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Genetic Analysis of the *PAX9* Gene Polymorphism in the Etiology of Impacted Maxillary Canines

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Abstract

Background: Maxillary canines are the second most frequently impacted teeth after third molars, with a prevalence of approximately 2% in the general population. The etiology of canine impaction is multifactorial, involving both environmental and genetic factors. Among the candidate genes associated with tooth development, *PAX9* plays a crucial role in odontogenesis and tooth bud positioning. Genetic variations in *PAX9* have been linked to dental anomalies such as agenesis and impaction; however, evidence regarding their association with maxillary canine impaction remains limited, particularly in the Indian population. ***Aim:*** This study aimed to evaluate the association between *PAX9* gene polymorphism (SNP rs4904210) and maxillary canine impaction in an Indian population sample. ***Materials and Methods:*** A case-control study was conducted on 60 subjects (30 cases with impacted maxillary canines and 30 controls with normally erupted canines) aged 10–40 years. Venous blood samples were collected, and DNA was isolated using standard protocols. The *PAX9* gene was amplified using Polymerase Chain Reaction (PCR), followed by Restriction Fragment Length Polymorphism (RFLP) analysis with the enzyme HpaII for genotyping. Statistical analyses, including Hardy-Weinberg equilibrium and chi-square tests, were performed to determine the association between genotypes and the occurrence of canine impaction. ***Results:*** The study population showed no significant differences in age or gender distribution between cases and controls ($p > 0.05$). Among the cases, unilateral impaction was more common (73.3%) than bilateral (26.7%). Both case and control groups were in Hardy-Weinberg equilibrium. No statistically significant differences in genotype or allele frequencies of *PAX9* (rs4904210) were observed between the two groups, indicating no genetic association with maxillary canine impaction. ***Conclusion:*** The present study found no significant association between *PAX9* polymorphism (rs4904210) and maxillary canine impaction in the Indian population studied. Although *PAX9* plays a critical role in tooth development, this specific SNP does not appear to influence canine impaction risk. Future studies with larger, ethnically diverse samples and broader genetic analysis are warranted to better understand the multifactorial etiology of maxillary canine impaction.

Keywords: Maxillary canine impaction, *PAX9* gene, polymorphism, rs4904210, genetic association, odontogenesis, Indian population.

INTRODUCTION

Maxillary canines are the second most frequently impacted teeth after third molars, with a prevalence of about 2% in the general population.[1] This condition is observed twice as often in females than in males, with impaction occurring more than twice as frequently in the maxilla compared to the mandible. Bilateral impactions are reported in nearly 8% of cases. Among impacted maxillary canines, approximately one-third are located labially, while two-thirds are palatally displaced.[2]

The etiology of canine impaction is multifactorial, involving both environmental and genetic influences. Jacoby [3] demonstrated that 85% of palatally impacted canines had sufficient arch space for eruption, whereas only 17% of labially impacted canines exhibited adequate space. These findings suggest that labial impactions are closely related to arch length discrepancies, while palatal impactions are more likely to have a genetic basis.

Palatally impacted canines are often associated with other dental anomalies, including tooth agenesis, oligodontia, and peg-shaped lateral incisors.[4] Such anomalies typically present during the developmental phase between 4 and 8 years of age and often cluster within families, supporting a strong genetic component. Much like dental agenesis, palatal impaction of canines has been shown to follow patterns of familial inheritance, highlighting the importance of genetic regulation in tooth eruption and positioning.[5]

Studies have identified several candidate genes implicated in tooth development, including MSX1, PAX9, AXIN2, WNT10A, EDA, EDAR, BMP4, FGF8, IRF6, and PITX2. [6] These genes encode signaling molecules, transcription factors, and mediators essential in pathways such as WNT/β-catenin, TGFβ, and BMP, which regulate odontogenesis. Among these, mutations in MSX1 and PAX9 are most commonly associated with dental developmental disorders, including agenesis and impaction. [7]

