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Association of rs 5934997 Single Nucleotide Polymorphism in AMELX Gene with Dental Caries Risk in a Group of Egyptian Children (Case Control Study)

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Abstract

Dental caries is a very common, intricate, and multidimensional illness which is influenced by the host's microbiological, genetic, environmental, and socioeconomic factors. Research conducted on humans has indicated that caries may be caused by genetic variations in the genes that form enamel and how those variations interact with the levels of Streptococcus mutans. To determine the relationship between selected single nucleotide polymorphism (SNP) in AMELX gene and (streptococcus mutans) with caries susceptibility at a group of children from Egypt. This case control study, rs 5934997 in AMELX gene was studied in 54 children with deciduous teeth from 4 to 6 years. Children are allocated in 2 groups, Group A patients (children with dental caries) and group B controls (caries free children). Oral swab and saliva samples were collected for molecular and microbiological evaluation. SNP marker (rs5934997) was genotyped. *Streptococcus mutans* count revealed that Group A were significantly more than Group B. Positive correlations which were statistically significant found between TT genotype of rs5934997 in AMELX and presence of dental caries as comparison between both groups revealed that mutant TT genotype in group A (48.1%) was significantly higher than group B (0%). Regarding allele frequency C allele in group B (control) 77.78%was significantly greater than group A (patients) 31.48%. Significant association of both CT and CC genotypes with *Streptococcus mutans* count was found in both groups. rs5934997 marker SNP in AMELX gene may be considered as one of the risk factors for occurrence of dental caries in children from Egypt.

Keywords: Single nucleotide polymorphism, children, caries, AMELX gene.

Full length article **Corresponding Author*: *Nehall A. Abdel lattif*, e-mail: nihalalaa28487@gmail.com

1. Introduction

Dental decay is a very common, intricate, and multidimensional illness which is influenced by the host's microbiological, genetic, environmental, and socioeconomic factors. It is the consequence of the dentine and dental enamel's ongoing localized demineralization. Caries can affect people of any age, but research indicates that early exposure to the cariogenic *Streptococcus mutans* in newborns and early children may exacerbate existing lesions and increase the risk of developing new ones. Furthermore, although caries is becoming less common in some countries, it still affects 60–90% of children globally, with developing nations being the most affected [1-2]. Oral Streptococci represent 80% of the initial invaders during early formation of biofilm but only account for 20% of the oral biofilm's supragingival bacteria [1]. Dental caries may be more common in some patients than in others, even though the same environmental factors are present for them; these variations may be caused by genetic components in dental caries etiopathogenesis. Recent research has increased the likelihood that genetic factors and dental caries are related. Dental caries arises from complex interactions between environmental and genetic factors [3]. There is proof that there is a hereditary component to caries susceptibility, and research on humans has indicated that genetic variants in the

genes that form enamel and their interaction with the levels of *Streptococcus mutans* may be responsible for caries [4]. Research assessing genetic variation and its relationship to dental decay has been suggested in the hopes that novel findings will result in creative methods for preventing the disorder [3]. Dental enamel, the hardest biological tissue on human teeth is essential and fundamental component of the tooth. It is primarily responsible for overcoming food resistance, preventing masticatory pressures. Ameloblasts are cells that form enamel and are derived from ectoderm [5]. Genetics governs both odontogenesis and amelogenesis; genetic diversity affects the microhardness, size, morphology, color, and shade of enamel as well as the likelihood of caries [6]. We hypothesize that human caries susceptibility is partly due to genetic variants in genes involved in enamel formation. As a result, we looked into genetic markers in previously identified genes related to enamel formation. It has been reported that the development of dental caries is influenced by three common genes: amelogenin X (AMELX), enamelin (ENAM), and lactotransferrin (LTF) [7]. The amelogenin is encoding by AMELX gene. Amelogenins are required for the hydroxyapatite prism to form properly, for the prisms to elongate during the development of enamel, and for the production of normal enamel thickness [7]. The primary protein of dental enamel, amelogenin, is produced by the crucial gene AMELX during amelogenesis secretion stage. Enamel microstructure exhibits unique variations due to AMELX polymorphisms. As a result, these polymorphisms are essential for controlling enamel thickness and mineralization [7].

Therefore, we aimed to evaluate the role of genetics in dental caries through studying the association of polymorphisms of this gene and dental caries risk.

This study was aimed to evaluate the association between AMELX gene single nucleotide polymorphisms, the microbial causing agent of dental caries (streptococcus mutans) and dental caries susceptibility in a group of children from Egypt.

2. Materials and methods

A total sample size of 54 (27 in each group) was adequate to identify an effect size = 0.38, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for two-sided hypothesis test [8]. Study participants were chosen from children visiting the outpatient clinic of Pedodontics and Oral Health Department, Faculty of Dental Medicine for Girls, Al-Azhar University and children attending day nurseries located in Cairo, Egypt. A total of 54 subjects (age 4-6 years old) participated in this study. They were screened depending on the following eligibility criteria.

2.1. Inclusion criteria [8]

1. Age range of children should be from 4-6 years.
2. Children with dental caries and incipient carious lesions whom were diagnosed ≥ Code 1 according to ICDAS scoring system, and they were classified as the study group (“cases”). Children who were free of the disease (Code 0), and had no filled teeth, will be classified as (“controls”).
3. Children whose parents gave their informed consent.
4. Egyptian children.

2.2. Risks and Benefits of the study

Benefits for the child:

After conducting the study, the parent was informed of the extent of his child’s susceptibility to caries, and the patient was completely treated for caries in all of his teeth.

Scientific benefits of the study:

Based on the results of the samples for each child, it was determined whether the child needed additional preventive measures to avoid tooth decay.

Risks of the Study:

No risks or side effects for the study.

Written consent was given to the Parents of children for an oral swab and saliva sample as well as a dental examination (Fig.1). Each child voluntarily participated in the study.

