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**Research Article**

# ASSOCIATION OF GABRB3 (RS2059574) AND GAD67 (RS2177433) GENE POLYMORPHISMS WITH NONSYNDROMIC CLEFT LIP AND PALATE IN SOUTH INDIAN POPULATION USING DNA SEQUENCING

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**A B S T R A C T**

***Background:*** Non-syndromic cleft lip with or without cleft palate (CL/CP) is a common congenital facial malformation without any other structural or developmental abnormalities.
***Objectives:*** To evaluate the association of GABRB3 gene variant rs2059574 and GAD67 gene variant rs2177433 gene polymorphisms with non syndromic cleft lip and palate in South Indian population.
***Methods:*** DNA samples of 25 subjects with non syndromic cleft lip and palate and 25 unrelated controls, collected from the department were used for the study. **Group A: -** DNA samples of 25 subjects with Nonsyndromic cleft lip and palate (P1-P25)**Group B: -** DNA samples of 25 unrelated controls (C1- C25).The extracted DNA samples were subjected to Polymerase chain reaction and later these amplified products were subjected to DNA sequencing. Results were documented in the form of electropherograms.
***Results:*** The results indicated that there is a strong association between the presence of GABRB3gene variant rs2059574 with the incidence of Nonsyndromic cleft lip and palate. This study suggests that the likelihood of Non syndromic cleft lip and palate is higher in subjects having TT ($p<0.001$) genotype for GABRB3 rs2059574.
***Conclusion:*** We can conclude that GABRB3gene variant rs2059574 can be considered as genetic markers for Non syndromic cleft lip and palate for our population.



## INTRODUCTION

Craniofacial abnormalities are among the most common of all birth defects, and the most frequent of these are oral clefts (OC) [1]. Oral clefts can occur in isolation (nonsyndromic) or as a feature of certain syndromes (syndromic). On the basis of anatomical, embryological, and genetic observations, nonsyndromic OC (NSOC) typically are classified into two subgroups: cleft lip with or without cleft palate (CL ±P; MIM 119530) and isolated cleft palate (CP; MIM 119540) [2]. Its prevalence varies according to the ethnicity (Africans: 0.3:1000; Europeans:1.3:1000; Asians: 2.1:1000; Native Americans: 3.6:1000) and socioeconomic level [3].

Recent success in genome-wide linkage and association studies has identified novel loci significantly associated with Cleft lip and palateand this knowledge should eventually result in improved prevention, treatment, and prognosis for individuals with this condition[4].

Gamma aminobutryic acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain, where it acts at GABA-A receptors, which are ligand-gated chloride channels composed of five homologous subunits. The inhibitory neurotransmitter, aminobutyric acid plays a role in normal embryonic development, particularly facial development and aminobutyric acid receptor type A β-3 subunit (GABRB3) knockout mice have been shown to have a cleft palate[5]. Glutamic acid decarboxylase (GAD) is a key enzyme that synthesizes GABA from glutamic acid, and the two GAD isozymes are derived from two distinct genes, GAD65 and GAD 67[6]. Microarray and immunohistologic analysis have shown that levels of GABA, glutamic acid decarboxylase(GAD), and GABA receptor type A β-3 subunit (GABRB3) in developing palatal tissues change dramatically during palatogenesis [7].

A new approach for sequencing the DNA is the Automated DNA sequencing procedure where each nucleotide is labeled with fluorescent dyes. Thus, when the DNA fragments are placed on the electrophoresis gel and passed through a laser beam, the DNA sequence is detected more precisely and accurately on an electropherogram using labeled ddNTPs (Dideoxyribo Nucleotide Tri Phosphate) unlike other sequencing techniques[8].
As orthodontists are intimately involved in the successful therapeutic management of the patients affected with cleft lip

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with or without cleft palate and its associated tooth anomalies, it becomes essential that they keep contemporary knowledge on the etiology of these conditions.

It is, therefore, necessary to study these genetic variations to elaborate our understanding of the genetic control in various craniofacial determinants. In the present study, the focus of interest is to study the relationship of GABRB3 rs2059574 and GAD67 rs2177433 with Non Syndromic Cleft Lip/Palate in our population. This will help us in understanding the etiology of Non syndromic Cleft Lip / Palate so as to predict its occurrence and also to target at the molecular level for correction of such problems.

