# Analyzing de novo mutations in case-parent trios with cleft lip only

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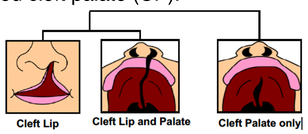
## Introduction

- Orofacial clefts (OFCs) are the most common craniofacial malformation with an overall incidence of 1/1000 live births.
- Nonsyndromic OFCs are phenotypically and etiologically heterogenous.
- OFCs are generally classified into isolated cleft lip (CL), cleft lip with cleft palate (CLP), and isolated cleft palate (CP).



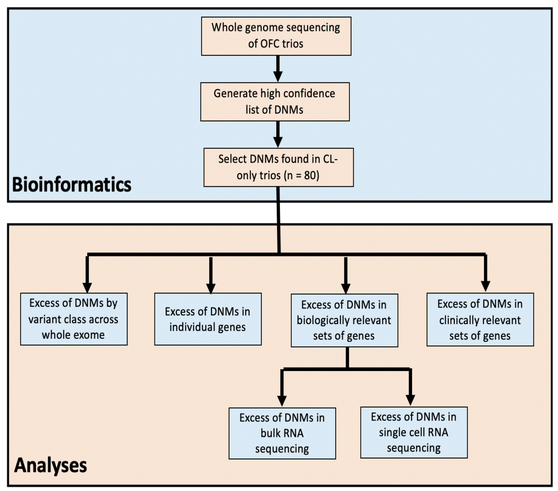

- CL occurs at a different time point in development, so it may have a different etiology from the other subtypes. Little research has been conducted on genetic risk factors for this specific subtype.
- *De novo* mutations (DNMs) spontaneously arise during embryonic development and have not been thoroughly studied as genetic risk factors for CL-only cases

## Methods

**Gabriella Miller Kids First Pediatric Research Program**: The Kids First program is a large-scale resource to help uncover the biology of childhood cancer and structural birth defects.

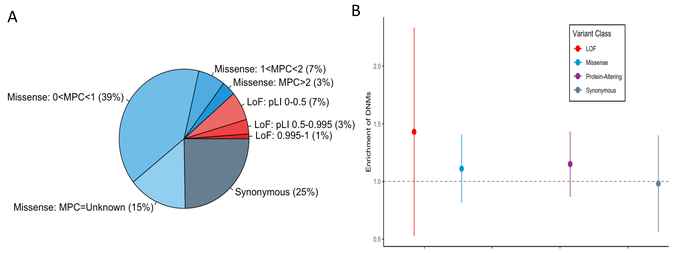
**OFC cohort**: 376 European trios with cleft lip with or without cleft palate (CL/P) were selected from a larger nonsyndromic OFC cohort

**Sequencing**: Trios were whole-genome sequenced at the Broad Institute on the Illumina HiSeq X Ten platform. Variants were called using a pipeline following the GATK Best Practice guidelines.



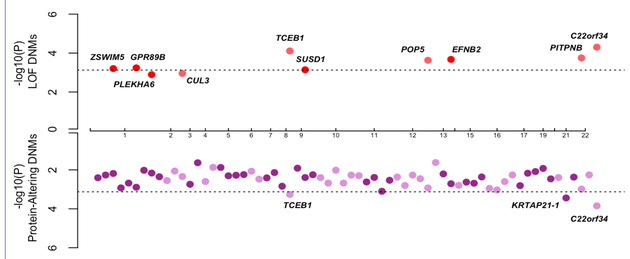

## Results

### Figure 1. DNMS by variant class



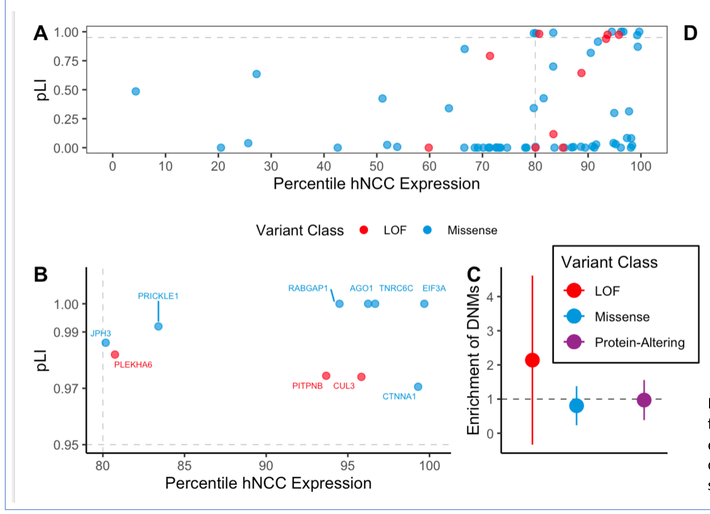

This shows the overall distribution of rare, coding DNMs (n = 89) by variant class in the CL-only trios (n = 80). A significant enrichment of loss of function (LoF; nonsense, frameshift, splice) and missense DNMs was not observed, likely due to the small cohort size.

