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Insights from Studies with Oral Cleft Genes Suggest Associations between WNT-pathway Genes and Risk of Oral Cancer

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ABSTRACT

Oral squamous cell carcinoma (OSCC) accounts for more than 90% of the malignant neoplasms that arise in the mucosa of the upper aerodigestive tract. Recent studies of cleft lip/palate have shown the association of genes involved in cancer. WNT pathway genes have been associated with several types of cancer and recently with cleft lip/palate. To investigate if genes associated with cleft lip/palate were also associated with oral cancer, we genotyped 188 individuals with OSCC and 225 control individuals for markers in *AXIN2, AXIN1, GSK3β, WNT3A, WNT5A, WNT8A, WNT11, WNT3*, and *WNT9B*. Statistical analysis was performed with PLINK 1.06 software to test for differences in allele frequencies of each polymorphism between cases and controls. We found association of SNPs in *GSK3B* (p = 0.0008) and *WNT11* (p = 0.03) with OSCC. We also found overtransmission of *GSK3B* haplotypes in OSCC cases. Expression analyses showed up-regulation of *WNT3A*, *GSK3B*, and *AXIN1* and down-regulation of *WNT11* in OSCC in comparison with control tissues (P < 0.001). Additional studies should focus on the identification of potentially functional variants in these genes as contributors to human clefting and oral cancer.

KEY WORDS: WNT pathway genes, cleft lip/palate, oral cancer, squamous cell carcinoma,polymorphism.

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INTRODUCTION

Oral cancer accounts for roughly 2% of all cancers diagnosed annually in the United States. Approximately 35,000 people will be diagnosed with oral cancer each year, and about 7600 will die from the disease (National Institute of Dental and Craniofacial Research, 2010). Oral-facial clefts comprise a large fraction of all human birth defects, affecting approximately 1 in every 500 to 1000 births worldwide, and are notable for their significant lifelong morbidity and complex etiology (Murray, 2002).

Several authors have proposed that cancer and congenital malformations such as cleft lip and palate may occasionally have a common etiology (Nishi *et al*., 2000; Zhu *et al*., 2002; Bille *et al*., 2005; Menezes *et al*., 2009). The underlying concept is that the same genes may have functions during normal development, as well as in cancer development.

Several genes compose the Wnt signaling pathway, each with a specific role contributing to the complex cascade of events that eventually lead to the normal morphogenesis of the face (Mani *et al*., 2010). WNT genes have been recently suggested as candidate genes for cleft lip/palate, due to their essential functions during embryonic development (Chiquet *et al*., 2008). Moreover, mutations in *AXIN2*, a gene belonging to the Wnt signaling pathway, have also been detected in families segregating cancer and tooth agenesis (Lammi *et al*., 2004). Single-nucleotide polymorphisms (SNPs) in *AXIN2* have been associated with cleft lip/palate in a case-control study (Letra *et al*., 2009), and in a family study where the incidence of cancer in cleft families was notably higher than in control families (Menezes *et al*., 2009). To further investigate the hypothesis that genes may have roles in cleft lip/palate and in cancer development, we examined WNT pathway genes that were previously investigated or associated with oral clefts for association with oral squamous cell carcinoma.

MATERIALS & METHODS

Study Population

The study was approved by the University of Pittsburgh Institutional Review Board (IRB#010356). In total, 413 individuals of white ancestry from Pittsburgh, PA, were ascertained at the University of Pittsburgh Hillman Cancer Institute. The study population consisted of 188 affected individuals

