Published in final edited form as:
J Prev Med Public Health. 2009 January ; 42(1): 1–4. doi:10.3961/jpmph.2009.42.1.1.

Differential Parental Transmission of Markers in *BCL3* among Korean Cleft Case-parent Trios

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Abstract

Objectives—Isolated cleft lip with or without cleft palate (CL/P) is among the most common human birth defects, with a prevalence of approximately 1 in 700 live births. The B-Cell Leukemia/lymphoma 3 (*BCL3*) gene has been suggested as a candidate gene for CL/P based on association and linkage studies in some populations. This study tests for an association between markers in *BCL3* and isolated, non-syndromic CL/P using a case-parent trio design, while considering parent-of-origin effects.

Methods—Forty case-parent trios were genotyped for two single nucleotide polymorphisms (SNPs) in the BCL3 gene. We performed a transmission disequilibrium test (TDT) on individual SNPs, and the FAMHAP package was used to estimate haplotype frequencies and to test for excess transmission of multi-SNP haplotypes.

Results—The odds ratio for transmission of the minor allele, OR (transmission), was significant for SNP rs8100239 (OR=3.50, p=0.004) and rs2965169 (OR=2.08, p=0.027) when parent-of-origin was not considered. Parent-specific TDT revealed that SNP rs8100239 showed excess maternal transmission. Analysis of haplotypes of rs2965169 and rs8100239 also suggested excess maternal transmission.

Conclusions—*BCL3* appears to influence risk of CL/P through a parent-of-origin effect with excess maternal transmission.

Keywords

BCL3; Oral cleft; Maternal transmission effects; Parent-of-origin

INTRODUCTION

Oral clefts are one of the most common birth defects in humans, and represent a significant public health problem both in terms of the medical and economic burden for affected individuals and their families. Non-syndromic cleft lip with or without palate (CL/P) is ‘complex’ or ‘multifactorial’ in its etiology, in that both genes and environmental risk factors determine risk [1,2]. Although several candidate genes have been extensively studied in different populations (tranforming growth factor alpha, interferon regulatory factor 6, retinoic acid receptor alpha, etc), only a few genes have been shown to contain mutations that appear

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causal (msh homeobox 1, poliovirus receptor-related 1, etc.), and these are rare and often show incomplete penetrance [3-5].

The B-cell leukemia/lymphoma 3 (BCL3) gene is located on chromosome 19q13, where studies of multiplex families have yielded evidence for linkage to nonsyndromic orofacial clefts [6,7]. Several other studies also observed an association between markers in the BCL3 gene and CL/P [6,8]. Although these studies suggested candidate genes, there have not been many studies on whether the BCL3 gene is a risk factor for CL/P in Asian populations.

It is important to consider parent-of-origin effects when studying birth defects because maternal genotype controls the in utero environment of the developing fetus, and separating maternal genotypic effects from imprinting effects remains an important question [9,10]. Maternal parent-of-origin effects have been suggested for several genes associated with non-syndromic CL/P [11-14]. In this paper, we tested for an association between markers in BCL3 and the risk of CL/P in 40 Korean case-parent trios, with specific consideration of parent-of-origin effects.

METHODS

I. Sample Description

As part of an international study of oral clefts, we enrolled 40 unrelated Korean patients aged 6 months to 19 years and their parents through the department of plastic surgery, Yonsei university medical center (Seoul, Korea) from January 2003 to March 2004. Parents of the cases were interviewed regarding family history, medical history, and exposure to suspected risk factors. The patient and his/her medical records were examined to confirm the classification of non-syndromic CL/P. There were 22 male cases and 18 female cases. The mother's and father's mean age at proband's birth were 30.5 and 33.4. The institutional review boards of Yonsei university and the Johns Hopkins Bloomberg school of public health approved this study. All parents received adequate information about this study and gave written informed consent.

II. SNP Selection, DNA, & Genotyping

Single nucleotide polymorphisms (SNPs) were selected in a region surrounding BCL3 on chromosome 19q13, with a goal of identifying one SNP per 5 kb of physical distance. Variants with "SNP scores" (an assessment of design quality of the Illumina assay based on a proprietary algorithm) above 0.6, high validation levels in dbSNP (this included validation levels where the submitter had validated the SNP on multiple platforms), and high heterozygosity levels (particularly in multiple populations) were given higher priority during the selection process. From seven selected SNPs, two SNPs were found to be polymorphic in the Korean population (Table 1).

