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Parental craniofacial morphology in cleft lip with or without cleft palate as determined by cephalometry: a meta-analysis

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Structured Abstract

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Objective – To integrate findings from previous cephalometric studies comparing the craniofacial complex of unaffected parents with cleft lip with or without cleft palate (CL/P) children to controls with no history of the disease.

Design – Meta-analysis of case-control cephalometric data.

Inclusion criteria – Studies were selected if the unaffected parents of children with CL/P were included and were not combined with parents of children with isolated CP; quantitative data were obtained through cephalometry; the cephalometric variables used were not unique to a study; a case-control design was used; and the means and standard deviations for all variables were reported or could be calculated for both the experimental and the control group.

Outcome measure – Using raw data obtained from nine studies, mean weighted effect sizes with 95% confidence intervals were calculated for 28 cephalometric variables (mothers and fathers combined) or 18 variables (mothers and fathers separately). Heterogeneity statistics for the effect sizes were also calculated.

Results – In general, unaffected parents of children with CL/P possessed significantly wider interorbital, nasal cavity and upper facial dimensions, narrower cranial vaults, longer cranial bases, longer and more protrusive mandibles, shorter upper faces and longer lower faces compared with controls. Increased width of the nasal cavity was the most robust finding. Significant effect size heterogeneity was observed in roughly half of the variables examined.

Conclusion – Unaffected parents of children with CL/P are characterized by a suite of consistent, yet subtle, craniofacial differences, which could indicate an underlying genetic liability.

Key words: cephalometry; clefting; face shape hypothesis; meta-analysis; parental phenotype

Introduction

It has been suggested that certain heritable aspects of craniofacial form might serve as a pre-disposing factor in the pathogenesis of non-syndromic orofacial clefting (1–3). Historically, this 'face-shape hypothesis' has been supported by three lines of evidence. In the late 1960s, Trasler (4) described differences in the shape of the craniofacial complex during embryogenesis in two strains of mice with varying susceptibility to spontaneous cleft lip (CL). Specifically, strains with high rates of clefting were characterized by more centrally located medial nasal processes, thereby increasing the risk of failed fusion with adjacent facial processes. Secondly, epidemiological data (5,6) on differences in orofacial cleft incidence across once-geographically distinct populations suggest, at least indirectly, that face shape might account for some of the observed variation. For example, the broad upper face, brachycephalic head shape and elliptical palate shape that characterize some Asian-derived populations (7–9) has been hypothesized to contribute to the elevated incidence of clefting observed in these groups (10,11). Once again, the basis of this hypothesis is that early in embryological development, the position of the facial processes relative to one another increases the likelihood that they will fail to contact one another and fuse.

In order to test the face-shape hypothesis in humans, the principle approach has been to describe the craniofacial morphology of unaffected family members within cleft families. The central assumption underlying this approach is that the presence of craniofacial differences in unaffected relatives serves as a phenotypic proxy for underlying genetic risk. Besides anecdotal reports, the earliest studies to examine the craniofacial morphology of unaffected CL/P relatives were either case reports or primarily qualitative in nature (12–17). Nevertheless, these studies noted the presence of a number of unusual facial and palatal variations in unaffected relatives. Fraser and Pashayan (1) published the first major quantitative study to compare craniofacial features in a sample of unaffected parents of probands with orofacial clefts to those of controls with no history of the disease. They identified numerous craniofacial differences in unaffected parents including increased facial height, increased upper facial width and maxillary retrusion.

In the years following Fraser and Pashayan's publication, numerous additional studies have documented the craniofacial form of the unaffected relatives (parents and sibs) of non-syndromic cleft individuals (see Tables 1 and 2). Despite the fact that all such studies have identified differences in the craniofacial complex of unaffected cleft relatives vs. controls, specific results have been so inconsistent across studies that a clear picture has yet to emerge as to exactly how these unaffected relatives differ from the general population. It is quite likely that at least some of the variation in study results can be explained by methodological inadequacies; for example, many studies have utilized questionable control samples (e.g. convenience samples comprised of dental students), a variety of different methods of assessment have been used (e.g. cephalometry, direct anthropometry, photogrammetry) and in general the choice of measurement variables has been rather haphazard. Another key factor may be population; to date, studies comparing unaffected relatives to controls have been carried out in Japan, Chile, Costa Rica, Saudi Arabia, Canada, USA, Scotland, Czech Republic and India. Not only are there clear etiological differences among these populations, but it is also reasonable to assume that differences in craniofacial morphology across such geographically disparate groups will impact case-control outcomes to some degree.

The identification of clinically unaffected, but morphologically and genetically informative, family members has the potential to boost the power of gene mapping approaches and to improve recurrence risk estimates (18,19). Before this can take place, however, a clearer understanding of the craniofacial phenotype in unaffected CL/P relatives must be achieved. To this end, this paper will utilize a meta-analytic approach to integrate findings from previous cephalometric studies and attempt to identify which, if any, parental craniofacial differences are consistent across studies.

