Prediction of liability to orofacial clefting using genetic and craniofacial data from parents

Peter A Mossey, Reynir Arngrimsson, John McColl, Gill M Vintiner, J Michael Connor

Abstract

***Background*—Cleft lip with or without cleft palate (CL(P)) and isolated cleft palate (CP) are separate clinical entities and for both polygenic multifactorial aetiology has been proposed. Parents of children with orofacial clefting have been shown to have distinctive differences in their facial shape when compared to matched controls.**

***Objective*—To test the hypothesis that genetic and morphometric factors predispose to orofacial clefting and that these markers differ for CL(P) and CP.**

***Methods*—Polymorphisms at the transforming growth factor alpha (TGFα) locus in 83 parents of children with non-syndromic orofacial clefts were analysed, and their craniofacial morphology was assessed using lateral cephalometry.**

***Results*—Parents of children with CL(P) and CP showed an increased frequency of the TGFα/*TaqI* C2 allele (RR=4.10, p=0.009) relative to the comparison group. Also the TGFα/*Bam*HI A1 allele was more prevalent in the CP parents.**

***Multivariate statistical analysis*—Using stepwise logistic regression analysis the TGFα/*TaqI* C2 polymorphism provides the best model for liability to orofacial clefting. To determine the type of clefting a model involving interaction between the parental TGFα/*Bam*HI and TGFα/*Rsa*I genotypes showed the best fit. Using genotype only to predict the clefting defect in the children according to parental genotype, 68.3% could be correctly classified. By adding information on craniofacial measurements in the parents, 76% of CP and 94% of CL(P) parents could be correctly classified.**

***Conclusions*—This study provides a model for prediction of liability to orofacial clefting. These findings suggest that different molecular aberrations at the TGFα locus may modify the risk for CP and CL(P).**

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Keywords: orofacial clefts; TGFα; cephalometrics

Department of Dental Health, University of Dundee Dental School, Park Place, Dundee DD1 4HR, UK
P A Mossey

Department of Medical Genetics, The University, Glasgow, UK
R Arngrimsson
J M Connor

Department of Statistics, The University, Glasgow, UK
J McColl

Institute of Child Health, Guilford Street, London, UK
G M Vintiner

Correspondence to:
Dr Mossey.

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Cleft lip with or without cleft palate (CL(P)) and isolated cleft palate (CP) are caused by primary defects in the fusion of craniofacial processes that form the primary and secondary palate respectively, but differ in respect to timing. Primary palate fusion takes place at about the fifth week of embryonic life by a highly regulated process of mesenchymal proliferation and epithelial breakdown in three facial prominences, the medial nasal, lateral nasal, and maxillary prominences, whereas elevation and fusion of the secondary palate occurs at about eight weeks. Furthermore, epidemiological and family studies indicate that CL(P) and CP are separate aetiological entities,[1-3] and for both multifactorial inheritance has been proposed. The precise roles played by genes and environment has not been elucidated, in particular the nature and number of genes involved is not known.[4-8] Thus in current medical practice genetic counselling is based on empirical figures which show recurrence risk, depending on the type of orofacial clefting found in the family, varying from 2-6% if one child or one parent is affected to 9% if two children are affected and 15-17% if a parent and a child both have clefts.[2]

Evidence for a genetic contribution comes from both human and animal studies. In humans, the importance of the TGFα locus in orofacial clefting has been highlighted in several studies, where association with polymorphisms within the TGFα gene locus has been shown.[9-16] Possible interaction between polymorphisms of this gene and environmental factors such as smoking has also been suggested.[7 8] The importance of TGFα is further indicated by gene expression studies in murine palatogenesis in mice where spatial and temporal expression of the gene is highly regulated during secondary palatogenesis.[17]

Fraser and Pashayan[18] in 1970, and a number of others since then,[19-23] have reported significant differences in parental cranial and facial shape compared to controls. They generally imply that the deviations from "normal" or control craniofacial morphology may represent the extreme limit of normal variability, and that the genes responsible for the deviation from normal contribute to the manifestation of clefting in their offspring. It is feasible, therefore, that cephalometric studies could enable the identification of phenotypes which could be used to identify people who possess the cleft lip/palate susceptibility genotype. In the quest for genetic predictors of orofacial clefting, most of the previous research effort has been focused on affected subjects. The contribution of heredity is acknowledged but the nature of this with respect to maternal and paternal contribution remains virtually unexplored. The aim of this study was to identify parental characteristics (phenotypic or genotypic) which were associated with an increased risk of having a child with CL(P) or CP.

