Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

Novel Mutations in PAX9 Gene Associated with Dental Anomalies - A Review

Kiren.J, Vijayashree Priyadharsini.J, Vinay Sivaswamy

Kiren.J
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Email Id : kirenjackson@gmail.com

Vijayashree Priyadharsini. J
Assistant Professor, Department of Microbiology,
Saveetha dental college and hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Email id: vijayashreej.sdc@saveetha.com

Vinay Sivaswamy
Senior lecturer, Department of Prosthodontics
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Email id: ai.vynter@gmail.com

Corresponding author:
Dr.Vijayashree Priyadharsini. J
Assistant Professor,
Department of Microbiology,
Saveetha dental college and hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
162, PH Road, Chennai-600077,
Tamil Nadu, India
Contact Number: +9199441125984
Email id: vijayashreej.sdc@saveetha.com

ABSTRACT

Dental anomalies such as tooth agenesis are caused by disturbances and gene mutations that occur during odontogenesis. Human genetic variations have long been of researcher's interest as it is partly responsible for the inter-individual response to drugs, infections and several other phenotypes related to development and progression of the disease. Tooth development is an intricate process which involves complex interplay of genes acting in symphony to exhibit the trait. Mutations or genetic errors in the DNA sequences encoding proteins

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

involved in the process of odontogenesis have been identified in recent years. Some of the genes of prime importance are the homeobox genes which are known to play an important role in tooth development. Several genes such as *PAX, MSX, AXIN, DLX* have been implicated in the process of odontogenesis. Animal models used in earlier studies reported that *PAX9*-deficient knockout mice exhibit missing molars due to an arrest of tooth development at the bud stage. The aim of this review was to prepare an exhaustive collection of *PAX9* mutation panel reported to be associated with dental anomalies. The literature review will also provide a comprehensive understanding of variations observed in *PAX9* gene in association with several common dental anomalies.

Keywords: Homeobox genes, Mutation, Orofacial cleft, *PAX9*, Polymorphism, Tooth agenesis

INTRODUCTION:

Homeobox genes are transcription factors that are key regulators of developmental processes such as regional specification, patterning and differentiation. The first homeobox genes were identified in *Drosophila* and homologous genes were remarkedly found in all animal species, fungi and plants. The dentition is derived from the first branchial arch, where complex interactions between the stomodeal epithelium (derived from the ectoderm) and the underlying mesenchyme derived from cranial neural crest (CNC, migrating from the neuroectoderm during early neurulation) drive the development of the appendages. Odontogenesis begins from the thickening and invagination of the stomodeal epithelium forming the dental placode. The dental placode gets invaginated into the dental mesenchyme and forms a tooth bud. The mesenchyme, then proliferates and condensates around the tooth bud. At the cap stage, the invaginated epithelium expands laterally and covers the condensed mesenchyme that will become the dental papilla. Different structures can be distinguished in the dental epithelium, including the enamel knot, which is known as an organizing center of tooth morphogenesis. At the late bell stage, cytodifferentiation starts: the cells of the internal enamel epithelium differentiate into ameloblasts (enamel-producing cells) while adjacent cells in the dental papilla differentiate into odontoblasts (dentine-producing cells). Our team has rich experience in research and we have collaborated with numerous authors over various topics in the past decade (1)(2–24).

Even though the origin of mandibular (lower jaw) and maxillary (upper jaw) teeth are histologically and morphologically identical, they involve different developmental pathways. The patterning of murine dentition was determined by the complex and specific distribution of homeobox genes in the first arch mesenchyme before the initiation of tooth formation [1] In dentition, these genes play an important role in the specific determination of incisors versus molars while they can also discriminate between maxillary and mandibular teeth. [2] During embryogenesis, *PAX* genes encode a family of transcription factors that play key roles. *PAX9* gene expression marks the prospective sites of tooth development and is maintained in the developing tooth mesenchyme thereafter. Preliminary analyses conducted [3] show that *Pax9* is vital for the tooth development to proceed beyond the bud stage.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

The basic steps in genetic mapping of a disease gene include identification of the mode of inheritance; genetic mapping of the disease gene; identification and screening of candidate genes; and evaluation of the functional consequences of the mutation(s) identified. The categories of mutations that can occur in human DNA are single base- pair change, deletion, insertion, inversion and chromosomal abnormalities. A disease mutation in a gene can ultimately affect the function of the encoded protein in many ways like transcription, mRNA stability, translation, or protein stability, localization, or function [4].

Paired-box gene 9 (*PAX9*) mutation is potentially associated with impaction among patients. The relationship between *PAX 9* polymorphism and the occurrence of maxillary canine impaction was analysed in a study [5]. The results of this study are presented in Table 1. The transcription factors involved in the development of dentition in humans is Paired box 9 (*PAX9)*. Mutations in *PAX9* gene influence the number, position and morphology of the teeth in an affected person. Numerous mutations have been reported by several researchers globally to discuss the association of *PAX9* mutations or polymorphisms with syndromic or nonsyndromic dental anomalies. The most common consequence of PAX9 gene mutation is the autosomal-dominant isolated (non-syndromic) oligodontia or hypodontia [6]. A comprehensive meta-analysis by Zhang et al, [7] evaluated the association between paired box 9 (*PAX9*) gene polymorphisms and tooth agenesis among isolated humans. The results of this study are summarized in Table 1.

