ROLE OF PAX9 AND MSX1 GENE POLYMORPHISM IN CONGENITALLY MISSING LATERAL INCISORS – A SYSTEMATIC REVIEW

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Covering letter

To,

The Editor

Sub: Submission of manuscript for publication

Dear Sir,

We intend to publish an article entitled **"ROLE OF PAX9 AND MSX1 GENE POLYMORPHISM IN CONGENITALLY MISSING LATERAL INCISORS – A SYSTEMATIC REVIEW**" in your esteemed journal as an Original article.Once the article is published, the undersigned author(s) hereby assign(s) all copyright of the manuscript **"ROLE OF PAX9 AND MSX1 GENE POLYMORPHISM IN CONGENITALLY MISSING LATERAL INCISORS – A SYSTEMATIC REVIEW**" to your esteemed journal. The undersigned author(s) warrant(s) that this is an original article and that it does not infringe any copyright or other third-party proprietary rights, it is not under consideration for publication by another journal and has not been published previously, be it in print or electronically. I (we) hereby sign this statement and accept full responsibility for the publication of the aforesaid article.

Thanking you,

Yours sincerely,

(Ashwin Mathew George)

Running Head: Role of AX9 and MSX1 gene pleomorphism in lateral incisor agenesis

ABSTRACT:

Aim:
The aim of this study was to evaluate and review articles and researches done on the polymorphism of PAX9 and MSX1 genes in congenitally missing lateral incisors.

Materials and methods:
A thorough search of the electronic database through Cochrane library, PubMed central, LILACS and BMC and manual hand searching of orthodontic journals was done till March 2020. The keywords included in the search were: "Genetics", "lateral incisors", "agenesis", "PAX9", "MSX1". The studies were selected as per the PRISMA guidelines. Articles were shortlisted based on the exclusion and inclusion criterias. Of all the obtained results, 12 studies were selected based on the inclusion criteria and they were analysed for the role of polymorphism of PAX9 and MSX1 genes in the congenitally missing lateral incisors.

Results:
Results of this systematic study show an association of PAX9, EDA and WNT10A gene pleomorphism with congenitally missing lateral incisors. MSX1 gene however is not shown to have an association with lateral incisor agenesis.

Conclusion:
This systematic review has provided a clear view on the role of the pleomorphism of different genes on the agenesis of lateral incisors, which will help the reader understand the problem better and handle it with better precision. Future studies are required to further conduct more randomised controlled trials, which will provide more concrete evidence to the claims.

Clinical significance:
Maxillary lateral incisors are the third most common congenitally missing teeth in the human dentition, preceded only by the third molars and mandibular second premolars The cause of lateral incisor agenesis is thought to be rooted to a genetic etiology.

KEYWORDS:

Agenesis, genetics, genotyping, lateral incisors, MSX1, PAX9.

INTRODUCTION:

"The eye sees only what the mind is prepared to comprehend" were the words of a French philosopher Henri Bergson. Rightly so, this holds true for almost all medical and dental related problems that require attention for treatment. Only by knowing what is wrong in the first place can a dental professional provide a remedy or a solution. It becomes imperative for a dentist to know the exact etiology of every dental defect as it will aid in formulation of a valid and appropriate treatment plan. Many of these problems are seeded by their etiology and just by rectifying the etiology, the problem is often addressed and solved.

One of the most commonly encountered forms of anomalies in dentistry is aplasia of permanent teeth accounting for 2.6% to 11.3% of the cases.(1) It is more often manifested in the form of hypodontia or oligodontia.(2) Missing teeth are usually managed either by virtue of a prosthesis or by closing spaces using orthodontic therapy. Lateral incisors are the second most common tooth found to be congenitally missing, preceded by the mandibular second premolars.(3) Maxillary lateral incisor agenesis (MLIA)

specifically accounts to about 0.8% - 4.25% of the population affected globally.(4) Its positioning in the esthetic zone of the oral cavity makes it a necessity to address the agenesis of lateral incisors with prompt action.

