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DLX1 and MMP3 contribute to oral clefts with and without positive family history of cancer

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ABSTRACT

Objective: It has been suggested that oral clefts and cancer share a common genetic background. This study aimed to investigate the epidemiological and molecular association between oral clefts and cancer.

Methods: One hundred forty-eight nuclear families with oral clefts and 162 subjects with no birth defect were recruited. Data on self-reported family history of cancer among first, second, and third degree relatives of each patient were collected via a structured questionnaire. We also investigated the association between polymorphisms in the genes *AXIN2*, *BMP2*, *BMP4*, *BMP7*, *DLX1*, *DLX2*, and *MMP3* and oral cleft with and without history of cancer. Markers in these genes were genotyped using real time PCR. Chi-square and *t*-test were used to assess the differences about self-reported family history of cancer between oral cleft and non-cleft individuals. The transmission disequilibrium test (TDT) was used to analyze the distortion of the inheritance of alleles from parents to their affected offspring.

Results: Families with oral clefts had an increased risk of having a family history of cancer ($p = 0.01$; odds ratio = 1.79; 95% confidence interval, 1.07–1.87). TDT results showed an association between *DLX1* and cleft lip and palate, in which the A allele was undertransmited ($p = 0.022$). For *MMP3*, G was undertransmited among affected progeny ($p = 0.019$) in cleft palate subgroup.

Conclusion: Oral clefts were associated with positive self-reported family history of cancer and with variants in *DLX1* and *MMP3*. The association between oral clefts and cancer raises interesting possibilities to identify risk markers for cancer.

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1. Introduction

Oral clefts are one of the most common types of congenital malformation with an average birth prevalence of 1/700 live births,[1] and its incidence rate is variable depending on ethnicity and geographic origin.[2] Oral clefts result from a combination of genetic and environmental factors that result in the failure of the developing facial process to fuse, merge, or interact. It has been estimated that anywhere from 3 to 14 genes contribute to oral clefts.[3]

Previous studies have shown that a high risk of oral clefts may exist in families where a cancer case has been identified,[4–6] while others provide data about increased risk of cancer for relatives of individuals with oral clefts.[7–10] Some of these recent studies addressed the fact that oral clefts and cancer sometimes share the same genetic background.[8,10]

Wnts are a family of signalling molecules that play important roles in various aspects of craniofacial development.[11] Mutations in *AXIN2*, a gene belonging to the Wnt signalling pathway, have been detected in families that segregate cancer and tooth agenesis (the most common craniofacial anomaly).[12]

Bone morphogenetic proteins (BMPs) are secreted signalling molecules belonging to the transforming growth factor-β (TGF-β) superfamily of growth factors. Studies have shown that *BMP2*, *BMP4*, and *BMP7* genes are expressed during embryogenesis of the many structures in the orofacial complex.[13–15] In recent years, evidence has accumulated of BMPs' important functions in tumour development.[16,17]

DLX genes constitute a family of homeobox transcription factors involved in control of cell differentiation and morphogenesis. The mouse and human *DLX* gene system is formed by three bi-gene clusters: *DLX1* and *DLX2*; *DLX3* and *DLX4*; *DLX5* and *DLX6*. All DLX genes play a role in the control of craniofacial embryogenesis.[18] DLX genes may also be involved in the neoplastic process.[19]

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that mediate the degradation of practically all extracellular matrix molecules contributing to both normal and pathological tissue remodelling.[20] MMPs are also the main proteolytic enzymes involved in cancer invasion and metastasis.[21,22] Among all MMPs, MMP3 has shown the most extensive distribution during palatal shelf morphogenesis.[23,24] In addition, genetic variations in *MMP3* have been associated with oral clefts.[25,26]

Furthermore, genetic studies of oral cleft have shown the association of genes that are also involved in cancer, raising a hypothesis that some genes might be involved in embryonic development and later in cancer development.[27,28] Therefore, in this study we examined whether families of oral cleft subjects have an increased risk for cancer. Furthermore, we investigated the association between single nucleotide polymorphisms in genes involved in palatogenesis and tumorigenesis in nuclear families with clefts.

2. Materials and methods

2.1. Sample

The Human Ethics Committee of the Health Department of the city of Rio de Janeiro (113/09) approved this study. Informed consent was obtained from all participating individuals or parents/legal guardians. Individuals with non-syndromic oral clefts, treated at the Nossa Senhora do Loreto Hospital, and their parents were recruited as a studied group. Healthy unrelated individuals, treated at the Department of Pediatric Dentistry at the Federal University of Rio de Janeiro, were recruited as a control group. Both institutions are located in Rio de Janeiro city (Brazil) within 10 km each other to avoid selection bias. The collection of the both groups was performed in the same period – between January 2012 and May 2013.

The ethnicity definition was ascertained based on self-reported information. The population included in this study resides in the metropolitan area of Rio de Janeiro, Brazil, which is comprised of a mix of Caucasians (mainly of European descent; 53.6%) and African descendants (obviously mixed Europeans, 33.6%; or not obviously mixed Africans, 12.3%). The remaining 0.5% of the population is of Amerindian or Asian descent (IBGE, 2007). The determination of the cleft phenotype (cleft lip only, cleft lip with cleft palate, and cleft palate only) was based on the description in the clinical records.

2.2. Data collection

Data on self-reported family history of cancer and history of oral clefts among first, second, and third degree relatives were collected from each patient via a structured questionnaire. If at least one of the family members had cancer, that participant was considered to have a family history of cancer. A positive family history of oral cleft was defined as having at least one relative affected with cleft in addition to the index case.

2.3. DNA sample and genotyping

Molecular analyses were performed for the individuals with oral clefts and their parents. Genomic DNA for molecular analysis was extracted from buccal cells based on the modified reported method.[29] Genetic polymorphisms in *AXIN2*, *BMP2*, *BMP4*, *BMP7*, *DLX1*, *DLX2*, and *MMP3* were genotyped by real-time polymerase chain reactions using the Taqman assay[30] (Agilent Technologies, Stratgene Mx3005P). The characteristics of the studied SNPs are presented in Table 1. These selected genes were independently associated with both craniofacial anomalies and cancer.

2.4. Statistical analyses

Epidemiological data were analyzed using the Statistical Package for the Social Sciences (SPSS 16.0). Student's *t* test and X^2 test at a significance level of 0.05 were used to assess the differences between individuals with and without oral clefts. Odds ratio calculations and X^2 test at a significance level of 0.05 were used to determine if any cleft types were associated with cancer. Genetic data was analyzed using Plink. The transmission disequilibrium test (TDT) was used to check the distortion of the inheritance of alleles from heterozygous parents to their affected offspring,[31] and thus to test for association between each marker and susceptibility for oral cleft subgroups.