Table 1. Summary of Candidate Genes and SNPs Studied

Gene	CHR	SNP	Base Pair Position	Base Change	SNP Type	MAF
WNT3A	1	rs708111	228191365	A/G	5′ upstream	0.47 (G)
WNT3A	1	rs3094912	228209815	T/A	Intron	0.47 (A)
WNT3A	1	rs752107	228247351	T/C	UTR 3	0.31 (T)
WNT3A	1	rs1745420	228251732	C/G	3′ upstream	0.13 (C)
WNT5A	3	rs566926	55520778	A/C	Intron	0.2 (A)
GSK3B	3	rs13314595	119631098	C/T	Intron	0.24 (T)
GSK3B	3	rs4072520	119635393	A/C	Intron	0.24 (A)
GSK3B	3	rs7620750	119643181	A/G	Intron	0.24 (A)
GSK3B	3	rs6769435	119684407	A/C	Intron	0.25 (C)
GSK3B	3	rs6771023	119693611	C/T	Intron	0.25 (C)
GSK3B	3	rs9879992	119712721	A/G	Intron	0.25 (G)
WNT8A	5	rs2040862	137419989	C/T	Intron	0.16 (T)
WNT11	11	rs1533767	75905800	A/G	Exon	0.22 (A)*
AXIN1	16	rs214249	348687	A/C	Intron	0.4 (C)
AXIN1	16	rs7359414	362638	G/T	Intron	0.48 (T)
WNT3 (NSF)	17	rs142167	44795234	A/G	Intron	0.2 (G)
WNT3	17	rs199498	44865603	C/T	Intron	0.17 (C)
WNT3	17	rs111769	44871987	C/T	Intron	0.35 (T)
WNT3	17	rs9890413	44901449	A/G	Intergenic	0.32 (G)*
WNT9B	17	rs2165846	44941366	A/G	Intron	0.44 (G)
WNT9B/GOSR2	17	rs197915	44990522	A/G	Intergenic	0.4 (G)*
AXIN2	17	rs7591	63525082	A/T	UTR 3	0.42 (A)
AXIN2	17	rs7224837	63528123	A/G	Intron	0.13 (G)
AXIN2	17	rs4128941	63531331	A/G	Intron	0.06 (A)
AXIN2	17	rs11867417	63537898	C/T	Intron	0.3 (T)
AXIN2	17	rs4791171	63541497	A/G	Intron	0.3 (A)
AXIN2	17	rs3923087	63549261	C/T	Intron	0.21 (T)
AXIN2	17	rs3923086	63549488	A/C	Intron	0.39 (A)
AXIN2	17	rs2240307	63554307	A/G	Exon	0.02 (G)
AXIN2	17	rs2240308	63554591	A/G	Exon	0.47 (G)
AXIN2	17	rs740026	63561681	A/G	Intergenic	0.46 (A)
AXIN2	17	rs1017020	63563906	C/A	Intergenic	0.11 (C)

MAF, minor allele frequency according to HapMap CEPH.
*According to Applied Biosystems.

(98 male, 90 female), aged 33-92 (average, 62.5 yrs), diagnosed with oral squamous cell carcinoma, and 225 unrelated control individuals (109 male, 116 female), aged 29-85 (average, 60.3 yrs), without oral squamous cell carcinoma or family history of oral squamous cell carcinoma. Data on smoking and ethanol consumption were not consistently available for all study participants. Participants signed an informed consent and were examined clinically and through their medical records for determination of their individual oral cancer status.

Single-nucleotide Polymorphism Selection and Genotyping

We selected 32 SNPs spanning 9 WNT pathway genes that have been previously suggested as candidate genes for cleft/lip palate based on studies with animal models or association studies in humans (Juriloff *et al*., 2005, 2006; Lan *et al*., 2006; Liu *et al*., 2007; Chiquet *et al*., 2008; Menezes *et al*., 2009, 2010) to test for association with oral squamous cell carcinoma in our population. Details of the selected SNPs and genes investigated are presented in Table 1.

Genomic DNA was obtained from blood samples as previously described (Andrade Filho *et al*., 2010). Genotyping of selected polymorphisms was carried out with Taqman assays and reagents in an ABI 7900 automatic instrument (Applied Biosystems, Foster City, CA, USA). We used PLINK 1.06 software (Purcell *et al*., 2007) to test for differences in allele frequencies of each polymorphism between cases with oral squamous cell carcinoma and controls. Haplotype analysis was also performed with PLINK. We considered significant the P-values adjusted with Bonferroni correction as implemented in PLINK.

