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Asymmetry of the craniofacial skeleton in the parents of children with a cleft lip, with or without a cleft palate, or an isolated cleft palate

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SUMMARY The objective of this study was to evaluate asymmetry of the parental craniofacial skeleton of subjects with a cleft lip, with or without cleft palate [CL(P)], and isolated cleft palate (CP).

The postero-anterior (PA) cephalograms of 52 parents of children with CL(P) and 40 parents of children with CP from a sample of 196 children with non-syndromic clefts in the west of Scotland were analysed. A conventional cephalometric asymmetry analysis was used to evaluate size-related right:left asymmetry comprising eight linear distances, nine angular, and three facial area measurements. Right:left ratios of the mean values identified the direction of the asymmetry and two-sample *t*-tests determined statistical significance. A shape-related asymmetry analysis was also undertaken. The configurations of landmarks were optimally superimposed and scaled using Procrustes algorithms. Euclidean distance matrix analysis (EDMA) was then compared and the shape of the left and the right landmark configurations were statistically tested using a non-parametric bootstrap technique.

For the parents of CL(P) children, size-related asymmetry was identified and the area of the craniofacial polygon was statistically significantly larger on the right than on the left side. EDMA detected the presence of shape-related asymmetry (T statistic = 1.304; $P = 0.003$). For the parents of CP children, although size-related asymmetry was identified, EDMA did not identify shape-related asymmetry (T statistic = 1.281; $P = 0.065$).

Size and shape directional asymmetries are characteristic features of the parental craniofacial skeleton in CL(P). Although directional size asymmetry is present in the parental craniofacial skeleton in CP, shape asymmetry is not a characteristic feature.

Introduction

Aetiologic heterogeneity is involved in cleft lip, with or without cleft palate [CL(P)], and isolated cleft palate (CP) with relative contributions from genetic and environmental sources (Blanton *et al.*, 2004). Genetic contributions are minimal in some cases, heavily weighted to one parent in others, and approximately equal where each parent possesses the same degree of predisposing factors (Ward *et al.*, 1989). Features predisposing to CL(P) and CP are specified by the parental genome and may be identifiable in the parental phenotype. This is the premise of investigations of the craniofacial morphology of the parents of children with CL(P) and CP (McIntyre and Mossey, 2002b; Weinberg *et al.*, 2006). Moreover, the correlation between the craniofacial morphology of children and their respective parents (Saunders *et al.*, 1980; Suzuki and Takahama, 1991; Johannsdottir *et al.*, 2005) means the craniofacial skeleton of the parents of children with CL(P) and CP provides an opportunity to investigate craniofacial skeletal asymmetry as a heritable predisposing factor in CL(P) and CP.

Craniofacial asymmetry is most obvious in unilateral CL(P), where nasolabial distortion is accompanied by underlying hard tissue asymmetries such as alveolar defects, secondary palatal clefts, and lateral expansion of the piriform aperture (Zemann *et al.*, 2002). However, subtle asymmetries are also present in bilateral CL(P) and CP, particularly where the right or the left palatal shelf fuses with a section of the nasal septum (Kilpeläinen and Laine-Alava, 1996). Furthermore, the expression of craniofacial morphogenes involved in the aetiopathogenesis of CL(P) and CP (McIntyre and Mossey, 2002a) may produce asymmetries at other craniofacial regions.

Coupled with the consistent finding of the left-sided predilection of CL(P) across ethnic groups, there are reports of deviations in symmetry in CL(P) families including dermatoglyphic asymmetry (Woolf and Gianas, 1976; Kobliansky *et al.*, 1999; Neiswanger *et al.*, 2002) and non-right-handedness (Rintala, 1985; Wentslaff *et al.*, 1997). It would therefore be useful to detect the phenotypic predisposition in clefts by an assessment of the craniofacial skeleton if asymmetry was consistently identified.

There are few published investigations of parental craniofacial asymmetry in clefting. McIntyre and Mossey (2002a) found that a heterogeneous group of parents of children with CL(P) and CP exhibited shape-related but not size-related asymmetry. Yoon *et al.* (2003) suggested that a unilaterally increased nasomaxillary width in parents may play a key role in the development of ipsilateral CL(P) in

their offspring. More recently, Neiswanger *et al.* (2005) found asymmetry in ear size in Chinese families with CL(P). Further studies are required before any specific morphogenes coding for particular asymmetric phenotypic features of CL(P) and CP can be investigated.

