PVR/CD155 Ala67Thr Mutation and Cleft Lip/Palate

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Abstract: The 19q13 locus has been linked to cleft lip and palate by our group and independently by others. Here we fine mapped the region in an attempt to identify an etiological variant that can explain cleft lip and palate occurrence. A total of 2739 individuals born with cleft lip and palate, related to individuals born with cleft lip and palate, and unrelated were studied. We used linkage and association approaches to fine map the interval between D19S714 and D19S433 and genotypes were defined by the use of TaqMan chemistry. We confirmed our previous findings that markers in PVR/CD155 are associated with cleft lip and palate. We studied the mutation Ala67Thr further and calculated its penetrance. We also attempted to detect PVR/CD155 expression in human whole saliva. Our results showed that markers in PVR/CD155 are associated with cleft lip and palate and the penetrance of the Ala67Thr is very low (between 1% and 5%). We could not detect PVR/CD155 expression in adult human whole saliva and PVR/CD155 possibly interacts with maternal infection to predispose children to cleft lip only.

Key Words: Association, dental abnormalities, linkage, oral clefts, poliovirus receptor, tooth agenesis

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Cleft lip with or without cleft palate (CL/P) is a common congenital disorder in humans, classified as nonsyndromic (isolated) or syndromic based on the presence of other congenital structural defects or developmental delay. Approximately 70% of all CL/P cases are isolated and multifactorial in origin, with both genetic and environmental components.[1] The average birth prevalence of isolated CL/P is 1/700 live births, depending on population ancestry. In general, Asian and American Indian populations have a higher birth prevalence of CL/P (1/500), followed by White (1/1000), and last African populations (1/2500). Estimates suggest that anywhere from 3 to 14 interacting loci may contribute to the etiology of CL/P[2] and many more may have stronger gene effects that explain cases in a particular family.[3]

Differences in the etiology or epidemiology of a complex trait such as CL/P may remain undetected, because of the high variability in disease phenotype. Thus, our work on the etiology of isolated CL/P has focused on increasing the sophistication of the clinical descriptions, rather than aiming to study many thousands of people. The use of potential subclinical features, or subphenotypes, has been suggested to allow for the identification of "unaffected" individuals with equivalent genetic risks as affected individuals carrying disease-causing alleles. We have suggested using dental development as a tool for the creation of more complete cleft phenotypes. Individuals with cleft lip and palate present considerably more dental anomalies outside the cleft area than do individuals without clefts. Tooth agenesis in particular is the most prevalent dental anomaly seen in association with CL/P, although supernumerary teeth, tooth impaction, tooth malposition, and the combination of more than one of these abnormalities are also common findings.[4]

Then, we performed a genome linkage scan for CL/P and dental anomalies in a Filipino population and suggested the interval 19p13.12–19q13 may contain a gene that contributes to clefts but not to dental anomalies. The LOD scores increased from 3.11 (using clefts as affected status) to 3.91 (excluding individuals presenting dental anomalies).[5] This result was exciting because we previously studied the 19q13 region (motivated by a positive linkage report with 19q13[6]) and found an intronic marker (rs35385129) in the gene that codes for CD155, the poliovirus receptor (*PVR*) gene, that was associated with clefts in Filipinos and Latin Americans.[7] CD155 (cluster of differentiation 155) is a protein of type I transmembrane glycoprotein in the immunoglobulin family and is the only known receptor for poliovirus, hence the poliovirus receptor gene name *PVR*.[8] CD155 plays a role in the establishment of intercellular adherent junctions between epithelial cells.[9] Since the epithelium undergoes apoptosis during the leveling and union of connective tissue in the areas of the face that are affected by clefts, we thought of CD155 as a possible contributor to clefts in humans. Our initial result of an association between the 19q13 locus and clefts was independently studied in Italians and North Americans, and the same general results were observed despite differences in allele frequencies.[10,11] We have recently replicated the association between isolated CL/P and the 19q13 locus in a recent genome-wide association study (GWAS) of 6480 subjects (823 unrelated cleft cases, 1700 unrelated controls, and 1319 case-parent cleft trios) with European, Asian, African, and

Central and South American ancestry.[12] These independent association findings with 19q13 and clefts collectively support a contribution of the locus to CL/P risk.

Here, we analyzed the association of markers in the 19p13.12–19q13 region in individuals affected by isolated CL/P with and without dental anomalies to determine if we can identify a variant that can be used for risk predictions.

