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Maternal age and oral clefts: A reappraisal

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Objective. The aim of the study was to test the hypothesis that increased maternal age is associated with a higher risk of having a child with oral clefts.
Study design. A meta-analysis of 8 population-based studies with information regarding live birth and oral clefts was performed.
Results. No association between increased maternal age and isolated oral clefts was found.
Conclusion. Oral clefts occurrence is not correlated with increasing maternal age, and inclusion of data mixing isolated and syndromic cases can confound the analysis and must be avoided.
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Maternal age is a known risk factor for the occurrence of some chromosome abnormalities, but a consensus about whether maternal age presents an increased risk for having a child with an oral cleft cannot be reached.

The majority of the studies before 1970 suggested an association between increased maternal age and oral clefts. Since 1970, a mixture of positive and negative studies has appeared (see Electronic Appendix for references: http://genetics.uiowa.edu/publications.html). A California study reported that women over 39 years are twice as likely as those 25 to 29 years old to have a child with either cleft palate (CP) or cleft lip with or without cleft palate (CL/P).[1] Another study using data from residents in the San Francisco East Bay area found an association between CP and mothers 24 years old or younger.[2] However, studies performed in Canada, Iran, the Netherlands, and South America did not find an association between increased maternal age and either CL/P and CP.[3-7] The largest study to date, using data from 3 registries of congenital anomalies based on a total of more than 5 million births, reported a U-shaped maternal age relationship for CL/P.[8] Also, an epidemiologic study in China found an association between increased maternal age and bilateral CL/P in males and all types of CL/P in females.[9]

The current analysis revisits these and additional studies with data related to oral clefts and maternal age. The goal is to determine whether older mothers have an increased risk of having a child with an oral cleft.

METHODS

Two basic approaches were used to obtain literature in any language regarding maternal age and oral clefts. First, Medline, Embase, and Science Citation Index databases were searched for studies published from 1966 to 2000 using the keywords "cleft lip and palate," "oral clefts," "orofacial clefts," "maternal age," and "paternal age." A total of 4346 papers were identified. We selected all papers that presented information regarding maternal age and oral clefts. We then scanned through the reference list of the studies obtained in the database searches. Sixty-four papers were selected by using the criteria described (Electronic Appendix: http://genetics.uiowa.edu/publications.html).

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All population-based studies with information regarding the number of live births and CL/P and CP cases by maternal age (<20, 20-24, 25-29, 30-34, 35-39, and ≥40 years) were included in this study. Studies that used maternal age intervals differing from these and studies not specifying the frequency of cases or live births by the maternal age intervals were excluded from the analysis. Details regarding the ascertainment of cases in the individual studies can be found in the original publications.

The association between maternal age groups and both CL/P and CP were measured by the odds ratio (OR) and its associated 95% CI. All statistical analyses were conducted with Epi Info version 6.03.[10] We first reanalyzed each set of study data. Table I shows ORs for the 8 studies included. Then we used the Stouffer method of integrating 1-tailed exact *P* values.[11] This analysis was conducted by using the Meta-Analysis 5.3 package (Table II).[12]

The second step involved the compilation of data from the 8 studies selected for the analysis. The case group comprised the total number of CL/P and CP cases analyzed separately. The control group consisted of the sum of the total live births described in the studies selected. Data regarding paternal age, birth rank, socioeconomic status, or ethnicity by maternal age were not included in the majority of the studies selected, and all analyses were conducted without any adjustment.

