Original Article

# A study on genetic and mutans streptococcal transmissibility of dental caries

Shailja Chatterjee[1], Satyawan G. Damle[2], Nageshwar Iyer[3]

[1]Department of Oral and Maxillofacial Pathology, Yamuna Institute of Dental Sciences and Research, Yamuna Nagar, Haryana, [2]Former Vice-chancellor, M. M. (Deemed to be) University, Mullana, Ambala, Haryana, [3]Former Principal, MM College of Dental Sciences and Research, M. M. (Deemed to be) University, Mullana, Ambala, Haryana, India

## Abstract

**Background:** Dental caries is characterized by an interplay between environmental and genetic factors.

**Aim:** The aim of this study was to analyse the transmissibilities of high caries risk chromosomal loci at 5q 12.1-13.3 and low caries risk chromosomal loci at 13q31.1 and Streptococcus mutans (S. mutans) in family units.

**Materials:** This prospective cohort study was performed on 56 families grouped into four: (a) Group I: 18 families of children with caries affected primary teeth; (b) Group II: 21 families of children with caries in permanent teeth; (c) Group III: 6 families of children with no caries in primary teeth and (d) Group IV: 12 families of children with no caries in permanent teeth. Blood, saliva and plaque samples were collected from consenting study participants. Isolated DNAs were subjected to polymerase chain reactions using suitable primers. Data collected was analysed with ANOVA and Chi-squared test.

**Results:** Wide expression of chromosome loci 5q12.1-13.3 was obtained in both blood and saliva samples. For chromosome loci 13q31.1, no expression was found in saliva samples, hence indicating its local absence. For the GtfB expression, transmissibility was common for a single band expressing S. mutans.

**Conclusion:** This study reflects upon newer findings in the field of genetic research on dental caries.

**Keywords:** 5q12.1-13.3, 13q31.1, dental caries, GtfB, genetic, transmissibility

**Address for correspondence:** Dr. Shailja Chatterjee, Oral Cancer Screening and Diagnostic Centre, 3/1323, Chatterjee Cottage, Janak Nagar, Saharanpur, Uttar Pradesh – 247 001, India.
E-mail: shailjachatterjee@gmail.com

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## INTRODUCTION

Dental caries has been technically considered a biofilm-induced disease which is an interplay between various resident oral microorganisms and host-origin genetic factors.[1] Thus, dental caries may be alternatively defined as a transmissible dental tissue infection associated with modifiable carbohydrates with saliva acting as a critical regulatory factor.[2] It has been suggested that the development of resistance or susceptibility towards dental caries has environmental phenotype-related and genotypic influence.[3] Streptococcus mutans (S. mutans) plays an important role in initiation of dental caries. It acts by adhering to tooth enamel and is an important constituent of the biofilm responsible for initiation of dental caries by glucan synthesis on tooth surfaces.[4] The existence of a biofilm community requires well adaptation to dynamic

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conditions required for microbial growth and diversification of various species.[5] S. mutans colonizes dental hard tissue structures and is considered main etiological microorganism responsible for dental caries. The increased capacity of this microorganism to produce a biofilm in the presence of a sucrose molecule (a carbohydrate molecule) along with its capability in the metabolism of wide range of carbohydrates and its extreme tolerance towards fluctuating pH and availability of nutrients are essential requirements for its persistence and survival which would eventually lead to dental caries.[6] Hence, the initial acquisition along with the transmission of Mutans streptococci takes via the mother-to-child transmission routes. Maternal transmission rates have been reported to range between 24 and 100% in mother-child dyads. Transmission of the organism has also been reported between family members, parent-child and among siblings.[7]

Therefore, a constant homeostasis between oral microbiota and host factors is a requisite. Thus, the underlying mechanism of this disease is little less understood leading to pitfalls in disease management. Environmental factors responsible for pathogenicity of S. mutans are: (1) their ability to survive in low pH milieu; (2) capacity to produce acid and (3) extracellular polysaccharides. Formation of a biofilm is important in growth and transmission of oral microbiota.[8] The biofilm adheres itself to the salivary pellicle which colonizes the adherent surface by binding by utilizing adhesins located on fimbriae surfaces.[9]

By utilizing next-generation sequencing techniques such as: metagenomic and metatranscriptomic analysis, the polymicrobial nature has been deciphered. Other cariogenic organisms identified using molecular biological methods include Bifidobacterium dentium, B. longum, B. adolescentis, Scardovia wiggsiae, Lactobacillus spp., Prevotella spp. etc.[10]

Epidemiological studies have identified that genetics also plays a major role in determining high caries scores among population. The genotype of an individual helps in determining environmental feasibility for a microorganism in order to produce or create favourable environmental niche.

An individual's genotype plays an important role in determining a favourable environment for pathogenic organisms. The genetic constitution of an individual leads to influencing the production of salivary antibodies and proteins. When there is prevalence of genes responsible for less quantity of saliva production, plaque accumulation leads to an increased risk of dental caries development. This results in a disturbance in oral microbiome. There is evidence to suggest that variations in plaque biomass, local pH and host immunobiological response strongly influence a subject's susceptibility to dental caries.[1]

However, considering various measures for analysing dental caries such as the Decayed, Missing, Filled Teeth (DMFT) and Decayed, Missing, Filled Surface (DMFS) indices it can be suggested that those do not reflect optimal methods. Thus, neither the DMFT/S nor deft/s indices scores determine actual caries destruction rates; however, they reflect morbidity of tooth.[4-6] Goodman *et al.* (1959)[7] were the first to establish the role of genetics in aetiology of dental caries after studying a total of 38 same-sex monozygotic and dizygotic twins. They observed heritable characteristics for oral microflora, rate of salivary flow, pH of saliva and activity of salivary amylase enzyme.[7] Also, Lovinaa (2012) claimed a high correlation rate of dental caries, malocclusion and periodontal health and genetic constitution in 30 twins of which 9 were monozygotic and 21 were dizygotic.[8] Hamid (2015) reported that there is 45 to 64% caries heritability in primary dentition as compared to permanent dentition. However, studies investigating only dental caries susceptibility are insufficient in demonstrating the genetic role as behavioural and environmental factors also contribute to the co-variances and may present as genetic affliction.[11-13]

Bretz (2003) concluded that twin studies can be conducted to analyse effects of genetics on phenotypic variations in dental caries.[4] Monozygous pairs of twins have been shown to exhibit small variances in dental caries, thus, impressing upon the genetic aspect of this disease.[13]

