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# Screening of the locus 8q24 in patients with cleft lip and palate

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## Introduction

Cleft lip and/or palate (CLP) is a common birth defect of complex etiology. Clefts are the major birth defect disruptors of facial structures and affect 1 in 700 births and require surgical, nutritional, dental, speech, and behavioral interventions. About 70% of individuals are born with an isolated cleft and no other structural or cognitive abnormalities.

Family and population studies have confirmed a genetic component underlying nonsyndromic CLP. However, the precise genes involved in human CLP remain unknown. The first genome wide association study (GWAS) published on CLP reported a highly significant association with markers in a gene desert located at the chromosome region 8q24.21. The study characterized a region of 640Kb on 8q24.21 as containing one or more common variants associated with CL/P in European populations. This novel locus for CLP was confirmed by two different replication studies with European populations. We report the ongoing analysis of the 8q24.21 region using different approaches such as GWAS, direct sequencing in samples of patients with CLP derived from European and Asian populations as well as studies with animal models.

## GWAS

Genome wide association studies (GWAS) have become widely used for their unbiased approach to identifying candidate genes or loci for complex traits such as cleft lip and palate. Several GWAS have been recently completed for orofacial clefting. Three studies have identified loci in European populations including 8q24, VAX1, and NOG. Using a family based design, Beaty et. al. (submitted) replicated the association with 8q24 and identified novel associations with markers near genes *MAFB* and *ABCA4*.

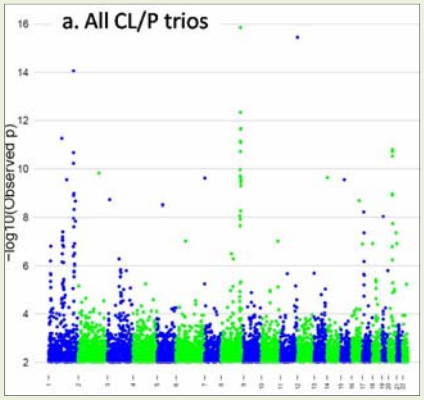



**Figure 1.** Manhattan plot of significant p-values ($p<10^{-16}$) in the 8q24.21 region.

## Sequencing

We sequenced highly conserved region in the locus 8q24 and potential regulatory elements. We identified a number of new and also known variants and compared the frequency between cases and controls for both populations.

The SNP rs6990893 showed a significant difference between cases and controls (p-value = 0.001) for the European population.

rs6990893

| CASES | # | (%) |
|---|---|---|
| GG | 136 | 0.25 |
| AG | 245 | 0.44 |
| AA | 173 | 0.31 |
| total | 554 | |

rs6990893

| CONTROLS | # | (%) |
|---|---|---|
| GG | 68 | 0.33 |
| AG | 101 | 0.49 |
| AA | 36 | 0.18 |
| total | 205 | |

**p=0.001**

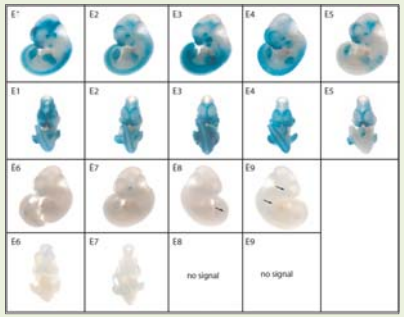
We identified new variants in the region of interest, most of them found only in cases. Although the frequency of the new mutations were not significantly different between cases and controls when analyzed by themselves, the overall number of mutations identified in this region showed borderline significance (p=0.04) .

**New variants in 8q24**

| Position | Conservation | Cases (494) | Controls (205) | p-value |
|---|---|---|---|---|
| 130057312 (T>C) | 8/9 | 1 | 0 | 0.52 |
| 130057369 (C>T) | 9/9 | 4 | 0 | 0.2 |
| 130057460 (C>T) | 7/9 | 9 | 2 | 0.42 |
| 130057487 (C>T) | 6/9 | 1 | 0 | 0.42 |
| 130057522 (G>A) | 5/9 | 1 | 0 | 0.42 |
| 130057563 (G>A) | 9/9 | 1 | 0 | 0.42 |
| 130057582 (G>A) | 8/8 | 1 | 0 | 0.42 |
| 130057659 (A>G) | 9/9 | 1 | 0 | 0.42 |
| 130057678 (G>A) | 9/9 | 1 | 0 | 0.42 |
| TOTAL | | 20 | 2 | **0.04** |

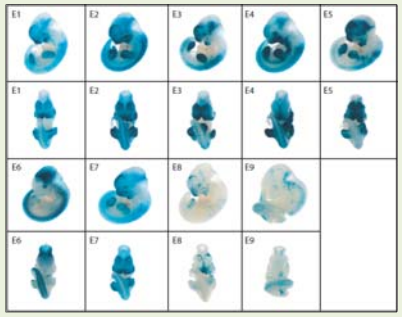
## Animal model

We have subsequently designed and tested the wild type major allele G for rs6990893, for enhancer activity at E11.5 in mouse embryos. 7 out of 9 transgenic were positive for a complex but reproducible pattern, which included craniofacial staining.




Wild type major allele G

To test the effect of the rare variant (minor allele, A), we have cloned that DNA from a homozygous patient and tested its enhancer activity in vivo. 9 out of 9 transgenics are positive for the same complex pattern than the WT version of the construct however, the transcriptional activation of the reporter gene LacZ is stronger. Further experiments should be performed to confirm this observation



Minor allele A

## Conclusions

The association of gene desert regions with human disease highlights the importance in examining genomic regions outside of transcribed DNA that may contain regulatory elements; the presence of mutations in such regions can lead to disregulation of target genes involved in human disease such as CLP. The identification of these genetic variants will help to understand the role of this new candidate region in cleft etiology and lead to the identification of new regulatory regions that play a role in craniofacial development. We identified potential craniofacial enhancers elements in the 8q24 region and further experiments are being conducted to confirm whether variants in this region can disturb regulation of genes that play a role during craniofacial development.