In the last step, only nonsyndromic forms of CL/P and CP were compiled for the analysis. Studies that included cases of CL/P and CP with other anomalies, without indicating the number of cases by maternal age, were excluded in this step.[13-16]

RESULTS

From the 8 studies selected for this analysis, 4 originally reported no association between increased maternal age and oral clefts.[6,17-19] We confirmed 2 of the 4 studies that reported a positive association between increased maternal age and oral clefts. The first one showed an increased risk for mothers older than 40 years for CL/P.[15] The other one showed a positive association between mothers older than 30 years and CP and between mothers older than 40 years and CL/P (Table I).[16]

Table II shows the results of the heterogeneity analysis. The original studies did not provide appropriate statistics to allow calculations of effect sizes; therefore, the meta-analysis was reduced to a combination of probabilities. One-tailed *P* values were calculated comparing maternal age categories 25-29 years and ≥40 years (for reference 19, the comparison group was 35-39 years). For CL/P, the combination of all studies suggests rejection of the hypothesis of the null effect of maternal age higher than 40 years over the risk of having a child with oral clefts (unweighted and weighted solutions are concordant). The corresponding effect size *r* for each solution indicates that the variance observed between the studies is mainly the result of sampling error than systematic factors. However, the test of homogeneity indicates heterogeneity between the studies. This is partly explained because some studies have negative outcomes. Also 4 studies combined information of isolated cases and cases with other anomalies. Therefore, conclusions drawn from the compilation of the data must be cautiously exercised. The Fail-Safe *N* shows the number of nonsignificant “file drawer studies” necessary to invalidate a significant overall result at a certain predefined level. This number indicates how many no-effect findings would have to exist in the file drawers (either because of reporting bias or publication bias) to invalidate a significant overall *P*. For CL/P, 20 unpublished studies or studies with incomplete data showing no effects of the increased maternal age over the risk for oral clefts would have to be tracked down in the file drawers to overturn the combined significance of .00002 of the 8 available studies. When only the information about isolated cases of CL/P is compiled, the unweighted and weighted solutions are discordant. Therefore, the hypothesis of the null effect of the maternal age higher than 40 years over the risk of having a child with CL/P can be confirmed. The test of homogeneity indicates homogeneity between the studies. The Fail-Safe *N* became negative and should be ignored. For CP, both data from all studies and data for only isolated cases showed that the unweighted and weighted solutions are discordant. Therefore, the hypothesis of the null effect of the maternal age higher than 40 years over the risk of having a child with CP can be confirmed. The test of homogeneity indicates homogeneity between the studies with data for only isolated cases and heterogeneity for the combination of all studies.

Table III shows odds ratios by maternal age from the 8 studies selected for the analysis. The data show that women 20-24, 30-34, and over 40 years are more likely to have a child with CL/P. For CP, women 20-24 and over 30 years show significant results.

Table IV shows data from the 4 studies that included a separate description by maternal age for isolated cases of CL/P and CP or included only isolated cases with CL/P and CP.[6,17-19] The data show that there is no association between increased maternal age and isolated CL/P or CP.

Table I. Frequencies and odds ratios (95% CIs) for the references selected for this study (references are listed in chronological order)*

