DOCTOR OF DENTAL SCIENCE

A Preliminary Investigation into Orofacial Clefts in the Central and Western Regions of Saudi Arabia

Sabbagh, Heba J.

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ORIGINAL ARTICLE

Parental Consanguinity and Nonsyndromic Orofacial Clefts in Children: A Systematic Review and Meta-analyses

Heba J. Sabbagh, B.D.S., M.S.c., Mona Hassan Ahmed Hassan, B.D.S., M.S.c., Ph.D., Nicola P. T. Innes, B.D.S., B.M.Sc., Ph.D., Alaa Al Baik, B.D.S., Peter A. Mossey, B.D.S., Ph.D.

Objective: **To assess whether individuals born to consanguineous parents had a higher frequency of nonsyndromic orofacial clefts compared with those with no parental consanguinity.**

Design: **A prespecified plan for a search strategy, inclusion/exclusion criteria, and data extraction from studies reporting consanguinity in relation to nonsyndromic orofacial clefts (NSOFC) was carried out. Papers reporting observational studies with control populations were included, without language restrictions, and these reports were assessed for quality. Sensitivity analyses using subgroups, homogeneity evaluation, and assessment of publication bias were carried out, and meta-analyses of extracted data were performed.**

Results: **Sixteen studies fulfilled the selection criteria and were included in the meta-analyses. There were statistically significant relationships between consanguinity and NSOFC for all 16 studies combined ($P = .0003$), with odds ratio (OR) = 1.83 and 95% confidence interval (CI) = (1.31, 2.54); 10 case-control studies ($P = .006$), with OR = 2.06 and 95% CI = (1.23, 3.46); six cross-sectional studies ($P = .03$), with OR = 1.34 and 95% CI = (1.02, 1.76); first cousins consanguineous marriages ($P = .04$), with OR = 1.40 and 95% CI = (1.01, 1.93); cleft palate alone ($P = .01$), with OR = 1.89 and 95% CI = (1.14, 3.13); and cleft lip with or without cleft palate cases ($P = .002$), with OR = 1.56 and 95% CI = (1.18, 2.07).**

Conclusion: **Although there was a high level of study heterogeneity, the evidence is consistent in suggesting that consanguinity is a risk factor for NSOFC, with an overall OR of 1.83 (95% CI, 1.31 to 2.54), implying that there was almost twice the risk of a child with NSOFC being born if there was parental consanguinity.**

KEY WORDS: *cleft lip, cleft palate, consanguinity, etiology, meta-analysis*

Nonsyndromic orofacial clefts (NSOFC), including cleft lip (CL), cleft lip with or without cleft palate (CL±P), and isolated cleft palate (CP), are among the most common birth defects in the world (Mossey and Little, 2009). Although NSOFC is known to be a multifactorial disorder with both genetic and environmental risk factors involved, its etiology is not fully understood. One proposed risk factor is parental consanguinity (Mossey and Castilla, 2003).

Ms. Sabbagh is Lecturer, King Abdulaziz University, Jeddah, Saudi Arabia, and PhD student, Dundee University, Scotland, United Kingdom. Dr. Hassan is Professor of Biostatistics, Department of Preventive Dental Sciences, Faculty of Dentistry, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia, and Department of Biostatistics, High Institute of Public Health, Alexandria University, Alexandria, Egypt. Dr. Innes is Senior Lecturer, University of Dundee, Dundee, Scotland, United Kingdom. Mr. Al Baik is Resident, King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia. Dr. Mossey is Consultant Orthodontist and Associate Dean of Research, University of Dundee, Dundee, Scotland, United Kingdom.

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Address correspondence to: Ms. Heba J. Sabbagh, 38 Thorter Row, City Quay, DD1 3BW, Dundee, Scotland, U.K. E-mail: hjsabbagh@gmail.com.

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Consanguinity is known to be a risk factor for autosomal recessive disorder–related birth defects, increasing the chance of an infant being born with a homozygous genotype for a disease-associated allele (Radha Rama et al., 1987; Ozalp et al., 1990). However, as NSOFC is a multifactorial disease, the genetic basis of the disease differs. In addition, most NSOFC cases (more than 60%) do not have a reported family history of NSOFC (Sabbagh et al., 2012). Moreover, countries with high consanguinity prevalence, such as the Middle East and North Africa, with the highest prevalence of consanguineous marriages in the world, have a prevalence of NSOFC similar or less than European countries with rare consanguineous marriages (Mossey and Castilla, 2003).

Consanguinity varies in prevalence and type, both geographically and between different cultures (Bittles, 2001). Consanguineous relationships are generally considered to be those between first cousins, first cousins once removed, and second cousins, although consanguineous marriages also occur between uncles and nieces in India (Bittles, 1998). In Western societies, it is rare, but in India and Middle Eastern countries, it can involve more than 50% of the population (El-Hazmi et al., 1995).

Although parental consanguinity is generally accepted as a risk factor for some birth defects, the association between consanguinity and orofacial clefting is still unclear (Fraser and Biddle, 1976; Tadmouri et al., 2009; Alsahafi, 2010).

The aim of this study was to carry out a systematic review of the literature and, if appropriate, meta-analyses, to study and clarify the association between consanguinity and NSOFC.

Material and Methods

Literature Search

A research protocol comprising a search strategy, inclusion/exclusion criteria, and a plan for data extraction from studies was prepared. Institutional review board approval was taken for the research from the Saudi Ministry of Health. The search strategy included key words listed separately or combined. These key words included *consanguinity*, *orofacial cleft*, *cleft lip AND cleft palate*. Another search was carried out with the same key words combined with the list of countries and regions with known high consanguinity prevalence to be greater than 20% of the population (Bittles, 1998). These countries/regions were India, Pakistan, Bangladesh, Sri Lanka, Tunisia, Egypt, Sudan, Algeria, Guinea, Tanzania, Nigeria, Turkey, Middle East, Jordan, Palestine, Saudi Arabia, United Arab of Emirates, Bahrain, Kuwait, Lebanon, Iraq, Daizu, Kirgiz, Tajik, and Uzbek. The search was run, with no language restrictions, in three search engines (PubMed, Scopus, Google Scholar) covering 1980 to 2012. Google Scholar was used in an attempt to detect data that may be available in local journals but not listed in Medline. The search was run in January 2012, and the search strategy is available on request from the corresponding author. In addition, researchers were contacted who were known to have published and have interest in the field.

