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The role of AMY1 gene copy number variation in dental caries susceptibility: insights from a Turkish population

Ömer Hatipoğlu[1] and Faruk Saydam[2*]

Abstract

Objective Dental caries is a multifactorial disease influenced by environmental, behavioral, and genetic factors. Recent studies suggest that variations in the AMY1 gene, which encodes salivary amylase, may contribute to caries susceptibility. This study investigates the relationship between AMY1 gene copy number variation (CNV) and dental caries in a Turkish population.

Method A total of 154 participants (63% female; mean age 19.6 ± 1.4 years) were included. Epithelial cells in inner cheek tissues were collected from volunteers using swabs, and the collected samples were preserved and stored in a DNA stabilization solution. The demographic characteristics of the volunteers were recorded, and DMFT and DMFS index scores were documented on the provided forms. The AMY1 gene CNVs were determined using a Real-time polymerase reaction device. The TaqMan chemistry, which comprises quantitative real-time PCR reactions utilizing a dual TaqMan kit, was utilized in this analysis process. Statistical analyses included the Kruskal-Wallis and Mann-Whitney U tests for group comparisons, Spearman's correlation analysis, and binomial logistic regression to evaluate associations between AMY1 CNVs and dental caries indices.

Results No statistically significant differences were observed between AMY1 CNVs and DMFT or DMFS indices ($p > 0.05$). However, significant associations were found between daily tooth brushing frequency and caries indices (Cramer's $V = 0.219$, $p < 0.05$), as well as between preferred beverage consumption and caries indices (Cramer's $V = 0.219$, $p < 0.05$). Other factors, including gender, dental floss and mouthwash use, and tongue brushing, did not show statistically significant associations ($p > 0.05$).

Conclusion Although AMY1 CNVs were not significantly associated with caries indices, the findings highlight the complex interplay of genetic, behavioral, and dietary factors in caries development. This study emphasizes the critical role of oral hygiene and dietary habits in caries prevention and underscores the need for further research on genetic contributions to oral health.

Clinical trial number Not applicable.

Keywords α-Amylase, Copy number variation, Dental caries, Genetics

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Introduction

Dental caries is a prevalent chronic disease caused by the destruction of tooth structure by acid-forming bacteria influenced by environmental and genetic factors [1]. Its multifactorial etiology includes factors such as dental plaque, high carbohydrate consumption, inadequate oral hygiene, suboptimal saliva properties, insufficient fluoride intake, and the bacterial composition of the oral cavity [2]. Saliva, a biological fluid rich in proteins like mucin, immunoglobulins, lactoferrin, and amylase, plays a critical role in oral health by promoting remineralization, neutralizing acids, and preventing microbial adhesion [3].

The amylase enzyme is a key component of the digestive system, specifically in the breakdown of starch. This enzyme is present in both human saliva and pancreatic secretions, with salivary glands responsible for approximately half of the amylase activity in serum and the exocrine pancreatic glands responsible for the remainder [4]. Alongside its digestive function, amylase plays a crucial role in the adhesion of various bacterial species within the streptococcus genus to the dental biofilm [5, 6]. Research has suggested that the acid products formed as a result of the hydrolysis of starch into glucose and maltose may contribute to the development of dental caries [7]. High levels of amylase activity have been associated with an increased risk of dental caries [8–10]. Alpha-amylase-coated bacteria can produce acid during starch fermentation, which, when combined with the acid produced by the conversion of starch to glucose, contributes to the formation of dental caries by causing demineralization in dental tissues.

Amylase enzymes are produced via two distinct genes, namely salivary amylase alpha 1 (AMY1) and pancreatic amylase alpha 2 (AMY2) genes [11]. One of the most abundant proteins in human saliva (accounting for approximately 50%) is α-amylase, encoded by AMY1, located in a gene cluster at 1p21. The AMY1 gene consists of three repetitive structures, namely AMY1A, AMY1B, and AMY1C, which, apart from a few small indel variations [12], are over 99.9% identical in DNA sequence [13]. The number of repeats in the AMY1 gene structure, which is effective in determining amylase levels in saliva and serum, may vary from 1 to 20 repeats based on differences among populations and individual groups [14]. Typically, individuals with low numbers of repeats, such as 1–4 repeats, reside in societies with a hunter-gatherer character, and these individuals have low amylase production [15], low carbohydrate digestion capacity [16], increased insulin resistance [17], a higher risk of infection [17], increased gluten sensitivity [15], and a greater risk of obesity [18]. Conversely, individuals with over 9 repeats in the AMY1 gene reside in agricultural areas, and these individuals have high amylase production [15], a high carbohydrate digestion capacity [16], low insulin resistance [17], a lower risk of infection [19], a lower gluten sensitivity [15], and a lower risk of obesity [18].