Table 1 Composition of parental and control cephalometric and genetic study samples classified according to gender and clefting status

	Cephalometric study			Genetic study		
	Parents		Control	Parents		Control*
Subjects	CP	CL(P)		CP	CL(P)	
Female	17	25	50	18	21	—
Male	18	23	49	17	20	—
Totals (%)	35 (42.2%)	48 (57.8%)	99	35 (45.3%)	41 (54.7%)	62
All	83		99	76		62

*Gender ratio of genetic controls unknown.

Methods

SUBJECTS

The subjects in this study were the parents of a completely ascertained sample of 256 children with cleft lip/palate born in the west of Scotland between 1 January 1980 and 31 December 1984. The study sought only the parents of non-syndromic cases of both CL(P) and isolated CP. Ethical approval for the study was obtained. A careful history was taken to determine that the parents used were in fact the biological parents of the cleft proband. Parents of those with syndromic clefting as well as non-white and edentulous subjects were excluded, leaving 196 parental "pairs". All these parents were invited to participate and 136 replied. Of these 83 subjects, 40 fathers and 43 mothers attended the study clinic (table 1). No bias with respect to age and social class was apparent in the final study sample compared to the total sample.

The cephalometric control information was derived from evaluation of existing records from normal adult subjects among the archives of the Dental Hospital and School, Glasgow.[23] The criteria used in the selection of this control group so as to match the parental sample as closely as possible were gender, age, racial background, previous dental history, and type of malocclusion. A total of 49 male and 50 female radiographs was used as the cephalometric control material.

GENOTYPING

DNA was extracted from lymphocytes using standard laboratory procedures[24] and three polymorphisms within the TGFα gene, as previously described,[25] were studied. Genotyping was carried out by DNA amplification at the TGFα locus using PCR and restriction endonuclease digestion using *Bam*HI, *Rsa*I, and *Taq*I restriction enzymes. The *Bam*HI polymorphism is situated in exon VI and detects a single nucleotide substitution allowing distinction between two alleles, A1 and A2. The *Rsa*I and *Taq*I sites are both in intron V, the former created by a single base pair transition showing the two alleles B1 and B2, whereas the latter restriction site represents a four base pair deletion, C1 representing the insertion and C2 the deletion. The three sets of alleles were visualised by subjecting the digested PCR products to horizontal electrophoresis on a 2.5% agarose gel.

In the absence of genetic control data for the west of Scotland population, the control data for the present study were derived from the UK study by Holder *et al.*[11] Use of such data can be justified by the fact that allele frequency at the TGFα locus in control subjects in populations as diverse as Australia, America, and Britain were found to be remarkably similar (table 2).

CEPHALOMETRIC MEASUREMENT

Measurements of the craniofacial parameters in the parental and control radiographs was carried out by the same investigator (PAM) without knowledge of the status of the subject. Cephalometric tracings of the lateral cephalograms were prepared on acetate overlays. Subsequent digitisation was performed on a GTCO Company backlit digitising screen using a digipad 5 digitiser. The PC DIG software program was used to compute the desired parameters and individual, mean, and superimposed tracings were produced using a Hewlett Packard ColorPro printer. (The computer software program used in this study was devised by Dr John McWilliam, Karolinska Institute, Stockholm, Sweden, 1987.) Measurements were verified by double digitisation of all the radiographs and repeated tracing of 20% of the material as recommended by Houston.[26] The PC DIG program allowed measurement of cephalometric areas and, in the context of the present study, six separate

Table 2 TGFα allele frequencies from previous population controls

	Allele frequency					
Reference	A1	A2	B1	B2	C1	C2
(1) Murray *et al*	0.190	0.810	0.290	0.710	0.940	0.060
(2) Ardinger *et al*[9]	0.130	0.870	0.270	0.730	0.950	0.050
(3) Chenevix-Trench *et al*[10]	—	—	—	—	0.945	0.055
(4) Holder *et al*[*11]	0.130	0.870	0.360	0.640	0.960	0.040
(5) Qian *et al*[25]	0.076	0.924	0.293	0.707	0.930	0.070
(6) Sassani *et al*[14]	—	—	0.262	0.738	0.911	0.089

Control characteristics
(1) American whites, n=69.
(2) American whites, n=102.
(3) Australian whites, n=100.
(4) British whites, n=62.
(5) Alcasian whites, n=99.
(6) American whites, n=98.
*Control data used in the present study.

