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Meta-Analysis

# Association between SATB2 gene polymorphism and cleft palate only risk in eastern Guangdong population and a meta-analysis

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**Abstract:** To characterize the associations between the cleft palate (CPO) and single nucleotide polymorphisms (SNPs) of special AT-rich sequence-binding protein 2 (SATB2). We recruited 241 CPO and performed a case-control study with 242 controls. Concurrently, 103 of the patients and their normal parents were recruited to perform a case-parent trio study. Sixteen selected SNPs were genotyped. Furthermore, A meta-analysis was used to enhance the robustness of our conclusions. The case-control study provided no support for the hypothesis that any of the 16 selected SNPs played a significant role in CPO. In the meta-analysis, we also did not find that the SATB2 was associated with nonsyndromic cleft palate risk, in Asians or in Caucasians. The 16 selected SNPs do not contribute to the development of CPO.

***Key words:*** Special AT-rich sequence binding protein 2 (SATB2); Nonsyndromic cleft palate (NSCP); Gene polymorphism; Meta-analysis.

## Introduction

Orofacial clefting, one of the most common forms of congenital malformation, displays the substantial phenotypic variation. Cleft lip with/without cleft palate (CL/P) and cleft palate only (CPO) are the two most common forms of orofacial clefting (1, 2). Nonsyndromic cleft palate (NSCP) is one of the most common congenital malformations worldwide, and both genetic and environmental factors are involved in its etiology (3). The etiology of cleft palate is complex and largely unclear, but it is currently believed to be also caused by both genetic and environmental factors and their interaction (4-7). The development of cleft palate is a programmed event that includes the expression of genes, regulation of transcription, and the participation of related factors (8).

Treatment requires a multidisciplinary approach after birth up to adulthood (9). Although nonsyndromic familial forms of cleft palate have been shown, approximately 70% of all patients are isolated nonsyndromic entities without clear Mendelian inheritance patterns (9, 10). Both population and family studies suggest that genetic factors play a critical role in the etiology of NSCP (11, 12). Several genes have been identified for syndromic forms of CP, but few have been identified as an influencing risk for NSCP (13). The etiology of this complex trait has been widely studied in order to search for the risk factors and to design strategies for prevention.

Special AT-rich sequence-binding protein 2 (SATB2), a DNA-binding protein and a member of the family of matrix attachment region-binding transcription factors, is involved in transcriptional regulation and chromatin remodeling, and has developmental roles in craniofacial, neural, and osteoblastic differentiation (14, 15). Several independent studies have reported the effects of SATB2 gene polymorphisms on NSCP (16-18). In this study, we characterized the association between cleft palate and SNPs of SATB2 in the eastern Guangdong population. Furthermore, we performed a meta-analysis to assess the relationship between the SATB2 rs4673313 T/C gene polymorphism and NSCP risk.

## Materials and Methods

### Patient samples

The Ethics Committee of the Second Hospital of Shantou University Medical College approved this study. The subjects of this study were recruited from the Cleft Lip and Palate Treatment Center of the Second Hospital of Shantou University Medical College in Guangdong, China. Two hundred forty-one patients with nonsyndromic cleft palate (including 103 complete trio families) were selected, and the informed consent was obtained. Patients with known teratogenic exposure