## MATERIALS AND METHODOLOGY

The polymorphism in GABRB3 rs2059574 and GAD67 rs2177433 gene variants was detected using the Polymerase Chain Reaction (PCR) test followed by DNA Sequencing. Automated DNA sequencing procedure was selected for the sequencing of DNA where each nucleotide was labeled with fluorescent dyes. Thus when the DNA fragments were placed on the electrophoresis gel and passed through a laser beam, the DNA sequence was detected more precisely and accurately on an electropherogram, unlike other sequencing techniques.

Saliva samples from 25 cases with non-syndromic cleft lip with/without palate and 25 unrelated controls were collected from Department of Orthodontics and Dentofacial Orthopedics, D.A.P.M.R.V. Dental College and Swasthya Foundation, Mysore after taking the written informed consent. These were divided into two groups:

***Group A:*** Twenty five subjects with Non syndromic cleft lip/ palate (P1- P25)

***Group B:*** Twenty five controls (C1- C25)

***Inclusion criteria for Group-A subjects***

1. The presence of Non-syndromic cleft lip/ palate on clinical examination.

***Exclusion criteria for Group-A subjects****:* Cleft lip/palate associated with any:-

1. History of developmental disabilities, including learning disabilities and attention deficits, hearing impairment, and speech deficits or abnormalities may be the first indication of an underlying syndromic genetic disorder.
2. Family history of orofacial clefts and related conditions, including any additional major associated anomalies (e.g., cardiac defects and eye and brain anomalies).
3. History of maternal illnesses.
4. Medication (e.g., anticonvulsants and retinoic acid derivatives), vitamin (before and after conception) during pregnancy.
5. Tobacco use, Smoking during Pregnancy.
6. Ethanol intake during pregnancy.

## METHODOLOGY

The method was divided into four steps:

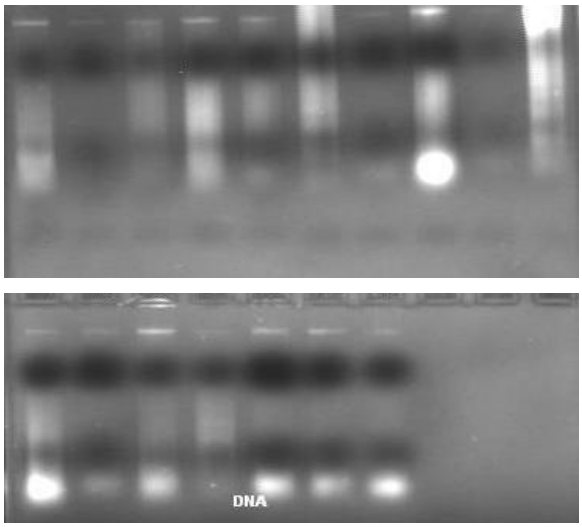
1. Step 1: Collection and storage of saliva samples,
2. Step 2: Isolation of Genomic DNA,
3. Step 3: Polymerase Chain Reaction Test (PCR),
4. Step 4: DNA sequencing

***Step 1- Collection and Storage of Saliva Samples***

- Collection of saliva (5ml) in phosphate buffer
- Transportation to laboratory
- Storage of samples in liquid nitrogen

***Step 2: Isolation of Genomic DNA Protocol***

- Saliva sample transferred to 30 ml centrifuge tubes and Centrifuged at 6000 rpm for 5 minutes at RT (Room temperature)
- Supernatant discarded and 2ml of DNazollysis buffer added and Incubated at 65°C for 1 Hour and centrifuged at 10,000 rpm for 10 minutes
- An equal volume of isopropanol added and mixed gently and centrifuged at 10,000 rpm for 15-20minutes and the supernatant discarded
- 500 µl of 70% Ethanol added and centrifuged at 10,000rpm for 5 minutes
- The supernatant was drained out and air dried the pellet
- Added 50 µl of 1X TE (TRIS EDTA) to the pellet and resuspended by finger flicking




***STEP 3: Polymerase Chain Reaction (PCR) test***

The Polymerase chain reaction (PCR) is an in vitro technique which allows the amplification of a specific deoxyribonucleic acid (DNA).