Materials and methods

Literature search and selection

The term meta-analysis describes a set of statistical techniques for integrating results across a sample of independent studies. Meta-analysis differs from

Table 1. **General characteristics of the nine studies included in the present meta-analysis**

Study	Cephalometry method	Cleft type	Relative sample	Control sample	Population	Ascertainment notes
Coccaro et al. (21)	Lateral	CL/P	Total, 40; ♂, 20; ♀, 20	Total, 40; ♂, 20; ♀, 20	USA	Experimental and control group identified from children enrolled in growth studies at NIH
Kurisu et al. (22)	Lateral, PA	CL/P, CP*	Total, 223; ♂, 92; ♀, 131	Total, 114†; ♂, 56; ♀, 58	USA	Experimental group identified from cleft cases served by the Lancaster Cleft Palate Clinic. Not clear how control sample was ascertained
Shibasaki et al. (23)	Lateral	CL/P	Total, 118; ♂, 58; ♀, 60	Total, 60; ♂, 30; ♀, 30	Japan	Experimental group identified from cleft cases served by the Tohoku University Dental Center. Control sample was comprised of university dental faculty and students
Nakasima and Ichinose (24)	Lateral, PA	CL, CL/P, CP‡	Total, 180§	Total, 110§	Japan	Experimental group identified from cleft cases retrospectively from files at the Kyushu University Orthodontic Department. Controls were comprised of dental students
Sato (25)	Lateral, PA	CL/P, CP*	Total, 86; ♂, 43; ♀, 43	Total, 60; ♂, 30; ♀, 30	Japan	Experimental group identified from cleft cases served by the Tokyo Dental College Oral and Maxillofacial Surgery Department. Not clear how control sample was ascertained
Blanco et al. (26)	PA	CL/P	Total, 44¶; ♂, 13; ♀, 31	Total, 20¶; ♂, 8; ♀, 12	Chile	Experimental group identified from cleft probands served by the dental and orthodontic faculty of the University of Chile Cleft Clinic or from those receiving support from a local humanitarian foundation. Control group was comprised of local faculty and a selection of their unaffected patients
Raghavan et al. (27)	Lateral, PA	CL/P	Total, 38§	Total, 24§	India	Not clear how experimental or control group was ascertained
AlEmran et al. (28)	PA	CL/P	Total, 80; ♂, 40; ♀, 40	Total, 67; ♂, 32; ♀, 35	Saudi Arabia	Experimental group identified from cleft cases served by the King Kahlid University Plastic Surgery Clinic and the King Saud University Cleft Lip and Palate Clinic. Majority of control group were dental students
Suzuki et al. (29)	Lateral, PA	CL/P, CP**	Total, 65; ♂, 25; ♀, 40	Total, 826; ♂, 413; ♀, 413	Japan	Experimental group identified from cleft cases served by the Kyushu University Orthodontic Clinic. Eligible parents were from families with at least one other affected individual. Not clear how control sample was ascertained

*Results from CP group not included in meta-analysis.
†Figures refer to Lancaster control group.
‡Results from CL and CP groups not included in meta-analysis.
§Mid-parent values used.
¶Unaffected sibs not included.
**Small number of CP families mixed in with CL/P group.

Table 2. **List of studies excluded from present meta-analysis and reason(s) for exclusion**

Study	Reason(s) for exclusion from meta-analysis
Fukuhara and Saito (12)	No quantitative data included; case report
Fukuhara and Saito (13)	No quantitative data included; case report
Rusconi and Brusati (15)	No quantitative data included; case report
Mills et al. (16)	No cephalometric data included
Niswander (17)	No quantitative data included
Fraser and Pashayan (1)	No cephalometric data included
Figalová and Šmahel (30)	No cephalometric data included
Erickson (31)	No cephalometric data included
Nakasima and Ichinose (32)	No measures overlapped with other studies
Procházková and Tolarová (33)	Only data from CP cases included
Ward et al. (2)	Effect sizes could not be calculated because raw means and standard deviations were not reported
Ward et al. (34)	Effect sizes could not be calculated because raw means and standard deviations were not reported; case report
Procházková and Vinšová (35)	Only data from CP cases included
Mossey et al. (36)	No normative control group included
Mossey et al. (37)	Effect sizes could not be calculated because raw means and standard deviations were not reported
Mossey et al. (3)	Effect sizes could not be calculated because raw means and standard deviations were not reported
McIntyre and Mossey (38)	Review article, no original data
McIntyre and Mossey (39)	No control group included
McIntyre and Mossey (40)	Only raw data for measurements with statistically significant values reported
Ward et al. (20)	Review article, no original data
Perkiomaki et al. (43)	Effect sizes could not be calculated because raw means and SDs were not reported
Yoon et al. (42)	No control group included; effect sizes could not be calculated because raw means and standard deviations were not reported
Yoon et al. (43)	Effect sizes could not be calculated because raw means and standard deviations were not reported
McIntyre and Mossey (44)	Effect sizes could not be calculated because raw means and standard deviations were not reported
Chatzistavrou et al. (45)	Discordant twin study, no parents included