Genetics is one of the contributing factors to many malocclusion, other related conditions and features of the body. Tooth development as such is also determined by genetics. Infact, about 200 genes are expressed during the stages of tooth development.(5) Amongst the genes responsible for agenesis of teeth in humans, PAX9 (paired box gene 9) and MSX1 (muscle segment homeobox 1) are the most commonly found genes to be associated with tooth agenesis. Other genes responsible for congenitally missing teeth are EDA (ectodysplasin A), AXIN2 (axis inhibition protein 2) and WNT10A (Wingless-Type MMTV Integration Site Family, Member 10A).(6,7)

Anodontia or tooth agenesis can either occur as an isolated incident or as a feature associated with a syndrome. This explains the genetic component of the congenitally missing dental counterpart. Dental agenesis is usually of autosomal dominant inheritance type, although cases of autosomal recessive and X linked inheritance have also been reported.(4,8)

Hence, the purpose of this review was to provide a systematic approach for the review of literature that is pertinent to the genes such as PAX9 and MSX1 and their influence on lateral incisor agenesis.

MATERIALS AND METHODS:

The systematic review has been written as per the guidelines provided by the PRISMA checklist. All the articles published until the year of March 2020 about the role of genetics on the agenesis of lateral incisors were searched on the electronic databases such as PubMed, Cochrane library, BMC and LILACS. The articles were gathered by utilizing the advanced search column provided in these databases. The following keywords were used to search for the articles pertaining to the topic of this systematic review: “genetics”, “lateral incisors”, “agenesis”, “PAX9”, “MSX1”.
Randomised controlled trials, prospective studies, retrospective studies, cross sectional studies, cohort studies, literature reviews and case reports on the role of genetics on the agenesis of lateral incisors were included for this systematic review.
The abstracts of the studies were checked prior to shortlisting it for the systematic review. Duplicates were eliminated from the study. Only those articles that fulfilled the requirements for this study were retrieved. The retrieved articles were further analysed and only those articles that satisfied the inclusion criteria were analysed in depth for this systematic review, to collect relevant data. The study design, method of data collection and assessment of all the selected studies were recorded.

Inclusion criteria:

- Genetic studies evaluating the role of PAX9, MSX1 and any other gene on the agenesis of permanent lateral incisors.
- Prospective studies, retrospective studies, cross sectional studies, cohort studies and case reports.
- Studies with proper statistical analysis.

Exclusion criteria:

- Animal studies
- Prevalence studies
- Review articles

- Studies conducted on syndromic cases and cleft patients.

A total of 202 articles were obtained from the search engines by using the keywords: “genetics”, “lateral incisors”, “agenesis”, “PAX9”, “MSX1”. 3 articles were obtained from manual search from various dental journals.

Out of these, 42 articles resulted once the duplicates were removed. After assessing the articles for its eligibility, 12 articles were selected. Again 6 articles were removed as they did not satisfy the inclusion criteria. Finally, 6 articles were selected for appraisal in this study.
The characteristics of the studies involved were designed for the type of study, study setting, criteria for participants, sample size, variables and outcomes measured (Figure 1).

RESULTS:

The various stages of the process of study selection followed in this systematic review is shown in (Table 1). For this study, 205 articles were selected by searching through four electronic databases and other sources, 42 articles were examined for the removal of duplicates by screening of titles and abstracts. Only 12 articles seemed to be eligible for inclusion in the study and were hence included for full text reading. 6 articles were removed due to the reasons mentioned in (Table 2). 6 articles were finally included from 205 reports as they fulfilled the required inclusion criteria of the study.

DISCUSSION:

Previously, our team had conducted numerous clinical trials (9), in vitro studies (10–15)(16,17) and a couple of prospective studies (18–22) over the past 5 years. Now we are focusing on systematic reviews to provide higher levels of evidence in the field of research. The idea for this systematic review stemmed from the current interest in our community on the topic of lateral incisor agenesis.

The studies included in this systematic review evaluated the role of PAX9 and MSX1 gene pleomorphism and its role in agenesis of permanent lateral incisors. Almost all the studies revealed a positive correlation between the PAX9 gene and lateral incisor agenesis, except one study conducted by Mostowska A et al. where he concluded that MSX1 and PAX9 gene were not involved in the agenesis of lateral incisors, but it is the mutation of the gene WNT10A that is shown to have a positive correlation. On the other hand, almost all the studies show that MSX1 gene plays no role or has no significance in the agenesis of lateral incisors. Whereas, WNT10A and EDA gene has been shown to have an influence on the missing lateral incisors in multiple studies. A brief overview of all the findings of the final shortlisted studies in this systematic review is tabulated in (Table 1).