**Table 1.** Allele frequency analysis.

| RS | Allele | Case n=482 (%) | Control n=484 (%) | Chi² | *P*-value | Odds Ratio (OR) | 95% CI |
|---|---|---|---|---|---|---|---|
| rs2305262 | T | 308 (63.9) | 307 (63.4) | | | | |
| | C | 174 (36.1) | 177 (36.6) | 0.023 | 0.879 | 1.021 | 0.785-1.327 |
| rs13392032 | C | 89 (18.5) | 94 (19.4) | | | | |
| | G | 393 (81.5) | 390 (80.6) | 0.144 | 0.704 | 0.940 | 0.681-1.296 |
| rs6435017 | T | 102 (21.2) | 111 (22.9) | | | | |
| | C | 380 (78.8) | 373 (77.1) | 0.441 | 0.507 | 0.902 | 0.665-1.223 |
| rs4673313 | T | 260 (53.9) | 265 (54.8) | | | | |
| | C | 222 (46.1) | 219 (45.2) | 0.064 | 0.800 | 0.968 | 0.751-1.247 |
| rs1868427 | T | 395 (82.0) | 395 (81.6) | | | | |
| | C | 87 (18.0) | 89 (18.4) | 0.019 | 0.892 | 1.023 | 0.738-1.418 |
| rs17266097 | T | 83 (17.2) | 98 (20.2) | | | | |
| | C | 399 (82.8) | 386 (79.8) | 1.454 | 0.228 | 0.819 | 0.593-1.133 |
| rs260761 | C | 398 (82.6) | 403 (83.3) | | | | |
| | G | 84 (17.4) | 81 (16.7) | 0.082 | 0.775 | 0.952 | 0.681-1.332 |
| rs260758 | A | 54 (11.2) | 66 (13.6) | | | | |
| | G | 428 (88.8) | 418 (86.4) | 1.314 | 0.252 | 0.799 | 0.544-1.173 |
| rs1374360 | T | 149 (30.9) | 146 (30.2) | | | | |
| | C | 333 (69.1) | 338 (69.8) | 0.064 | 0.801 | 1.036 | 0.788-1.362 |
| rs7593422 | A | 292 (60.6) | 299 (61.8) | | | | |
| | T | 190 (39.4) | 185 (38.2) | 0.145 | 0.703 | 0.951 | 0.734-1.232 |
| rs1992949 | T | 72 (14.9) | 71 (14.7) | | | | |
| | C | 410 (85.1) | 413 (85.3) | 0.014 | 0.907 | 1.022 | 0.716-1.457 |
| rs4459679 | T | 289 (60.0) | 295 (61.0) | | | | |
| | G | 193 (40.0) | 189 (39.0) | 0.099 | 0.753 | 0.959 | 0.741-1.242 |
| rs16831466 | A | 114 (23.7) | 124 (25.6) | | | | |
| | C | 368 (76.3) | 360 (74.4) | 0.504 | 0.478 | 0.899 | 0.671-1.205 |
| rs2167006 | A | 221 (45.9) | 219 (45.2) | | | | |
| | T | 261 (54.1) | 265 (54.8) | 0.035 | 0.851 | 1.025 | 0.795-1.320 |
| rs2881208 | T | 285 (59.1) | 279 (57.6) | | | | |
| | C | 197 (40.9) | 205 (42.4) | 0.219 | 0.640 | 1.063 | 0.823-1.373 |
| rs1446636 | T | 86 (17.8) | 89 (18.4) | | | | |
| | G | 396 (82.2) | 395 (81.6) | 0.049 | 0.826 | 0.964 | 0.695-1.337 |

RS, reference SNP ID; 95%CI, 95% confidence interval.

and other recognized syndromes, as well as children with other major or multiple minor defects and/or developmental delay, as determined from demographic details, perinatal history, teratogenic exposure and family history, were excluded. Two hundred forty-two unaffected individuals from the same geographic region, who had other congenital diseases or no craniofacial anomaly and no family history of craniofacial malformation, were included in the study as controls (Table 1). Informed consent was obtained for each study participant for both blood collection and subsequent genotyping.

### Sample and baseline data

Peripheral blood samples (10 ml from each case) were collected by venipuncture. Total genomic DNA was extracted from blood samples, SNPs were genotyped by the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS), and genetic analysis was performed. T-test was used to detect the difference in baseline data between the groups, and there was no significant difference between the groups regarding sex and age ($P$>0.05).

### Search strategy for the relationship between SATB2 rs4673313 T/C gene polymorphisms and nonsyndromic cleft palate risk

A comprehensive literature search was first conducted in the PubMed and Cochrane Library databases, using a combination of the following items: "polymorphism OR polymorphisms OR single nucleotide polymorphism OR SNP genotype OR genotypes OR allele OR alleles" and "special AT-rich sequence binding protein 2 OR SATB2", and "cleft lip OR cleft palate". The search time was updated to June 1, 2018. Moreover, we included the additional studies extracted from references in the retrieved articles.

### Eligibility criteria

In this current meta-analysis, only the studies meeting the following criteria were recruited: (1) report published in English; (2) unrelated case-control studies; (3) tested for association of the SATB2 rs4673313 T/C gene polymorphism with NSCP risk; and (4) sufficient information to pool the odds ratios (ORs) and 95% confidence intervals (CIs). Reports that failed to meet the criteria mentioned above were excluded from the final analysis.