traditional narrative literature review in that the goal is to quantitatively synthesize previous research and arrive at an overall estimate of effect size (ES) for a given independent variable. The first step in meta-analysis is the identification and subsequent selection of suitable studies based on a set of *a priori* inclusion criteria. Study identification was divided into two phases. In the first phase, a MEDLINE search with no limits was performed using various combinations of the keywords 'craniofacial', 'cephalometry', 'cleft' and 'parent'. This initial electronic search yielded just over 260 articles, the titles and abstracts of which were evaluated for potential relevance. Of the 260 articles initially identified, only 23 were deemed pertinent. In the second phase, the references from these 23 articles along with a single book chapter (20) were reviewed for any additional articles missed during the initial computerized database search. Finally, the references

from any articles obtained during the second phase were reviewed. In the end, our search process resulted in the identification of 34 relevant articles. Of these, only nine articles (21–29) met our inclusion criteria: 1) unaffected parents of children with cleft lip with or without cleft palate (CL/P) were included and were not combined with parents of children with isolated cleft palate (CP); 2) quantitative data were obtained through cephalometry; 3) the cephalometric variables used were not unique to a study; 4) a case-control design was used; and 5) means and standard deviations for all variables were reported or could be calculated for both the experimental and control group. Case reports were also excluded from analysis.

Basic characteristics of the nine studies included in the meta-analysis are outlined in Table 1. The 25 studies not included in the meta-analysis (1–3,12,13,15–17,20,30–45) are listed in Table 2 along with the reason(s) for their exclusion. For each of the nine studies included, an index of methodological quality was calculated based on nine study properties related to technical rigor, ascertainment strategy and sample quality (see Table 3). Index scores range from 0 to 9, with a score of nine indicating the highest methodological quality. As Table 2 shows, with the exception of Blanco et al. (26), the nine studies were fairly uniform in terms of overall methodological quality.

Statistical methods

Numerous statistical guidelines exist for carrying out meta-analyses (46–49); however, a uniform and generally agreed upon set of procedures is lacking. The statistical procedures used in the present study follow the model outlined by Kline (50), which is a traditional pretest/post-test (or case-control) meta-design based on the calculation of cumulative measures of ES. Briefly, ESs are a family of standardized indices designed to quantify the magnitude of a treatment effect. For simple case-control designs, one of the most common measures of ES is the standardized mean difference statistic (d) (51). The only data required for the calculation of d are the experimental and control group means, SD and sample sizes.

First, the individual ES statistic d was calculated for each cephalometric variable in each of the nine studies. In each of the seven studies where fathers and mothers were treated separately, individual ESs for each variable were calculated for each parent separately and for the parents combined. Once d was calculated for each variable in each study, the individual ESs were combined across studies to produce a weighted average ES (d_w), which is a single cumulative estimate of overall effect taking into account sampling error. For the present analysis, d_w was only calculated for variables present in at least *three* studies. Given this restriction, 28 cephalometric variables were included when both

Table 3. **Factors used in the assessment of methodological quality**

	Study								
Study property	A	B	C	D	E	F	G	H	I
1. Relatives and controls from same ethnic background?	1	1	1	1	1	1	1	1	1
2. Control group a random, representative sample?	0	1	0	0	0	0	0	0	0
3. Relatives and controls of similar age?*	1	0	0	0	0	0	1	1	1
4. Sample size in relative and control group roughly equivalent?	1	0	1	1	1	0	1	1	0
5. Samples of sufficient size for statistical analysis?†	1	1	1	1	1	0	1	1	1
6. Relatives of isolated CP cases treated separately or not included?	1	1	1	1	1	1	1	1	0
7. Proportion of relatives from simplex vs. multiplex families reported?	0	0	0	0	1	0	0	0	1
8. Assessment of measurement error?	0	0	0	0	0	0	1	1	1
9. Instrument/observers for both groups the same?	1	1	1	1	1	1	1	1	1
Total	6	5	5	5	6	3	7	7	6

A, Coccaro et al. (21); B, Kurisu et al. (22); C, Shibasaki et al. (23); D, Nakasima and Ichinose (24); E, Sato (25); F, Blanco et al. (26); G, Raghavan et al. (27); H, AlEmran et al. (28); I, Suzuki et al. (29); 0, no or unclear; 1, yes.
*Mean age in both groups within 10 years; †Sample size at least 10 in both groups.

parents were combined; however, only 18 variables were available for each parent separately. Although formal guidelines do not exist, traditionally, an ES is considered weak if it falls between 0.20 and 0.49, moderate if it falls between 0.50 and 0.79, and large if >0.80 (52). To determine whether or not d_w was statistically different from zero (i.e. no effect), 95% confidence intervals were calculated.

Two additional statistics were included in order to facilitate ES interpretation. The first statistic, termed fail-safe N (N_{fs}), was originally developed to counteract some of the biases inherent in the meta-analytic approach; namely, the 'file drawer problem', which describes the tendency for studies with non-positive statistical findings to remain unpublished (53–55). Similarly, it addresses the fact that numerous available studies, while relevant, may not meet the researcher's inclusion criteria. The N_{fs} answers the question, 'How many hypothetical studies would need to be added to a meta-analysis in order to change an ES from its present value to some ostensibly trivial value?' Thus, N_{fs} provides another means to gauge the degree of significance a given ES warrants. Secondly, the heterogeneity statistic (Q) was calculated (48–50), which provides an estimate of ES variability across studies. The larger the value of Q, the less certain we can be that a set of individual ESs represents a homogeneous sample. The statistical significance of Q is determined from critical values derived from a chi-square distribution with k-1 degrees of freedom. For variables with statistically significant heterogeneity scores, the influence of potential moderator variables (e.g. population, methodological quality) on ES variability was explored using the general linear models procedure (regression) in SPSS (SPSS Inc., Chicago, IL, USA). The formula used to calculate each of the above statistics can be found in the Appendix.