2.3. Identifying caries phenotype

Caries was identified by performing a visual inspection, a probe and dental mirror was used to be sure of the change. It was performed from July to August 2023 by 1 calibrated and trained pedodontist. Caries's condition was evaluated by international Caries Detection and Assessment System II (ICDAS II) [9-10]. Patient were split into two groups. (27 patients each group) following ICDAS II Scoring System. **Group (A):** Subjects with dental caries whom were diagnosed with at least one tooth from Code 1 to Code 6 according to ICDAS II Scoring System, and they were categorized as "cases" or the study group. **Group (B):** Subjects who were free of decay (Code 0 according to ICDAS II Scoring System), and had no filled teeth, were classified as (“controls”).

Dental evaluation looks for the presence of teeth with carious cavities according to ICDASII Scoring System. Oral swabs for DNA extraction and saliva samples for microbiological culture were collected during the examination.

2.4. Saliva sample collection

Each child had a sample of their unstimulated saliva taken. Before saliva collection, each child was instructed to fast for at least an hour without eating, drinking, or using any oral hygiene products. Each child's saliva was collected in a labeled graduated centrifuge tube (a Citotest centrifuge tube), which was then brought to the microbiology department for microbial culturing. 0.2 units of bacitracin per milliliter and 20% sucrose were added to mitis salivarius agar to create mitis salivarius-bacitracin (MSB) agar. An inoculated platinum loop was used to remove streaks of saliva from the specimen onto MSB agar plates after the MSB agar plates had been prepared. After inoculation all plates were put in a candle jar and incubated anaerobically at 37℃ in Binder BF400 incubator (Binder Tuttlingen, Germany) for 24 hours. After incubation the colonies on MSB were identified according to colony morphology where they formed dark blue colonies with raised rough surfaces and differentiated by gram staining [(11)]. For the gram staining of mutans streptococci showed up as chains of gram-positive cocci (Fig.2). The viable count technique was used to count the number of cells, and the subsequent equation was used: Number of colonies/ml (CFU/ml) = Number of colonies counted times the reciprocal of the dilution times reciprocal of the cultured volume (ml)

جامعه الازهر
كليه طب الاسنان
فرع البنات

الموافقة المستنيرة للمتطوعين

عنوان البحث باللغة العربية: تقييم العلاقة بين جين **AMELX** ، المكورات العقدية الطافرة ، وقابلية تسوس الأسنان في مجموعة من الأطفال المصريين

الهدف من إجراء البحث: الدراسة لتقييم العلاقة بين بعض الاشكال المتعددة النوكليوتيدية المنفردة لجين و قابلية تسوس الاسنان في مجموعة من الاطفال المصريين بالإشتراك مع العامل الميكروبي المسبب لتسوس الأسنان (العقدية الطافرة).

مقدمة عن وما سيتم اجراؤه على المريض بالتفصيل (خطة العمل):

الجزء الاول: سيتم توفير مسحات فموية لكل طفل في عبوات معقمة ثم فرك الجزء الداخلي من الفم 10 مرات على الأقل من كلا جانبي الخدين ثم تم وضع المسحة داخل أنبوب إيبندورف سعة 1.5 مل ووضعها عند +4 درجات مئوية في ثلاجة محمولة.

سيتم إستخلاص الحمض النووي الجيني من الخلايا الشدقية ثم إجراء التنميط الجيني من خلال تحليل تعدد أشكال النوكليوتيدات المفردة (SNPs) في جين AMELX.

الجزء الثاني:سيتم جمع عينات اللعاب غير المحفزة من كل طفل.

الفائدة المباشرة للشخص المتطوع: سيستفيد كل متطوع من كشف كامل للأسنان وتشخيص لكل التسوس ثم إزالته وحشو الضروس المعنية بالتجربة.

الفائدة العلمية والفائدة العامة المرجوة من البحث: تهدف هذه الدراسة الي تقييم العلاقة بين تعدد أشكال النوكليوتيدات المفردة لجين **AMELX** ، والمكورات العقدية الطافرة ، وقابلية تسوس الأسنان لدى مجموعة من الأطفال المصريين. بناءا على نتيجة العينات لكل طفل يتم تحديد اذا كان الطفل يحتاج سيل وقاية اضافية لتجنب تسوس الاسنان و ذلك بناءا على مدي قابليته للتسوس.

الأعراض الجانبية ودرجة المخاطر المتوقع حدوثها وكيفية التعامل معها: لا يوجد أعراض جانبية او خطورة من البحث.

المعرفة الكاملة للمريض بخطوات البحث. قراءة [ ] شرح شفهي [√]. أخرى[ ]

1. لقد اطلعت بعناية وفهمت الغرض من إجراء البحث وطبيعة هذه الدراسة، وأنا أفهم ما هو ضروري لإنجاز هذه الإجراءات.
2. سوف يتم اجراء العلاج في كلية طب الأسنان جامعة الازهر بنات أو أي جهة معتمده مثل الجامعات الأخرى أو المراكز البحثية.
3. قد أعلمني الطبيب الباحث بالبدائل العلاجية الممكنة لهذا البحث.
4. لقد أبلغني الطبيب الباحث بجميع المخاطر المحتملة لهذا البحث وكيفية التعامل معها.
5. أوافق على التصوير والتسجيل، وجميع أنواع الأشعة والتي يتعين القيام بها في هذا الدراسة، بشرط عدم الكشف عن هويتي.
6. -لقد قدمت تقريرا دقيقا عن تاريخ حالة (نجلي/نجلتي) الصحية. وأبلغت الطبيب بجميع أنواع ردود الأفعال الصحية أو الحساسية غير العادية من الأدوية والأغذية أو لدغ الحشرات أو مواد التخدير والغبار أو أي ردود أفعال حدثت لي من أي مواد أخرى، ونزيف غير طبيعي أو أي ظروف أخرى ذات صلة على صحتي.
7. أقر بأني غير مشترك في أي بحث آخر منذ بداية هذا البحث وحتى انتهائه وأني سأعلم الطبيب لو دخلت أي بحث آخر طوال فترة البحث.

بعد معرفة المعلومات المتاحة الخاصة بالبحث يتفضل الشخص المتطوع او المسئول عنه بالإختيار بحرية ما بين الاشتراك من عدمه في حال الموافقة يتفضل بملء البيانات الموضحة. من حق المتطوع الإنسحاب من البحث بدون إبداء الأسباب مع مراعاة حق استرجاع الباحث لأي أجهزة أو أدوات طبية مستعملة بغرض البحث بحوزة المتطوع (تسمى من قبل الباحث).

