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Spectrum of Dental Phenotypes in Nonsyndromic Orofacial Clefting

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Abstract

Children with oral clefts show a wide range of dental anomalies, adding complexity to understanding the phenotypic spectrum of orofacial clefting. The evidence is mixed, however, on whether the prevalence of dental anomalies is elevated in unaffected relatives and is mostly based on small samples. In the largest international cohort to date of children with nonsyndromic clefts, their relatives, and controls, this study characterizes the spectrum of cleft-related dental anomalies and evaluates whether families with clefting have a significantly higher risk for such anomalies compared with the general population. A total of 3,811 individuals were included: 660 cases with clefts, 1,922 unaffected relatives, and 1,229 controls. Dental anomalies were identified from in-person dental exams or intraoral photographs, and case-control differences were tested using χ^2 statistics. Cases had higher rates of dental anomalies in the maxillary arch than did controls for primary (21% vs. 4%, $P = 3 \times 10^{-8}$) and permanent dentitions (51% vs. 8%, $P = 4 \times 10^{-62}$) but not in the mandible. Dental anomalies were more prevalent in cleft lip with cleft palate than other cleft types. More anomalies were seen in the ipsilateral side of the cleft. Agenesis and tooth displacements were the most common dental anomalies found in case probands for primary and permanent dentitions. Compared with controls, unaffected siblings (10% vs. 2%, $P = 0.003$) and parents (13% vs. 7%, $P = 0.001$) showed a trend for increased anomalies of the maxillary permanent dentition. Yet, these differences were nonsignificant after multiple-testing correction, suggesting genetic heterogeneity in some families carrying susceptibility to both overt clefts and dental anomalies. Collectively, the findings suggest that most affected families do not have higher genetic risk for dental anomalies than the general population and that the higher prevalence of anomalies in cases is primarily a physical consequence of the cleft and surgical interventions.

Keywords: genetic susceptibility, nonsyndromic cleft lip with or without cleft palate, tooth agenesis, microdontia, supernumerary teeth, tooth abnormalities

Introduction

Compared with the general population, children with nonsyndromic clefting present with an increased rate of dental anomalies. These anomalies influence the shape, size, number, symmetry, abnormal eruption, and malposition of teeth both inside and outside the cleft area (Eerens et al. 2001; Vieira et al. 2003; Letra et al. 2007; Rawashdeh and Abu Sirdaneh 2009; Walker et al. 2009; Wu et al. 2011). The range and severity of such anomalies vary greatly between cases and often result in more intricate oral rehabilitation procedures.

Classifying the etiology of dental anomalies in affected cases into those of genetic origins, those due to the physical consequences of the cleft itself, and/or those introduced during surgical repairs is extremely challenging. For example, primary repairs can affect dental development both inside and outside the cleft areas in the maxilla, since flaps are elevated from both sides of the oral cavity to repair a unilateral cleft lip or a cleft palate (Fisher 2005). Dental anomalies can also be caused by long-range disturbances to the intraoral environment due to deficiencies in mesenchymal tissue, blood supply, or perturbations in molecular signaling between the dental lamina and surrounding mesenchyme (Ranta 1986). These can be influenced by genes or by physical effects of the cleft and its severity, or surgical repair procedures. Indeed, studies have

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found that dental anomalies increase with cleft severity (Stahl et al. 2006; Letra et al. 2007; Aizenbud et al. 2011). Mutations in genes influencing both palatogenesis and dental development could also partly account for the greater prevalence of dental anomalies in individuals with clefts. A genome-wide linkage study of families with clefting suggested genetic differences between those with and without dental anomalies (Vieira et al. 2008b). Also, previous associations between cleft candidate genes/loci, including *MSX1, PAX9, IRF6, ANKS6, ERBB2, ABAC4-ARHGAP29*, 8q24, and 6q14, and specific patterns of dental anomalies in individuals with clefts (Vieira et al. 2004; Modesto et al. 2006; Vieira et al. 2008a; Letra et al. 2012; Yildirim et al. 2012) support the hypothesis of a common genetic link between oral clefts and dental anomalies. However, they may also be partly explained by mechanical effects of the cleft and surgery if these variants affect cleft type and severity.