Genomic DNA samples were prepared from peripheral blood using the previously described protein precipitation method [15]. DNA concentration was determined using the PicoGreen® dsDNA Quantitation Kit (Molecular Probes Inc., Eugene, OR, USA), and all DNA samples were stored at −20°C. A 4 μg aliquot of each genomic DNA sample was dispensed into a bar-coded 96-well microtiter plate at a concentration of 100 ng/μl, and was subsequently genotyped for SNP markers using the Illumina Golden-Gate™ chemistry with Sentrix® Array Matrices (Illumina, San Diego, USA) [16] at the SNP center of the genetic resources core facility (GRCF), a part of the McKusick-Nathans institute of genetic medicine, Johns Hopkins school of medicine. Two duplicates and four centre d'etude du polymorphisme humain (CEPH) controls were included on each plate to evaluate genotyping consistency within and between

plates, and to insure correct orientation. Genotypes were generated on a BeadLab 1000 system (Illumina, San Diego, USA) [17].

III. Statistical Analysis

The minor allele frequency (MAF) was computed among parents, and pairwise linkage disequilibrium (LD) was computed as the R-square value for all SNPs using the Haploview program (Broad institute, Cambridge, USA) [18]. The standard transmission disequilibrium test (TDT) described by Speilman et al. [19] was used to test for excess transmission of individual alleles. Parent-of-origin effects were examined using Clayton's extension of the TDT incorporated into STATA 8.2 (Stata Corporation, College station, USA), which stratifies the standard TDT into separate allelic tests for fathers and mothers [20].

The FAMHAP package (IMBIE, Bonn, Germany) was used to estimate haplotype frequencies and to test for excess transmission of multi-SNP haplotypes [6]. The FAMHAP package calculates maximum likelihood estimates (MLEs) of haplotype frequencies (for up to 20 SNPs) from nuclear families with varying numbers of children via the expectation-maximization algorithm, and is robust when handling missing SNPs [21]. This program provides a haplotype-based test for nuclear family data. This test statistic is based on Monte-Carlo simulations, in which the set of transmitted and non-transmitted genotypes/haplotypes is randomly permuted for each replicate [22,23]. In this analysis, the chi-square statistic for marker combinations is replaced by the maximum chi-square over single haplotypes (maximum TDT statistic). The program gives an empiric p-value, corrected for the multiple haplotypes being considered. This haplotype analysis was also carried out separately for maternal and paternal transmission.

RESULTS

Five of the seven SNPs were monomorphic, leaving only two SNPs with reasonable heterozygosity (Table 1). The R-square value between SNP rs2965169 and rs8100239 was 0.38. Only trios with complete data were used for the TDT. When all markers were screened using the TDT without considering the parent of origin, the odds ratio of transmission for the minor allele, OR (transmission), was significant for both SNP rs2965169 (OR=2.08, p=0.027) and SNP rs8100239 (OR=3.50, p=0.004)(Table 2).

Parent-of-origin effects were investigated by stratifying informative transmissions (T) and non-transmissions (NT) by parental source for these two SNPs (Table 3). This analysis revealed that SNP rs8100239 showed excess maternal transmission, significant at the p=0.004 level (OR=11.0).

Table 4 shows the results of haplotypes analysis for rs2965169 and rs8100239. In these Korean trios, haplotypes showed evidence of excess transmission of the 2-1 haplotype to CL/P children (p=0.018 for overall transmission). This can be largely attributed to excess maternal transmission (p=0.038).

DISCUSSION

Our study of CL/P case-parent trios showed significant evidence of linkage and disequilibrium for SNP rs8100239 in BCL3. In screening for parent-of-origin effects, we found suggestive evidence of excess maternal transmission of this SNP. Haplotypes of rs8100239 and rs2965169 also showed significant deviation from the expected levels when transmitted from mothers, but not from fathers.

