0.81	563	1.44
Control (N = 824)	274	27.2	136 (2)	18.6	28	4.7	569	0.69	1007	1.22

* $\frac{\text{No. of spontaneous abortion}}{\text{No. of total birth} - \text{No. of induced abortion}} \cdot 100$

Ectopic pregnancies were included into spontaneous abortions, their absolute numbers are shown in brackets

sib-occurrence of MCAs. After the birth of index patients with MCA which is often lethal, the parents wanted more children or at least they terminated their pregnancies less often.

Summing up the results of genetic approach, the multifactorial threshold model seems to be the most plausible explanation of the origin of CL(P) with considerable polygenic liability (77%). On the other hand, CP and MCA groups may represent several entities of different origin.

Teratology

Owing to the high value of h^2 in the groups of CL(P) and CP, single and decisive environmental factors could not be expected. The triggering and suppressing external effects, i.e., in a narrow sense the teratogens and the possible maternal factors may, however, be important.

First, the *circumstances of conception* were studied, because these may be important from the teratological point of view. Only the MCA group had a significantly higher proportion of unplanned pregnancies than the control group (Table VII). Among unplanned pregnancies the failure of the calendar method (0.6–3.5 *vs* 2.4), coitus interruptus (7.0–10.8 *vs* 10.1), oral contraceptives (1.1–2.9 *vs* 1.3), condom-pessarium (0.3–1.1 *vs* 0.1), IUD (0.0–0.3 *vs* 0.0) did not show significant differences between groups of facial clefting and total matched controls. The time interval (number of female cycles) between the discontinuation of contraception or the beginning of sexual intercourse and the conception was studied in the cases of planned pregnancies (Table VIII). Owing to the high and different proportion of unknown figures,

of the mothers of index patients

Subsequent pregnancies								Total pregnancy°		Grand total°	
Induced abortion		Spontaneous abortion*		Stillbirth**		Livebirth°					
No.	%	No.	%	No.	%	No.	$\bar{x}$	No.	$\bar{x}$	No.	$\bar{x}$
115	28.8	30 (1)	10.5	5	2.0	250	0.40	400	0.63	1190	1.89
34	31.8	13	17.8	5	8.3	55	0.31	107	0.60	327	1.83
59	19.4	45 (2)	18.4	2	1.0	198	0.51	304	0.78	867	2.21
214	34.9	49 (3)	12.3	5	1.4	345	0.42	613	0.74	1620	1.97

** $\frac{\text{No. of stillbirth}}{\text{No. of total births}} \cdot 100$ ° $\frac{\text{No. of given pregnancy outcomes}}{\text{No. of index patients}} = \bar{x}$

it was difficult to evaluate this variable. The unknown percentage was significantly higher in the MCA group. The CP group had a considerably higher per cent of late conception (after the 10th month).

The duration of *working during pregnancy* and the possible dangerous occupational exposures (radiation, microbial, chemical, noise) were also studied, but no considerable differences were found between the study and control groups. Thus, we could not confirm the relation between facial clefting and organic solvent exposure during pregnancy [16].

Next, the so-called teratogens were analysed. In general, the occurrence of diagnostic *abdominal X-rays*, mechanical trauma and psychological

TABLE VII

Proportion of planned pregnancies which ended in birth of index patients and matched controls

Group	Planned		Unplanned		Unknown	
	No.	%	No.	%	No.	%
CL(P) (N = 630)	499	79.2	110	17.5	21	3.3
CP (N = 179)	146	81.6	25	14.0	8	4.4
MCA (N = 392)	297	75.8	82	20.9	13	3.3
Control (N = 824)	671	81.4	137	16.6	16	1.9

TABLE VIII

Time interval (number of female cycles) between beginning of reproductive activity and conception. (In brackets the percentage figures are shown)