Few studies have examined dental anomalies among unaffected relatives compared with controls or to norms. Since unaffected relatives likely carry more cleft risk genes than the general population (Weinberg et al. 2006), evaluating their risk for dental anomalies is useful for discerning the potential relationship(s) between genetic predisposition to clefting and dental anomalies. A clear understanding of these relationships could shed light on whether the dental anomalies in affected cases are caused by cleft-related genetic mechanisms or develop as a physical consequence of the cleft and/or are a secondary effect of surgical repairs. The evidence is highly mixed, however. Some studies found no significant differences (Woolf et al. 1965; Mills et al. 1968; Anderson and Moss 1996; Haria et al. 2000), concluding that unaffected relatives have no elevated risk of dental anomalies, which are also common in the general population (28%–40%) (Haugland et al. 2013). Others instead have reported significant increases in agenesis, asymmetric dental development, microdontia, and supernumerary teeth (Schroeder and Green 1975; Eerens et al. 2001; Kuchler et al. 2011; Aspinall et al. 2014).

These mixed findings leave the question of whether unaffected relatives have an elevated risk for dental anomalies open for empirical investigation. The inconsistencies could be partly related to the small samples of previous studies, which make the analyses more prone to higher type I error that may have exaggerated statistical significance in certain cases, as well as lower power for finding real differences for specific dental anomalies. Evaluating various types of dental anomalies is important as these could vary in their genetic etiologies and because not all dental anomalies may necessarily be related to cleft genes.

This study characterizes the spectrum of cleft-related dental anomalies in the largest international consortium with dental data for children with nonsyndromic clefting, their parents and siblings, and controls. This large sample allows us to more conclusively test the hypothesis that families with clefting have a significantly higher risk for dental anomalies compared with the general population. Also, this sample allows us to more definitively evaluate the risk of dental anomalies in unaffected relatives than previous research and to identify small signals possibly obscured in previous studies due to modest sample sizes. Furthermore, it allows us to investigate specific hypotheses about sidedness of dental anomalies—whether anomalies are higher on the left side, where clefts more commonly occur—dental arch (e.g., mandible vs. maxilla), and anomaly type. We evaluated unaffected parents and siblings, as well as the primary and permanent dentitions, separately. We also thoroughly investigated dental anomalies among affected cases compared with controls, taking advantage of the large sample, which allowed us to evaluate specific dental anomalies and affected areas and to examine differences by cleft type.

Methods

Sample

A total of 3,811 subjects were recruited from multiple sites in the United States, including Colorado, Iowa, Pennsylvania, and Texas, and internationally from Guatemala, Hungary, Nigeria, Argentina, and the Philippines (Appendix Table 1). Internal review board (IRB) approval was attained at each site by the appropriate IRB process and committee. This study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

The total sample included 660 case probands with nonsyndromic cleft lip with our without cleft palate and cleft palate alone; 1,922 unaffected relatives, including parents and siblings; and 1,229 control individuals, including control probands, their parents, and siblings (Appendix Table 1). Exclusion criteria for controls included a positive family history for orofacial clefts or syndromes and a history of facial trauma or surgery. Also, edentulous individuals were excluded from the study.

Questionnaires recording dental history, including dental extractions and orthodontic treatment, were collected on all subjects. Two additional types of data were collected: in-person dental exams or intraoral photos. In-person dental exams were performed for a subset of subjects by oral cavity inspection using dental mirrors and explorers. Sites were provided with cameras (Canon EF 100-mm f/2.8 macro USM lens, Canon macro MR-14EX ring flash; Canon, Tokyo, Japan) and supplies for intraoral photo collection. Prior to photographs, all removable appliances/prostheses were removed. A minimum of 6 photographs were taken per subject to appropriately display the entire oral cavity. The photo rater (BJH) was blinded to study site, sex, cleft status (in the absence of obvious clefting), and family relations.