Asymmetries are classified as fluctuating (FA), directional (DA), and antisymmetry (AA). FA is part of craniofacial skeletal variability within populations and indicates overall developmental stability. DA presents as a left-right sided discrepancy and is explainable by early embryonic regulation by homeobox genes (Pirttiniemi, 1998). Moreover, DA left–right differences are associated with the genetic inheritance pattern of laterality and early childhood developmental patterns (Pirttiniemi, 1998). Clearly, DA is a feature of CL(P) because of the predominance of left-sided clefts (Paulozzi and Lary, 1999), superimposed on the background population FA. AA is a systematic deviation from symmetry, but despite population variability, the bimodal distribution is centred on zero.

Cephalometric analyses have assessed size-related asymmetry using postero-anterior (PA) cephalograms, most combining linear distances, angles, and area measurements (McIntyre and Mossey, 2002a; Yoon *et al.*, 2003). However, shape asymmetry is arguably of greater relevance in the search for CL(P) and CP morphogenes. Mathematical shape is the information that remains when the properties of size, location, and orientation are eliminated from biological data (Kendall, 1989). Conventional cephalometric asymmetry analyses (CCAA) are therefore unable to produce shape information despite their ability to derive information about size-related asymmetries. Biological shape data have proven to be important in the phenotypic identification of the morphogenetic features of Pfeiffer, Saethre-Chotzen, Carpenter, Crouzon, and Apert syndromes (Young *et al.*, 1986; Richtsmeier, 1987, 1988; Richtsmeier and Lele, 1990, 1993). Procrustes transformation and Euclidean distance matrix analysis (EDMA) is one morphometric method of evaluating shape-related asymmetry (Hay *et al.*, 2000), by comparing the shapes of the right and left landmark configurations using a series of ratios of Euclidean distances arranged into a matrix for analysis. Notwithstanding, morphology is a combination of size and shape (Klingenberg, 2002) and investigations of asymmetry should involve an assessment of both size- and shape-related asymmetry.

The aims of this study were to identify if regions of the parental craniofacial skeleton of CL(P) and CP subjects demonstrate directional skeletal asymmetry using CCAA to evaluate size-related asymmetry, while a combination of a Procrustes transformation and subsequent EDMA (Lele and Richtsmeier, 1990) evaluated shape-related asymmetry.

The null hypothesis tested is that the parents of children with CL(P) and CP demonstrate craniofacial skeletal symmetry.

Subjects and methods

The biological parents of all children with non-syndromic CL(P) and CP born in the West of Scotland between 1 January 1980 and 31 December 1984 were invited to volunteer for a project having ethical approval for obtaining PA cephalograms. Of 196 parental pairs, 136 parents replied. Thirty-two subjects defaulted for record collection. The participants were confirmed verbally as the biological parents of the index case. Twelve of the 104 volunteers were excluded because of previous trauma or poor quality PA cephalograms leaving 92 parental PA cephalograms for study. There were 40 parental pairs, plus eight fathers and four mothers. Fifty-two were from parents of children with CL(P) and 40 from parents of children with CP. The high CL(P) to CP ratio approximating 1:1 is representative of the West of Scotland population (Fitzpatrick *et al.*, 1994) compared to ratios of 2:1 in other European centres (Jensen *et al.*, 1988). The mean parental age was 37.2 years and was representative of the population compared with UK census data (1981 census, 1991).

The PA cephalograms were obtained by one radiographer using an Orthoceph 10 cephalometer (Siemens Plc; Siemens House, Bracknell, Berkshire, UK). The source–transporionic axis distance was 152 cm and the transporionic axis–film distance 12 cm. The subjects were positioned with the transporionic axis and Frankfort plane horizontal to the floor (Grummons and Kappeyne, 1987), while the ear-rods and nasal rest were used to eliminate rotational errors. The standard cephalometer settings were 74 kV, 15 mA, 0.64 second exposure time for males and 73 kV, 15 mA, 0.5 second exposure time for females, with magnification standardized at 10 per cent. The films were scanned at 600 dpi and displayed on a flat screen personal computer monitor with a pixel size of 0.051 mm, smaller than the 0.1 mm maximum as recommended by Quintero *et al.* (1999). The x, y coordinates of 29 skeletal landmarks (Figure 1) were digitized by one investigator (GTM) under identical conditions using an automated routine. In order to evaluate individual landmark intraoperator reproducibility, 25 per cent ($n = 24$) of the images were redigitized 1 month later by the same investigator (Houston, 1983). Random and systematic errors were calculated using the coefficient of reliability and a two-sample t-test where the level of significance was 0.95 for the random error values and $P < 0.1$ for systematic errors. Consequently, five landmarks [CG, IO(R), IO(L), Cond(R), and Cond(L)] were excluded to leave 24 reproducible landmarks for analysis.