Reference (study location)	*Maternal age*					
	<20	*20-24*	*25-29*	*30-34*	*35-39*	*≥40*
6 (The Netherlands)						
Population	1696	16,237	32,018	18,334	4,607	534
CL/P	2 OR = 1.18 (0.28-4.93)	16 OR = 0.99 (0.54-1.8)	32 OR = 1.0 (−)	19 OR = 1.04 (0.59-1.83)	4 OR = 0.87 (0.31-2.46)	1 OR = 1.87 (0.25-13.74)
CP	2 OR = 6.29 (1.27-31.2)	5 OR = 1.64 (0.5-5.38)	6 OR = 1.0 (−)	6 OR = 1.75 (0.56-5.41)	0	0
19 (Russia)[†]						
Population	10,032	60,423	37,889	22,552	12,005	—
CL/P	6 OR = 0.73 (0.3-1.75)	45 OR = 0.91 (0.58-1.44)	31 OR = 1.0 (−)	17 OR = 0.92 (0.51-1.67)	8 OR = 0.81 (0.37-1.77)	—
CP	5 OR = 0.82 (0.31-2.16)	27 OR = 0.74 (0.42-1.28)	23 OR = 1.0 (−)	9 OR = 0.66 (0.3-1.42)	5 OR = 0.69 (0.26-1.81)	—
18 (United States)						
Population	24,291	71,504	55,083	32,503	16,080	5816
CL/P	24 OR = 0.98 (0.61-1.59)	71 OR = 0.99 (0.69-1.41)	55 OR = 1.0 (−)	32 OR = 0.98 (0.63-1.52)	14 OR = 0.87 (0.48-1.56)	2 OR = 0.34 (0.08-1.41)
CP	7 OR = 0.69 (0.29-1.6)	22 OR = 0.73 (0.41-1.32)	23 OR = 1.0 (−)	12 OR = 0.88 (0.43-1.77)	7 OR = 1.04 (0.44-2.43)	2 OR = 0.29 (0.07-1.26)
16 (United States)						
Population	1,240,100	3,060,500	2,126,000	1,214,100	644,700	190,200
CL/P	916 OR = 0.88 (0.81-0.95)	2,595 OR = 1.01 (0.95-1.07)	1,792 OR = 1.0 (−)	1,040 OR = 1.02 (0.94-1.1)	568 OR = 1.05 (0.95-1.15)	226 OR = 1.41 (1.23-1.62)
CP	411 OR = 0.95 (0.84-1.07)	1,120 OR = 1.05 (0.96-1.15)	741 OR = 1.0 (−)	481 OR = 1.14 (1.01-1.27)	295 OR = 1.131 (1.15-1.5)	111 OR = 1.67 (1.37-2.04)
17 (Hungary)						
Population	107,560	330,968	218,855	108,242	45,716	12,979
CL/P	46 OR = 0.72 (0.51-1.01)	202 OR = 1.03 (0.83-1.29)	129 OR = 1.0 (−)	74 OR = 1.15 (0.87-1.54)	34 OR = 1.26 (0.86-1.84)	13 OR = 1.69 (0.96-3.0)
CP	13 OR = 0.51 (0.28-0.95)	44 OR = 0.57 (0.38-0.85)	51 OR = 1.0 (−)	11 OR = 0.43 (0.22-0.83)	8 OR = 0.75 (0.35-1.58)	2 OR = 0.66 (0.16-2.71)
15 (United States)						
Population	39,666	99,169	83,511	58,458	24,010	8350
CL/P	42 OR = 0.97 (0.67-1.4)	101 OR = 0.93 (0.7-1.24)	91 OR = 1.0 (−)	79 OR = 1.24 (0.91-1.67)	32 OR = 1.22 (0.81-1.83)	23 OR = 2.52 (1.59-3.99)
14 (Australia)						
Population	3979	16,120	16,187	10,627	5517	1779
CL/P	5 OR = 0.84 (0.32-2.22)	23 OR = 0.96 (0.54-1.7)	24 OR = 1.0 (−)	20 OR = 1.26 (0.7-2.29)	13 OR = 1.58 (0.8-3.12)	4 OR = 1.55 (0.52-4.37)
CP	1 OR = 0.27 (0.03-2.05)	7 OR = 0.46 (0.19-1.14)	15 OR = 1.0 (−)	4 OR = 0.4 (0.13-1.22)	7 OR = 1.36 (0.55-3.35)	0
13 (England)						
Population	1057	4223	4056	2640	1556	432
CL/P	1 OR = 0.51 (0.06-3.84)	2 OR = 0.24 (0.05-1.13)	8 OR = 1.0 (−)	3 OR = 0.58 (0.15-2.17)	2 OR = 0.65 (0.14-3.07)	1 OR = 1.17 (0.15-9.41)

CL/P, Cleft lip with or without cleft palate; *CP,* cleft palate only; *OR,* odds ratio.

*Crude ORs were calculated by the authors of the current study. Most of the original report conclusions come from frequency comparisons between cases and population.

[†]This study did not report any case and population frequency of mothers older than 40 years.