Anomalies

To harmonize variables between intraoral photo and in-person dental exam forms, anomalies were restricted to hypoplasia; microdontia; impacted, rotated, and displaced teeth; supernumerary teeth; and agenesis (see definitions in Appendix Table 2). Anomalies were further separated by primary and permanent dentitions (Appendix Tables 3 and 4) and categorized into right maxilla, left maxilla, total maxilla, and total mandible. The outcomes were indicators for having at least 1 dental

anomaly in the above anomaly type and dental area. Aside from evaluating the mandibular arch separately, no further distinction was made between anomalies inside and outside of cleft maxillary regions. This is because primary repair surgeries, even in unilateral cases, could have an effect on both sides of the upper dentition. Cases and controls were compared on the "any anomaly" outcomes, both including and excluding dental rotation and displacement variables, given the large incidence of dental malposition in both cases and controls in the study sample. Sex differences were tested for the any "anomaly without malposition or displacement" variable only. Last, proband cases (children with clefts) were compared with proband controls, siblings of cases with siblings of control probands, and parents of cases with control parents.

Image/Oral Cavity Analysis

On the forms (Appendix Figures 1 and 2), appropriate teeth were marked as either primary or permanent, and each tooth was marked as "present or missing." Under the missing category, the options "agenesis" or "other" were chosen in accordance with specific definitions designed for this study and detailed in the Appendix. Analyses were completed on all primary teeth (A-T) and permanent teeth from first molar to first molar in each arch. The second and third permanent molars were excluded due to their inconsistent visualization in intraoral photographs.

Calibration

Calibration and training for intraoral dental exams and photos were performed at the University of Pittsburgh for all the different sites prior to the start of data collection.

The photo rater (BJH) was calibrated against 2 experienced dentists (LMMU and ARV). Data from 15 subjects randomly chosen were used for calibration. Each subject was rated 2 times by each rater (BJH, LMMU, and ARV). Intrarater reliability for BJH was 100% agreement, with $\kappa = 0.95$. Interrater reliability between all 3 raters was 97.1% to 97.3% (κ = 0.91–0.93).

Statistical Methods

Descriptive analyses were completed for all variables in the sample. Case-control comparisons were performed by using χ^2 tests. After Bonferroni correction for 324 independent tests, a P value $<1.5 \times 10^{-4}$ was selected as the threshold for significance. All tests were performed with SAS (version 9.3; SAS Institute, Inc., Cary, NC, USA).

Results

Proband Case-Control Comparisons

Results in the primary dentition showed significantly more case probands with at least 1 dental anomaly in the maxilla ($P = 1 \times 10^{-9}$) compared with controls (Table 1). Differences excluding displacement and rotations were still significant ($P = 3 \times 10^{-8}$) in the maxilla. No significant differences were found in the mandibular primary dentition.

Table 1. Probands: Primary Dentition with at Least 1 Dental Anomaly.

Anomaly	Case Proband (n = 466), n (%)	Control Proband (n = 156), n (%)	P Value
Right maxilla			
Hypoplasia	16 (3)	4 (2)	0.61
Microdontia	5 (1)	0	0.33
Impacted	0	0	NA
Rotated	96 (21)	18 (11)	0.005
Displaced	52 (11)	4 (2)	4E-04
Agenesis	23 (5)	0	0.001
Supernumerary	13 (3)	0	0.03
Any anomaly	145 (31)	24 (14)	2E-05[a]
Any anomaly[b]	53 (11)	4 (2)	2E-04
Left maxilla			
Hypoplasia	16 (3)	5 (3)	1.00
Microdontia	8 (2)	0	0.12
Impacted	0	0	NA
Rotated	107 (23)	21 (13)	0.005
Displaced	67 (14)	1 (<1)	1E-08[a]
Agenesis	39 (8)	0	6E-06[a]
Supernumerary	13 (3)	0	0.03
Any anomaly	163 (35)	27 (16)	3E-06[a]
Any anomaly[b]	66 (14)	5 (3)	2E-05[a]
Maxilla			
Hypoplasia	24 (5)	6 (4)	0.53
Microdontia	10 (2)	0	0.07
Impacted	0	0	NA
Rotated	142 (30)	29 (17)	0.001
Displaced	97 (21)	5 (3)	3E-09[a]
Agenesis	55 (12)	0	3E-09[a]
Supernumerary	25 (5)	0	7E-04
Any anomaly	209 (45)	36 (22)	1E-09[a]
Any anomaly[b]	96 (21)	6 (4)	3E-08[a]
Mandible			
Hypoplasia	4 (<1)	1 (<1)	1.00
Microdontia	3 (<1)	0	0.57
Impacted	3 (<1)	0	0.57
Rotated	108 (23)	29 (17)	0.15
Displaced	14 (3)	3 (2)	0.58
Agenesis	1 (<1)	0	1.00
Supernumerary	3 (<1)	0	0.57
Any anomaly	120 (26)	31 (19)	0.07
Any anomaly[b]	14 (3)	1 (<1)	0.13