تعهد الطلب المسئول عن البحث بالحفاظ على سرية المعلومات الخاصة بالشخص المتطوع بالمشاركة في البحث مع ذكر الطرق المستخدمه لذلك مثل استبدال الإسماء بارقام كوديه أو اخفاء معالم الوجه عند التصوير الفوتوغرافي ان امكن (الخ....)

في حالة وجود شكاوى: يتم التواصل مع منسق لجنة الاخلاقيات في القسم كما يمكن التواصل مع اللجنة مباشرة عن طريق البريد الإلكتروني ethics.committee@azhar.edu.eg

اسم المتطوع: تاريخ الميلاد:
اسم ولي الأمر أو المرافق (عند اللزوم): الرقم القومي:
العنوان: الهاتف:
توقيع الباحث: ط. نهال علاء التاريخ:
توقيع المشرف على البحث (في حالة الرسائل): أ.د/ إعتماد شوربيه د/ محمد حسن مصطفي التاريخ:

هذا البحث تمت موافقة اللجنة عليه برقم
PEDOD-102-2Q

من حق المتطوع الاحتفاظ بنسخه مصورة من الموافقة المستنيرة للبحث الذي تطوع فيه

Figure 1. Informed Consent

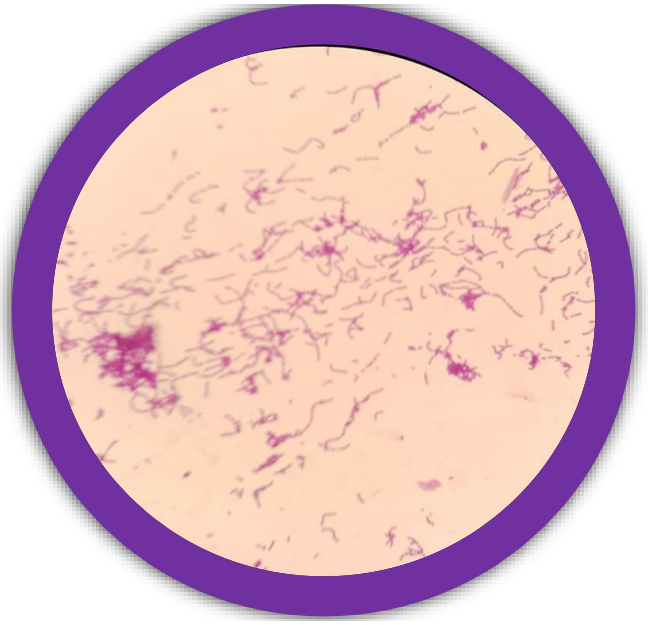

Fig.2: *Streptococcus mutans* under light microscope

2.5. Oral swab collection

Each subject received a sterile pack of oral swab. Rubbing the cheek from the inside was done for 15 seconds (approximately 20 brushes) for each side of the cheeks and after that the swab was put inside the 1.5 mL Eppendorf tube that contains DNA extraction lysis buffer. After that, we cut off the plastic stick and the Eppendorf tube was put at +4 °C in a movable refrigerator till extraction of DNA.

2.6. DNA Extraction

Extraction of genomic DNA from the swab was done the same day of collection by the column system using Quick-DNA™ Miniprep Kit, ZYMO RESEARCH.USA following manufacturer's guidelines then DNA samples frozen in -20°C until analysis.

2.7. Molecular Analysis and Genotyping

Genotyping was done for one known to date from the literature single nucleotide polymorphism (SNP) in AMELX (rs5934997), that has been implicated in the susceptibility to caries in previous studies. rs5934997 genotyping was performed by real-time polymerase chain reaction (PCR) using TaqMan probe assay system (Applied Biosystems), in which 2 probes – each for one allele in a two-allele system –distinguish between two polymorphic variants. The 96-well optical reaction plates were used for quantitative real-time PCRs using the Light Cycler® 480 Instrument II (Roche), according to the instructions of the manufacturer instructions. For each sample reactions were done in a 10 µL total volume of reaction, using 5 µL of TaqMan genotyping master mix, 1.25 µL of×20 probe and 5µL of DNA (up to 50ng of high quality DNA per reaction). The cycling conditions began with an initial denaturation at 94°C for 15 minutes then 40 denaturation cycles at 94°C for 20 seconds. Annealing/extension at 55°C for 1 min. The outcomes were displayed as an increase in either just one of the two dyes—FAM or VIC, separately—or from both dyes simultaneously. The presence of one allele (homozygous) or two alleles (heterozygous) was indicated [12].

2.8. Statistical analysis

Microsoft Excel 2016 was used to conduct the statistical analysis along with SPSS 20®, Graph Pad Prism®, and. The chi-square test was used to calculate the genotypes that were obtained for the study using the TaqMan probe assay. Using the Mann-Whitney test, the frequency of allele and genotype for SNP were assessed and compared between the study and control groups. Odds ratio values (OR) were were assessed and p < 0, 05 considered as statistically significant. Shapiro Wilk and Kolmogorov Normality Test were used to determine the normality of all quantitative data, which were then presented as minimum, maximum, means, and standard deviation (SD) values. All qualitative data were displayed as percentages (%) and frequency (N).

Statically tests used: Shapiro Wilk and Kolmogorov were used for normality exploration, Mann Whitny`s test to compare between different groups regarding quantitative data and Chi square test in qualitative data comparisons.

2.9. Ethical Committee approval

The Research Ethics Committee (REC) of the Faculty of Dental Medicine for Girls at Al-Azhar University approved the study, (approval no:REC-PE-23-16-b)

3. Results

A total of 54 children were enrolled in the study (27 in each group). Group A (children with dental caries) included 14 females (51.9%) and 13 males (48.1%) with average age 5.29 ± 0.82. on the other hand, group B (caries free children) 15 were males (55.6%) and 12 females (44.4%) with average age 6.63 ± 2.73.

3.1. Microbiology results

Results of *Streptococcus mutans* count in terms of (CFU) showed that Group A was significantly more than Group B as P‹ 0.0001. The mean *S. mutans* count was (40.41 ±21.98) and (6.75 ± 2.86) in Group A and Group B respectively.