Vieira (2012) identified: (a) low caries causing or susceptibility gene loci such as: 5q13.3, 14q11.2 and Xq27.1 and (b) high caries causing susceptibility genetic loci such as: 13q31.1 and 14q24.3.[9] in another genetic study, Li *et al.* (2015)[10] in their study examined seven polymorphisms: rs2274328, rs17032907, rs2274333, rs10864376, rs6680186, rs11576766 and rs3765964 in intron or exon 2/3 regions. Subjects with TT genotype: rs2274327 demonstrated significantly lower CA VI levels when compared to those with CT or CC genotypes ($P < 0.05$). Also, an association between rs2274333 polymorphism and salivary CA VI concentration was obtained. This study demonstrated an association between CA VI genetic polymorphism and susceptibility towards dental caries among Chinese subjects. Hence, it was seen that short nuclear polymorphisms act by altering enzymatic properties.[12] Wendell (2010) demonstrated a significant association between genes regulating the taste pathway: TAS2R38, TAS1R2 and GNAT3 and dental caries. Additionally, studies have

demonstrated that 40%–60% of susceptibility towards dental caries is determined genetically.[14]

There are multiple genetic alterations along with a variety of environmental or exogenous factors subsidized to dental caries.[13] It was as early as in 1946, when Klein made a conclusive remark that the susceptibility of dental disorders has strong underlying familial carriers or pathways which may be genetically related with probable sex-linked traits.[15]

Based upon available evidence, the aim of current study is to study and analyse the effects of low caries susceptibility (5q12.1-13.3) and high caries susceptibility (13q31.1) genes on and transmissibility of S. mutans in family units.

## MATERIALS AND METHODS

This study was designed as a prospective cohort genetic study to assess and evaluate the expressions of human gene loci: 5q12.1-13.3 (suggestive of low caries susceptibility) and 13q31.1 (suggestive of high caries susceptibility) and Mutans streptococcus (MS) among family units comprising parents and a child. The study was conducted after ethical approval from the following research approval committees: (a) Institutional Research Committee and (b) Institutional Ethical Committee.

The study cohort was classified into four groups:

**Group I:** Children with caries affecting primary dentition (≤6 years) and their parents (<30 years) comprising 18 family units.

**Group II:** Children with caries affecting permanent dentition (12–18 years) and their parents (<40 years) comprising 21 family units.

**Group III**: Children without dental caries in primary dentition (≤6 years) and parents (<30 years) comprising 6 family units.

**Group IV:** Children without dental caries in permanent dentition (12–18 years) and parents (<40 years) comprising 12 family units.

Thorough clinical examinations of oral cavities were performed using dental chair illumination, mouth mirror and probes to record dental caries status.

### Sample collection

For identification of genetic loci on chromosomes 5q12.2-13.3 and 13q31.1, blood and saliva samples were collected from consenting study participants. An informed consent form was signed by each subject in the presence of two impartial and unrelated witnesses who also signed the form.

### Collection of blood samples

For blood collection, venous puncture was performed and blood sample (2 ml) was collected in EDTA-coated vacutainers and stored at -20°C. While collecting of blood sample, two impartial witnesses were present and videography of the procedure was performed. Blood sample was collected from antecubital vein as per guidelines given by **(Indian Council of Medical Research) ICMR, Ministry of Health and Family Welfare** (**MHFW)** and **Supreme Court of India**.

**a. Collection of saliva samples:**

Stimulated saliva (1 ml) was obtained after instructing the subject to chew paraffin wax and to allow pooling of saliva in floor of the mouth. The collected sample was then drooled into a wide-mouthed sterile vial and tightly capped. Sample was stored at -20°C.

**b. Collection of plaque samples for MS isolation:**

Study participants were instructed not to eat or drink any sugar-containing beverage 2 h prior to sampling. A sterile cotton-tipped swab was used for collecting tooth-associated plaque from buccal surfaces of teeth. The collected sample was then placed in Todd-Hewitt broth at -20°C.

Primers used in the study were based upon PubMed Blast tool and the sequences used were:

a. **For Chromosome 5q 12.1-13.3 (btf3):** The forward primer sequence was: 5'-CTTTAGCTGCCATCTTGCGT-3' (GC%- 50) while of the reverse primer was 5'-GCCTCCAGAGCCTTTTG-3' (GC%- 57.89).
b. **For Chromosome 13q31.1 (SLITRK**): The forward primer sequence was: 5'-GGAGTCTGCATTCCACCACA-3' (GC%- 55) and reverse primer sequence was 5'-AGAGGAGAGTAGGTTGCAGGT-3' (GC%- 52.38).
c. **For Glucosyltransferase B:** The forward and reverse primer sequences used were: 5'-AGCCATGCGCAATCAACAGGTT-3' and 5'-CGCAACGCGAACATCTTGATCAG-3'.

**Procedure for isolation of genomic DNA from blood samples:** 200 µl of collected blood sample was taken in an anticoagulant-coated Eppendorf tube; to this, 600 µl of RBC lysis buffer (Qiagen Tokyo, Japan) was added. This step was followed by incubation in ice for 5 min. The solution was centrifuged at 10,000 rpm for 15 min at 4°C. After discarding the supernatant, 100 µl WBC lysis buffer (Qiagen Tokyo, Japan) was added. The obtained solution was kept at -20°C for 5 min. Then, 300 µl WBC and 100 µl RBC lysis buffers

were added and mixed by vortexing. Then, 4 µl of Proteinase K solution (Sigma, Tokyo, Japan) was added which was kept at room temperature for 10 min. Following this, one-tenth of this solution was pipetted and sodium acetate was added. This solution was incubated at -20°C for 2 min followed by centrifuging at 10,000 rpm for 5 min. The obtained supernatant was then transferred into a fresh Eppendorf tube and equal volume of phenol was added. This was then centrifuged at 10,000 rpm for 5 min. An aqueous layer was obtained which was then transferred to a fresh Eppendorf tube. Double the volume of 100% cold ethanol was added to the aqueous layer and centrifuged at 10,000 rpm for 5 mins. The supernatant obtained was discarded and 200 µl T-E buffer was added. The solution was stored at -20°C for 24 h which resulted in DNA precipitation.

**Procedure for isolation of genomic DNA from saliva samples:** 1 ml of saliva was collected and to this, 4 ml phosphate buffer saline (PBS) was added and centrifuged at 1800 × g for 5 min.