Group \ Number of cycles	1	2	3	4–5	6–9	10–12	12–	Subtotal	Unknown
CL(P) (N = 630)	111 (28.3)	84 (21.4)	49 (12.5)	45 (11.5)	43 (11.0)	25 (6.4)	35 (8.9)	392	107 (21.4)
CP (N = 179)	32 (29.4)	18 (16.5)	11 (10.1)	13 (11.9)	13 (11.9)	8 (7.4)	14 (12.8)	109	37 (25.3)
MCA (N = 392)	62 (28.3)	39 (17.8)	32 (14.6)	28 (12.8)	29 (13.2)	10 (4.6)	19 (8.7)	219	173 (44.9)
Control (N = 824)	169 (30.0)	97 (17.2)	94 (16.7)	71 (12.6)	54 (9.6)	27 (4.8)	51 (9.1)	563	108 (16.1)

stress (Table IX) was higher in the critical period of CAs studied, i.e., roughly in the first trimester of gestation. This was the case in the next two trimesters of pregnancy as well, except for psychological stress. Thus the separation of true impacts from the recall bias was difficult. The role of psychological stress was discussed several times in the aetiology of facial clefting [12] but this has not been confirmed in human beings.

Maternal disorders including microbial infections were evaluated independently for the duration of pregnancy (Table X). Influenza or influenza-like diseases (so-called "flu") during pregnancy were mentioned more frequently by the mothers of index patients in all groups. There are, however, two important arguments against the role of flu in the aetiology of facial cleftings. First, the flu occurred after the critical period

TABLE IX

Occurrence of so-called physical teratogens and psychological stress

Group	Diagnostic abdominal X-ray			Mechanical trauma			Psychological stress		
	Month		Total	Month		Total	Month		Total
	1–3	4–9		1–3	4–9		1–3	4–9	
CL(P) (N = 630)	1 (0.2)	9 1.4	10 1.6	18 2.9	27 4.3	45 7.1	51 8.1	30 4.8	81 12.9
CP (N = 179)	0 —	1 (0.6)	1 (0.6)	0 —	11 6.1	11 6.1	10 5.6	13 7.3	23 12.8
MCA (N = 392)	1 (0.3)	4 (1.0)	5 1.3	5 1.3	10 2.6	15 3.8	24 6.1	19 4.8	43 11.0
Control (N = 824)	0 —	2 (0.2)	2 0.2	5 0.6	6 0.7	11 1.3	29 3.5	42 5.1	71 8.6

TABLE X

Maternal disorders during pregnancy (If there were several diseases, only the most serious one was considered)

Group	No occurrence	Rubella	Flu	Urinary infection or disease	Respiratory infection or disease	Unindentified fever	Liver disease	Mumps	Hypertension	Epilepsy	Anaemia	Others	Total
CL(P)	359	1	175	39	11	7	6	5	7	5	10	5	271
(N = 630)	57.0	0.2	27.8	6.2	1.7	1.1	1.0	0.8	1.1	0.8	1.6	0.8	43.0
CP	99	1	56	7	2	0	1	0	3	1	8	1	80
(N = 179)	55.3	0.6	31.3	3.9	1.1	—	0.6	—	1.7	0.6	4.5	0.6	44.7
MCA	222	6	119	17	9	3	1	1	6	1	5	2	170
(N = 392)	56.6	1.5	30.4	4.3	2.3	0.8	0.3	0.3	1.5	0.3	1.3	0.5	43.4
Control	648	1	92	25	14	4	8	3	13	2	9	5	176
(N = 824)	78.6	0.1	11.2	3.0	1.7	0.5	1.0	0.4	1.6	0.2	1.1	0.6	21.4

of facial cleftings in the majority of cases. Furthermore, the detailed analyses of the correlation between the time of influenza epidemics in Hungary and the monthly distribution of CL(P) and CP did not give a positive result (Fig 6), i.e., birth prevalences of facial clefting did not increase after influenza-epidemics. Previously, Leck [19] and Leck et al [20] found a high rate of facial clefting in index patients born 6–9 months after influenza epidemics, although not in any for whom the related epidemic was the initial outbreak of A2 influenza. However, later Leck [21] wrote: "it is difficult to believe that maternal exposure to infection caused the defects in these children, since the epidemics apparently happened when many of those concerned had already passed the stage at which facial clefting is laid down".

Furthermore, 6 rubella infections and/or diseases were noteworthy in the group of MCAs [13]. Out of 9 mumps infections 2, 3, 2 and 2 occurred in the 2nd, 3rd, 4th and 6th month of gestation, respectively. Finally, within the CL(P) group 5 mothers had epilepsy and were treated with anticonvulsants during pregnancy. It is well known that facial clefting is a principal component CA in both the fetal hydantoin and the fetal trimethadione syndromes.