Lateral incisors are one of the most commonly missing group teeth in the human dentition. Maxillary lateral incisor agenesis (MLIA) can be considered as a separate clinical and genetic entity as per Pinho, who conducted studies on families with missing lateral incisors. As per his study, the probability of a first degree family member, with a proband relative having MLIA, to exhibit the same condition is as much as 15 folds as compared to the normal counterpart. They have also stated that the most common manifestation in these affected proband’s relatives is only MLIA, which is then followed by microdontia of the opposite side.(23)

PAX9 (paired box genes 9) and MSX1 (muscle segment homeobox genes 1) are two genes of significant importance to the development of teeth. They belong to a subfamily of homeobox genes which are localised to areas of condensing embryonic connective tissues / ectomesenchyme in the tooth bud.(24,25) Experiments on mices involving targeted deletion of a specific gene have helped researchers get into the

depths of the genetic cause for tooth agenesis. Such targeted deletion of selected genes in a knockout mice has led to defect or lack of tooth development, hinting at the role of that specific gene in tooth development.(26) These findings show the need for MSX1 and PAX9 gene in the normal development of dentition. MSX1 has been shown to have an influence on the agenesis of mandibular second premolar and mandibular first premolar. On the other hand, PAX9 is involved with the agenesis of incisors, second premolars and all permanent molars, with second molar agenesis being its distinguishing feature from an MSX1 defect.(27)

EDA (ectodysplasin A), a protein that belongs to the TNF ligand family has also been shown to have an association with tooth agenesis. Unlike PAX9 and MSX1, EDA is more specific to the development of incisors and canines. This is evident as the defect in EDA gene has shown the agenesis of central incisors, lateral incisors and canines, leaving the molars and premolars intact.(28)

WNT10A (Wingless-Type MMTV Integration Site Family, Member 10A) is a gene that encodes the Wnt-10a protein in humans. These genes provide instructions which are vital for the production of certain proteins that act as chemical signaling pathways in the body. Recent studies have shown WNT10A gene's association with the agenesis of lateral incisors.(29)

Alves-Ferreira et al in his study had taken 8 genes, namely: MSX1, PAX9, AXIN2, EDA, SPRY2, TGFA, SPRY4, and WNT10A and had performed one of the largest case control study in order to identify the gene responsible for MLIA susceptibility. In his study, he had found PAX9, EDA, SPRY2, SPRY4, and WNT10A to be risk factors for MLIA. MSX1-TGFA, AXIN2-TGFA, and SPRY2-SPRY4 gene pairs were shown to have a strong synergistic association with MLIA.(30)

Wang J et al in his study, tried to gain insight into the role of mutations of PAX9, MSX1 and AXIN2 in oligodontia phenotypes in a Chinese family. As per his findings, he reported that the mutation of A240P of PAX9 gene in the proband and the family member was responsible for oligodontia. No mutations were seen in MSX1 and AXIN2 genes of the proband or the relatives, suggesting their lack of association.(31)

Pinho T et al., evaluated the possible association between mutations in MSX1, PAX9, and the MLIA phenotype in patients of Portuguese origin in which nucleotide alterations were not detected in the coding region of MSX1 gene. Polymorphism was however found in the PAX9 gene, leading to transition of G718 to C, implying a change of alanine 240 for proline.(32)

The association of the EDA gene was shown by Han D et al., in his study, that showed mutation at 8 positions in the exon 8 of the EDA gene. Congenitally missing maxillary and mandibular central and lateral incisors with a higher possibility of persistence of maxillary and mandibular first permanent molar, suggested the presence of EDA mutation.