***Primer Sequence***

For GABRB3 rs2059574
**GARB3FP**:5'TGGTCCAAGCATAAATCATGC'3
**GARB3RP**:5'GAGGCAGACATGCCACCTC'3
For GAD67 rs2177433
**GAD67FP**:5'CACT GTTTACTGAC AATATGATCG'3
**GAD67RP**:5'CGATTATGCCCATCCATGAGC'3

**Step 4: DNA Sequencing:**

DNA sequencing was performed using Frederick Sanger's dideoxy sequencing method in an automated ABI sequencer machine based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication.

***Statistical Methods***

Z test has been used to find the significance of association of GABRB3*and*GAD67gene polymorphism with non-syndromic cleft lip and palate.

Z – test: It can be applied for qualitative as well as quantitative data. Here it was applied to test the difference between two proportions (cases and controls).

***Z-test for proportions formula***

$$Z = \frac{\hat{P}1 - \hat{P}2}{SEDp}$$

$$SEDp = \sqrt{\hat{P}(1 - P\hat{})(1/n1 + 1/n2)}$$ and

$$P = \frac{X1+X2}{n1+n2}$$

p1, proportion1= x1/ n1
p2, proportion2= x2/ n2
x1 =number of cases with the 3 genotypes of each gene.
x2 =number of controls with the 3 genotypes of each gene.
n1 =total number of cases
n2 =total number of controls

***Statistical interpretation***

Highly Statistically significant p≤0.001
Statistically Insignificant p≥0.05

***Statistical software:*** The Statistical software namely SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc

## RESULTS

In the present study, the relationship between GABRB3 rs2059574 and GAD67 rs2177433 variants with cleft lip with or without cleft palate was evaluated in 50 subjects consisting of group A (P1-P25) as cases and group B (C1-C25) as controls using polymerase chain reaction(PCR) test followed by DNA sequencing.

***Results for GABRB3 rs2059574 variants***

For GABRB3 rs2059574threegenotype can be possible:

| | |
|---|---|
| T/T | HOMOZYGOUS MUTANT ALLELE |
| A/T | HETEROZYGOUS MUTANT ALLELE |
| A/A | NORMAL HOMOZYGOUS ALLELE |

In group A
12 out of 25 cases showed the presence of TT genotype.
8 out of 25 cases showed the presence of AT genotype.
5 out of 25 cases showed the presence of AA genotype.
In group B
2 out of 25 controls showed the presence of TT genotype.
6 out of 25 controls showed the presence of AT genotype.
17 out of 25 controls showed the presence of AA genotype.
After statistical analysis (Z- test) (Table 3):

- There was a statistically significant difference in TT, AT and AA genotype frequencies between cases and controls
- AA genotype was found to be highly statistically significant with the controls (GROUP B ) (p=0.001 )
- TT genotype was found to be highly significant with the cases (GROUP A ) (p=0.001)
- AT genotype was found to be statistically insignificant with the cases and controls (p=0.53)

***Results for variants***

For **GAD67 rs2177433** three genotypes can be possible

| | |
|---|---|
| T/T | HOMOZYGOUS MUTANT ALLELE |
| A/T | HETEROZYGOUS MUTANT ALLELE |
| A/A | NORMAL HOMOZYGOUS ALLELE |

In group A
3 out of 25 cases showed the presence of TT genotype.
6 out of 25 cases showed the presence of AT genotype.
16 out of 25 cases showed the presence of AA genotype.
In group B
4 out of 25 controls showed the presence of TT genotype.
7 out of 25 controls showed the presence of AT genotype.
14out of 25 controls showed the presence of AA genotype