Quantitative Real-time PCR

Frozen tumor samples (n = 10) and normal mucosal tissues (n = 4) collected after surgeries for tumor biopsies and amygdalectomies, respectively, were obtained from the University of Pittsburgh Hillman Cancer Institute. Total RNA was isolated with the RNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocol. The mucosa was carefully dissected from subjacent muscle or lymphoid tissue before RNA extraction, to completely isolate the normal squamous

epithelium. The RNA pellet was dried under a vacuum and resuspended in 30-50 µL of DEPC-treated water. We confirmed the integrity of RNA samples by analyzing 1 µg of total RNA on 1.2% (w/v) denaturing formaldehyde-agarose gels. Prior to DNA synthesis, RNA was treated with deoxyribonuclease I, Amplification Grade (Invitrogen, Carlsbad, CA, USA) for 15 min at room temperature to avoid DNA contamination. DNase I was inactivated by incubation with 25 mM EDTA at 65°C for 10 min. Complementary DNA was synthesized by the use of 3 µg of RNA through a reverse-transcription reaction as previously described (Garlet *et al*., 2004). Real-time polymerase chain-reaction (qPCR) quantitative messenger RNA (mRNA) analyses were performed in an ABI 7900 automatic instrument (Applied Biosystems, Foster City, CA, USA) with SYBR-green chemistry (Applied Biosystems) and specific primers (Appendix Table). The determination of the relative levels of gene expression was performed by the cycle threshold (C_T) method, in reference to the internal control gene beta-actin, as described elsewhere (Garlet *et al*., 2004). Reaction conditions were 95°C for 10 min, 40 cycles at 94°C for 1 min, annealing at 56°C for 1 min, and at 72°C for 2 min. Results are depicted as the mean average mRNA expression from triplicate measurements normalized by the internal control gene beta-actin. Statistical analyses included analysis of variance followed by Bonferroni correction in GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA). A $P \leq 0.05$ was considered statistically significant.

Immunohistochemical Localization of GSK-3B

We used immunohistochemistry to investigate the presence of GSK-3B in human oral squamous cell carcinoma samples. Briefly, paraffin-embedded oral squamous cell carcinoma tissues obtained from the archives of the Department of Diagnostic Sciences at the University of Pittsburgh School of Dental Medicine were sectioned into 5-µm slices, deparaffinized, and rehydrated by standard techniques. Immunohistochemical localization of GSK-3B was performed by the immunoperoxidase (avidin-biotin-peroxidase) method. Antigen retrieval was performed with citrate buffer, pH 6.0, at 96°C for 20 min. Sections were incubated in a rabbit polyclonal GSK-3B antibody (SC-9166; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:100 dilution at 4°C overnight, followed by incubation in biotinylated secondary antibody for 1 hr at room temperature. Negative controls were obtained by substitution of the primary antibody with PBS.

RESULTS

The most significant genetic association results are summarized in Table 2. Genotype and allele distributions were within Hardy-Weinberg equilibrium (data not shown). SNPs in 2 genes, *GSK3B* (rs9879992) and *WNT11* (rs1533767), showed association with oral squamous cell carcinoma ($p = 0.0007$ and $p = 0.03$, respectively). Alternatively, a SNP in *AXIN2* (rs3923087) also showed association with oral squamous cell carcinoma ($p = 0.001$), although toward a protective effect [OR = 0.57 (95%CI: 0.41-0.8)]. Borderline association was also observed for SNPs located in *WNT3A* and *AXIN1* genes (Table 2).

The results of the haplotype analysis further support the associations found for the individual SNPs (Table 3). We observed overtransmission of *GSK3B* haplotypes containing the associated SNP rs9879992: rs6771023-rs9879992 ($p = 0.0008$) and rs6769435-rs6771023-9879992 ($p = 3.00e-005$).

Results of Expression Analyses

To support the potential association of *WNT3A, WNT11, GSK3B, AXIN1*, and *AXIN2* genes with the pathogenesis of oral squamous cell carcinoma, we investigated their expression in diseased *vs*. healthy tissues (Fig.). *WNT3A*, *GSK3B*, and *AXIN1* mRNA expression was significantly higher in cases with oral squamous cell carcinoma compared with control tissues ($P < 0.001$), whereas expression of *AXIN2* and *WNT11* mRNA was similar in both groups, with *WNT11* showing even lower expression in tumor tissues (Fig.).