The only study in this systematic review to show an association between MSX1 gene pleomorphism and MLIA is the one conducted by Boeira Junior et al, in which polymorphism in *6C>T was identified in all three affected family members. Since *6C>T polymorphism is quite common, additional genes must be evaluated to determine the true validity of the study.(33)

Mostowska et al in his study showed the association of WNT10A with MLIA as mutation was seen in 5 out of 20 patients. He found no association of MSX1 and PAX9 gene with MLIA in his study.(29) However, the association of PAX9 and MSX1 gene with MLIA cannot be ruled out as two intronic variants of PAX9 have been shown to have an association with MLIA in another study.(30)

Recent literature reveals there are Cervicovertebral anomalies in patients with congenitally bilateral absent maxillary lateral incisors(BAMLIs)(34). Based on lateral cephalometric and orthopantomographic radiographs, it was observed that deficiency of the atlas bone (PADA); atlanto-occipital ligament calcification, known as "ponticulus posticus" (PP); and interclinoid ligament calcification, known as "sella turcica bridging" were present and this association may be based on the development of neural crest cells and/or homeobox genes in the development of the craniofacial region.The relationship between cervicovertebral anomalies and BAMLIs predicts the presence of tooth deficiency in later life and can be used to diagnose these skeletal anomalies and/or normal variants for early detection measures.

With advancements in technology and newer cutting edge screening procedures available at our disposal, research in the field of genetics has the scope of providing immense amount of information on the etiology of not just the development of dentition, but almost any sort of anomalies, which can be traced back to its roots and be effectively dealt with.

CONCLUSION:

Etiology of any defect or abnormality is as important as the defect itself and it is of utmost importance to have adequate knowledge on the genetic etiology of all aspects of dental development and its association with craniofacial abnormalities.
This systematic review has provided a clear view on the role of the pleomorphism of different genes on the agenesis of lateral incisors, with mutations in the PAX 9, EDA and WNT10A genes as the main contributing factors. The role of the MSX 1 gene did not show any significant contribution. Future studies are required to further conduct more randomised controlled trials, which will provide more concrete evidence to the claims.

CLINICAL SIGNIFICANCE:

Maxillary lateral incisors are the third most common congenitally missing teeth in the human dentition, preceded only by the third molars and mandibular second premolars The cause of lateral incisor agenesis is thought to be rooted to a genetic etiology.

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FIGURES AND TABLES:

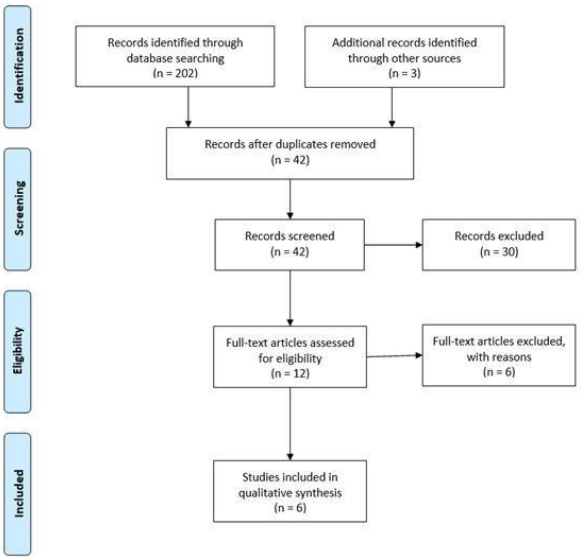

Figure 1: Prisma flow chart

Table 1: Characteristics of articles included in the systematic review

	AUTHOR	STUDY DESIGN	MISSING TOOTH	SAMPLE	GROUPS	AIM	METHOD	RESULT
1	Alves-Ferreira M et al	Case - control study	12, 22, 31, 32, 41, 42, 35, 45, 18, 28, 38, 48,	306 unrelated Portuguese individuals;	Case - 102 individuals with MLIA Control – 204 normal individuals.	Identification of Genetic risk Factors for Maxillary lateral Incisor Agenesis	MLIA of subjects was confirmed with radiographs. Candidate gene and SNP selection was done.	Involvement of PAX9, EDA, SPRY2, SPRY4 and WNT10A as risk factors of MLIA. No significant allelic, genotypic and haplotypic associations were found for