METHODS

Subjects

Two different cohorts with nonsyndromic CL/P were initially used in this study and have been previously described (Philippines[5,13] and Brazil).[4,14–22] The first is a family-based cohort consisting of 74 cleft families (expanded from the 46 families included in the original genome-wide linkage scan)[5] comprising 280 nuclear families (1118 individuals) from the Philippines that was used for fine-mapping the linked region. The second is an independent cohort to replicate any findings from the fine-mapping consisting of 328 cases with CL/P and 282 unrelated unaffected individuals from the southeast region of Brazil (these individuals are all white and descendants of Portuguese individuals who migrated around the 1500s during colonization of Brazil). Only individuals presenting isolated CL/P were included in the study. Individuals with syndromic forms of clefting or presenting a cleft of the palate only were not considered for the analyses. Information on dental anomalies outside the cleft area was available for both cohorts and were used to provide further evidence that different contributors play a role when clefts is associated with dental anomalies.

The University of Iowa and the University of Pittsburgh Institutional Review Boards approved this study in conjunction with local approval in the Philippines and in Brazil. Genomic DNA samples were collected from saliva and/or blood with informed consent from patients and/or their legal guardians.

To test the hypothesis that maternal infection is a potential interacting factor, a third CL/P cohort was investigated. This population was selected through the Latin-American Collaborative Study of Congenital Malformations (ECLAMC). Established in 1967, ECLAMC utilizes 70 different hospitals and volunteer physicians to collect data on births occurring in Latin America. From January 1998 to April 2002, ECLAMC collected blood spots on filter cards from 434 patients with oral clefts (355 with CL/P and 69 with cleft palate only), and their mothers from 8 countries in South America: Argentina, Brazil, Bolivia, Chile, Ecuador, Paraguay, Uruguay, and Venezuela. Patients known to have a syndrome or other major or multiple minor defects were excluded from the analysis.[23] Cleft palate only cases were also not included in this study. All samples were collected with signed consent and possessed local and/or University of Iowa and University of Pittsburgh IRB approval.

Finally, to test if CD155 is expressed in whole saliva, we obtained saliva samples from 143 unrelated individuals born with or without CL/P living in 12 Argentinian Patagonia sites (San Carlos de Bariloche, El Bolsón, Esquel, El Maitén, Maquinchao, Ingeniero Jacobacci, Rio Colorado, Choele Choel, Valcheta, Sierra Grande, Santo Antonio Oeste, and General Roca). Both the Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno" (CEMIC) (IRB#543) and University of Pittsburgh (IRB#405013) Institutional Review Boards approved the study of these samples and appropriate written informed consent was obtained from all participants.[32] For this particular study group, 2 saliva samples were taken from each participant, one for DNA and another for RNA extraction. Samples were collected using specific Oragene Self-Collection kits (DNA Genotek Inc, Kanata, Canada) and kept at room temperature until processed.

This study conforms to the STROBE guidelines.

Association Analyses

We tested 7 single nucleotide polymorphisms around 2 satellite markers (D19S714 and D19S433) in chromosomal region 19p13.12–19q13 that showed the most compelling results on the genome scan in the Filipinos.[5] Briefly, marker D19S714 (19p13) presented a LOD score of 3.11 (for families with clefts as the only assigned affection status), while marker D19S433 (19q12) presented a LOD score of 3.91 (for cleft families whose probands presented without additional dental anomalies under a recessive model). We also investigated 2 single nucleotide polymorphisms in the *PVR/CD155* because of its previous association with CL/P in Filipinos.[7]

Markers were selected to have high heterozygozity levels in both Asian and White populations. Genotyping was carried out using TaqMan chemistry[24] on an automated 7900HT instrument (Applied Biosystems, Foster City, CA). Assays and reagents were supplied by Applied Biosystems. Details of the selected markers are presented in Table 1.

Statistical Analyses

Analyses were stratified by population. For the Filipino and Brazilian cohorts, alleles at each marker were tested for association in 3 different sets of analyses, based on affection status. First, we performed the analyses considering the affected cleft probands with and without dental anomalies. Second, we considered the affected cleft probands without dental anomalies. Third, we considered the affected probands with dental anomalies.