Size asymmetry

CCAA, comprising eight linear distance, nine angular, and three facial area measurements, were used to measure right:left size-related directional asymmetry. These were calculated from the coordinate data using a spreadsheet. The linear distance variables measured the transverse component of the anterior and posterior cranial base, the orbital, zygomatic, nasal, and maxillary regions. The angular measurements represented the right and left zygomatic bones, the maxillary halves, and the right and left sides of

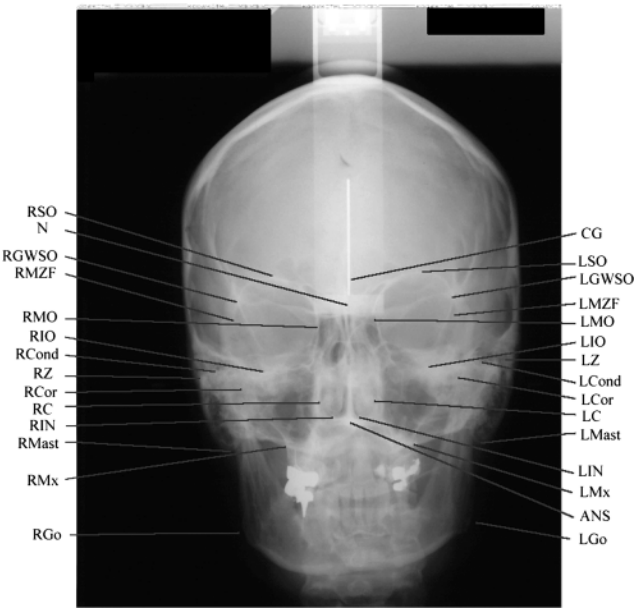

Figure 1 Landmarks on postero-anterior cephalograms used in the study. SO(R), most superior point on the inner cortical plate of the right orbital rim; GWSO(R), intersection of the right greater wing of sphenoid and inner cortex of the supero-lateral orbital rim; (R)ZF, most medial point of the right zygomatico-frontal suture; SO(L), most superior point on the inner cortical plate of the left orbital rim; GWSO(L), intersection of right greater wing of sphenoid and the inner cortex of the supero-lateral orbital rim; MZF(L), most medial point of the left zygomatico-frontal suture; MO(R), most medial point on the inner cortical plate of the right orbital septum and the anterior cranial base—nasion; MO(L), most medial point on the inner cortical plate of the left orbital rim; IO(R), most inferior point on the inner cortical plate of the right orbital rim; IO(L), most inferior point on the inner cortical plate of the left orbital rim; Z(R), zygion—most lateral point on the right zygomatic arch; Cond(R), condylar—most superior point on the right mandibular condyle; Cor(R), most superior point on the right mandibular coronoid process; Mast(R), most inferior point on the right mastoid process (apex); Z(L), zygion—most lateral point on the left zygomatic arch; Cond(L), condylar—most superior point on the left mandibular condyle; Cor(L), most superior point on the left mandibular coronoid process; Mast(L), most inferior point on the left mastoid process (apex); MX(R), maxillare—most medial point on the right maxillary buttress; MX(L), maxillare—most medial point on the left maxillary buttress; C(R), most lateral point on the inner cortex of the right anterior nasal aperture; IN(R), most inferior point on the inner cortex of the right anterior nasal aperture; ANS, anterior nasal spine—the centre of the intersection of the nasal septum and the palate; IN(L), most inferior point on the inner cortex of the left anterior nasal aperture; C(L), most lateral point on the inner cortex of the left anterior nasal aperture; Go(R), right gonion—the most outward inferior point on the angle of the mandible; Go(L), left gonion—the most outward inferior point on the angle of the mandible.

the nasal cavity. The areas of the right/left polygons, right/left maxillozygomatic complexes, and right/left nasal cavities were also calculated. Right:left ratios of the mean values identified the direction of the asymmetry and two-sample *t*-tests determined statistical significance (Table 1).

Shape asymmetry

Morphometric asymmetry analysis (MAA) was used to evaluate shape-related asymmetry. Uniformly scaled right and left landmark configurations were produced using the thin plate spline small program (ftp://life.bio.sunysb.edu/morphmet/tpssmalw32.exe). Procrustes algorithms simultaneously scale the configurations of the 24 landmarks to uniform size, translating them to superimpose the centroids (the geometric midpoint), and iteratively rotate them to minimize the squared differences between landmarks (Auffray *et al.*, 1999). This produces the position of 'optimal fit' of the landmark configurations being tested. These uniformly scaled landmark configurations were then used to investigate right:left shape asymmetry using EDMA. The FORM procedure of the EDMA software (Cole, 1999) performed a form difference analysis on the mean x, y coordinates of the landmark configurations. This program generates a form matrix for each left and right landmark configuration by calculating all possible Euclidean distances between landmark pairs. Each pair of homologous Euclidean distances from the form matrices are then systematically compared as a ratio, producing the Form Difference Matrix (FDM), sorted to rank the elements according to increasing value. The sorted FDM allows the identification of the elements of the FDM corresponding to the regions of greatest shape asymmetry. The T statistic for form-difference testing was calculated as the ratio of the largest to the smallest of the elements of the FDM. This represents the overall right:left shape difference for both the CL(P) and CP groups. The statistical significance of T was assessed by comparing the observed value to the distribution of T values using a non-parametric bootstrap procedure (Richtsmeier and Lele, 1993), based on 1000 resamples (pseudosamples). The proportion of bootstrapped T values greater than T are represented as a P value.