NA, not applicable.
[a]Indicates significance (P < 1.5E-04).
[b]Any anomaly without malposition (displacement and rotation).

Tests for specific anomalies in the maxillary primary dentition revealed that agenesis and tooth displacements were significantly increased, whereas supernumerary teeth were marginally elevated in cases compared with control probands. Also, tooth rotations were elevated but not significant (P = 0.001).

For the permanent dentition, dental anomalies overall were significantly more common in the maxilla of case probands ($P = 4 \times 10^{-62}$) (Table 2). Specifically, agenesis was the most significant finding ($P = 3 \times 10^{-55}$), followed by tooth displacement, microdontia, impactions, hypoplasia, and supernumerary

Table 2. Probands: Permanent Dentition with at Least 1 Anomaly.

Anomaly	Case Proband (n = 497), n (%)	Control Proband (n = 683), n (%)	P Value
Right maxilla			
Hypoplasia	34 (7)	19 (3)	0.002
Microdontia	23 (5)	4 (<1)	4E-06[a]
Impacted	16 (3)	2 (<1)	5E-05[a]
Rotated	229 (46)	266 (39)	0.01
Displaced	145 (29)	84 (12)	8E-13[a]
Agenesis	112 (23)	10 (2)	4E-34[a]
Supernumerary	9 (2)	1 (<1)	0.003
Any anomaly	308 (62)	304 (45)	3E-09[a]
Any anomaly[b]	164 (33)	35 (5)	3E-37[a]
Left maxilla			
Hypoplasia	43 (9)	19 (3)	1E-05[a]
Microdontia	36 (7)	5 (<1)	1E-11[a]
Impacted	17 (3)	1 (<1)	4E-06[a]
Rotated	244 (49)	277 (41)	0.004
Displaced	159 (32)	80 (12)	2E-17[a]
Agenesis	129 (26)	14 (2)	2E-37[a]
Supernumerary	7 (1)	0	0.002
Any anomaly	324 (65)	315 (46)	1E-12[a]
Any anomaly[b]	191 (38)	38 (6)	2E-46[a]
Maxilla			
Hypoplasia	63 (13)	29 (4)	1E-07[a]
Microdontia	49 (10)	6 (<1)	2E-13[a]
Impacted	29 (6)	3 (<1)	9E-09[a]
Rotated	280 (56)	325 (48)	0.003
Displaced	206 (41)	129 (19)	3E-17[a]
Agenesis	180 (36)	19 (3)	3E-55[a]
Supernumerary	16 (3)	1 (<1)	9E-06[a]
Any anomaly	366 (73)	364 (53)	1E-14[a]
Any anomaly[b]	252 (51)	56 (8)	4E-62[a]
Mandible			
Hypoplasia	12 (2)	18 (2)	0.85
Microdontia	1 (<1)	0	0.42
Impacted	6 (1)	4 (<1)	0.34
Rotated	305 (61)	366 (54)	0.009
Displaced	90 (18)	148 (22)	0.14
Agenesis	19 (4)	13 (2)	0.07
Supernumerary	2 (<1)	1 (<1)	0.58
Any anomaly	324 (65)	392 (57)	0.008
Any anomaly[b]	38 (6)	33 (5)	0.05

[a]Indicates significance (P < 1.5E-04).
[b]Any anomaly without malposition (displacement and rotation).

