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Oral clefts and life style factors – A case–cohort study based on prospective Danish data

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Abstract. This study examines the association between oral clefts and first trimester maternal lifestyle factors based on prospective data from the Danish National Birth Cohort. The cohort includes approximately 100,000 pregnancies. In total 192 mothers gave birth to child with an oral cleft during 1997–2003. Information on risk factors such as smoking, alcohol consumption, tea, coffee, cola, and food supplements was obtained during pregnancy for these and 828 randomly selected controls. We found that first trimester maternal smoking was associated with an increased risk of oral clefts (odds ratio (OR): 1.50; 95% confidence interval (CIs): 1.05, 2.14). Although not statistically significant, we also saw associations with first trimester consumption of alcohol (OR: 1.11; CIs: 0.79, 1.55), tea (OR: 1.31; CIs: 0.93, 1.86), and drinking more than 1 l of cola per week (OR: 1.40; CIs: 0.92, 2.12). Furthermore supplementation with ≥400 mcg folic acid daily during the entire first trimester (OR: 0.75; CIs: 0.46, 1.22) suggested an inverse associated with oral clefts, similar to our results on coffee drinking. No effects were found for smaller doses of folic acid, vitamin A, B6 or B12 in this study. The present study found an association between oral clefts and smoking and, although not conclusive, supports an association of oral cleft with alcohol.

Key words: Alcohol, Caffeine, Oral cleft, Smoking, Vitamin

Abbreviations: CI = confidence interval; CL(P) = Cleft lip with/without cleft palate; CP = Cleft Palate; OR = Odds Ratio

Introduction

Oral clefts comprise cleft lip with/without cleft palate (CL(P)) and cleft palate only (CP) and affects children worldwide. It is found in 1–2 per 1,000 live births [1, 2] and have major consequences for the children, their families, and the society. Family studies suggest that CL(P) and CP are two genetic distinct malformations [3]. The etiology of both defects is thought to be multi-factorial with genes playing an important role [4, 5]. Numerous syndromes including oral clefts are known and mutations in single genes for MSX1 [6] and IRF6 [7–9] have recently been associated with non-syndromic CL(P) and CP. Among environmental risk factors the only consistently replicated finding is a modest effect of smoking [10–17]. Results on alcohol intake during pregnancy are much more diverse, possibly because alcohol intake during pregnancy varies substantially over time and between populations.

Many other risk factors have also been suspected: folic acid antagonists, illnesses, and infections. Furthermore, a protective effect of folic acid [18–22], multivitamins [19, 23] and vitamin B6 and B12 [24–28] has been suggested. Most studies have, however, been case-control studies with retrospectively obtained exposure data subject to differential recall and an effective prevention against oral clefts cannot be implemented yet.

The aim of the present study is to use prospectively collected data to study associations between early pregnancy exposures (smoking, alcohol habits, tea and coffee consumption and food supplement use) and risk of oral clefts.

Material and methods

The present study is a case–cohort study in the Danish National Birth Cohort.

(1) *The Danish National Birth Cohort* was established during 1996–2002 and covered all regions in Denmark. About 100,000 pregnant women accepted the invitation at the first antenatal visit to the general practitioner in gestational weeks 6–14 (mean = 10th week). About half of all general practitioners in Denmark took part in the recruitment, and approximately 30% of all pregnant women in Denmark were enlisted in the cohort. Besides being pregnant, the criteria for inclusion in the cohort were as follows: (1) an address in Denmark, (2) intention to carry the pregnancy to term, and (3) the ability to speech Danish fluent enough to participate in four telephone interviews.

At enrolment the pregnant women completed a questionnaire on intake of medicine and supplementations during the first weeks of the pregnancy. Information on exposures was collected by computer-assisted telephone interviews shortly after (mean week of gestation = 17th week, 90% range 12–27). About 9% of the pregnant women participated more than once.

(2) *The Danish National Patient Registry* [29] was established in 1977 and includes International Classification of Diseases, 10th Revision (ICD-10) codes on diagnoses and surgeries on all individuals who have had contact with any of the Danish hospitals. All individuals with an address in Denmark on and after 1 April 1968 are registered with a unique 10 digit personal identification number (PIN) which includes a built-in check code disclosing most invalid numbers. Through the PIN, children born to women in the birth cohort can be traced in the register in order to identify cases with an oral cleft.