Comparison between group A and B was done by using Independent t test which showed that Group A was significantly higher than group B in male, female, and overall as P<00001.

3.2. Molecular results

The molecular results for the SNP assay was displayed as a rise in the fluorescent signal from either both dyes simultaneously or just 1 of the 2 dyes (FAM or VIC, separately) (Fig.3) It indicated whether there was a homozygous (single allele) or heterozygous (two alleles) condition.

3.3. Genotyping

Rs5934997 genetic marker in *AMELX* gene

Molecular analysis revealed the presence of SNP in group A as homozygote mutant TT genotype in 13 patients out of 27 (48.1%), heterozygote CT genotype in 11 patients (40.7%) and homozygote normal CC genotype in only three patients (11.1%). With T and C allele frequency as 68.52% and 31.48% respectively. On the other hand group B revealed the absence of TT genotype, 12 out of 27 (44.4) were CT genotype and 15 out of 27 (55.6%) were CC genotype. Comparison between both groups revealed that the homozygote mutant genotype TT was significantly higher in group A (48.1%) than group B with p value P<0.0001. In group A, homozygote normal CC genotype (11.1%) was significantly lower than in group B (55.6%) with p value P=0.0007. There was insignificant difference between group A (40.7%) and group B (44.4%) regarding CT genotype. Distribution of different rs 5934997 genotypes in the two groups were showed in table (1) and (Fig. 4). Comparison of both groups regarding the alleles frequency revealed that The T mutant allele in group A (68.52 %) was significantly more than in group B (22.22%) with p value P=0.0007 .The C normal allele in group A (31.48 %) was significantly lower than group B (77.78%) with p value P<0.0001. Distribution of different rs 5934997 genotyping alleles in both groups were presented in table (2) and (Fig. 5). Regarding Distribution of different genotypes in rs 5934997of both groups among gender comparison between both groups revealed that in males TT mutant genotype was significantly higher in group A (53.8%) than group B (0%) with p value P<0.0001. The same in females TT mutant genotype was significantly higher in group A (42.9%) than group B (0%) with p value P=0.0002. Regarding CC homozygote normal genotype in males group A (7.7%) was significantly lower than group B (66.7%) with p value P<0.0001.While in females CC normal genotype was significantly lower in group A (14.3%) than group B (41.7%) with p value P=0.02.There was insignificant difference between males and females regarding CT genotypes.

3.4. The association results between different genotypes and Streptococcus mutans count.

Comparison between group A and B was done by using Independent t test which proved the significant association of both CT and CC genotypes with *Streptococcus mutans* count thus CT heterozygote was significantly higher in group A (34.64 ± 12.61) than group B (7.33 ± 2.90) with p value P<0.0001.CC Homozygote normal genotype CC Homozygote normal genotype was significantly in group A (75.0 ± 30.41) higher than group B (6.07 ± 2.55) with p value P<0.0001. Mean and standard deviation of *Streptococcus mutans* count regarding group A and Group B were presented in (table 3) and (Fig 6).

4. **Discussion**

Dental caries is a contagious condition with numerous host and environmental contributing factors due to its multifactorial nature. Through their impacts on the mineralization and structure of enamel, whether direct or indirect, these factors may have an impact on the susceptibility to caries [11]. More than 600 distinct bacterial species can be found in the oral cavity, with *Streptococcus mutans* being the most common colonizer that causes dental caries [13]. Numerous epidemiological research have conclusively shown that *S. mutans* is positively correlated with the caries prevalence [14]. Dental caries arises from complex interactions between environmental and genetic variables. The role of genetics in enamel development has become more apparent in recent years. Numerous studies show that human dental caries susceptibility is influenced by genes involved in enamel development [3-9]. According to genetic studies, caries may be caused by genetic variants in the genes that form enamel and how those variants interact with the levels of *Streptococcus mutans* [4]. Our study is carried out on children aged from 4 to 6 years as in primary teeth, demineralization is more rapid than in permanent teeth and the progression rate of caries varies between primary and permanent dentition [11]. Ranging in age from four to six years, the case and control populations under examination were well matched. Additionally, because all of the children in the group were from Egypt, there were no differences in their demographic, ethnic, or regional makeup. This study is restricted to children from Egypt and as we know, our study is the first conducted on such a homogeneous population in Egypt regarding the genetic component of dental decay. Significant advancements have also been made in human genome mapping, which displays the location of specific genes on chromosomes. Consequently, it became possible to identify the genes that contribute to an increased susceptibility to caries [6]. Data from the literature suggests a potential link between dental caries occurrence and the genes that produce distinct enamel proteins [12]. Dental enamel is the most highly mineralized tissue and is derived from epithelial cells. Despite its hardness, dental caries, an infectious disease, can quickly erode enamel. Once the enamel is impacted by the carious process, it might not recover. Ameloblasts in the secretory stage secrete matrix proteins specific to enamel, including amelogenin, enamelin, and ameloblastin, which are essential for appropriate mineralization of enamel [15]. The synthesis of tooth enamel is the result of numerous chemical and physiological processes, including mineral growth, protein secretion, folding, assembly, degradation and also gene expression. The extracellular proteins that are positioned between the ameloblasts and dentin regulate the orientation, organization, and start of crystal formation in the enamel.

The cells that make enamel, known as ameloblasts, originate from epithelial tissue. Proteins that are produced and secreted by ameloblasts during the secretory stage are exchanged for calcium and phosphate during the maturation stage of the enamel matrix. Amelogenin and non amelogenin proteins comprise the majority of secretory proteins.