### Data extraction

The following information from each included study was extracted independently by two investigators. From the corresponding genotype distribution, frequencies of the C allele were calculated for the case and control groups. The results were compared, disagreement was resolved by discussion, and the results of search and extracted data for this meta-analysis were the same between two investigators.

### Statistical analysis

Strength of the associations of the SATB2 rs4673313 T/C gene polymorphism with the trisk of NSCP was assessed using ORs and 95% CIs. Significance of the pooled OR was evaluated using the Z-test, and a $P$ < 0.05 was defined as the significance threshold. Heterogeneity was evaluated using the $\chi^2$-based Q statistic, as well as the $I^2$ statistic, with a $P$ < 0.10 defined as the significance threshold. The pooled statistic was counted by the fixed effects model, but a random effects model was used when the $P$-value of the heterogeneity test was less than 0.1 (19-21). All statistical tests were performed using Revman 5.3 (Cochrane Collaboration, Oxford, UK).

## Results

### Results from case-control analysis

#### *Allele frequencies*

The 16 selected SNPs were in Hardy–Weinberg equilibrium. Allele frequency analysis showed that the distribution of alleles at all loci was not notably significant between these two groups ($P$>0.05, Table 1).

#### *Genotype frequency distribution*

The results of the genotypic frequency analysis at each site are shown in Table 2. The analysis results showed that the distribution of all genotypes in the two groups was not statistically significant ($P$>0.05).

#### *Linkage disequilibrium*

Five sites rs1374760, 260758, 4459679, 7593422, and 260761 are closely linked to form a haploid domain (Block 1), while rs1446636, rs4673313, rs2167006, rs2305262, rs1868427, 17266097, rs16831466, rs1992949, and rs6435017 constitute another haploid domain (Block 2; Figure 1).

#### *Haplotype analysis*

Haplotype analysis showed that the distribution of all haplotypes between the two groups was not statistically significant ($P$>0.05, Table 3).

### Results from meta-analysis

#### *Study characteristics*

Two studies reporting the relationship between SATB2 rs4673313 T/C gene polymorphism and NSCP risk were recruited into this meta-analysis (22). The data of interest was extracted, and the frequencies of the C allele of SATB2 rs4673313 for the case and control groups were calculated.

