

Association between Genetic Polymorphisms in Enamel Formation Genes and Environmental Factors with Caries Experience

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Mine Oluşum Genlerindeki Genetik Polimorfizmler ve Çevresel Faktörler ile Çürük Deneyimi Arasındaki İlişki

ABSTRACT

Objective: The etiology of dental caries involves both environmental and host-related factors. This study aims to evaluate the effect of enamel formation genes (AMELX, ENAM, and AMBN), environmental factors, and their combined influence on caries experience in adults..

Methods: A Case-Control Study was conducted including 114 adults aged 20-65 years. Data regarding oral hygiene habits were obtained through patient declaration. Data regarding caries, plaque and saliva were obtained through clinical examination. Genomic DNA was isolated from saliva. Patients were divided into two groups according to caries experience. Environmental factors (age; amount of plaque; tooth brushing duration; consumption of sugary drinks and foods; use of fluoride toothpaste, fluoride mouthwash and dental floss; Streptococcus mutans and Lactobacillus counts) and host factors (saliva flow rate, pH, buffering capacity and AMELX, ENAM and AMBN) were examined. Real-time PCR was used to evaluate genotype distributions. Data were analyzed using chi-square test and logistic regression with SPSS.

Results: No statistically significant differences were observed in AMELX, ENAM or AMBN Allele and genotype frequencies between groups in selected subjects in Turkey. There was no significant association between the groups with regard to salivary flow rate or consumption of sugary drinks and food. All other factors were found to be associated with high caries experience.

Conclusion: When environmental factors and genotypes were evaluated together using multivariate regression analysis, it was determined that increased age, high plaque levels, and the absence of fluoride mouthwash use increased the likelihood of high caries experience.

Keywords: AMELX, ENAM, AMBN, Genetic Polymorphism, Caries experience

Öz

Amaç: Çürüğün etiyolojisi hem çevresel hem de konakçı faktörleri içermektedir. Bu çalışma, mine oluşum genleri (AMELX, ENAM ve AMBN), çevresel faktörler ve bunların birlikte etkisinin yetişkinlerde çürük deneyimi üzerindeki etkisini değerlendirmeyi amaçlamaktadır.

Yöntemler: 20-65 yaş arası 114 yetişkinin dahil edildiği bir vaka kontrol çalışması yürütüldü. Ağız hijyeni alışkanlıklarına ilişkin veriler hasta bildirimi yoluyla elde edildi. Çürük, plak ve tükürük ile ilgili veriler klinik muayene yoluyla elde edildi. Tükürükten genomik DNA izole edildi. Hastalar çürük deneyimine göre iki gruba ayrıldı. Çevresel faktörler (yaş; plak miktarı; diş fırçalama süresi; şekerli içecek ve yiyecek tüketimi; florürlü diş macunu, florürlü gargara ve diş ipi kullanımı; Streptococcus mutans ve Lactobacillus sayıları) ve konakçı faktörler (tükürük akış hızı, pH, tamponlama kapasitesi ve AMELX, ENAM ve AMBN) incelendi. Genotip dağılımlarını değerlendirmek için gerçek zamanlı PCR kullanıldı. Veriler ki-kare testi ve lojistik regresyon kullanılarak SPSS ile analiz edildi.

Bulgular: Türkiye'den seçilen bireylerde AMELX, ENAM veya AMBN alel ve genotip frekansları arasında gruplar arasında istatistiksel olarak anlamlı bir fark gözlenmedi. Gruplar arasında tükürük akış hızı veya şekerli yiyecek ve içecek tüketimi açısından da anlamlı bir ilişki bulunmadı. Diğer tüm faktörlerin yüksek çürük deneyimi ile ilişkili olduğu tespit edildi.

Sonuç: Çevresel faktörler ve genotipler birlikte çok değişkenli regresyon analizi ile değerlendirildiğinde; artan yaş, yüksek plak seviyeleri ve florürlü gargara kullanımının olmamasının yüksek çürük deneyim olasılığını artırdığı belirlendi.

Anahtar Kelimeler: AMELX, ENAM, AMBN, Genetik Polimorfizm, Çürük deneyimi.