Analysis for the Filipino cohort was performed using the Family Based Association Test (FBAT)[25] to test for linkage disequilibrium between allele markers and CL/P in the Filipino families. Analysis for the Brazilian case-control cohort was performed using PLINK software v. 1.06[26] to test for differences in allele frequencies between cleft and control groups, as well as between cleft subphenotypes and controls. We performed the same sets of analyses comparing cases with and without dental anomalies with unaffected control individuals without dental anomalies. We took advantage of the complete description of the cleft for this population, and also performed these analyses according to cleft type/side. Bonferroni correction was applied and P values ≤ 0.007 were considered statistically significant.

For the ECLAMC population, the likelihood ratio test (LRT) of Weinberg[27] was applied under the assumption that the distribution of paternal and maternal alleles was the same. Parameters R1 and R2 and model likelihoods were estimated.

Maternal Infection

Prenatal infection exposure and prenatal viral infection exposure data were used to stratify the ECLAMC results[7] and explore possible existing gene-environment interactions (Table 2). Exposure information was collected from the ECLAMC registration forms, which contained information regarding the infant, pregnancy, parents, and family history for clefting. The forms were completed at birth by a member of the ECLAMC medical staff (usually a pediatrician).[28] Prenatal infection exposure included fever, flu, pharyngitis, urinary tract infection, sore throat, bronchitis, cold, tuberculosis, toxoplasmosis, vaginal discharge, candidiasis, pneumonia, HIV, sinusitis, human papilloma virus, syphilis, mumps, vaginal infection, Chagas disease, otitis, cold sores, and appendicitis. Prenatal viral infection exposure data included only fever, flu, cold, sore throat, bronchitis, sinusitis, HIV, mumps, and cold sores.

Estimation of Penetrance

We obtained the frequency of *PVR/CD155* A67T mutation from the 1000 genomes database (The International Genome Sample

TABLE 1. Results of Association Analyses in Cleft Lip/Palate Families and Unrelated Individuals With and Without Dental Anomalies as an Additional Affection Status

					Philippines, Families (N = 74)		
Marker ID	**Base Change***	**SNP Function**	**Region**	**Gene/Nearby Gene**	**Cleft Probands With and Without Dental Anomalies *P* Value**	**Cleft Probands Without Dental Anomalies *P* Value**	**Probands With Dental Anomalies *P* Value**
rs11668164	C/G	Intron	19p13.12 near D19S714	*CYP4F8*	0.10	0.12	0.44
rs4809211	A/C	Intergenic		—	0.69	0.50	†
rs2077080	C/G	Intron		*CYP4F3*	0.39	0.53	0.64
rs1543284	G/A	Intergenic		—	0.37	0.18	0.73
rs10413555	G/T	Intron	19q12 near D19S433	*URI1*	0.62	0.14	0.28
rs10414287	G/T	Intron		*URI1*	0.58	0.3	0.22
rs2080887	G/A	Intron		*URI1*	0.44	0.22	0.75
rs1058402	G/A	Missense		*PVR (A67T)*	0.27	0.06	†
rs35385129	A/C	Missense	19q13.31	*PVR (R391S)*	0.41	**0.02**	0.73
rs10421283	G/A	Downstream		*CEACAM19*	Not typed	Not typed	Not typed
rs2927438	G/A	Intergenic		*BCL3*	Not typed	Not typed	Not typed
rs419010	A/G	Intron		*PVRL2*	Not typed	Not typed	Not typed
rs2075620	A/G	Intron	19q13.32	*CLPTM1*	Not typed	Not typed	Not typed
rs875255	G/C	Intron		*CLPTM1*	Not typed	Not typed	Not typed

Marker ID: The unique identification number used in the genomic databases to identify a single nucleotide polymorphism, SNP. Using this ID number, one can obtain the sequence surrounding the single base-pair change, its location in a given chromosome and in regard to genes, and additional details about its known function. These genetic markers are changes in DNA that have arisen due to mutation and are spread throughout the genome and over the centuries, have become common in populations. They are used to help identify chromosomal regions in which a possible deleterious mutation exists that is physically close to where the genetic marker, in our case the SNP being studied, is.

Base change: This field indicates the actual mutation. The first letter is the ancestral allele, and the second letter describes to which allele the ancestral allele mutated to.