Results

The complete results of the meta-analysis are shown in Table 4 and represented graphically in Figs 1–3. Table 5 provides an additional summary of the results. With both parents combined, 12 variables (43%) had mean weighted ESs significantly different from zero (i.e. their 95% CI did not include zero). Specifically, unaffected parents possessed narrower heads, wider faces, longer cranial bases, shorter upper faces, longer lower faces, longer inferior mandibular rami and more protrusive mandibles (S–N–Pg angle). Nevertheless, the vast majority of significant variables (83%) had either a small or a very small ES magnitude; only two significant variables relating to nasal cavity width had ES magnitudes in the moderate range (0.50–0.79). This general pattern is reflected by the small N_{fs} values observed for variables with significant ESs. Overall, 21 variables (75%) were found to be larger in the unaffected parental group versus the control group. The same pattern held true when only variables with significant ESs ($n = 12$) were considered; only two variables, maximum head width and upper facial height, were reduced in the combined parental group. Regarding heterogeneity, just over 50% of the 28 variables under consideration demonstrated statistically significant heterogeneity scores; four of these variables (maximum head width, relative face width, relative nasal cavity width and anterior cranial base length) also had significant ES values.

Considering just the unaffected fathers of cleft probands, four variables (22%) were shown to have small yet statistically significant ESs (Table 4 and Fig. 2). Three of these variables were craniofacial widths (lateral and medial orbital width and nasal cavity width), all of which were larger in the male parental sample compared with male controls. Conversely, upper facial height was reduced in male parents. In addition to those differences noted in unaffected fathers, unaffected mothers demonstrated significantly reduced head width, increased total and anterior cranial base length, and an increased mandibular protrusion (S–N–B angle) (Table 4 and Fig. 3). Mothers, however, did not demonstrate the reduced upper facial height observed in fathers. Regarding heterogeneity, unaffected fathers had twice as many variables with significant Q values compared with unaffected mothers.

Potential moderator variables, population and methodological quality, were not related to ES variability in any meaningful way.

Discussion

The purpose of this study was to utilize a meta-analytic approach to combine results across previous cephalometric studies comparing the craniofacial complex of unaffected parents with CL/P offspring to unaffected

Table 4. **Mean effect size and heterogeneity statistics for unaffected parents compared with controls**

	Parents combined				Unaffected fathers				Unaffected mothers			
Variable	n	d_w ± 95% CI	N_{fs}	Q	n	d_w ± 95% CI	N_{fs}	Q	n	d_w ± 95% CI	N_{fs}	Q
Maximum head width (Eu–Eu)	7	−0.29 ± 0.11*	3	47.77***	5	−0.01 ± 0.20	–	5.41	5	−0.21 ± 0.18*	1	4.07
Forehead width (F–F)	3	0.15 ± 0.18	–	40.22***	–	–	–	–	–	–	–	–
Lateral orbital width (Lo–Lo)	3	0.39 ± 0.15*	3	0.73	3	0.36 ± 0.23*	2	2.19	3	0.41 ± 0.20*	3	3.12
Medial orbital width (Mo–Mo)	5	0.39 ± 0.12*	5	3.92	3	0.47 ± 0.23*	4	0.56	3	0.42 ± 0.20*	3	1.77
Face width (Zy–Zy)	6	0 ± 0.13	–	17.71**	4	0.07 ± 0.25	–	4.19	4	0.01 ± 0.22	–	5.73
Nasal cavity width (Nc–Nc)	5	0.56 ± 0.14*	9	3.96	3	0.48 ± 0.26*	4	1.08	3	0.50 ± 0.23*	5	4.74
Maxillary width (J–J)	6	−0.03 ± .12	–	24.89***	4	0.03 ± 0.20	–	6.38	4	0.08 ± 0.19	–	7.60
Mandible width (Go–Go)	7	0.09 ± 0.11	–	23.52***	5	0.04 ± 0.20	–	9.51*	5	0.09 ± 0.18	–	9.02
Relative face width (Zy–Zy/Eu–Eu)	3	0.41 ± 0.16*	3	24.06***	–	–	–	–	–	–	–	–
Relative nasal cavity width (Nc–Nc/Eu–Eu)	3	0.71 ± 0.16*	8	7.52*	–	–	–	–	–	–	–	–
Maximum head length (Gl–Op)	4	0.11 ± 0.16	–	8.42*	–	–	–	–	–	–	–	–
Total cranial base length (N–Ba)	4	0.21 ± 0.17*	1	4.75	3	−0.01 ± 0.27	–	3.12	3	0.42 ± 0.24*	3	2.36
Anterior cranial base length (S–N)	5	0.18 ± 0.14*	1	16.58**	4	0.15 ± 0.21	–	7.98*	4	0.28 ± 0.19*	2	6.74
Posterior cranial base length (S–Ba)	4	0.05 ± 0.17	–	4.15	3	−0.07 ± 0.27	–	2.13	3	0.21 ± 0.24	–	1.76
Total facial height (N–Me or N–Gn)	5	−0.06 ± 0.12	–	13.04*	4	0.04 ± 0.22	–	17.64***	4	−0.12 ± 0.19	–	1.02
Upper face height (N–Ans)	5	−0.41 ± 0.16*	5	6.22	3	−0.37 ± 0.34*	3	1.55	3	−0.30 ± 0.32	–	1.73
Lower face height (Ans–Me)	3	0.45 ± 0.18*	4	1.75	–	–	–	–	–	–	–	–
Mandibular depth (Go–Me or Go–Gn)	3	0.23 ± 0.20*	1	3.53	–	–	–	–	–	–	–	–
Mandible length (Ar–Pg or Ar–Gn)	4	0.11 ± 0.16	–	2.94	–	–	–	–	–	–	–	–
Mandibular ramus height (Ar–Go)	4	0.08 ± 0.16	–	7.71	–	–	–	–	–	–	–	–
Cranial base angle (N–S–Ba)	6	0.07 ± 0.13	–	28.47***	5	0.10 ± 0.19	–	9.93*	5	0.01 ± 0.18	–	20.38***
A-N-B Angle	4	−0.11 ± 0.14	–	14.47**	4	−0.06 ± 0.20	–	16.21**	4	−0.15 ± 0.18	–	3.90
Facial angle (NPg–FH)	3	0.05 ± 0.15	–	2.85	–	–	–	–	–	–	–	–
Angle of convexity (NA–APg)	5	−0.02 ± 0.15	–	40.98***	4	−0.13 ± 0.24	–	13.00**	4	−0.04 ± 0.22	–	21.26***
S–N–A angle	4	−0.02 ± 0.16	–	3.85	3	0 ± 0.25	–	5.02	3	−0.11 ± 0.23	–	0.45
S–N–B angle	4	0.14 ± 0.16	–	31.35***	3	−0.15 ± 0.25	–	11.23**	3	0.29 ± 0.23*	1	28.68***
S–N–Pg angle	3	0.27 ± 0.20*	1	4.48	–	–	–	–	–	–	–	–
Gonial angle (Ar–Go–Me)	5	0.12 ± 0.13	–	12.59*	3	0.24 ± 0.25	–	5.35	3	0.17 ± 0.23	–	0.85

