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SHORT COMMUNICATION

Studies of genes involved in craniofacial development and tumorigenesis: *FGF3* contributes to isolated oral clefts and may interact with *PAX9*

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Abstract

Objective. Previous studies suggest individuals born with oral clefts and their families have a higher susceptibility for cancer, which raises the hypothesis that these two conditions share common molecular pathways. This study evaluated the association between oral clefts and polymorphisms in genes that play a role in craniofacial and tumor development. ***Materials and methods.*** Four hundred and ninety-seven subjects born with oral clefts and 823 unaffected subjects were recruited. Twenty-nine markers in 13 genes were genotyped by the Taqman method. Chi-square was used to compare allele and genotype frequencies. Bonferroni correction for multiple testing was used and the established alpha was 0.0003. This study also used logistic regression to test if genetic variants were associated with oral clefts using positive family history of cancer and age as covariates. ***Results.*** There was no association between family history of cancer and oral clefts ($p = 0.51$). None of the 1320 study participants had a diagnosis of cancer at the time of participation in the study. The marker rs4980700 in *FGF3* was associated with oral clefts ($p = 0.0002$). Logistic regression analysis also provided evidence for gene–gene interaction between *FGF3* (rs4980700) and *PAX9* (rs2073242), increasing the risk for isolated oral clefts ($p = 0.0003$). ***Conclusion.*** *FGF3* is associated with oral clefts and may interact with *PAX9*.

Key Words: *cleft lip, cleft palate, cancer, genetics*

Introduction

Isolated oral clefts are considered clinically and genetically heterogeneous conditions that include cleft lip with or without cleft palate and cleft palate only. These defects affect ~1.7 per 1000 live born babies, with ethnic and geographic variation [1].

Individuals born with oral clefts have a shorter life-span and may have a higher incidence of cancer [2–7]. Families of individuals born with oral clefts report more often cancer [8–11] and, conversely, isolated oral clefts were reported more often in families in which one individual was diagnosed with cancer [12,13].

Recent studies have demonstrated that genetic factors, including genes involved in tumorigenesis, play an important role in the etiology of oral clefts [9,11,14,15]. In this study we selected genes that have been independently implicated in both craniofacial development and cancer and tested for evidence of association with isolated oral clefts.

Methods

From July 2009 to December 2011 we recruited biologically unrelated individuals to participate in this study. All the participants, or their parents or

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Table I. Studied genes and markers.

Gene	Chromosome	Variants (rs#)	MAF	Base change	Role of the gene in the craniofacial development	Role of the gene in the tumorigenesis	References
AXIN2	17q23-q24	2240308	0.48	A/G	Previous evidence of association with oral clefts in humans	Suggested as a tumor suppressor gene	[9,17–19]
		740026	0.33	A/G			
DLX1	2q32	743605	0.32	A/G	In mice, the functions of Dlx1 and Dlx2 are crucial for the initial formation of the posterior palatal shelves. In addition, Dlx genes lie upstream of multiple signaling molecules and transcription factors important for later stages of palatogenesis	DLX genes are expressed in several types of cancer	[20–22]
DLX2	2q32	788173	0.37	C/T			
EDAR	2q11-q13	13029834	0.31	A/G	Involved in the development of craniofacial glands	Involved in mediating cell death	[23,24]
		7585138	0.31	C/T			
		12992554	0.32	C/T			
		17269487	0.31	A/G			
FGF3	11q13	4631909	0.47	C/T	Previous evidence of association with oral clefts in humans	FGF signaling is involved in autonomous tumor growth and tumor vascularization. There are many evidences supporting the role of FGF signaling in the pathogenesis of many cancers. Deregulated expression and abnormal activation of ligands and receptors has been described in several tumor types	[25,26]
		12574452	0.32	A/G			
		1893047	0.42	A/G			
		7932320	0.48	C/T			
		4980700	0.43	A/G			
FGF10	5p13-p12	1448037	0.36	C/T			
		900379	0.48	C/T			
		11750845	0.37	C/T			
		1893047	0.42	A/G			
		593307	0.36	A/G			
FGFR2	10q26	2981582	0.40	A/G			
		2592600	0.42	A/T			
GLI2	2q14	1992901	0.42	A/G	*Gli2* and *Gli3* mouse mutants exhibit an incomplete penetrance for oral clefts. Previous evidence of association with oral clefts in humans exist for *GLI2*	The involvement of *GLI* in tumor development has been well investigated and they play a role in several types of cancers	[27,28]
		929387	0.41	A/G			
GLI3	7p13	846266	0.37	C/T			
		2237435	0.38	A/C			
LHX6	9q33.2	12532	0.42	A/G	Mouse embryos deficient for *Lhx6* presented clefting of the secondary palate	The *LHX6* isoform *LHX6.1* is suggested to be a tumor suppressor gene in the cervix	[29,30]
MSX1	4p16.2	2073242	0.38	A/G	Previous evidence of association with oral clefts in humans	Msx1 was identified as a p53-interacting protein and shows function as a p53 regulator. The over-expression of homeobox Msx1 induced apoptosis of cancer cells	[31–33]
PAX9	14q13.3	4904155	0.38	C/G	Previous evidence of association with oral clefts in humans	PAX genes exhibit several attributes involved with cancer including a role in cancer cell survival and widespread expression in cancer	[33–35]
		2595110	0.16	A/G			
PITX2	4q25	2595110	0.16	A/G	Pitx2-deficient mice die at an early embryonic stage and show severe defects in the palate.	PITX2 methylation is considered a promising prognostic biomarker in breast and prostate cancer.	[36,37]