Table 3. Unaffected Sibling: Primary Dentition with at Least 1 Anomaly.

Anomaly	Unaffected Siblings (n = 285), n (%)	Control Siblings (n = 86), n (%)	P Value
Right maxilla			
Hypoplasia	10 (3)	1 (<1)	0.70
Microdontia	1 (<1)	0	1.00
Impacted	0	0	NA
Rotated	44 (11)	10 (11)	1.00
Displaced	8 (2)	3 (3)	0.43
Agenesis	1 (<1)	0	1.00
Supernumerary	0	0	NA
Any anomaly	57 (15)	14 (16)	0.74
Any anomaly[a]	12 (3)	1 (1)	0.48
Left maxilla			
Hypoplasia	8 (2)	1 (<1)	1.00
Microdontia	0	0	NA
Impacted	0	0	NA
Rotated	48 (12)	11 (12)	1.00
Displaced	12 (3)	4 (5)	0.51
Agenesis	1 (<1)	0	1.00
Supernumerary	1 (<1)	0	1.00
Any anomaly	61 (16)	15 (17)	0.75
Any anomaly[a]	10 (3)	1 (1)	0.70
Maxilla			
Hypoplasia	13 (3)	1 (1)	0.48
Microdontia	1 (1)	0	1.00
Impacted	0	0	NA
Rotated	63 (16)	15 (17)	0.87
Displaced	17 (4)	4 (5)	1.00
Agenesis	1 (<1)	0	1.00
Supernumerary	1 (<1)	0	1.00
Any anomaly	80 (21)	19 (22)	0.77
Any anomaly[a]	16 (4)	1 (1)	0.33
Mandible			
Hypoplasia	11 (3)	1 (1)	0.70
Microdontia	2 (<1)	0	1.00
Impacted	0	0	NA
Rotated	89 (23)	22 (26)	0.67
Displaced	22 (6)	1 (<1)	0.10
Agenesis	0	0	NA
Supernumerary	2 (<1)	0	1.00
Any anomaly	106 (28)	24 (28)	1.00
Any anomaly[a]	15 (4)	1 (1)	0.33

NA, not applicable.
[a]Any anomaly without malposition (displacement and rotation).

teeth. Dental rotations were elevated in the maxilla ($P = 0.003$) and mandible ($P = 0.009$) but not significantly. No other differences were found in the mandibular permanent dentition.

Siblings Case-Control Comparisons

When comparing unaffected siblings of children with clefts to control siblings, most outcome rates were comparable. No differences were found in the primary dentition (Table 3). In the permanent dentition, the rate of any dental anomalies in the maxilla was elevated but not significantly different from controls ($P = 0.003$) (Table 4).

Parent Case-Control Comparisons

Similar to the sibling comparisons, no prominent differences were found between parents of affected children and control parents. A small trend for an increase in the rate of any anomaly in the maxilla ($P = 0.001$) and mandible ($P = 0.006$) was observed among unaffected parents (Table 5).

Analyses by Cleft Type and Laterality

Results by cleft type in the primary dentition showed that any anomaly ($P = 7 \times 10^{-6}$), agenesis ($P = 1 \times 10^{-7}$), and tooth displacement ($P = 3 \times 10^{-5}$) were significantly elevated in cleft lip

Table 4. Unaffected Sibling of Proband: Permanent Dentition with at Least 1 Anomaly.