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INTRODUCTION

The etiology of caries lesions has been investigated for many years. Environmental factors, such as cariogenic diet, poor oral hygiene, dental plaque, insufficient fluoride exposure, and colonization levels of cariogenic bacteria, and host factors, such as salivary flow rate, salivary buffering capacity, and genetics, play a role in the etiology of caries lesions.¹ Although the pathogenesis of caries lesions has been known for a considerable time, this disease is still an important oral health problem in most countries.² While environmental factors have been reported to affect the development of dental caries, susceptibility may vary between individuals exposed to the same environmental factors. It has been suggested that these differences are caused by genetic factors in the etiopathogenesis of dental caries.³ According to the results of genetic studies, the genes that may affect dental caries susceptibility are classified as those associated with enamel formation, immune response, salivary proteins, and taste receptors.² Genetic variation in the genes involved in enamel formation has been reported to cause structural changes in tooth enamel that may lead to mineral losses.⁴ The genes associated with mineralization, namely amelogenin (AMELX), enamelin (ENAM), tuftelin (TUFT1), ameloblastin (AMBN), and kallikrein (KLK4), are involved in enamel mineralization, while dentin sialophosphoprotein (DSPP) is involved in dentin mineralization.^{5,6} Ninety percent of the organic matrix of enamel is composed of the proline-rich protein amelogenin. It governs the organization and growth of enamel crystals. Ameloblastin is a cell adhesion protein responsible for controlling cell differentiation.⁷ Enamelin, one of the proteins effective in mineralization, is found in the mature organic matrix and takes part in enamel formation.⁸ Therefore, mutations in the above-mentioned genes may increase the susceptibility of surfaces to caries by causing abnormal protein production. In addition, when the amounts of these proteins are reduced in developing teeth, defective mineralization can occur, which can affect both bacterial adhesion and enamel resistance to acid pH.⁹ Identification of the genes that affect susceptibility may contribute to the prevention of dental caries.² An early screening test for genetic susceptibility to caries may be developed.⁶ In addition, the investigation of genetic factors may facilitate the identification of individuals at high caries risk. It can help in the planning of preventive treatments on a per-person basis.^{2,10} According to the results of a meta-analysis published in 2020, three of the studies evaluating the effect of genes on dental caries etiology were performed in adults. Therefore, the aim of the study was to evaluate the impact of genetic and environmental factors and their interactions on caries experience in adults in selected subjects in Turkey. The null hypotheses of the study are as follows: 1) AMELX, ENAM, and AMBN gene polymorphisms are not associated with high caries experience. 2) Environmental factors (age; amount of plaque; tooth brushing duration; use of fluoride toothpaste, fluoride mouthwash, and dental floss; consumption of sugary drinks and food; and *Streptococcus mutans* and *Lactobacillus* counts) and host-related factors (saliva flow rate, saliva pH, and salivary buffering capacity) are not associated with a high caries experience.

METHODS

Subject

The study protocol was approved by the decision of Atatürk University Faculty of Medicine Clinical Research Ethics Committee (Date:13.03.2019 Number: B.30.2.ATA.0.01.00/138) and was supported Ataturk University Scientific Research Projects Foundation (TDH-2019-7313). It was conducted by the Declaration of Helsinki. Informed consent forms prepared accordingly were signed by each person included in the study. Patients within the age range 20 to 65 who

presented to the Atatürk University Faculty of Dentistry Restorative Dentistry Clinic for dental treatment and were regarded as adults according to the criteria of the World Health Organization (WHO1998) were selected for the research group¹¹. Power analysis was performed using the software package G*Power 3.1. Sample size was calculated from the goodness of fit tests(contingency tables) with $\alpha=0.05$ power $(1-\beta)=0.85$ and effect size $w=0.30$, and was determined to be 100 samples to account for possible dropout.¹² Fourteen replacement patients were added to compensate for possible data loss (due to a problem that may occur in genomic DNA extraction). A total of 114 Turkish patients were included in the study.¹³

Inclusion Criteria

Age between 20 and 65 years, regarded as adults according to WHO criteria (1998). Patients presenting to the university's Faculty of Dentistry Restorative Dentistry Clinic for dental treatment.