**Table 1** Tabulated Results For Sixty Subjects Showing Variation In Presence of Genotypes of Gabrb3 Rs2177433 Gene Variant Among Cases And Controls

| GROUP A Cases | GENOTYPE TT | GENOTYPE AT | GENOTYPE AA | GROUP B controls | GENOTYPE TT | GENOTYPE AT | GENOTYPE AA |
|---|---|---|---|---|---|---|---|
| 1. | ABSENT | ABSENT | PRESENT | 1. | ABSENT | ABSENT | PRESENT |
| 2. | PRESENT | ABSENT | ABSENT | 2. | ABSENT | ABSENT | PRESENT |
| 3. | PRESENT | ABSENT | ABSENT | 3. | ABSENT | PRESENT | ABSENT |
| 4. | ABSENT | ABSENT | PRESENT | 4. | ABSENT | ABSENT | PRESENT |
| 5. | PRESENT | ABSENT | ABSENT | 5. | ABSENT | ABSENT | PRESENT |
| 6. | ABSENT | PRESENT | ABSENT | 6. | ABSENT | PRESENT | ABSENT |
| 7. | PRESENT | ABSENT | ABSENT | 7. | ABSENT | ABSENT | PRESENT |
| 8. | PRESENT | ABSENT | ABSENT | 8. | ABSENT | ABSENT | PRESENT |
| 9. | ABSENT | ABSENT | PRESENT | 9. | PRESENT | ABSENT | ABSENT |
| 10. | ABSENT | PRESENT | ABSENT | 10. | ABSENT | ABSENT | PRESENT |
| 11. | PRESENT | ABSENT | ABSENT | 11. | ABSENT | ABSENT | PRESENT |
| 12. | PRESENT | ABSENT | ABSENT | 12. | ABSENT | ABSENT | PRESENT |
| 13. | ABSENT | ABSENT | PRESENT | 13. | ABSENT | ABSENT | PRESENT |
| 14. | PRESENT | ABSENT | ABSENT | 14. | ABSENT | PRESENT | ABSENT |
| 15. | ABSENT | PRESENT | ABSENT | 15. | ABSENT | PRESENT | ABSENT |
| 16. | ABSENT | PRESENT | ABSENT | 16. | ABSENT | ABSENT | PRESENT |
| 17. | PRESENT | ABSENT | ABSENT | 17. | ABSENT | ABSENT | PRESENT |
| 18. | PRESENT | ABSENT | ABSENT | 18. | PRESENT | ABSENT | ABSENT |
| 19. | ABSENT | PRESENT | ABSENT | 19. | ABSENT | ABSENT | PRESENT |
| 20. | PRESENT | ABSENT | ABSENT | 20. | ABSENT | ABSENT | PRESENT |
| 21. | ABSENT | PRESENT | ABSENT | 21. | ABSENT | PRESENT | ABSENT |
| 22. | PRESENT | ABSENT | ABSENT | 22. | ABSENT | PRESENT | ABSENT |
| 23. | ABSENT | ABSENT | PRESENT | 23. | ABSENT | ABSENT | PRESENT |
| 24 | ABSENT | PRESENT | ABSENT | 24 | ABSENT | ABSENT | PRESENT |
| 25. | ABSENT | PRESENT | ABSENT | 25. | ABSENT | ABSENT | PRESENT |

**Table 2** The Presence of TT, AT, AA Genotype OF Gabrb3 rs2059574 GENE Variants Among Cases And Controls

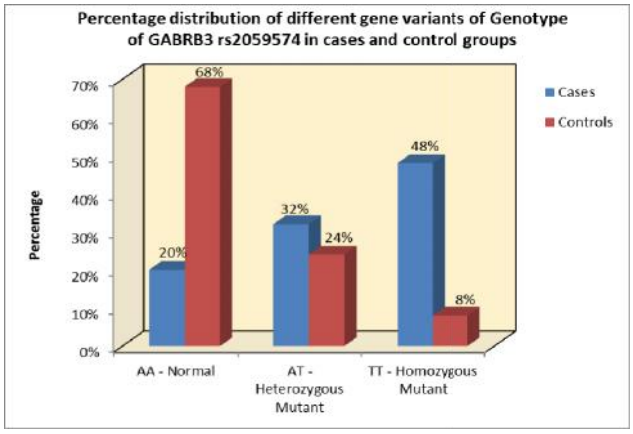
| Genotype of GABRB3 rs2059574 Gene variant | GROUP A (CASES) | GROUP B (CONTROLS ) | TOTAL |
|---|---|---|---|
| AT | 8 | 6 | 14 |
| TT | 12 | 2 | 14 |
| AA | 5 | 17 | 22 |
| Total | 25 | 25 | 50 |

- There was no statistically significant difference in AA genotype frequency between cases and controls(p=0.56)
- TT genotype was found to be statistically insignificant with the cases and controls. (p=0.68)
- AT genotype was found to be statistically insignificant with the cases and controls (p=0.75 )