The PAX9 gene, located on chromosome 14, consists of four exons, with most mutations reported in exon 2 and less frequently in exon 4. [8] These mutations are strongly linked to non-syndromic hypodontia and oligodontia, particularly affecting molars. Deletion or mutation of PAX9 alleles results in selective tooth agenesis, often through haploinsufficiency. [9] Additionally, single nucleotide polymorphisms (SNPs) in PAX9 and MSX1 have been associated with impacted canines, with PAX9 being especially relevant due to its role in odontogenesis and determination of tooth bud position. [10]

Despite global research progress, genetic studies on impacted maxillary canines remain limited, particularly in the Indian population. To date, no published data exists on the association between PAX9 gene polymorphisms and maxillary canine impaction in India.[11] Given the potential clinical relevance of genetic predisposition, further exploration in this area may provide valuable insights into the etiology and management of canine impaction.[12]

This study therefore aims to evaluate the genetic association between PAX9 gene polymorphisms and impacted maxillary canines in an Indian population sample.

MATERIAL AND METHODOLOGY

This case-control study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, The Oxford Dental College, Bengaluru, after obtaining the Institutional Ethical Committee clearance.

The inclusion criteria for the study will consist of patients aged between 10 and 40 years, encompassing both males and females, presenting with either unilateral or bilateral maxillary impacted canines. Individuals will be excluded from the study if they present with multiple impacted permanent teeth, cleft lip and palate, or craniofacial syndromes, as these conditions may confound the genetic and clinical evaluation of maxillary canine impaction.

A total of 60 subjects (cases: 30 and controls: 30) were included in the study based on the inclusion and exclusion criteria. After explaining the procedure, written informed consent was taken from all the subjects/parents. Then, 3 milliliters of venous blood were collected by a qualified person from each subject in Ethylenediaminetetraacetic acid (EDTA) tube (Fig. 1, 2 & 3). These tubes were transported in ice packs (Fig. 4) to maintain a temperature of 2 to 8°C to the laboratory for DNA isolation (Fig.5), amplification, and SNP genotyping. Finally, the genotypic data obtained was sent for statistical analysis. This study was performed as per declaration of Helsinki (revised 2013) for experiments involving human subjects and STREGA guidelines.