Our institution is passionate about high quality evidence based research and has excelled in various fields ((19,20,25–33)A study was conducted to perform screening for mutations and/or polymorphisms in the critical regions of *PAX9* and *MSX1* genes. The screening results are shown in Table 1. The authors hypothesize that the lower expression of *PAX9* protein could have contributed to the development of tooth agenesis among affected subjects. [8] Mutations in *AXIN2, PAX9* and *MSX1* have been determined in families with dental agenesis. The absence of one or more primary or permanent teeth is considered dental agenesis in children. Data for congenital tooth agenesis prevalence vary between 0.3 and 11.3% for both males and females. The prevalence of congenital tooth agenesis was found to be higher in females than in males. [9]

Mutations in nine genes (*MSX1, PAX9, AXIN2, WNT10A, EDA, EDAR, EDARADD, NEMO* and *KRT17*) have been associated with non-syndromic oligodontia till today. The first and second genes to be identified in non-syndromic oligodontia were *MSX1* and *PAX9*. These two gene proteins are vital for the formation of the odontogenic potential of the mesenchyme. A study group [10] investigated six genes (*MSX1, PAX9, AXIN2, WNT10A, EDA and EDARADD*) in a patient with sporadic non-syndromic oligodontia. An extensive analysis of publicly accessible databases revealed 15 causative genes responsible for non-syndromic TA (tooth agenesis). Among 198 different mutations, about 15 genes are responsible for non-syndromic TA. The findings have shown new lights on the discovery of novel molecular mechanisms associated with tooth agenesis. [11]

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

Tooth agenesis affects the function and esthetics. In the patterning and morphogenesis of tooth and taste buds, *PAX9* plays a critical role. Mutations of *PAX9* occur in conditions like tooth agenesis. Familial analysis of nonsyndromic tooth agenesis of multiple Chinese populations were carried out using DNA sequencing. The genotype and phenotype investigations revealed 9 novel (as mentioned in Table 2) and 2 known heterozygous mutations in the *PAX9* gene among 120 probands. The clinical characteristics and the results of this study are presented in Table 2. The tooth agenesis was attributed to *PAX9* haploinsufficiency or loss of function of the genes, which was confirmed by functional analysis.[12] The discovery of genetic mutations that unlock the causes of non-syndromic tooth agenesis are being carried out. Interaction of several genes are involved in tooth development in relation to tooth epithelium and mesenchyme odontogenesis. Mutation of candidate genes *PAX9* and *MSX1* are identified as the main causes of hypodontia and oligodontia. A knockout mice model exhibiting PAX9 deficiency was reported to present with missing molars which resulted due to the arrest of bud phase during tooth development. [13] Murakami et al, stated that *PAX9* and *MSX1* play crucial roles in the development of permanent teeth at the bud stage, and their loss-of-function variants have been associated with congenital tooth agenesis [14]. Sequencing the coding regions of the *PAX9* and *MSX1* genes from nine patients with non-syndromic tooth agenesis was done. The clinical presentation resulted in this study is shown in Table 2.

PAX9 and tooth agenesis

Tooth agenesis may occur either in association with genetic syndromes, based on the presence of inherited abnormalities, or as a non-syndromic trait, with both familiar and sporadic cases. A study was conducted by direct Sanger sequencing of *PAX9* and *MSX1* genes. The results of the study are shown in Table 2. It is confirmed that the *WNT10A* played a major role in tooth agenesis and genetic heterogeneity of this disease. *WES* analysis may be an effective approach to search for genetic variants in familial or sporadic tooth agenesis. [15]

A study was conducted to find any association between *PAX9* promoter polymorphisms and the development of hypodontia. The findings of the study are shown in Table 2. The researchers concluded that the promoter polymorphisms viz., *rs2073247* and *rs2073244* of *PAX9* might play a role in the development of hypodontia among the Jordanian population.[16] An association between mutations in *MSX1, PAX9, EDA, AXIN2, WNT10A, WNT10B* and *LRP6* and human tooth agenesis have been identified by researchers using Sanger sequencing of the candidate genes (as shown in Table 2). The 2 novel mutations identified were further analyzed using structure modeling using computational tools. The mutations were found to result in conformational changes in the *MSX1* homeodomain.[17]. The results reported are shown in Table 2. A study [18] was performed with an objective to elucidate the genetic background of non-syndromic hypodontia (NSH). The NHS group was individually and in groups with frontal and lateral agenesis were assessed for single nucleotide variations. The results of the study are summarised in Table 2. Rodrigues et al, in their research article stated that tooth agenesis may occur in the form of an isolated familial or sporadic anomaly or in association with other genetic diseases like cleft lip/palate. A cross-

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

sectional, multi-centre, genetic study was conducted among orthodontic Brazilians patients to assess if genetic polymorphisms in tooth agenesis (TA)-related genes are associated with craniofacial morphological patterns. The results of the study are summarised in Table 2. The researchers concluded that the genetic polymorphism rs1893047 in *FGF3* might contribute to variations in the craniofacial sagittal pattern. [19].