Drug ingestion during pregnancy was also analysed (Table XI). The per cent of no drug use was significantly lower in the MCA group ($\chi^2 = 4.12$; $p < 0.05$). The recorded occurrence of drugs usual in prenatal care, i.e. different vitamins, iron and calcium preparations did not show any significant difference among the groups, and this may be an argument against the recall bias. Anticonvulsants were used by the mother of 11 index patients with CL(P) and caused a significant increase ($\chi^2 = 9.11$; $p < 0.01$). (It is disturbing that only 5 epilepsies were mentioned among the

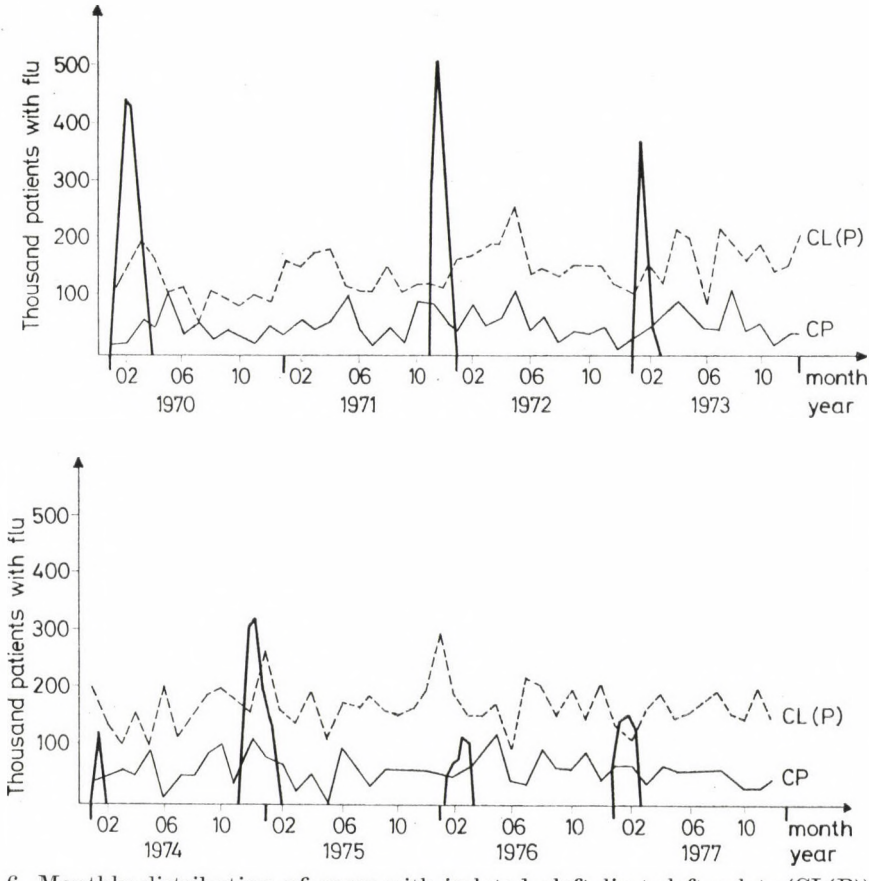

FIG. 6. Monthly distribution of cases with isolated cleft lip±cleft palate (CL(P)) and cleft palate (CP) and the time of influenza epidemics in Hungary, 1970-1976

maternal disorders.) All anticonvulsants had been taken in the first month of pregnancy as well. As it was mentioned previously, three fetal hydantoin syndromes were excluded. The higher frequency of isolated CL(P) after the ingestion of hydantoin (4), trimethadione (3) and primidone (2) indicated that in general practice mainly CL(P) is diagnosed after anticonvulsant treatment.

The use of antibiotics ($\chi^2 = 20.65$; $p < 0.001$), chemotherapeutics ($\chi^2 = 10.84$; $p < 0.01$), antipyretics ($\chi^2 = 40.20$; $p < 0.001$), analgesics-antispasmodics ($\chi^2 = 28.59$; $p < 0.001$) and sedatives ($\chi^2 = 9.41$; $p < 0.001$) was also more frequent in the mothers of CL(P) index patients than those of the control group.