The supernatant was decanted and pellet was resuspended in 180 µl PBS. 20 µl protease and 200 µl buffer were added to the sample and mixed by vortexing for 15 s. The solution was incubated at 56°C for 10 min. 200 µl ethanol (100%) was added and mixed by vortexing. QIAamp spin column in a 2 ml collection tube was added, cap was closed and centrifuged at 8,000 rpm for 1 min. The QIA amp spin column was placed in a 2 ml collection tube and filtrate was discarded. The QIAamp spin column was carefully opened and 500 µl of buffer AW1 was added. This was centrifuged at 800 rpm for 1 min. The QIAamp spin column was placed in a 2 ml collection tube, and collection tube containing the filtrate was discarded. The QIAamp spin column was opened and 500 µl of buffer AW2 was added. This was centrifuged at a full speed for 3 min. The QIAamp spin column was placed in a 1.5 ml microcentrifuge tube and the filtrate in the collection tube was discarded. It was then carefully opened. DNA was eluted with 150 µl buffer AE or distilled water. This was incubated at room temperature for 1 min and then centrifuged at 6000 × g (8000 rpm for 1 min). The DNA pellet was isolated.

### MS culture and DNA isolation

The basic challenge which underlies oral bacterial genotypic assessment for a particular individual is determined by the number of colonies of bacteria that may be required for the representation of sampling of the genotypic diversity of the microorganism.[16]

Clinical samples were plated on Mitis Salivarius Bacitracin agar, followed by incubation at 37°C in 10% $CO_2$. Representative morphological S. mutans colonies were collected from each sample and sub-cultured on Mitis Salivarius agar. Purity and identity of isolated organisms was confirmed by Gram's staining and colony morphology on Mitis Salivarius agar. Pure cultures were stored at -70°C in skimmed milk medium. Aliquots from skin milk were plated on brain heart infusion (BHI) agar incubated at 37°C in 10% $CO_2$. Colonies grown in BHI agar were then inoculated at 37°C in 10% $CO_2$. Colonies were picked up from this media and subjected to DNA extraction. The bacterial genomic DNA was isolated as follows: Frozen saliva samples were quickly thawed at 37°C, and 250 µl of each sample was centrifuged; pellets obtained were resuspended in 570 µl of 20 mg/ml 31 lysozyme (Sigma, Tokyo, Japan) solution which contained 50 mM Tris HCl (pH 8.0) and 20 mM EDTA; this suspension was incubated at 37°C for 30 min. 30 µl of 20 mg/ml Proteinase K solution (Qiagen Tokyo, Japan) was added and the sample was incubated at 55°C for 10 min. Approximately 0.8 g of acid-washed glass beads (diameter 150 × 212 µm, Sigma) and 1 µl of 100 mg/ml RNase A (Qiagen) were added. The samples were vigorously shaken in 2 ml Safe-Lock micro test tubes (Eppendorf, Tokyo, Japan) by a Mixer Mill MM300 (Qiagen) at 30 Hz for 10 min. 600 µl of buffer AL from a DNeasy Tissue kit (Qiagen) was added and incubated at 70°C for 30 min. The beads were spun down and the supernatant was transferred to new tubes. To the supernatant, one-third of the volume of ethanol was added and mixed. DNA was isolated from the solution using a DNeasy tissue column according to the Qiagen instructions.

DNA concentration was determined by measuring OD values at 260 nm and 280 nm using an UV spectrophotometer.

### Polymerase chain reaction

Gene specific primers targeting chromosomes 5q12.1-13.3 and 13q31.1 and microbial gtfB gene were used to confirm and identify the isolates. Procedure for performing the polymerase chain reaction is as under: 25 µl of reaction mixture containing 1X reaction buffer Taq polymerase, 1.5 mM $MgCl_2$, 0.1 mM deoxynucleoside triphosphate, 0.2 µM primer, 1.5 U Taq polymerase along with 2.5 µl DNA sample. PCR amplification was performed using a thermocycler (Touchgene Gradient, UK) maintained at optimal temperatures, i.e., denaturation was performed at 95°C for 5 min followed by amplification cycles (45 cycles each) at 95°C for 30 s, 36°C for 30 s and 72°C for 1 min.

The PCR products were separated by electrophoresis in 1.5% agarose gel. DNA was stained using 0.5 µg of

ethidium bromide and bands obtained were visualized under UV illumination.

Bands obtained were identified under UV illumination and their size was determined based on their electrophoretic movement on 1.2% agarose gel stained with ethidium bromide. A DNA ladder (SMOBiO Inc.) of 50 bp (25 kb) demonstrating 26 individual DNA fragments containing 4 enhance bands (3k, 1.2k, 500 and 200 bp) was used as a reference.

Statistical analysis was performed by tabulating the data collected and applying statistical tools – ANOVA (Analysis of Variance) and Chi-squared test using SPSS software version XIX. Probability was found to be statistically significant if the value was less than 0.05 and of extreme significance for values lesser than 0.01.

## RESULTS

I. Assessment of demographics, lifestyle and clinical parameters:
   a. Age distribution: The mean ± SD values (years) in male children with primary and permanent dentitions were: 4.57 ± 0.64 and 13.7 ± 1.128, respectively while for female child participants, it was 4.3 ± 0.94 and 13.69 ± 1.109, respectively. The mean age ± SD for fathers and mothers was 38.29 ± 6.67 and 34.42 ± 6.27 years, respectively [Tables 1 and 2].
   b. Gender-wise distribution: In the current study, there was no significant difference between respective genders of children who participated in the study ($P > 0.05$). On applying the Chi-squared test in children with primary dentition, a negative correlation ($\Phi = -1.5$) was obtained between gender and distribution of dental caries; however, on applying the same test, in children bearing permanent dentition, a positive correlation ($\Phi = +0.2$) was noted though no significant $P$ value ($P = 1$) was obtained. On comparing the caries scores, higher prevalence of dental caries was seen in female children (with primary dentition) when compared to males (23.72 ± 38.66 and 10.69 ± 13.11, respectively). However, only a marginal difference in caries experience was noted when comparing both genders [Table 3].
   c. Sugar exposure: Sugar exposure was calculated by analysing a week-long diet diary. On comparison of distribution of sugar exposures, the mean ± SD was found to be 3.15 ± 1.699 and 2.81 ± 1.69 in fathers and mothers, respectively. A non-significant difference of $P = 0.47$ was calculated. Higher exposure to sugar consumption was seen among male children who had primary teeth when compared to those with permanent dentition ($P = 0.03$ and 0.8, respectively). No significant difference was found between male and female subjects with either primary or permanent dentitions (males, $P = 0.86$; 0.3 and females, $P = 0.83, 0.45$, respectively). In a similar manner, no significant difference was noted in sugar exposures between the parents ($P = 0.86$) [Tables 4 and 5].
   d. DMFS/dfs scores: Mean DMFS score values 12.94 ± 14.43 and 9.22 ± 11.57 was observed among fathers and mothers, respectively while the mean ± SD dfs scores in children with primary dentition was (1.69 ± 13.117, males and 26.1 ± 39.89, females; respectively) whereas in children with permanent dentition, mean DMFS scores of 3.1 ± 3.65 and 4.64 ± 10.681, respectively were seen. Plaque index: Oral biofilm covers initial mucosal and tooth surfaces and comprises a complex community of microorganisms which may turn pathogenic depending upon the oral environmental conditions. Current study shows mean ± SD plaque scores of 4.68 ± 2.77 and 4.47 ± 2.9 in fathers and mothers, respectively with no significant difference between plaque and DMFS scores ($r = -0.08$, $P = 0.59$ (fathers) and $r = 0.104$, $P = 0.22$ (mothers), respectively. When comparing the plaque scores in both the studied dentition groups, higher plaque scores were observed in male children with primary