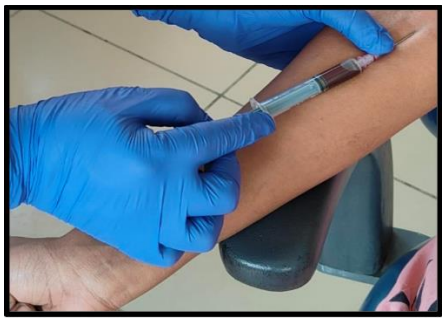

Fig. 1. Blood sample collection

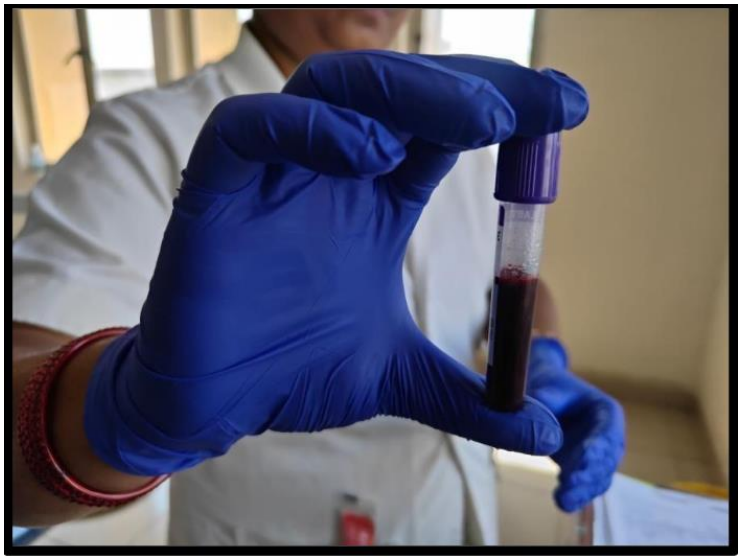

Fig. 2: Transferred blood in the EDTA tube

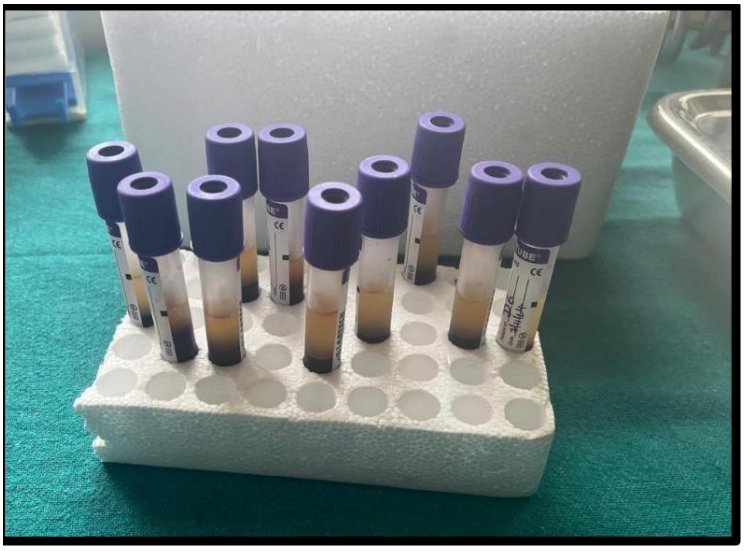

Fig. 3: EDTA tubes with blood samples

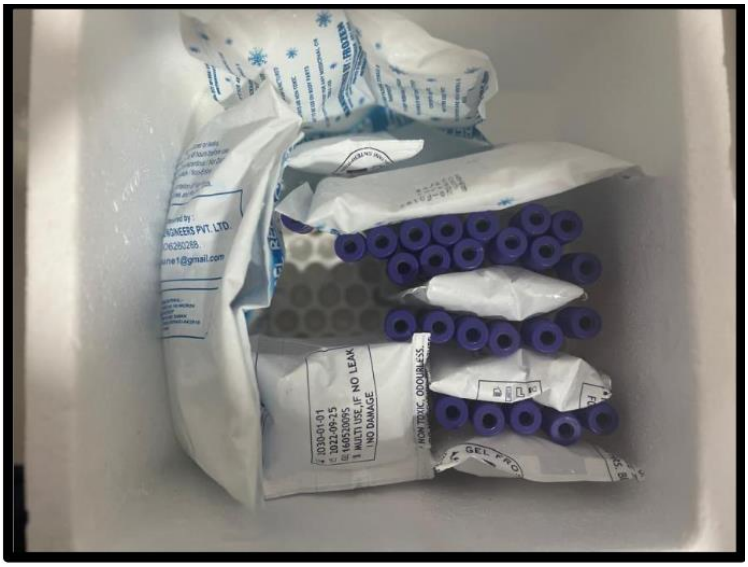

Fig. 4: Storage of blood samples in ice packs for transport to lab

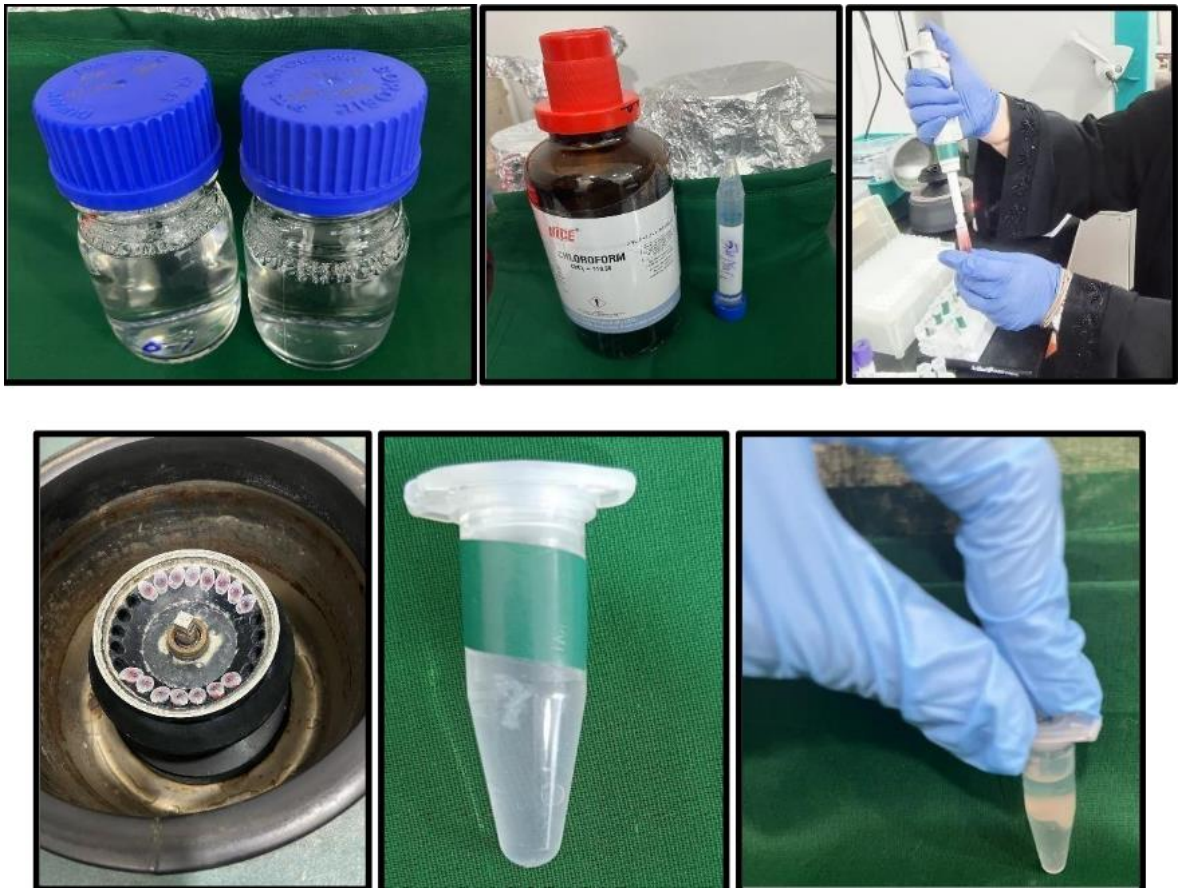

Fig. 5: DNA isolation from blood

Fig. 6: Components of PCR

DNA isolation:

2 ml of blood samples were collected in an EDTA coating tube. The RBC lysis buffer is used to lysis the RBC from whole blood. The repeated washing step with lysis buffer ensures the removal of RBC. The WBCs were collected at the bottom of the tube. The pellet dissolved by adding lysis buffer of 400 µl, 100 µl 5M of NaCl with 600 µl of chloroform. The lysate solution was centrifuged at 7000PM for 3 minutes. After centrifugation, the aqueous layer in a fresh tube was collected and ice-cold ethanol was added to an equal volume of the aqueous layer for precipitation. The sample was centrifuged at 12,000 rpm for 10 minutes. The supernatant was discarded and Pellet was found at the bottom. The pellet was washed with 70% ethanol and centrifuged at the same condition. After air drying the

sample, the elution buffer was added to it. Eluted DNA was checked with Nanodrop one at A260/280 for quantity and purity.

Polymerase Chain Reaction