In non-syndromic agenesis, gene mutations are said to be the cause [20]. The mutations of *PAX9, MSX1*, and *AXIN2* genes are responsible for tooth development. In a case-control study of 306 unrelated Portuguese individuals, single nucleotide polymorphisms in the *PAX9* gene were associated with a high risk of maxillary lateral incisor agenesis. Non-syndromic tooth agenesis is most likely caused by mutations of *MSX1, PAX9, AXIN2, and WNT10A* genes. As the phenotypes of both oligodontia and Regional Odontodysplasia (RO) co-occur in one Finnish family, the study was conducted [21] to investigate the genetic aetiology of the two conditions. A mutation screening of the genes was performed for the family members of a RO patient and family history of oligodontia. The study results are shown in Table 2. In humans, heterozygous mutations in *PAX9* have been associated with non-syndromic tooth agenesis, predominantly in the molars [22]. Novel mutations identified by the group was a triplet deletion and a missense mutation in *PAX9* gene identified in two Japanese patients who presented with non-syndromic tooth agenesis. The results are discussed in Table 2. The decline in the gene expression was suggestive of haploinsufficiency of *PAX9* gene.

Missing permanent molars and second premolars are found to be caused by defects in mutations of *PAX9* and/or *MSX1* genes. It was also found in few studies that *PAX9* and *MSX1* gene mutations may change tooth size. All of these factors were investigated in the study conducted by [23]. The study results are summarised in Table 2. *PAX9, MSX1, AXIN2, WNT10a*, and *EDA* genes mutation have been associated with tooth agenesis during tooth morphogenesis. Hlouskova et. al, also conducted a similar study to investigate the relationship between the *PAX9* gene variants and tooth agenesis in the Czech population. The results of the study are summarised in Table 2. The authors conclude that tooth agenesis among these patients is caused by mutations in regions different from *PAX9* exons analysed in this study. [24]

Pax9 gene and orofacial clefting

Children with oral clefts show dental anomalies, adding complexity to understanding the phenotypic spectrum of orofacial clefting. A study conducted by Howe et al, [25] showed that cases had higher rates of dental anomalies in the maxillary arch than did controls for primary and permanent dentitions but not in the mandible. The other clinical presentations seen in this study are summarised in Table 3. Cleft palate and/or lip is the most common human craniofacial malformations and caused by multiple genetic and environmental factors. A study was conducted on a well-characterized Pax9−/− mouse model with a consistent cleft palate phenotype to test small-molecule Wnt agonist therapies. The functional interactions and results are summarised in Table 3. [26] In the Pax9-mediated regulation of development of the secondary palate, Canonical Wnt signaling is vital. Jia et al, demonstrated that reduced expression of Axin2, a target of canonical Wnt signaling, was accompanied by Pax-9 deficient embryos who developed characteristics of cleft palate. The data also identified a

crucial role for canonical Wnt signaling in acting downstream of Pax9 to regulate palate morphogenesis. [27]

Pax9 gene and tooth impaction

Maxillary canines are the second-most commonly impacted teeth. Most of the impacted maxillary canines are impacted in the palates. About 40% of cases with palatal impaction of maxillary canines presented with agenesis of third molars. Polymorphisms in the *MSX1* and *PAX9* genes are found to be associated with Sporadic agenesis of third molars. A study conducted by [28], to evaluate the association between polymorphisms of *PAX9, MSX1* and palatally impacted canines. The study results are seen in Table 3. [28] The potential role of *PAX9* in squamous cell differentiation and carcinogenesis of the oro-esophageal epithelium have been discussed in previous studies. Not only does *PAX9* have an effect on tooth development, the decreased expression has been found to be associated with oro-esophageal squamous cell carcinoma. The down-regulation of the same has been associated with alcohol drinking.

Taken together, several studies have recently populated the polymorphism and mutation data of *PAX9* gene which could be of importance to the clinicians to trace the pattern of inheritance and deduce the probability of acquiring the disease. The team of researchers of our institution have carried out studies encompassing several topics pertaining to the clinical studies and genetic aspects of tooth agenesis, syndromes associated with orofacial clefting, periodontal and other dental infections [30-44]. The data available from the resources helped us in preparing the structure of the present literature review. More research into this field would open up new avenues for translating the experimental data into clinically relevant applications.

CONCLUSION:

This review has provided a comprehensive source of information related to *PAX9* gene functions and its association with several dental anomalies. The number, position and morphology of the teeth are influenced by mutations in *PAX9* gene in an affected individual. Numerous mutations reported in the gene so far have been associated with different types of dental agenesis and other dental defects. In the present review, the authors have summarized to the best of their knowledge, all known *PAX9* mutations associated with some common dental anomalies. The authors believe that this review would benefit dental students, dental practitioners in the application and dental practice.