Table 3 Number of chromosomes with each allele in the CP, CL(P), and control group and the relative risk associated with these TGFα polymorphisms

		No of alleles		RR (95% CI)	χ^2	p value
*Bam*HI		A1	A2			
	Control	16	104			
	CP	15	45	2.17 (1.00–4.71)	3.82	0.05
	CL(P)	6	68	0.57 (0.36–1.32)	1.24	0.26
	All parents	21	113	1.21 (0.61–2.14)	0.27	0.60
*Rsa*I		B1	B2			
	Control	42	78			
	CP	18	40	0.81 (0.42–1.59)	0.36	0.55
	CL (P)	25	49	0.92 (0.50–1.70)	0.07	0.79
	All parents	43	89	0.87 (0.52–1.48)	0.25	0.61
*Taq*I		C1	C2			
	Control	115	5			
	CP	53	9	3.91 (1.33–11.45)	6.17	0.01
	CL (P)	68	10	3.38 (1.17–9.78)	5.06	0.02
	All parents	121	19	3.61 (1.38–9.48)	6.82	0.01

Relative risk (RR) and 95% confidence interval (95% CI) as the odds ratio in the parental sample compared with the control sample for alleles A1, B1, and C2 .

area measurements were chosen to augment the linear and angular cephalometric analysis devised.[27] In order to delineate and compute areas on a lateral cephalogram using the PC DIG program, perimeter landmarks of the structures to be investigated were digitised sequentially. Area was automatically calculated in square centimetres.

The choices of linear, angular, and area measurements used in this study were based on the need to describe comprehensively all anatomical regions of the head and face, avoiding the use of parameters that were highly correlated with each other, and where possible landmarks were chosen on the basis of reliability.[27] Thirty seven independent variables were used in the study to achieve a comprehensive description of all parts of the craniofacial skeleton. Full details on how craniofacial parameters were chosen and adjusted for multivariate discriminant analysis are described in Mossey *et al.*[27]

STATISTICAL ANALYSES

Comparison between allele and genotype frequencies was performed using the chi-squared test and the relative risk estimation was calculated as odds ratio devised by Woolf.[28] The two sample *t* test was used to estimate the difference in craniofacial measurements between the parental and control samples. Significance levels (p values) were corrected for multiple testing. One way analysis of variance (ANOVA) was used to estimate which of the cephalometric parameters gave best discrimination between parents of CP and CL(P) patients. Multivariate stepwise discriminant analysis was used to identify those cephalometric parameters found to be most useful to discriminate between the study groups. Logistic regression models were used to determine the relationship between genotypes and liability to orofacial clefting. The factors introduced into the models were the status, as parent of a child with orofacial clefting or a normal control subject. Secondly, the genotypes representing the three polymorphisms for each of these subjects were entered in a stepwise manner each as a separate factor. A forward stepwise approach was adopted and a lattice of hypotheses produced. The resulting logistic regression has a chi-squared statistic distribution. A second logistic regression model was set up in the same way to determine which of the following factors, parental status and the three TGFα genotypes, would best discriminate between a parent predisposed towards producing a child with CP rather than CL(P). Finally, cephalometric variables found to be significantly different by the ANOVA analysis in the CP compared to the CL(P) parental sample were introduced to the second model with and without the genetic data.

As indicated above, it was possible to carry out a comparison of cephalometric and genotypic differences between parents of children with CP versus CL(P) and of each cleft sample with their respective control groups. However, a joint analysis of the genetic and morphometric variations was not possible in this study because the control subjects for each comparison were not the same subjects.

Results

ALLELE AND GENOTYPE FREQUENCIES

Study of the allele frequencies showed a significant difference in the prevalence of the C2 allele of the TGFα/*Taq*I polymorphism among parents of patients with orofacial clefting compared to the control group (RR=3.61, p<0.01) (table 3). This was found in both the CP (3.91, p<0.01) and the CL(P) (3.38, p<0.02) parental sample. When the allele distribution at the *Bam*HI and *Rsa*I TGFα gene restriction sites were investigated no significant difference in allele frequencies was observed between case parents and controls. The prevalence of the A1 allele of the *Bam*HI polymorphism was, however, slightly increased in the CP parental sample (RR=2.75, p<0.05) (table 4).