**Table 1: Table demonstrating age-distribution in children**

| | Male child | | | Female child | | |
|---|---|---|---|---|---|---|
| | Primary dentition | Permanent dentition | Overall | Primary dentition | Permanent dentition | Overall |
| Sample size | 13 | 20 | 33 | 10 | 14 | 24 |
| Mean age (years) | 4.57 | 13.7 | 10.19 | 4.3 | 13.69 | 9.6 |
| Minimum age (years) | 3 | 12 | 3 | 2 | 12 | 2 |
| Maximum age (years) | 5 | 15 | 15 | 5 | 15 | 15 |
| Standard deviation (years) | 0.64 | 1.128 | 4.733 | 0.948 | 1.109 | 4.868 |
| Standard error | 0.177 | 0.252 | 0.823 | 0.3 | 0.307 | 1.015 |
| Confidence level (95%) | 0.387 | 0.631 | 1.678 | 0.678 | 1.731 | 2.105 |

dentition (5.92 ± 3.81) when compared to children with permanent dentition (4,47 ± 1,945). Similar observations were made in female children also (5.78 ± 2.08 and 3.065 ± 2.24, respectively in primary and permanent dentitions). Overall, there was no statistical difference observed between dfs scores and plaque scores (r = 0.284, *P* = 0.78). Similarly, no significance was observed in males (r = -0.15, *P* = 0.5) and female (r = 0.47, *P* = 0.09) in children with permanent dentition. In the present analysis, on comparing both genders regardless of the type of dentition, an extremely significant difference (r = 0.66; *P* = 0.0005) was obtained between plaque index and caries index scores in female study participants whereas no significant difference (r = 0.067, *P* = 0.7) was noted among male children [Tables 6 and 7].

II. Genetic parameters studied are as follows [Figures 1, 2 and 3]-
   a. Chromosome 5q12.1-13.3 in blood samples of family units of children with primary dentition: Positive correlation was observed between father-child (Φ = +0.86) and mother-child (Φ = +0.86) in carious and non-carious primary dentition bearing children (Φ = +1 and + 1, respectively) in 2.5 bp band expression and a significant difference was observed. Similarly, positive correlation was noted between father-child and mother-child in carious (Φ = +1 and + 0.09, respectively) and primary dentition with no dental caries (Φ = +1 and + 1, respectively). However, a significantly different *P* value (*P* = 0.046) was observed on a comparison between caries status, family members and band expression. On analysing the 1.2 bp band expression, positive correlation was noted between father-child and mother-child pairs in carious (Φ = +0.4 and 0.44, respectively and non-carious primary dentition families (Φ= +1 and +1, respectively). No statistical significance was noted on comparison of any of the variables. Positive correlation (Φ = +1 and + 0.63, respectively) was seen between 900 bp band expression in carious primary children and non-carious primary dentition (Φ = +1 and + 1, respectively) although, in this case as well, significant *P* values were obtained. The 3 bp band expression showed positive correlation between father-child and mother-child pairs (Φ = +1 and + 0.69, respectively) of carious primary

**Table 2: Table demonstrating age-distribution in parents**

| | Father | Mother |
|---|---|---|
| Sample Size | 57 | 57 |
| Mean Age (Years) | 38.29825 | 34.42105 |
| Minimum Age (Years) | 26 | 21 |
| Maximum Age (Years) | 58 | 50 |
| Standard Deviation (Years) | 6.67609 | 6.27053 |
| Standard Error | 0.88427 | 0.830552 |
| Confidence Level (95%) | 1.771405 | 1.663796 |

**Table 3: Table demonstrating gender-wise distribution in children**

| Gender | Primary dentition | Permanent dentition | Overall |
|---|---|---|---|
| Male | 13 | 20 | 33 |
| Female | 11 | 13 | 24 |
| Total | 24 | 33 | 57 |
| Chi-squared | | 0.05 | |
| Df | | 1 | |
| *P* | | 0.823 (*P*>0.05) | |

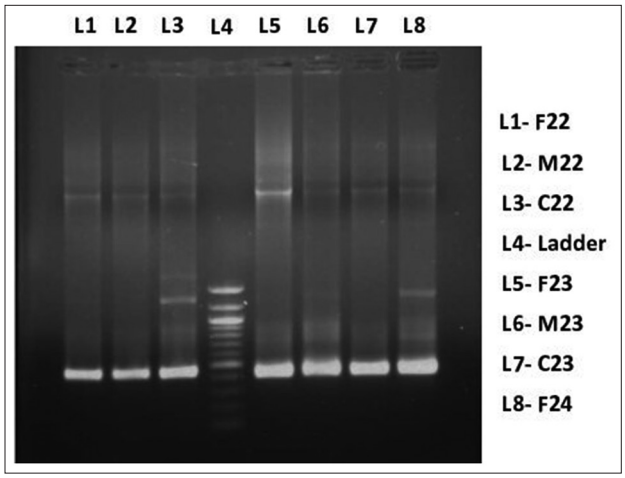


**Figure 1:** Chromosome 5q 12.1-13.3 expression in blood samples. On right is demonstrated labelling for different lanes with codes for individual family member