Anomaly	Unaffected Siblings (n = 623), n (%)	Control Siblings (n = 121), n (%)	P Value
Right maxilla			
Hypoplasia	16 (3)	0	0.09
Microdontia	11 (2)	0	0.23
Impacted	7 (1)	1 (<1)	1.00
Rotated	325 (52)	66 (55)	0.69
Displaced	90 (14)	27 (22)	0.04
Agenesis	5 (<1)	0	1.00
Supernumerary	2 (<1)	0	1.00
Any anomaly	359 (58)	69 (52)	0.92
Any anomaly[a]	39 (6)	1 (<1)	0.01
Left maxilla			
Hypoplasia	16 (3)	2 (2)	0.75
Microdontia	16 (3)	0	0.09
Impacted	8 (1)	0	0.37
Rotated	338 (54)	64 (53)	0.84
Displaced	103 (17)	21 (17)	0.79
Agenesis	11 (2)	0	0.23
Supernumerary	3 (<1)	0	1.00
Any anomaly	374 (60)	69 (57)	0.55
Any anomaly[a]	49 (8)	2 (2)	0.01
Maxilla			
Hypoplasia	26 (4)	2 (2)	0.29
Microdontia	17 (3)	0	0.09
Impacted	10 (2)	1 (<1)	1.00
Rotated	391 (63)	74 (61)	0.77
Displaced	149 (24)	35 (29)	0.25
Agenesis	14 (2)	0	0.14
Supernumerary	5 (<1)	0	1.00
Any anomaly	426 (68)	77 (63)	0.34
Any anomaly[a]	65 (10)	3 (2)	0.003
Mandible			
Hypoplasia	17 (3)	1 (<1)	0.33
Microdontia	1 (<1)	0	1.00
Impacted	3 (<1)	0	1.00
Rotated	466 (75)	92 (76)	0.82
Displaced	149 (24)	21 (17)	0.13
Agenesis	7 (1)	1 (<1)	1.00
Supernumerary	3 (<1)	0	1.00
Any anomaly	488 (78)	94 (78)	0.90
Any anomaly[a]	29 (5)	1 (2)	0.21

[a]Any anomaly without malposition (displacement and rotation).

Table 5. Unaffected Parents of Probands: Permanent Dentition with at Least 1 Anomaly.

Anomaly	Unaffected Parents (n = 1,186), n (%)	Control Parents (n = 347), n (%)	P Value
Right maxilla			
Hypoplasia	52 (4)	9 (3)	0.16
Microdontia	19 (2)	1 (<1)	0.06
Impacted	6 (<1)	1 (<1)	1.00
Rotated	535 (45)	142 (41)	0.18
Displaced	160 (13)	49 (14)	0.79
Agenesis	28 (2)	4 (1)	0.20
Supernumerary	5 (<1)	0	0.59
Any anomaly	616 (52)	165 (48)	0.16
Any anomaly[a]	106 (9)	15 (4)	0.004
Left maxilla			
Hypoplasia	46 (4)	6 (2)	0.06
Microdontia	18 (2)	1 (<1)	0.09
Impacted	7 (<1)	1 (<1)	0.69
Rotated	539 (45)	149 (43)	0.43
Displaced	156 (13)	54 (16)	0.25
Agenesis	37 (3)	6 (2)	0.20
Supernumerary	3 (<1)	1 (<1)	1.00
Any anomaly	637 (54)	167 (48)	0.08
Any anomaly[a]	107 (9)	15 (4)	0.003
Maxilla			
Hypoplasia	70 (6)	12 (3)	0.08
Microdontia	26 (2)	2 (<1)	0.06
Impacted	10 (<1)	1 (<1)	0.47
Rotated	661 (56)	180 (52)	0.22
Displaced	243 (20)	79 (23)	0.37
Agenesis	51 (4)	8 (2)	0.11
Supernumerary	8 (<1)	1 (<1)	0.69
Any anomaly	749 (63)	198 (57)	0.04
Any anomaly[a]	157 (13)	24 (7)	0.001
Mandible			
Hypoplasia	35 (3)	6 (2)	0.26
Microdontia	2 (<1)	0	1.00
Impacted	3 (<1)	0	1.00
Rotated	801 (68)	218 (63)	0.11
Displaced	342 (29)	92 (27)	0.42
Agenesis	18 (2)	0	0.02
Supernumerary	1 (<1)	0	1.00
Any anomaly	841 (71)	229 (66)	0.08
Any anomaly[a]	59 (5)	6 (2)	0.006

[a]Any anomaly without malposition (displacement and rotation).

with cleft palate compared with other cleft types. In contrast, supernumerary teeth rates were significantly elevated in cleft lip cases ($P = 1 \times 10^{-5}$) (Appendix Table 5).