Identification of the study population

Cases (n = 220) were identified through two independent sources: (1) maternally reported oral clefts in the two post pregnancy interviews in the birth cohort and (2) a discharge diagnosis of oral clefts or an ICD-10 code for reconstructive surgery on lips or palate in The National Patient Register. In addition to the exclusion of twins (n = 18), one case that had received reconstructive surgery in the palate due to a haemangioma, but was not registered with oral cleft in any of the other sources, was excluded.

Controls (n = 880) were selected randomly among participants at baseline (the first interview) in the birth cohort. Twins and pregnancies not leading to births (miscarriages and elective terminations) were excluded. By chance none of the remaining 828 controls were registered with oral clefts in the birth cohort or the National Patient Register.

The diagnoses and specific cleft type of the 202 non-twin cases were validated through the two National Institutes of Defects of Speech to which every newborn child with a cleft is reported. These two institutes provide lifelong dental treatment and coordinate all treatment between dentists, surgeons, and speech-therapists. The cleft diagnosis of 190 cases was verified through these institutional data sources and additionally two were verified over the telephone by the mothers of the remaining 12 cases (one with microform cleft lip and one with submucous cleft palate). The 192 cases were grouped into CL(P) (n = 134) and CP (n = 58).

Detailed information on associated anomalies was obtained through The Danish Facial Cleft Register [2] for cases born before 2002 (n = 147) that registers all individuals born with an oral cleft in Denmark during 1936–2001. The registry enables subclassification of cases into syndromic (n = 24) and non-syndromic (n = 123) oral clefts.

The male–female sex ratio was 1.18 among cases and 0.90 among controls. The sex distribution for each subtype of cleft (1.73 for CL(P) and 0.49 for CP) was in accordance with known distributions. Females are usually overrepresented in the CP group compared to males, while males constitute the majority of the CLP group.

Analysis and statistics

We used two logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) in order to analyse associations of oral clefts with the different environmental exposures. The first was adjusted for parental ages and social class based on occupation and education (Social class, 2005), the second for parental ages, social class and additionally for smoking (yes/no), and alcohol consumption (units/week) since these were the factors which seemed to be associated with an increased risk of oral cleft. All analyses were done separately for all cases as one group referred to as 'Case' and for CL(P) and CP and additionally for each outcome including non-syndromic cases only. The risk factors were analysed both as binary, continuous, and categorical variables.

Use of food supplements were recorded through the enrolment form and data on daily supplementation of vitamin A, B6, B12 and folic acid for each week from conception until enrolment was therefore available. Gestational age at enrolment, however, varied. Thus, for some of the women this was before the end of their first trimester. E.g. for women reporting supplementation of 400 mcg folic acid during pregnancy weeks 4–9 at the enrolment in week 9, data were missing for weeks 10–12. We assumed, however, a continuous supplementation of 400 mcg folic acid during pregnancy week 10–12 and used a "last value carry forward strategy" to estimate the

Table 1. Odds ratios (OR) and 95% confidence intervals (CI) for environmental risk factors to all oral clefts and two subtypes of oral cleft