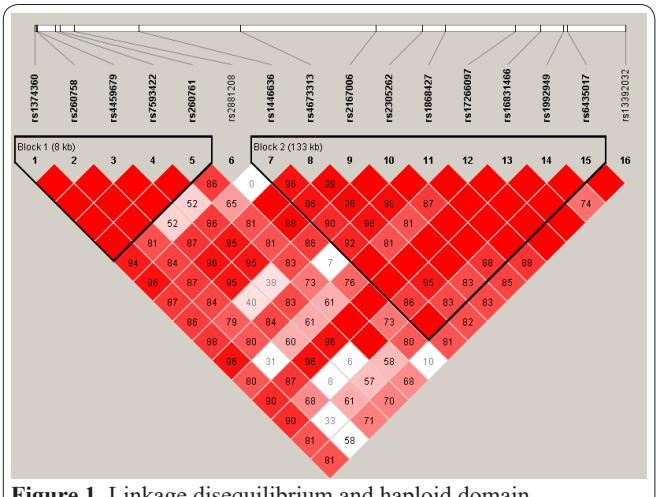


**Figure 1.** Linkage disequilibrium and haploid domain.

**Table 2.** Analysis of genotypic frequency.

| rs | Genotype | Case n=241(%) | Control n=242(%) | Chi2 | P | Odds Ratio | 95% CI |
|---|---|---|---|---|---|---|---|
| rs2305262 | TT | 97(40.2) | 100(41.3) | | | 1.000 | |
| | CT | 114(47.3) | 107(44.2) | | | 1.098 | 0.748-1.613 |
| | CC | 30(12.4) | 35(14.5) | 0.650 | 0.723 | 0.884 | 0.504-1.55 |
| rs13392032 | GG | 161(66.8) | 156(64.5) | | | 1.000 | |
| | CG | 71(29.5) | 78(32.2) | | | 0.882 | 0.597-1.302 |
| | CC | 9(3.7) | 8(3.3) | 0.465 | 0.793 | 1.090 | 0.41-2.897 |
| rs6435017 | CC | 149(61.8) | 143(59.1) | | | 1.000 | |
| | CT | 82(34.0) | 87(36.0) | | | 0.905 | 0.619-1.321 |
| | TT | 10(4.1) | 12(5.0) | 0.451 | 0.798 | 0.800 | 0.335-1.909 |
| rs4673313 | TT | 75(31.1) | 77(31.8) | | | 1.000 | |
| | CT | 110(45.6) | 111(45.9) | | | 1.017 | 0.673-1.538 |
| | CC | 56(23.2) | 54(22.3) | 0.065 | 0.968 | 1.065 | 0.652-1.739 |
| rs1868427 | TT | 163(67.6) | 161(66.5) | | | 1.000 | |
| | CT | 69(28.6) | 73(30.2) | | | 0.934 | 0.629-1.385 |
| | CC | 9(3.7) | 8(3.3) | 0.182 | 0.913 | 1.111 | 0.418-2.952 |
| rs17266097 | CC | 167(69.3) | 153(63.2) | | | 1.000 | |
| | CT | 65(27.0) | 80(33.1) | | | 0.744 | 0.502-1.104 |
| | TT | 9(3.7) | 9(3.7) | 2.162 | 0.339 | 0.916 | 0.354-2.368 |
| rs260761 | CC | 166(68.9) | 167(69.0) | | | 1.000 | |
| | CG | 66(27.4) | 69(28.5) | | | 0.962 | 0.645-1.436 |
| | GG | 9(3.7) | 6(2.5) | 0.668 | 0.716 | 1.509 | 0.525-4.334 |
| rs260758 | GG | 189(78.4) | 182(75.2) | | | 1.000 | |
| | AG | 50(20.7) | 54(22.3) | | | 0.892 | 0.577-1.378 |
| | AA | 2(0.8) | 6(2.5) | 2.284 | 0.319 | 0.321 | 0.064-1.611 |
| rs1374360 | CC | 120(49.8) | 114(47.1) | | | 1.000 | |
| | CT | 93(38.6) | 110(45.5) | | | 0.803 | 0.551-1.171 |
| | TT | 28(11.6) | 18(7.4) | 3.749 | 0.153 | 1.478 | 0.775-2.817 |
| rs7593422 | AA | 93(38.6) | 94(38.8) | | | 1.000 | |
| | AT | 106(44.0) | 111(45.9) | | | 0.965 | 0.653-1.427 |
| | TT | 42(17.4) | 37(15.3) | 0.435 | 0.805 | 1.147 | 0.678-1.943 |
| rs1992949 | CC | 172(71.4) | 177(73.1) | | | 1.000 | |
| | CT | 66(27.4) | 59(24.4) | | | 1.151 | 0.765-1.733 |
| | TT | 3(1.2) | 6(2.5) | 1.462 | 0.482 | 0.515 | 0.127-2.09 |
| rs4459679 | TT | 90(37.3) | 91(37.6) | | | 1.000 | |
| | GT | 109(45.2) | 113(46.7) | | | 0.975 | 0.659-1.444 |
| | GG | 42(17.4) | 38(15.7) | 0.276 | 0.871 | 1.118 | 0.66-1.892 |
| rs16831466 | CC | 141(58.5) | 130(53.7) | | | 1.000 | |
| | AC | 86(35.7) | 100(41.3) | | | 0.793 | 0.545-1.153 |
| | AA | 14(5.8) | 12(5.0) | 1.652 | 0.438 | 1.076 | 0.48-2.411 |
| rs2167006 | TT | 75(31.1) | 76(31.4) | | | 1.000 | |
| | AT | 111(46.1) | 113(46.7) | | | 0.995 | 0.659-1.504 |
| | AA | 55(22.8) | 53(21.9) | 0.059 | 0.971 | 1.052 | 0.642-1.723 |
| rs2881208 | TT | 92(38.2) | 82(33.9) | | | 1.000 | |
| | CT | 101(41.9) | 115(47.5) | | | 0.783 | 0.525-1.168 |
| | CC | 48(19.9) | 45(18.6) | 1.577 | 0.455 | 0.951 | 0.574-1.574 |
| rs1446636 | GG | 164(68.0) | 162(66.9) | | | 1.000 | |
| | GT | 68(28.2) | 71(29.3) | | | 0.946 | 0.636-1.407 |
| | TT | 9(3.7) | 9(3.7) | 0.075 | 0.963 | 0.988 | 0.382-2.552 |

RS, reference SNP ID; 95%CI, 95% confidence interval.