BCL3, a proto-oncogene that encodes a transcription factor involved in cell cycle regulation, has been suggested as a candidate gene for CL/P [6,24]. The BCL3 gene has been associated

with oral clefts in some association studies [6,8], but not others [25,26]. A possible reason for these conflicting results is that the susceptibility loci may have different contributions in different populations [6]. The present study suggests that BCL3 is associated with oral clefts in Koreans.

Excess maternal transmission could reflect genomic imprinting or maternal genotype effects. Maternal genotypic effects for non-syndromic cleft lip with/without palate (CL/P) have also been reported for several other candidate genes (5,10 methylenetetrahydrofolate reductase (MTHFR) and cystathionine beta synthase), but these have yet to be confirmed [3,12]. Recently, Reuter et al. [27] found parent-of-origin effects in the transforming growth factor beta 3 gene among central Europeans with nonsyndromic cleft lip and palate, and found a lower risk of maternal transmission compared to paternal transmission. Our results for rs8100239 in BCL3 (analyzed both alone and as a haplotype) showed evidence of excess maternal transmission, which could reflect an imprinting effect or a maternal genotype effect.

Several studies have also suggested that the BCL3 gene may be a modifier or may exert an additive effect [6,28]. In a family-based association study in a Brazilian population, Gaspar et al. [28] observed an interaction between the maternal MTHFR and offspring's BCL3 genotypes.

The case-parent trio design offers the advantage of testing directly for maternal vs. paternal effects, and allows the separation of these effects from the effects of the fetal genotype vs. parental origin in a robust manner [20,29,30]. Another advantage of this study design is that it minimizes issues of confounding that plague traditional case-control designs. The small sample size of the present study may not provide a reliable result (e.g. OR=11.0 in Table 3), and further replication studies are necessary. Another limitation of this study was that only two SNPs of seven SNPs were polymorphic in this study because we selected SNPs from Caucasian samples. Further studies should be conducted with more polymorphic SNPs for the Korean population.

The present study suggests maternal transmission effects for markers in BCL3 and risk of non-syndromic CL/P. Further work will be needed to confirm this suggestion that maternal transmission of alleles in BCL3 influences the risk of CL/P, and to determine its ultimate impact on risk.

Acknowledgments

This research was supported by the Korean Research Foundation (2004-041-E00104).

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Table 1

SNP minor allele frequencies among parents of CL/P cases in Korea

No	SNP name	Physical Location	Minor allele	Minor allele Frequency
1	rs2965169	49942996	C	0.45
2	rs8100239	49944944	A	0.25

Table 2

Number of Transmitted (T) or Non-Transmitted (NT) minor alleles in 40 CLP cases for TDT and estimated odds ratios of transmission OR* (transmission) ignoring parent-of-origin

No	SNP Name	TDT			
		T	NT	P-value	OR*
1	rs2965169	27	13	0.027	2.08
2	rs8100239	21	6	0.004	3.50

T: transmitted, NT: not transmitted

*OR (transmission): odds ratio of transmission for the minor allele.

Table 3

Number of Transmitted (T) or Non-Transmitted (NT) minor alleles to 40 CLP cases from TDT and estimated odds ratio considering parent-of-origin

No	SNP Name	Paternal				Maternal			
		TDT		p-value	OR*	TDT		p-value	OR*
		T	NT			T	NT		
1	rs2965169	11	2	0.013	5.50	10	5	0.197	2.00
2	rs8100239	8	3	0.132	2.67	11	1	0.004	11.0

T: transmitted, NT: not transmitted, TDT: transmission disequilibrium test

*OR (transmission): odds ratio of transmission for the minor allele.

Table 4

Analysis of haplotypes using rs2965169 and rs8100239 in BCL3 with analyses using the program FAMHAP

Haplotype	Frequency	Overall			Paternal			Maternal		
		T	NT	Maximum TDT (p-value)	T	NT	Maximum TDT (p-value)	T	NT	Maximum TDT (p-value)
11	0.008	1.01	0.0	6.021	0.0	0.0	2.04	1.01	0	5.32
12	0.539	12.0	27.0	(0.018)	6.0	12.0	(0.224)	6.0	15.0	(0.038)
21	0.250	18.0	6.0		8.0	4.0		10.0	2.0	
22	0.203	13.0	11.0		7.0	5.0		6.0	6.0	

T: transmitted, NT: not transmitted, TDT: transmission disequilibrium test