n, number of studies included; d_w, Mean weighted effect size statistic (positive score indicates parental sample larger than control sample); N_{fs}, Fail-safe N (only calculated for variables with significant effect sizes); Q, heterogeneity statistic.
*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$.

population controls. Our results suggest that a wide variety of craniofacial differences characterize unaffected parents. When data from unaffected mothers and fathers were pooled to produce mid-parent values, the combined parental sample was shown to possess wider interorbital, nasal cavity and upper facial dimensions, narrower cranial vaults, longer cranial bases, longer and more protrusive mandibles, shorter upper faces and longer lower faces compared with controls. Of these changes, increased nasal cavity width was the strongest and most consistent finding in unaffected parents (Table 5). Although fewer variables were considered, unaffected fathers and mothers separately showed patterns of craniofacial deviation similar to the combined parental sample, particularly for craniofacial width dimensions. Unaffected mothers, however, possessed slightly more craniofacial differences than unaffected fathers. In general, our results suggest that while the craniofacial phenotype of unaffected parents indeed differed from controls, the

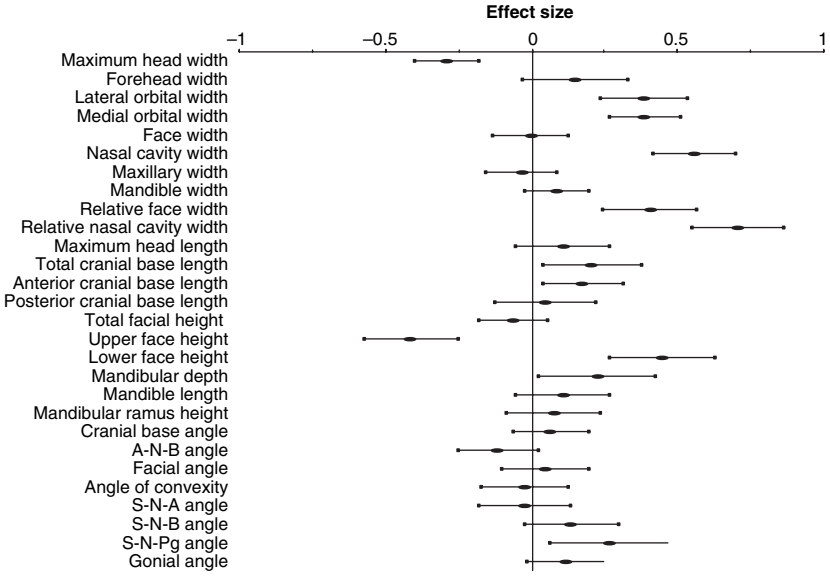

Fig. 1. Mean weighted effect size estimates and 95% confidence intervals for the combined parental data. Mean effect sizes placed to the right of the center line indicate values larger in the unaffected parents compared with controls.