		n of exposed		All oral clefts *n* = 192				Cleft lip with/without cleft palate *n* = 134				Cleft palate *n* = 58			
		Cases	Controls	OR*	OR**	CI lower	CI upper	OR*	OR**	CI lower	CI upper	OR*	OR**	CI lower	CI upper
Smoking[a]															
Binary	Yes/no	62	218	**1.50**	1.52	1.05	2.14	1.48	1.49	0.97	2.24	1.53	1.60	0.83	2.82
Continuous	Cig/day (0–20cig)	52	207	1.04	1.03	1.00	1.08	1.03	1.03	0.98	1.08	1.05	1.05	0.99	1.12
Categorical	0 cigarettes/day	127	618												
	1–9	40	147	1.33	1.28	0.88	2.00	1.19	1.17	0.73	1.93	1.67	1.66	0.85	3.25
	10–19	10	55	0.91	0.92	0.45	1.85	0.90	0.92	0.39	2.04	0.96	0.91	0.28	3.25
	20 +	2	5	1.97	2.27	0.35	11.20	1.47	1.52	0.13	16.26	3.09	4.41	0.37	25.66
Alcohol[b]															
Binary	Yes/no	77	353	1.11	1.18	0.79	1.55	1.11	1.09	0.75	1.64	1.10	1.49	0.62	1.95
Continuous	Units/week (0–7units)	77	353	1.02	1.02	0.88	1.19	0.99	0.96	0.83	1.17	1.10	1.18	0.87	1.40
Categorical	0 units/week	101	475												
	1–2	64	305	1.06	1.16	0.74	1.50	1.05	1.07	0.70	1.59	1.06	1.46	0.59	1.92
	3 +	13	48	1.43	1.31	0.74	2.79	1.48	1.20	0.68	3.19	1.36	1.68	0.45	4.15
Coffee[c]															
Binary	Yes/no	71	370	0.86	0.95	0.61	1.21	0.83	0.87	0.56	1.24	0.93	1.22	0.52	1.64
Continuous	Cups/day (0–24cups)	71	370	0.97	0.98	0.90	1.06	0.98	0.99	0.89	1.09	0.95	0.96	0.85	1.07
Categorical	0 cups/day	108	458												
	1–4	62	308	0.89	1.00	0.63	1.27	0.86	0.89	0.57	1.30	0.97	1.37	0.54	1.76
	5 +	9	62	0.68	0.63	0.32	1.44	0.66	0.71	0.27	1.62	0.70	0.38	0.21	2.31
Tea[c]															
Binary	Yes/no	120	508	1.31	1.30	0.93	1.86	1.28	1.26	0.86	1.93	1.39	1.43	0.76	2.52
Continuous	cups/day (0–20cups)	120	508	1.05	1.05	0.99	1.11	1.02	1.01	0.95	1.09	**1.10**	**1.13**	1.01	1.20
Categorical	0 cups/day	59	320												
	1–4	98	421	1.29	1.27	0.90	1.85	1.34	1.31	0.89	2.03	1.18	1.16	0.63	2.23
	5 +	22	87	1.41	1.46	0.81	2.45	1.00	1.04	0.49	2.05	**2.47**	**2.87**	1.10	5.55
Cola															
Binary	yes/no	115	538	0.98	0.92	0.70	1.37	0.93	0.87	0.63	1.39	1.10	1.10	0.61	1.98
Categorical	< 1 l/week	142	701												
	≥1 l/week	37	127	1.40	1.40	0.92	2.12	1.46	1.38	0.91	2.35	1.22	1.39	0.59	2.52
Vitamins[d]															
Folic acid															
Binary	Yes/no	111	508	0.87	0.88	0.63	1.22	0.79	0.76	0.54	1.16	1.08	1.42	0.61	1.92
Continuous	Mcg (0–5200 mcg)	111	508	1.0002	1.0002	0.9997	1.0006	1.0003	1.0003	0.9999	1.0007	0.9991	0.9991	0.9976	1.0006
Categorical	0 mcg/day	81	320												
	< 400	83	360	0.95	0.95	0.66	1.35	0.78	0.74	0.51	1.18	1.39	1.87	0.78	2.50
	≥400	28	148	0.75	0.72	0.46	1.22	0.90	0.80	0.53	1.53	0.32	0.35	0.09	1.09