We observed some interesting correlations with regard to the expression levels of the investigated genes. We noted that *GSK3B* expression levels showed positive correlation with *WNT3A* ($p = 0.01$, $r^2 = 0.2042$), *AXIN1* ($p = 0.03$, $r^2 = 0.1693$), and *AXIN2* ($p = 0.04$, $r^2 = 0.1537$) levels. In addition, *AXIN1* levels showed positive correlation with *WNT3A* ($P < 0.0001$, $r^2 = 0.8011$), *WNT11* ($p = 0.0004$, $r^2 = 0.3847$), and *AXIN2* ($p < 0.0001$, $r^2 = 0.7880$) levels. Further, *AXIN2* expression showed positive correlation with *WNT3A* ($p < 0.0001$, $r^2 = 0.8671$) and *WNT11* ($p < 0.0001$, $r^2 = 0.7035$), and *WNT3A* expression was positively correlated with *WNT11* ($p < 0.0001$, $r^2 = 0.4680$).

We also investigated the GSK3-B protein expression in oral squamous cell carcinoma tissues, due to the significant results found in the SNP association analysis. We detected the marked presence of GSK3-B in the cytoplasm of tumor cells (Appendix Fig.).

DISCUSSION

In this study, we investigated the association of Wnt signaling pathway genes that had previously been suggested as candidates for oral-facial clefting for their association with oral squamous cell carcinoma. We found association of *GSK3B* and *WNT11* genes with oral squamous cell carcinoma. This study provides evidence that variations in WNT genes shown to be involved in oral-facial clefting may also increase an individual's susceptibility of developing oral squamous cell carcinoma.

The importance of WNT signaling for proper craniofacial development in the mouse embryo has been reported by several authors (Juriloff *et al*., 2005, 2006; Clevers, 2006; Lan *et al*., 2006). Activation of Wnt signaling has also been demonstrated as the underlying etiology of some oral cancers (Liu and Millar, 2010). Increasing evidence also exists to support the hypothesis that some genes display essential functions during embryogenesis and at some point may be involved in cancer development (Christensen *et al*., 2004; Bille *et al*., 2005; Menezes *et al*., 2009). We have previously reported that individuals with oral-facial clefts present a family history of cancer much more frequently than do individuals without clefts. Further, we verified that variations in *AXIN2*, a gene that belongs to the WNT pathway and which, when mutated, increases susceptibility to colon

Table 2. Results of Association Tests with WNT Genes in OSCC and Control Individuals

