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Role of Paired Box9 (PAX9) (rs2073245) and Muscle Segment Homeobox1 (MSX1) (581C>T) Gene Polymorphisms in Tooth Agenesis

Research Article

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Abstract

Objective: The objectives of this study were to investigate the relationship of PAX9 (rs2073245) and MSX1 (581C>T) with tooth agenesis in local population.

Method: Venous blood samples were taken from 20 subjects and 20 controls. DNA was extracted and polymerase chain reaction and amplification was conducted and subjected to the restriction enzymes for PAX9 (rs2073245) and MSX1 (581C>T) gene polymorphisms respectively. The results obtained were subjected to Z test for statistical analysis.

Results: The association between CC ($P < 0.001$) and GG ($P < 0.01$) genotypes of PAX9 and CT ($P < 0.001$) and CC ($P < 0.01$) genotypes of MSX1 gene was found to be statistically significant. In all the controls the three genotypes of PAX9 (rs2073245) and two genotypes CC and CT gene variant of MSX1 (581C>T) were absent.

Conclusion: The above findings suggest that PAX9 (rs2073245) and MSX1(581C>T) may be implicated as genetic markers for tooth agenesis in local population.

Keywords: *PAX9 (rs2073245); MSX1 (581C>T); Tooth Agenesis; Genotypes; Genetic Markers*

Introduction

Agenesis of one or more teeth is the most common anomaly of dental development in humans. Tooth agenesis may occur as a syndromic form, associated with genetic diseases, such as ectodermal dysplasia. However, it mostly occurs as a non-syndromic form. It can be inherited in an autosomal-dominant, autosomal recessive or X-linked mode.

Amongst the genetic factors, the Paired Box Gene 9 (PAX9) and Muscle Segment Homeobox 1 (MSX1) genes have shown to have a strong correlation to tooth morphogenesis. PAX9 is widely expressed in the neural crest derived mesenchyme, involved in craniofacial and tooth development. PAX9 is mapped onto chromosome 14q12-q13 and mutations in this gene can lead to non-syndromic tooth agenesis. MSX1, is located at chromosome 4 and has considerable evidence suggesting that it plays a role in dental development. It has been reported that both MSX1 and PAX9 have many polymorphic variants that might have association with tooth agenesis.

Hence, this study was conducted to establish the role of PAX9 (rs2073245) and MSX1 (581C>T) polymorphism in tooth agenesis.

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Objectives of the Study

1. To evaluate the relationship of PAX9 (rs2073245) and MSX1 (581C>T) with tooth agenesis.
2. To determine and compare the degree of association of each gene polymorphism with tooth agenesis.
3. To test whether any of these gene polymorphisms can be used as a genetic marker for tooth agenesis in our population.

Materials and Methodology

Venous blood samples from 40 subjects were taken after written informed consent.

These were divided into two groups:

- Group A: 20 subjects with one or more tooth agenesis (excluding only third molars agenesis).
- Group B: 20 subjects without tooth agenesis with all the complement of teeth including third molars.

Inclusion and Exclusion Criteria

Inclusion criteria for Group-A subjects:

• Sporadic Tooth agenesis of one or more teeth.

Exclusion criteria for Group-A subjects:

- Agenesis of only third molars.
- Presence of any known syndrome.
- Missing teeth as a result of trauma, previous extractions.

Methodology

The method was divided into four steps:

- Step 1: Collection and storage of blood samples,
- Step 2: Isolation of Genomic DNA,
- Step 3: Polymerase Chain Reaction Test (PCR),
- Step 4: Digestion with Restriction Enzyme Alw26I and Taq1 for PAX9 (rs2073245) and MSX1 (581 C>T) respectively (Restriction Fragment Length Polymorphism).

Step 1: Collection and Storage of Blood Samples

2 ml of Venous blood sample was collected in EDTA coated tubes and transported to the laboratory for storage of samples in liquid nitrogen (-70^{0}C).

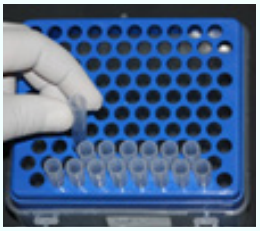

Figure 1: *EDTA coated tubes.*

Step 2: Isolation of Genomic DNA

Blood and tris Hcl buffer solution to maintain pH and proteinase K which acts as a proteolytic enzyme and causes cell lysis were taken in a microcentrifuge tubes. Sodium dodecyl sulphate (SDS 10%) was added which acts as a detergent for cell lysis and the tubes were then incubated at 37^0C for 30 minutes.