Table 1. continued

		n of exposed		All oral clefts *n* = 192				Cleft lip with/without cleft palate *n* = 134				Cleft palate *n* = 58			
		Cases	Controls	OR*	OR**	CI lower	CI upper	OR*	OR**	CI lower	CI upper	OR*	OR**	CI lower	CI upper
Categorical	0 mcg/day	81	320												
	0–200	37	196	0.75	0.76	0.48	1.17	0.60	0.58	0.35	1.03	1.15	1.50	0.56	2.33
	200–400	46	164	1.18	1.19	0.78	1.80	0.98	0.93	0.60	1.63	1.70	2.31	0.86	3.33
	≥400	28	148	0.75	0.78	0.46	1.22	0.90	0.87	0.53	1.53	0.32	0.34	0.09	1.09
Vitamin A															
Binary	Yes/no	102	480	0.82	0.81	0.59	1.13	0.73	0.69	0.50	1.06	1.06	1.29	0.60	1.86
Continuous	mcg/day (0–3200 mcg)	102	480	0.9998	0.9998	0.9993	1.0002	0.9999	0.9999	0.9994	1.0004	0.9994	0.9994	0.9986	1.0002
Categorical	0 mcg/day	90	348												
	< 800	65	327	0.78	0.78	0.54	1.12	0.60	0.58	0.39	0.94	1.28	1.69	0.71	2.33
	≥800	37	153	0.90	0.86	0.57	1.40	1.00	0.95	0.61	1.63	0.60	0.47	0.24	1.50
Categorical	0 mcg/day	90	348												
	0–400	40	173	0.93	0.93	0.61	1.43	0.69	0.63	0.41	1.18	1.60	2.22	0.82	3.11
	400–800	25	154	0.62	0.63	0.38	1.02	0.51	0.52	0.28	0.93	0.93	1.12	0.41	2.10
	≥800	37	153	0.90	0.86	0.57	1.40	1.00	0.95	0.61	1.63	0.60	0.47	0.24	1.50
Vitamin B6															
Binary	Yes/no	104	487	0.83	0.82	0.60	1.15	0.75	0.72	0.51	1.10	1.03	1.25	0.58	1.80
Continuous	mg/day (0–64.5 mg)	104	487	0.9552	0.9552	0.9008	1.0129	0.9741	0.9741	0.9213	1.0300	0.8779	0.8779	0.7664	1.0056
Categorical	0 mg/day	88	341												
	< 1.2	17	81	0.80	0.85	0.44	1.46	0.44	0.45	0.18	1.05	1.78	2.50	0.79	4.04
	≥1.2	87	406	0.84	0.82	0.59	1.17	0.81	0.77	0.55	1.20	0.88	1.01	0.48	1.61
Categorical	0 mg/day	88	341												
	0–1.2	17	81	0.80	0.85	0.44	1.46	0.44	0.45	0.18	1.06	1.78	2.50	0.79	4.04
	1.2–2.4	53	234	0.86	0.85	0.58	1.28	0.88	0.82	0.56	1.38	0.79	0.94	0.39	1.63
	≥2.4	34	172	0.80	0.79	0.51	1.25	0.72	0.70	0.43	1.22	0.99	1.12	0.46	2.13
Vitamin B12															
Binary	Yes/no	102	483	0.82	0.81	0.59	1.14	0.74	0.70	0.51	1.08	1.04	1.27	0.59	1.83
Continuous	mcg/day (0–36 mcg)	102	483	1.0022	1.0022	0.9501	1.0572	1.0072	1.0072	0.9443	1.0744	0.9875	0.9875	0.9100	1.0716
Categorical	0 mcg/day	90	345												
	< 2	46	222	0.76	0.73	0.51	1.15	0.68	0.64	0.42	1.10	0.97	1.09	0.49	1.95
	≥2	56	261	0.87	0.88	0.60	1.28	0.79	0.75	0.51	1.23	1.10	1.43	0.57	2.12

Table 1. continued

		n of exposed		All oral clefts $n = 192$				Cleft lip with/without cleft palate $n = 134$				Cleft palate $n = 58$			
		Cases	Controls	OR*	OR**	CI lower	CI upper	OR*	OR**	CI lower	CI upper	OR*	OR**	CI lower	CI upper
Categorical	0 mcg/day	90	345												
	0–2	46	222	0.76	0.73	0.51	1.15	0.68	0.64	0.42	1.10	0.97	1.09	0.49	1.95
	2–4	11	57	0.86	0.88	0.43	1.71	0.75	0.76	0.32	1.74	1.17	1.39	0.39	3.52
	≥4	45	204	0.88	0.89	0.58	1.32	0.80	0.75	0.50	1.28	1.08	1.44	0.53	2.19

[a]Smoking: Women were questioned more thoroughly about smoking than the other variables. The binary data for smoking represents answers to "Have you been smoking during your pregnancy?" Analyses of the continuous and categorical data were based on mean daily number of cigarettes during first trimester which was estimated on the basis of exact amount of smoking in each pregnancy week.