Gene	SNP	A1	MAF_A	MAF_U	P	OR (95%CI)
WNT3A	rs708111	A	0.53	0.47	0.08	1.28 (0.97-1.69)
WNT3A	rs3094912	T	0.46	0.53	0.04	0.75 (0.57-0.99)
WNT3A	rs752107	T	0.31	0.39	0.02	0.7 (0.52-0.94)
WNT3A	rs1745420	C	0.11	0.13	0.29	0.8 (0.52-1.23)
WNT5A	rs566926	T	0.27	0.29	0.42	0.88 (0.65-1.20)
GSK3B	rs13314595	T	0.22	0.19	0.19	1.25 (0.89-1.76)
GSK3B	rs4072520	A	0.22	0.20	0.42	1.15 (0.82-1.62)
GSK3B	rs7620750	A	0.22	0.18	0.24	1.23 (0.87-1.75)
GSK3B	rs6769435	C	0.21	0.19	0.44	1.15 (0.81-1.62)
GSK3B	rs6771023	C	0.23	0.19	0.14	1.29 (0.92-1.82)
GSK3B	rs9879992	G	0.27	0.17	0.0007	1.78 (1.27-2.50)
WNT8A	rs2040862	T	0.19	0.20	0.59	0.91 (0.64-1.29)
WNT11	rs1533767	A	0.33	0.26	0.03	1.4 (1.03-1.90)
AXIN1	rs214249	G	0.38	0.37	0.69	1.06 (0.80-1.41)
AXIN1	rs7359414	T	0.45	0.51	0.08	0.78 (0.59-1.03)
WNT3 (NSF)	rs142167	G	0.24	0.24	0.90	0.98 (0.71-1.35)
WNT3	rs199498	C	0.24	0.21	0.35	1.17 (0.84-1.62)
WNT3	rs111769	T	0.42	0.39	0.53	1.09 (0.83-1.45)
WNT3	rs9890413	G	0.30	0.31	0.71	0.94 (0.70-1.27)
WNT9B	rs2165846	G	0.43	0.38	0.17	1.22 (0.92-1.62)
WNT9B	rs197915	G	0.41	0.44	0.46	0.9 (0.68-1.19)
AXIN2	rs7591	A	0.42	0.42	0.9	0.98 (0.74-1.30)
AXIN2	rs7224837	G	0.09	0.11	0.3	0.78 (0.48-1.26)
AXIN2	rs4128941	A	0.03	0.05	0.13	0.57 (0.27-1.19)
AXIN2	rs11867417	T	0.37	0.34	0.52	1.10 (0.82-1.48)
AXIN2	rs4791171	T	0.33	0.30	0.29	1.17 (0.87-1.58)
AXIN2	rs3923087	T	0.1885	0.29	0.001	0.57 (0.41-0.80)
AXIN2	rs3923086	A	0.4601	0.47	0.82	0.97 (0.73-1.28)
AXIN2	rs2240307	A	0.484	0.47	0.76	1.04 (0.79-1.37)
AXIN2	rs2240308	A	0.4798	0.5	0.58	0.92 (0.69-1.22)
AXIN2	rs740026	A	0.4472	0.4	0.18	1.21 (0.91-1.61)
AXIN2	rs1017020	C	0.1317	0.1	0.21	1.31 (0.85-2.01)

A1: Minor allele (based on whole sample).
MAF_A : minor allele frequency in cases.
MAF_U : minor allele frequency in controls.

cancer, were also associated with a cleft phenotype in those families (Menezes *et al*., 2009). Additional studies in mice and humans support the involvement of WNT genes in the etiology of oral-facial clefts. In an inbred A/WySn mouse strain characterized for the presence of cleft palate, *Wnt3* and *Wnt9B* genes were found to be located in the *clf1* cleft locus, suggesting their possible involvement in the cleft phenotype (Juriloff *et al*., 2005). Recently, variations in WNT genes were associated with non-syndromic oral-facial clefts in different populations, further supporting the notion that WNT genes may be involved in the etiology of oral clefts in humans as well (Chiquet *et al*., 2008; Menezes *et al*., 2010).

GSK3B is part of the Wnt signaling pathway and plays a major role in epithelial cell homeostasis (Kim *et al*., 2007), and because *GSK3B* interacts with many different pathways, its specific developmental roles remain unclear. Our results showed a significant association of an intronic SNP in *GSK3B* (rs9879992) with oral squamous cell carcinoma. Analysis of *GSK3B* combined marker haplotypes also showed association of haplotypes containing the associated SNP. In addition, we observed *GSK3B* to be highly expressed in the oral squamous cell carcinoma biopsies. It has been proposed that the manipulation of the activated *GSK3B* may provide hope for treating oral cancer (Mishra, 2010). Interestingly, a role for *GSK3B* in craniofacial defects has also been demonstrated, since homozygous null mice display cleft palate, incomplete fusion of the ribs at the midline and bifid sternum, and delayed sternal ossification (Liu *et al*., 2007).