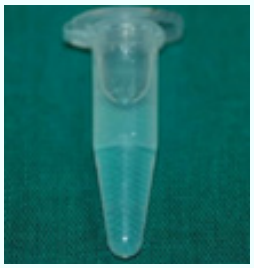

Figure 2: *Microcentrifuge tubes.*

Figure 3: *Incubator.*

Then the solution was phenol treated to remove cell proteins followed by chloroform treatment to remove phenol. Ethanol (100%) treatment to concentrate and precipitate the DNA is carried out which is kept in the ultracentrifuge (12000 rpm) for five minutes at room temperature and the genomic precipitate is obtained.

Figure 4: *Ultracentrifuge machine.*

The isolated genomic DNA precipitate is then used as a template for the polymerase chain reaction (PCR) test.

Primers used for PCR of PAX9 (rs2073245):

F 5'-CTGAATCCTGTGTGCACAAG-3'

R 5'-GAGAAATATTTTCGTGAATTTGAGA-3'

Primers for MSX1 (581C>T):

MSX2.1F: GGCTGATCATGCTCCAATGCT

MSX2.4R: GCACCAGGG CTGGAGGAATC

Step 3: Polymerase Chain Reaction Test

The obtained genomic DNA precipitate is taken in a PCR tube and Taq polymerase enzyme is added with Tris HCl buffer (to maintain pH) and PCR primers (250 mmol/l). Distilled water is added till the total volume is 20 µl and it acts as a reaction medium.

Figure 5: *PCR tubes in a PCR machine.*

PCR Machine is programmed for repetitive following cycles of 5 minutes duration for 35 times:

- Stage I) - Separation/Denaturation: The double stranded DNA denatured into single strand (1minute, 95 degree C)
- Stage II) - Priming/Annealing: Primers anneal to the end of strands (1 minute at 58 degree C)
- Stage III) - Polymerisation/Elongation: Formation of a complementary strand (1 minute at 72 degree C).

The obtained amplified PCR Products of PAX9 (rs2073245) (103 base pairs (bp)) and 557 bp of MSX 1 are taken in PCR tubes with Tris HCl buffer solution restriction enzyme Alw26I for PAX 9 and restriction enzyme Taq1 for MSX1 and distilled water.

After the digestion of PCR products with specific restriction enzyme Alw26I/Taq 1 takes place, the digested PCR Products are subjected to GEL ELECTROPHORESIS (1.4% agarose gel with ethidium bromide) for separation of base pairs

Figure 6: *Agarose gel electrolytic chamber with running PCR products.*

UV transilluminator is used to visualize specific bands of base pairs of digested PCR products of PAX9 (rs2073245) and MSX1 (581C>T) and gel documentation is attached to the computer.

Figure 7: *U.V transilluminator.*

Figure 8: *Gel documentation attached to a computer.*

Results

The presence of CC, GG and CG genotype of PAX9 (rs2073245) gene variant among cases and controls.

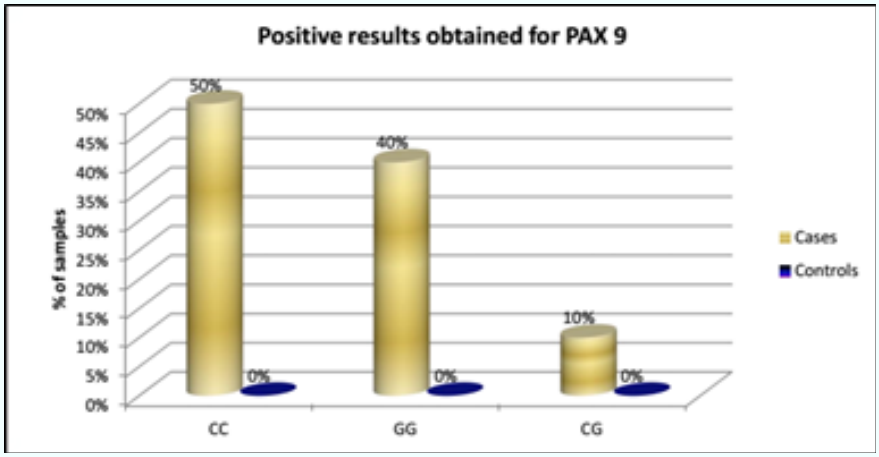

Figure 9

Genotype	Case (N = 20)		Control (N = 20)		Difference in proportion	z	P-value
	n	%	n	%			
CC	10	50%	0	0%	0.28	3.65	< 0.001*
GG	8	40%	0	0%	0.16	3.16	0.002*
CG	2	10%	0	0%	-0.20	1.45	0.147