The use of antiemetics was significantly lower in the CL(P) group, though this type of drug was supposed to play a role in the aetiology of facial clefting [15, 26]. In the CP group antibiotics ($\chi^2 = 6.43$; $p < 0.05$)

Table XI

Drug ingestion during pregnancy

Group	No drug use	Drug use during pregnancy	Hormones						Antibiotics					
			Oral contraceptives	Oestrogens	Progesterone	Methandro-stenolone	Prednisolone	Insulin	Penicillins	Streptomycin Isoniazid	Chlor-amphenicol	Oxytetracycline	Moroxydine	Nalidixic acid
CL(P)	196	434	25	13	73	1	0	0	55	2	4	11	0	1
N = 630	31.1		*5.8*	*3.0*	16.8	0.2	—	—	*12.7*	0.5	0.9	*2.5*	—	0.2
CP	58	121	1	2	30	0	0	1	17	0	1	0	0	0
N = 179	32.4		0.8	1.7	24.8	—	—	0.8	*14.0*	—	0.8	—	—	—
MCA	96	296	17	10	74	0	1	0	45	1	1	9	0	0
N = 392	24.5		*5.7*	*3.4*	25.0	—	0.3	—	*15.2*	0.3	0.3	*3.0*	—	—
Control	248	576	15	6	117	0	1	0	31	1	4	5	0	1
N = 824	30.1		2.6	1.0	20.3	—	0.2	—	5.4	0.2	0.7	0.9	—	0.2

Group	Chemotherapeutics				Antipyretic and Anti-inflammatory Agents			Analgesics				Antiemetics		Antihyper-tensive Agents	
	Sulfonamides	Nino-furantoin	Metronidazole	Natamycin	Salicylates	Amino-phenazone	Oxyquinoline sulphonic acid	Codeine and its salts	Amobarbital	Combinations	Spasmolytics	Dimen-hydrinate	Thioethyl-perazine	Reserpine	Reserpine + hydrochloro-thiazide
CL(P)	15	15	3	0	66	24	6	6	1	35	28	75	12	2	0
N = 630	*3.5*	*3.5*	0.7	—	*15.2*	*5.5*	*1.4*	1.4	0.2	8.1	6.5	17.3	2.8	0.5	—
CP	1	3	0	0	13	3	1	0	0	5	7	24	8	2	0
N = 179	0.8	*2.5*	—	—	*10.7*	2.5	0.8	—	—	4.1	5.8	19.8	6.6	1.7	—
MCA	6	5	1	2	47	12	4	2	0	14	23	52	9	1	1
N = 392	2.0	1.7	0.3	0.7	*15.9*	*4.1*	1.4	0.7	—	4.7	2.3	17.6	3.0	0.3	0.3
Control	10	5	2	0	36	8	0	3	0	12	17	119	24	6	0
N = 824	1.7	0.9	0.3	—	6.3	1.4	—	0.5	—	2.1	3.0	20.7	42	1.0	—

Group	Sedatives–Tranquillizers												
	Barbital	Promethazine	Metofenazin	Chlorpromazine	Chlordiazepoxide	Amitriptyline	Nitrazepam	Levopromazine	Glutethimid	Diazepam	Methylpentynol	Meprobamate	Phenobarbital
CL(P)	5	48	4	2	4	0	1	0	1	16	1	12	5
N = 630	*1.2*	11.1	0.9	0.5	0.9	—	0.2	—	0.2	3.7	0.2	2.8	1.2
CP	1	16	1	0	2	0	0	0	0	3	0	2	0
N = 179	0.8	13.2	0.8	—	1.7	—	—	—	—	2.5	—	1.7	—
MCA	5	33	0	2	5	1	2	1	0	13	1	5	8
N = 392	*1.7*	11.1	—	0.7	1.7	0.3	0.7	0.3	—	*4.4*	0.3	1.7	2.7
Control	1	57	0	2	3	0	1	0	0	16	0	3	5
N = 824	0.2	9.9	—	0.3	0.5	—	0.2	—	—	2.8	—	0.5	0.9