Results

Parents of children with CL(P)

The results of the CCAA for the parents of children with CL(P) are shown in Table 2. None of the linear distance measurements were statistically significantly different between the right and left sides of the craniofacial complex. Although all three angles in the triangle depicting the inferior half of the maxillozygomatic complex statistically significantly differed between the right and left sides of the craniofacial skeleton ($P < 0.05$), the only area measurement that was statistically significantly asymmetric was the area of the craniofacial polygon, where the right side was larger than the left ($P < 0.05$).

The sorted FDM of the MAA for the parents of children with CL(P) is shown in Table 3. Figure 2 displays the ratios of the Euclidean distances as lines between the respective homologous landmarks in 10 per cent groupings. Although 78 out of 79 ratios (99 per cent) were within the 0.9–1.0 and 1.0–1.1 groupings, involving less than a 10 per cent difference in morphology between the right and left sides, the T statistic was 1.304, with only 0.3 per cent of the bootstrapped T values being greater than T. Thus, a

Table 1 Variables selected for conventional cephalometric asymmetry analysis.

	Region described	Right	Left
Linear distances	Anterior cranial base	GWSO(R)–N	N–GWSO(L)
	Inner orbital width	MO(R)–N	N–MO(L)
	Facial width	Z(R)–N	N–Z(L)
	Facial width	Z(R)–ANS	ANS–Z(L)
	Mastoid width	MAST(R)–ANS	ANS–MAST(L)
	Maxillary width	MX(R)–ANS	ANS–MX(L)
	Nasal width	C(R)–ANS	ANS–C(L)
	Width of nasal floor	IN(R)–ANS	ANS–IN(L)
Angles	Maxillozygomatic complex	ANS–MZF(R)–Z(R)	ANS–MZF(L)–Z(L)
	Maxillozygomatic complex	ANS–Z(R)–MZF(R)	ANS–Z(L)–MZF(L)
	Maxillozygomatic complex	MZF(R)–ANS–Z(R)	MZF(L)–ANS–Z(L)
	Maxillozygomatic complex	ANS–Z(R)–MX(R)	ANS–Z(L)–MX(L)
	Maxillozygomatic complex	ANS–MX(R)–Z(R)	ANS–MX(L)–Z(L)
	Maxillozygomatic complex	Z(R)–ANS–MX(R)	Z(L)–ANS–MX(L)
	Nasal cavity	N–C(R)–ANS	N–C(L)–ANS
	Nasal cavity	N–ANS–C(R)	N–ANS–C(L)
	Nasal cavity	C(R)–N–ANS	C(L)–N–ANS
Areas	Right/left polygon	SO(R)–N–ANS + SO(R)–GWSO(R)–ANS + GWSO(R)–MZF(R)–ANS + MZF(R)–Z(R)–ANS + Z(R)–MX(R)–ANS	SO(L)–N–ANS + SO(L)–GWSO(L)–ANS + GWSO(L)–MZF(L)–ANS + MZF(L)–Z(L)–ANS + Z(L)–MX(L)–ANS
	Right/left maxilla + zygoma	ANS–MZF(R)–Z(R) + ANS–Z(R)–MX(R)	ANS–MZF(L)–Z(L) + ANS–Z(L)–MX(L)
	Right/left nasal cavity	N–C(R)–ANS	N–C(L)–ANS

Table 2 Conventional cephalometric asymmetry analysis [means, standard deviations, right (R)/left (L) ratios, and two-sample *t* -test results] of parents of children with cleft lip with or without cleft palate.