MAF, Minor allele frequency.

guardians if the potential subject was a minor, provided written informed consent. The Federal University of Rio de Janeiro Research and Ethics Committee approved the study (#150A/2009), as well as the University of Pittsburgh Institutional Review Board (PRO12080056).

A total of 1320 individuals were included in this study. The affected group consisted of 497 individuals born with oral clefts (mean age = 15.04 years with ages ranging from 1 month to 60 years) receiving dental care at a public hospital that is the center of oral clefts rehabilitation in Rio de Janeiro, Brazil. This group was stratified according to sub-type of oral clefts, since our hypothesis is that different groups of genes can lead to different phenotypes. Three hundred and eighty-two individuals were born with cleft lip with palate (CLP), 90 subjects with cleft lip only (CL) and 75 with cleft palate only (CP). None of the affected individuals were diagnosed with median cleft. The comparison group consisted of 823 individuals born without any birth defects (mean age = 17.14 years with ages ranging from 2–77 years) recruited at the Dental Clinics of the Federal University of Rio de Janeiro. Individuals with any syndrome and unaffected subjects with positive family history of oral clefts were excluded.

The determination of the cleft type (CL, CLP and CP) and cleft laterality was based on clinical examination and confirmed with descriptions present in the clinical files. Self-reported history of cancer data was also collected via a structured questionnaire. Data about family history of cancer of the index case and/or first-degree relatives (parents, siblings and offspring) of the index case were included. If at least one of the family members had cancer, that subject was considered to have a family history of cancer. None of the 1320 study participants had a diagnosis of cancer at the time of participation in the study.

Genomic DNA for molecular analysis was extracted from oral cells using an established method [16]. Twenty-nine markers in 13 genes were selected (Table I) and were genotyped by real-time polymerase chain reactions using the Taqman method performed with the Stratagene Mx3005P real time PCR system (Stratagene, La Jolla, CA) and an ABI PRISM® 7900HT Sequence Detection System (Foster City, CA). Pre-designed probes were supplied by Applied Biosystems (Foster City, CA).

Statistical analysis

The data was subsequently processed and analyzed using the Epi Info 3.3.2 statistical software package (http://www.cdc.gov/epiinfo). Cleft groups were analyzed as a total group as well as in stratified sub-groups based on cleft type (CL, CLP and CP) and cleft laterality. The Student's *t*-test was used to compare age differences between affected and unaffected individuals. Hardy-Weinberg equilibrium was evaluated using Chi-square test. Chi-square or Fisher's exact tests were used to compare allele and genotype distributions between affected and unaffected individuals. Logistic regression analysis was implemented using positive history of cancer and age as covariates. Bonferroni correction was used and alpha was established at 0.0003 (0.05/174). To look for evidence of gene–gene interactions, logistic regression analysis was implemented with the presence of particular genotypes of genes, positive history of cancer and age as covariates.

Results

Affected and unaffected individuals had similar ethnic background distributions ($p = 0.1$) and individuals born with oral clefts were younger than unaffected individuals ($p = 0.002$). Fifty-six (6.9%) unaffected individuals reported positive family history of cancer, while 22 (4.5%) affected individuals reported positive family history of cancer (Table II). In order to verify if there was an association between oral clefts and cancer we performed a binary logistic regression and, after adjustment for age, this difference was insignificant ($p = 0.51$; OR = 0.83, 95% CI = 0.49–1.42).