Single Nucleotide Polymorphisms (SNPs) within the gene structure denote variations in single nucleotides with a frequency of 1% or more in normal individuals of the population, while copy number variations (CNVs) are genetic character traits that manifest as differences in the copy numbers of a gene. It is hypothesized that widespread CNVs in humans, similar to single nucleotide polymorphisms, may influence phenotypic diversity and disease susceptibility. However, it is believed that CNVs may have a more significant impact on the phenotype than single nucleotide polymorphisms [20].

Recent research has begun to uncover the genetic underpinnings of dental caries, with a pilot study in Lithuania identifying a potential correlation between AMY1 gene CNV and caries incidence, particularly on smooth surfaces [21]. This pioneering study highlights the importance of genetic contributions to caries risk; however, the findings remain preliminary and require validation through studies involving diverse populations. Despite its initial promise, the Lithuanian study was limited by its sample size and geographic focus, underscoring the need for further investigation. In contrast, our study builds on this research by focusing on a genetically distinct Turkish population, offering insights into how AMY1 CNV relates to caries in a different ethnic and genetic context. By focusing on a genetically distinct cohort, this research seeks to enhance our understanding of AMY1 CNV's role in caries susceptibility and contribute to the growing body of evidence supporting personalized approaches to oral health management.

Materials and methods

Study protocol

The determination of the sample size for the study was performed using the G*Power package program (G*Power Version 3.0.10, Franz Faul, Üniversität Kiel, Germany). The effect size was estimated based on the occlusal caries DMFS values (with a cut-off value of 9) in the CMV groups of a similar previous study [21]. Using this information, an effect size of 0.416 was calculated. To achieve a statistical power of 99% with an alpha level of 0.05, the minimum required sample size was determined to be 136 participants. The final sample size of the study was 154 participants, exceeding the minimum requirement.

The current investigation underwent evaluation by the Clinical Research Ethics Committee of the Faculty of Medicine at Sutcu Imam University, during its meeting held on 02.10.2019. The study received approval, as it was deemed suitable with the committee's decision, numbered 2019/254. Following the approval of the Clinical

Research Ethics Committee, the collection of samples from volunteers commenced. All participant data were anonymized and coded before analysis to ensure confidentiality. Identifiable information was removed, and the data were stored securely in compliance with ethical standards and institutional guidelines.

A total of 155 volunteers were recruited from the Restorative Dentistry Department Clinic of Sutcu Imam University Faculty of Dentistry. The volunteers provided their consent to participate in the study by signing the patient consent form, which was created in accordance with the ethics committee approval. Epithelial cells in inner cheek tissues were collected from the volunteers using swabs, and the collected swab samples were placed in a DNA stabilization preservation and storage solution (Swab Collection and DNA Preservation System, Norgen Biotek Corp., Ontario, Canada). The swab samples were stored at room temperature until they were transferred to the Department of Medical Biology, Molecular Genetics Research Laboratory at Recep Tayyip Erdogan University Faculty of Medicine. The samples were transferred under appropriate conditions for genotype analysis.

Intra-rater reliability

Prior to the commencement of the examinations, the researcher (O.H) was initially calibrated in accordance with the WHO Basic Surveys Calibration Protocol for the detection of caries, coding of findings, and recording of results. To evaluate intra-rater reliability for continuous variables, ten subjects who were not a part of the study sample were assessed for the DMFT and DMFS index at two-week intervals. Concordance correlation coefficients of 0.93 and 0.86 for DMFT and DMFS were obtained, which are considered acceptable based on the threshold of ≥ 0.75, which aligns with guidelines that indicate substantial to almost perfect agreement [22].