All titles from the search were reviewed for relevance independently by two authors (H.J.S. and A.A.B.). Reference lists of all articles were reviewed for other studies. The abstracts of titles considered likely to be relevant were reviewed, and all articles that did not discuss NSOFC risk factors or describe orofacial cleft epidemiology were excluded. Authors were contacted to provide clarity on any aspects of the data as necessary. The full-text articles for remaining abstracts were screened, independently and in duplicate by the same two authors, for inclusion based on predefined criteria.

Inclusion criteria:

- Case-control studies comparing consanguinity in NSOFC against control group
- Cross-sectional studies comparing the prevalence of consanguinity in NSOFC cases with the prevalence of consanguinity in noncleft patients in the same community or the general population

Exclusion criteria:

- Case series or epidemiological studies in which results were not compared with controls
- Studies with unclear sampling, methodology, or results
- Studies that discussed congenital abnormalities as a whole and did not specify NSOFC cases in the analysis

Consensus agreement was reached for the included studies.

Data Extraction

The following data were extracted:

- Study design and setting
- Sample size, description, and base population characteristics
- Prevalence and type of consanguineous marriages in cases and controls
- Prevalence and type of NSOFC cases

If NSOFC cases could not be attributed to consanguinity or nonconsanguinity groups or where there was missing information, the study was excluded. In cross-sectional studies, the prevalence of consanguinity in the general population was considered to be the control.

Quality Assessment

The quality of the included articles was assessed independently by two of the authors (H.J.S. and A.A.B.) using the Newcastle-Ottawa Quality Assessment Scale (NOS; Wells et al., 2010). The scale has a minimum score of 0 and a maximum score of 9. Studies achieving >6 (equivalent to $>67\%$ of the maximum score) were regarded as "good" quality (Wong et al., 2008), 3 to 6 as "fair," and <3 poor quality. If the studies provided the appropriate data, they were not excluded on the basis of quality. Sensitivity analysis was conducted, and the Kappa statistic was used to measure the degree of agreement between the authors' judgments. A Kappa of 0.83 was obtained, which indicated good agreement. Disagreements were resolved by consensus.

Sensitivity Analysis

To assess the stability of the results, subgroup analyses were carried out based on the quality of the study (NOS >6 versus ≤ 6), year of publication (pre- versus post-2000), type of study (case control versus cross-sectional), and the level of consanguinity in the general population (high versus low). Also, these areas of subgrouping were thought to be possible sources of heterogeneity.

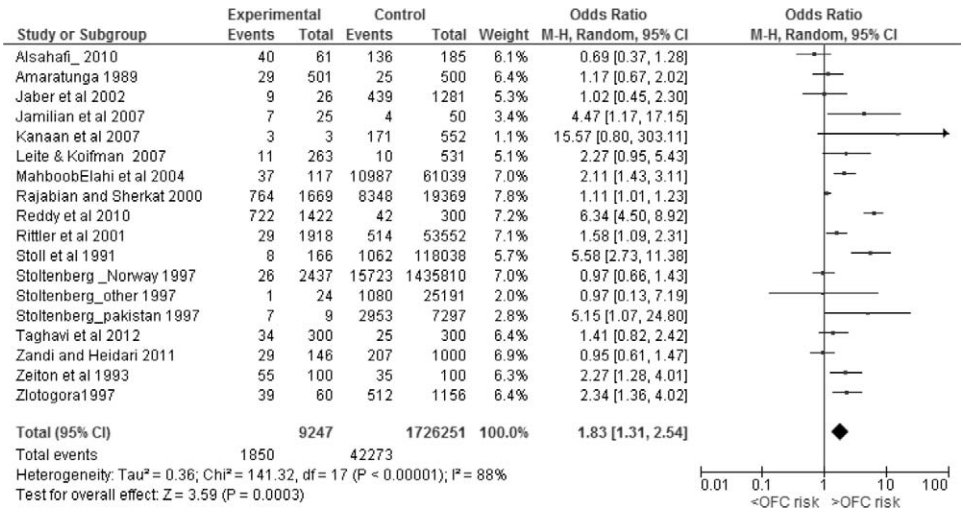

FIGURE 1 Forest plot for the meta-analysis of the association between consanguinity and the risk of NSOFC for all 16 studies combined.

Evaluation of Heterogeneity

A statistical test of heterogeneity was carried out. This test assumes that differences in the results of the individual studies are due to chance alone (i.e., that all study results are homogenous). This method provides a statistic with a chi-square distribution and n-1 degrees of freedom, where n is the number of studies. A P value $>.05$ indicates that the assumption of statistical homogeneity is not violated. Based on the chi-square test, an inconsistency coefficient was computed (I^2 statistic), whereby a value of more than 50% indicated significant heterogeneity.

Publication Bias

We used funnel plots to visually assess publication bias (i.e., the greater likelihood of studies with significant results being published compared with studies with nonsignificant and null results). In the funnel plot, the log odds ratio (OR) was plotted on the horizontal axis and the precision (1/SE) on the vertical axis. In the absence of publication bias, because of sampling variability, the graph should have the shape of a funnel, with the tip pointed up and centered on the true effect size. If there is bias against the publication of small studies with adverse and null results, the corner of the pyramidal part corresponding to adverse treatment effects will be distorted or missing. Egger's test was used to assess the significance of publication bias. If the P value of Egger's regression intercept was $<.05$, the trim and fill method was used to quantify the degree of publication bias. This method tests what the results might look like if all relevant studies had been included. The impact of bias is considered minimal when the two versions of the analysis would yield similar estimates of the effect size. The impact could be considered modest when the effect size would change substantially but the key finding (that consanguinity increases or decreases the risk of NSOFC) would remain in force. The impact of publication bias could be considered severe when the basic conclusion of the analysis (that consanguinity increases or decreases the risk of NSOFC) is called into question (Egger et al., 2001; Rothstein et al., 2006). In this meta-analysis, studies liable to cause severe publication bias were excluded.