All genotypes were in Hardy-Weinberg equilibrium (data not shown). Table III presents the allele and genotype distribution of the studied SNPs among cleft sub-groups. No markers showed statistically significant differences in allele or genotype distributions, but a trend for association was found for *AXIN2* rs2240308 with CP ($p = 0.008$), *FGF10* rs144837 with CLP ($p = 0.003$), *GLI2* rs2592600 with all types of clefts and with unilateral clefts ($p = 0.006$ and $p = 0.008$, respectively), *LHX6* rs989798 with CP ($p = 0.006$) and *MSX1* rs12532 with CP ($p = 0.005$).

Genotypes were also tested in a logistic regression using age and positive family history of cancer as covariates and the summary results are seen in Table IV. The marker rs4980700 in *FGF3* was associated with clefts ($p = 0.0002$). This same marker presented a borderline association for unilateral clefts ($p = 0.0004$) and a trend for association with CLP ($p = 0.003$) and bilateral clefts ($p = 0.005$). Logistic regression analysis was also used to look for evidence of gene–gene interaction, increasing the risk of clefts, using cancer and age as covariates, and evidence of interaction between *FGF3* (rs4980700) and *PAX9* (rs2073242) was found ($p = 0.0003$).

Discussion

While earlier studies suggest individuals born with clefts and their family members report more often cancer [2–7], the results of the present study contradict those findings. This study agrees with at least one

Table II. Reported cancer types among individuals born with or without isolated oral clefts. Data is presented as obtained from subjects. Individuals reporting multiple types of cancer in the same person could not indicate which tumor was primary and which ones were metastatic.

Type of reported cancer in first degree relatives	Cases	Controls
No Cancer Reported	475	767
Bladder and kidney	0	1
Brain and nervous system	0	2
Brain and breast	0	1
Bone	0	1
Breast	4	7
Breast and lymphoma	0	1
Breast, uterus and intestine	0	1
Breast, uterus, intestine and stomach	0	1
Cervical/uterus	2	2
Esophagus	1	0
Head and neck	1	0
Hodgkin's lymphoma	0	1
Intestine (colon)	0	3
Larynx	1	0
Leukemia	1	0
Lymphoma	0	1
Lymphoma and larynx	0	1
Liver	2	1
Liver and intestine	0	1
Lung	2	2
Lung and brain	1	0
Lung and liver	0	1
Neuroblastoma	0	2
Neuroendocrine	0	1
Pancreas	0	1
Prostate	1	2
Prostate and brain	0	1
Skin	0	5
Skin and breast	1	1
Spleen	0	1
Stomach	1	2
Throat	0	2
Throat, lung and leukemia	1	0
Thyroid	0	3
Wilms tumor (nephroblastoma)	0	1
Unknown	3	6

previous investigation [38] that also did not find an association between oral clefts and cancer. The explanation for these negative results, however, may lie with the fact that we relied on self-reported family history of cancer and that affected and unaffected individuals were draw differently. While affected individuals were the patients from a hospital that is a center for craniofacial anomalies treatment in the Rio de Janeiro state; the group of unaffected individuals comprised patients, students and employees of the hospital. Our analyses were corrected by age to minimize the possible effect of having the lower chance of identifying cancer in the family of the patient group in comparison to controls, since they were relatively younger. However, the effect of age in our study cannot be completely discarded. The confounding role of different social/cultural factors and education levels cannot be excluded. Differences between affected and unaffected individuals could lie in the capacity of the study participant to provide information about cancer history. In addition, differences between exposure to carcinogenic agents, as well as access to healthcare and cancer diagnosis may exist between these two groups.

The list of genes selected for this study (Table I) was by no means meant to be exhaustive. There are other genes that could have been investigated under the same premise of our study. *TP63* when mutated causes a syndromic form of clefting (ectrodactily, ectordemal dysplasia and orofacial clefts) [39]. Immunohistochemistry evaluations of ductal and cribriform acinar prostatic adenocarcinomas by TP63/HMWCK may help identify remnants of basal cells that likely represent intraductal spread of tumor [40]. Another example is *SKI*, a proto-oncogene that functions as a repressor of TGF-beta signaling, that has been associated with isolated oral clefts [27]. Another limitation of our study is the inability to analyze the data by reported cancer type. One can hypothesize that specific genetic associations may be dependent on specific types of cancer. Much larger sample sizes are likely needed to perform comparisons that will have enough statistical power to detect genetic associations by cancer type. Another limiting factor is the lack of data on the age of onset of cancer, which can also help discriminate cancer types, survival rates and severity of these conditions.

