Association of Mitochondrial Ribosomal Protein L53 (MRPL53) with Nonsyndromic Orofacial Clefts in Multiple Populations

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Introduction
- Orofacial clefts (OFCs) are the most common craniofacial birth defects with a global incidence of 1 per 700 live births (Butali et al., 2014).
- Complex etiology of OFCs include many genetic and environmental factors, such as maternal smoking, alcohol, and folic acids (Adeyemo & Butali, 2017).
- About 70% are nonsyndromic OFCs, meaning they are not accompanied by any other craniofacial anomalies. (Gowans et al., 2016).

The MRPL53 gene is highly expressed in embryonic structures such as tongue and skeletal muscle. Also, dysregulation of the gene could disrupt craniofacial development, leading to OFCs (Masotti et al., 2018).

The objective was to explore the MRPL53 gene to determine if it plays a role in orofacial clefting.

Methods
- Collection of Samples ➔ Primer Design ➔ DNA Amplification
- Sequencing Analysis ➔ Sanger Sequencing ➔ Gel Electrophoresis

Identifying Variants ➔ Re-sequencing ➔ Segregation Analysis

Results
Two rare, known missense variants were discovered in the third exon of MRPL53 from the Puerto Rican CLP population.

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- Figure 1. Cleft palate only (CPO) (left); cleft lip and palate (CLP) (right) Image credit: Apollo Hospital
- Figure 2a. Chromatograms showing homogenous wild type CC (top), heterozygous first variant CT (left), and heterozygous second variant CT (right).
- Figure 2b. Amino acid change p.Arg73Cys.
- Figure 2c. Pedigree chart showing the mutation is inherited from the father.
- Figure 2d. Pedigree chart showing the mutation is inherited from the mother.

Segregation analysis showed that in the first variant the father was heterozygous, while in the second variant the mother was heterozygous.

Conclusions
- Found two rare, known missense mutations in a total of three individuals. Through a chi-squared test for homogeneity, it was found that the variation was significantly enriched in the observed sample compared to the American population, indicating the rarity of the mutation.
- According to SIFT, both missense mutations were predicted as deleterious, while PolyPhen showed the first mutation as probably damaging and the second as possibly damaging.
- This study creates an association between MRPL53 and nonsyndromic orofacial clefts in humans and demonstrates the complex etiology of OFCs.

Future Implications
- Three assays need to be conducted to confirm that the mutations are relevant to OFCs and result in a negative phenotype.
  1. Zebrafish assay: In this assay, zebrafish embryos will be injected with mutant DNA to determine the phenotype of the mutation.
  2. Migration assay: Mutated cells will be “scratched” and the rate at which they heal the scratch will be recorded every six hours.
  3. Real-time PCR: This experiment reveals the different expression levels of the mutation in comparison to the wild type.

Figure 3a. Chromatograms showing homogenous wild type TT (left) and heterozygous variant CT (right).
- Figure 3b. Amino acid change p.Ser92Pro.
- Figure 3c. Proline disrupts α-helix which can cause severe effects on structure of the protein.
- Figure 3d. Pedigree chart showing the mutation is inherited from the mother.

Since proline is larger than serine, there may be bumps in protein folding. Also, the new amino acid is more hydrophilic thus potentially leading to changes in hydrogen bonds and other interactions.

Figure 4. Zebrafish embryo. Image credit: Butali Laboratory

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References

The Cleft Palate Hospital Image credit: Butali Laboratory

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Figure 4. Zebrafish embryo. Image credit: Butali Laboratory

Ultimately, the research can be used to fully understand the etiology of OFCs. After obtaining this information, scientists can:
- Predict the chance of inheriting OFCs and thus provide counseling for families with the disease.
- Provide gene therapy through CRISPR.