**Table 3.** Haplotype analysis.

| Block | Haplotype | Freq. | Case, Control Ratio Counts | Case, Control Frequencies | Chi Square | *P*-value |
|---|---|---|---|---|---|---|
| Block 1 | | | | | | |
| | CGGTC | 0.388 | 190.0 : 292.0, 185.0 : 299.0 | 0.394, 0.382 | 0.145 | 0.703 |
| | TGTAC | 0.303 | 149.0 : 333.0, 144.0 : 340.0 | 0.309, 0.298 | 0.154 | 0.695 |
| | CGTAG | 0.169 | 84.0 : 398.0, 79.0 : 405.0 | 0.174, 0.163 | 0.210 | 0.646 |
| | CATAC | 0.124 | 54.0 : 428.0, 66.0 : 418.0 | 0.112, 0.136 | 1.314 | 0.252 |
| Block 2 | | | | | | |
| | GCATTCACC | 0.246 | 114.0 : 368.0, 124.0 : 360.0 | 0.237, 0.256 | 0.504 | 0.478 |
| | GCATTCCCC | 0.186 | 98.4 : 383.6, 80.9 : 403.1 | 0.204, 0.167 | 2.192 | 0.139 |
| | TTTTCCCCC | 0.170 | 81.0 : 401.0, 82.9 : 401.1 | 0.168, 0.171 | 0.020 | 0.888 |
| | GTTCTTCCT | 0.170 | 75.9 : 406.1, 87.9 : 396.1 | 0.158, 0.182 | 0.999 | 0.318 |
| | GTTCTCCTC | 0.140 | 69.5 : 412.5, 66.0 : 418.0 | 0.144, 0.136 | 0.124 | 0.725 |
| | GTTCTCCCT | 0.033 | 19.0 : 463.0, 13.0 : 471.0 | 0.039, 0.027 | 1.189 | 0.276 |
| | GCATTTCCT | 0.018 | 7.1 : 474.9, 10.1 : 473.9 | 0.015, 0.021 | 0.522 | 0.470 |
| | GTTTCCCCC | 0.010 | 6.0 : 476.0, 4.1 : 479.9 | 0.013, 0.008 | 0.402 | 0.526 |

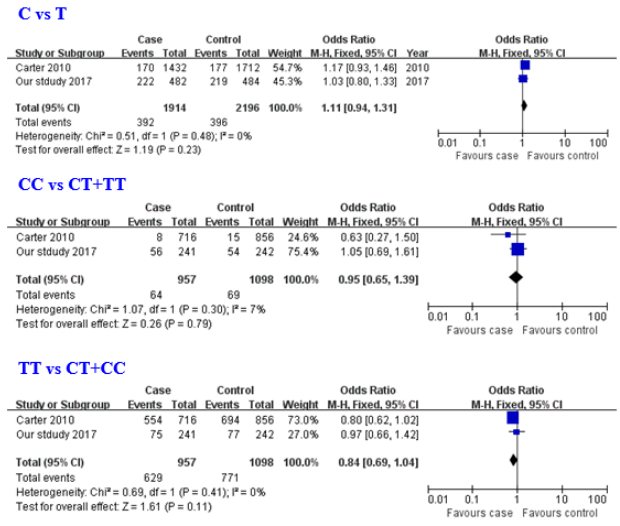


**Figure 2.** Association between SATB2 rs4673313 T/C gene polymorphism and nonsyndromic oral clefts risk.

### *The SATB2 rs4673313 T/C gene polymorphism is not associated with NSCP risk*

In this meta-analysis, we found that the SATB2 rs4673313 T/C gene polymorphism was not associated with NSCP risk (C allele: OR = 1.11, 95% CI: 0.94-1.31, *P*=0.23; CC genotype: OR = 0.95, 95% CI: 0.65-1.39, *P* = 0.79; TT genotype: OR = 0.84, 95% CI: 0.69-1.04, *P*=0.11; Figure 2 and Table 4). In the sub-group analysis according to ethnicity, this meta-analysis indicated that the SATB2 rs4673313 T/C gene polymorphism was not associated with NSCP risk in either the Asian population or Caucasians (Table 4).