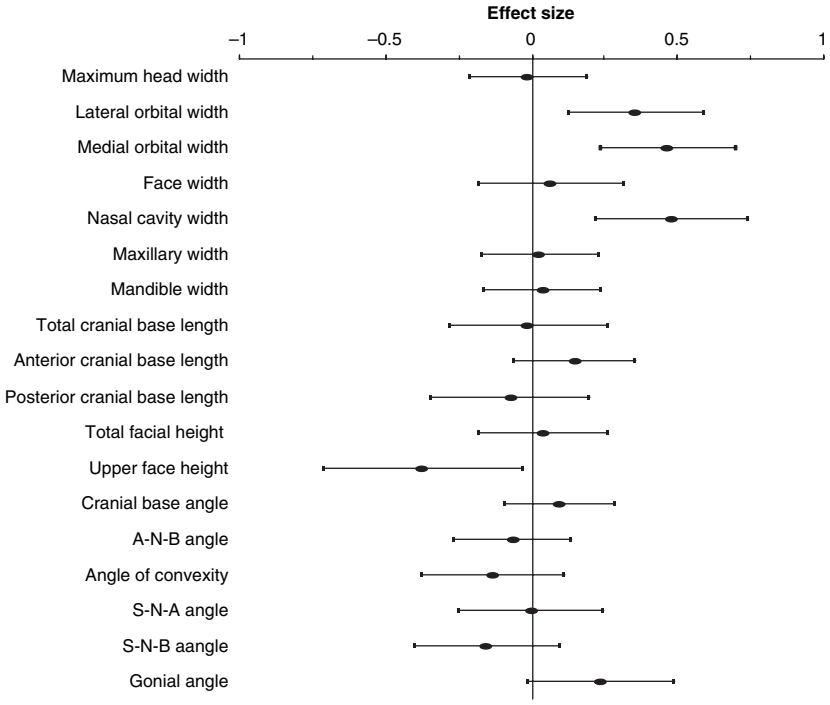

Fig. 2. Mean weighted effect size estimates and 95% confidence intervals for the male parental data. Mean effect sizes placed to the right of the center line indicate values larger in the unaffected fathers compared with controls.

discrepancies were quite subtle. This is evidenced by the fact that the overwhelming majority of cephalometric variables were associated with either weak (0.20–0.49) or very weak (0–0.19) ES statistics and small N_{fs} values; in fact, only two of the 28 variables included (nasal cavity width and relative nasal cavity width) had an ES in the moderate range (0.50–0.79). No variables were associated with a large ES (>0.80). This is not surprising, given the fact that the experimental group is comprised entirely of 'unaffected' parents, at least from a clinical point of view. Therefore, we should expect the differences to be subtle.

Furthermore, the large number of variables with significant heterogeneity scores indicates that the difference between unaffected parents and unaffected controls was quite erratic across studies. This was especially true for the combined parental data, perhaps because of the greater number of studies included. Such ES variability among studies reveals itself plainly as inconsistent or even paradoxical results, as noted in

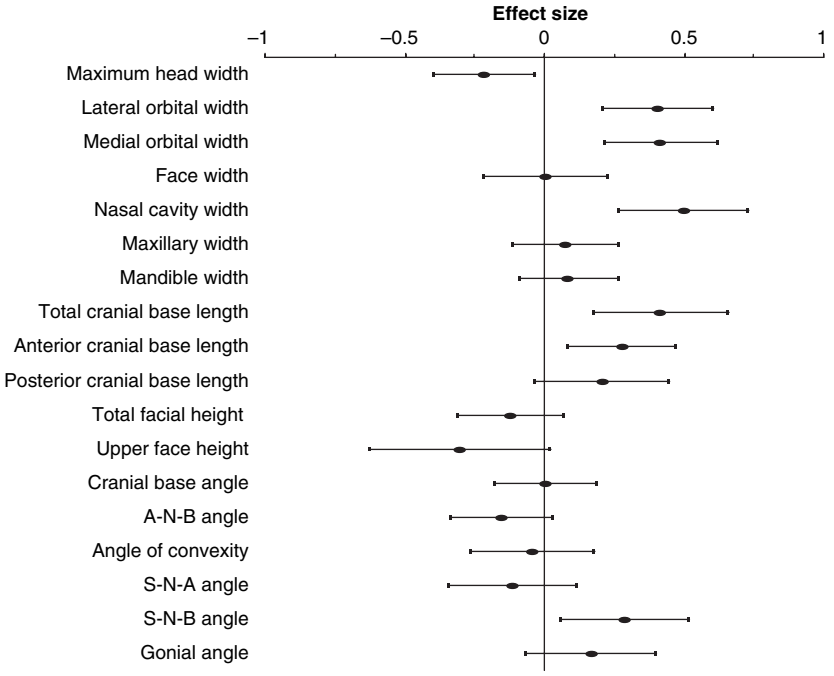

Fig. 3. Mean weighted effect size estimates and 95% confidence intervals for the female parental data. Mean effect sizes placed to the right of the center line indicate values larger in the unaffected mothers compared with controls.