**Table 3** The Table Denotes The Statistical Significance of The Genotype of GABRB3 rs2059574 Gene Variant When Cases And Controls Are Compared Using Z-Test

| Genotype of GABRB3 rs2059574 Gene Variant | Cases | | Controls | | Difference in Proportions | 95% CI of the Diff | | Z | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | | Lower | Upper | | |
| AA - Normal | 5 | 20% | 17 | 68% | -0.48 | 0.20 | 0.76 | -3.149 | 0.001** |
| AT - Heterozygous Mutant | 8 | 32% | 6 | 24% | 0.08 | -0.17 | 0.33 | 0.630 | 0.53 |
| TT - Homozygous Mutant | 12 | 48% | 2 | 8% | 0.40 | 0.15 | 0.65 | 3.150 | 0.001** |

- The difference in the proportion of positive results between cases and controls for genotype TT was found to be statistically highly significant (p<0.001)
- The difference in the proportion of positive results between cases and controls for genotype AT was found to be statistically significant (p=0.03 )
- The difference in the proportion of positive results between cases and controls for genotype AA was found to be statistically significant (p<0.001 )




**Graph 1**

NSCL/P is a common congenital anomaly with significant medical, psychological, and social impacts. Substantial evidence indicates that this anomaly represents a complex disease in which clinical and genetic heterogeneities are observed [3].

The genetic basis of oral clefting remains an open question, and the identification of risk factors for NSCL/P has been the subject of intensive research.

Sequencing of human and other genomes has been the center of interest in the biomedical field over the past several decades and is now leaning towards an era of personalized medicine.

**Table 4** Tabulated Results For Sixty Subjects Showing Variation In Presence of Genotypes of GAD67 rs2177433 Gene Variant Among Cases And Controls

| GROUP A Cases | GENOTYPE TT | GENOTYPE AT | GENOTYPE AA | GROUP B controls | GENOTYPE TT | GENOTYPE AT | GENOTYPE AA |
|---|---|---|---|---|---|---|---|
| 1. | ABSENT | ABSENT | PRESENT | 1. | ABSENT | ABSENT | PRESENT |
| 2. | ABSENT | ABSENT | PRESENT | 2. | ABSENT | ABSENT | PRESENT |
| 3. | ABSENT | ABSENT | PRESENT | 3. | ABSENT | PRESENT | ABSENT |
| 4. | ABSENT | ABSENT | PRESENT | 4. | ABSENT | ABSENT | PRESENT |
| 5. | ABSENT | PRESENT | ABSENT | 5. | ABSENT | PRESENT | ABSENT |
| 6. | ABSENT | PRESENT | ABSENT | 6. | ABSENT | ABSENT | PRESENT |
| 7. | ABSENT | ABSENT | PRESENT | 7. | PRESENT | ABSENT | ABSENT |
| 8. | ABSENT | ABSENT | PRESENT | 8. | ABSENT | ABSENT | PRESENT |
| 9. | ABSENT | ABSENT | PRESENT | 9. | ABSENT | ABSENT | PRESENT |
| 10. | ABSENT | PRESENT | ABSENT | 10. | ABSENT | ABSENT | PRESENT |
| 11. | ABSENT | ABSENT | PRESENT | 11. | PRESENT | ABSENT | ABSENT |
| 12. | PRESENT | ABSENT | ABSENT | 12. | ABSENT | ABSENT | PRESENT |
| 13. | ABSENT | ABSENT | PRESENT | 13. | ABSENT | ABSENT | PRESENT |
| 14. | ABSENT | ABSENT | PRESENT | 14. | ABSENT | PRESENT | ABSENT |
| 15. | ABSENT | ABSENT | PRESENT | 15. | PRESENT | ABSENT | ABSENT |
| 16. | ABSENT | PRESENT | ABSENT | 16. | ABSENT | ABSENT | PRESENT |
| 17. | ABSENT | ABSENT | PRESENT | 17. | ABSENT | PRESENT | ABSENT |
| 18. | ABSENT | ABSENT | PRESENT | 18. | ABSENT | ABSENT | PRESENT |
| 19. | PRESENT | ABSENT | ABSENT | 19. | PRESENT | ABSENT | ABSENT |
| 20. | ABSENT | ABSENT | PRESENT | 20. | ABSENT | ABSENT | PRESENT |
| 21. | ABSENT | PRESENT | ABSENT | 21. | ABSENT | PRESENT | ABSENT |
| 22. | ABSENT | ABSENT | PRESENT | 22. | ABSENT | ABSENT | PRESENT |
| 23. | PRESENT | ABSENT | ABSENT | 23. | ABSENT | PRESENT | ABSENT |
| 24 | ABSENT | PRESENT | ABSENT | 24 | ABSENT | PRESENT | ABSENT |
| 25. | ABSENT | ABSENT | PRESENT | 25. | ABSENT | ABSENT | PRESENT |