## Discussion

In this study, we detected 16 selected SNPs (rs2305262, rs13392032, rs6435017, rs4673313, rs1868427, rs17266097, rs260761, rs260758, rs1374360, rs7593422, rs1992949, rs4459679, rs16831466, rs2167006, rs2881208, and rs1446636) and found all to be in Hardy-Weinberg equilibrium. The case-control study and transmission disequilibrium test provide no support for the hypothesis that the 16 selected SNPs play a significant role in CPO development in the eastern Guangdong population.

FitzPatrick *et al.* (23) showed by high-resolution FISH mapping of two de novo CPO-associated translocations involving 2q32-q33 that one breakpoint interrupts the transcription unit of the gene encoding the DNA-binding protein SATB2, and reported that mutation analysis of 70 unrelated patients with CPO did not reveal any coding region variants. Subsequently, Van Buggenhout *et al.* reported that four patients with syndrome cleft palate had an interstitial deletion of chromosome 2q32 → 2q33 in a region where the SATB2 gene was located (24). Several studies on syndrome cleft palate have demonstrated an important role for the SATB2 gene (25-28).

However, evidence for an association between SATB2 gene polymorphisms and nonsyndromic cleft

**Table 4.** Meta-analysis of the association of the SATB2 rs4673313 T/C gene polymorphism with oral cleft risk.

| Genetic contrasts | Group and subgroups | studies | Q test *P*-value | Model selected | OR (95%CI) | *P* |
|---|---|---|---|---|---|---|
| C vs. T | Overall | 2 | 0.48 | Fixed | 1.11(0.94,1.31) | 0.23 |
| | Caucasian | 1 | - | Fixed | 1.17(0.93,1.46) | 0.17 |
| | Asian | 1 | - | Fixed | 1.03(0.80,1.33) | 0.80 |
| CC vs. (CT+TT) | Overall | 2 | 0.30 | Fixed | 0.95(0.65,1.39) | 0.79 |
| | Caucasian | 1 | - | Fixed | 0.63(0.27,1.50) | 0.30 |
| | Asian | 1 | - | Fixed | 1.05(0.69,1.61) | 0.81 |
| TT vs. (CT+CC) | Overall | 2 | 0.41 | Fixed | 0.84(0.69,1.04) | 0.11 |
| | Caucasian | 1 | - | Fixed | 0.80(0.62,1.02) | 0.07 |
| | Asian | 1 | - | Fixed | 0.97(0.66,1.42) | 0.87 |

palate is lacking. Carter *et al.* (22) performed a study to detect the effects of SATB2 gene polymorphisms in 892 patients with NSCP and 902 normal controls, and reported that SATB2 gene variations do not contribute to the development of NSCP in the Irish population. Mossey *et al.* performed a study to investigate gene-environment and gene joint effects, in a large multi-center study of case-parent triads, and found that the rs1348813 polymorphism of the SATB2 gene does not contribute to NSCP in European populations (17). Gurramkonda *et al.* (29) performed a study to detect the role of rs137853127, rs200074373 and rs1992950 polymorphism of the SATB2 gene in 173 patients with NSCP and 176 normal controls, and reported that SATB2 gene variations do not contribute to the development of NSCP in the south Indian population. Similar conclusions have been reached in a study of southeast Asian populations (Malaysia, Taiwan and Singapore); Beaty *et al.* showed there is no correlation between the SATB2 gene and cleft palate (30). In this study, we first assessed the relationship between cleft palate and SNPs of SATB2 in eastern Guangdong population.

In this study, there was not enough of a sample number for meta-analysis on other 15 SNPs. Thus, we performed the meta-analysis for only SATB2 rs4673313 T/C gene polymorphism. For another word, this report first showed the association data for the relationship between other 15 SNPs and NSCP risk. However, more studies should be conducted to confirm them.

We also performed a meta-analysis, and we did not find that the SATB2 rs4673313 T/C gene polymorphism is associated with NSCP risk in the overall population, in Asians or in Caucasians. In previous analyses, no meta-analyses were used to assess this relationship. However, more studies in this topic should be conducted in the future to provide additional confirmation.

Sixteen selected SATB2 SNPs do not contribute to the development of CPO in the eastern Guangdong population. By meta-analysis, we also do not find that the SATB2 rs4673313 T/C gene polymorphism associates with NSCP risk in the overall population, in Asians or in Caucasians. Additional well-designed experiments should be performed to confirm this relationship in future studies.