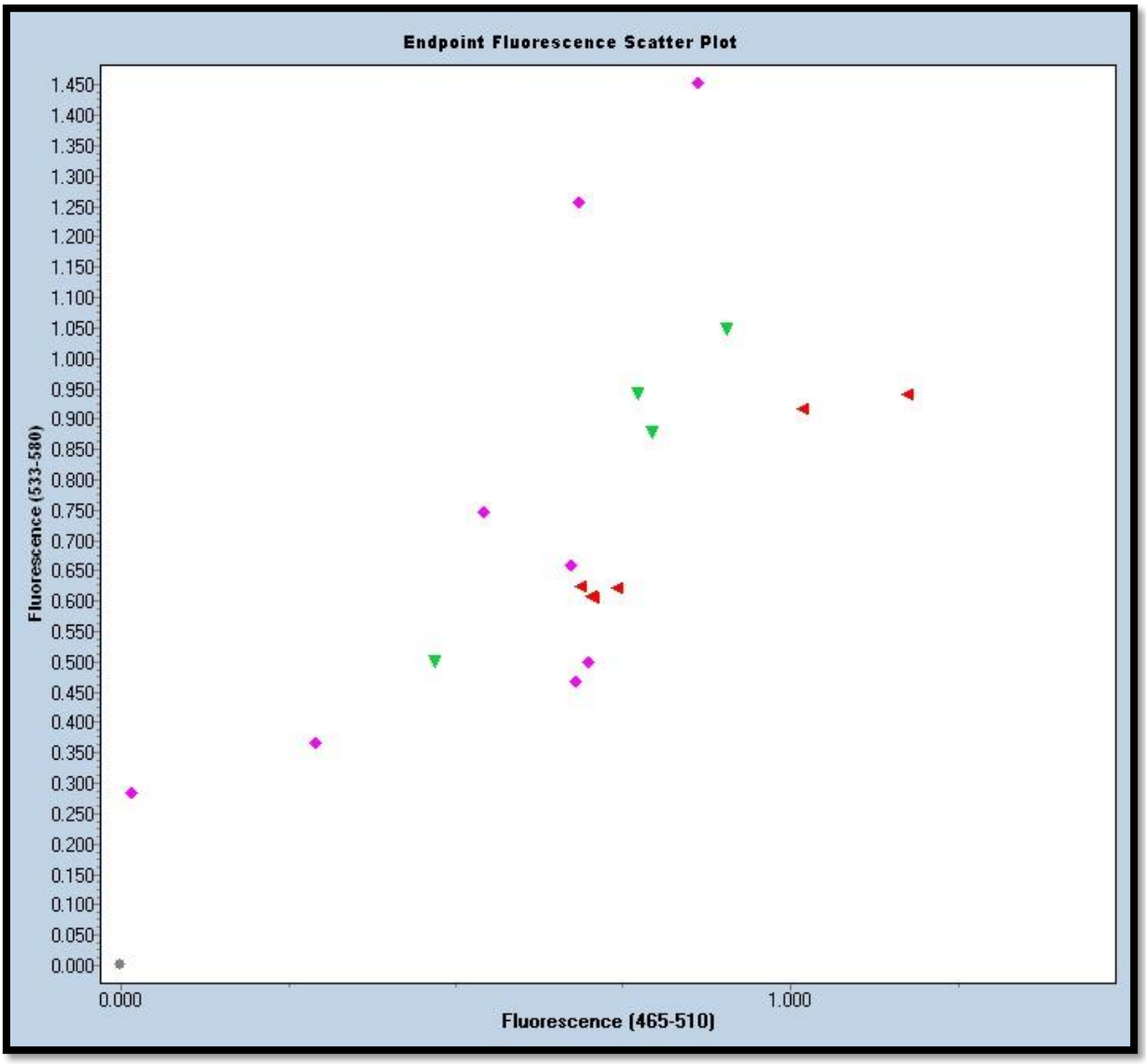

Fig. 3: Results showed on end point fluorescence scatter plot for rs5934997

Table 1. Distribution of different genotypes in rs5934997 marker in both groups, comparison between them using Chi square test

	Genotype		**Group A**		**Group B**		**P-value**
			N	%	N	%	
rs 5934997	**CC**	homozygote normal	3	11.1%	15	55.6%	0.0007**
	CT	heterozygote	11	40.7%	12	44.4%	0.76 ns
	TT	homozygote mutant	13	48.1%	0	0.0%	<0.0001 **

N: frequency, %: percentage, Ns: non-significant difference as P>0.05.
*Significant difference as P<0.05.
** Highly significant difference as P<0.001.