Table 1

The presence of CC, GG and CG genotype of MSX1 (581C>T) Gene variant among cases and controls.

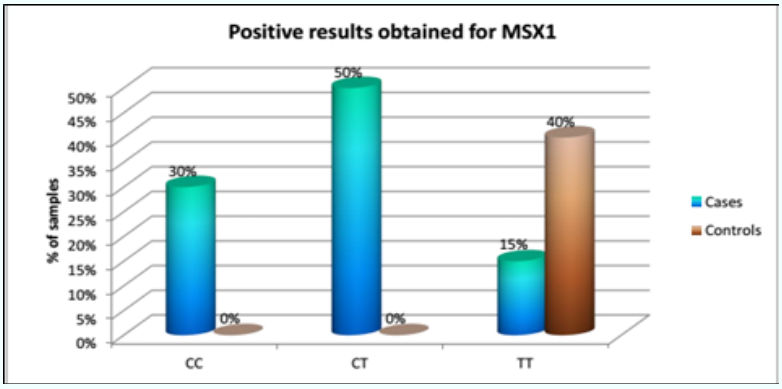

Figure 10

Genotype	Case (N = 20)		Control (N = 20)		Difference in proportion	z	P-value
	n	%	n	%			
CC	6	30%	0	0%	0.28	266	0.008*
GG	10	50%	0	0%	0.16	3.65	< 0.001*
CG	3	15%	8	40%	-0.20	-1.77	0.077

Table 2

Z test has been used to find the significance of association of PAX9 and MSX 1 gene polymorphism with tooth agenesis.

Discussion

The important role of genetics has been increasingly recognized in recent years with respect to the understanding of dental anomalies, especially tooth agenesis. Polymorphism in genes is a mechanism by which individuals may exhibit variations within the range of what is considered biologically normal. Gene polymorphisms are also associated with disease susceptibility. Most polymorphisms are single nucleotide exchanges that occur at a high frequency in the human genome and may affect the function of genes.

In accordance with the various studies performed earlier, it can be concluded that at present, 14 mutations and one deletion of the PAX9 gene have been associated with familial, non-syndromic tooth agenesis.MSX1 in conjunction with PAX9, has a synergistic effect on the process of odontogenesis therefore a deficiency of MSX1 can result in tooth agenesis.

The above findings suggest that PAX9 (rs2073245) CC and GG genotype and MSX1 (581C>T) CT and CC genotype may be implicated as genetic marker for tooth agenesis in our population. This can be confirmed by further studies with a larger sample size. In contrast PAX9 (rs2073245) CG genotype and MSX1 (581C>T) TT did not show any significant statistical association with tooth agenesis.

This study could bring about new possibilities of early diagnosis and foresight of orthodontic or prosthetic treatment. Once these genetic markers have been established with increased sample size they can be used as powerful tools for screening the population. In the near future, with advances in science a correction at molecular level remains a possibility [1-7].

Conclusion

The results of this study indicated that:

1. There is no significant association between PAX9 (rs2073245) genotype CG [p value = 0.147] and MSX1 (581C>T) genotype TT [p value = 0.077] with tooth agenesis in the subjects included in this study.
2. There is a statistically significant association between PAX9 (rs2073245) CC genotype [p value < 0.001] and GG genotype [p value = 0.002] and MSX1 (581C>T) CC genotype [p value = 0.008] and CT genotype [p value < 0.001] and subjects with tooth agenesis included in this study.
3. The expression of the various genotypes of PAX9 gene polymorphism (rs2073245) shows ethnic variation.
4. The study affirms the association between novel heterozygous transition of MSX1 (581C>T) genotype CT with tooth agenesis.

Disclosure

The author reports no conflicts of interest in this work.

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