DISCUSSION

A meta-analysis has several potential biases, including publication bias, bias in location of studies (English language bias, database bias, citation bias, multiple publication bias), bias in provision of data, and poor methodologic quality.[20] Among these, this study could eliminate only the multiple publication bias. Several studies could not be included because they did not

Table II. Results of meta-analysis for probabilities (Stouffer method)

	CL/P		*CP*	
	All cases	*Only isolated cases*	*All cases*	*Only isolated cases*
Number of studies	8	4	6	4
Total sample size of mothers older than 40 years	8488	877	2,363	290
Unweighted solution				
Average *z* value	3.09	1.17	1.24	−0.75
P value (1-tailed)	.0009	.12	.10	.22
Corresponding effect size	0.033	0.039	0.025	−0.04
Weighted solution				
Average *z* value	4.02	1.72	3.68	−0.50
P value (1-tailed)	.00002	.04	.0001	.30
Corresponding effect size	0.043	0.058	0.075	−0.02
Fail-Safe *N* (*P* = .05)	20	−1	−2	−3
Fail-Safe *N* (*P* = .01)	6	−2	−4	−3
Test of homogeneity				
χ^2	23.20	2.36	14.15	0.60
Degrees of freedom	7	3	5	3
P value	.001	.50	.01	.89

Table III. Odds ratios for CL/P and CP including all cases

	CL/P		*Controls*	*CP*		*Controls*
Maternal age	*N*	*OR (95% CI)*	*N*	*N*	*OR (95% CI)*	*N*
<20	1142	0.87 (0.81-0.94)	1,428,381	439	0.92 (0.82-1.03)	1,387,658
20-24	3055	1.19 (1.12-1.25)	3,064,723	1325	1.29 (1.19-1.41)	2,961,331
25-29	2162	1.0 (−)	2,573,599	859	1.0 (−)	2,486,032
30-34	1284	1.12 (1.05-1.2)	1,359,214	523	1.17 (1.05-1.3)	1,298,116
35-39	675	1.07 (0.98-1.16)	754,191	322	1.28 (1.13-1.45)	728,625
≥40	270	1.46 (1.29-1.68)	220,090	115	1.58 (1.3-1.91)	211,308
Total	8488	—	9,400,198	3583	—	9,073,070

Data from the 8 studies selected. Among these, 4 studies included cases with at least one associated anomaly. The frequency of the associated cases by the maternal age intervals could not be accessed in the original reports.
CL/P, Cleft lip with or without cleft palate; *CP,* cleft palate only; *OR,* odds ratio.

report either the frequency of cases or population for the maternal age intervals proposed in this study. As a matter of fact, all but one study not selected for this work reported the data for both cases and population in different age categories.[21] This shows how difficult it was to perform this meta-analysis.

Even though our data could not be adjusted for possible confounding factors, differences found in Tables III and IV might be related to the inclusion of studies that also used nonisolated cases.[13-16] Older mothers are known to be at a higher risk for trisomy 13, 18, or 21. One of the clinical characteristics of these chromosomal abnormalities is CL/P or CP. Thus, syndromic cases might confound some studies.

The results from the compilation of the raw data were reproduced by a formal meta-analysis technique (Table II), and all this information together is strongly suggestive that increased maternal age is not associated with an increased risk for nonsyndromic oral clefts.

The current data set is comparable with that of the largest study to date.[9] This study reported a very weak maternal age effect for CL/P. A slight U-form dependence of maternal age is seen with an increased risk in women below 25 and above 40 years. The authors suggested that their results might be partly explained by undetected chromosome anomalies.