Variable	*Right (mm)*	*Left (mm)*	*Ratio (R:L)*	*P value*
GSWO–N	56.8 (2.1)	57.2 (2.3)	0.992	0.342
MO–N	16.2 (2.0)	16.1 (2.0)	1.01	0.648
Z–N	87.2 (4.2)	88.0 (4.8)	0.99	0.351
Z–ANS	87.7 (4.7)	88.8 (5.4)	0.988	0.311
MAST–ANS	70.1 (4.2)	71.6 (4.1)	0.979	0.088
MX–ANS	36.5 (2.2)	37.5 (2.5)	0.973	0.039
C–ANS	20.9 (2.1)	21.4 (2.3)	0.974	0.261
IN–ANS	10.3 (1.6)	11.2 (2.0)	0.922	0.025
Variable	*Right (°)*	*Left (°)*	*Ratio (R:L)*	*P value*
Maxilla + zygoma				
ANS–MZF–Z	83.1(9.6)	83.8 (10.7)	0.991	0.733
ANS–Z–MZF	71.5 (6.8)	70.4 (7.6)	1.015	0.442
MZF–ANS–Z	25.3 (4.4)	25.7 (4.4)	0.984	0.634
ANS–Z–MX	26.9 (2.4)	18.6 (2.8)	1.446	**
ANS–MX–Z	105.2 (5.6)	130.4 (8.1)	0.806	**
Z–ANS–MX	47.8 (4.8)	30.9 (6)	1.546	**
Nasal cavity				
N–C–ANS	97.2 (6.1)	95.5 (6.5)	1.017	0.161
N–ANS–C	62.2 (5.1)	63.3 (5.1)	0.982	0.254
C–N–ANS	20.5 (2.7)	21.1 (3)	0.971	0.298
Variable	*Right (mm^2)*	*Left (mm^2)*	*Ratio (R:L)*	*P value*
Polygon area	5697 (523)	4864 (376)	1.171	***
Maxilla + zygoma area	2443 (623)	2519 (276)	0.969	0.153
Nasal cavity area	557 (79)	576 (86)	0.967	0.24

**$P \leq 0.01$; ***$P \leq 0.001$.

statistically significant morphological difference existed between the right and left sides of the craniofacial complex in the parents of children with CL(P), $P = 0.003$). There were no ratios below 0.9 or greater than 1.2. The median ratio was 1.001, between MX and C.

Parents of children with CP

The results of CCAA for the parents of CP subjects are shown in Table 4. All three angles in the triangle depicting the inferior half of the maxillozygomatic complex statistically significantly differed on the right and left sides ($P < 0.05$), while the

Table 3 Asymmetry of parents of children with a cleft lip, with or without cleft palate. Euclidean distance matrix analysis sorted to rank the elements according to increasing value.

Euclidean distance	Ratio	Euclidean distance	Ratio
COR–MAST	0.901	MZF–MX	1.001
IN–ANS	0.933	MZF–GO	1.001
MZF–MAST	0.972	COR–C	1.002
Z–MAST	0.972	COR–IN	1.002
MZF–Z	0.976	SO–C	1.002
SO–MO	0.977	MO–MX	1.002
MX–ANS	0.978	MZF–C	1.002
C–ANS	0.979	Z–IN	1.003
COR–MX	0.980	MO–C	1.003
ANS–GO	0.981	GWSO–ANS	1.003
MO–MAST	0.984	N–IN	1.004
COR–GO	0.984	Z–MX	1.005
MAST–ANS	0.985	N–ANS	1.005
SO–N	0.986	SO–IN	1.005
N–MAST	0.986	SO–MX	1.006
MAST–C	0.988	SO–GO	1.006
GWSO–MAST	0.989	GWSO–Z	1.006
IN–GO	0.989	MZF–IN	1.006
SO–MAST	0.990	MO–ANS	1.007
COR–ANS	0.991	MZF–MO	1.007
GWSO–MO	0.993	SO–Z	1.007
MAST–IN	0.993	GWSO–C	1.007
MX–GO	0.994	MZF–N	1.008
MO–Z	0.994	MO–IN	1.009
Z–ANS	0.994	GWSO–GO	1.010
C–GO	0.995	MO–N	1.010
N–Z	0.996	GWSO–MX	1.010
MX–IN	0.997	GWSO–IN	1.010
MAST–MX	0.997	Z–GO	1.013
N–GO	0.997	N–COR	1.017
N–C	0.998	SO–GWSO	1.020
GWSO–N	0.998	MO–COR	1.024
N–MX	0.999	C–IN	1.026
MO–GO	1.000	MZF–COR	1.034
Z–COR	1.000	SO–COR	1.039
Z–C	1.000	MAST–GO	1.050
MZF–ANS	1.000	GWSO–COR	1.052
SO–ANS	1.000	SO–MZF	1.058
MX–C	**1.001**	GWSO–MZF	1.175

T statistic (maximum/minimum): 1.304 ($P = 0.003$).
Median ratio in bold.

