# Assessment of Candidate Genetic Variants in Orofacial Clefts

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**Background**: Orofacial clefts remain one of the most prevalent birth defects, both in the United States and globally. Non-syndromic oral clefts present more frequently, occurring in as high as 70% of all cleft cases. Previous studies have established strong genetic links to the development of orofacial clefts, but how the numerous unique genetic alterations contribute to the expression of orofacial clefts remains understudied. In this study, we investigated how copy number variants (CNVs), as a genetic alteration, were associated with non-syndromic cleft lip and/or palate.

**Methods**: Data on CNVs in non-syndromic cleft cases from two distinct population groups, Filipino and European, were extracted from a previous study on orofacial clefts. Using an in-house Linux server, BEDTOOLS, Unix code, and several R packages, a computational pipeline was implemented to: (1) identify overlapping CNV gains and losses in patients (i.e., CNV regions shared by two or more patients), (2) assess whether patient CNV overlaps occur by chance, by comparing the Z-scores of patient CNV overlaps with the Z-scores of a random sample of (1,000) CNV overlaps, (3) prioritize genes encompassed by patient overlapping CNVs, (4) assess the likelihood of patient overlapping CNVs to occur in regions of the genome with high variability, and, (5) perform gene ontology analysis to determine biological pathways enriched in genes prioritized in patient overlapping CNVs.

**Results**: Filipino cleft palate only (CPO) patients shared more overlaps with CNV gains (2448) than CNV losses (474). Comparing Z-scores and p-values for both groups observed, along with the randomly selected 1,000 CNV overlaps, showed that both losses and gains that appeared in patient CNVs did not occur by chance (Z-score = 595.2892, p = 0.0068 for CNV gains; Z-score = 361.7982, p =1.820e-08 for CNV losses). Specifically, there were 183 and 61 genes impacted by CNV gains and losses respectively, for Filipino CPO patients. Of these, 48 have been reported in previous studies (18 were homologs associated with cleft phenotypes in mice).

**Conclusions**: Z-score comparisons showed that patient CNV overlaps differ significantly, suggesting that at least some of the genomic regions of the overlaps contain genes relevant to orofacial clefts. In addition, the approach used to prioritize genes identified some cleft-associated genes that were known previously, further validating the pipeline. The prioritized genes with no known association with lip or palate observed in prior scientific literature are novel candidates to explore and confirm in future studies designed to isolate genetic contributions to the development of oral clefts.