Statistical Meta-analysis

Meta-analysis was performed using RevMan 5.1 software for statistical analysis (Nordic Cochrane Centre, Cochrane Collaboration, 2011) and Egger's test using a trial copy of Biostat's Comprehensive Meta-Analysis Software Version 2.0 (Borenstein et al., 2005). The Mantel-Haenszel method was used for combining studies to calculate summary OR and 95% confidence intervals (CIs) for consanguinity versus nonconsanguinity. The ORs were pooled with a fixed effect model for homogeneous studies and a random effects model for heterogeneous studies. The significance was set at $P <$ .05. The ORs with their 95% confidence limits for the individual studies and summary estimate of effect were graphically displayed in a Forest plot. The ORs are expressed by a solid square proportional to the weight of the study, while a horizontal line indicates the 95% confidence limits (Fig. 1). When the value of a confidence limit exceeds the extreme values of the scale, it is indicated by an arrow. Similarly, when the estimated exposure effect is out of the range of the scale, the solid square is not reported, and its position with respect to the scale is indicated by the arrow. The cumulative results of the meta-analysis are expressed together with their 95% CIs by a diamond-shaped symbol.

TABLE 1 Baseline Data of Included Studies

Reference	*Site and Country*	*Duration*	*Study Design*	*Consanguinity in CL±P and CP*	*Consanguinity in Controls*	*Consanguinity in General Population*	*Type of Consanguinity*	*Statistically Significant Association*
Alsahafi (2010)	KFAH, KAUH, KFSH, NGH, Jeddah, Saudi Arabia	—	Matched case-control	40/61 (65.6%) 30/61 (48.8%)	136/185 (73.5%)* 112/185 (60.5%)*		All types First cousin	Not significant $P > .05$ Not significant $P > .05$
Amaratunga (1989)	Oral surgery clinic, University of Peradeniya, and survey in district of Kandy, Sri Lanka	1985 to 1987	Population based Matched case-control Retrospective and prospective	29/501(5.8%) CL±P: 23/395 (5.8%) CP: 6/106 (5.66) 11/501 (2.2%) CL±P: 9/395 (2.3%) CP: 2/106 (1.7%)	25/500 (5%) 11/500 (2.2%)		All types First cousin	Not significant $P > .05$ Not significant $P > .05$
Jaber et al. (2002)	Grade 1 and 2 in all seven primary schools in the town of Taibe Arab town in Israel	1995	Population based Cross-sectional	9/26 (34.6%) 7/26 (27%)		439/1281(34%) 248/1281 (19.3%)	All types First cousins or double	Not significant $P > .05$† Not significant $P > .05$†
Jamilian et al. (2007)	Maternity hospital, Tehran, Iran	1998 to 2005	Hospital based Matched case-control Retrospective	7/25 (31.8%)	4/50 (8%)	—	Not mentioned	Significantly higher in NSOFC $P < .05$†
Kanaan et al. (2008)	Three major areas, Bekaa, Lebanon		Hospital base Cross-sectional	3/3 (100%)	171/552 (31%		First cousins	Not significant $P > .05$†
Leite et al. (2009)	The Nossa Senhora do Loreto Municipal Hospital, in Rio de Janeiro, Brazil		Hospital based, matched case-control	11/263 (4.2%) CL±P: 10/208 (4.8%) CP: 1/66 (1.5%)	10/531 (1.9%)	—	First cousins and uncle/nephew and aunt/niece	Not significant $P > .05$†
Elahi et al. (2004)	Birth registry in the city of Abbotabad, Pakistan	1998 to 2001	Population based Matched case-control Retrospective and prospective	37/117 (32%)* −7 syndromic		10,987/61,039 (18%)*	First and second cousin	Significantly higher in NSOFC $P < .05$
Rajabian and Sherkat (2000)	Two referral centers, plastic surgery and a maternity hospital, Iran	1976 to 1991	Hospital based Cross-sectional	764/1669 (45.8%) 423/1669 (25.3%)	8348/19,369 (43.1%) 4745/19,369 (24.5%)	—	All types First cousins	Significantly higher in NSOFC $P < .05$† Significantly higher in NSOFC $P < .05$†
Reddy et al. (2010)	Three districts in Andhra Pradesh. District medical and health officer and district education officer. The state of Andhra Pradesh, south India	2001	Population based Case-control Retrospective	722/1422 (50.8%)	42/300 (14%)	—	All types, including uncle/niece	Significantly higher in NSOFC $P < .05$†
Rittler et al. (2001)	114 maternity hospitals, in nine South American countries	1967 to 1997	Population based Case-control	29/1918 (1.5%) CL±P: 25/1660 (1.5%) CP: 4/287 (1.4%)		514/53,552 (0.96%)	All types	Significantly higher in NSOFC $P < .05$†
Stoll et al. (1991)	Registry of congenital malformations Alsace, France	1979 to 1987	Population based Matched case-control	8/166 (4.8%)* 2/166 (1.2%)*		1062/118,038 (0.9%)	All types First cousins	Significantly higher $P < .05$†

TABLE 1 Continued

Reference	*Site and Country*	*Duration*	*Study Design*	*Consanguinity in CL±P and CP*	*Consanguinity in Controls*	*Consanguinity in General Population*	*Type of Consanguinity*	*Statistically Significant Association*
Stoltenberg et al. (1997)	Birth registry, Norway	1967 to 1993	Population based Cross-sectional Retrospective	Norwegian: 26/2437 (1.06%) 6/2437 (0.2%)		15,723/1,435,810 (1.09%) 1992/1,435,810 (0.14%)	All First cousins	Not Significant $P > .05$†
				Pakistanis: 7/9 (77.7%) 6/9 (66.6%)		2953/7297 (40.5%) 2,268/7297 (31.1%)	All First cousins	Significantly higher in NSOFC $P < .05$†
				Other origins: 1/24 (4.26) 1/24 (4.16)		1080/25,191 (4.3%) 657/25,191 (2.6%)	All First cousins	Not significant $P > .05$†
Taghavi et al. (2012)	Bahrami Hospital, Tehran, Iran	2005 to 2010	Hospital base Case-control	34/300 (11.3%)	25/300 (8.3%)		Not mentioned	Not significant $P > .05$†
Zandi and Heidari (2011)	All neonatal department in all hospitals of Hamedan City, Iran	1993–2008	Hospital based Case-control	29/146 (22.5%)	207/1000 (20.7%)	—	All types	Not significant $P > .05$†
Zeiton et al. (1993)	Alexandria University Hospital, Egypt	—	Matched case-control	55*/100 30*/100	35‡100 14‡100	—	All types First cousins	Significantly higher in NSOFC $P < .05$†
Zlotogora (1997)	Genetics clinic, Hadassah Medical Center, Palestine	1995	Cross-sectional	39/60 (65%) CL±P: 23/40 CP: 16/20 20/60 (33.3%)		512/1156 (44.3%) 261/1156 (22.6%)	All types First cousins	Significantly higher in NSOFC $P < .05$† —