**Table 4: Table demonstrating sugar exposure in children**

| | Male child | | | Female child | | |
|---|---|---|---|---|---|---|
| | Primary dentition | Permanent dentition | Overall | Primary dentition | Permanent dentition | Overall |
| Sample size | 13 | 20 | 33 | 11 | 14 | 24 |
| Mean exposure | 3.45 | 2.13 | 2.65 | 2.9 | 2.23 | 2.54 |
| Minimum exposure | 0 | 0 | 0 | 1.2 | 1 | 1 |
| Maximum exposure | 14.5 | 3.2 | 14.5 | 5.5 | 4 | 5.5 |
| Standard deviation | 3.55 | 0.87 | 2.37 | 1.22 | 1.035 | 1.151 |
| Standard error | 0.986 | 0.196 | 0.413 | 0.367 | 0.287 | 0.235 |
| Confidence level (95%) | 2.149 | 0.410 | 0.841 | 0.819 | 0.625 | 0.486 |

dentition category. No correlation was seen in children with no caries' families. On comparing the 1.5 bp band expression, positive correlation was observed between father-child and mother-child pairs (Φ = 0.4 and 0.69, respectively). However, no correlation was seen in families with no caries in primary dentition. Extremely significant *P* values (*P* = 0.0002, 0.6, 0.0013 and 0.006, respectively) were obtained on comparison of dental caries status, band expression and parents. A significant *P* value (*P* = 0.03) was noted on comparing caries and band status. The 1 bp band showed positive correlation between mother-child and father-child parental pairs in children with primary teeth affected with caries (Φ = +0.89 and 0.69, respectively) and also, with non-carious primary dentition (Φ = +0.63 and + 1, respectively). A significant *P* value (*P* = 0.04) was observed between band and caries status while no significant differences were observed among all the studied variables [Table 8a].

b. Chromosome 5q12.1-13.3 expression in blood samples of family units of children with permanent dentition: On analysing the 4 bp band expression, a positive correlation was seen between father-child (Φ = +0.84) and mother-child (Φ = +0.84) with carious teeth. Similarly, positive correlation was observed in families with non-carious teeth (Φ = +1; father-child pairs and Φ = +0.52, mother-child pairs, respectively). In a similar manner, positive correlation was noted between father-child and mother-child pairs in carious (Φ = +0.69 and + 0.69, respectively). The 3 bp band expression showed positive correlations between father-child and mother-child pairs in family units of children with carious (Φ = +0.09 and + 0.69, respectively) as well as non-carious teeth (Φ = +1 and + 1, respectively). No statistical significance was obtained in both the studied band expressions. On studying the 2.5 bp band expression, positive correlation was seen between father-child and mother-child pairs with carious permanent dentitions (Φ = +0.58 and + 0.58, respectively) while in those with non-carious dentitions, again positive correlations (Φ = +1 and + 1, respectively) were observed. An extremely significant probability value was obtained on statistical comparisons between the presence or absence of caries and band expression (*P* = 0.0006). Similarly, the 2 bp band expression also demonstrated positive correlations between father-child and mother-child pairs in both carious as well as in those without any caries (Φ = +0.84, +0.73, +0.17, +0.26, respectively). However, no significant correlations were observed. Although, extremely significant statistical significance was obtained with 1 bp band expression and caries status (*P* < 0.001) and on correlating caries status with band

**Table 5: Table demonstrating sugar exposure in parents**

| | Father | Mother |
|---|---|---|
| Sample size | 57 | 57 |
| Mean sugar exposure | 3.15 | 2.81 |
| Minimum sugar exposure | 1 | 0.2 |
| Maximum sugar exposure | 9 | 12 |
| Standard deviation | 1.699 | 1.691 |
| Standard error | 0.225 | 0.224 |
| Confidence level (95%) | 0.451 | 0.448 |

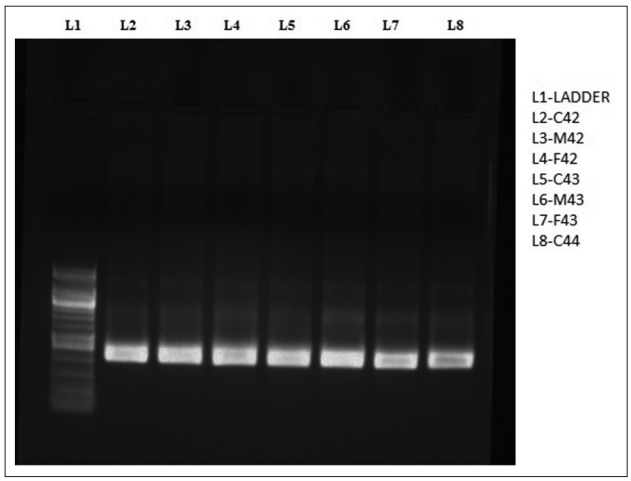


**Figure 2:** Chromosome 13q31.1 expression in blood sample. Towards right side is shown labelling for different lanes

**Table 6: Table demonstrating caries scores in children**

| | Male child | | | Female child | | |
|---|---|---|---|---|---|---|
| | Primary dentition | Permanent dentition | Overall | Primary dentition | Permanent dentition | Overall |
| Sample size | 13 | 20 | 33 | 10 | 14 | 24 |
| Mean dfs/DMFS | 11.69 | 3.1 | 6.09 | 26.1 | 4.64 | 13.58 |
| Minimum dfs/DMFS | 0 | 0 | 0 | 0 | 0 | 0 |
| Maximum dfs/DMFS | 44 | 12 | 44 | 128 | 41 | 128 |
| Standard deviation | 13.117 | 3.654 | 9.308 | 39.898 | 10.681 | 28.357 |
| Standard error | 3.638 | 0.817 | 1.62 | 12.616 | 2.854 | 5.788 |
| Confidence level (95%) | 7.926 | 1.710 | 3.300 | 28.541 | 6.167 | 11.974 |

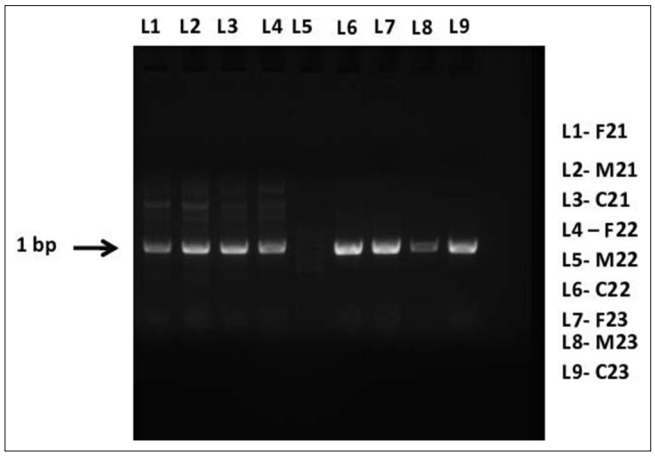


**Figure 3:** 1 bp band expression for GtfB in Streptococcus mutans

expression and family member ($P < 0.0001$). Also, positive correlation was noted between father-child and mother-child pairs with carious as well as non-carious teeth (Φ = +1, +0.89, +1 and + 0.89, respectively). No statistical significance was observed between the 900 bp expressions [Table 8a].