Exclusion Criteria

Presence of neurological, mental, systemic, or genetic diseases. Severe physical disabilities or acute/chronic upper respiratory tract diseases. Dental fluorosis or use of chlorhexidine-containing preparations within the previous three months. Current orthodontic treatment.

Intraoral and Radiographic Examination

The individuals included in the study were assigned to the groups according to the DMFT scores calculated using the World Health Organization (WHO) criteria.¹¹ All teeth were examined in detail visually and radiographically by the same dentist. The participants in the study were divided into two extreme groups based on their DMFT (decayed, missing, and filled teeth) scores, as done in other studies, to reduce heterogeneity.^{14,15} conducted in adults: a group with high caries experience (DMFT ≥ 14 , n = 55) and a group with low caries experience (DMFT ≤ 5 , n = 59). According to the Silness and L  e plaque index, which is accepted worldwide in determining oral hygiene, tooth no. 12, 24, 16, 32, 44 and 36 were evaluated.¹⁶

Environmental Factors

Environmental factors, such as age, amount of plaque, tooth brushing duration, consumption of sugary drinks and foods, and the use of fluoride toothpaste, fluoride mouthwash, and dental floss, as well as *Streptococcus mutans* and *Lactobacillus* counts, were examined.

Host-Related Factor

Host-related factors, such as saliva flow rate, pH, buffering capacity, and the genetic polymorphisms of AMELX, ENAM, and AMBN, were examined.

Salivary and Microbiological Tests

Salivary flow rate, pH, buffering capacity, and the counts of *Streptococcus mutans* and *Lactobacillus* were determined using the KariesScreen Test +P (Aurosan GmbH, Essen, Germany).

Salivary Tests

Participants were instructed to chew paraffin (Aurosan GmbH, Essen, Germany) for 5 minutes without swallowing the saliva produced during this period. The saliva was collected into a millimeter-graded container (KariesScreen Test +P, Aurosan GmbH, Essen, Germany). The total volume of saliva was measured and divided by the collection time (5 minutes) to calculate the stimulated salivary flow rate in mL per minute, which was then recorded.

A portion of the collected saliva was applied to the first test strip. The salivary pH was determined by comparing the color change on the strip with the scale provided by the manufacturer. To evaluate buffering capacity, 1 mL of stimulated was mixed with 3 mL of 0.005 N HCl. After 5 minutes, the second test strip was immersed in the

mixture. The value representing the buffering capacity was determined based on the color change observed on the test strip and compared with the scale provided by the manufacturer (pH > 6.0: very good; pH > 5.0: good; pH < 5.0: poor; pH < 4.0: very poor buffering capacity). The values were recorded.

Microbiological Tests

The counts of *S. mutans* and *Lactobacillus* were determined using the dip-slide method.¹⁷ For the counts of *S. mutans* and *Lactobacillus*, media containing NaHCO₃ tablets were washed with stimulated saliva and incubated at 37°C for 48 hours. The microbial counts were recorded as colony forming units/mL (CFU/mL) using the scale provided by the manufacturer (<10³ CFU/mL: very low; <10⁵ CFU/mL: low; 10⁵–10⁶ CFU/mL: high; >10⁶ CFU/mL: very high).

Genomic DNA Extraction and Analysis of Gene Polymorphisms

Saliva samples were transferred to plastic tubes (Transport Swab, Biologix Group Limited, Shandong, China). The samples were stored at +4 °C until extraction was performed. AMELX, ENAM, and AMBN gene polymorphism analysis was performed by real-time PCR (ABI 7500 Fast Real-Time PCR). The genes and their polymorphisms examined in the study are shown in Table 1.

Table 1. Genes examined in the study and their polymorphisms

Gene	Protein Coded	Reference number	sequence
AMELX	Amelogenin	rs17878486	
ENAM	Enamelin	rs12640848	
AMBN	Ameloblastin	rs17733915	

Statistical Analysis

Statistical analysis of the data was performed using SPSS 20 (IBM, Chicago, IL, New York). All genotypes were in Hardy-Weinberg equilibrium. The chi-squared test was used to compare the genotype and allele distributions of the AMELX, ENAM, and AMBN genes between the low and high caries experience groups. The differences between the groups in terms of age, amount of plaque, tooth brushing duration, consumption of sugary drinks and foods, and the use of fluoride toothpaste, fluoride mouthwash, and dental floss, as well as *Streptococcus mutans* and *Lactobacillus* counts, were evaluated using logistic regression analysis. Finally, genes and some environmental and host factors were combined in the same model and evaluated using multivariate regression analysis.