* The number was calculated from the percentage presented by the authors from data in the paper.
† The .05 level was used as the cutoff value of statistical significance for all studies.
‡ The number was not measured in the article; it was obtained through personal communication with the authors.

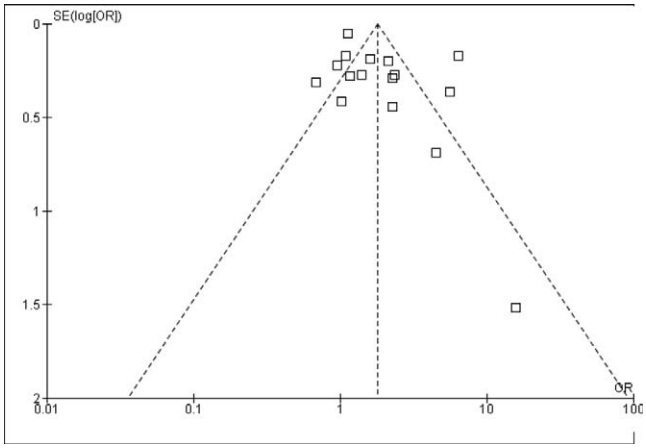

FIGURE 2 Funnel plot for demonstration of publication bias for meta-analysis of the association between consanguinity and the risk of NSOFC for all 16 studies combined.

Results

Literature Search

The search strategy produced the following hits: PubMed (608) hits, Scopus (389) hits, Google Scholar (4067) hits. After reviewing these titles for relevance and removing duplicate articles, 146 studies remained. Abstracts of these titles were obtained and reviewed. Eighty articles did not discuss NSOFC-related factors and were excluded. The full text of the remaining 66 articles were obtained and reviewed to determine whether they met the inclusion criteria. Forty-nine articles were excluded either because they did not include a control group (n = 23), the data included syndromic cases or other craniofacial diseases in their sample and it was not possible to determine the number of NSOFC cases (n = 5), or the articles were discussing topics other than NSOFC and consanguinity as a risk factor (n = 21). Furthermore, three case-control studies were excluded because either there were no data on the number of controls with consanguinity (Czeizel and Tusnády, 1984, #85; Calzolari, 1988, #83) or it was not clear what the number of affected individuals was within the NSOFC subdivision (Czeizel and Tusnády, 1984; Calzolari et al., 1988; Glinka et al., 1996). This resulted in 14 articles.

Four of the six authors of articles (Stoll et al., 1991; Rajabian and Sherkat, 2000; Elahi et al., 2004; Reddy et al., 2010; Zandi and Heidari, 2011; Taghavi et al., 2012) who were contacted for gray literature and inquiries about data responded. An additional two case-control studies, one carried out in Egypt (Zeiton et al., 1993), published in a local journal, and one in Saudi Arabia, which was part of a successful completed Master's thesis at Boston University (Alsahafi, 2010), were included, having been obtained through personal communication. This resulted in 16 articles, in which 10 were case-control studies. In Stoltenberg et al. (1997), the data were categorized according to country of origin and divided into children with Norwegian parents, children of immigrants from Pakistan, and children of immigrants from other countries. These three subgroups were analyzed separately (see Table 1). Also, in Kanaan et al. (2008), the sample was considered a source of bias as only three cases of NSOFC were found, all of which had first cousin parents. However, as the study met the inclusion criteria, it was not excluded from the meta-analysis, but it was categorized under the low-quality studies to compensate for bias (Fig. 7).

Quality Assessment

Of the 16 studies, 11 had a "good" quality score (greater than 6 of 9 on the NOS), with five of the studies scoring less than 6 ("fair" or "poor") on the NOS scale for quality.

Sensitivity Analysis

The sensitivity analysis showed stability and reliability of the meta-analyses results. This was observed by the consistency of the results of the meta-analyses in the different studies subgrouping, which showed significant relationships between consanguinity and NSOFC in all situations (see Figs. 3, 6, 7, and 8).

Evaluation of Study Heterogeneity

Heterogeneity analysis of different areas of subgrouping (type of NSOFC, type of study, quality of the study, year of publication, and the level of consanguinity in the general population [high versus low]) showed significant heterogeneity of more than 50% in all subgrouping except in cleft type. There was a very low level of heterogeneity in studies with CL±P subgrouping, measured as 0.0% (Table 2).

Publication Bias

Egger's test showed a statistically significant publication bias for the all-studies model only ($P = .037$). However, it is ranked as mild since the ORs after inclusion of imputed studies showed minimal changes (from 1.80 to 1.78; Table 2).