c. Chromosome 5q12.1-13.3 analysis in saliva samples in family units of children with primary dentitions: On studying the band expression at 3 bp, positive correlations were obtained between father-child and mother-child pairs with carious and non-carious dentitions (Φ = +0.24, +0.69, +0.44 and 0.6, respectively). An extremely significant *P* value ($P = 0.0002$) was obtained on comparison between caries status, band expression and family unit studied. A significant association was found between dental caries and 300 bp band expression ($P = 0.01$) through positive correlation ($P = 0.2$) was found to exist between father-child pair with no caries in child's teeth. A positive correlation was observed between father-child and mother-child pairs of children having carious teeth (Φ = +0.57 and + 0.57, respectively) whereas no correlation was seen between parent-child pairs with no caries. Extreme significant difference ($P = 0.0016$) was seen on comparing caries status, 1.2 bp band expression and family member. Positive correlation was noted between father-child and mother-child pairs with carious teeth (Φ = +0.6 and + 0.6, respectively) while no correlation was seen between parent-child pairs with no carious teeth. Thus, an extreme significant *P* value ($P = 0.0012$) was seen between caries presence and band presence. On studying the 1.7 bp band, positive correlations of Φ = +1 was obtained between parent-child pairs in both the groups with caries or without caries while an extreme significant difference ($P = 0.0012$) was noted between dental caries and band expression. The 1.8 bp band expression demonstrated no correlation between band expression and dental caries status. Significant difference was noted between dental caries and band expression. The 500 bp band demonstrated significant association with individual family member compared with a *P* value of 0.03. However, no significance could be derived with caries status [Table 8b].

d. Chromosome 5q12.1-13.3 analysis in saliva samples in family units of children with permanent dentitions: The band analysis of 10 bp expression demonstrated no correlation in family units with children having carious teeth but, on the other hand, in the non-carious group, negative correlation (Φ = -0.09) was noted between father-child and positive correlation (Φ = +0.43) was observed between mother-child pairs. Significant differences were noted on comparing caries status, band expression and family unit ($P = 0.01$) in families of children with carious dentitions, and positive correlation (Φ = +1) was noted between mother-child pairs and no correlation was seen among families with children with no dental caries. An extremely significant difference ($P = 0.0012$) was observed between caries status and band expression. On analysing the 500 bp expression, no correlations were observed between parent-child pairs and band expression; no correlation was observed between parent-child pairs and band expression, however, significant *P* value ($P = 0.03$) was noted between these two parameters ($P = 0.03$). On analysing the 1 bp band expression, positive correlation existed between father-child and mother-child pairs (Φ = +0.69 and + 0.69, respectively) along with a significant association of $P = 0.03$ between caries and band expression [Table 8b].

III. Chromosome 13q31.1 expression in blood: A uniform band expression at 99 bp with no correlation among the

**Table 7: Table demonstrating caries scores in parents**

| | Father | Mother |
|---|---|---|
| Sample size | 57 | 57 |
| Mean DMFS | 12.94 | 9.22 |
| Minimum DMFS | 0 | 0 |
| Maximum age DMFS | 65 | 51 |
| Standard deviation | 14.437 | 11.571 |
| Standard error | 1.912 | 1.532 |
| Confidence level (95%) | 3.83 | 3.07 |

**Table 8: Table demonstrating genetic parameters studies**

| a) Chromosome 5q12.1-13.3 in blood samples | | | | | |
|---|---|---|---|---|---|
| **Bands expressed** | **Correlation between father and child (ϕ)** | **Correlation between mother and child (ϕ)** | **Correlation between father and child (ϕ)** | **Correlation between mother and child (ϕ)** | ***P*** |
| | **Carious paediatric primary dentition** | **Carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | |
| 2.5 bp | +0.86 | +0.86 | +1 | +0.09 | 0.046 |
| 1.2 bp | +0.4 | +0.44 | +1 | +1 | 0.99 |
| 900 bp | +1 | +0.63 | +1 | +1 | 0.77 |
| 3 bp | +1 | +0.69 | - | - | 0.6 |
| 1.5 bp | 0.4 | +0.69 | - | - | 0.002 |
| 1 bp | +0.89 | +0.69 | +0.63 | +1 | 0.04 |
| | **Carious paediatric permanent dentition** | **Carious paediatric permanent dentition** | **Non-carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | ***P*** |
| 4 bp | +0.84 | +0.84 | +1 | +0.52 | 0.6 |
| 3 bp | +0.09 | +0.69 | +1 | +1 | 0.98 |
| 2.5 bp | +0.58 | +0.58 | +1 | +1 | 0.0006 |
| 2 bp | +0.84 | +0.73 | +0.17 | +0.26 | 0.56 |
| 1 bp | +1 | +0.89 | +1 | +0.89 | 0.001 |
| 900 bp | - | - | - | - | - |
| **b) Chromosome 5q12.1-13.3 in saliva samples** | | | | | |
| **Bands expressed** | **Correlation between father and child (ϕ)** | **Correlation between mother and child (ϕ)** | **Correlation between father and child (ϕ)** | **Correlation between mother and child (ϕ)** | ***P*** |
| | **Carious paediatric primary dentition** | **Carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | |
| 3 bp | +0.24 | +0.69 | +0.44 | 0.6 | 0.0002 |
| 1.2 bp | +0.6 | +0.6 | - | - | 0.0012 |
| 1.7 bp | +1 | +1 | +1 | +1 | 0.0012 |
| 1.8 bp | - | - | - | - | 0.3 |
| 500 bp | - | - | - | - | 0.03 |
| | **Carious paediatric permanent dentition** | **Carious paediatric permanent dentition** | **Non-carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | ***P*** |
| 10 bp | - | - | -0.09 | +0.43 | 0.01 |
| 3 bp | +0.09 | +0.69 | +1 | +1 | 0.98 |
| 2.5 bp | +0.38 | -0.22 | +0.41 | +0.41 | 0.0012 |
| 500 bp | - | - | - | - | 0.03 |
| 1 bp | +0.69 | +0.69 | - | - | 0.64 |
| 900 bp | - | - | - | - | - |
| **c) Chromosome 13q31.1 in blood samples** | | | | | |
| **Bands expressed** | **Correlation between father and child (ϕ)** | **Correlation between mother and child (ϕ)** | **Correlation between father and child (ϕ)** | **Correlation between mother and child (ϕ)** | ***P*** |
| | **Carious paediatric primary dentition** | **Carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | |
| 99 bp | - | - | - | - | 1 |
| 99 bp | - | - | - | - | 1 |
| **d) Glucosyltransferase B expression in Mutans Streptococcus** | | | | | |
| **Bands expressed** | **Correlation between father and child (ϕ)** | **Correlation between mother and child (ϕ)** | **Correlation between father and child (ϕ)** | **Correlation between mother and child (ϕ)** | ***P*** |
| | **Carious paediatric primary dentition** | **Carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | |
| 2 bp | +1 | +1 | - | - | 1 |
| 1 bp | - | - | - | - | 0.02 |
| | **Carious paediatric permanent dentition** | **Carious paediatric permanent dentition** | **Non-carious paediatric primary dentition** | **Non-carious paediatric primary dentition** | ***P*** |
| 1 bp | - | - | - | - | 0.12 |

studied parameters: band, caries presence or absence and family unit in both carious and non-carious family units [Table 8c].