### Figure 2. Genes with an excess of DNMs



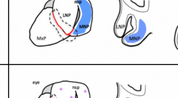
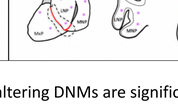

We identified a total of 8 genes with a significant excess of LoF DNMs and 3 genes with a significant excess of protein-altering (LoF + missense) DNMs using an exome-wide significance threshold ($p < 7.5X10^{-4}$ ). Genes *TCEB1* and *C22orf34* had significant excess in both variant classes.

### Figure 3. DNMs identified in genes highly expressed in human neural crest cells



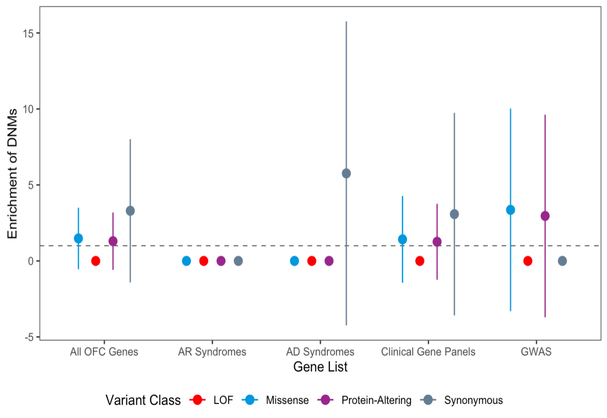

Plot A shows a ranking by pLI score and expression level of gene in RNA-sequencing data from human neural crest cells. Plot B shows genes in the top 20$^{th}$ percentile of hNCC expression with a pLI score ≥ 0.95. Plot C shows the enrichment values for DNMs by variant class in genes in the top 20$^{th}$ percentile of hNCC expression with a pLI score ≥ 0.95. Plot D shows a protein-interaction network for genes in our category of interest.

### Figure 4. Single cell RNA sequencing analysis

| Subcluster | Location | Observed | Expected | Enrichment | pValue |
|---|---|---|---|---|---|
| E0E11 |  | 1 | 0.7 | 1.5 | 0.49 |
| E9 |  | 1 | 0.8 | 1.32 | 0.53 |
| M3 |  | 2 | 0.3 | 6.62 | 0.04 |
| M6 |  | 1 | 0.3 | 2.9 | 0.29 |
| M7 |  | 1 | 0.3 | 3.78 | 0.23 |

Protein-altering DNMs are significantly enriched in marker genes for mesenchymal subcluster 3. Depiction of spatial locations of subclusters in the frontal view, anterior section, and posterior section; adapted from Li, *et al.*

### Figure 5. Analysis of DNMs in sets of clinically relevant genes




Enrichment of DNMs ± two standard errors for all CL-only trios in clinically relevant gene sets. We did not observe any LoF DNMs in any gene set.

## Conclusions and Future Directions

- Overall, we found evidence for five genes of interest that may contribute to the etiology of cleft lip only.

| Genes of interest | | *AGO1* | *EFNB2* | *CUL3* | *PITPNB* | *PLEKHA6* |
|---|---|---|---|---|---|---|
| Identified evidence supporting role in CL only etiology | Missense DNM identified | X | | | | |
| | LoF DNM identified | | X | X | X | X |
| | In the top 20th percentile for expression with a pLI score ≥0.95 | X | | X | X | X |
| | In the list of marker genes for the M3 subcluster | | X | | | |
| | Achieved individually significant p-values regarding LoF DNMs | | X | | X | |
| | Evidence in the literature | Family microRNA with transcriptional gene silencing | Mouse studies found association with neural crest defects. Damanging missense DNM for criofrontonasal syndrome. | Associated with autism, schizophrenia, and hypertension. Essential regulator of nerual crest specification. | Deletion mutation believed to be pathogenic for CL/P | Novel fusion gene in Langerhans cell histiocytosis |

- Future experiments should compare high significant CL genes to genes for CL/P to determine if the the etiologies are different

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