Meta-analyses

All studies with controls or comparison groups (Table 1) were included in the overall meta-analyses (Fig. 1), with publication bias indicated by the funnel plot shown in Figure 2. A significant relationship between consanguinity and NSOFC was found for all studies combined

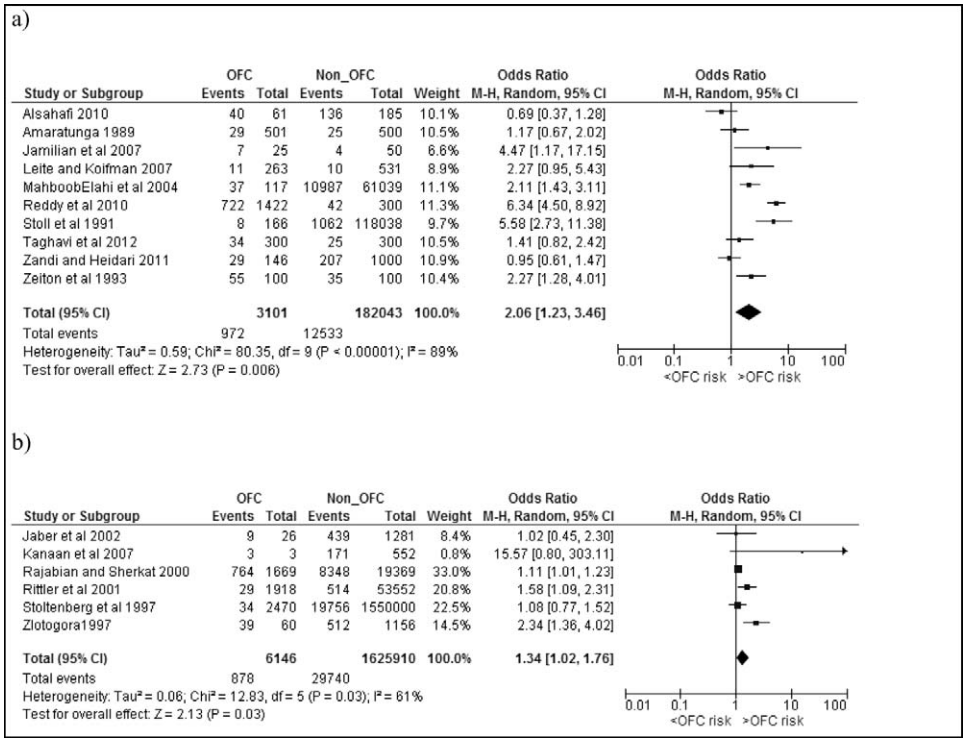

FIGURE 3 Forest plots for meta-analyses of the association between consanguinity and NSOFC according to the type of study. a: For case-control studies. b: For cross-sectional studies combined.

($P = .0003$), with OR = 1.83 and 95% CI = (1.31, 2.54); in case-control studies ($P = 0.006$), with OR = 2.06 and 95% CI = (1.23, 3.46; Fig. 3a); in cross-sectional studies ($P = .03$), with OR = 1.34 and 95% CI = (1.02, 1.76; Fig. 3b); in first cousins consanguineous marriages ($P = .04$), with OR = 1.40 and 95% CI = (1.01,1.93; Fig. 4); in CP ($P = .01$), with OR = 1.89 and 95% CI = (1.14, 3.13; Fig. 5a); and in CL±P cases ($P = .002$), with OR = 1.56 and 95% CI = (1.18, 2.07; Fig. 5b).

Discussion

There were 16 studies that met the inclusion criteria for this literature review, and the overall meta-analysis (Fig. 1) revealed a clear relationship between consanguinity and NSOFC, with almost twice the risk of an NSOFC child being born to consanguineous parents. Although the extent of risk varied, it held consistently for each data subgroup that was analyzed, whether this was study type (case-control studies only and cross-sectional studies only), type of consanguineous relationship, or type of NSOFC (CP or CL±P).

Various subgroup analyses were carried out to validate the reliability of the results and to study possible effects of bias by looking at the results of the meta-analyses where potential areas of increased risk of bias were removed. The hypotheses to support the subgroups' analyses were the following: for study quality, it was thought that lower-quality studies may have altered the overall result (Fig. 7); for date of publication, studies carried out before 2000 may

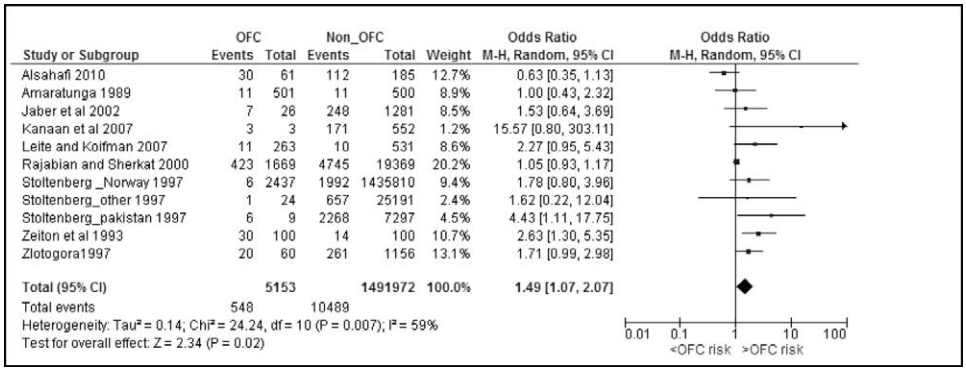

FIGURE 4 Forest plot for meta-analysis of the association between first cousins' consanguinity and the risk of NSOFC.

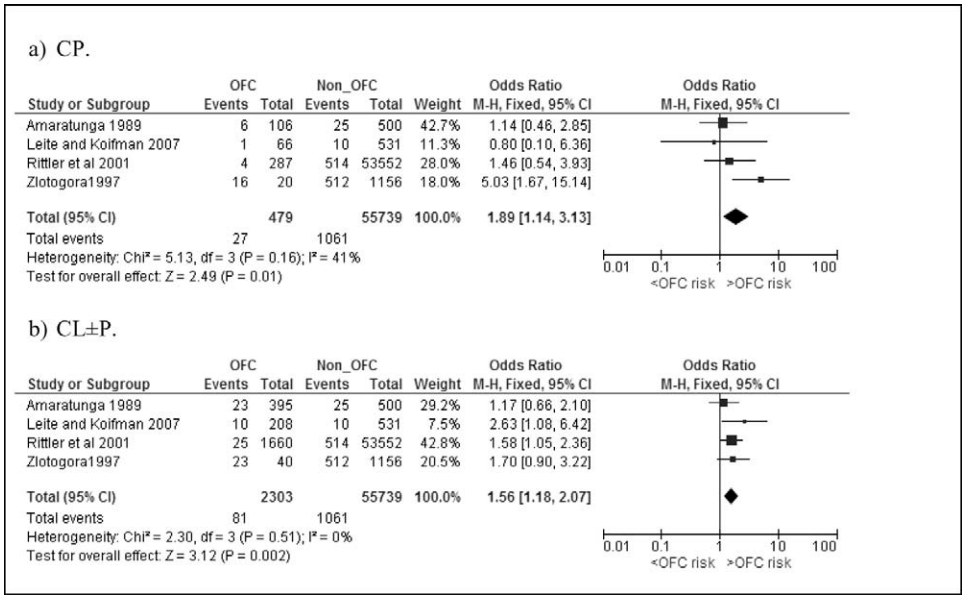

FIGURE 5 Forest plot for the meta-analysis of the association between consanguinity and NSOFC according to the type of cleft. a: CP. b: CL±P.