Further studies showed contrasting differences in the genotypes in these samples. The C2 allele was found to be rare in both parental samples and the control sample. Only two subjects were found to be homozygous for this allele and both of these were fathers of a child with CL(P). There was a significantly higher prevalence of the C2 allele, in either the homozygous or heterozygous state, in both parents of CL(P) patients (RR=3.79, p<0.05) and parents of children born with isolated CP (RR=4.50, p<0.01) (table 4). Only in the CP parental group was an increased prevalence of the A1 allele found among heterozygous or homozygous subjects (RR=2.75, p<0.03). When the genotype frequencies were compared in the CP and CL(P) groups, a difference in the prevalence of the A1 allele was observed. The parents of CP patients were found to have a significantly higher prevalence of the A1 allele and A1A2 genotype than the CL(P) group (χ^2=7.29, p=0.003).

When the genotype pattern in respect to the *Bam*HI and *Rsa*I sites were compared, parents of CP patients were found to have predominantly the A1A2/B1B2 genotype (12/14), while parents of children with CL(P) had predominantly the A2A2/B1B2 genotype (13/17). In the control group the distribution of these combined genotypes was A1A2/B1B2 (n=12, 52%) and the A2A2/B1B2 (n=11, 48%) (table 7). Applying the chi-squared statistic, a

Table 4 Genotype distribution for BamHI, RsaI, and TaqI alleles and relative risk (RR) associated with possession of the TGFα BamHI A1 or TaqI C2 alleles in parents of children with orofacial clefts compared to control subjects

				Relative risk (R)			
	A1A1	A1A2	A2A2	A1A2	95% CI	χ^2	p value
(A) *Bam*HI genotypes							
Holder *et al*[11]	0	16	44				
CP	0	15	15	2.75	1.11-6.78	4.82	0.028*
CL(P)	0	6	31	0.53	0.19-1.50	1.42	0.23
All clefts	0	21	46	1.26	0.58-2.71	0.04	0.56
For A1A2 CP *v* CL(P)						7.29	0.003**
	B1B1	B1B2	B2B2				
(B) *Rsa*I genotypes							
Holder *et al*[11]	10	22	27				
CP	2	13	14			1.74	0.42
CL(P)	4	15	19			0.78	0.68
All clefts	6	28	33			1.82	0.40
	C1C1	C1C2	C2C2	C2†			
(C) *Taq*I genotypes							
Holder *et al*[11]	55	5	0				
CP	22	9	0	4.50	1.40-14.02	6.73	0.009**
CL(P)	29	8	2	3.79	1.25-11.55	6.35	0.04*
All clefts	51	17	2	4.10	1.50-11.18	7.76	0.02*

Homozygosity for C2C2: χ^2=1.55; p=0.21.
χ^2 repeated with C1C2 and C2C2 combined in one group:
(i) CP: χ^2=5.23; p=0.022*
(ii) CL(P): χ^2=5.51; p=0.019*
(iii) All clefts: χ^2=7.34; p=0.006**
**p<0.01, *p<0.05
†Relative risk for possession of C2 allele with C1C2 and C2C2 combined in one group.

significant difference between parental and control groups was observed (χ^2=11.89, p<0.001). All of the variation producing this difference is in the *Bam*HI polymorphism.

The parental distribution of alleles was studied further by looking at the fathers and mothers separately. The TGFα/*Bam*HI A1 allele was found significantly more often among mothers (16/60) compared to the fathers (5/53) (χ^2=3.86, p<0.05) and this variance could be attributed to differences among parents of CL(P) patients rather than those of CP. In the CL(P) group, none of the fathers possessed a genotype with this allele (A1A2 or A1A1), so for an offspring with CL(P) the A1 allele was always transmitted from the mother. It was also noted that when the father had transmitted the A1 allele to his affected child (n=5) the outcome was always an offspring with isolated CP, but with such small numbers this needs to be interpreted with caution.

CEPHALOMETRIC MEASUREMENTS

To compare the cranial and facial shape of parents and control subjects, 37 cephalometric parameters were measured in males and females and the data were analysed separately using the two sample *t* test. The fathers of children with orofacial clefts were shown to have significantly smaller mandibular, symphyseal, and maxillary areas on lateral skull radiographs and reduction in palatal length (ANS-PNS) (table 5, fig 1). In addition, the cranial base angle (N-S-Ba) was more acute and although the cross sectional area of the cranium was smaller in the fathers compared to the control males the occipital subtenuce was larger (table 5, fig 1). Using stepwise discriminant analysis, 83.3% of fathers were correctly classified as being at risk for having a child with orofacial clefting. The mothers had larger anterior facial height (N-Gn) and total facial length (S-Gn). The anterior cranial base (N-S) and the clivus length (S-Ba) were also larger than in the control females (table 5, fig 1). As in the paternal group, reduced cross sectional area of the cranium and increase in the occipital subtenuce length was observed. Using these parameters in a stepwise discriminant analysis 95.1% of mothers were correctly classified as being at risk for having a child with orofacial clefting.