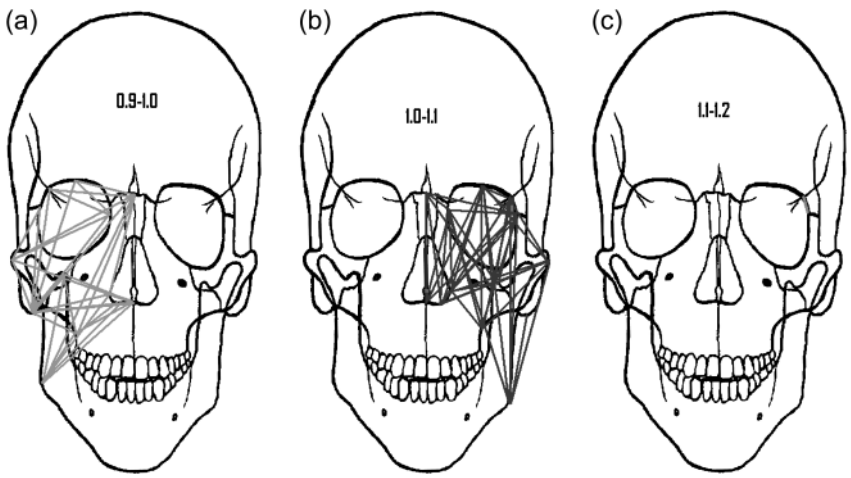

Figure 2 Aysemmetry in the parents of children with cleft lip (palate): Euclidean distance matrix analysis ratios. The smaller ratios are depicted on the right side of the craniofacial complex and the larger ratios on the left.

only area measurement that was statistically significantly asymmetric was the area of the craniofacial polygon, where the right side was larger than the left ($P < 0.05$).

The FDM of the MAA for the parents of children with CP is shown in Table 5. The T statistic was 1.281 ($P = 0.065$), and thus, although a morphological difference was present between the right and left sides of the craniofacial complex in CP, this was not statistically significant. This is because 6.5 per cent of the bootstrapped T values were greater than the observed value of T.

Discussion

Statistically significant size and shape asymmetries were detected in the parents of children with CL(P), while only a size asymmetry was identified in the parents of those with CP. The null hypothesis was rejected and the alternative hypothesis supported. The existence of DA in both the parents of children with CL(P) and CP was confirmed by the statistically significantly larger polygon on the right side for both parental groups. These findings are logical considering the significantly asymmetric nature of unilateral CL(P) and the less asymmetrical presentation of CP.

There was only one EDMA ratio that showed a difference greater than 10 per cent in the right and left landmark configurations of the parental CL(P) group (Figure 2; GWSO–MZF: 1.175). This threshold has been suggested as being clinically significant (McIntyre and Mossey 2002a). Although ratios between 0.900 and 1.100 characterize minor right/left asymmetries, the overall shape difference between the right and left sides of the parents of CL(P) children was statistically significant ($P = 0.003$). The median ratio estimates the general size difference represented by the separate asymmetry FDM. Values close to 1.000 confirm that the right and left morphologies for each group were correctly scaled in advance of conducting EDMA (Richtsmeier and Lele, 1990).

PA cephalograms were selected for this study because they provide a significant amount of biological information in relation to the relatively low ionizing radiation dose (Melsen and Baumrind, 1995). Although the three-dimensional nature of craniofacial asymmetries can be assessed using computerized tomography, the increased ionizing radiation dose was not justifiable.

CCAA are the customary methods of evaluating craniofacial skeletal asymmetry on PA cephalograms. Most use constructed reference planes for comparison of variables on the respective sides of the craniofacial skeleton. An imaginary straight cephalometric midline does not represent the biological midline, especially in subjects with craniofacial scoliosis and craniofacial microsomia (Trahar *et al.*, 2003). In addition, as slight head rotation in the cephalometer alters the relationship of landmarks to this midline, the use of a constructed midline may produce inaccurate results (Athanasiou *et al.*, 1996). The landmarks N and ANS were therefore selected to represent the biological midline as they are highly reproducible and were appropriate for assessment

Table 4 Conventional cephalometric asymmetry analysis [means, standard deviations, right (R)/left (L) ratios, and two-sample t -test results] parents of children with cleft palate.