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**Interest Conflict**

The authors declare no competing interests.

**Author's Contribution**:

Conceptualization: Wancong Zhang, Lungang Shi, Shijie Tang.
Performing this original study and the meta-analysis: Wancong Zhang, Linwang Tan, Yue Xing, Xing Lang, Jianda Zhou, Shijie Tang.
Data curation: Wancong Zhang, Linwang Tan, Yue Xing, Hanxing Zhao, Lungang Shi.
Formal analysis: Wancong Zhang, Linwang Tan, Yue Xing, Hanxing Zhao, Jianxiong Cai.
Funding acquisition: Shijie Tang.
Project administration: Wancong Zhang, Shijie Tang.
Writing this manuscript: Wancong Zhang, Linwang Tan.
All authors have read and approved the manuscript, and ensure that this is the case.

## References

1. Ishorst N, Francheschelli P, Bohmer AC, Khan MFJ, Heilmann-Heimbach S, Fricker N, et al. Nonsyndromic cleft palate: An association study at GWAS candidate loci in a multiethnic sample. Birth Defects Res. 2018.
2. Mangold E, Bohmer AC, Ishorst N, Hoebel AK, Gultepe P, Schuenke H, et al. Sequencing the GRHL3 Coding Region Reveals Rare Truncating Mutations and a Common Susceptibility Variant for Nonsyndromic Cleft Palate. Am J Hum Genet. 2016;98(4):755-62.
3. Bohmer AC, Golz L, Kreusch T, Kramer FJ, Potzsch B, Nothen MM, et al. Investigation of dominant and recessive inheritance models in GWAS data of nonsyndromic cleft lip with or without cleft palate. Birth Defects Res. 2017.
4. da Silva HPV, Oliveira GHM, Ururahy MAG, Bezerra JF, de Souza KSC, Bortolin RH, et al. Application of high-resolution array platform for genome-wide copy number variation analysis in patients with nonsyndromic cleft lip and palate. J Clin Lab Anal. 2018.
5. Gowans LJJ, Oseni G, Mossey PA, Adeyemo WL, Eshete MA, Busch TD, et al. Novel GREM1 Variations in Sub-Saharan African Patients With Cleft Lip and/or Cleft Palate. Cleft Palate Craniofac J. 2018:1055665618754948.
6. Howe BJ, Cooper ME, Wehby GL, Resick JM, Nidey NL, Valencia-Ramirez LC, et al. Dental Decay Phenotype in Nonsyndromic Orofacial Clefting. J Dent Res. 2017;96(10):1106-14.
7. Liu D, Schwender H, Wang M, Wang H, Wang P, Zhu H, et al. Gene-gene interaction between MSX1 and TP63 in Asian case-parent trios with nonsyndromic cleft lip with or without cleft palate. Birth Defects Res. 2018;110(4):317-24.
8. Meng L, Bian Z, Torensma R, Von den Hoff JW. Biological mechanisms in palatogenesis and cleft palate. J Dent Res. 2009;88(1):22-33.
9. Schoen C, Aschrafi A, Thonissen M, Poelmans G, Von den Hoff JW, Carels CEL. MicroRNAs in Palatogenesis and Cleft Palate. Front Physiol. 2017;8:165.
10. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet. 2011;12(3):167-78.
11. Sivertsen A, Wilcox AJ, Skjaerven R, Vindenes HA, Abyholm F, Harville E, et al. Familial risk of oral clefts by morphological type and severity: population based cohort study of first degree relatives. BMJ. 2008;336(7641):432-4.
12. Grosen D, Chevrier C, Skytthe A, Bille C, Molsted K, Sivertsen A, et al. A cohort study of recurrence patterns among more than 54,000 relatives of oral cleft cases in Denmark: support for the multifactorial threshold model of inheritance. J Med Genet. 2010;47(3):162-8.
13. Duan SJ, Huang N, Zhang BH, Shi JY, He S, Ma J, et al. New insights from GWAS for the cleft palate among han Chinese population. Med Oral Patol Oral Cir Bucal. 2017;22(2):e219-e27.
14. Sliwinska-Jewsiewicka A, Kowalczyk AE, Krazinski BE, Godlewski J, Kwiatkowski P, Kiewisz J, et al. Decreased Expression of SATB2 Associates with Tumor Growth and Predicts Worse Outcome in Patients with Clear Cell Renal Cell Carcinoma. Anticancer Res. 2018;38(2):839-46.
15. Berg KB, Schaeffer DF. SATB2 as an Immunohistochemical Marker for Colorectal Adenocarcinoma: A Concise Review of Bene-