[b]Alcohol: Total amount of units per week were estimated from information on number of beer, number of glass of vine, and number of glass of strong alcohol consumed per week.

[c]Coffee and Tea: When women were asked about number of daily consumed cups of coffee and tea, the size of a cup were specified to 100–125 ml. No differentiations into types of coffee or tea were done.

[d]Use of food supplements were recorded through the enrolment form. Data on daily supplementation of vitamin A, B6, B12 and folic acid derived from food supplements for each week from conception until enrolment was therefore available and the mean amount for first trimester estimated.

OR*: Adjusted for parental age and social class.

OR**: Non-syndromic oral cleft. CIs are corresponding to model 1.

mean daily supplementation during the first trimester.

Supplementations were categorised according to three different methods:

(1) Fulfilment of the folic acid recommendation (400 mcg folic acid) and supplementation with vitamin A, vitamin B6, and vitamin B12 in an amount corresponding to the recommended dietary allowance for these vitamins (> 800 mcg vitamin A, 1.2 mg vitamin B6, and 2 mcg vitamin B12): (A) zero supplementation, (B) less than the recommended dose, and (C) fulfilment.

(2) Into four categories of mean daily supplementation during the first trimester with an approximately equal number of women in each category to further explore a possible dose effect relationship.

(3) In attempt to be more specific with respect to the exposure periods analyses were restricted to supplementation in the critical periods when the normal closure of the lip (fetal developmental day 7–49) and the palate (fetal developmental day 50–84) occurs.

Cut-off levels for exposure during these periods were 200 and 400 mcg of folic acid, 800 and 3,000 mcg of vitamin A, 1.2 and 2.4 mg of vitamin B6, and 2 and 4 mcg of vitamin B12. Women reporting no supplements during this period were defined as none-exposed. (These data and the corresponding result are not included here).

All analyses were replicated for the 123 non-syndromic oral cleft cases on which information concerning associated anomalies was available from the Danish Facial Cleft Register [1, 2].

Results

Logistic regression analyses (Table 1) of the binary *smoking* variable "have you smoked during pregnancy?" showed a tendency of an association between smoking (although not statistical significant) and an increased prevalence of CL(P) (ORs: 1.48; CIs: 0.97, 2.24) and CP (OR: 1.53; CIs: 0.83, 2.82). None of these were, however, statistical significant. No clear dose–response relations were found when analysing the categorised mean numbers of daily cigarette smoking, although we do not have sufficient information to exclude such an association. Furthermore a possible association between passive smoking by the partner and oral clef was analysed. No association was found (results not shown).

An increased risk of both CL(P) and CP in the children whose mothers had consumed *alcohol* during the first trimester was identified (CL(P): OR 1.11; CIs: 0.75, 1.64 and CP: OR 1. 10; CIs: 0.62, 1.95). An increased prevalence, was, however, only seen for mother with an intake of 3 units/week or more (36+ grams of alcohol).

Coffee intake indicated a protective dose–effect relationship with oral clefts, although not statistically significant. The ORs for the most extreme doses (5 + cups daily) were 0.66 and 0.70 for CL(P) and CP, respectively. *Tea* intake**,** however, seemed to be associated with an increasing risk of CP. OR for the most extreme case-mother group (≥5 cups of tea per day) was 2.47 (CIs: 1.10, 5.55) for CP.

Also high amounts of *cola* consumption seemed to be associated with a moderately increased risk of both CL(P) and CP, although not statistically significant. The effect was reduced (but still larger than 1 in the multivariate analyses when adjusting additionally for smoking and alcohol.

Supplements

Relatively few mothers were consuming the recommended 400 mcg of folic acid daily in all 12 weeks. Analyses indicated a non-significant, protective effect of this dose on the risk of both CL(P) and CP.