The PCR (Polymerase Chain Reaction) (Fig. 6) and RFLP (Restriction fragmentation length Polymorphism) (Fig. 8) methods were used for genotyping analysis. The PAX9 primers Forward 5'TCTCCATCAGTGAGCGACAG 3' and reverse 5'AAGGGACCCATCACAAAGTG 3' were used to amplify the DNA and its amplicon size was 230bp. The PCR was performed in 20 ul containing 50ng of genomic DNA, 5 pmol/ul of each primer, and PCR master mix [Takara, Shiga, Japan]. PCR is carried out in a thermal cycler (Fig.7) with a condition that includes an initial denaturation at 95°C for 5 min followed by 39 cycles of denaturation at 95°C for 35seconds, primer annealing at 64°C for 35 seconds, extension at 72°C for 35 seconds and a final extension at 72°C for 5 min. The Amplicon size of PCR was 230bp. The amplified PCR product was checked on a 1% agarose gel electrophoresis. (Fig. 9)

Fig. 7: Polymerase chain reaction by thermal cycler

Fig. 8: Restriction enzyme for restriction fragmentation length polymorphism

RFLP (Restriction Fragmentation Length Polymorphism):

The NAT2 amplified PCR product sized 230bp was digested using HpaII respectively for *rs2073244*.The PCR product was digested with restriction enzymes [New England Biolabs, UK] at 37°C for respective enzymes for 1 hr. The digested product was electrophoresed in 2 % agarose with 0.5 µg/ml ethidium bromide and photographed using a gel documentation system. The digested products sizes were CC-97+133 bp, GG- 230 bp, GC-97+133+230 bp.