fits and Pitfalls. Arch Pathol Lab Med. 2017;141(10):1428-33.
16. Mohamad Shah NS, Salahshourifar I, Sulong S, Wan Sulaiman WA, Halim AS. Discovery of candidate genes for nonsyndromic cleft lip palate through genome-wide linkage analysis of large extended families in the Malay population. BMC Genet. 2016;17:39.
17. Mossey PA, Little J, Steegers-Theunissen R, Molloy A, Peterlin B, Shaw WC, et al. Genetic Interactions in Nonsyndromic Orofacial Clefts in Europe-EUROCRAN Study. Cleft Palate Craniofac J. 2017;54(6):623-30.
18. Vieira AR, Avila JR, Daack-Hirsch S, Dragan E, Felix TM, Rahimov F, et al. Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. PLoS Genet. 2005;1(6):e64.
19. Rindone JP, Mellen CK. Meta-analysis of trials comparing cefazolin to anti-staphylococcal penicillins in the treatment of methicillin-sensitive Staphylococcus aureus bacteremia. Br J Clin Pharmacol. 2018.
20. Wang R, Shen J, Yang R, Wang WG, Yuan Y, Guo ZH. Association between heme oxygenase-1 gene promoter polymorphisms and cancer susceptibility: A meta-analysis. Biomed Rep. 2018;8(3):241-8.
21. You S, Saxena A, Wang X, Tan W, Han Q, Cao Y, et al. Efficacy and safety of intravenous recombinant tissue plasminogen activator in mild ischaemic stroke: a meta-analysis. Stroke Vasc Neurol. 2018;3(1):22-7.
22. Carter TC, Molloy AM, Pangilinan F, Troendle JF, Kirke PN, Conley MR, et al. Testing reported associations of genetic risk factors for oral clefts in a large Irish study population. Birth Defects Res A Clin Mol Teratol. 2010;88(2):84-93.
23. FitzPatrick DR, Carr IM, McLaren L, Leek JP, Wightman P, Williamson K, et al. Identification of SATB2 as the cleft palate gene on 2q32-q33. Hum Mol Genet. 2003;12(19):2491-501.
24. Van Buggenhout G, Van Ravenswaaij-Arts C, Mc Maas N, Thoelen R, Vogels A, Smeets D, et al. The del(2)(q32.2q33) deletion syndrome defined by clinical and molecular characterization of four patients. Eur J Med Genet. 2005;48(3):276-89.
25. Britanova O, Depew MJ, Schwark M, Thomas BL, Miletich I, Sharpe P, et al. Satb2 haploinsufficiency phenocopies 2q32-q33 deletions, whereas loss suggests a fundamental role in the coordination of jaw development. Am J Hum Genet. 2006;79(4):668-78.
26. Mc Cormack A, Taylor J, Gregersen N, George AM, Love DR. Delineation of 2q32q35 deletion phenotypes: two apparent "proximal" and "distal" syndromes. Case Rep Genet. 2013;2013:823451.
27. Docker D, Schubach M, Menzel M, Munz M, Spaich C, Biskup S, et al. Further delineation of the SATB2 phenotype. Eur J Hum Genet. 2014;22(8):1034-9.
28. Tegay DH, Chan KK, Leung L, Wang C, Burkett S, Stone G, et al. Toriello-Carey syndrome in a patient with a de novo balanced translocation [46,XY,t(2;14)(q33;q22)] interrupting SATB2. Clin Genet. 2009;75(3):259-64.
29. Gurramkonda VB, Syed AH, Murthy J, Lakkakula BV. SATB2 gene variants in non-syndromic cleft lip with or without cleft palate in Indian population. J Oral Biol Craniofac Res. 2015;5(3):161-4.
30. Beaty TH, Hetmanski JB, Fallin MD, Park JW, Sull JW, McIntosh I, et al. Analysis of candidate genes on chromosome 2 in oral cleft case-parent trios from three populations. Hum Genet. 2006;120(4):501-18.