AUTHOR'S CONTRIBUTIONS:

All the authors contributed to the design and implementation of the research, and to write the review and approved the final manuscript.

ACKNOWLEDGMENT:

The authors would like to acknowledge the help and support rendered by the department of Microbiology of Saveetha Dental College and hospitals and the management for constant assistance with the research.

CONFLICT OF INTEREST:

None

REFERENCES:

1.Thomas BL, Sharpe PT. Patterning of the murine dentition by homeobox genes. European Journal of Oral Sciences. 1998;106(Suppl 1):48–54.

2. Duverger O, Morasso MI. Role of homeobox genes in the patterning, specification, and differentiation of ectodermal appendages in mammals. J Cell Physiol. 2008;216(2):337- 346. doi:10.1002/jcp.21491

3. Laura Bull, Genetics, Mutations, and Polymorphisms - Madame Curie Bioscience Database, 2013.

4. Peters H, Neubüser A, Balling R. Pax genes and organogenesis: Pax9 meets tooth development. Eur J Oral Sci. 1998;106 Suppl 1:38- 43. doi:10.1111/j.1600-0722.1998.tb02151.x

5. Vitria EE, Tofani I, Kusdhany L, Bachtiar EW. Genotyping analysis of the Pax9 Gene in patients with maxillary canine impaction. F1000Res. 2019;8:254. Published 2019 Mar 5. doi:10.12688/f1000research.17147.1

6. Bonczek O, Balcar VJ, Šerý O. PAX9 gene mutations and tooth agenesis: A review. Clin Genet. 2017;92(5):467- 476. doi:10.1111/cge.12986

7. Zhang W, Qu HC, Zhang Y. PAX-9 polymorphism may be a risk factor for hypodontia: a meta-analysis. Genet Mol Res. 2014;13(4):9997- 10006. Published 2014 Nov 28. doi:10.4238/2014.November.28.4

8. Šerý O, Bonczek O, Hloušková A, et al. A screen of a large Czech cohort of oligodontia patients implicates a novel mutation in the PAX9 gene. Eur J Oral Sci. 2015;123(2):65- 71. doi:10.1111/eos.12170

9. Citak M, Cakici EB, Benkli YA, Cakici F, Bektas B, Buyuk SK. Dental anomalies in an orthodontic patient population with maxillary lateral incisor agenesis. Dental Press J Orthod. 2016;21(6):98- 102. doi:10.1590/2177-6709.21.6.098-102.oar

10. Liu H, Ding T, Zhan Y, Feng H. A Novel AXIN2 Missense Mutation Is Associated with Non-Syndromic Oligodontia. PLoS One. 2015;10(9):e0138221. Published 2015 Sep 25. doi:10.1371/journal.pone.0138221

11. Yu M, Wong SW, Han D, Cai T. Genetic analysis: Wnt and other pathways in nonsyndromic tooth agenesis. Oral Dis. 2019;25(3):646- 651. doi:10.1111/odi.12931

12. Wong SW, Han D, Zhang H, et al. Nine Novel PAX9 Mutations and a Distinct Tooth Agenesis Genotype-Phenotype. *J Dent Res*. 2018;97(2):155- 162. doi:10.1177/002203451772932.

13. Fauzi NH, Ardini YD, Zainuddin Z, Lestari W. A review on non-syndromic tooth agenesis associated with PAX9 mutations. Jpn Dent Sci Rev. 2018;54(1):30- 36.doi:10.1016/j.jdsr.2017.08.001

14. Murakami A, Yasuhira S, Mayama H, Miura H, Maesawa C, Satoh K. Characterization of PAX9 variant P20L identified in a Japanese family with tooth agenesis. PLoS One. 2017;12(10):e0186260. Published 2017 Oct 12. doi:10.1371/journal.pone.0186260