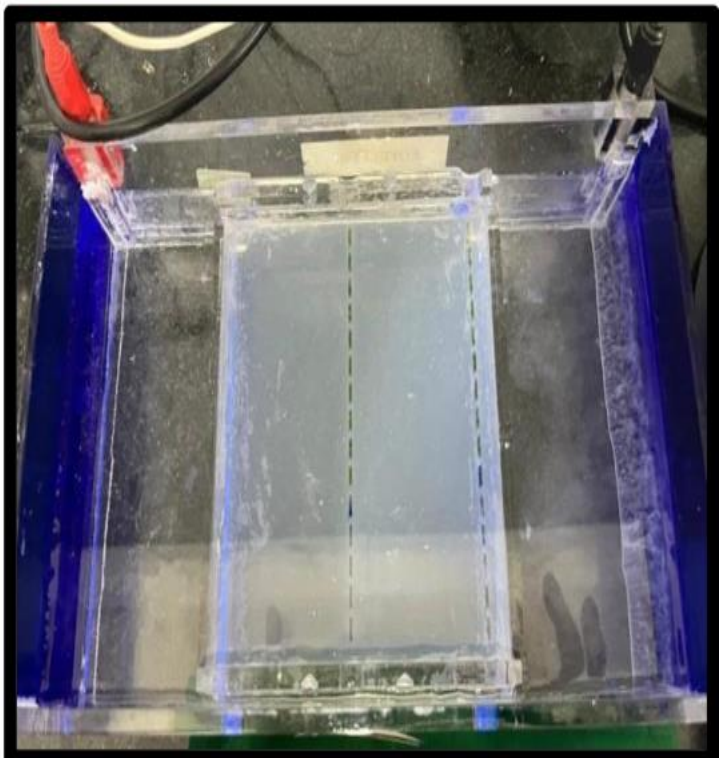

Fig. 9: Agrose Gel Electrophoresis

RESULTS

Table 1 shows a total of 60 subjects, evenly distributed between cases (n=30) and controls (n=30). The subjects were categorized into three age groups: 13 to 20 years, 21 to 30 years, and 31 to 36 years. The majority of participants (51.7%) were in the 13 to 20 years age group, with a slightly higher proportion among cases (56.7%) compared to controls (46.7%). The 21 to 30 years age group comprised 38.3% of the total population, with 33.3% in cases and 43.3% in controls. The 31 to 36 years age group had the smallest representation, accounting for only 10% in both cases and controls. A chi-square test was conducted to assess the association between age group distribution and case/control status, yielding a chi-square value of 0.682 and a p-value of 0.711. Since the p-value is greater than 0.05, the difference in age distribution between cases and controls is not statistically significant, indicating that age is not a confounding factor in this study.

The mean age of the participants was compared between the cases and controls using an independent sample t-test. The cases group (n=30) had an age range of 13 to 36 years, with a mean age of 21.27 years and a standard deviation (S.D.) of 6.63. The controls group (n=30) had a slightly different age range of 14 to 36 years, with a mean age of 21.60 years and an S.D. of 5.63. The mean difference between the two groups was -0.33 years, indicating that the cases group had a marginally lower mean age than the controls. However, the p-value of 0.835 suggests that this difference is not statistically significant ($p > 0.05$), indicating that there is no meaningful difference in age distribution between the two groups.

The study population consisted of 60 subjects, evenly distributed between cases (n=30) and controls (n=30). Among them, 37 (61.7%) were females and 23 (38.3%) were males. In the cases group, females comprised 66.7% (n=20), while males made up 33.3% (n=10). In the controls group, females accounted for 56.7% (n=17), and males 43.3% (n=13). A chi-square test was performed to determine if there was a significant association between gender and group classification. The test yielded a chi-square value of 0.635 and a p-value of 0.426. Since the p-value is greater than 0.05, the difference in gender distribution between the cases and controls is not statistically significant. This suggests that gender is not a confounding factor in this study.

