Original Article

Gene Polymorphisms of FGF2 (Rs 1476214) and FGF18 (Rs 4043716) in Non-syndromic Cleft Lip and Palate in Local Population

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ABSTRACT

Background: Cleft Lip and palate (CLP) are instantly recognizable disruptions of normal facial structure.. Genetic and Environmental factors have a role in aetiology of orofacial clefts. FGFs (Fibroblast Growth Factors) and their receptors (FGFRs) plays an important role in development of craniofacial structures. So this study is done to evaluate the single nucleotide polymorphisms of FGF2 and FGF18 with Non-syndromic cleft lip and palate in local population.

Methodology: 25 subjects with nonsyndromic cleft lip and palate and 25 unrelated controls, collected from the department and the extracted DNA samples were subjected to Polymerase chain reaction and later they were subjected to DNA sequencing. Results were documented in the form of electropherograms.

Results: The results show strong association between the presence of FGF2 gene variant rs1476214 and FGF18 gene variant rs4043716 with the Nonsyndromic cleft lip and palate. This study also suggests that chances of Non syndromic cleft lip and palate is highest in subjects having GG ($p < 0.001$) genotype for FGF2 gene variant rs1476214 and AG ($p < 0.001$) genotype for FGF18 gene variant rs4043716.

Conclusion: This study concludes that that FGF2 gene variant rs1476214 and FGF18 gene variant rs4043716 can be considered as one of the genetic marker of Non syndromic cleft lip and palate for our population.

Key words: Non-syndromic Cleft Lip and Palate, FGF2 gene variant rs1476214 and FGF18 gene variant rs4043716.

INTRODUCTION

Isolated, non-syndromic cleft lip and palate represents one amongst the most common human birth defects. It is a polygenic, multifactorial disorder with both genetic and environmental factors contributing to the etiology of this condition.[1-3]

Non syndromic or isolated cleft lip and palate occur in a wide geographical distribution, with an average Asian populations having a higher birth prevalence of clefting whites are intermediate, and African populations have the least.In India,the highest rates are reported in the states of Andhra Pradesh, Karnataka and Tamil Nadu, with Kerala being an exception because of the strict avoidance of consanguineous marriage amongst the large Christian population.[27]

Cleft lip or palate is caused by genetic variations in more than one gene because several processes are involved in lip and palate formation including cell proliferation, differentiation, adhesion and apoptosis.The clinical manifestations of these defects are diverse. This includes isolated clefts of the lip to complete bi-lateral clefts of the lip, alveolus and palate.[28]

Recent success in genome-wide linkage and association studies has identified gene loci significantly associated with CLCP. Researchers are presently striving to identify the etiologic variants at these novel loci to understand the developmental disturbances leading to Cleft lip and palate.[30]

It is therefore necessary to study about the genetic variations controlling various craniofacial deformities. In this study, the focus of interest is to find out the relationship of FGF2 (rs1476214) and FGF18(rs 4043716) gene variants with Non Syndromic Cleft Lip and Palate in local population. This will help us to target at the molecular level for correction of such problem.

MATERIAL AND METHODS

2 ml venous blood samples from 25 subjects withnon syndromic cleft lip and palate and 25 unrelated controls who visited Department, were taken after the written informed consent.

Group A: 25 subjects with Non syndromic cleft lip/palate (P1-P25).

Group B: 25 unrelated controls (C1-C25).

Inclusion Criteria

- Non syndromic cleft lip/palate on clinical examination.

Exclusion Criteria

- Cleft lip/palate associated with -developmental disabilities, including learning disabilities and attention deficits, speech defects and hearing impairmentmay be the first indication of an underlying syndromic genetic disorder;
- Family history of clefts.
- Medication (e.g., anticonvulsants/retinoic acid derivatives).
- Alcohol use and Smoking during Pregnancy.

The polymorphism in FGFR 2 (rs1476214) and FGF 18 (rs4043716) gene variants were detected using the Polymerase Chain Reaction (PCR) and DNA Sequencing.

Automated DNA sequencing procedure was used for the sequencing of DNA where each nucleotide was labelled with fluorescent dyes. DNA sequence were detected more precisely and accurately on a electropherogram **(Figure 3)** unlike other sequencing techniques.