Table 5 Craniofacial measurements found to be significantly different between parents of children with orofacial clefting and controls using two sample t test

Variable	Cases	Controls	(95% CI)	p value
Males	*n=47*	*n=49*		
Area-symphysis (cm^2)	3.1±0.4	3.6±0.4	(0.22, 0.59)	0.0001
Area-mandible (cm^2)	30.1±3.4	33.1±3.1	(1.20, 3.95)	0.0003
Area-maxilla (cm^2)	3.9±0.7	4.5±0.5	(0.17, 0.72)	0.0015
ANS-PNS (mm)	51.4±3.9	53.1±2.8	(0.68, 3.40)	0.0037
Area-cranium (cm^2)	219.9±14.3	235.2±16.0	(8.5, 20.7)	<0.001
Occipital subtenuce (mm)	30.7±4.2	28.8±4.5	(−4.25, −0.08)	0.04
Females	*n=47*	*n=50*		
Mandibular length (mm)	106.4±4.7	104.2±4.8	(−4.44, −0.58)	0.011
Anterior face height (mm)	108.2±5.1	106.6±5.9	(−4.87, −0.16)	0.036
Facial length (mm)	113.4±5.1	111.2±5.0	(−4.92, −0.91)	0.005
Anterior cranial base (mm)	64.5±3.0	63.3±2.4	(−2.56, −0.27)	0.016
Clivus length (mm)	42.4±2.0	41.3±3.1	(−2.35, −0.10)	0.033
Sella width (mm)	10.1±0.9	9.7±1.2	(−0.92, −0.00)	0.048
Area-cranium (cm^2)	203.5±14.1	216.4±12.2	(6.1, 17.10)	0.0001
Occipital subtenuce (mm)	29.5±3.3	25.8±4.3	(−5.33, −1.90)	0.0001

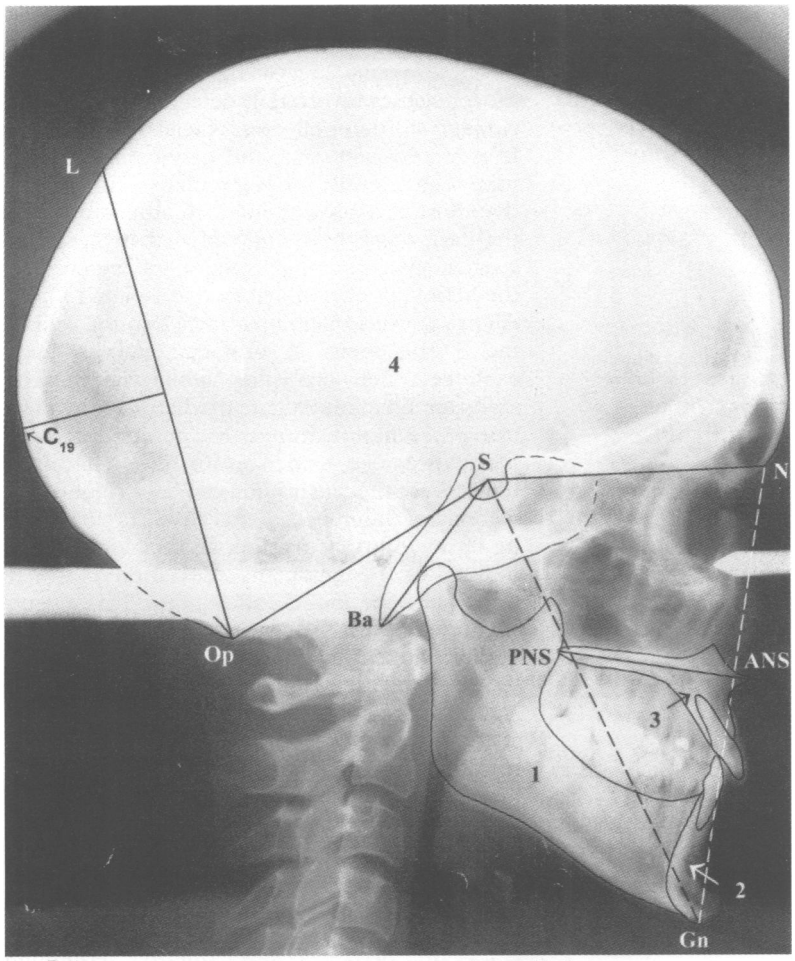


Figure 1 Lateral skull cephalogram illustrating the cephalometric parameters which were significantly different in fathers and mothers compared to controls.