RESULTS

The associations between environmental and host factors with caries experience are shown in Table 2. Out of the 114 participants included in the study, 59 were categorized in the low caries experience group (DMFT ≤ 5), while 55 were in the high caries experience group (DMFT ≥ 14). When comparing the groups in terms of gender distribution, no significant difference was observed. The mean age and standard deviation for the high and low caries experience groups were found to be 35 (±12.6) and 27 (±5.7), respectively. The mean age of the high caries experience group was significantly higher than that of the low caries experience group ($P < .001$).

The mean DMFT scores and standard deviations for the high and low caries experience groups were 15.8 (±2.59) and 2.63 (±1.95), respectively. High plaque levels ($P < .001$), high *Lactobacillus* counts ($P < .05$), high *S. mutans* counts ($P < .001$), and a salivary pH of <7 ($P < .05$) were associated with high caries experience. Good salivary buffering capacity was found to be protective against high caries experience ($P = .001$).

No significant differences were found in salivary flow rate, consumption of sugary foods and drinks, and caries experience. However, the absence of fluoridated toothpaste use increased the likelihood of high caries experience by 7,658 times ($P = .002$), the absence of fluoridated mouthwash use by 6,766 times ($P < .05$), the absence of dental floss use by 2,312 times ($P = .032$), and brushing for less than 1 minute increased the likelihood by 11,786 times ($P = 0.006$). The genotype and allele frequency distributions of the AMELX rs17878486, ENAM rs12640848, and AMBN rs17733915 genes are presented in Table 3. No statistically significant differences were found between the low and high caries experience groups in terms of AMELX, ENAM, or AMBN genotypes or allele distributions.

Table 2. Logistic regression analysis for the association between environmental factors, host-related factors, oral health habits and caries experience

	Sample n=114	Low Caries experience Group n=59	High Caries Experience Group n=55	OR(95% CI)	P value
Mean Age	31(10.4)	27 (5.7)	35 (12.6)	1.097 (1.044-1.154)	<.001*
Gender					
Female	53	31	22	Reference	
Male	61	28	33	1.661 (0.790-3.491)	.181
Plaque Quantity					
Low	83	55	28	Reference	
High	31	4	27	13,259 (4,222-41,641)	<.001*
S. Mutans count					
Low	26	19	7	Reference	
High	88	40	48	4.703 (2.125-10.410)	<.001*
Lactobacillus count					
Low	63	43	20	Reference	
High	51	16	35	3.257 (1.244-8.531)	.016*
Buffering capacity of the saliva					
Good	66	43	23	Reference	
Poor	48	16	32	0.267 (0.122-0.586)	.001*
Saliva pH					
pH>7	93	54	39	Reference	
pH<7	21	5	16	4.431 (1.497-13.117)	.007*
Saliva flow rate					
Good	89	47	42	Reference	
Sufficient	14	7	7	1.119 (0.362-3.455)	.845
Insufficient	11	5	6	1.343 (0.382-4.723)	.646
Tooth brushing duration					
3 minute	22	15	7	Reference	
1-3 minute	57	32	25	1.728 (0.611-4.891)	.303
>1 minute	22	10	12	2.338 (0.694-7.872)	.170
<1 minute	13	2	11	11.786 (2.041-68.061)	.006*
Consumption of sugary drinks and food (daily)					
None	15	9	6	Reference	
1-2 times	73	38	35	1.382 (0.446-4.279)	.575
3-4 times	17	8	9	1.687 (0.414-6.878)	.465
More than 4	9	4	5	1.875 (0.352-9.981)	.461
Use of Fluoride toothpaste					
Yes	95	56	39	Reference	
No	19	3	16	7.658 (2.089-28.075)	.002*
Use of Fluoride mouthwash					
Yes	14	12	2	Reference	
No	100	47	53	6.766 (1.440-31.801)	.015*
Use of dental floss					
Yes	47	30	17	Reference	
No	67	29	38	2.312 (1.074-4.977)	.032*

* $P < .05$, statistically significant; OR (95% CI)= Odds ratios; 95% confidence intervals; bold forms indicated statistical significance

Some environmental and host factors that were statistically significant in the univariate regression analysis were selected, and their combined effect with genotypes was evaluated using multivariate regression analysis (Table 4). An increase in age by 1.101 times, high plaque levels by 7.834 times, and the absence of fluoridated mouthwash use by 7.834 times were found to increase the likelihood of high caries experience ($P < .05$).