Table 5. **Summary of craniofacial cephalometric variables with statistically significant ($p < 0.05$) non-zero effect sizes**

Variables with weak ES (d_w = 0.20–0.49)		Variables with moderate ES (d_w = 0.50–0.79)	
ES Homogeneous*	ES Heterogeneous*	ES Homogeneous	ES Heterogeneous
Parents combined			
↑ Lateral orbital width	↓ Maximum head width	↑ Nasal cavity width	↑ Relative nasal cavity width
↑ Medial orbital width	↑ Relative face width		
↑ Total cranial base length	↑ Anterior cranial base length		
↓ Upper face height			
↑ Lower face height			
↑ Mandibular depth			
↑ S–N–Pg angle			
Unaffected fathers			
↑ Lateral orbital width			
↑ Medial orbital width			
↑ Nasal cavity width			
↓ Upper face height			
Unaffected mothers			
↓ Maximum head width	↑ S–N–B angle	↑ Nasal cavity width	
↑ Lateral orbital width			
↑ Medial orbital width			
↑ Total cranial base length			
↑ Anterior cranial base length			

*Determined from the *Q* statistic.
↑ Larger in the parental sample.
↓ Smaller in the parental sample.

previous assessments of the literature (20,38) but never quantified. Based on previously described differences in cleft etiology and craniofacial shape across diverse ethnic groups, we hypothesized that ES variability would be related to the population from which the study samples were derived. However, no statistical relationship was identified. Likewise, variability in ES was not related to the overall methodological quality of the studies. Therefore, the factors moderating ES variability in the present set of studies are as yet unknown. It must be noted, however, that the small number of studies available for any given variable likely resulted in a serious loss of statistical power. Perhaps, with a larger sample of studies such relationships would have emerged.

Although many studies did not meet the inclusion criteria for the present meta-analysis (see Table 2), it is nevertheless useful to compare selected results obtained here to those of excluded studies. As in the present analysis, many of these excluded studies report one or more increased middle to upper facial width in their unaffected parental sample (1,30,34,42,43). Consequently, these findings are in accordance with the embryological data on craniofacial form in high-risk cleft animal models (4,11). Despite this, our results for facial width largely conflict with those of McIntyre and Mossey (40), who found reduced nasomaxillary, bizygomatic and interorbital width dimensions in their sample of unaffected parents from Scotland. In this same study, however, they did describe increases in superolateral facial breadth. Although they used a slightly different study design (discordant unaffected twin vs. control), Chatzistavrou et al. (45) also reported reduced nasal cavity width in their sample of unaffected sibs compared with controls, which is in direct conflict with the most significant finding of the present study. Interestingly, in an earlier report, Johnston and Hunter (56) described a bimodal distribution in nasal cavity width in the unaffected twins of cleft probands, with some twins showing reduced nasal cavity dimensions and others showing increased dimensions. Liu et al. (57) suggested that these statistically identified subsets could represent distinct etiological subgroups. If anything, this finding firmly underscores the importance of exploring pre-existing craniofacial variation prior to statistical comparison when dealing with a complex disease like non-syndromic CL/P, where there is likely to be etiological heterogeneity even within populations (2,20).

In terms of maximum head width, our results are in general agreement with excluded studies describing reduced calvarial size in unaffected CL/P relatives (3,30,32,43). Findings are less clear with regard to cranial base changes. While there is some agreement with the present study showing cranial base elongation in unaffected relatives (2,3), at least one study has found the opposite (41). Regarding mandibular differences, Mossey et al. (3) found some evidence in unaffected mothers for increased mandibular length, while no difference was noted by Fraser and Pashayan (1). Many previous studies, however, report decreased facial convexity in unaffected relatives (1,2,14,31,34), which could be the result of a relative increase in mandibular length, reduction in maxillary AP length or a combination of the two. Finally, with regard to facial height changes, the present study found that unaffected parents had shorter upper faces and longer lower faces compared with controls. This is similar to the phenotypic pattern reported in some previous studies (2,40). Both Fraser and Pashayan (1) and Mossey et al. (3) reported increased total facial height in their parental samples, although it is not entirely clear how this increase was distributed among the facial segments. In a more recent study of Costa Rican relatives by Yoon et al. (43), the exact opposite pattern was observed; parents had increased upper facial height and decreased lower facial height. Figalová and Šmahel (30) similarly found that upper facial height was increased in their sample of unaffected parents, while Perkiomaki et al. (41) reported no significant differences in any measures of facial height.

Thus, it is clear that when the results of the present meta-analysis are considered within the context of the entire literature, no single trait is capable of differentiating between unaffected CL/P parents and population controls with perfect consistency. Nevertheless, taking into account the results of this study along with those of additional excluded studies, the most reliable findings are increased facial width dimensions, reduced calvarial dimensions and/or changes in facial profile due to altered mandibular position. It is perhaps noteworthy that these findings echo the conclusions of Ward et al. (20), who suggest in their comprehensive review of the literature that increased interorbital width and rotated mandibular position represent the most

salient craniofacial characteristics of unaffected cleft relatives described to date. The major weakness in the present meta-analysis was the small sample of studies that met our inclusion criteria. Furthermore, of the nine studies that were included, often only a handful considered the same variables. We limited our meta-analysis to variables present in at least three studies; however, it could be argued that such a small sample may not be representative of studies in general. The fact that results from addition excluded studies correspond for the most part to the findings of the present meta-analysis provides some validation.