WNT11 also appears to be involved with oral squamous cell carcinoma risk. We found association of a synonymous coding mutation in *WNT11* (rs1533767, Pro136Pro) so far not predicted to have any deleterious effect on protein function. Using

Table 3. Most Significant Sliding Window Haplotype Association Results Observed for Markers in *GSK3β* and *WNT3A* Genes Located in Chromosomes 3 and 1, Respectively, and OSCC in a Pittsburgh, PA, USA, Population

<table>
<tr><th colspan="6">GSK3β</th></tr>
<tr><td>rs13314595</td><td>rs4072520</td><td>rs7620750</td><td>rs6769435</td><td>rs6771023</td><td>rs9879992</td></tr>
<tr><td colspan="2">p = 0.2 (TA)</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td colspan="2">p = 0.2 (AA)</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td colspan="2">p = 0.2 (AC)</td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td colspan="2">p = 0.2 (CC)</td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td colspan="2">p = 0.0008 (TG)</td></tr>
<tr><td colspan="3">p = 0.17 (TAA)</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td colspan="3">p = 3.00E-05 (ATG)</td></tr>
<tr><td colspan="6">p = 0.00005 (CCGATG)</td></tr>
<tr><th colspan="6">WNT3A</th></tr>
<tr><td>rs708111</td><td>rs3094912</td><td>rs752107</td><td>rs1745420</td><td></td><td></td></tr>
<tr><td colspan="2">p = 0.06 (AA)</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td colspan="2">p = 0.1 (AC)</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td colspan="2">p = 0.03 (CG)</td><td></td><td></td></tr>
<tr><td colspan="3">p = 0.08 (AAC)</td><td></td><td></td><td></td></tr>
<tr><td></td><td colspan="3">p = 0.05 (ACG)</td><td></td><td></td></tr>
<tr><td colspan="4">p = 0.06 (AACG)</td><td></td><td></td></tr>
</table>

the software FAS-ESS (http://genes.mit.edu/fas-ess/), we verified that this mutation is involved in splicing regulation and the ancestral allele G attributes splicing silencing properties. Moreover, the results of *WNT11* mRNA expression analysis showed significant down-regulation in the tumor tissues when compared with healthy tissues as measured by quantitative real-time PCR, suggesting a role for *WNT11* as a tumor suppressor gene during the development of oral squamous cell carcinoma. These findings are further supported by a recent study where loss of *WNT11* expression was shown to promote the malignant phenotype *via* both canonical and non-canonical Wnt signaling pathways (Toyama *et al*., 2010).

AXIN2 is a negative regulator of the WNT-beta-catenin pathway. We observed the association of *AXIN2* with a lower risk of oral squamous cell carcinoma, thus suggesting that the associated variant may have a protective effect in these cases. We also detected lower *AXIN2* mRNA expression in the tumor tissues when compared with healthy control tissues. Our results corroborated those from previous studies showing that epigenetic silencing of *AXIN2* is relevant for hypermethylation and histone deacetylation, leading to a decreased mRNA/protein expression of *AXIN2* gene in lung cancer (Tseng *et al*., 2008). We may speculate that a similar mechanism may occur during oral squamous cell carcinoma development.

In addition to the individual genetic and expression results for *GSK3B, WNT11,* and *AXIN2* that implicate a possible role for these genes in the etiology of oral squamous cell carcinoma, the correlation of their expression levels revealed interesting findings that warrant further investigations. Except for the exonic *WNT11* mutation, both *GSK3B* and *AXIN2* SNPs are located in introns and are less likely to be considered disease-causing variants. Instead, they are likely to be in linkage disequilibrium with the true causal variant, and may contribute indirectly to the disease risk. A substantial proportion of disease-associated SNPs is not located within or near known genes, suggesting that non-coding DNA variation may impart functional effects (Notaridou *et al*., 2011). In regard to gene expression in the tumor tissues, several positive correlations were observed between the associated genes and additional genes in the WNT pathway. *WNT3A, GSK3B*, and *AXIN1* were all significantly up-regulated in oral squamous cell carcinoma cases in comparison with controls, whereas *AXIN2* was slightly up-regulated and *WNT11* was down-regulated. Further, the positive correlations observed for *GSK3B* with *WNT3A, AXIN1* and *AXIN2*, where *GSK3B* and *WNT3A* are considered "tumor-promoters", and *AXIN1* and *AXIN2* are considered "tumor-suppressors", highlight the importance of studying genetic pathways instead of one single gene as causative for complex diseases such as cancer and craniofacial anomalies.