The fibroblast growth factor (*FGF*) family represents a group of heparin binding, multifunctional polypeptides with mitogenic activity, which is involved in diverse processes including embryonic development and autonomous tumor growth and vascularization. The Fgf signal is involved in palatogenesis in multiple stages. Both FGFs and FGF receptors can be placed in sequential stages of human palatal shelf fusion from palatal shelf elevation to the completion of fusion [41] and play important roles in suture and synchondrosis regulation. In agreement with our results, previous studies demonstrated that this same marker in *FGF3* may be involved in the oral cleft etiology [25–42]. Menezes et al. [25] reported an association between *FGF3* and unilateral clefts, a trend we also observed in our study ($p = 0.0004$).

Table III. Genotype and allele associations.

		All cleft *p*-value		CL *p*-value		CLP *p*-value		CP *p*-value		Unilateral *p*-value		Bilateral *p*-value	
Gene	*rs#*	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype
AXIN2	*2240308*	0.081	0.025	0.633	0.288	0.327	0.217	0.008	0.013	0.920	0.396	**0.018**	0.068
	740026	0.315	0.213	0.681	0.899	0.696	0.319	0.054	0.140	0.966	0.210	0.120	0.269
DLX1	*743605*	0.870	0.640	0.777	0.346	0.755	0.834	0.947	0.993	0.041	0.354	0.678	0.867
DLX2	*788173*	0.042	0.116	0.440	0.755	0.182	0.403	0.055	0.056	0.207	0.415	0.726	0.659
EDAR	*13029834*	0.868	0.899	0.985	0.519	0.838	0.647	0.977	0.955	0.947	0.624	0.802	0.591
	7585138	0.138	0.218	0.308	0.195	0.148	0.042	0.964	0.934	0.148	0.143	0.309	0.597
	12992554	0.169	0.848	0.201	0.555	0.173	0.340	0.441	0.969	0.122	0.227	0.337	0.710
	17269487	0.310	0.811	0.318	0.131	0.844	0.354	0.526	0.996	0.313	0.693	0.377	0.878
FGF3	*4631909*	0.989	0.492	0.765	0.773	0.899	0.465	0.541	0.734	0.581	0.549	0.637	0.451
	12574452	0.500	0.594	0.509	0.801	0.654	0.345	0.779	0.724	0.583	0.378	0.661	0.897
	1893047	0.417	0.048	0.356	0.099	0.727	0.271	0.524	0.399	0.430	0.096	0.941	0.552
	7932320	0.870	0.660	0.138	0.529	0.889	0.803	0.796	0.961	0.619	0.493	0.269	0.536
	4980700	0.812	0.801	0.865	0.964	0.837	0.836	0.965	0.934	0.446	0.616	0.064	0.178
FGF10	*1448037*	0.235	0.125	0.738	0.757	0.042	0.003	0.584	0.868	0.123	0.076	0.572	0.506
	900379	0.235	0.370	0.953	0.918	0.295	0.499	0.209	0.331	0.505	0.611	0.454	0.754
	11750845	0.538	0.073	0.558	0.135	0.646	0.328	0.866	0.415	0.539	0.232	0.783	0.343
	1893047	0.417	0.048	0.356	0.099	0.727	0.271	0.524	0.399	0.430	0.096	0.941	0.552
	593307	0.991	0.660	0.109	0.296	0.216	0.301	0.170	0.401	0.760	0.956	0.672	0.096
FGFR2	*1219648*	0.658	0.905	0.332	0.257	0.665	0.699	0.170	0.348	0.898	0.656	0.817	0.161
	2981582	0.800	0.681	0.939	0.662	0.859	0.384	0.780	0.919	0.778	0.947	0.914	0.099
GLI2	*2592600*	0.016	0.006	0.033	0.106	0.115	0.013	0.175	0.389	0.018	0.008	0.645	0.451
	1992901	0.087	0.160	0.924	0.423	0.082	0.140	0.234	0.278	0.327	0.405	0.148	0.180
GLI3	*929387*	0.371	0.429	0.037	0.097	0.665	0.292	0.707	0.791	0.580	0.458	0.340	0.443
	846266	0.752	0.600	0.465	0.103	0.499	0.784	0.892	0.594	0.508	0.750	0.658	0.558
LHX6	*989798*	0.159	0.100	0.448	0.517	0.750	0.187	0.006	0.025	0.513	0.115	0.360	0.842
MSX1	*12532*	0.015	0.033	0.286	0.527	0.013	0.041	0.005	0.012	0.088	0.066	0.322	0.341
PAX9	*2073242*	0.046	0.072	0.043	0.083	0.173	0.387	0.361	0.143	0.027	0.082	0.861	0.735
	4904155	0.019	0.049	0.170	0.125	0.044	0.049	0.242	0.424	0.040	0.112	0.227	0.411
PITX2	*2595110*	0.894	0.400	0.837	0.967	0.544	0.634	0.339	0.014	0.695	0.542	0.727	0.783