Differences in the ascertainment of the cases in the different studies could have affected the results of the current study. Three studies, all from the United States, collected data from birth certificates.[15,16,18] This kind of study is known to miss cases and will also be suboptimal in identifying syndromes, especially in the older

Table IV. Odds ratios for CL/P and CP using data from isolated cases exclusively

Maternal age	CL/P		CP		Controls
	N	OR (95%)	N	OR (95% CI)	N
<20	78	0.76 (0.57-0.96)	27	0.63 (0.41-0.96)	143,579
20-24	334	0.97 (0.82-1.14)	98	0.68 (0.52-0.9)	479,132
25-29	247	1.0 (−)	103	1.0 (−)	343,845
30-34	142	1.09 (0.89-1.34)	38	0.7 (0.48-1.01)	181,631
35-39	60	1.07 (0.8-1.41)	20	0.85 (0.53-1.37)	78,408
≥40	16	1.15 (0.69-1.91)	4	0.69 (0.25-1.88)	19,329
Total	847	—	290	—	1,245,924

Original reports described the frequency of isolated cases independently from associated or syndromic cases or reported data from isolated forms of clefting exclusively.
CL/P, Cleft lip with or without cleft palate; *CP,* cleft palate only; *OR,* odds ratio.

studies. Two studies used data from birth defects registries from The Netherlands and Hungary.[6,17] Three studies from Liverpool (England), Tasmania (Australia), and Moscow (Russia) used data from hospital records.[13,14,19]

One important confounding factor might be the income status associated with racial distribution in some populations. The 3 studies from the United States have a high percentage of white women among cases and live births, but no data regarding income status is given.[15,16,18] The same absence of income data occurs in the studies from England, Australia, Hungary, Russia, and The Netherlands.[6,13,14,17,19] Among all 64 studies selected in the current study, only 1 studied income status.[22] This study showed a statistical difference between cases and controls. Difficulties in obtaining a consistent measurement of socioeconomic status, especially across different populations and different countries, might explain why this variable was not extensively studied.

Birth order is another variable reported to be associated with CL/P and CP. The Russian study showed a positive association with increased number of previous births but no association with increased maternal age.[19] The largest study from the United States suggested that both increased maternal age and higher birth order contribute independently to CL/P and CP.[16]

One substantive confounder of maternal age is paternal age. Several studies provide data regarding paternal age (see Electronic Appendix for references). Because it is well recognized that a number of single gene disorders correlate with new mutations associated with increasing paternal age, such as achondroplasia, Apert syndrome, and neurofibromatosis, it is possible that there is a similar effect for cleft lip and palate. Recently, an autosomal dominant mutation in the MSX1 gene was described as causing a phenotype consistent with nonsyndromic clefting.[23] This would support the observation that an increase in maternal age might underlie an increasing rate of mutations associated with advancing paternal age and that the association with maternal age is a surrogate for mutations arising in older fathers. If new mutations can be identified as the cause of CL/P and CP in 1 or more genes, such as MSX1, then this hypothesis can be tested directly, as is already the case for Apert syndrome.[24,25] Other possible variables to test include differences in nutrition and cigarette smoking habits. There is an indication that folic acid supplementation can protect children at risk for some congenital malformations, including CL/P and CP.[26,27] Analysis of various maternal age groups that have used prenatal folic acid supplementation could clarify if there is any difference in the action of folic acid between age groups. On the other hand, because cigarette smoking increases the risk for CL/P and CP whether or not it is associated with specific genetic markers, stratifying the data by maternal age could show if there is any difference in gene-environment interactions among age groups.[28-31]

Caution should be exercised with regard to drawing conclusions that the data cannot support.[4] Because, at present, only a small percentage of birth defects are preventable, knowledge of any risk factor that increases the risk for a common birth defect such as oral clefts is relevant. We do not have reasons to believe that increased or decreased maternal ages increase the risk of having a child with nonsyndromic forms of CL/P or CP. Special attention to folic acid intake and prenatal care might provide extra protection to at least a percentage of all pregnancies.

In conclusion, a meta-analysis using data from 8 studies that investigated a possible association between maternal age and CL/P and CP suggests there is no association between increased maternal age and isolated forms of CL/P and CP.