SNP function: The column describes the location of the SNP in regard to a given gene. Intron indicates the SNP is located between 2 exons. Intergenic indicates the SNP is located between 2 genes. Downstream suggests the SNP is at the 3′ end of a gene. Missense is a type of mutation in which a nucleotide change in a coding exon will cause a change of 1 amino acid in the protein chain.

Region: It describes the chromosomal location, according to classic cytogenetic banding, in the chromosome 19 we studied.

Gene/nearby gene: This field describes the gene that is the closest to the studied SNP.

*Ancestral allele listed first, based on NCBI dbSNP build 149.

†Number of families was not informative.

Bold indicates *P* value lower than 0.05.

Resource, http://www.internationalgenome.org/) for several population groups to compare with the frequencies of the same mutation we obtained in our previous work.[7] We calculated penetrance of the *PVR/CD155* A67T mutation, using the Bayes theorem to estimate disease risk using the following formula:

$$\text{Penetrance} = \frac{\text{Baseline risk X affected allele frequency}}{\text{Population allele frequency}}$$

RESULTS

Determination of Affection Status

In the Filipino families, 240 individuals were affected with CL/P whereas 41 individuals were affected with CL/P and additional dental anomalies. Among the 866 unaffected family members, 96 had dental anomalies. In the Brazilian cohort, there were 328 individuals with CL/P of which 144 presented with dental

TABLE 2. Distribution of ECLAMC Cases by Cleft Type According to Prenatal Infection Exposure and *PVR* rs35385129 Genotypes

	Cleft Lip and Palate	**Genotypes**		**Cleft Lip Only**	**Genotypes**		**Cleft Lip With or Without Cleft Palate**	**Genotypes**	
Likely Viral Infection	46	AA	36	23	AA	29	69	AA	55
		AC	9		AC	6		AC	13
		CC	1		CC	1		CC	1
Likely Non-Viral Infection	45	AA	36	19	AA	14	64	AA	51
		AC	9		AC	4		AC	12
		CC	1		CC	1		CC	1
No Infection	150	AA	119	72	AA	69	222	AA	176
		AC	30		AC	3		AC	43
		CC	1		CC	0		CC	3

Ten cases with syndromic or unknown cleft type, and 69 cleft palate only cases were excluded from analysis.

The data were divided based on the existing information in the records in infections likely of viral origin versus infections likely of non-viral origins. This distinction is important since PVR was originally described as a virus receptor, due to its involvement in the cellular poliovirus infection in primates. The role of this protein in the immune system remains unclear, but it appears that this gene is specific to the primate lineage, and serves as a cellular receptor for poliovirus in the first step of poliovirus replication. Stratifying the analysis based on the likely viral origin of the maternal infection in our data aimed to decrease heterogeneity in the analysis, since the data were limited to what was recorded.

TABLE 3. Association Analysis Results for Polymorphisms in Candidate Genes Located in Chromosome 19q and Cleft Subphenotypes With and Without Dental Anomalies in Cases (n = 328) and Controls (n = 282)

	CL/P		
Marker ID	**With and Without Dental Anomalies**	**Without Dental Anomalies**	**With Dental Anomalies**
rs1058402	0.005	0.0009	0.75
rs35385129	0.11	0.46	0.17
rs10421283	0.21	0.71	0.13
rs2927438	0.24	0.17	0.8
rs419010	0.34	0.36	0.1
rs2075620	0.44	0.52	0.58
rs875255	0.80	0.29	0.35
		CL/P Unilateral	
rs1058402	**0.02**	0.006	0.83
rs35385129	0.95	0.54	0.62
rs10421283	0.23	0.57	0.21
rs2927438	0.26	0.11	0.86
rs419010	0.07	0.43	0.03
rs2075620	0.31	0.41	0.47
rs875255	0.10	0.44	0.05
		CL/P Bilateral	
rs1058402	0.05	0.01	0.74
rs35385129	0.57	0.42	0.55
rs10421283	0.58	0.53	0.11
rs2927438	0.66	0.14	0.84
rs419010	0.59	0.41	0.11
rs2075620	0.83	0.91	0.68
rs875255	0.83	0.77	0.15
		CL/P Left	
rs1058402	**0.003**	0.02	0.13
rs35385129	0.88	0.86	0.71
rs10421283	0.18	0.67	0.08
rs2927438	0.34	0.08	0.60
rs419010	0.13	0.49	0.09
rs2075620	0.56	0.70	0.63
rs875255	0.20	0.32	0.32

Bonferroni correction P value = 0.007; $P < 0.05$ are bolded.

