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

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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.

Key words: 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 a 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 (transformation growth factor alpha, interferon regulatory factor 6, retinoic acid receptor alpha, etc.), only a few genes have been shown to contain mutations that appear 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’étude 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 Spielman 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 allele 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. Haplotype frequencies and to test for excess transmission of multi-SNP 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.

Table 1. SNP minor allele frequencies among parents of CL/P cases in Korea

<table>
<thead>
<tr>
<th>No</th>
<th>SNP Name</th>
<th>Physical Location</th>
<th>Minor allele</th>
<th>Minor allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs2965169</td>
<td>4994296</td>
<td>C</td>
<td>0.45</td>
</tr>
<tr>
<td>2</td>
<td>rs8100239</td>
<td>4994494</td>
<td>A</td>
<td>0.25</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>No</th>
<th>SNP Name</th>
<th>T</th>
<th>NT</th>
<th>P-value</th>
<th>OR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs2965169</td>
<td>27</td>
<td>13</td>
<td>0.027</td>
<td>2.08</td>
</tr>
<tr>
<td>2</td>
<td>rs8100239</td>
<td>21</td>
<td>6</td>
<td>0.004</td>
<td>3.50</td>
</tr>
</tbody>
</table>

Table 3. Parent-of-origin effects for SNP rs2965169 and rs8100239

<table>
<thead>
<tr>
<th>SNP Name</th>
<th>TDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2965169</td>
<td>2.08 (0.027)</td>
</tr>
<tr>
<td>rs8100239</td>
<td>3.50 (0.004)</td>
</tr>
</tbody>
</table>

Table 4. Haplotype analysis for SNP rs2965169 and rs8100239

<table>
<thead>
<tr>
<th>SNP Name</th>
<th>Haplotype</th>
<th>TDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2965169</td>
<td>2-1</td>
<td>11.0 (0.018)</td>
</tr>
<tr>
<td>rs8100239</td>
<td>2-1</td>
<td>3.50 (0.004)</td>
</tr>
</tbody>
</table>
BCL3 and Oral Clefts

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

<table>
<thead>
<tr>
<th>No</th>
<th>SNP Name</th>
<th>Paternal</th>
<th>Maternal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>NT</td>
</tr>
<tr>
<td>1</td>
<td>rs2965169</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>rs8100239</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Paternal</th>
<th>Maternal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>NT</td>
<td>Maximum TDT (p-value)</td>
</tr>
<tr>
<td>11</td>
<td>0.008</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>12</td>
<td>0.539</td>
<td>12.0</td>
<td>37.0</td>
</tr>
<tr>
<td>21</td>
<td>0.250</td>
<td>18.0</td>
<td>6.0</td>
</tr>
<tr>
<td>22</td>
<td>0.203</td>
<td>13.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>

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

Maternal transmission of alleles in BCL3 influences the risk of CLP, and to determine its ultimate impact on risk.

ACKNOWLEDGEMENTS

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

REFERENCES