To identify which of the cephalometric parameters could best distinguish between parents having a child with CP and those with a child with CL(P), a three group (CP, CL, and CLP), one way analysis of variance (ANOVA) was carried out by comparing the results from the cephalometric measurements in these three groups. Not one parameter differed between the CLP and CL groups. However, three parameters, the mandibular ramus length, mandibular area, and cranial area, turned out to be significantly larger in CP parents compared to CL(P) parents (table 6).

Table 6 Cephalometric parameters which show a significant difference between parents of children with CP and CL(P)

Parameter	*CP*	*CL(P)*	*95% CI*	*p value*
Mandibular				
Area (cm^2)	31.13±3.09	29.77±2.82	0.03–2.67	0.045*
Ramus length (mm)	68.70±4.57	65.67±4.38	1.04–5.02	0.0033**
Cranium				
Area (cm^2)	214.9±0.6	208.5±6.6	0.006–0.06	0.018*

Table 7 BamHI/RsaI genotype interaction for CP, CL(P), and control groups

	B1B2		
Genotype	*CP*	*CL(P)*	*Control*
A1A2	12	4	12
A2A2	2	13	11
BamHI/RsaI interaction	χ^2	*p value*	
CP *v* control	4.30	0.038*	
CL(P) *v* control	3.34	0.067	
CP *v* CL(P)	11.89	0.0006***	

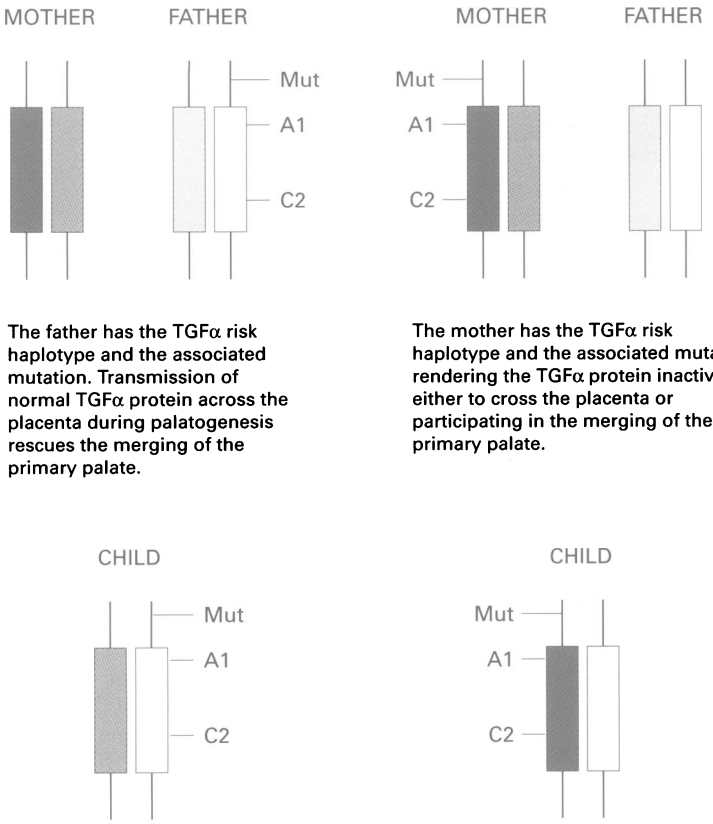
*= $p<0.05$.
***=$p<0.001$.

LIABILITY MODELS USING GENOTYPIC DATA

The first logistic regression model to determine the relationship between the parental genotypes and the liability to have a child with clefting (either CP or CL(P)) was set up. The factors used were the TGFα genotypes produced by the *Bam*HI, *Rsa*I, and *Taq*I restriction enzymes and the parental gender. With this model forward stepwise approach was adopted and a lattice of hypotheses created. The effect of *Taq*I genotypes, either homozygous for the C2 allele or heterozygous (that is, C1C2), were entered first and this was shown to be the best discriminating factor in predicting the risk of having a child with orofacial clefts (CP or CL(P)). There was only one subject in the entire study homozygous for this allele, and in the heterozygous state there were 17 out of 51 parents and five out of 60 controls. On the basis of the C2 allele alone as a discriminator, therefore, 33% of parents would be correctly classified as being predisposed to having orofacial clefting in their offspring, whereas 8% of controls would be wrongly classified. This is in line with previous observations on the TGFα gene, that it is a modifier gene rather than a major gene in the aetiology of orofacial clefting.