After statistical analysis (Z- test) (Table 6):

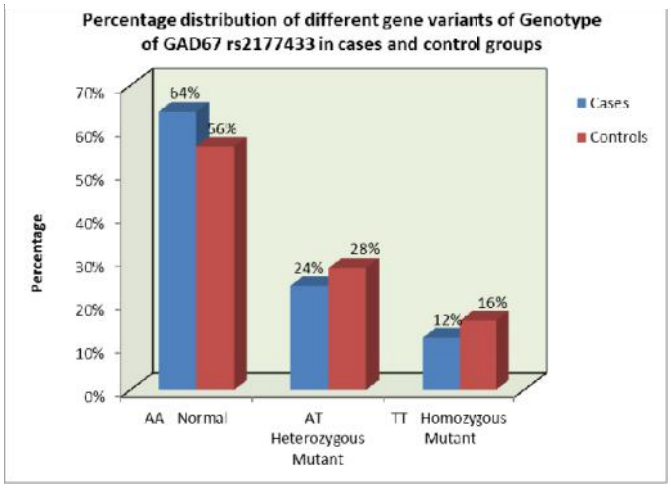
**Table 5** The Presence of AT,TT,AA Genotypes of GAD67 rs2177433 Gene Variant Among Cases And Controls

| Genotype of GAD67rs2177433 Gene Variant | Cases | | Controls | | Difference in Proportions | 95% CI of the Diff | | Z | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | n | % | N | % | | Lower | Upper | | |
| AA - Normal | 16 | 64% | 14 | 56% | 0.08 | -0.19 | 0.35 | 0.577 | 0.56 |
| AT - Heterozygous Mutant | 6 | 24% | 7 | 28% | -0.04 | -0.20 | 0.28 | -0.322 | 0.75 |
| TT - Homozygous Mutant | 3 | 12% | 4 | 16% | -0.04 | -0.15 | 0.23 | -0.408 | 0.68 |

**Table 6** The Table Denotes The Statistical Significance of The Genotypes of GAD67 rs2177433 Gene Variant When Cases and Controls Are Compared Using Z-Test

| Genotype of GAD67 rs2177433 Gene variant | Group A (Cases ) | Group B (Controls ) | Total |
|---|---|---|---|
| AT | 6 | 7 | 13 |
| TT | 3 | 4 | 7 |
| AA | 16 | 14 | 30 |
| Total | 25 | 25 | 50 |

- The difference in the proportion of positive results between cases and controls for genotype AA was not statistically insignificant (p=0.56 )
- The difference in the proportion of positive results between cases and controls for genotype AT was not statistically significant (p=0.75 )
- The difference in the proportion of positive results between cases and controls for genotype TT was not statistically highly significant (p=0.68 )




**Graph 2**

During this time, DNA sequencing methods have evolved from the labor intensive slab gel electrophoresis to automated multicapillary electrophoresis systems using fluorophore labeling[9].

Dye-terminator based DNA sequencing allowed the use of four dideoxynucleotide chain terminators, tagged with dyes of different fluorescent emission wavelengths, in a single sequencing reaction which is depicted by a graph called as Electropherogram or Chromatogram. This graph contains a sequence of peaks in four colors which are universally color coded for each nucleotide (thymine-red, adenine-green, guanine-black and cytosine-blue). Any change in normal nucleotide sequencing will be shown by different color peaks and if it is homozygous it will be shown as a single peak while if it is heterozygous it will be shown as double peak[9].