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Appendix. Electronic Appendix (http://genetics.uiowa.edu/publications.html). Studies identified by the selection method used and results of the association between oral clefts and increased maternal/paternal age originally reported

Bibliographical entry and study location	*Included or not*	*Number of subjects CL/P*	*CP*	*Study design and results reported*
Malpas (1937) – Liverpool, England	Yes	17	—	Population-based study. Case and population maternal age frequencies were compared. Association with increased maternal age.
Phair (1947) – Wis	No[a]	840	294	Population-based study. Case and population maternal and paternal age frequencies were compared. Suggestive association with both increased maternal and paternal ages.
de Voss (1952) – San Bernardino County, Calif	No[a,f]	42 total		Population-based study. Case and population maternal age frequencies were compared. No association with increased maternal age.
McEvitt (1952) – Detroit, Mich	No[e,f]	282 total		Subjects from The Straith Clinic. Case and population maternal and paternal age frequencies were compared. No association with both increased maternal and paternal ages.
MacMahon and McKeown (1953) – Birmingham, England	No[e]	168	105	Case-control study. Case and control frequencies were compared. Association between increased maternal age and CL/P.
Mazaheri (1957) – Lancaster, Pa	No[a]	661 total		Subjects from Lancaster Cleft Palate Clinic. Comparison of frequencies was performed by the χ^2 test. Association with increased maternal age.
Mazahari (1958) – Lancaster, Pa	No[a]	540 total		Case-control study. Comparison of frequencies was performed by the χ^2 test. Association with increased maternal age.
Shapiro et al. (1958) – Kings County (Brooklyn) New York, NY	No[c]	23	24	Asssociation not tested.
Oldfield (1959) – England	No[a]	643	—	Source of the cases not described. Case and population maternal age frequencies were compared. Association with increased maternal age.
Rank and Thomson (1960) – Tasmania, Australia	Yes	85	34	Population-based study. Comparison of frequencies was performed by the χ^2 test. Association with increased maternal age.
Fraser and Calnan (1961) – Oxford, England	No[a]	221	152	Case-control study. Case and control maternal and paternal mean ages compared. Suggestive association with increased paternal age. No association with maternal age.
Loretz et al. (1961) – California	Yes	368	—	Population-based study. Comparison of frequencies was performed by the χ^2 test. Association with increased maternal age.
Knox (1963) – Northumberland and Durham, England	No[a]	227	102	Population-based study. Case and population maternal and paternal age frequencies were compared. Suggestive association with increased maternal age. No association with paternal age.
Woolf (1963) – Utah	No[a]	411	—	Case-control study. Comparison of frequencies was performed by the χ^2 test. Association with both increased maternal and paternal ages.
Woolf et al. (1963) – Utah	No[a,b]	418	135	Population-based study. Comparison of frequencies was performed by the χ^2 test. Association between increased maternal age and CL/P.
Greene et al. (1964) – Calif, Hawaii, Pa, and Wis	No[a]	3,181	1,270	Population-based study. Maternal, paternal, and population age frequencies were compared. Association with both maternal and paternal ages.
Ingalls et al. (1964) – Philadelphia and Allentown, Pa	No[a]	69	29	Population-based study. Maternal and population age frequencies were compared. No association with increased maternal age.
Donahue (1965) – 17 states, USA	No[a]	6,070	—	Case-control study. Maternal, paternal, and control age frequencies were compared. Association with both increased maternal and paternal ages.
Greene et al. (1965) – 29 states, Baltimore, Md, and New York, NY	No[a]	1,381	622	Case-control study. Case and control maternal and paternal age frequencies were compared. Association with both increased maternal and paternal ages.
Milham Jr. and Gittelsohn (1965) – NY State	No[a]	2,714 total		Population-based study. Cases with multiple malformations analyzed together. Maternal and paternal age frequencies compared with population frequency. Suggestive association with both increased maternal and paternal ages.
Altemus (1966) – District of Columbia	No[a]	30	30	Author describes maternal age frequency by each cleft type. Suggestive association with decreased maternal age.