A second logistic regression model was set up to determine which of the genotypes with or without consideration of parental gender would best discriminate between parents predisposed towards producing a child with CP or CL(P). This indicated that an interaction between *Bam*HI and *Rsa*I genotypes should be introduced into the model. For the combined parental genotype A1A2/B1B2, the outcome is predominantly a child with CP (12/14), while the genotype A2A2/B1B2 predisposed to CL(P) (13/17) (table 7).

To investigate the influence of parental morphometric data on the discriminative ability of this second model, three cephalometric measurements found to distinguish best between parents with CP children and those having CL(P) children were entered into the model. This showed an improvement in the prediction values as 76% of CP parents and 94% of CL(P) parents could be correctly classified. The same three cephalometric variables were then entered into the logistic regression model in the same order without the genetic variables. This resulted in a reduction in the discriminative power of the model for CL(P) status from 94% to 78%, while for CP the prediction (72%) was only slightly reduced. This indicates the comparatively greater importance of genetic factors



The father has the TGFα risk haplotype and the associated mutation. Transmission of normal TGFα protein across the placenta during palatogenesis rescues the merging of the primary palate.

The mother has the TGFα risk haplotype and the associated mutation rendering the TGFα protein inactive either to cross the placenta or participating in the merging of the primary palate.

The child has inherited a polygenic background from both parents making it susceptible for developing orofacial clefts including the risk haplotype at the TGFα locus from the father. The child is thus unable to express this molecule during primary palatogenesis. The maternal rescue influences the merging of the primary palate but not the fusion of the secondary palate and the child develops isolated cleft palate (CP).

The child has inherited a polygenic background from both parents making it susceptible for developing orofacial clefts, including the risk haplotype at the TGFα locus from the mother. The child is thus unable to express this molecule during primary palatogenesis and because of the defect in the maternal TGFα protein it is unable to salvage the merging of the primary palate and the child develops cleft lip with or without cleft palate (CL(P)).

Figure 2 The influence of polymorphisms at the TGFα locus on the type of cleft (CP or CL(P)) explained by a maternal rescue model.

apart from possible morphogenetic ones in the aetiology of CL(P).

Discussion

Analysis of cephalometric craniofacial parameters in this study confirms that distinctive differences do exist between the parents of children with clefting deformities and controls. Furthermore, there are differences between the parents of CP children and those of CL(P) children with regard to craniofacial morphology.

This study indicates that polymorphisms at the TGFα locus shown by *Taq*I enzyme digestion (the C1, C2 alleles) predict the likelihood of a person in the population having a child with a cleft defect per se. Furthermore, digestion with *Bam*HI (producing A1, A2 alleles) enables discrimination between the likelihood of that birth defect being a CP or CL(P). The discrimination between the two types of birth defect can be further sharpened by incorporating cephalometric data. Three craniofacial parameters have been identified by this study as being particularly useful for this purpose. This represents a considerable simplification of the cephalometric analysis procedure normally required for analysis of craniofacial form.

These results suggest that there are certain morphogenes involved in determining the cleft susceptible parental craniofacial morphology. It is noteworthy that the genotypic markers were consistently more predictive for CL(P) than for CP. This is in line with other studies in the past which have concluded that there is a greater environmental component involved in the aetiology of non-syndromic isolated CP.[1 2] All previous association studies into the role of the TGFα locus in orofacial clefting have examined cleft probands, while the present study aimed to investigate predictors of liability using parental features. Examining parental DNA provides evidence for the hereditary nature of the gene defects and highlights distinctive differences between CL(P) and CP in terms of transmission at this locus. The finding of a significant association between CL(P) and the *Taq*I C2 allele of the TGFα gene is consistent with previous studies.[7-12 14 15] Only one previous study looked exclusively at the TGFα locus in an isolated CP sample[13] and the significant association between the *Taq*I C2 allele reported in their sample of 52 patients was reproduced in the present parental study. The present study is the first to report a significant association between CP and the *Bam*HI A1 allele, although Ardinger *et al*[9] did report an association between CL(P) probands and the same A1 allele, as did Stoll *et al*[12] in a sample of bilateral CL(P) probands. The contrasting differences in the prevalence of the B*am*HI genotype found among parents of CP and CL(P) patients has not been described before.