TABLE 4. Results of Haplotype Analyses of *PVR* Markers in Cleft Lip/Palate Families With and Without Dental Anomalies

Affection Status	**Haplotype Marker Alleles rs1058402—rs35385129**	**Estimate of Frequency**	***P* value***
All cleft probands	2–2 (A-C)	0.695	**0.03**
	2–1 (A-A)	0.223	0.39
	1–2 (G-C)	0.076	0.06
	1–1 (G-A)	0.006	—
Cleft probands without dental anomalies	2–2 (A-C)	0.688	**0.001**
	2–1 (A-A)	0.226	**0.04**
	1–2 (G-C)	0.080	0.06
	1–1 (G-A)	0.006	—
Cleft probands with dental anomalies	2–2 (A-C)	0.638	0.84
	2–1 (A-A)	0.314	0.95
	1–2 (G-C)	0.048	—
	1–1 (G-A)	—	—

*$P < 0.05$ are bolded and indicate statistical differences between groups.

anomalies, and 282 unrelated controls of which 23 presented dental anomalies.

Association Analyses

Data for all SNPs were consistent with Hardy-Weinberg proportions in both affected and unaffected individuals (data not shown). Table 1 describes the results of association analyses (before multiple test correction) in the Filipinos. No over-transmission was found for marker alleles in the interval 19p13–19q12. Nevertheless, both SNPs in the *PVR/CD155* showed a trend for association with CL/P (rs35385129, $P = 0.02$; and rs1058402, $P = 0.06$) in cleft probands without dental anomalies. For the Brazilian cohort, *PVR* SNP (rs1058402) showed association with CL/P ($P = 0.0009$) in cleft cases without dental anomalies (Table 3). Haplotype analysis further suggests CL/P without dental anomalies is associated with *PVR* (Table 4).

Maternal Infection

To generate evidence that maternal infection is an underlying mechanism that increases the risk for having a child born with clefts based on the presence of the *PVR/CD155* rs35385129 risk allele, we stratified the ECLAMC dataset by prenatal infection exposure during pregnancy and found significant association for the *PVR/CD155* marker and the cleft lip only group ($P = 0.03$).

Estimation of Penetrance

We have determined that the frequency of the *PVR/CD155* rs1058402 risk allele (A67T) was 12% in Iowa, 7% in the Philippines, and 2.8% in Brazil (present study and reference[7]). We obtained frequencies of the same variant for multiple populations from the 1000 Genomes (Asians 12.6%; Europeans 4.27%; Africans 6.98%; Mexicans 10.23%, and North American Indians 18.3%). There is a clear gradient for the rare allele of *PVR/CD155* rs1058402 to be more frequent in Asians, American Indians, and Mexicans, intermediate in Africans, and less frequent in Europeans. Among these groups, Europeans resemble well the population we previously studied from Iowa. Since the Asians are a combination of Chinese and Japanese, they do not resemble Filipinos well. Similarly, Mexicans do not appear to be an appropriate group to be compared with South Americans. The frequency of *PVR/CD155* rs1058402 risk allele in the 1006 Europeans tested in the 1000 Genomes was 4.27% (43 in 1006). When compared with the frequency we found in individuals born in Iowa with CL/P (12%, 11 in 93 subjects), we determined that Iowans inheriting the risk allele of *PVR/CD155* rs1058402 are 2.6 times more likely to be born with CL/P (odds ratio = 2.6, 95% confidence intervals 1.32–5.31, chi-square 8.09, $P = 0.004$). For Brazilians, odds ratios were calculated from our data (odds ratio = 1.17, 95% confidence intervals 0.56–2.46). We calculated the penetrance of the risk allele of *PVR/CD155* rs1058402 (A67T) and found it to be 4.26% for Iowans and 1.36% for Brazilians.

DISCUSSION

CD155 is a protein that mediates natural killer cell effector functions and binds to CD96 and CD226. These complexes accumulate at the cell-cell contact site, and form a mature immunological synapse with the target cell. This is what is thought to trigger adhesion.[29] Since the protein allows for epithelial cells to adhere, failure of this process could explain the occurrence of cleft lip and

palate. The gene that codes for CD155, *PVR*, is located in a locus that has been multiple times implicated in the etiology of CL/P, both by hypothesis-driven investigations (linkage,[6] association)[7,10,11] and hypothesis-free approaches (linkage,[5] association).[12] Here we provide further evidence supporting the role of the same locus on the etiology of CL/P. We believe *PVR/CD155* is a contributing gene and that the missense mutation A67T is a low penetrance variant more easily detected in North Americans of North European descent.