DISCUSSION

The etiology of dental caries is influenced by the complex relationship between genetic and environmental factors. Very few studies have investigated the effect of genes on caries etiology had been conducted in adults.¹⁸ In a recent meta-analysis, it was emphasized that further studies were needed to increase the level of evidence and support the current conclusions regarding the genetic influence on caries etiology.¹⁹ Based on a literature review, this study was designed to investigate some of the gene polymorphisms previously identified in relation to caries to assess their association with caries experience in Turkish adults.

The first hypothesis, stating that AMELX, ENAM, and AMBN gene polymorphisms are not associated with high caries experience, was accepted. However, as environmental factors such as age, plaque amount, tooth brushing duration, the use of fluoride toothpaste, fluoride mouthwash, and dental floss, as well as Streptococcus mutans and Lactobacillus counts, and host-related factors such as saliva pH and salivary buffering capacity were found to be associated with high caries experience, the second hypothesis was partially rejected.

Gerreth et al.²⁰ had reported a significant positive association between the AMELX rs17878486 T allele and TT genotype in Polish children and the occurrence of developmental enamel defects in primary dentition. Similarly, Patir et al.²¹ had found that the AMELX rs17878486 polymorphism in Turkish children was associated with an increased susceptibility to caries by altering enamel structure. In a study conducted on South Indian children, it was reported that the analyzed polymorphisms of the AMELX gene had not revealed any significant association with early childhood caries (ECC).²²

In this study, no significant difference was observed in the distribution of AMELX rs17878486 genotypes and alleles between the low and high caries experience groups. This was thought to stem from

Table 3. The chi-square test for genotype and allele distribution between caries experience groups

Polymorphism	Genotype frequency			p	Allele Frequency		P
AMELXrs17878486	CC	TC	TT		C	T	
Low Caries experience Group n=59	6 (%10.2)	20 (%33.9)	33 (%55.9)	0.157	32(%27.1)	86 (%72.9)	.444
High Caries Experience Group n=55	1 (%1.8)	23 (%41.8)	31 (%56.4)		25(%22.7)	85 (%77.3)	
ENAM rs12640848	AA	AG	GG		A	G	
Low Caries experience Group n=59	25(%42.4)	27 (%45.8)	7 (%11.9)	0.705	77(%63.6)	41 (%36.4)	.467
High Caries Experience Group n=55	24(%43.6)	27 (%49.1)	4 (%7.3)		75(%68.2)	35 (%31.8)	
AMBN rs17733915	CC	CT	TT		C	T	
Low Caries experience Group n=59	22 (%40)	21 (%38.2)	12 (%21.8)	0.144	65(%59.1)	45 (%40.9)	.101
High Caries Experience Group n=55	34(%57.6)	14 (%23.7)	11 (%18.6)		82(%69.5)	36 (%30.5)	

* $P < .05$, statistically significant.

Table 4. Multivariate regression analysis combining environmental factors and genotypes

	OR(%95CI)	P value
Age	1.101 (1.024-1.185)	.010*
Plaque Quantity		
Low	Reference	
High	7.834 (1.753-35.002)	.007*
Use of Fluoride toothpaste		
Yes	Reference	
No	2.022 (0.430-9.517)	.373
Use of Fluoride mouthwash		
Yes	Reference	
No	8.553 (1.062-68.863)	.044*
S. Mutans count		
Low	Reference	
High	2.325 (0.799-6.761)	.121
Lactobacillus count		
Low	Reference	
High	1.760 (0.525-5.901)	.360
Buffering capacity of the saliva		
Good	Reference	
Poor	1.695 (0.592-4.853)	.325
AMELX		
CC	Reference	
TC	0.078 (0.004-1.720)	.106
TT	0.151 (0.008-2.823)	.206
ENAM		
AA	Reference	
AG	1.484 (0.515-4.283)	.465
GG	0.434 (0.068-2.757)	.376
AMBN		
CC	Reference	
CT	1.957 (0.609-6.287)	.259
TT	0.834 (0.198-3.508)	.804