Group	Anticonvulsants										
	Phenytoin	Phenacemide	Pirimidone	Mephenytoin	Morpholep	Sultiame	Trimethadione	Together	Vitamins	Iron and iron salts	Calcium
CL(P)	2	0	2	2	1	1	3	11	36	25	0
N = 630	0.5	—	0.5	0.5	0.2	0.2	0.7	*2.5*	8.3	5.8	—
CP	0	0	0	0	0	0	0	0	7	3	0
N = 179	—	—	—	—	—	—	—	—	5.8	2.5	—
MCA	0	1	0	0	0	0	1	2	21	10	2
N = 392	—	0.3	—	—	—	—	0.3	0.7	7.1	3.4	0.7
Control	1	0	1	0	0	0	0	2	41	36	3
N = 824	0.2	—	0.2	—	—	—	—	0.3	7.1	6.3	0.5

Significant increases are indicated by italics

and antipyretics ($\chi^2 = 4.45$; $p < 0.05$), in the group of MCAs antipyretics ($\chi^2 = 38.12$; $p < 0.001$), some antibiotics ($\chi^2 = 17.78$; $p < 0.01$), sedatives ($\chi^2 = 19.03$; $p < 0.001$), some hormones ($\chi^2 = 14.00$; $p < 0.001$) (mainly hormonal supportive therapy) were reported more often.

These differences of several types of drug use during pregnancy may be embarrassing for experts because they indicate mainly the effect of recall bias. The analysis of time-distribution of the above-mentioned drugs with a significant increase during pregnancy showed that the majority had been taken *after* the critical period of facial clefting.

Alcohol consumption during pregnancy did not show any significant difference among the groups (Table XII). Only the per cent of hard drinkers was higher in the CP group ($\chi^2 = 9.41$; $p < 0.01$).

The possible aetiological role of *smoking* was also raised in the literature [11, 26]. The per cent of non-smoker mothers was significantly lower in the CL(P) group ($\chi^2 = 6.31$; $p < 0.05$) (Table XIII). Accordingly,

TABLE XII

Maternal alcohol consumption

Group	Alcohol consumption							
	Total abstinence	Only before conception	Only 0–3 months		Whole pregnancy		Hard drink	
			occasionally	habitually	occasionally	habitually	No.	%
CL(P)	442	28	12	0	144	4	70	11.1
(N = 630)	70.2	4.4	1.9	—	22.9	0.6		
CP	99	23	7	0	48	2	31	17.3
(N = 179)	55.3	12.8	3.9	—	26.8	1.1		
MCA	272	14	6	1	101	3	46	11.7
(N = 392)	69.4	3.6	1.5	0.3	25.8	0.8		
Control	555	6	6	0	251	6	87	10.6
(N = 824)	67.4	0.7	0.7	—	30.5	0.7		

TABLE XIII

Maternal smoking

Group	No smoking	Only before conception	Only 0–3 months			Whole pregnancy			Total
			0–10	11–20	21–	0–10	11–20	21–	
CL(P)	418	30	30	4	0	100	25	23	148
(N = 630)	66.3	4.8	4.8	0.6	0.0	15.9	4.0	3.7	23.5
CP	130	16	9	0	0	12	10	2	24
(N = 179)	72.6	8.9	5.0	0.0	0.0	6.7	5.6	1.1	13.4
MCA	302	7	14	3	0	35	16	15	66
(N = 392)	77.0	1.8	3.6	0.8	0.0	8.9	4.1	3.8	16.8
Control	597	30	32	4	6	89	43	23	155
(N = 824)	72.5	3.6	3.9	0.5	0.7	10.8	5.2	2.8	18.8