The parental origin of the molecular aberration may also be important. The preliminary observation made in this study that the *Bam*HI genotype A1A2 is only found among fathers of children with CP and mothers with CL(P) will need further investigation, but this preliminary study supports a maternal rescue hypothesis in the development of orofacial clefting.[29] If the parents have transmitted polygenic predisposing mutations for orofacial clefting to the offspring and either of them is a carrier of a modifying molecular aberration associated with the A1A2 genotype and the C2 allele, the effect of transmission of this allele and the associated molecular aberration may be influenced by the parental gender. For example, if the father is the transmitting parent the outcome could be modified by maternal rescue in the form of normal maternal protein being transferred over the placenta (fig 2). This would result in an isolated CP, while if the mother was the transmitting carrier the maternal protein would also be defective, the maternal rescue would fail, and a different defect, that is CL(P), would be found in the child. The first step to test this hypothesis would be to perform a transmission disequilibrium test[30] and haplotype relative risk analysis[31 32] on a larger data set which includes parents and offspring with orofacial clefts.

This study for the first time provides tangible morphometric and genetic evidence for a distinctive difference between the two types of

orofacial clefting at a locus selected because of the temporal and spatial expression of its protein product during murine palatogenesis. Transforming growth factors (including TGFα) are suspected of playing a crucial role in human craniofacial morphogenesis and the finding of cephalometric differences between the parents of children with orofacial clefting and a control implies that these "craniofacial microforms" confer susceptibility. Identification of the sites of the craniofacial skeleton that characterise predisposition to CP and CL(P) might also provide clues as to the pathogenetic mechanisms involved in orofacial clefting.

FUTURE STUDIES

It is important to appreciate the subtlety of these craniofacial morphometric differences. While this study and a number of previous studies have reported statistically significant differences between the parents of children with orofacial clefting and a matched control, these differences tend to be small and often no greater than the variation in the normal population. It is also widely recognised that conventional cephalometric analysis using lines and angles is a crude morphometric instrument. As such it would be misleading to suggest that these parental cephalometric differences alone are sufficient to be of diagnostic value in predicting risk of orofacial clefting. This problem might, however, be overcome by the use of techniques which can measure shape differences such as Procrustes, thin plate spline, and finite element analysis.[33] These techniques could concentrate on identification of morphometric differences in areas where previous studies suggest the differences lie.

Much larger samples, preferably obtained through multicentre collaboration, are required for meaningful and statistically powerful data even when these are subdivided for analysis into cleft type and gender. Consistency of association between studies carried out in populations with varying genetic backgrounds, lifestyles, and environmental exposures is an important criterion in judging whether an association is causal. It may also be that the morphometric hypothesis suggesting craniofacial shape predisposes to orofacial clefting is too unidimensional in the implication of cause and effect. There may well be a morphogenetic factor but the morphometric manifestation may not be a consistent one, even within the same population group, as other genetic and environmental factors are also operating.

The evidence from the present and previous studies[8-16] collectively indicates that the TGFα locus undoubtedly contributes towards the liability to orofacial clefting. It is, however, more likely to be a modifier gene rather than a major aetiological gene and other genetic factors are important. Future morphological and genetic studies will aim to establish further predisposing markers for both CP and CL(P). This study may present a model for investigating the role and possible interaction of candidate genes involved in the fusion of both the primary and secondary palate in the development of orofacial clefting, as well as their interaction with environmental risk factors. It will also be necessary to investigate phenotypic/genotypic correlations in both CP and CL(P) groups in the expectation that this will further the present state of knowledge on the aetiopathogenesis of orofacial clefting. The evidence would seem to indicate that morphogenes responsible for the distinctive parental craniofacial morphology may also be predisposing to orofacial clefting in the offspring.

The information gained from the study of liability not only offers an improvement in the accuracy of genetic counselling to prevent recurrence, but also the possibility of identification of genetically susceptible subjects. Since orofacial clefting is a polygenic multifactorial disorder, subsequent research will need to be directed towards (1) improving knowledge about the role of environmental teratogens and (2) understanding the role of genetic susceptibility to environmental factors, that is, gene/environment interaction. Identification of genetically susceptible subjects and knowledge of the relevant environmental teratogens would assist in the planning of interventions in the periconceptional period making prevention of orofacial clefting theoretically possible.

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