Note: All groups were compared with controls.

Animal models demosntrated that FGFs interact with Pax9 during craniofacial development [35,43]. *Fgf3* expression is strongly affected by combined reduction of *Pax9* and *Msx1* gene dosages [35] and interestingly our study provides statistical evidence that oral clefts can be the result of *FG3* and *PAX9* interaction ($p=0.0003$). An association between oral clefts and *FGF3* rs4980700 was observed after analysis took into consideration positive family history of cancer. In addition, *FGF* genes contribute to ~3% of total oral clefts cases [43]. It is possibly that *FGF3* plays a role in oral clefts only in specific groups of individuals that also report cancer more often in their families.

FGF3 haploinsufficiency is the proposed cause of otodental syndrome (MIM 166750), an autosomal dominant entity characterized by globodontia of canines and molars and sensorineural hearing impairment. In addition, ocular coloboma is present in some families (oculo-otodental syndrome) and this phenotype was associated with microdeletions that not only included *FGF3*, but also would lead to haploinsuffciency of *FADD* (Fas-associated death domain) [44]. We are not aware of reports suggesting associations between these conditions and cleft lip and palate or cancer. However, at least one family segregating ocular coloboma and microphthalmos with cleft lip and palate in an autosomal dominant fashion has been reported [45].

Additional trends for association could be seen in the data. While concerned with the issue of

Table IV. Summary results of the logistic regression.

Variables			Multivariate analysis		
Oral clefts sub-groups	Gene	Reference	Genotype	*p*-value	OR (95% CI)
All cleft	*AXIN2* rs2240308	AA	AG	0.008	1.72 (1.14–2.59)
			GG	0.021	1.62 (1.07–2.45)
	EDAR rs7585138	CC	CT	0.015	1.63 (1.10–2.44)
			TT	0.056	1.50 (0.98–2.30)
	FGF3 rs4980700	AA	AG	0.001	0.48 (0.31–0.75)
			GG	0.0002*	0.43 (0.27–0.70)
	FGF3 rs12574452	AA	AG	0.015	1.53 (1.08–2.18)
			GG	0.299	1.23 (0.83–1.83)
	FGF3 rs1893047	AA	AG	0.016	1.53 (1.08–2.18)
			GG	0.299	1.23 (0.83–1.83)
	FGF10 rs11750845	CC	CT	0.045	1.34 (1.00–1.79)
			TT	0.679	0.90 (0.56–1.45)
	FGF10 rs1448037	CC	CT	0.009	1.55 (1.11–2.16)
			TT	0.201	1.33 (0.85–2.09)
	FGF10 rs1448037	TT	CT	0.024	0.52 (0.30–0.92)
			CC	0.522	0.83 (0.49–1.43)
	GLI2 rs2592600	AA	AT	0.940	0.98 (0.75–1.29)
			TT	0.004	0.60 (0.41–0.85)
CLP	*EDAR* rs7585138	CC	CT	0.015	1.63 (1.10–2.44)
			TT	0.056	1.56 (0.98–2.30)
	FGF3 rs4980700	AA	AG	0.003	0.48 (0.29–0.78)
			GG	0.002	0.43 (0.25–0.74)
	FGF10 rs1448037	CC	CT	0.009	1.55 (1.11–2.17)
			TT	0.195	1.34 (0.85–2.09)
	FGF10 rs1448037	TT	CC	0.024	0.52 (0.30–0.92)
			CT	0.522	0.83 (0.49–1.43)
	GLI2 rs2592600	AA	AT	0.435	1.13 (0.83–1.53)
			TT	0.037	0.64 (0.42–0.97)
	MSX1 rs12532	AA	AG	0.038	1.36 (1.01–1.84)
			GG	0.034	1.53 (1.03–2.27)
	PAX9 rs4904155	CC	CG	0.722	0.94 (0.71–1.26)
			GG	0.018	0.59 (0.38–0.91)
CL	*FGF3* rs4980700	AA	AG	0.056	0.46 (0.21–1.02)
			GG	0.038	0.40 (0.16–0.95)
	FGF3 rs1893047	AA	AG	0.040	2.29 (1.03–5.06)
			GG	0.316	1.58 (0.64–3.86)
	GLI3 rs846266	CC	CT	0.042	0.59 (0.36–0.98)
			TT	0.955	0.98 (0.51–1.86)
	PAX9 rs4904155	CC	CG	0.023	0.55 (0.33–0.92)
			GG	0.320	0.72 (0.37–1.37)
CP	*DLX2* rs743605	CC	CT	0.031	0.52 (0.29–0.94)
			TT	0.100	0.53 (0.25–1.12)
	LHX6 rs989798	CC	CT	0.017	0.49 (0.27–0.88)
			TT	0.118	0.31 (0.07–1.34)