The methodology consisted of five steps:
Step 1: Collection and storage of samples
Step 2: Extraction of Genomic DNA,
Step 3: Column purification of Genomic DNA,
Step 4: Polymerase Chain Reaction Test (PCR) **(Figure 1)**
Step 5: DNA sequencing **(Figure 2)**

Figure 1 PCR machine

Figure 2 ABI DNA sequencer

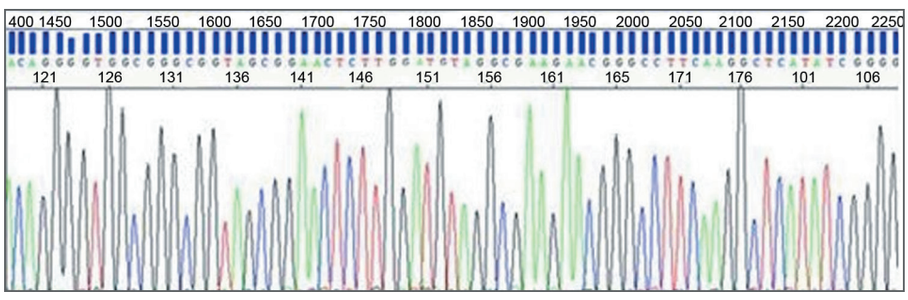


Figure 3 Image of electropherogram

Statistical Analysis

Z test has been used to findout the significance of association of *FGF2, FGF18* gene polymorphism with non-syndromic cleft lip and palate. SPSS 11.0 and Systat 8.0 software were used for the analysis of the data .

RESULTS

In this study, the association between FGF2 (rs1476214) and FGF18 (rs4043716) genes with CLCP was evaluated in 50 subjects, group A (P1-P25) as cases and group B (C1-C25) as controls using polymerase chain reaction(PCR) test and DNA sequencing.

Results for FGF2 RS 1476214 Variants

For FGF2 (rs1476214) three genotype can be possible:

A/A	Normal Homozygous Allele
G/G	Mutant Homozygous Allele
A/G	Mutant Heterozygous Allele

In group A,

2 out of 25 cases showed AA genotype.
5 out of 25 cases showed AG genotype.
18 out of 25 cases showed GG genotype.

In group B,

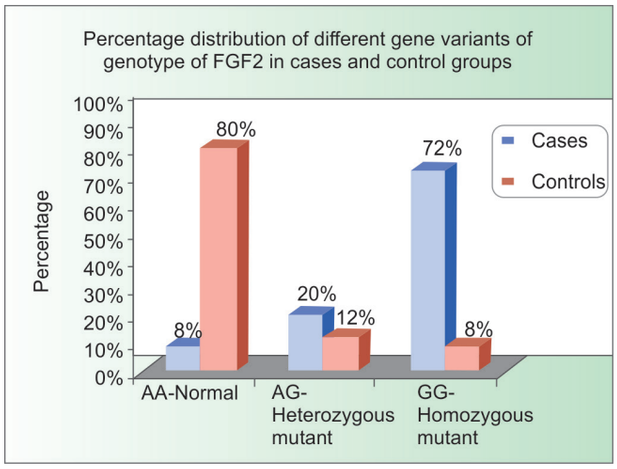
20 out of 25 controls showed AA genotype.
3 out of 25 controls showed AG genotype.
2 out of 25 controls showed GG genotype **(Graph 1)**.

Results for FGF 18 RS Variants

For FGF 18 (rs4043716) three genotype can be possible

In group A ,

1 out of 25 cases showed AA genotype.
19 out of 25 cases showed AG genotype.
5 out of 25 cases GG genotype.



**Highly statistically significant

Graph 1 Bar diagram showing the percentage distribution of different genotypes of FGF2 in case and control groups

Table 1

The table denotes the statistical significance of the genotype when cases and controls are compared using *Z*-test

- AA genotype was highly statistically significant with the controls (GROUP B) ($p < 0.001$)
- AG genotype was statistically insignificant with the cases (GROUP A) ($p = 0.44$)

In group B ,

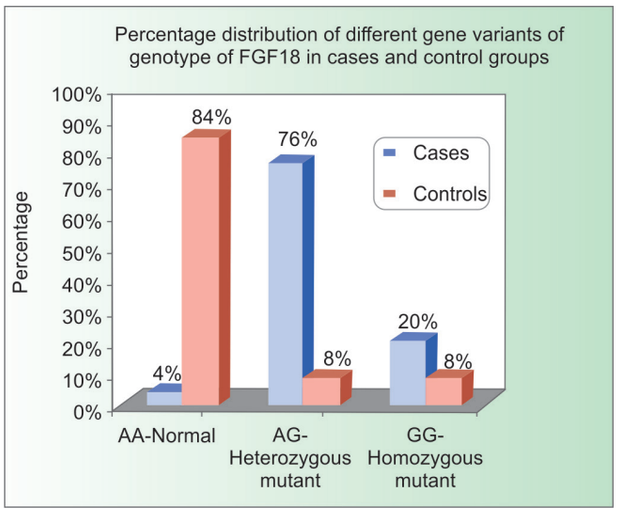
21 out of 25 controls showed the presence of AA genotype.
2 out of 25 controls showed AG genotype.
2 out of 25 controls showed GG genotype **(Graph 2)**.