* $P < .05$, statistically significant; OR (95% CI)= Odds ratios; 95% confidence intervals; bold forms indicated statistical significance

the fact that this study had focused on adults and permanent teeth, while the other studies had investigated primary dentition in children.²³ Although studies conducted in adults are more limited, one study found no significant association between AMELX polymorphism and susceptibility to dental caries.¹³ Therefore, AMELX may have different effects on susceptibility to dental caries in primary and permanent dentition. Enamelin, the largest protein in the enamel matrix during enamel formation, is encoded by the ENAM gene. Mutations in the ENAM gene have been reported to cause autosomal dominant amelogenesis imperfecta.²⁴ A significant relationship between the ENAM rs12640848 gene and caries risk was identified by Shimizu et al.²⁵ while Abbasoğlu et al.²⁶ reported that the ENAM rs12640848 GG genotype was associated with a protective effect against ECC. Additionally, ENAM rs12640848 has been linked to being caries-free.²⁷ However, in the present study, a caries-free group was not included, which may have contributed to the lack of association observed. Another study was found to support these findings, reporting no significant relationship between the ENAM gene and caries experience.⁵ Similarly, in a study investigating the ENAM (rs12640848) genetic polymorphism in Riyadh, Saudi Arabia, no significant association was observed between this polymorphism and either an increased risk or protection against ECC.²⁸ Furthermore, in a study conducted by Borilova et al.²⁹ on Czech children, no association was detected between ENAM rs12640848 and dental caries in either primary or permanent dentition. The differences in the results were explained by populations with different genetic backgrounds and varying environmental factors. First, genetic variations among different populations may influence the role of ENAM rs12640848 in caries susceptibility. Second, differences in environmental factors, such as dietary habits, fluoride exposure, and

oral hygiene practices, could affect the manifestation of genetic predispositions. The ameloblastin protein was found to regulate the elongation of enamel crystals during tooth development.⁶ Shaffer et al.⁴ found a significant association between AMBN rs17733915 and caries experience, where carrying at least one copy of the rare amelogenin marker allele was linked to greater caries experience after adjusting for age.⁵ In this study, no statistically significant differences were identified in the distribution of AMBN rs17733915 genotypes or alleles between the groups. It was reported that meta-analyses of AMBN rs17733915 had been thought-provoking. Further studies are needed to understand how variations in genes involved in enamel formation influence the risk of dental caries and how they interact with environmental exposures.⁴ The analyzed polymorphisms were shown to have no significant association with caries in the Turkish cohort. However, this did not rule out the presence of a genetic component influencing caries susceptibility.

Based on the fact that gene polymorphisms had been found to remain stable over time, patients aged 20 to 65, who were considered adults according to WHO criteria (1998), were included in the study. To minimize heterogeneity, participants were classified into two distinct groups based on their caries experience: those with a DMFT score below 5 were assigned to the low caries experience group, while those with a DMFT score above 14 were placed in the high caries experience group.¹⁵ Similar grouping strategies based on the DMFT index have been applied in previous studies examining the association between gene polymorphisms and caries.³⁰ However, it has also been recognized that while the DMFT index provides a standardized method for classification, it offers limited insight into the disease process itself.²⁵ For this reason, additional comparisons were made between the groups for *S. mutans* and *Lactobacillus* counts, saliva flow rate, pH, and buffering capacity. Researchers have suggested that obtaining *S. mutans* and *Lactobacillus* counts is a critical step for monitoring the effectiveness of caries prevention strategies in acute periods.³¹ In this study, significantly higher *S. mutans* and *Lactobacillus* counts were observed in individuals with high caries experience.

