ABSTRACT

This thesis describes the association of the transcription factor gene polymorphisms with non-syndromic cleft lip with or without cleft palate (NSCLP). The thesis is based on a case-control study on south Indian NSCLP. The subjects included 173 (77 women and 99 men) non-syndromic cleft lip with or without cleft palate (NSCLP) subjects as cases and 176 ((77 women and 96 men) age and sex matched healthy subjects without a family history of cleft were recruited as controls. Out of 173 NSCLP cases, 144 individuals have cleft lip and palate (CLP) and 29 individuals have cleft palate only (CPO). Overall 20 important single nucleotide polymorphisms in seven (IRF6, TFAP2A, MAFB, ABCA4, SATB2, TBX22 and MSXI) candidate genes were selected. The genotyping was done by both PCR-RFLP method and KASPar SNP genotype method. Hardy-Weinberg equilibrium (HWE) was assessed for all polymorphisms in both the case and control groups by using the chi-square test. Allele frequencies were estimated by the gene counting method. Comparison of genotypes and allele frequencies among the study and the control group was analyzed by the chi-square test. The Odds ratio and 95% confidence intervals were calculated using wild type genotypes or alleles as reference group. Pair wise linkage disequilibrium (LD) was computed as both of D' and r² for all SNPs by using the Haploview. Gene-gene interactions among variants were assessed by using Multifactor Dimensionality Reduction software.

Genotyping of 20 SNPs from seven genes revealed that the Hardy-Weinberg expectations were fulfilled in controls for all SNPs except TBX22 rs7055763 SNP in females. IRF6 rs223571 and rs223575 SNPs showed
significant differences in genotype or allele frequencies between controls and cases with NSCLP and are associated with increased risk of NSCLP and \textit{IRF6} rs642961 was not associated with NSCLP. \textit{TFAP2A} (rs3798691, rs1675414 and rs303050) polymorphisms were not shown significantly associated with NSCLP. The distribution of genotypic frequencies for \textit{MAFB} rs13041247 and rs11696257 SNPs were similar and acted as surrogates in both cases and controls. Both SNPs were significantly associated with a reduced risk of NSCLP. The \textit{ABCA4} variants are not found to be associated with NSCLP at either the genotype or allele levels. Significantly higher frequency of \textit{MSX1} gene rs11726039 C variant allele and the TC/CC genotypes were found in controls rather than in the NSCLP group. The minor alleles and genotype frequencies of other \textit{MSX1} gene SNPs (rs868257, rs6446693, rs1907998 and rs6832405) were similar between the control and NSCLP groups.

As the \textit{TBX22} gene SNPs were located on chromosome X, the association analysis was performed separately in men and women. Significant association of rs7055763 and rs41307258 with NSCLP was found only in women. Both polymorphisms increased the risk for NSCLP in heterozygous and homozygous variant condition, but it is not significant. Both the SNPs were not associated with risk for NSCLP in men. The mutations in \textit{SATB2} exon 6 (rs137853127 and rs200074373) were monomorphic and the intronic variant (rs1992950) was polymorphic and the genotype distribution was not statistically significant between NSCLP and controls.
No linkage disequilibrium (LD) was found between variants of IRF6, TFAP2A, ABCA4, MSX1 and SATB2 genes. The MAFB variants are in strong LD. Pair wise linkage disequilibrium between TBX22 gene rs7055763 and rs41307258 was strong and significant. None of the haplotypes examined were significantly associated with NSCLP at p<0.05.

Genotype-genotype interaction analysis had also shown significant synergetic interaction between SATB2 rs1992950 and IRF6 rs2235375 SNPs. A midway point between synergy and redundancy interaction was observed between TFAP2A rs1675414 and SATB2 rs1992950 SNPs and TFAP2A rs1675414 and IRF6 rs2235375 SNPs. Further, the results were discussed in the light of the current hypothesis regarding the association of transcription factors and oral clefts.