PVR has its name because it was found to act as a receptor for poliovirus, and for this reason, we tested for statistical evidence that maternal infection could be an interacting factor with *PVR/CD155* genetic variation to increased CL/P risk. The statistical evidence was specific for cases with cleft lip only in the sample tested. It was not possible to consider in this analysis the type of viral association, which may include DNA and RNA viruses. We know viruses operate through distinct receptors and in many instances the poliovirus receptor conformation may be irrelevant since viruses may infiltrate through other types of receptors. There is also the possibility that the role of CD155 in clefts is related to disruption of epithelial cell-cell adhesion during development. We were hoping to be able to detect *PVR/CD155* expression in whole saliva (see Supplemental Digital Content, http://links.lww.com/SCS/A284) with the idea this expression could be assayed as a surrogate of what possibly may have occurred during development but did not detect any under the conditions we experimented. CD155 is transcriptionally activated by the sonic hedgehog protein. Downstream effectors of the sonic hedgehog signal such as GLI proto-oncogenes (GLI1 and GLI3) and cis-acting elements such as AP-2 also activate the CD155 core promoter.[30,31] Cell adhesion mediation has been demonstrated for several members of the CD155-related family of genes and the pattern of developmental expression of CD155 that matches known adhesion molecules suggest a physiological function of CD155 involving the mediation of cell adhesion.[30]

We estimated the penetrance of *PVR/CD155* A67T mutation in our sample, and found that the penetrance is very low and potentially negligible in all of the study populations. Hence, when we interpret these 3 decades of research on the genetic etiology of cleft lip and palate, we have a combination of genes with very small effects and instances where single gene mutations with strong effects explain the defect in a single family.[3] This possibly explains the results of the several genome-wide association studies published so far for cleft lip and palate: few loci and/or genetic markers reach former statistical significance and even less are replicated. We believe these inconsistencies depend more on the dataset and sample sizes, rather than on the phenotype, since the presence of clefts is easily determined at birth. These individual small gene effects, with such low levels of penetrance as exemplified in our data, are unlikely to be useful for clinical management. Furthermore, our understanding of the mechanisms leading to clefts has also not been improved substantially by these genome-wide association study results. Another aspect of this is that the use of such strict multiple comparison corrections (such as Bonferroni) often leads to the disregard of true association signals since they do not reach formal statistical significance. We have shown this effect in our previous work, in which a known cleft-associated variant did not reach statistical significance because of the alpha threshold arbitrarily established by the Bonferroni method.[13] We can also predict that whole genome sequencing will be able to detect additional rare variants that may explain the etiology of cleft lip and palate but these also may be relevant to a few specific families. Studying other potential mechanisms leading to clefts in humans such as changes in DNA methylation may provide a new venue for the future. We would also emphasize that improving the clinical phenotype characterization by including CL/P subphenotypes in genetic analyses gives us an opportunity to better understand gene effects. Our results of the association between *PVR* and clefts consistently show that this association is driven by cases that are not affected by additional dental anomalies outside the area affected by the cleft. This is consistent with our previous work that showed that some genes appear to be associated with clefts in families segregating both clefts and dental anomalies, whereas other genes are associated with the subset of families that segregate only clefts and individuals do not have dental anomalies.[5,13] This hypothesis can only be tested in designs that include older individuals with dentition present. Study designs that include neonates, such as the ECLAMC cohort in this study, do not allow for this type of analysis, as well as inclusion of other physical traits better measured at older ages (ie, facial features). Family-based studies, in comparison with case-control designs, may be better suited for studying family history and concomitant diseases such as cancer, but have the disadvantage of being costlier since each proband requires additional samples in excess to just one ''control.'' We believe the field should rethink its effort on the topic, and explore epigenetics and mechanistic approaches rather than hypothesis-free association studies with samples characterized just by the presence or absence of the defect.

In summary, we provide further evidence that the 19q13 locus, and in particular PVR/CD155 contribute to isolated forms of cleft lip and palate in which individuals do not have additional dental anomalies outside the cleft area.

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