have had less robust methodologies as, around this time, previous studies, together with the event of systematic reviews, were leading to improved study design (Fig. 6); and finally, for the prevalence of consanguinity in the study population, it was considered possible that targeting populations with high consanguinity only might in itself be regarded as a source of bias (Fig. 8).

The best place to study the effect of consanguinity on the occurrence of a disease is in countries where there is high consanguinity prevalence. In this review, the search strategy included the names of countries known to have high rates of consanguinity. The search yielded 12 studies with control or comparison groups, which were carried out in countries with high prevalence of consanguinity (Fig. 8a). Six of these

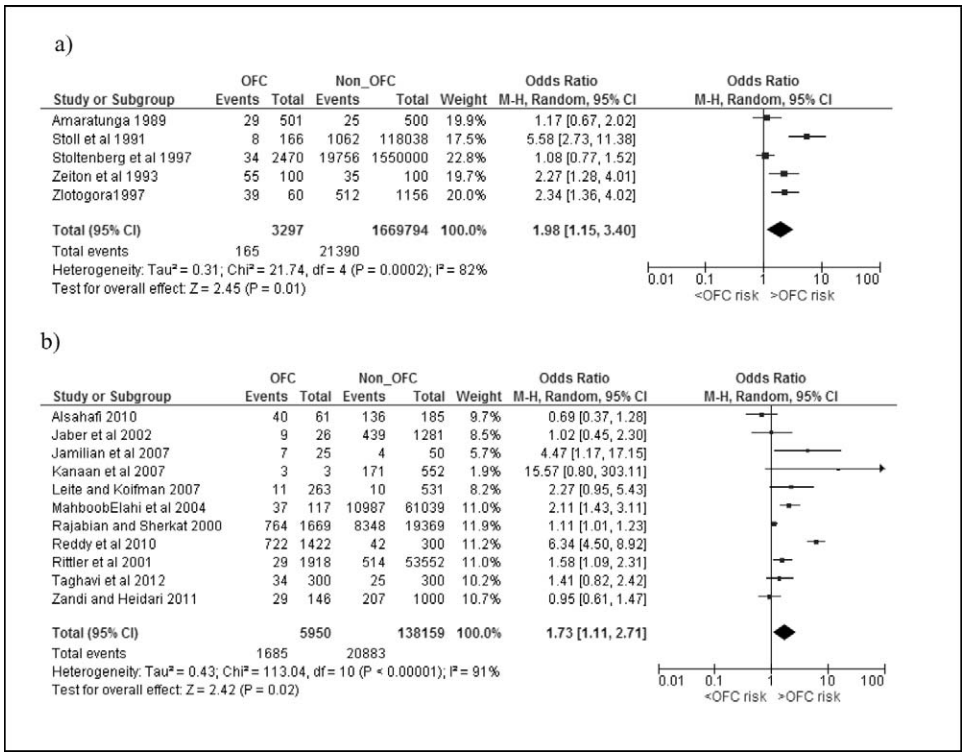

FIGURE 6 Forest plot for meta-analysis of the association between consanguinity and NSOFC according to the study's date of publication. a: Studies published before 2000. b: Studies published 2000 or after.

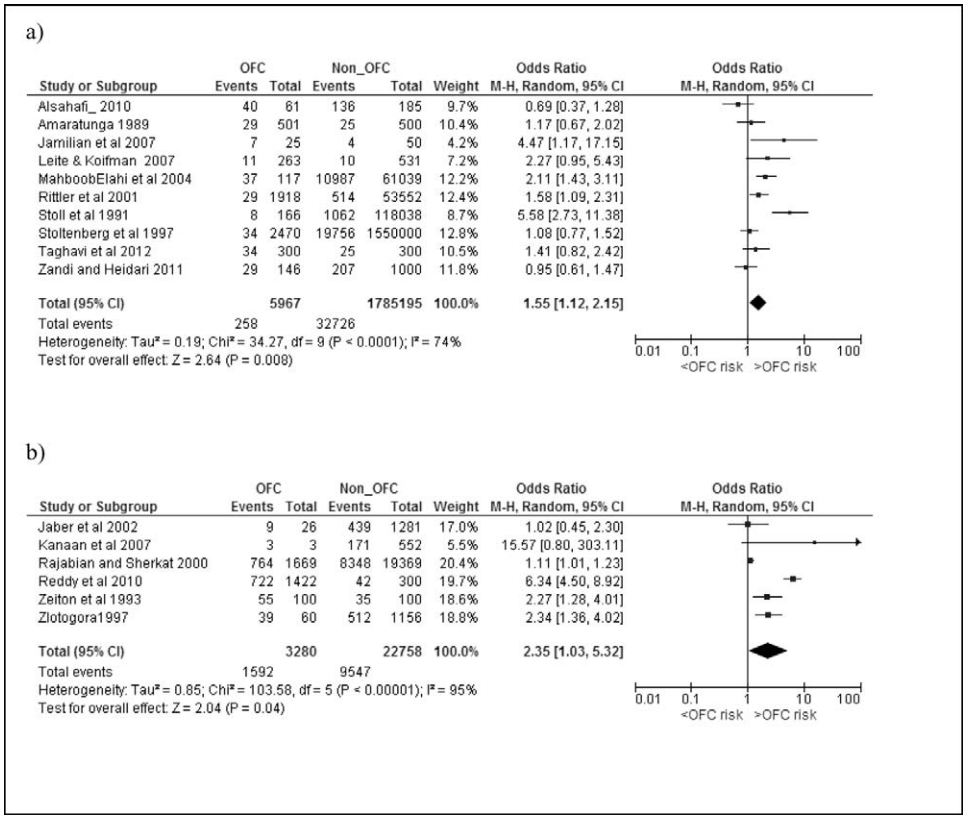

FIGURE 7 Forest plots for meta-analyses of the association between consanguinity and the risk of NSOFC for studies according to NOS quality score (Wells et al., 2010). a: Studies with good quality (scored more than 6). b: Studies with fair or poor quality (scored 6 or less).