Among the cases group (n=30), the distribution based on the number of sides involved shows that 22 individuals (73.3%) had unilateral involvement, while 8 individuals (26.7%) had bilateral involvement.

For Cases, the chi-square statistic is 2.29, which is less than the critical value of 3.841. Therefore, the Cases group is in Hardy-Weinberg equilibrium. For Controls, the chi-square statistic is 0.260, which is also less than the critical value of 3.841. Hence, the Controls group is also in Hardy-Weinberg equilibrium.

Table 1 – Distribution of the subjects based on age groups

Age Groups		Groups		Total
		Cases	Controls	
13 to 20 yrs	Count	17	14	31
	%	56.7%	46.7%	51.7%
21 to 30 yrs	Count	10	13	23
	%	33.3%	43.3%	38.3%
31 to 36 yrs	Count	3	3	6
	%	10.0%	10.0%	10.0%
Total	Count	30	30	60
	%	100.0%	100.0%	100.0%
Chi-square value- 0.682				
p value- 0.711				

Table 2 – Comparison of the mean age between the groups using independent sample test

Groups	N	Minimum	Maximum	Mean	S.D	Mean diff	p value
Cases	30	13.0	36.0	21.27	6.63	-0.33	0.835
Controls	30	14.0	36.0	21.60	5.63		

Table 3 – Distribution of the subjects based on gender

Gender		Groups		Total
		Cases	Controls	
Females	Count	20	17	37
	%	66.7%	56.7%	61.7%
Males	Count	10	13	23
	%	33.3%	43.3%	38.3%
Total	Count	30	30	60
	%	100.0%	100.0%	100.0%
Chi-square value- 0.635				
p value- 0.426				

Table 4 – Distribution based on number of sides involved

Cases	Frequency	Percent
Bilateral	8	13.3
Unilateral	22	36.7

Table 5 – Hardy Weinberg equilibrium analysis

Groups	GG	GC	Grand Total
Cases	13 (43.33%)	17 (56.66%)	30 (100%)
Controls	21 (70%)	9 (30%)	30 (100%)
Total	**34 (56.66%)**	**26 (43.33%)**	**60 (100%)**

DISCUSSION

Developmental irregularities in the positioning of tooth buds and the eruption sequence of permanent teeth may result in anomalies such as ectopic eruption, rotation, and impaction, which can compromise arch length, occlusion, and treatment outcomes. Among these, impaction poses a significant clinical challenge due to its complexity and unpredictable prognosis. Maxillary canines are the second most frequently impacted teeth after third molars, with their impaction often requiring prolonged orthodontic and sometimes surgical intervention. [11,12]

The etiology of maxillary canine impaction is multifactorial, involving local, environmental, and genetic influences. Local causes include inadequate arch space, prolonged retention of deciduous teeth, trauma, pathological obstructions such as odontomas or cysts, and misalignment of adjacent teeth. [1,13,14] Systemic conditions and genetic predispositions may further increase susceptibility. Increasingly, genetics is being recognized as a critical determinant in tooth development and eruption, with more than 200 genes implicated in odontogenesis. [14]

Among these, PAX9 has received significant attention. This transcription factor plays a key role in odontogenic signaling pathways and is associated with various dental anomalies such as tooth agenesis, alterations in size and shape, and abnormal eruption patterns. [12] Given its importance, polymorphisms in PAX9, such as SNP rs4904210, have been investigated for possible associations with maxillary canine impaction.

Our study aimed to explore whether SNP rs4904210 of the PAX9 gene is associated with maxillary canine impaction. The study included 60 participants—30 cases with impacted maxillary canines and 30 controls with normally erupted dentition. The majority were aged 13–20 years (51.7%), followed by 21–30 years (38.3%), with no significant age-related differences between cases and controls. Gender distribution also showed no significant association, suggesting that neither age nor gender acted as confounding variables.