Advances in genetic testing, gene therapy, pharmacogenomics, mechanogenomics, and stem cell therapy are likely to produce the most dramatic changes in orthodontic treatment. Blood specimens are used extensively to monitor the general state of health and for analysis of many specific diagnostic analytes. Drawing blood can be impractical for people with blood-injection injury type phobia and those who require daily monitoring of biomarker levels. Non-invasive technology is thus become increasingly important and would be ideal for point-of-care diagnosis[10].

According to a study by Thomas.V.Hansen *et al* in 2007, the efficacy of DNA extraction from saliva is 84% compared to blood which is 96%. Saliva is one of the most preferable and practical specimens for health monitoring as it is readily available as well as easily collected and stored[11].

According to the interpretation of the electropherogram and statistical analysis, in our population, GABRB3 variant rs2059574 , showed statistically significant differences in genotype frequencies between cases and controls, with TT ($p<0.001$) and AT ($p=0.03$) genotypes found more in cases, with AA genotype ($p<0.001$) found more in controls. (Table no. 2 & 3 and Graph no.1).

GAD67 gene variant rs2177433 showed no statistically significant difference in genotype frequencies between cases and controls, with AA($p=0.56$) genotype, with TT ($p=0.68$) genotype and AT ($p=0.75$)genotype. Our study showed a highly significant difference in presence of genotypes in cases and controls in both the GABRB3 variant rs 2059574 (TT and AA) but GAD67 gene variant rs2177433 showed an insignificant difference.

This is in accordance with a study done by Hiroki Inoue *et al*, which concluded the associations that the *GABRB3* gene is involved in the pathogenesis of cleft lip with or without cleft palate in the Japanese population[12].

Previous studies have investigated the role of the GABRB3 variants in orofacial clefts, but the results have been inconsistent. The study was performed by Tanabe *et al*. (2000) with one group of Japanese patients affected by NSCL/P. Those authors evaluated the CA dinucleotide-repeat polymorphism in GABRB3 and concluded that the GABRB3 gene is not involved with the pathogenesis of oral clefts in Japanese patients, but it should be taken into account that their results were not submitted to any statistical analysis[13] Scapoli *et al*. (2002) investigated the same CA dinucleotide repeat marker in 38 Italian families with NSCL/P and showed significant evidence of the linkage disequilibrium[14].

The results of our study are contrary to the study done byKiyoshi Kanno *et al* (2004) in the Japanese population, suggesting GAD67 is involved in the pathogenesis of NSCLP in the Japanese population. Asignificant association between five-locus haplotype and CL/CP was detected in both case-control studies and transmissiondisequilibrium tests (TDT). The results suggested that GABA is necessary not only for palate formation but also for lip formationin humans. The contradictory results are probably due to genetic hetero-genecity, incomplete penetrance, limited sample sizes, and different study designs[15].

In the near future, with rapid advances in the science of gene manipulation, the correction or alteration of genetic defects at the molecular level remains a possibility. Gene manipulation can be employed to control the expression of any gene in several orthodontically relevant issues. In turn, we may witness the introduction of both preventative and in vivo fetal therapy for these debilitating conditions.

The findings of this study indicate that GABRB3 rs2059574 and GAD67 rs2177433 polymorphisms may be one of the genetic markers for cleft lip and palate in our population. Further studies, targeting a large sample size are required for better insight and complex genetics of Non syndromic cleft lip and palate.

## CONCLUSION

1. This study indicates that there is a strong association between the presence of GABRB3 rs2059574 and there is no association between the presence of GAD67 rs2177433 with the incidence of Nonsyndromic cleft lip and palate.
2. This study suggests that the likelihood of Non syndromic cleft lip and palate is higher in subjects having TT ($p<0.001$) genotype for GABRB3 rs2059574.
3. This study suggests that the incidence of Nonsyndromic cleft lip and palate is lesser in subjects having AA($p<0.001$) and AT($p=0.44$) genotype of GABRB3 gene variant rs2059574 and AT($p =0.75$), TT($p=0.68$) & AA($p=0.56$)genotype of GAD67 gene variant rs2177433.
4. The findings of this study suggest that GABRB3 gene variant rs2059574 can be considered as genetic markers for Non syndromic cleft lip and palate for our population.

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