Similarly, few of the women took supplements of 800 mcg vitamin A, 1.2 mg B6, and 2 mcg B12 daily as is recommended. A tendency for a protective effect on CL(P) was found for vitamins B6 and B12 and on CP for vitamin A but without any sign of a dose–effect . No overall evidence of a protective effect of these nutrients was found in this study, not even after restricting the analyses to the critical time periods (data not shown).

Except for cola no changes appeared when adjusting additionally for alcohol and smoking, and virtually identical results were found in all analyses restricting the cases to non-syndromic oral clefts (data are not shown). No results changed when analyses were adjusted for week of gestation for data collection.

Discussion

The strength of the present study is the prospectively collected data from a large number of women during pregnancy at the time when the normal oro-facial development occurs and when they are unaware of any fetal defects. Consequently, the exposure data are not subject to differential maternal recall bias.

Furthermore the validity of the cleft diagnoses is very high owing to the Danish Facial Cleft Register [30]. The birth prevalence of CL(P) was 1.4 per 1,000 live births, and exactly as expected [1, 2]. For CP, however, the birth prevalence (0.6 per 1,000 live births) was less than the expected 0.9 per 1,000 live births. This probably reflects that not all submucous cleft palate had been identified at the time of this study. Delayed ascertainment of this subtype is known from previous studies [2]. The low prevalence might also partly be explained by a decreasing trend in the occurrence of CP in Denmark. Indeed CP often occurs as one part of a syndrome and prenatal screening may lower the occurrence of CP at birth.

Data collection regarding the analysed possible risk factors was done early in pregnancy. If CLP outcomes was known to a major part of the women at the time of data collection this might introduce bias. Routine ultrasound examinations were, however, only implemented a few places in Denmark during 1996–2001 and the number of women aware of oral cleft outcome is therefore probably small.

The present case–cohort study revealed a statistically significant OR on 1.50 of smoking for oral clefts and indicated a small association between high maternal alcohol consumption early in pregnancy and oral clefts. Furthermore we found an increased prevalence of CP with intake of tea was found.

Even though 192 cases with prospectively obtained exposure data is a large study population compared with other studies, numbers rapidly decreased when we divided the cases into different subtypes of oral clefts and focused on relatively rare exposures. Several of the analyses, especially those of CP, therefore suffer from lack of statistical power as indicated by wide confidence intervals.

Information on associated anomalies was available for cases born before year 2002. Separate analyses for non-syndromic cases (n = 123) as recommended by the International Consortium for Oral Cleft Genetics [31] were therefore possible, but revealed virtually identical results as when syndromic cases were included. Furthermore the study indicates that although CL(P) and CP may have some distinct genetic components they may be shared environmental etiologies. Our results do not provide any strong support for keeping these diseases separate in analyses on environmental exposures.

Smoking

Our findings on smoking and oral clefts is in agreement with two meta-analyses where OR was found to be approximately 1.3 [11, 17]. The results were also in agreement in regard to CL(P) but not CP with a previous large Danish case control study [32]. This included 95% of oral clefts born in Denmark in 1991–1994 and three controls for each case. Differences in study design (prospectively versus retrospectively obtained data) might explain the difference for CP but also other explanations exist. Comparing smoking habits in the previous and the present Danish studies with general smoking habits in Denmark indicate that both a self-selection into the Danish National Birth Cohort, where smoking pregnant women participated to a lesser degree than non-smoking pregnant women, as well as a general decrease in smoking among pregnant women during the 1990s has occurred [33].

Gene–environment interaction studies have suggested a threefold increase of oral clefts in smoking pregnant women carrying Glutathione *S*-transferase Theta 1-null gene (GSTT1) compared with non-smoking women carrying the wild type genotype, and a 5-fold increase if the foetus were carrying the GSTT1-null gene too [34]. This indicates that the smoking related increased risk of oral clefts might at least partly be due to genetic determined bio-transformation of toxic compounds from the smoke. Smoking might, however, also be just an indication of an addictive behaviour that has some other biologic correlate with oral clefting. Studies of nicotine dependence strongly suggest that genes influence the addictive behaviour of smoking [35–37].