DISCUSSION

The etiology of CLCP occupy both major and minor genetic influences with erratic connections from environmental factors. Its complexity is exaggerated by the large number of candidate genes and loci that seems to be involved. Collectively CLCP

Genotype of FGF2 gene variant	*Cases*		*Controls*		*Diffrence in proportions*	*Z*	*P value*
	N	*%*	*N*	*%*			
AA	2	8%	20	80	-0.72	-5.128	<0.001**
AG	5	20%	3	12	0.08	0.771	0.44
GG	18	72%	2	8	0.64	4.619	<0.001**

AA	Normal Homozygous Allele
GG	Mutant Homozygous Allele
A/G	Mutant Heterozygous Allele



Graph 2 Bar diagram showing the percentage distribution of different genotypes of FGF 18 in case and control groups

Table 2

The Table denotes the statistical significance of the genotype when cases and controls are compared using *Z*-test

- AA genotype was found to be highly statistically significant with the controls (GROUP B) ($p < 0.001$)
- AG genotype was highly statistically significant with the cases (GROUP A) ($p < 0.001$)
- GG genotype was neither significant with cases (GROUP A) nor controls (GROUP B) (p = 0.22)(as shown in **Table 2**)

has a major clinical impact requiring surgical, orthodontic, speech, hearing and psychological treatments or therapies.

Polymorphism is a mechanism by which individuals may exhibit variations within the range of what is considered biologically normal.. Most polymorphisms are single nucleotide exchanges that occur at a high frequency in the human genome and may affect the function of genes. Thus genes involved in craniofacial development are plausible candidates for oral clefts.

Fibroblast growth factors (FGF) signalling involves almost all structures of the craniofacial morphology from the development and outgrowth of the facial primordia and is now implicated in the genetic basis of non-syndromic CLCP.[23] Fibroblast growth factors (FGFs) and their receptors (FGFRs) comprise a large, complex system of growth factor signalling. The human FGF family comprises of 22 members.The FGF signalling is known to have an important role in neural crest induction, skeletogenesis and epithelial–mesenchymal interactions. FGF signalling in the mouse is closely integrated with other pathways such as Bmp, Shh, Tgfb, Sox, Msx, Dlx, Egf and Wnt which are also known to be required for normal craniofacial development.[23]

Several studies shows the role of FGF gene family in the etiology of cleft lip/palate. A study done by Leibbrandt et al in 2007 concluded that FGF8 is essential for survival and normal development of the neural crest derived facial mesenchyme and suggest that other FGF receptors in addition to FGFR1 are involved in the reception of the FGF8 signal.[20]

In present study, the FGF2 gene variants rs1476214 and FGF18 gene variants rs4043716 were checked in a sample of 50 subjects comprising of 25 cases (P1-P25) with non-syndromic cleft lip and palate and 25 unrelated controls (C1-C25) **(Table 1)** and **(Table 4)**.

DNA sequencing of human and other genome has been the center of interest in the biomedical field over the past several decades and is now leading toward the era of personalized medicine. DNA sequencing allows the use of four dideoxynucleotide chain terminator, tagged with dyes of different fluorescent emission wavelengths in a single sequencing reaction which is depicted by a graph called as Electropherogram and Chromatogram.

According to the interpretation of the electropherogram and statistical analysis, in our population, FGF2 gene variant rs 1476214, showed highly statistically significant differences in genotypes between cases and controls, with GG ($p < 0.001$) genotypes found more in cases, with AA genotype ($p < 0.001$) found more in controls. FGF18 gene variant rs4043716 showed highly statistically significant differences with AG ($p < 0.001$) genotypes found more in cases, with AA ($p < 0.001$) genotype found more in controls.

Our study showed a highly significant difference in both the FGF2 gene variants rs1476214 and FGF18 gene variants rs4043716. This is in accordance with a study done by Riley et al in Philippines population, which concluded the associations between NS CLP and SNPs in FGF3, FGF7, FGF10, FGF18, and FGFR1. This data suggests that FGF signalling pathway may contribute upto 3–5% of NS CLCP.[19]

The results of this study indicate that FGF2 gene variants rs1476214 and FGF18 gene variants rs4043716 polymorphisms may be a genetic marker for cleft lip and palate in our population. A larger sample size are required for a better understanding of complex genetics of Non syndromic cleft lip and palate.

CONCLUSION

The conclusions from this study are:

1. This study indicates that there is a strong association between FGF2 gene variant rs1476214 and FGF18 gene variant rs4043716 and incidence of Non-syndromic cleft lip and palate.
2. FGF2 gene variant rs1476214 and FGF18 gene variant rs4043716 can be considered as genetic markers for Non syndromic CLCP for our population.

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