Variable	*Right (mm)*	*Left (mm)*	*Ratio (R:L)*	*P value*
GSWO–N	57.6 (2.7)	57.0 (2.9)	1.01	0.337
MO–N	16.6 (2.1)	15.8 (2.2)	1.051	0.093
Z–N	87.8 (4.4)	88.0 (5.2)	0.997	0.852
Z–ANS	89.6 (3.4)	90.6 (6.0)	0.989	0.461
MAST–ANS	70.9 (5.2)	72.9 (4.4)	0.972	0.071
MX–ANS	37.7 (2.9)	37.9 (2.7)	0.994	0.108
C–ANS	22.2 (2.5)	22.1 (3.1)	1.007	0.809
IN–ANS	11.7 (2.0)	11.4 (2.0)	1.028	0.491
Variable	*Right (°)*	*Left (°)*	*Ratio (R:L)*	*P value*
Maxilla + zygoma				
ANS–MZF–Z	84.4 (9.5)	85 (9)	0.992	0.774
ANS–Z–MZF	71.4(7.5)	70 (6.9)	1.02	0.407
MZF–ANS–Z	24.1 (4)	24.8 (4.3)	0.971	0.43
ANS–Z–MX	27.4 (2.5)	19.2 (3)	1.427	**
ANS–MX–Z	100.5 (6.9)	127.6 (8.8)	0.787	**
Z–ANS–MX	52 (6.7)	33.1 (6.4)	1.57	**
Nasal cavity				
N–C–ANS	96.2 (7.3)	96.1 (7)	1.001	0.973
N–ANS–C	62.2 (6.2)	62.5 (6.3)	0.995	0.861
C–N–ANS	21.4 (2.6)	21.2 (2.6)	1.009	0.743
Variable	*Right (mm^2)*	*Left (mm^2)*	*Ratio (R:L)*	*P value*
Polygon area	5880 (712)	4952 (484)	1.187	***
Maxilla + zygoma area	2504 (319)	2594 (322)	0.965	0.213
Nasal cavity area	602 (105)	602 (133)	1	0.984

**$P \leq 0.01$; ***$P \leq 0.001$.

Table 5 Asymmetry of parents of children with a cleft palate (CP) Euclidean distance matrix analysis sorted to rank the elements according to increasing value.

Euclidean distance	Ratio	Euclidean distance	Ratio
		GWSO–Z	**1.001**
COR–MAST	**0.927**		
MX–IN	**0.960**	GWSO–IN	**1.001**
MZF–Z	**0.968**	Z–GO	**1.001**
MAST–IN	**0.969**	GWSO–MAST	**1.001**
MAST–C	**0.971**	MO–GO	**1.002**
MX–ANS	**0.974**	SO–ANS	**1.002**
MAST–ANS	**0.979**	COR–MX	**1.003**
C–IN	**0.982**	SO–MX	**1.004**
SO–MO	**0.982**	GWSO–MO	**1.004**
COR–GO	**0.982**	GWSO–C	**1.004**
Z–COR	**0.983**	N–Z	**1.004**
ANS–GO	**0.985**	Z–MAST	**1.004**
MZF–MAST	**0.985**	GWSO–GO	**1.004**
MX–C	**0.986**	N–C	**1.005**
Z–IN	**0.987**	N–MX	**1.005**
IN–GO	**0.987**	MZF–ANS	**1.006**
MAST–MX	**0.988**	MZF–MX	**1.007**
COR–IN	**0.988**	MO–IN	**1.007**
Z–C	**0.989**	N–ANS	**1.007**
MX–GO	**0.991**	Z–MX	**1.008**
C–GO	**0.992**	GWSO–ANS	**1.009**
SO–MAST	**0.992**	MO–C	**1.010**
SO–GWSO	**0.993**	MO–MX	**1.011**
SO–IN	**0.994**	GWSO–MX	**1.015**
MO–MAST	**0.994**	SO–N	**1.015**
SO–Z	**0.994**	MO–ANS	**1.016**
Z–ANS	**0.995**	C–ANS	**1.017**
COR–C	**0.995**	GWSO–N	**1.020**
SO–C	**0.995**	MZF–MO	**1.020**
N–MAST	**0.995**	MZF–COR	**1.022**
MZF–GO	**0.996**	SO–COR	**1.022**
MZF–IN	**0.996**	N–COR	**1.026**
MO–Z	**0.997**	MO–COR	**1.029**
SO–GO	**0.998**	MZF–N	**1.030**
MZF–C	**0.998**	SO–MZF	**1.037**
N–GO	**0.999**	IN–ANS	**1.041**
COR–ANS	**1.000**	GWSO–COR	**1.042**
MAST–GO	**1.001**	MO–N	**1.055**
N–IN	1.001	GWSO–MZF	**1.188**

Median ratio in bold.

of facial asymmetry as they are located in the same antero-posterior plane as facial landmarks that in other planes would be subject to excessive geometric errors (Pirttiniemi *et al.*, 1996). Furthermore, the anterior facial location of N and ANS means they are unlikely to be distorted by cranial or dental asymmetries. This method possesses greater validity than simply calculating a series of linear distance measurements between landmarks and constructed reference planes.