IV. Chromosome 13q31.1 expression in saliva: This gene was found to be absent in saliva samples.

V. Microbiological parameters analysis by studying gtfB expression in MS [Table 8d]:
   (a) Band analysis in families of children with primary dentition: 2 bp expression presented with a positive correlation between father-child as well as mother-child pairs (Φ = +1, +1) in families of

children with dental caries but no correlation was seen between parent-child pairs in non-carious dentition children. In contrast, the 1.2 bp band demonstrated no correlation between parent-child pairs with or without caries affecting the teeth, while a significant *P* value ($P = 0.02$) was noted between caries, band expression and family unit. No correlations were obtained between expression of 1 bp band and parent-child in either carious or non-carious children families although significant difference was noted between caries status, band expression and family members ($P = 0.02$).

(b) Band analysis in families of children with permanent teeth: Only one band at 1 bp location of gtfB was found in collected biofilm samples. On studying its expression, it was observed that positive correlations were seen between father-child and mother-child pairs among families with non-carious dentitions ($\Phi = +0.07$ and + 0.53, respectively).

## DISCUSSION

Dental caries is an interplay between nature and nurture.[2] It is an homeostatic imbalance occurring between various environmental, behavioural and genetic factors which may be both protective and associated with the risk of caries development.[13] It is a complex oral pathological lesion where a variety of factors which include- environmental, microbiological and human genomic factors.[14] It is the result of a biofilm-dependent disease in which S. mutans plays an important role. Dysbiosis and homeostatic imbalances occurring within plaque-associated biofilms are the initiating events for dental caries.[15]

There appears to be a close association between the oral microbiological community ecosystem and human genetic constitution. The American Academy of Pediatric Dentists has also recommended that an assessment of caries risk includes: a child's biological age along with biological constitution and clinical findings.[16] There are few studies which have linked the occurrence of dental caries with its susceptibility. The present study is unique as it has demonstrated distinct findings in terms of extrinsic environmental factors and remarkable variations in genetic band expression for both human and microbiological genes that have been studied.

In our study, no significant difference was found between gender and caries experience. This is supported by Arangannal who had demonstrated no statistically significant difference between gender and prevalence of dental caries.[16]

In the present study, no significant difference in dental caries prevalence was observed between male and female subjects in either primary or permanent dentition groups (males, $P = 0.86$; 0.3 and females, $P = 0.83$, 0.45, respectively). Although, Sachdeva (2015) reported a prevalence of 33.85% which was associated with predilection for male subjects,[17] Toutoni (2015) reported no association of gender with dental caries.[18]

In the present study, no significant difference was noted in sugar exposures and dental caries. Supporting evidence has been reported by investigators such as Kaur *et al*.[19] who also did not find any significant difference between dental caries and number of sugar exposures.

In this study, mean DMFS scores of 12.94 ± 14.43 and 9.22 ± 11.57 were seen among fathers and mothers, respectively while the mean ± SD dfs scores in children with primary dentition were 1.69 ± 13.117 in males and 26.1 ± 39.89 in females; respectively. However, in permanent dentition, mean DMFS scores of 3.1 ± 3.65 and 4.64 ± 10.681, respectively were seen among males and females. These findings have been corroborated by Das *et al*.[20] who reported the prevalence of dental caries in permanent dentition as 25.39% in male and 30.86% in female children (overall, 28.06%). While a highly significant statistical difference of $P = 0.05$ was found in primary dentition, the overall prevalence was found to be 78.9%. According to Hiremath in 2016 in a Bengaluru-based study, the overall prevalence was found to be 78.9%.[21] Mohammadi in their study reported a mean DMFT score of 0.50 ± 1.04 and 0.66 ± 1.12 for boys and girls, respectively and this was found to have a statistical significance of *P* value of 0.014, whereas the mean DMFT among male and female children was found to be higher in boys compared to girls ($P = 0.55$).[22]

No statistical difference was observed between caries scores and plaque index ($r = 0.284$, $P = 0.78$) in children's dentitions. Similarly, no significance was observed in male ($r = -0.15$, $P = 0.5$) and female ($r = 0.47$, $P = 0.09$) children with permanent dentition. These findings are in conformance with Toi *et al's*.[23] who reported no statistically significant difference between mean bacterial counts and caries index. Although, in the present analysis, on comparing both the genders regardless of the type of dentition, an extremely significant difference ($r = 0.66$; $P = 0.0005$) was noted between plaque index and caries index scores in female children, no significant difference ($r = 0.067$, $P = 0.7$)

was noted among male children. In contrast, Madeline *et al*.[24] found a significant association between plaque and prevalence of dental caries.

Dental caries is an intricate interplay between genetic and numerous extraneous environmental factors. Host-associated genetic factors have shown a strong association with metabolism of carbohydrates in oral microbial organisms.[22,25,26]

On analysis of the chromosomal locus at 5q12.1-13.3 in blood and saliva, it was seen that the genetic expression was spread across multiple bands demonstrating the variabilities in expression in both primary and permanent dentition bearing children of various family units. However, on analysing the high-risk gene located at chromosome locus 13q31.1 in blood samples, a ubiquitous expression was observed regardless of a child's caries status. Interestingly, no expression of this gene was noted in the saliva samples, thus indicating that it has no protective local response against dental caries. Our study findings are in contrast to Kuchler who studied 72 Filipino families for caries experience by genotyping 61 single nucleotide polymorphisms (SNPs) in chromosome 13q31.1 isolated from saliva. A statistically significant association was seen between dental caries experience and 13q31.1. It was observed that when a subject carried the G allele of re17074565, the transcription factor involved was GAT3.[26] Significant association was also observed in our study between GATA3 band expression and the presence of dental caries for 1 bp band in the blood samples.