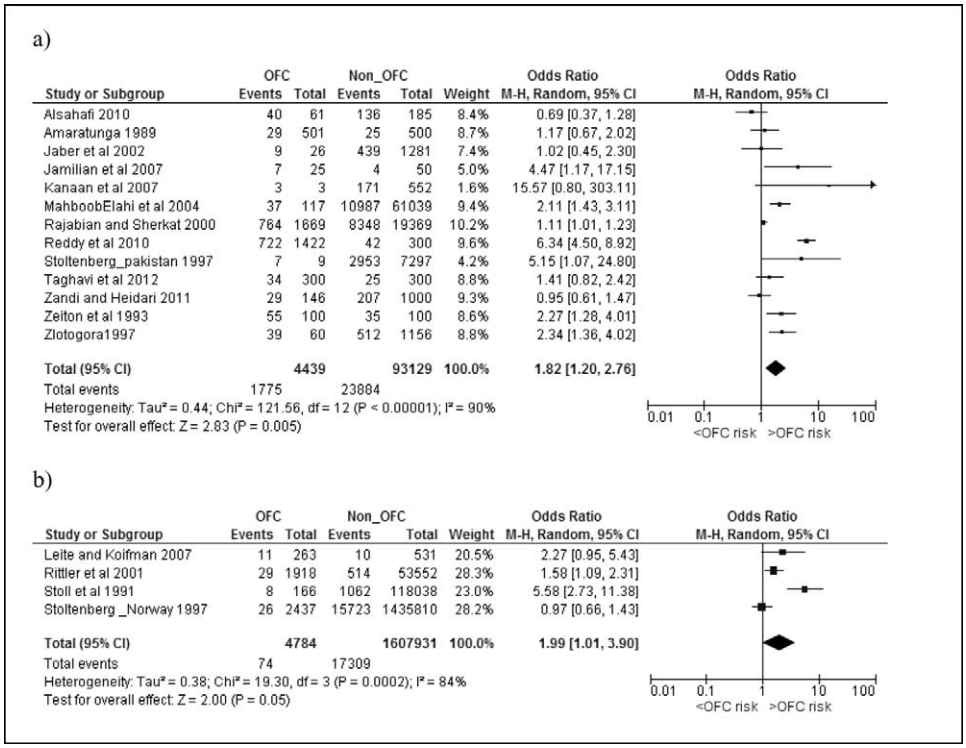

FIGURE 8 Forest plots for meta-analyses of the association between consanguinity and the risk of NSOFC according to the prevalence of consanguinity in the population. a: Studies in high consanguinity population. b: Studies in low consanguinity population.

TABLE 2 Studies Subgroupings' Homogeneity, Sensitivity, and Publication Bias

Groups of Studies	*No. of Studies*	*NSOFC Cases (Consanguineous/ Nonconsanguineous)*	*Non-NSOFC Cases (Consanguineous/ Nonconsanguineous)*	*Heterogeneity*		*Overall Effect Pooled*			*Egger's Intercept P*
				I^2 (%)	*P*	*OR*	*95% CI*	*P*	
All studies	16	1850/9247	42,273/1,726,251	88	.000	1.83	1.31, 2.54	.000	.037
Case control	10	972/3101	12,533/182,043	88	.000	2.06	1.23, 3.46	.001	.597
Cross-sectional	6	878/6146	29,740/1,625,910	61	.030	1.34	1.02, 1.76	.030	.173
First cousin	9	558/5153	10,513/1,573,674	62	.007	1.40	1.01, 1.93	.04	.135
CP	4	27/479	1061/55,739	41	.160	1.89	1.14, 3.13	.010	.896
CL±P	4	81/2303	1061/55,739	0	.510	1.56	1.18, 2.07	.002	.508
Publication before 2000	5	165/3297	21,390/1,669,794	82	.000	1.98	1.15, 3.40	.010	.070
Publication 2000 or after	11	1685/5950	20,883/138,159	92	.000	1.73	1.11, 2.71	.007	218
Quality score >6	10	258/5967	32,726/1,785,195	74	.000	1.55	1.12, 2.15	.008	.155
Quality score ≤6	6	1592/3280	9547/22,758	95	.000	2.35	1.03, 5.32	.040	.287
Studies in high consanguinity prevalence in their general population	13	1775/7393	20,944/90,176	90	.000	1.82	1.2,2.76	0.005	0.183
Studies in low consanguinity prevalence in their general population	4	74/4758	17309/1592208	84	0.000	1.99	1.01,3.90	0.05	0.200

12 studies with controls reported a significant relationship between consanguinity and NSOFC (Zeiton et al., 1993, Zlotogora, 1997; Rajabian and Sherkat, 2000; Elahi et al., 2004; Jamilian et al., 2007; Kanaan et al., 2008; Alsahafi, 2010; Reddy et al., 2010). Meta-analysis confirmed this risk ($P = .005$) and quantified it as OR = 1.74 and CI = (1.14, 2.66).