Among cases, unilateral impaction was more common (73.3%) than bilateral impaction (26.7%), which aligns with previous epidemiological findings. Hardy–Weinberg equilibrium analysis confirmed that genotype distributions for SNP rs4904210 were within expected frequencies, and allele frequencies did not differ significantly between cases and controls. These results indicate that this particular SNP is not significantly associated with maxillary canine impaction in the studied population.

Our findings are consistent with those of Vitria *et al.,*[15], who identified four SNPs in exon 3 of PAX9, with missense mutations potentially affecting dental development. Although variations were noted in patients with and without impaction, no statistically significant correlation was observed with PAX9 polymorphisms, aligning with our study. Similarly, Pan *et al.,*[16] examined PAX9 polymorphisms in subjects with tooth agenesis but reported no significant association, further suggesting that PAX9 variants alone may not explain dental developmental anomalies such as impaction.

Contrastingly, other studies have highlighted genetic links. Devi et al.[11] reported positive associations between PAX9 (rs2073247) and MSX1 (rs12532) polymorphisms with palatal impaction of maxillary canines, indicating that certain variants may predispose individuals to specific eruption patterns. Pereira *et al.,*[17] also emphasized that the C-allele at rs4904210 may influence dental development, though the present study did not replicate this finding. These inconsistencies across populations suggest potential ethnic and environmental influences that modify genetic susceptibility.

Broader genetic studies also support a role of multiple genes in dental anomalies. Klein *et al.,*[18] demonstrated that genes governing tooth size, shape, and eruption patterns—such as PAX9, MSX1, and AXIN2—are implicated in canine displacement and impaction. Similarly, Trybek *et al.,*[19] highlighted those polymorphic and haplotype variants of PAX9, MSX1, AXIN2, and IRF6 significantly influence odontogenesis and may predispose individuals to anomalies including impaction.

Taken together, the current evidence suggests that while PAX9 is undoubtedly important in tooth development, its individual SNPs such as rs4904210 may not exert a direct or isolated effect on canine impaction. Instead, impaction likely arises from a complex interplay of multiple genetic factors and environmental influences. It is plausible that other variants within PAX9, in combination with polymorphisms in genes such as MSX1 and AXIN2, contribute more substantially to impaction risk. [20]

The clinical relevance of exploring genetic markers lies in their potential to improve predictive models for early diagnosis and prevention. Identifying at-risk individuals through genetic screening could allow timely interventions, such as space management or interceptive extraction of deciduous teeth, potentially reducing the severity of impaction cases. However, given the multifactorial nature of this condition, genetic testing alone may not suffice and must be integrated with clinical and radiographic evaluations.

Our study concludes that SNP rs4904210 of the PAX9 gene is not significantly associated with maxillary canine impaction. Nonetheless, this does not exclude the role of PAX9 or other genetic variants in the etiology of this condition. Larger studies across diverse populations, with broader genetic analyses encompassing multiple candidate genes, are necessary to clarify the contribution of genetic polymorphisms to maxillary canine impaction.

While impaction of maxillary canines is influenced by both local and genetic factors, the present findings indicate no significant association between SNP rs4904210 of PAX9 and this anomaly. Future research should adopt polygenic approaches to better elucidate the genetic underpinnings of dental impactions and enhance preventive orthodontic strategies.

CONCLUSION

An analysis of the Hardy-Weinberg Equilibrium (HWE) was conducted to evaluate whether the observed genotype frequencies of the PAX9 gene in individuals experiencing maxillary canine impaction differed significantly from those in individuals without such impaction. The outcome indicates that the genotype distributions in both the case and control groups conform to the principles of Hardy- Weinberg equilibrium. Since the observed genotype frequencies correspond with the expected Mendelian ratios, there is no significant deviation that would suggest a genetic association. Furthermore, the analysis revealed no statistically significant differences in allele frequencies between the cases and controls, reinforcing the conclusion that the PAX9 gene does not demonstrate a strong association with maxillary canine impaction within the population studied.

LIMITATIONS

The sample size utilized in our research, consisting of 60 participants, is significantly smaller when compared to previous studies conducted on the same subject matter. Furthermore, this study concentrated solely on the South Indian population, which presents difficulties in accurately defining this demographic due to its rich diversity in ethnicities and cultural backgrounds. To enhance the reliability of our findings, future research should consider employing a larger sample size and incorporating a broader range of genetic and environmental variables. This approach may be essential for substantiating our conclusions with a higher degree of confidence.