S. mutans is responsible for the synthesis of three types of Gtf: (a) Gtf B, which synthesizes both the insoluble forms of glucan; (b) GtfC, which synthesizes both the soluble and insoluble forms of glucan and (c) GtfD, which synthesizes soluble form of glucans. Both the GtfB and –C are required for extracellular polysaccharide formation which is a constituent of biofilm. In particular, GtfB is responsible for aggregation of S. mutans thus contributing to a large extent in the formation of a biofilm.[25] This particular microorganism causes fermentation of sorbitol and mannitol and converts sucrose to glucans. This group of microorganisms demonstrate genetic and serological heterogeneity.[27,28]

In our study, on analysing the gtfB expression, the band weighing 1 bp was found to be commonly expressed among all biofilm samples regardless of caries presence or absence. Wide variations in the expression of various bands were noted with specific expression of 2 bp and 1.2 bp GtfB gene expression. Thus, supporting earlier observations that S. mutans may transmit within family members. Thus, various patterns of transmission of S. mutans include: parent-to-child transmission, transmission within families and between siblings.[29] Foxman *et al*.[31] analysed similarities in salivary microbiome within families along with association between salivary microbiota and dental caries with age. It was noted that there was a change in salivary microbiome with progression of age. However, in our study, a 1 bp band was consistently found in both the age groups, hence suggesting that there was no alteration in salivary microbiota with dental caries.[30]

Genes can influence dental caries by impacting (a) dental hard tissues; (b) host immunobiological response; (c) metabolism of carbohydrates and frequency of consumption and (d) salivary characteristics such as flow, constituents and defensive mechanisms.[2] Renuka *et al*.[2] in their literature review reported that various caries phenotypes reported in primary dentition exhibit heritability with approximately 54%–70% of caries scores variations whereas, 35%–55% of genes influence caries in permanent dentition. One of the influencing factors is the genetically determined composition of dental hard tissues that are influenced by genes such as amelogenin and nonamelogenin proteins like ameloblastin, tuftelin and enamelin.[2,31]

Shaffer *et al*. calculated heritability estimate as phenotypic proportion of variance attributed to cumulative genetic effects. This study identified the heritable nature of various tooth decay patterns based upon their heritable as well as non-heritable nature.[32] Studies on twins have demonstrated intangible evidence on influence of genetic and environmental factors on specific disease traits. The monozygotic twins have 100% sharing of all genes whereas dizygotic twins share 50% of genetic information.[32] Anu conducted an analytical study on 30 pairs of twin children of which 17 and 13 were monozygotic and dizygotic, respectively. It was observed that DMFT, teeth spacing, irregularities in mandibular teeth, open-bite, mesiodistal distance between canines demonstrated higher positive correlation in monozygotic when compared with dizygotic twins. This study showed high concordance rate of 87.5%, 71.6% correlation and 87.8% heritability.[32]

An individual's genetic susceptibility towards development of dental caries can be observed under specified experimental states. There are mainly three categories of candidate genes impacting dental caries: (1) genes affecting amelogenesis; (2) genes affecting formation and composition of saliva and (c) genes influencing immunological response.[33,38]

Koohpeima *et al.* demonstrated a significant association between rs946252 gene polymorphism in AMELX and dental caries. The T allele of this gene was found to have a significant protective role for dental caries among adult Iranian population.[34]

Roy performed a PCR-based analysis on blood samples of 60 subjects to investigate the association between rs2252070 (Matrix metalloproteinase protein-13) and dental caries. The study results showed that single gene nucleotide polymorphism was associated with dental caries even among individuals with good oral health irrespective of age and gender.[35]

Zeng observed a significant association of dfs scores for smooth-surfaced caries with rs17124372 and numerous linked short nucleotide polymorphisms localized on 20q11.21 in region containing nine genes belonging to PLUNC gene family. Similarly, a significant association was noted between pit and fissure dfs score scan and rs7121800 ($P$ = 6.9E-6 and 1.6E-7, respectively).[36] Also, various other associations were located on different chromosomal loci in both pit and fissure and smooth surface dental caries. These included: genome-wide significance in loci localized on 3q26.1 (rs17236529) for dfs (pit and fissure caries), $P$ = 2.0E-9; 18q12.2 (for rs11082096 and $P$ values of 2.6E-7 and 1.7E-6 for dfs scores for pit and fissure and smooth surface caries) and Xq21.2 (rs5967638 with $P$ values of 1.3E-6 and 8.3E-7 for pit and fissure and smooth surface caries, respectively).[36]

Wang *et al.* performed a genome-wide association study for dental caries affecting permanent dentition. These investigators did not find any genetic association with any genomic significance. Majority of the study participants had no exposure to fluoride. Loci observed on FZD1 located on chromosome 7 and TLR2 on chromosome 4 were demonstrated to show association with dental caries, but no significant association was observed.[37]

Goodman reported sharing of amplitypes of S. mutans in children who were not related genetically. There was a low rate of transmission which was attributed to short duration and less frequency of contact which may be due to less sharing of utensils and food items as compared to relatively younger age-group children.[7]

When a biofilm gets exposed to highly fermentable carbohydrates, cariogenic oral microorganism such as S. mutans, S. sobrinus and few species of Lactobacillus are naturally selected with continuous acidic exposure along with the limited capacity for buffering by the host leading to decalcification of teeth. This process gets modified via various environment-related factors like: oral hygiene status, fluoride exposure, gender, age and socioeconomic status.[37]

## CONCLUSION

Some of the multiple risk factors which subsidize to cariogenesis are: oral microflora, diet pattern, uptake of fluoride, oral hygiene maintenance, composition and rate of flow of saliva, position of teeth, anatomical traits, genetics and genetic-environmental interaction.[13,38] In conclusion, the present study has demonstrated numerous unique results for the chromosome locus at 5q12.1-13.3; the 1.7 bp band demonstrated common expression in all studied groups in saliva while in the blood samples, both the 2.5 bp and 2 bp band expressions showed their association with low dental caries susceptibility. Thus, there was a variation in alleleic locations on the studied chromosomal locus in blood and saliva samples. The high-risk caries susceptibility gene 13q31.1 demonstrated no statistical significance in blood while it was found to be absent in saliva samples. Clarke (1924) was the first person to identify 'S. mutans' as a causative pathogenic agent in dental caries. The GtfB is a bacterial cellular component responsible for bacterial adhesion. In this study, the GtfB genetic expression for MS demonstrated variations in expressions thereby indicating different subtypes of S. mutans.

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### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

There are no conflicts of interest.

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