In the permanent dentition, any anomaly ($P = 7 \times 10^{-18}$), agenesis ($P = 6 \times 10^{-19}$), and hypoplasia ($P = 7 \times 10^{-4}$) rates in the maxilla were higher for cleft lip with cleft palate compared with other cleft types (Appendix Table 6).

Differences in the rate of dental anomalies were also tested by cleft laterality of the case probands. An increase in the rate of dental anomalies on the ipsilateral side of the unilateral cleft was observed (Appendix Tables 5 and 6). No differences were found in the mandibular primary or permanent dentitions by cleft type or laterality.

Sex Differences in Dental Anomalies

Sex differences in the "any anomaly without dental malposition" variable were tested for the maxilla, mandible, and the oral cavity as a whole, and no significant differences were found (P values ranged from 0.07 to 1.0).

Discussion

The current work represents the largest study to date of dental anomalies in children with clefts, their relatives, and controls. We found that dental anomalies in the maxillary arch were more prevalent among case probands compared with controls in both dentitions. In the mandible, however, no significant

differences were seen. More dental anomalies were seen in the cleft lip with cleft palate group than other cleft types. A trend for increased anomalies on the ipsilateral side of the cleft compared with the opposite site was observed, lending support to the cleft environment as the main factor predisposing to dental anomalies in the case probands.

Moreover, we found no significant differences between unaffected parents and siblings compared with controls. Unlike for case probands, whose dental anomalies are highly related to the cleft environment, differences between unaffected relatives and controls could arguably be suggestive of some differences in genetic predisposition to clefting. We observed only a trend for an increased rate of any anomaly in the maxillary permanent dentitions of unaffected siblings (10% vs. 2%, $P = 0.003$) and parents (13% vs. 7%, $P = 0.001$) compared with controls, with no differences in prevalence between the maxillary right side and the left side, where clefts occur more often. Although our reported rates and rate differences between unaffected relatives and controls are within range of those reported in previous studies (rate differences ranging from 5.7% to 20.8%) (Schroeder and Green 1975; Kuchler et al. 2011), we do not consider these differences significant in our study after correcting for multiple testing. The significance of these differences reported in previous studies was potentially due to a type I error inflation.

Taken as a whole, these results suggest that most affected families do not have higher genetic risk for dental anomalies than the general population. However, some families may carry susceptibility to both overt clefts and dental anomalies, suggesting rare mutations in such cases. Furthermore, these results suggest that the higher prevalence of anomalies in cases is primarily a physical consequence of the cleft and surgical interventions.

Our results for overall differences in dental anomalies between cases and control probands are also in line with most previous studies (Letra et al. 2007; Vieira et al. 2008b; Rawashdeh and Abu Sirdaneh 2009; Akcam et al. 2010; Tannure et al. 2012; Riis et al. 2014). Despite the lack of radiographic access, which is challenging in an international sample, our estimates of the prevalence of dental anomalies are similar to studies based on radiographic images except for hypoplasia and supernumerary teeth. We found lower rates of hypoplasia (4%−13%) in case probands and controls than previous estimates (23%−38%; Chapple and Nunn 2001), possibly due to the lack of visibility in the photographs of all hypoplastic areas on the teeth. Similarly, we observed lower rates of supernumerary teeth in both the primary (0%−5%) and permanent (<1%−3%) dentitions of case probands and controls than did previous studies. Reported rates for case probands range from 6.6% to 34.9% in the primary (Tsai et al. 1998; Pegelow et al. 2012) to 4.4% to 32% in the permanent dentition (Pegelow et al. 2012; Riis et al. 2014). Rates for controls range within 1.2% to 3% (Anthonappa et al. 2013). Our estimates captured only erupted supernumerary teeth, due to the lack of radiographs. Similar to previous studies, however, we found more supernumerary teeth in individuals with cleft lip compared with other clefts (Stahl et al. 2006; Riis et al. 2014). Finally, our study also captured significant increases in dental displacements and a trend for increased rotations in the maxillary primary and permanent dentitions of our case probands, similar to previous studies (Letra et al. 2007).