								AXIN2, TGFA and MSX1 genes. Strong synergistic interaction between MLIA and the following gene pair: MSX1-TGFA, AXIN2-TGFA, and SPRY2-SPRY4.
2	Wang J et al	Prospective study	11,21,12,22,13,23,31,41, 32,42,33,43	6 subjects from the same family with 1 contrab and.	1 male proband and 5 of his other family members as per the family tree.	To gain insight into the role of mutations of PAX9, MSX1 and AXIN2 in oligodontia phenotypes in a Chinese family	6 subjects underwent complete oral examination and panoramic radiographs were taken. Retrospective data and blood samples were collected. PCR primer for PAX9, MSX1 and AXIN2 were designed.	Proband missed 4 permanent canines, 2 maxillary lateral incisors, 2 mandibular incisors. and 2 mandibular central incisors. Maternal grandfather lacked only 2 mandibular central incisors. None of

							PCR products were purified and sequenced and analyzed by the 3730 DNA analyzer.	the other family members has any missing teeth. Mutation of A240P of PAX9 in coding region in the proband and maternal family member. No mutation was seen in MSX1 and AXIN2.
3	Pinho T et al	Prospective study	Maxillary lateral incisor agenesis	52 subjects	12 probands 40 relatives (8 had MLIA); 23 males and 29 females. Control group – 91 normal	To evaluate a possible association between mutations in MSX1, PAX9, and the MLIA phenotype in patients of Portugue	Comprehensive clinical information, family history and radiographs were obtained; Random DNA samples of 91 Portuguese individuals were	Nucleotide alterations were not detected in the coding region of MSX1 gene. Polymorphism was found in the PAX9 gene, leading to transition of G718

					Portuguese individuals.	se origin	taken, which constituted the control group. Genomic DNA was extracted from peripheral blood smear and buccal smear for PCR. Mutation scanning was done by using a single-strand conformation polymorphism analysis.	to C, implying a change of alanine 240 for proline.
4	Han D et al	Prospective study	Maxillary and lateral mandibular incisors	17 subjects	1 proband patient; 16 family members	To evaluate the role of Novel EDA mutation in X-linked non syndromic hypodontia.	Retrospective data was collected from all the family members and the proband. Radiographs were taken to confirm the diagnosis of tooth agenesis.	Mutation was seen at 8 positions in the exon 8 of EDA gene. Congenital absence of maxillary and mandibular central and lateral

							Venous blood samples were obtained from subjects for PCR. Genomic DNA was isolated from peripheral blood lymphocytes by standard high-salt method.	incisor with a higher possibility of persistence of maxillary and mandibular first permanent molar, suggestive of the presence of EDA mutation.
5	Boeira et al	Case control study	Maxillary upper lateral incisors	14 subjects	1 proband, Case - 3 affected relatives, Control - 10 unaffected individual	Screening for mutations of MSX1 gene to investigate the relationship between genotype and phenotype.	Retrospective data and patient's panoramic radiographs were taken. Questionnaires were administered for better assessment of family medical history. Buccal epithelial cells were collected from all members by means of	Known polymorphism *6C>T was identified in all three affected family members. Ten control samples were negative for *6C>T polymorphism.

							cytology brush, DNA extraction was done for PCR and mutation analysis.	
6	Mostowska A et al	Cohort study	Unilateral and bilateral Maxillary lateral incisor agenesis	20 subjects	20 unrelated subjects	To evaluate the WNT10A, MSX1, and PAX9 mutation rates in a group of 20 Polish patients with MLIA.	20 unrelated subjects were recruited for this study with MLIA. Dental panoramic radiographs, intraoral photographs and clinical examination was done. Frequency of identifiable nucleotide variants were assessed in an additional cohort of 147 patients. Peripheral blood smears were	No mutation that were potentially aetiologic were identified in MSX1 and PAX9. However, mutation was seen in WNT10A gene in five of 20 patients.

							collected for PCR and analysis.	

Table 2: *Article excluded from the study*

S.No	AUTHOR	JOURNAL	REASON
1	Kantaputra et al.	European Journal of Medical Genetics (2017)	Agenesis of deciduous lateral incisor.
2	Seo et al.	Angle Orthodontist, Vol 83, No 6, 2013	Study on Cleft patients.
3	Mandeville L.C et al.	The Annals of Human Genetics	Prevalence study
4	Ruf S et al.	J Orofac Orthop 2013; 74:295-308	Review Article
5	Qamar et al.	POJ 2012:4(2) 69-72	Review Article
6	Tallon-Walton V. et al.	Eur J Oral Sci 2007; 115: 427–432	Does not have missing lateral incisor.