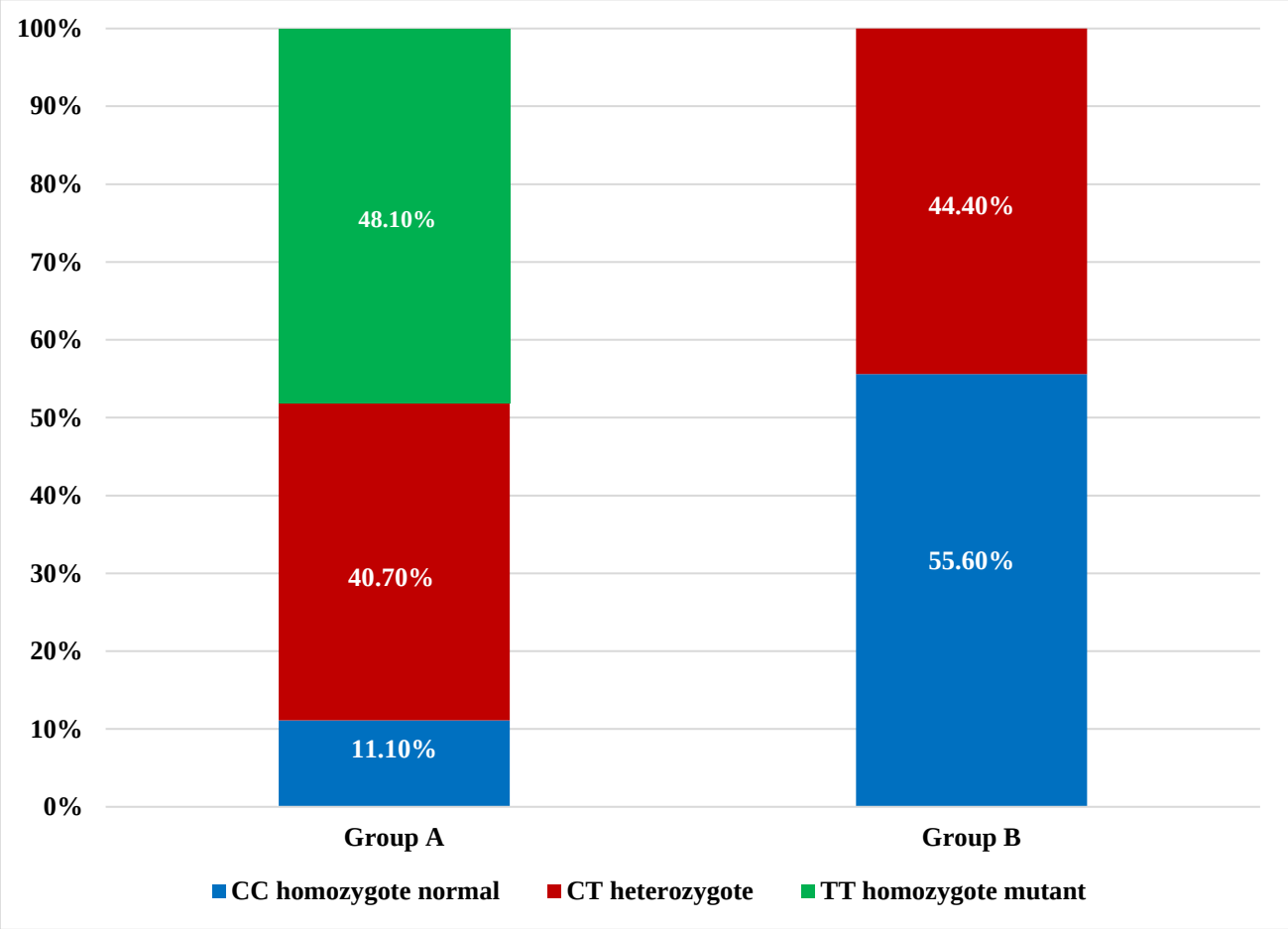

Figure 4. Bar chart showing Distribution of different genotypes in rs5934997 marker in both groups.

Table 2. Distribution of different alleles in rs5934997 marker in both groups, comparison between them using Chi square test

	Genotype		Group A		Group B		P value
			N	%	N	%	
rs 5934997	C	Normal	17	31.48%	42	77.78%	<0.0001 **
	T	Mutant	37	68.52%	12	22.22%	0.0007**

N: frequency, %: percentage, Ns: non-significant difference as P >0.05.
*significant difference as P<0.05.
** Highly significant difference as P<0.001

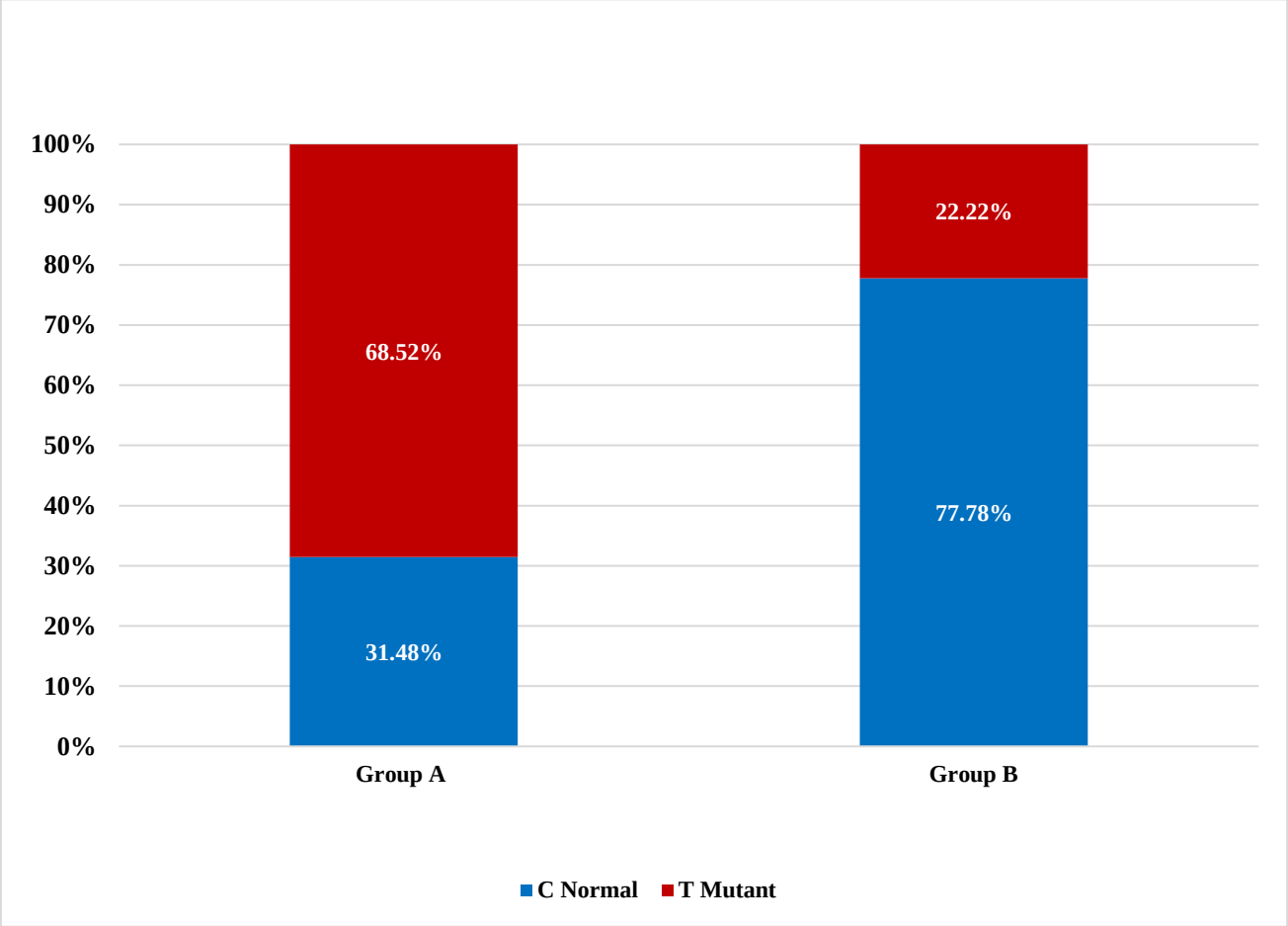

Figure 5. Bar chart showing Distribution of different alleles in rs5934997 marker in both groups.