15. Salvi A, Giacopuzzi E, Bardellini E, et al. Mutation analysis by direct and whole exome sequencing in familial and sporadic tooth agenesis. Int J Mol Med. 2016;38(5):1338- 1348. doi:10.3892/ijmm.2016.2742
16. Abu-Siniyeh A, Khabour OF, Owais AI. The role of PAX9 promoter gene polymorphisms in causing hypodontia: a study in the Jordanian population. Appl Clin Genet. 2018;11:145- 149. Published 2018 Nov 21. doi:10.2147/TACG.S183212
17. Yang L, Liang J, Yue H, Bian Z. Two novel mutations in MSX1 causing oligodontia. PLoS One. 2020;15(1):e0227287. Published 2020 Jan 8. doi:10.1371/journal.pone.0227287
18. Mártha K, Kerekes Máthé B, Moldovan VG, Bănescu C. Study of rs12532, rs8670 Polymorphism of Msh Homeobox 1 (MSX1), rs61754301, rs4904155 Polymorphism of Paired Box Gene 9 (PAX9), and rs2240308 Polymorphism of Axis Inhibitor Protein 2 (AXIN2) Genes in Nonsyndromic Hypodontia. Biomed Res Int. 2019;2019:2183720. Published 2019 Nov 5. doi:10.1155/2019/2183720
19. Rodrigues AS, Teixeira EC, Antunes LS, et al. Association between craniofacial morphological patterns and tooth agenesis-related genes. Prog Orthod. 2020;21(1):9. Published 2020 Apr 6. doi:10.1186/s40510-020-00309-5
20. Arandi NZ, Mustafa S. Maxillary lateral incisor agenesis; a retrospective cross-sectional study. Saudi Dent J. 2018;30(2):155- 160. doi:10.1016/j.sdentj.2017.12.006
21. Koskinen S, Keski-Filppula R, Alapulli H, Nieminen P, Anttonen V. Familial oligodontia and regional odontodysplasia associated with a PAX9 initiation codon mutation. Clin Oral Investig. 2019;23(11):4107- 4111. doi:10.1007/s00784-019-02849-5
22.Mitsui SN, Yasue A, Masuda K, et al. Novel PAX9 mutations cause non-syndromic tooth agenesis. J Dent Res. 2014;93(3):245- 249. doi:10.1177/0022034513519801
23. Kirac D, Eraydin F, Avcilar T, et al. Effects of PAX9 and MSX1 gene variants to hypodontia, tooth size and the type of congenitally missing teeth. Cell Mol Biol (Noisy-le-grand). 2016;62(13):78- 84. Published 2016 Nov 30. doi:10.14715/cmb/2016.62.13.14
24. Hlousková A, Bonczek O, Izakovicová-Hollá L, et al. Novel PAX9 gene polymorphisms and mutations and susceptibility to tooth agenesis in the Czech population. Neuro Endocrinol Lett. 2015;36(5):452- 457.
25. Howe BJ, Cooper ME, Vieira AR, et al. Spectrum of Dental Phenotypes in Nonsyndromic Orofacial Clefting. J Dent Res. 2015;94(7):905- 912. doi:10.1177/0022034515588281
26. Jia S, Zhou J, Fanelli C, et al. Small-molecule Wnt agonists correct cleft palates in Pax9 mutant mice in utero. Development. 2017;144(20):3819- 3828. doi:10.1242/dev.157750
27. Li C, Lan Y, Krumlauf R, Jiang R. Modulating Wnt Signaling Rescues Palate Morphogenesis in Pax9 Mutant Mice. J Dent Res. 2017;96(11):1273- 1281. doi:10.1177/0022034517719865
28. Devi MSA, Padmanabhan S. Role of polymorphisms of MSX1 and PAX9 genes in palatal impaction of maxillary canines. J Orthod. 2019;46(1):14- 19. doi:10.1177/1465312518820537
29. Xiong Z, Ren S, Chen H, et al. PAX9 regulates squamous cell differentiation and carcinogenesis in the oro-oesophageal epithelium. J Pathol. 2018;244(2):164- 175. doi:10.1002/path.4998

30. Smiline Girija AS, Vijayashree Priyadharsini J, Paramasivam Arumugam. CLSI based antibiogram profile and the detection of MDR and XDR strains of Acinetobacter baumannii isolated from urine samples. Medical Journal of the Islamic Republic of Iran. 2019; 33(3); 11- 16
31. Smiline Girija AS, Vijayashree Priyadharsini J, Paramasivam Arumugam. Prevalence of VIM and GIM producing Acinetobacter baumannii from patients with severe UTI. Acta microbiologica et immunologica Hungarica 2018; 16(8): 1- 12.
32. Smiline Girija AS, Vijayashree Priyadharsini J, Paramasivam Arumugam. Molecular characterization of plasmid encoded blaTEM, blaSHV and blaCTX-M among extended spectrum β-lactamases [ESBL's] producing Acinetobacter baumannii. British Journal of biomedical sciences. 2018; 16(8): 1-3
33. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020;43(2): 153- 154.
34. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of Acinetobacter baumannii as an oro-dental pathogen and its drug resistance gene profile - An in silico approach. Heliyon. 2018;4(12):e01051.
35. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Arch Oral Biol. 2018;94:93- 98.
36. Sohaib Shahzan M, Smiline Girija AS, Vijayashree Priyadharsini J. A computational study targeting the mutated L321F of ERG11 gene in C. albicans, associated with fluconazole resistance with bioactive compounds from Acacianilotica. J Mycol Med. 2019;29(4): 303- 309. doi:10.1016/j.mycmed. 2019.100899
37. Ashwin K S, Muralidharan N P. Vancomycin-resistant enterococcus (VRE) vs Methicillin-resistant Staphylococcus Aureus (MRSA). Indian J Med Microbiol 2015;33, Suppl S1:166-7
38. Smiline Girija AS, Vijayashree Priyadhasini J, Paramasivam Arumugam. Plasmid encoded dfrA-1, dfrA-5, sul 1 and sul 2 mediated trimethoprim sulfamethoxazole [TMP-SMX] resistance among Acinetobacter baumannii isolated from urine samaples of patients with severe UTI. Journal of Global Antimicrobial Resistance. 2019: 17: 145-`46
39. Renuka, S., & Muralidharan, N. P. (2017, February 1). Comparison in benefits of herbal mouthwashes with chlorhexidine mouthwash: A review. Asian Journal of Pharmaceutical and Clinical Research
40. Shahana, R.Y., Muralidharan, N.P., Efficacy of mouth rinse in maintaining oral health of patients attending orthodontic clinics, Research Journal of Pharmacy and Technology,9(11), 1991-1993
41. Marickar R, Geetha R V, Neelakandan P, Kurian M,Efficacy of contemporary and novel intracanal medicaments against Enterococcus faecalis, Journal of Clinical Pediatric Dentistry (2014) 39(1) 47-50
42. Ashwatha Pratha, A., Geetha, R.V., Awareness on hepatitis-B vaccination among dental students-A questionnaire survey, Research Journal of Pharmacy and Technology, vol 10(5)1360-1362