Even though numerous common genetic variants have now been shown to be associated with the risk of several cancer types, this study provides evidence that variation in WNT pathway genes, in particular *GSK3B*, might contribute to an individual's susceptibility to oral squamous cell carcinoma. We must note that in addition to individual predisposition to cancer, we should also consider environmental factors that may modulate each individual's risk to develop the disease, such as smoking and alcohol intake, among other factors. Unfortunately, data on these environmental variables were not conclusive to be included in our analyses, which was a limitation of this study. Nevertheless, our findings associating WNT genes such as *GSK3B* and *WNT11* and oral squamous cell carcinoma reinforce the suggested implications for this gene family in cancer. In addition, considering the recently reported associations of these genes in oral-facial clefts, the results of this study corroborate previous suggestions that the same genes may have different roles during different biological processes such as embryonic development and cancer. Additional studies should focus on the identification of potentially functional variants in these genes, while approaching cell-signaling networks as contributors to human clefting and oral cancer.

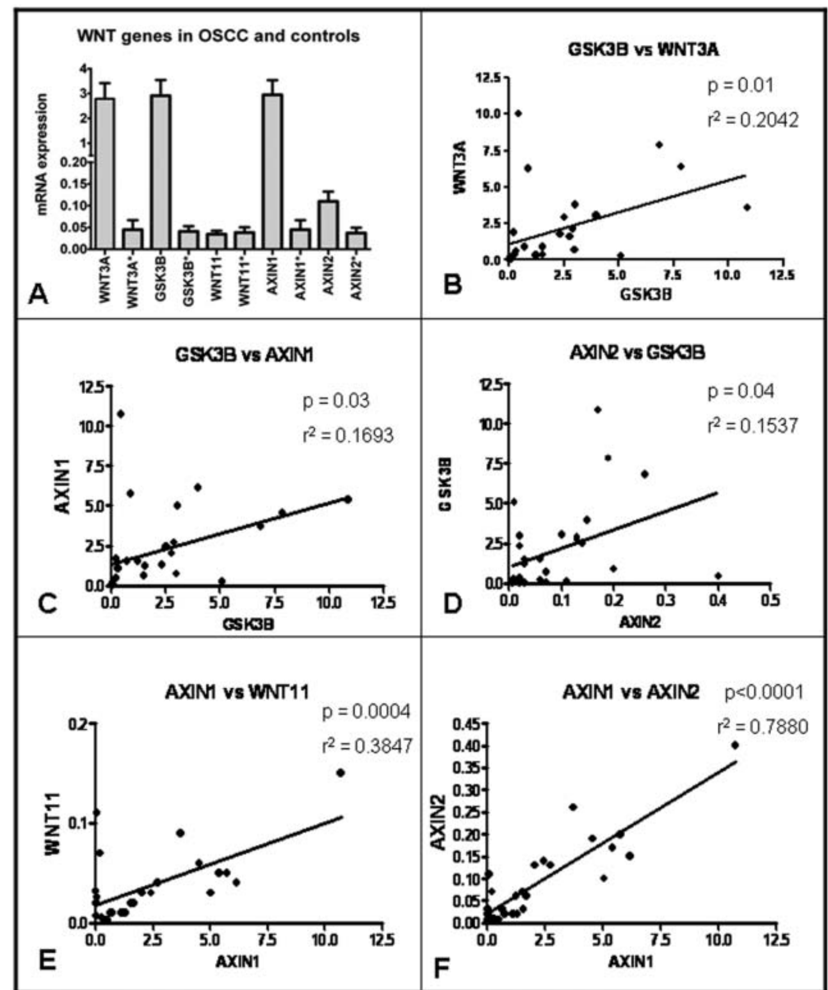

Figure. Summary of gene expression results. mRNA expression in tumor samples and controls **(A)**. Most significant positive correlations observed: *GSK3B vs. WNT3A* **(B)**; *GSK3B vs. AXIN1* **(C)**; *AXIN2 vs. GSK3B* **(D)**; *AXIN1 vs. WNT11* **(E)**; and *AXIN1 vs. AXIN2* **(F)**.

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