Table 3. Association between *Streptococcus mutans* number (CFU) and different genotypes regarding rs5934997 marker in both groups

***Streptococcus mutans* count (CFU)**			**Group A**		**Group B**		**P value**
			M	**SD**	**M**	**SD**	
rs 5934997	**CC**	Homozygote normal	75.00	30.41	6.07	2.55	<0.0001 **
	CT	Heterozygote	34.64	12.61	7.33	2.90	<0.0001 **
	TT	Homozygote mutant	37.31	20.49	----	-----	-----

M: mean, SD: standard deviation.
** highly significant difference as P<0.001

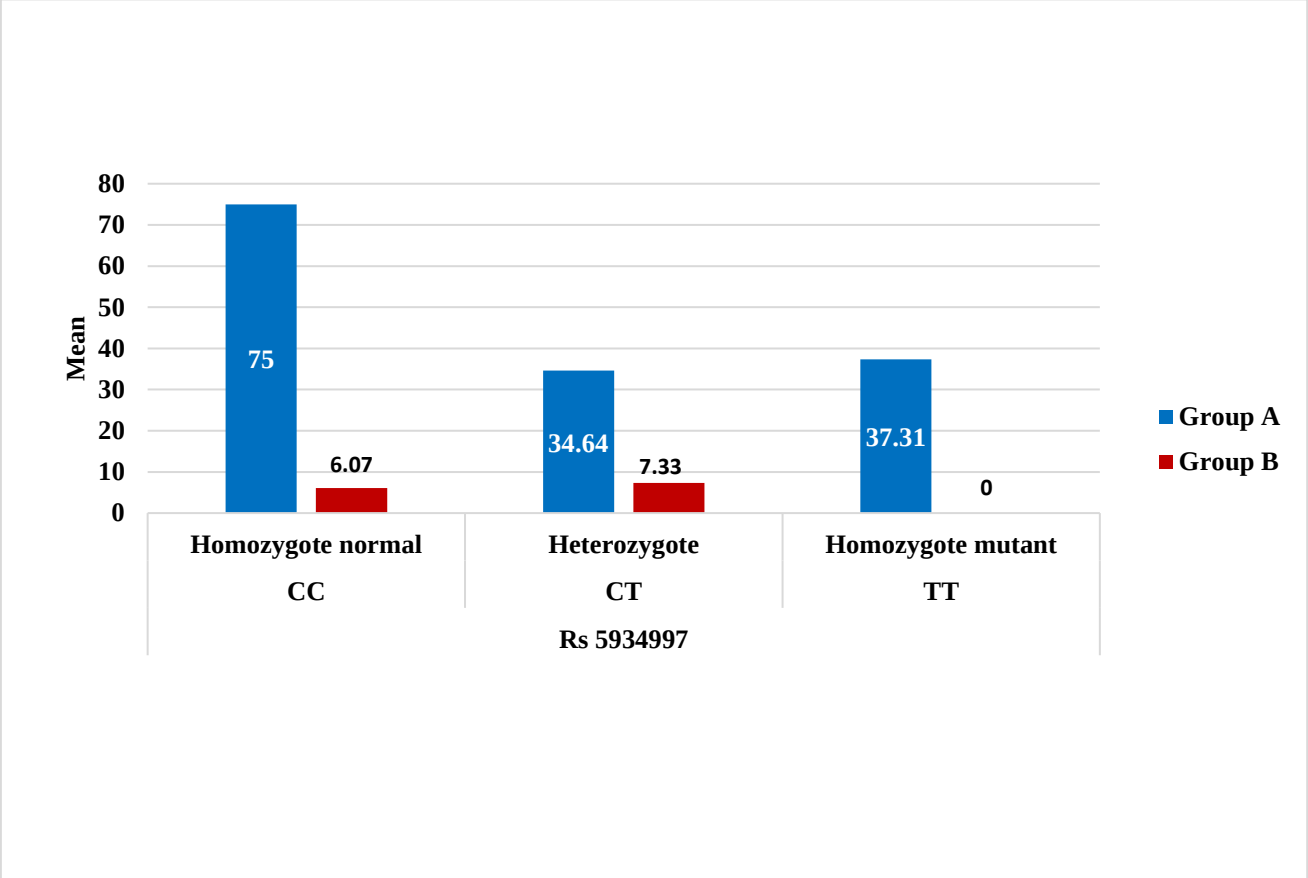

Figure 6. Bar chart showing Association between *Streptococcus mutans* count (CFU) and different genotypes regarding rs5934997 marker in both groups

Over 90% of the extracellular matrix protein content is made up of amelogenin, which is assumed to control the directed development and arrangement of enamel crystals. Although the Y and X chromosomes contain the genes that produce this protein, the X chromosome accounts for about 90% of all RNA transcripts [12]. Enamel formation requires two steps. The first stage involves the synthesis of enamel proteins, primarily amelogenin. AMELX is the gene that codes for amelogenin. The hydroxyapatite prism forms with the help of well-organized amelogenins, which are also necessary for the prisms' elongation during development of enamel and the creation of normal enamel thickness [7]. Extracellular matrix proteins called amelogenins are necessary for dental enamel development. The regulation of enamel thickness and mineralization seems to be significantly impacted by known mutations in the amelogenin X gene (AMELX). Amelogenin gene abnormalities are linked to X-linked amelogenesis imperfecta, a group of disorders marked by enamel hypoplasia [16]. Amelogenin, an important structural protein of the organic matrix protein found in enamel,is encoded by the X chromosome gene AMELX in a variety of isoforms. Previous studies employing genetically modified mice demonstrated that AMELX was essential for appropriate enamel formation [2].

Enamel microstructure exhibits unique variations due to AMELX polymorphisms. As a result, these polymorphisms are essential for controlling enamel thickness and mineralization [3]. It was discovered that Turkish children's susceptibility to caries may be influenced by variations in the AMELX gene. In the Korean population, a correlation was also observed between dental caries susceptibility and single nucleotide polymorphisms (SNPs) in AMELX [16]. Certain researches have demonstrated a relationship between mutations and dental caries susceptibility in the LTF, ENAM, and AMELX genes [3]. Furthermore, genetic variants in the genes that code for enamel proteins might potentially interact with environmental factors in some way. Therefore, much more research on this topic is needed. Single nucleotide polymorphisms are important genetic markers because they are the most prevalent form of genetic variation, occurring in the human genome on average every 1.3 kb. Furthermore, most SNPs are biallelic, which means that alleles only differ by a single nucleotide [6]. The SNP under investigation was found in intronic regions of the candidate gene; nevertheless, it may have an impact on the process of mRNA expression and change the functional activities of the encoded gene by influencing the splice sites, which would then result in a decrease in the protein's function [10].