43. Vaishali, M., Geetha, R.V., Antibacterial activity of orange peel oil on Streptococcus mutans and enterococcus- an in-vitro study, Research Journal of Pharmacy and Technology, vol 11(2) 513- 514.
44. Maajida Aafreen, M., Geetha, R.V., Thangavelu, L., Evaluation of anti- inflammatory action of laurus nobilis-an in vitro study, International Journal of Research in Pharmaceutical Sciences, vol10(2),1209-1213.

Table legends:
Table 1:Studies demonstrating the association of Paired box 9 (*PAX9*) gene polymorphisms or mutations with different dental abnormalities.
Table 2: Studies demonstrating the association of *PAX9* and tooth agenesis
Table 3: Studies demonstrating the association of *PAX9* and cleft lip/palate & other dental anomalies

Table 1: Studies demonstrating the association of Paired box 9 (*PAX9*) gene polymorphisms or mutations with different dental abnormalities.

Author Name / Year	Study population	Mutation/variation identified	Recessive/dominant-Phenotype	Clinical presentation/Results
Vitria et. al, 2019	Patients with and without maxillary canine impaction	Single nucleotide polymorphism (SNP) genotypes	Maxillary canine impaction	The presence of SNPs 3 and 4 is associated with increased likelihood of suffering from maxillary canine impaction. Patients with a CC genotype at SNP 3 and a CC genotype at SNP 4 were more likely to have maxillary canine impaction. All SNPs were located in exon 3 of *PAX 9* and in the region sequenced by the primer pair

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

Zhang et.al, 2014	Case-control population with a total of 855 hypodontia cases and 1201 healthy controls.	G allele and G carrier (AG + GG) of A1031G	Hypodontia	4 genetic sites of the PAX9 gene involved in hypodontia cases, of which 3 sites may be risk factors and 1 may have a protective role. -C allele and C carrier showed no significant association with oligodontia. -The G allele and G carrier in the PAX9 gene were not related factors. -The genotype (AG + GG) of IVS2-54 in the PAX9 gene may be a protective factor for oligodontia -No significant differences were found in the allele frequency of IVS2-54 in the PAX9 polymorphism between controls and subjects with sporadic tooth agenesis.
Sery et. al ,2015	270 individuals with tooth agenesis and in 30 healthy subjects of Czech origin	*PAX9* and *MSX1* genes	Oligodontia	Screening results revealed a previously unknown heterozygous g.9527G>T mutation in the *PAX9* gene in monozygotic twins with oligodontia and three additional affected family members. The same variant was not found in healthy relatives.

Table 2: Studies demonstrating the association of *PAX9* and tooth agenesis

Author Name / Year	Study population	Mutation/variation identified	Recessive/dominant-Phenotype	Clinical presentation/Results

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

Wong et. al, 2018	Chinese families with non-syndromic (NS) tooth agenesis	c.140G>C, c.167T>A, c.332G>C, c.194C>A, c.271A>T, c.146delC, c.185_189dup, c.256_262dup, c.592delG,2 known heterozygous mutations in the *PAX9* gene among 120 probands	Tooth agenesis	Families segregating a *PAX9* mutation reveal that all affected individuals were missing the mandibular second molar and maxillary central incisors are most susceptible to microdontia. A significant reduction of bitter taste perception was seen among 3 individuals harbouring *PAX9* mutations
Murakami et. al. 2017	nine patients with non-syndromic tooth agenesis	*P20L*, of *PAX9* in a single familial case involving three patients in two generations	Tooth agenesis	Defects were shown primarily in the first and second molars, which is typical for cases attributable to *PAX9* mutation.
Salvi et.al, 2016	16 individuals affected by tooth agenesis	None	Tooth agenesis	Two individuals were siblings and also carried a heterozygous functional variant in *EDAR*-associated death domain (*EDARADD*) (rs114632254), another disease-causing gene