As there is no standard CCAA the variables in Table 1 were selected to represent the craniofacial complex. This comprised the ratios of right to left linear distance, angular, and area measurements. Interestingly, no previous study has employed ratios although this method eliminates size variability between study participants. Nevertheless, information derived using geometric morphometric techniques such as Procrustes superimposition followed by EDMA may be of greater relevance in the elucidation of the parental craniofacial morphology in CL(P) and CP than that derived from a series of measurement-based computations in CCAA. It remains that parental cephalometric information derived using both traditional and geometric morphometric techniques should be synthesized in investigations of the CL(P) and CP genotype and phenotype (McIntyre and Mossey, 2003). This is because an evaluation of morphology should involve both assessments of size and shape (Klingenberg, 2002).

Nevertheless, this study has limitations. Controls were not available to estimate the level of asymmetry in the Scottish population. Notwithstanding the presence of FA within this population, overall AA will result where the level of craniofacial asymmetry is zero (Pirttiniemi, 1998). Retrospective studies can be associated with bias. This was minimized by selecting the experimental group from a completely ascertained sample of children with non-syndromic CL(P) and CP from a record registry. This eliminated subjectivity in the parental sample selection. Although the parental sample was not consecutive, including

both biological parents of consecutive births, the ratio of CL(P) to CP was similar to published data for the Scottish population (Fitzpatrick *et al.*, 1994). Epidemiologically, the parental group represented West of Scotland parents of children with CL(P) and CP. However, the CL(P) group were parents of children with both unilateral and bilateral CL(P), and the association between the parental craniofacial skeletal asymmetry and the laterality of the unilateral CL(P) defect in their children was not investigated.

The parents of children with unilateral and bilateral CL and CLP were grouped together. Mossey *et al.* (1998) found distinctive differences between CP and both the CL and CLP groups, but there were no differences between the latter two groups in this lateral cephalometric study of parents of children with clefts. As a result, it was deemed appropriate to keep the CL and CLP parents in one combined group. Although a more recent Norwegian study (Harville *et al.*, 2005) asks this question about CL and CLP differences, this is a single centre study and there is still no consistent rationale to separate CL and CLP on a genetic basis. Replication of the Norwegian findings is awaited before incorporating this into future research strategies. Similarly, there is as yet no evidence to genetically distinguish between unilateral and bilateral clefts and it was therefore legitimate to group these together.

The relevance of the findings of this study to the search for morphoregulatory clefting genes is twofold. Firstly, the parental craniofacial skeleton demonstrating directional asymmetry may confer a direct genetic susceptibility to CL(P) and CP by patterning aberrant embryonic craniofacial morphologies via early embryonic regulation by homeobox genes (Pirttiniemi, 1998) and predisposing the embryo to either CL(P) or CP. This may produce asymmetric discrepancies between the size of the embryonic facial processes and the craniofacial complex preventing contact and/or subsequent fusion of the facial processes during primary and secondary palatal development. This is accordance with the findings of Yoon *et al.* (2003). Indeed, homeotic genes such as morphoregulatory genes (controlling craniofacial morphogenesis; Slavkin, 2000) specify the geometry of orofacial form, and these could be of significance in the development of CL(P) and CP, by patterning aberrations in embryonic craniofacial morphology. Mounting evidence suggests that variants at the TGFβ and MSX1 loci may be important in the genetic contribution to orofacial clefting (Blanco *et al.*, 2001; Mitchell *et al.*, 2001), while other candidate genes are also under investigation (Vieira *et al.*, 2005). MSX1 has been implicated in the aetiopathogenesis of both CL(P) and CP (Lewanda and Jabs, 1994), while TGFβ3 has also been implicated in CL(P). Could it be that the parental craniofacial shape in CL(P) and CP is principally specified by a variant forms of MSX1, MSX2, JAG2, SKI, FOXE1, GLI2, JAG2, LHX8, MSX1, MSX2, SATB2, SKI, SPRY2, and TBX10, predisposing towards morphometric susceptibility in their offspring, with TGFβ3 (produced in the palatal mesenchyme; Kaartinen *et al.*, 1995; Ferguson, 2000), for example, being one local factor affecting primary palatogenesis or secondary palatal shelf fusion?

The finding of asymmetries relatively distant to the oronasal region suggests that overall craniofacial skeletal asymmetry in the non-cleft parental group is evidence of a CL(P) and CP microform. Logically, sufficient phenotypic and genotypic data will be required before CL(P) and CP morphogenes can be confirmed. Coupled with this, advances in high throughput genotyping make it possible to test panels of genes in an effort to establish their significance to parental craniofacial genotype and aetiopathogenesis of orofacial clefting (Park *et al.*, 2006). Further studies evaluating asymmetry in non-cleft individuals and individuals with CL(P) and CP using morphometric techniques are required, particularly to confirm if directional asymmetry is a feature of CL(P) and CP.

Conclusions

Size-related directional asymmetries were present in the parents of children with both CL(P) and CP. Shape-related asymmetry was present in the craniofacial skeleton of the parents of children with CL(P) but not in the parents of children with CP.

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