Appendix. Continued

Bibliographical entry and study location	*Included or not*	*Number of subjects CL/P*	*CP*	*Study design and results reported*
Bardhan (1967) – Delhi, India	No[a]	189	119	Author describes maternal and paternal age frequencies by each cleft type. Suggestive association with both increased maternal and paternal ages.
Hay (1967) – 29 states, Baltimore, Md, and New York, NY	No[a,b]	4,654	2,044	Population-based study. Maternal, paternal, and population age frequencies were compared. Association between both increased maternal and paternal ages and CL/P.
Meskin and Pruzansky (1968) – Minn	No[a]	232	208	Case-control study. Case and control maternal and paternal mean ages compared. Suggestive of association with both increased maternal and paternal ages.
Bardanouve (1969) – Mont	No[e]	242	121	Population-based study. Maternal age frequency for each cleft type is compared with the population frequency. Suggestive association with increased maternal age.
Bodnar (1969) – Szabolcs-Szatmar, Hungary	No[e]	164	—	Population-based study. Comparison of frequencies was performed by the χ^2 test. Association with increased maternal age.
Borçbakan (1969) – Turkey	No[a]	745	255	Author describes maternal age frequency for each cleft type. No association with increased maternal age.
Browne (1969) – Christchurch, New Zealand	No[a]	44	—	Subjects from Burwood Hospital. Comparison of cases and population frequencies was performed by the χ^2 test. No association with increased maternal age.
Fraser (1970)	No[d]	—	—	—
Czeizel and Tusnadi (1971) Budapest, Hungary	Yes	114	51	Population-based study. Comparison of frequencies was performed by the χ^2 test. No association with both increased maternal and paternal ages.
Woolf (1971) – Salt Lake City, Utah, and Phoenix, Ariz	No[b]	496	—	Association not tested.
Hay and Barbano (1972) − 29 states, Baltimore, Md, and New York, NY	Yes	7,137	3,159	Population-based study. Case and population maternal age frequencies were compared. Association with increased maternal age.
Perry and Fraser (1972) – Montreal, Canada	No[a]	350	89	Case-control study. Case and control maternal and paternal mean ages were compared. Suggestive of association with both increased maternal and paternal ages.
Emanuel et al. (1973) – King County (Seattle area), Wash	Yes	198	77	Population-based study. Comparison of frequencies was performed by the χ^2 test. No association with increased maternal age.
Ching and Chung (1974) – Hawaii	No[a]	341	195	Population-based study. Regression analysis showed a definite association between Hawaiian increased maternal and paternal ages and CP.
Saxén (1974) – Finland	No[a]	232	230	Case-control study. Case and control maternal and paternal age frequencies were compared. Association with both increased maternal and paternal ages.
Saxén (1975) – Finland	No[a,b]	85	105	Case-control study. Comparison of frequencies was performed by the χ^2 test. No association with both increased maternal and paternal ages.
Spry and Nugent (1975) – Adelaide, Australia	No[a]	368	184	Population-based study. Comparisons of observed and expected frequencies were performed by the χ^2 test. Association with both increased maternal and paternal ages and CP.
Leck (1976)	No[d]	—	—	—
Polednak (1976) – upstate NY	No[f]	897 total		Population-based study. CL/P and CP analyzed together. Comparison of frequencies was performed by the χ^2 test. No association with both increased maternal and paternal ages.
Burdi (1977)	No[d]	—	—	—
Gombos et al. (1980) – Naples, Italy	No[a]	29	30	Authors describe maternal age frequency for each cleft type. No association with increased maternal age.
Welch and Hunter (1980) – Manitoba, Canada	No[a]	264	124	Population-based study. Case and population maternal and paternal mean ages compared. Authors did not suggest anything from the maternal age comparison.