So, the objective of the article was to associate markers in AMELX gene and (mutans streptococci number) which are correlated with the susceptibility of caries also to study environment-genetic interactions on caries occurrence. In the present study we used saliva samples as a source for *S. mutans* because investigators either use dental plaque and/or saliva as two main sources. Dental plaque may be considered as more productive sample for S. mutans. However, saliva is also a convenient source for *S. mutans* because of its easy and noninvasive method. Also, saliva sample is a perfect source as *S. mutans* are detached in saliva [17]. In our present study, unstimulated saliva samples were used to quantify the number of *S. mutans* in both groups, the reason for that was because previous researches that used stimulated and unstimulated saliva samples went to a conclusion that the antioxidant capacity was greater in unstimulated saliva so it is more accurate in declaring the amount of the cariogenic bacteria [1]. Regarding *S. mutans* count the results of our study showed that in comparison between Group A (children with dental caries) and Group B (caries free children) Group A was significantly count greater in *S. mutans* count than Group B as P‹ 0.0001. Our results were in agreement with Thimmegowda et al. study, 2023 as their study showed that *S. mutans* count was significantly higher in caries children than in children without caries. Statistically significant results were seen at a $p = 0.01$ [1]. Also our results was in agreement with the study of Sounah and Madfa, 2020 as they stated that in saliva samples positive correlations between the amount of SM and the development of caries were observed [17]. Priti *et al*,2020 results showed that in their study, there was a highly significant difference between children with high caries and those without detectable caries in terms of mean salivary *S. mutans* counts. This was consistent with the findings of our investigation as well as the research carried out by Campus G et al. (2000) and Hegde PP et al. (2005) [11]. Our findings support the findings of Slayton et al. (2005), who reported a statistically significant positive correlation between *S. mutans* levels and dental caries in children who had caries and those who did not ($P < 0.0001$) [18]. One single Nucleotide Polymorphism (SNP) in *AMELX* (rs5934997) was chosen for this investigation based on relationships with dental caries and/or enamel abnormalities that have been previously reported in other populations. Between groups with various levels of caries experience, genotype and allelic frequencies were examined. Regarding rs 5934997 genetic marker and distribution of different genotypes in Group A the results revealed over presentation of TT mutant genotype (48.1%) in group A followed by CT genotype (40.7 %) and finally CC normal genotypes (11.1%) was high significant occurrence of both TT and CT genotypes as P=0.003 with the presence of T mutant allele in 37 children (68.52%) while C normal allele was seen in only 17 child (31.48%). As our work is a case – control study and while comparing between Group A as patients and Group B as controls regarding distribution of different genotypes the results revealed that TT genotype (homozygote mutant) was significantly higher in Group A (48.1%) than Group B (0%) as p ‹0.0001.While CC genotype (homozygote normal) in Group A (11.1%) was significantly lower than Group B (55.6%) as P=0.0007 which indicated the high significant association between the presence of homozygous mutant TT genotype and the occurrence of dental caries. Our genotype association was confirmed by the study of allele frequency analysis thus in comparison between both groups regarding distribution of different alleles among this genetic marker the results revealed that the high frequency of T mutant allele in Group A (68.52 %) than in group B (22.22%) as p value was P<0.0001 which indicate the T mutant allele was the most related to caries susceptibility. Our results were in accordance with the results of Kang et al.,2011 as they stated that there were significant associations between rs5934997 SNP and caries susceptibility in in Koreans. Also their results showed that TT genotypes increase caries susceptibility as TT genotype was the highest among other genotypes in higher caries experience group. Ultimately, they came to the conclusion that the Korean population's susceptibility to dental caries may be correlated with the SNPS of AMELX [14]. On the contrary of the results of our study was the results of the study of Muhammad and Ahmed, 2020 as their results revealed that the rs5934997 and C allele of AMELX was the most related to caries susceptibility and severity. Additionally, they reported that among the Iraqi Kurdish population, dental caries appears to be less common in T allele carriers and more common in C allele carriers [8]. Variation of the results of the previous studies may suggest that environmental and genetic factors are both significant contributors to the etiology of childhood dental caries. Numerous studies conducted on populations of various ethnicities have found that the distribution of alleles and genotypes differs significantly depending on demographic origin, which appeared to be a factor of great importance [6]. Regarding the association between different genotypes in Rs 5934997 and *Streptococcus mutans* count. Comparison of mean and standard deviation marker regarding group A and Group B revealed that in both CC and CT genotypes the *Streptococcus mutans* count in group A was significantly more than group B as P<0.0001. Reviewing literature, it is the initial molecular study in Egypt to examine the association between dental decay susceptibility, Streptococcus mutans, and AMELX gene polymorphisms. Despite other studies evaluating the relation between caries susceptibility with other genes like tuftelin gene polymorphism and Streptococcus mutans. So the results for the studies of Priti *et al*,2020 ; Slayton et al., 2005 were in accordance with our results as their results showed that the difference between the two groups regarding the mean salivary *S. mutans* counts was highly significant [11]. The major study limitation is the small sample size, which could have influenced the statistical calculations. In addition, no data existed in the community about the frequency of AMELX gene polymorphism in the country. Despite these limitations, these data provide insights into the relationship between AMELX SNPs and occurrence of dental caries as it is first study concerning the hereditary aspect of caries in Egypt, and so could form a basis for further studies in a large population including parents and siblings to concentrate on conducting additional research into the ways in which this gene-environment interaction works.

5. Conclusions

Under limitation of this study, it was determined that: rs5934997 marker SNP in AMELX gene may be regarded as a risk factor for the development of dental caries in Egyptian children.

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Recommendations

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Conflict of interest

There is no conflict of interest.

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