Abu-Siniyeh et. al, 2018	72 unrelated subjects with hypodontia and 72 normal healthy unrelated control individuals in Jordan	*PAX9* c.-912T>C (rs2073247) and c.-1031G>A (rs2073244) promoter polymorphisms	Hypodontia	Hypodontia group had a significantly higher -1031GG genotype (P<0.01) and a significantly lower -912TC genotype (P<0.01) compared with the control group. The transcriptional activity of *PAX9* gene is affected by polymorphisms in the promoter region of this gene and is associated with hypodontia phenotype.
Yang et .al,2020	Two unrelated individuals with non-syndromic tooth agenesis and their families	A missense mutation c.572 T>C and a frameshift mutation c.590_594 dup *TGTCC*	Tooth agenesis	There is a correlation between the observed phenotypes and alterations in hydrogen bond formation, thereby potentially affecting protein binding
Martha et al, 2019	97 NSH subjects (70 females and 27 males) from patients referred to orthodontic treatment, and matched to each NSH subject a control by age and sex.	None	Non-syndromic hypodontia	The variant genotype and variant T allele of the *MSX1* rs8670 SNP increased the risk of hypodontia in the studied population. The presence of the variant A allele of *AXIN2* rs2240308 is associated with frontal agenesis but not with lateral agenesis.
Rodrigues et. al ,2020	594 orthodontic Brazilians		Tooth agenesis	Genotypes and allele distributions for the FGF3 rs1893047 were significantly different according to the skeletal malocclusion. Carrying at least one G allele increased in more than two times the chance of presenting skeletal class III malocclusion. No association between another skeletal craniofacial pattern and some polymorphism assessed was

				found in the study.
Koskinen et, al. 2019	Family members of a Regional odontodysplasia patient and with family history of oligodontia	*MSX1*, *PAX9*, *AXIN2*, and *WNT10A*	Regional odontodysplasia	An initiation codon mutation of the *PAX9* gene was found in the proband and segregating with oligodontia in the family. The etiology of regional odontodysplasia (RO) may be genetic and the same genes can be involved both in RO and tooth agenesis. The results gave new insights into the aetiology of regional odontodysplasia

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

Mitsui et al, (2014)	Two unrelated Japanese patients with non-syndromic tooth agenesis	2 novel mutations in the paired domain of *PAX9*, a three-nucleotide deletion (73-75 delATC) and a missense mutation (*C146T*)	non-syndromic tooth agenesis	The individual with the 73-75del ATC mutation was missing all maxillary molars and mandibular second and third molars. The individual with the *C146T* mutation was missing the mandibular central incisors, maxillary second premolars, and first molars, along with all second and third molars. Both mutations affected amino acids that are highly conserved among different species and are critical for DNA binding. When both mutants were transfected to COS7 cells, nuclear localization of *PAX9* proteins was not affected.
Kirac et al, 2016	31 patients and 30 controls	*PAX9* and/or *MSX1*	Hypodontia	22 variations were detected in *PAX9* in which 18 of them are novel. In addition, 7 variations were found in *MSX1* in which 5 of them are novel and one of them lead to amino acid change. Statistically significant relations were found between detected variations and tooth sizes.
Hlousková et. al, 2015	Patients with tooth agenesis and controls among Czech population	*PAX9*	Tooth agenesis	Several novel variants in the *PAX9* gene were found. In subjects with full dentition, polymorphisms were observed.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6230 - 6249
Received 16 February 2021; Accepted 08 March 2021.

Table 3: Studies demonstrating the association of *PAX9* and Cleft Lip/Palate & other dental anomalies

Author Name / Year	Study population	Mutation/variation identified	Recessive/dominant-Phenotype	Clinical presentation/Results
Howe et. al, 2015	3,811 individuals; 660 cases with clefts, 1,922 unaffected relatives, and 1,229 controls.	None	Orofacial clefting, Agenesis, tooth displacement	Dental anomalies were more found in cleft lip with cleft palate than other cleft types. Agenesis and tooth displacements were the most common dental anomalies in case probands for primary and permanent dentitions. Compared with controls, unaffected siblings and parents showed an increase in anomalies of the maxillary permanent dentition.
Jia et. al,2017	well-characterized Pax9-/- mouse model	None	Cleft palate	The functional interactions between Pax9 and Dkk1 were shown by the genetic rescue of secondary palate clefts in Pax9-/-Dkk1f/+;Wnt1Cre embryos. The controlled intravenous delivery of small-molecule Wnt agonists (Dkk inhibitors) into pregnant Pax9+/- mice restored Wnt signaling. This has led to the growth and fusion of palatal shelves, as shown by an increase in cell proliferation and osteogenesis in utero,

				while other organ defects remain uncorrected
Devi et .al, 2019	Random population sample of fifty individuals with palatally impacted maxillary canines and 50 gender and age-matched controls	*MSX1* and *PAX9* genes	Palatal impaction of maxillary canines	Single nucleotide polymorphisms *[MSX1]* and *[PAX9]* showed a statistically significant association with palatal impaction of maxillary canines. The study results suggested that polymorphisms of genes *MSX1* and *PAX9* are positively associated with palatal impaction of maxillary canines

. Subramanyam D, Gurunathan D, Gaayathri R, Vishnu Priya V. Comparative evaluation of salivary malondialdehyde levels as a marker of lipid peroxidation in early childhood caries. Eur J Dent. 2018 Jan;12(1):67–70.