REFERENCES

1. Litsas G, Acar A. A review of early displaced maxillary canines: etiology, diagnosis and interceptive treatment. Open Dent J. 2011 Mar 16; 5:39-47.
2. Bishara SE, Ortho. D. Impacted maxillary canines: A review. Am J Orthod Dentofacial Orthop. 1992 Feb;101(2):159-71.
3. Jacoby H. The etiology of maxillary canine impactions. Am J Orthod. 1983 Aug;84(2):125-32.
4. Peters H, Balling R. Teeth. Where and how to make them. Trends Genet. 1999 Feb;15(2):59-65.
5. Vieira AR. Oral clefts and syndromic forms of tooth agenesis as models for genetics of isolated tooth agenesis. J Dent Res. 2003 Mar;82(3):162-65.
6. Kangas AT, Evans AR, Thesleff I, Jernvall J. Nonindependence of mammalian dental characters. Nature. 2004 Nov;432(7014):211-4.
7. Arte S, Nieminen P, Apajalahti S, Haavikko K, Thesleff I, Pirinen S. Characteristics of incisor-premolar hypodontia in families. J Dent Res. 2001 May;80(5):1445-50.
8. Parkin N, Elcock C, Smith RN, Griffin RC, Brook AH. The aetiology of hypodontia: The prevalence, severity and location of hypodontia within families. Arch Oral Biol. 2009 Dec;54: S52-S56.
9. Trybek G, Jaroń A, Grzywacz A. Association of polymorphic and haplotype variants of the MSX1 gene and the impacted teeth phenomenon. Genes (Basel). 2021 Apr 16;12(4):577.
10. Peck S, Peck L, Kataja M. Prevalence of tooth agenesis and peg-shaped maxillary lateral incisor associated with palatally displaced canine (PDC) anomaly. Am J Orthod Dentofacial Orthop. 1996

Oct;110(4):441-3.
11. Devi MSA, Padmanabhan S. Role of polymorphisms of MSX1 and PAX9 genes in palatal impaction of maxillary canines. J Orthod. 2019 Mar;46(1):14-9.
12. Ngan P, Hornbrook R, Weaver B. Early timely management of ectopically erupting maxillary canines. Semin Orthod. 2005 Sep;11(3):152-63.
13. Schindel RH, Duffy SL. Maxillary transverse discrepancies and potentially impacted maxillary canines in mixed-dentition patients. Angle Orthod. 2007 May;77(3):430-5.
14. Becker A, Sharabi S, Chaushu S. Maxillary tooth size variation in dentitions with palatal canine displacement. Eur J Orthod. 2002 Jun;24(3):313-8.
15. Vitria EE, Tofani I, Kusdhany L, Bachtiar EW. Genotyping analysis of the Pax9 Gene in patients with maxillary canine impaction. F1000Res. 2019 Mar; 8:254.
16. Pan Y, Wang L, Ma J, *et al.,* PAX9 polymorphisms and susceptibility to sporadic tooth agenesis: a case-control study in southeast China. Eur J Oral Sci. 2008 Apr;116(2):98-103.
17. Pereira TV, Salzano FM, Mostowska A, *et al.,* Natural selection and molecular evolution in primatePAX9gene, a major determinant of tooth development. Proc Natl Acad Sci U S A. 2006 Apr 11;103(15):5676-81.
18. Klein ML, Nieminen P, Lammi L, Niebuhr E, Kreiborg S. Novel mutation of the initiation codon of PAX9 causes oligodontia. J Dent Res. 2005 Jan;84(1):43-7.
19. Trybek G, Jaroń A, Gabrysz-Trybek E, *et al.,* Genetic factors of teeth impaction: Polymorphic and haplotype variants of PAX9, MSX1, AXIN2, and IRF6 genes. Int J Mol Sci. 2023 Sep 9;24(18):13889.
20. Pereira TV, Salzano FM, Mostowska A, *et al.,* Natural selection and molecular evolution in primatePAX9gene, a major determinant of tooth development. Proc Natl Acad Sci U S A. 2006 Apr 11;103(15):5676-81.