Appendix. Continued

Bibliographical entry and study location	Included or not	Number of subjects CL/P	CP	Study design and results reported
Bonaiti et al. (1982) – France	No[a]	104	44	Population-based study. Comparison of frequencies was performed by the χ^2 test. No association with both increased maternal and paternal ages.
Padron Caseres and Prytkov (1982) – Moscow, Russia	Yes	107	69	Population-based study. Comparison of frequencies was performed by the χ^2 test. No association with increased maternal age.
Balgir (1984)	No[d]	—	—	—
Amaratunga (1985) – central Sri Lanka area	No[a]	395	106	Case-control study. Comparison of frequencies was performed by the Fisher exact test. No association with increased maternal age.
Owens et al. (1985) – Liverpool, England	No[a]	166	153	Population-based study. Case and population maternal age frequencies were compared. No association with increased maternal age.
Lian et al. (1986) – Atlanta, Ga	No[a]	352	203	Population-based study. Paternal age tested by logistic regression corrected by maternal age and race (CL/P, OR = 1.0; CP not tested because of the small sample size). Maternal age not tested.
Womersley and Stone (1987) – Glasgow, Scotland	No[a]	65	44	Population-based study. Comparison of frequencies was performed by the χ^2 test. Association between increased maternal age and cleft lip alone.
Calzolari et al. (1988) – Emilia Romagna, Italy	No[a]	86	49	Population-based study. Comparison of frequencies was performed by the χ^2 test. No association with both increased maternal and paternal ages.
Jensen et al. (1988) – Denmark	No[a]	492	186	Population-based study. Maternal and paternal age means were analyzed by student *t* test. No association with both increased maternal and paternal ages.
Sayetta et al. (1989)	No[d]	—	—	—
Baird et al. (1991) – British Columbia, Canada	No[a]	669	444	Population-based study. Isolated cases studied together with cases with other anomalies. No association with increased maternal age.
Menegoto and Salzano (1991) – ECLAMC, South America	No[a]	741	115	Case-control study. Maternal and paternal age means were analyzed by student *t* test. No association with increased maternal and paternal ages.
Savitz et al. (1991) – San Francisco, Calif	No[a]	17	11	Population-based study. Variables tested by logistic regression. No association between both increased maternal and paternal ages and CL/P. Association for CP with increased paternal age and decreased maternal age.
Shaw et al. (1991), Calif	No[a]	335	173	Case-control study. Association with increased maternal age was reported although questionable odds ratios (CL/P, OR = 2.3 [0.99-5.2]; CP, OR = 2.7 [1.0-7.4]).
Stoll et al. (1991) – Alsace, France	No[a]	83	48	Case-control study. Comparison of frequencies was performed by the χ^2 test. No association with both increased maternal and paternal ages.
Cornel et al. (1992) – The Netherlands	Yes	120	48	Population-based study. Comparison of frequencies was performed by the χ^2 test. No association with increased maternal age.
Slavkin (1992)	No[d]			—
Baird et al. (1994), British Columbia, Canada	No[a]	475	227	Population-based study. Data analyzed for linear trends in the incidence rates of both defects by maternal age. No association with increased maternal age.
Robert et al. (1996) – central-east France, Sweden and Calif	No[a]	4,261	1,794	Population-based study. Association between CL/P and mothers 20-24 years of age (OR = 1.11 [1.04-1.19]) and over 40 years old (OR = 1.41 [1.13-1.76]), and CP and mothers 35-39 years of age (OR = 1.19 [1.02-1.38]).
Cooper et al. (2000) – Shanghai, China	No[a]	643	-	Population-based study. Association between bilateral CL/P in males and increased maternal age (OR = 1.33 [1.03-1.73]) and between all types of CL/P in females and increased maternal age + increased birth order (1.16 [1.05-1.27]).
Rajabian and Sherkat (2000) - Iran	No[a]	1,380	289	Case-control study. Comparison of frequencies was performed by the χ^2 test. No association with increased maternal age.

CL/P, Cleft lip with or without cleft palate; *CP,* cleft palate only; *OR,* odds ratio.
Reasons for not being included in this study: [a]maternal age frequency of the cases and/or the population was not reported; [b]same data used in previous report; [c]oral cleft data are mixed with other major congenital malformations; [d]literature review; [e]samples divided in age ranges different than was proposed in the current study; [f]CL/P and CP data mixed. -

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