Table IV. (*Continued*).

Variables			Multivariate analysis		
Oral clefts sub-groups	Gene	Reference	Genotype	*p*-value	OR (95% CI)
	MSX1 rs12532	AA	AG	0.004	2.47 (1.31–4.66)
			GG	0.010	2.75 (1.27–5.95)
	PITX rs2595110	AA	AG	0.017	0.46 (0.24–0.87)
			GG	0.245	1.72 (0.68–4.30)
Unilateral	*FGF3* rs4980700	GG	AA	0.0004	0.35 (0.20–0.63)
			AG	0.001	0.45 (0.27–0.74)
	FGF3 rs1893047	AA	AG	0.028	1.61 (1.05–2.47)
			GG	0.346	1.26 (0.77–2.05)
	FGF10 rs1448037	CC	CT	0.029	1.46 (1.03–2.06)
			TT	0.318	1.26 (0.79–2.01)
	FGF10 rs1448037	TT	CC	0.012	0.49 (0.27–0.85)
			CT	0.264	0.73 (0.42–1.26)
	GLI2 rs2592600	AA	AT	0.994	1.00 (0.73–1.36)
			TT	0.006	0.54 (0.35–0.84)
	MSX1 rs12532	AA	AG	0.020	1.43 (1.05–1.95)
			GG	0.251	1.28 (0.83–1.96)
	PAX9 rs4904155	CC	CG	0.260	0.84 (0.62–1.13)
			GG	0.037	0.63 (0.41–0.97)
Bilateral	*AXIN2* rs2240308	AA	AG	0.039	1.72 (1.02–2.88)
			GG	0.004	2.09 (1.25–3.49)
	FGF3 rs4980700	AA	AG	0.013	0.52 (0.31–0.87)
			GG	0.005	0.44 (0.25–0.78)
	FGF3 rs1893047	AA	AG	0.007	1.81 (1.17–2.81)
			GG	0.145	1.44 (0.88–2.35)
	GLI2 rs2592600	AA	AT	0.938	0.98 (0.71–1.36)
			TT	0.035	0.62 (0.39–0.96)
	GLI2 rs1992901	AA	AT	0.938	0.98 (0.71–1.36)
			TT	0.035	0.62 (0.39–0.96)
	MSX1 rs12532	AA	AG	0.056	1.36 (0.99–1.88)
			GG	0.006	1.77 (1.17–2.67)
	PAX9 rs4904155	CC	CG	0.667	0.93 (0.68–1.27)
			GG	0.039	0.61 (0.39–0.97)

OR (95% CI), Odds ratios; 95% confidence intervals.
*$p \leq 0.00028$.
Covariates included age and positive history for cancer.

multiple testing, we may inadvertently increase the likelihood of type II errors, and truly important differences can be deemed non-significant. For this reason, future work can further consider *AXIN2*, *DLX1*, *DLX2*, *EDAR*, *FGF10*, *GLI2*, *GLI3*, *LHX6*, *MSX1*, *PAX9* and *PITX2*. In summary, we report for the first time that *FGF3* and *PAX9* may interact in the formation of oral clefts and susceptible individuals may belong to families that have higher risk for cancer.

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