As mentioned above, the higher rates of dental anomalies in case probands can be a physical consequence of the cleft or surgery. Calcification of the maxillary primary teeth occurs sequentially from central incisors to second molars, beginning in utero at 14 weeks and culminating at 11 months of life with completion of the clinical crowns. Also, maxillary central and lateral permanent incisors begin calcification at 3 and 12 months after birth, respectively (Ash 1993). Therefore, the intraoral environment of the cleft itself could explain the occurrence of agenesis, supernumerary teeth, and microdontia in the upper primary and anterior permanent dentition, given their early initiation and calcification. The lack of fusion between the medio-nasal and maxillary prominences during the primary palate formation can result in insufficient mesenchyme to support the formation of tooth buds. Alternatively, the cleft can result in an extension of dental lamina, which can develop into extra teeth or can cause division of the tooth buds, resulting in supernumerary teeth. If the remaining tooth bud's tissue is defective or incapable to develop into a viable tooth, microdontia or agenesis could occur (Ranta 1986).

Given that the timing of the primary lip and secondary palate repair (3–6 and 9–12 months, respectively) (Ziak et al. 2010; Jeyaraj et al. 2014) coincides with the crown completion of anterior primary teeth and the calcification of upper permanent incisors, surgical manipulation and tissue scarring can also affect both stages in primary and permanent anterior teeth. Also, surgery can obliterate initiation and calcification of posterior permanent tooth buds or cause displacements and rotations of teeth, possibly explaining the occurrence of hypoplastic maxillary anterior teeth (both primary and permanent), agenesis of posterior permanent teeth (i.e., premolars), impactions, and dental malpositions (Olin 1964; Ranta 1986; Spauwen et al. 1993; Lekkas et al. 2000). As noted above, surgeries could affect anomalies both inside and outside cleft areas; therefore, counting anomalies only outside cleft area does not resolve the effect of surgery and could provide a biased estimate of the overall difference in rates of dental anomalies between affected probands and controls.

Cleft defects are the consequence of a cascade of events, including environmental and/or genetic factors, leading to different cleft types in addition to a number of microforms affecting the craniofacial complex and the dentition. Despite the many unknowns, it is clear that children with orofacial clefts exhibit a higher frequency of dental anomalies mostly caused by short and long range inherit or acquired disturbances of the physical environment surrounding the dentition. Although rare mutations in certain genes influencing both palatogenesis and dental formation may explain a small part of the added risk of dental anomalies in affected probands and their unaffected relatives carrying these mutations, it is not the case for most affected families.

Our findings support the need for comprehensive dental phenotyping in genetic studies of oral clefts. Even though results suggest that most affected families may not carry

mutations that predispose to an added risk of dental anomalies, identifying carriers still requires that dental anomalies are carefully characterized. Finding these rare mutations may be best achieved by sequencing candidate regions instead of association studies based on common variants. Dental phenotyping can also provide clues on the intersection between molecular mechanisms underlying the growth and development of the primary and secondary palates (4–8 weeks) and those that may subsequently trigger dental development (6–8 weeks and 20th week, initiation of the primary and permanent dentitions, respectively) (Nanci 2013). From a clinical perspective, characterization of cleft-related dental anomalies in cases and their unaffected relatives helps discern the primary etiologies of such defects by separating those secondary to the intraoral environment (i.e., the cleft itself or surgical repairs) from those due to genetic factors. Since our findings indicate that the increased prevalence of dental anomalies in cases is mostly due to cleft severity or surgery, research could be redirected toward innovative surgical approaches to minimize dental adverse effects and improve dental outcomes for these individuals.

Author Contributions

B.J. Howe, A.R. Vieira, J.M. Resick, N.L. Nidey, contributed to conception, design, and data acquisition, drafted and critically revised the manuscript; M.E. Cooper, contributed to conception, design, data analysis, and interpretation, drafted and critically revised the manuscript; S.M. Weinberg, L.M. Moreno Uribe, contributed to conception, design, data acquisition, and interpretation, drafted and critically revised the manuscript; G.L. Wehby, contributed to conception, design, and data interpretation, drafted and critically revised the manuscript; M.L. Marazita, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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