Alcohol

Fewer in the present study were consuming alcohol during pregnancy than in the previous 1991–1994 Danish case–control study [32]. The mean amount of alcohol consumption in the birth cohort (1.71 units/week) was, however, higher than among the participants in the 1991–1994 study (1.43 units/week). Our analyses suggested that high doses of alcohol may play a role for both CL(P) and CP. This was not seen in the previous 1991–1994 study [32], but a dose–response association was found with an even higher effect for CL(P) in a population based case–control study in Iowa [38]. Other previous studies support an association between CL(P) and maternal alcohol consumption during the first trimester of the pregnancy [39–42]. Not all were, however, statistically significant, and the recent study by Meyer et al. [43] including 642 CL(P) cases did not corroborate this association.

Probably due to the smaller number of CP cases, results from previous studies on this subtype is much more conflicting. In a multi-centre case-control study Lorente et al. [42] found an OR of 2.78 (CIs: 1.16, 6.65) for alcohol consumption during pregnancy. This is supported by some [38, 41] but not all studies [39, 40, 43].

Caffeine

Animal studies have found a teratogenic effect of caffeine, but there is no solid evidence to support caffeine as a risk factor for congenital malformations in humans [44–46] or oral clefts in specific [47]. We found no association between oral clefts and coffee although the present study suggested an association with a high consumption of tea and cola. The amount of caffeine in cola (8 mg/100 cc) is minimal compared to the amount in coffee (40 mg/100 cc) and we have no explanation for this finding. Unknown confounders might explain the different association.

Vitamins

Detailed data on vitamins and folic acid supplementation were obtained during the first trimester. The amount in each specific week from 4 weeks before and through the first trimester was known for vitamin A, B6, B12, and folic acid.

Although a protective effect of ≥400 mcg folic acid in the first 12 weeks was indicated no clear effect of the four nutritional supplements was seen.

Biologic explanations for a protective effect of folic acid have been suggested [48], and this is supported by animal models and studies of treatment with folic acid antagonist in humans [22, 49]. In the present study only few participants were actually taking the WHO recommended dose of *folic acid* (400 mcg per day in the first 12 weeks of gestation), and a lack of dose–effect relationship may be due to lack of statistical power or reflect that less than 400 mcg of daily folic acid is below the effective dose. In a recent Hungarian study [21] supplements with multivitamins including 1 mg of folic acid revealed no protective effect of folic acid whereas supplementation of 6 mcg in the critical periods did.

The recommendation of supplementing with 400 mcg folic acid from pregnancy planning and during the first trimester was first promoted in Denmark in 1997. The mean daily supplementation among all participating mothers increased from 149 mcg folic acid in 1997 to 258 in 2002. While this is a substantial improvement it falls far short of recommended guidelines.

A larger part of the women had been taking supplements of the three other vitamins analysed, and a protective tendency of *vitamin B6* and *B12* on the risk of CL(P) was found. The importance of B vitamins (besides folates) is supported by both animal studies [50, 51], and studies in humans [25–27]. Wong et al. [52] found a significantly decreased B6 level together with an increased homocysteine level in 35 case mothers compared to 56 control mothers and suggested that a protective effect of vitamin B6 might act through a lowering of hyperhomocysteinemia in the mother. Poor vitamin B6 and B12 status was, however, independent of maternal homocysteine levels associated with an increased risk of oral clefts in the study by Van Rooij et al. [25].

In conclusion, we used prospectively obtained data to evaluate five environmental risk factors (smoking, alcohol, coffee, tea, and cola) and the supplementation of four nutrients (vitamins A, B6, B12, and folic acid) and were able to corroborate a previously suggested association between oral clefts and smoking. All other findings were, however, not conclusive. An association between a high alcohol intake and oral clefts was suggested but the effect was small and not statistical significant. Furthermore no strong evidence for a protective effect of supplementation with the four studied nutrients was found.

Social class. http://www.dst.dk/HomeDK/Vejviser/dokumentation/Nomenklaturer/DISCO-88.aspx 2005

[Trends in smoking among the Danes] http://www.sst.dk/Borgerinfo/Tobak/Fakta_stat/Udvikling_i_danskernes_rygevaner.aspx?lang = da 4/2003

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