TABLE XIV

Symptoms of early toxaemia

Group	Pronounced nausea				Total	Continuous and strong vomitus				Total	Weight*			Together
	No occurrence	Months				No occurrence	Months				Gain 7 kg or more	0–6 kg	Loss	
		1–3	4–6	Whole			1–3	4–6	Whole					
CL(P)	344	148	90	48	286	533	54	26	17	97	561	63	6	69
(N = 630)	54.6	23.5	14.3	7.6	45.4	84.6	8.6	4.1	2.7	15.4	89.1	10.0	1.0	10.9
CP	97	52	13	17	82	145	19	6	9	34	158	19	2	21
(N = 179)	54.2	29.0	7.3	9.5	45.8	81.0	10.6	3.4	5.9	19.0	88.3	10.6	1.1	11.7
MCA	209	96	57	30	183	320	35	26	11	72	324	60	8	68
(N = 392)	53.3	24.4	14.5	7.7	46.7	81.6	8.9	6.6	2.8	18.4	82.7	15.3	2.0	17.3
Control	428	206	131	59	396	682	76	38	28	142	730	91	3	94
(N = 824)	51.9	25.0	15.9	7.2	48.1	82.8	9.2	4.6	3.4	17.2	88.6	11.0	0.4	11.4

* During the first six months of gestation

TABLE XV

Symptoms of late toxaemia

Group	Symptoms								Total	Weight gain		Together
	No occurrence	H	P	O	H + P	H + O	P + O	H + P + O		16–19 kg	20 kg or more	
CL(P)	389	26	33	142	0	18	15	7	241	61	54	115
(N = 630)	61.7	4.1	5.2	22.5	0.0	2.9	2.4	1.1	38.3	9.7	8.6	18.3
CP	111	7	5	39	3	4	8	2	68	16	12	28
(N = 179)	62.0	3.9	2.8	21.8	1.7	2.2	4.5	1.1	38.0	8.9	6.7	15.6
MCA	223	29	7	97	4	14	10	8	169	24	28	52
(N = 392)	56.9	7.4	1.8	24.7	1.0	3.6	2.6	2.0	43.1	6.1	7.1	13.3
Control	454	76	28	198	7	38	10	13	370	55	54	109
(N = 824)	55.1	9.2	3.4	24.0	0.8	4.6	1.2	1.6	44.9	6.8	6.6	13.2

Abbreviation:
H = Hypertension (>150 mm Hg)
P = Proteinuria
O = Oedema in leg

TABLE XVI

Occurrence of threatened abortion

Group	No occurrence	1–3 months				Total	4–9 months				Total
		V	U	V + U	H		V	U	V + U	H	
CL(P)	409	32	17	5	41	95	10	25	3	88	126
(N = 630)	64.9	5.1	2.7	0.8	6.5	15.1	1.6	4.0	0.5	14.0	20.0
CP	122	11	6	2	12	31	2	10	0	14	26
(N = 179)	68.2	6.1	3.3	1.1	6.7	17.3	1.1	5.6	—	7.8	14.5
MCA	220	14	16	7	29	66	7	33	3	63	106
(N = 392)	56.1	3.6	4.1	1.8	7.4	16.8	1.8	8.4	0.8	16.1	27.0
Control	577	29	8	4	51	92	13	31	0	111	155
(N = 824)	70.0	3.5	1.0	0.5	6.2	11.2	1.6	3.8	—	13.5	18.8

V = vaginal bleeding
U = uterine contraction
H = hospitalization

the rate of smokers during the first three months of gestation and the whole pregnancy was somewhat higher in the CL(P) group than in the total control group.

Finally, some categories of *pregnancy complication* were evaluated. The symptoms of early toxaemia, i.e. pronounced nausea as well as continuous and strong vomitus did not occur more frequently in the pregnancies of the mothers of index patients (Table XIV). The reported occurrence of single, pair and triplet symptoms of late toxaemia was not higher in the group of index patients (Table XV). There was only one exception: an extreme weight gain occurred more frequently in the CL(P) group.

The occurrence of *threatened abortion* was analysed on the basis of vaginal bleedings, uterine contractions, their combination, and hospitalization (Table XVI). While the mothers of index patients reported a higher rate of vaginal bleeding and uterine contraction in the first trimester, the combination of these symptoms, and mainly the hospitalization, had a similar occurrence in the study and the control groups. There was a higher rate of symptoms in the second and third trimesters in the MCA group ($\chi^2 = 22.30$; $p < 0.001$).

Consequently, a number of possible teratogens and maternal factors were found in this study, but our conclusions have to be limited, owing to the well-known difficulties of a retrospective epidemiological approach (ascertainment and recall bias). Nevertheless, the triggering impact of certain types of anticonvulsant seemed to be well-founded.

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