Studies that did not find statistically significant relationships between consanguinity and NSOFC were supported by other cross-sectional studies that were not included in this review as they did not match the inclusion criteria (i.e., they did not have comparison groups). Three were carried out in Saudi Arabia and one in Kuwait. They found a lower prevalence of consanguinity in NSOFC than in their general population (Kumar et al., 1991; Borkar, 1993; Al-Bustan et al., 2002; Aljohar et al., 2008). This was also supported by other studies in populations with low consanguinity (Czeizel and Tusnády, 1984; González et al., 2008). However, according to the author of the Kuwaiti study, this could have resulted from differences in sample recruitment time between the NSOFC study and the general population study (Al-Bustan et al., 2002). In the Saudi study, when the type of consanguinity was included in the analysis, first cousin consanguinity was more frequently associated with NSOFC than was found in the general population (Aljohar et al., 2008). In addition, in case-control studies in which there was no statistically significant relationship between consanguinity and NSOFC, the prevalence of consanguinity in NSOFC cases was still slightly higher than in the controls. Further research with robust methodology and reporting is needed to clarify this area.

Confounding Factors

The relation between consanguinity and NSOFC reported by the different studies could have been affected by confounding factors. Three of the matched-control studies carried out in Sri Lanka (Amaratunga, 1989), Iran (Jamilian et al., 2007), and a population-based matched-control study carried out in Pakistan (Elahi et al., 2004) included control groups in which the consanguinity prevalence values were much lower than those reported for the general population in the literature. Accordingly, the consanguinity prevalence figures in their control groups were 5%, 8%, and 18%, respectively, compared with other published data reporting rates of 22% in Sri Lanka (Bittles, 1998), 28% in Iran (Saadat et al., 2004), and 60% in Pakistan (Hussain and Bittles, 1998).

The presence of a positive family history of NSOFC is expected to affect the risk of NSOFC in infants from consanguineous parents. Therefore, it should be controlled in the sample selection and analyzed in the results. Aljohar et al. (2008) compared NSOFC infants with a positive family history of clefts and first cousin

parentage with those with unrelated parents and found a higher prevalence of orofacial cleft cases in the first group (31.9% compared with 24.4%). However, in the literature, no research has compared the two factors and their effect on NSOFC together with controls. It could be that the significant relationship found in the studies is affected by family history or other confounding factors rather than consanguinity, and again, further research to clarify this is needed.

Low levels of parental education (elementary school or less) was significantly related to consanguinity (Al Husain and Al Bunyan, 1997; Bittles, 2001; Kanaan et al., 2008). However, Rajabian and Sherkat (2000) found no significant relationship between level of the mother's education and NSOFC. Also, Stoltenberg et al. (1999) have found that parental education did not alter the effect of consanguinity on inheritance of NSOFC. However, Taghavi et al. (2012) reported a significant association between family history and low parental education and NSOFC while concurrently finding no significant association between consanguinity and NSOFC. The other studies did not consider educational attainment.

Type of Consanguinity

Different types of consanguinity result in differing proportions of gene sharing. In first cousin consanguinity, one in eight genetic materials is shared. However, first cousin once removed or second cousins results in 1 in 16 or 32 gene sharing, respectively (Jaber et al., 1998).

Not all of the included articles described the types of consanguinity. Where this information was given, it was usually not compared with the controls. In most of the articles, all types of consanguinity were grouped together or only first cousin consanguinity was included. Those articles in which both consanguinities were included and the individual results could be ascertained found a stronger association between first cousin marriages and NSOFC. However, in Jaber et al. (2002), although no significant relationship between consanguinity and NSOFC was found, there were more NSOFC-affected infants with first cousin parents (27%) than in the controls (19.3%). Aljohar et al. (2008) found that the prevalence of NSOFC in infants with first cousin parents (50%) was much higher than the prevalence of first cousin consanguinity in the general population (28.4%), which was not the case for all types of consanguinity (Al-Johar, 2008, #33). This finding was also supported by Hu et al. (1982), who found a higher prevalence of first cousin consanguinity in NSOFC (1.8%) cases than in the general population in China (0.77%). No case-control study compared the different types of consanguinity and their effect on NSOFC.

Reddy et al. (2010) included uncle/niece consanguineous marriages in their sample and found it to be major risk factor. However, although this study had a large sample size of NSOFC cases (1422) and was population based, the control group size was not large enough to allow grouping according to type of consanguinity.

Cleft Palate

Cleft palate prevalence and ascertainment is affected by many factors. Most of the studies did not describe clearly the inclusion and exclusion criteria for included CP sample. For example, CP could be a manifestation of Pierre Robin sequence or submucous CP and have differing and heterogeneous etiologies. In addition, CP is susceptible to ascertainment bias. This is because although submucous CP prevalence is as common as overt CP, it is more easily overlooked, remaining undiagnosed in many cases (Mossey and Little, 2002). Therefore, further investigation using prospective well-documented records to improve ascertainment of the results is required.

While these meta-analyses reveal a consistency of association between consanguinity and increased risk of NSOFC, they do not provide any proof of this, nor do they elaborate on possible biological mechanisms. However, the findings are consistent with the genetic cause of NSOFC being transmitted through a homozygous recessive mode of transmission in consanguineous unions, which increases the chance of allelic homozygosity at the same genetic loci. Two recently published studies in the human population provide useful supportive evidence. Mitchell et al. (2012) found a homozygous mutation in RIPK4 that was related to craniofacial manifestations as part of a pterygium syndrome in a consanguineous family, where the parents were heterozygous for this mutation. Also, Slavotinek et al. (2012) published the first report in the literature of a human mutation in VAX1. This was found in an infant of consanguineous Egyptian parents and presented with bilateral cleft lip and palate as part of the phenotype. Autosomal recessive genes contributing to clefts would be expected to be significant only in instances in which the genetic mutation was rare, and these exceptions are most interesting when we are seeking the etiology of cleft lip and palate. The effect of family history of CP and CL±P on the incidence and type of NSOFC, the coefficient of inbreeding, and the mode transmission of CL±P gene polymorphisms in homozygous and heterozygous transmission will also be important to clarify the exact role of consanguinity in orofacial clefts.

Conclusion

This systematic review of the literature yielded 16 studies, and meta-analysis demonstrated that consanguinity is a

risk factor for NSOFC ($P = .0003$). This gave an OR of 1.83 (95% CI = 1.31 to 2.54). More studies with better standardization of data are needed to investigate the relationship between different types of consanguinity, association with cleft subphenotype, severity of NSOFC, and the genetic mechanisms for both CL±P and CP.

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