The identification of superficially unaffected, yet morphologically divergent, individuals within CL/P families has direct relevance for understanding the etiology of isolated CL/P. Clearly some proportion of unaffected relatives is expected to be informative from a genetic perspective. Unfortunately, unlike most Mendelian disorders, simple pedigree analysis cannot reliably identify genetically informative individuals (i.e. gene carriers) in a complex disease such as isolated CL/P. As a result, genetic family studies of non-syndromic clefting have reduced power in large part because of the fact that a given family member's affection status may not correspond to their genetic risk. If, on the contrary, one makes the reasonable assumption that certain craniofacial variations disproportionately represented among a subset of unaffected relatives of cleft probands are indicative of underlying genetic susceptibility, then the identification and subsequent inclusion of those highly dysmorphic individuals into formal genetic analyses should improve statistical power. Mossey et al. (37) have already demonstrated this principle using genetic association analysis. They found that by combining genotypic (TGFa polymorphism) and morphometric information, prediction of cleft liability in parents improved over using either type of information alone. In another recent study, Weinberg et al. (18) incorporated data on occult *orbicularis oris* defects (another hypothesized sub-clinical phenotypic marker of genetic liability) in unaffected CL/P relatives into a genome-wide linkage approach. The results suggested that broadening the CL/P phenotype to include these sub-clinical markers results in increased statistical power to detect linkage, in comparison to when the more traditional, narrow phenotype definition (overt cleft = affected) was used. In future studies, we intend to incorporate morphometric data on unaffected family members into genetic linkage and association-based approaches of CL/P with a wide variety of candidate loci.

Conclusion

In summary, the major findings of the current meta-analysis are as follows: 1) in general, unaffected parents of children with CL/P were shown to have wider faces, narrower cranial vaults, longer cranial bases, longer and more protrusive mandibles, shorter upper faces and longer lower faces compared with controls; 2) the vast majority of these craniofacial differences were subtle in nature, as evidenced by weak to moderate ES statistics; 3) significant phenotypic heterogeneity was present in just over half of the variables examined; and 4) increased nasal cavity width was the strongest and most consistent finding across studies.

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Appendix: formulae used in meta-analysis

For each variable in each study, the following formula was used to calculate an individual effect size (d):

$$d = \frac{M_1 - M_2}{\sigma_p} \tag{1}$$

where M_1 is the mean value for the experimental group; M_2 the mean value for the control group; and σ_p the pooled standard deviation. To calculate σ_p, the following formula was used:

$$\sigma_p = \sqrt{\frac{(N_1 - 1)\sigma_1^2 + (N_2 - 1)\sigma_2^2}{N_1 + N_2 - 2}} \tag{2}$$

where N_1 is the sample size for the experimental group; σ_1 the standard deviation of the experimental group; N_2 the sample size for the control group; and σ_2 the standard deviation of the control group.

In order to calculate the weighted average effect size (d_w) for a group of studies, the following formula was used:

$$d_w = \frac{\sum_{i=1}^{k} w_i \, \mathrm{d}_i}{\sum_{i=1}^{k} w_i} \tag{3}$$

where d_i is the effect size index for the ith study; and w_i the weight for that particular effect size. Weights were calculated using the following formula:

$$w_i = \frac{1}{s_{di}^2} \tag{4}$$

where s_{di}^2 is the conditional (within-study) variance for an individual effect size, which can be calculated from the following formula:

$$s_{di}^2 = \left(\frac{d_i^2}{2df_w}\right) + \left(\frac{N}{n_1 n_2}\right) \tag{5}$$

where d_i is the effect size index for the ith study; df_w the within-study degrees of freedom ($n_1 + n_2$–2); N the combined sample size ($n_1 + n_2$); n_1 the sample size for the experimental group; and n_2 the sample size for the control group.

Once the weighted average effect size has been calculated, an approximate 95% confidence interval for that effect size can be obtained from the following formula:

$$95\%\ \mathrm{CI} = {}_{1.96}\sqrt{\frac{1}{\sum_{i=1}^{k} w_i}} \tag{6}$$

The value 1.96 is derived from the standard normal distribution. It corresponds to the two-tailed critical value (z) at the 0.05 level of significance.

The formula for calculating the fail-safe N is as follows:

$$N_{fs} = \frac{N(d_w - 0.20)}{0.20} \tag{7}$$

where N is the total number of studies included in the meta-analysis; and d_w the weighted average effect size. The value 0.20 is the value that d_w would equal if N_{fs} number of studies with such a negligible effect size were added to the meta analysis.

The heterogeneity statistic Q was calculated using the following formula:

$$Q = \sum_{i=1}^{k} w_i (d_i - d_w)^2 \tag{8}$$

where d_i is the effect size index for the ith study, w_i the weight for that particular effect size; and d_w the weighted average effect size calculated from all studies. The significance of Q is determined from a chi-squared distribution with $k - 1$ degrees of freedom, where k is the number of effect sizes (or studies) included in the meta-analysis.