2. Panchal V, Jeevanandan G, Subramanian E. Comparison of instrumentation time and obturation quality between hand K-file, H-files, and rotary Kedo-S in root canal treatment of primary teeth: A randomized controlled trial. J Indian Soc Pedod Prev Dent. 2019 Jan;37(1):75–9.

3. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol. 2018 Oct;117:91–5.

4. Abhinav RP, Selvarasu K, Maheswari GU, Taltia AA. The Patterns and Etiology of Maxillofacial Trauma in South India. Ann Maxillofac Surg. 2019 Jan;9(1):114–7.

5. Marimuthu M, Andiappan M, Wahab A, Muthusekhar MR, Balakrishnan A, Shanmugam S. Canonical Wnt pathway gene expression and their clinical correlation in oral squamous cell carcinoma. Indian J Dent Res. 2018 May;29(3):291–7.

6. Kavarthapu A, Thamaraiselvan M. Assessing the variation in course and position of inferior alveolar nerve among south Indian population: A cone beam computed tomographic study. Indian J Dent Res. 2018 Jul;29(4):405–9.

7. Ramesh A, Vellayappan R, Ravi S, Gurumoorthy K. Esthetic lip repositioning: A cosmetic approach for correction of gummy smile - A case series. J Indian Soc Periodontol. 2019 May;23(3):290–4.

8. Sweta VR, Abhinav RP, Ramesh A. Role of virtual reality in pain perception of patients following the administration of local anesthesia. Ann Maxillofac Surg. 2019 Jan;9(1):110–3.

9. Felicita AS. Orthodontic extrusion of Ellis Class VIII fracture of maxillary lateral incisor - The sling shot method. Saudi Dent J. 2018 Jul;30(3):265–9.

10. Rao TD, Kumar MPS. Analgesic efficacy of paracetamol vs ketorolac after dental extractions. J Adv Pharm Technol Res. 2018;11(8):3375.

11. Fluoride, fluoridated toothpaste efficacy and its safety in children - review. Int J Pharm Res [Internet]. 2018 Oct 1;10(04). Available from: http://www.ijpronline.com/ViewArticleDetail.aspx?ID=7041

12. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol Mech Methods. 2019 May;29(4):276–90.

13. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020 Feb;43(2):153–4.

14. Mehta M, Deeksha, Tewari D, Gupta G, Awasthi R, Singh H, et al. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact. 2019 Aug 1;308:206–15.

15. Padavala S, Sukumaran G. Molar Incisor Hypomineralization and Its Prevalence. Contemp Clin Dent. 2018 Sep;9(Suppl 2):S246–50.

16. Pandian KS, Krishnan S, Kumar SA. Angular photogrammetric analysis of the soft-

tissue facial profile of Indian adults. Indian J Dent Res. 2018 Mar;29(2):137–43.

17. Nair M, Jeevanandan G, R V, Emg S. Comparative evaluation of post-operative pain after pulpectomy with k-files, kedo-s files and mtwo files in deciduous molars -a randomized clinical trial. Braz Dent Sci. 2018 Oct 24;21(4):411.

18. Ke Y, Al Aboody MS, Alturaiki W, Alsagaby SA, Alfaiz FA, Veeraraghavan VP, et al. Photosynthesized gold nanoparticles from Catharanthus roseus induces caspase-mediated apoptosis in cervical cancer cells (HeLa). Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):1938–46.

19. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med. 2019 Apr;48(4):299–306.

20. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Arch Oral Biol. 2018 Oct;94:93–8.

21. Krishnan RP, Ramani P, Sherlin HJ, Sukumaran G, Ramasubramanian A, Jayaraj G, et al. Surgical Specimen Handover from Operation Theater to Laboratory: A Survey. Ann Maxillofac Surg. 2018 Jul;8(2):234–8.

22. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. Arab J Gastroenterol. 2018 Jun;19(2):56–64.

23. Palati S, Ramani P, Shrelin HJ, Sukumaran G, Ramasubramanian A, Don KR, et al. Knowledge, Attitude and practice survey on the perspective of oral lesions and dental health in geriatric patients residing in old age homes. Indian J Dent Res. 2020 Jan;31(1):22–5.

24. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from induce growth-inhibitory effect on melanoma cells (B16). Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3297–305.

25. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019 Dec;90(12):1441–8.

26. Pc J, Marimuthu T, Devadoss P. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. Clin Implant Dent Relat Res [Internet]. 2018; Available from: https://europepmc.org/article/med/29624863

27. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018 Oct;89(10):1241–8.

28. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. Clin Oral Investig. 2019 Sep;23(9):3543–50.

29. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019 Feb;48(2):115–21.

30. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: Randomized controlled trial. Clin Oral Investig. 2020;1–6.

31. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285–6.

32. R H, Hannah R, Ramani P, Ramanathan A, R JM, Gheena S, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene [Internet]. Vol. 130, Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2020. p. 306–12. Available from: http://dx.doi.org/10.1016/j.oooo.2020.06.021

33. Chandrasekar R, Chandrasekhar S, Sundari KKS, Ravi P. Development and validation of a formula for objective assessment of cervical vertebral bone age. Prog Orthod. 2020 Oct 12;21(1):38.