It had been reported that factors such as tooth morphology, buffering capacity, salivary flow, dietary habits, oral microbiome, oral hygiene, and a history of dental caries clearly played a significant role in the development of carious lesions.² Some researchers had stated that low saliva buffering capacity increased caries susceptibility in adults because it caused the acidic environment to persist in the mouth over a long period, allowing the growth of cariogenic bacteria. Therefore, the groups were compared in terms of salivary flow rate, salivary pH, and salivary buffering capacity. Significantly poor buffering capacity and salivary pH less than seven were detected in individuals in the high caries experience group. Due to the low salivary flow rate and frequent consumption of sugary drinks and foods, the pH level of the dental biofilm was observed to remain low for a long time, giving acid-tolerant oral pathogens an advantage among resident species.¹⁰ Reducing the consumption of sugary foods and drinks and eating these foods with the main meals was identified as an important factor for reducing the risk of caries.³² In the present study, the amount of sugary food and beverage consumption per day was not associated with caries experience. This discrepancy may be due to differences in dietary habits, variations in the frequency and timing of sugar consumption, or the influence of other protective factors such as fluoride exposure and oral hygiene practices. Even trace amounts of fluoride have been found to be crucial in caries prevention, as an increase in caries development has been reported in patients who discontinued the use of fluoride products.³³ According to our study results, not using fluoride

toothpaste and fluoride mouthwash significantly increased the probability of experiencing high caries ($P < .05$).

Some environmental factors that were found to be statistically significant in the univariate regression analysis were combined with gene polymorphisms in the multivariate regression analysis to evaluate their combined effect. It was determined that increased age, high plaque levels, and the absence of fluoride mouthwash use increased the likelihood of high caries experience. In studies evaluating genetic and environmental factors with logistic regression analysis, increasing age was reported to be a risk factor for caries.^{34,35} Taletar et al.¹⁴ had stated that an increase in the amount of plaque was associated with high caries experience. In the study by Wang et al.³⁵ frequent tooth brushing and the level of fluoride in the water were reported as protective factors for caries. Tannure et al.³⁴ reported that all individuals in their study used fluoride toothpaste, but no significant difference was found in the use of fluoride mouthwash between individuals with and without caries. In addition it was emphasized that the use of fluorine had not been evaluated together with genetic factors. It had been reported that the impact of environmental exposures varied based on an individual's genome and that other environmental factors, such as antimicrobial or pH-buffering mouthwashes or prescription topical fluorides, might benefit specific subgroups of the population.⁴ The presence of certain genotypes was suggested to cause individuals to respond differently to environmental factors such as fluoride or diet. It is believed that the use of fluoride mouthwash should be further investigated in future studies.

The limitation of our study was that information about environmental factors had been obtained from the statements of the participants. Therefore, more objective and reliable data collection methods should be considered in future studies. Future studies may include more closely matched age groups. In addition, although choosing one polymorphism per gene had a limitation, it should be taken into account that analyzing each polymorphism increases the cost. Finally, the presence or absence of white spot lesions and activity classification in individuals in the groups could be evaluated in further studies.

Our study findings indicated that the AMELX rs17878486, ENAM rs12640848, and AMBN rs17733915 gene polymorphisms were not associated with caries experience in Turkish adults. However, high plaque levels, elevated *Streptococcus mutans* and *Lactobacillus* counts, a salivary pH lower than 7, brushing duration of less than one minute, and the absence of fluoride toothpaste, fluoride mouthwash, and dental floss use increased the likelihood of high caries experience. In contrast, good salivary buffering capacity was identified as a protective factor against high caries experience. When environmental factors and genotypes were evaluated together, increased age, high plaque levels, and the absence of fluoride mouthwash use were found to increase the likelihood of high caries experience. Further studies are needed to assess the combined effects of different candidate genes, environmental factors, and fluoride mouthwash use on caries experience. Such studies may contribute to the development of individualized preventive treatments, which are essential for public oral health.

Ethics Committee Approval: The study protocol was approved by the decision of the Ataturk University Faculty of Medicine Clinical Research Ethics Committee (Date:13.03.2019 Number: B.30.2.ATA. 0.01.00/138).

Informed Consent: Forms were obtained in writing from each participant participating in the study.

Peer-review: Externally peer-reviewed.

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