Clinical and radiological examination

The demographic characteristics and oral hygiene behaviors of the study participants were meticulously recorded. Oral hygiene variables recorded were daily tooth brushing frequency (categorized as more than twice daily, once daily, or 2–3 times weekly), utilization of dental floss (none, weekly, daily), mouthwash use (none, weekly, daily), and tongue brushing habit (yes/no). Additionally, tooth brushing techniques (horizontal, vertical, or both), frequency of toothbrush replacement (monthly, every 3 months, every 6 months, or annually), and preferred beverages (water, tea/coffee, cola/juice, ayran) were documented. Dietary habits were also considered, classifying frequently consumed foods as carbohydrate-rich or protein-based.

During the clinical examination, all tooth surfaces were assessed for the presence of caries using a sterile dental Shepherd's Hook explorer and mirror by the same researcher (O.H). According to World Health Organization (WHO) criteria, lesions requiring clinical restoration and radiolucent areas clearly visible to be progressing from the enamel-dentin border to the dentin on radiographs were recorded as "caries lesions." Only white and brown color changes where the probe wasn't inserted were not considered decayed. Digital panoramic radiographs (Orthopantomograph® Op300 Panoramic, Instrumentarium Dental, Tuusula, Finland) were used for the detection of interfacial caries, which were routinely taken from patients examined at Sutcu Imam University Faculty of Dentistry Restorative Dentistry Clinic. In cases of superpositions that prevented caries diagnosis in digital radiography, two-bite radiographs were taken from the right and left posterior regions. Wisdom teeth were not evaluated. The DMFT score, based on the WHO criteria, was computed for each individual by adding the number of decayed (D), missing (M), and filled (F) teeth in their oral cavity. To calculate the DMFS score, surfaces were based instead of tooth numbers. DMFT and DMFS index scores were recorded on the provided forms.

DNA isolation

The DNA isolation process was conducted on swab samples stored at the Molecular Genetics Research Laboratory of Recep Tayyip Erdogan University Faculty of Medicine, Department of Medical Biology. The collected swab samples were stored in a DNA stabilization preservation solution at room temperature and later transferred to the laboratory under controlled conditions. The duration between sample collection and DNA isolation did not exceed four weeks. A commercial kit optimized for swab samples was used, and its sensitivity was adjusted to achieve optimal results. Subsequently, the purity and concentration of the isolated DNA samples were analyzed using a fluorometer with a fluorescent dye (Denovix QFX Fluorometer, Denovix Inc., DE, USA). All samples were optimized to 10 ng/ul by fluorometer analysis. Out of the 155 volunteers included in our study, DNA samples of 154 individuals were optimized, while one volunteer's DNA sample did not provide a sufficient amount of DNA for analysis. As a result, the data of 154 individuals were subjected to statistical analysis.

AMY1 gene copy number analysis

The determination of CNVs of the AMY1 gene was conducted through a Real-time polymerase reaction (PCR) device, (Roche Applied Science LightCycler® 480 II, Roche Diagnostics GmbH, Mannheim, Germany). The TaqMan chemistry, which comprises quantitative real-time PCR reactions utilizing a dual TaqMan kit, was utilized in this analysis process. To ascertain the copy number of the AMY1 gene, the AMY1 TaqMan™ Copy

Number Assay (Assay ID: Hs07226362_cn, Life Technologies, Carlsbad, CA, USA) kit was employed. The TaqMan™ Copy Number Reference Assay, human, RNase P (Life Technologies, Carlsbad, CA, USA) kit was used as the reference gene. To analyze the copy number of the AMY1 gene, each sample was subjected to Real-time PCR testing three times with a 20 µl reagent mixture. The gene copy number was assessed based on the triplicate reaction data. CopyCaller™ Software v2.0 (Life Technologies, Carlsbad, CA, USA) was employed to analyze the data. The diploid copy number of the AMY1 gene was determined using a standard curve generated from a previously determined calibrator DNA sample (NA18972; Coriell Cell Repositories, Camden, NJ).

Statistical analysis

The statistical analysis was conducted using the Jamovi software (Version 2.3.21). The data were presented as median (min-max) values, and normality of distribution was assessed using the Shapiro-Wilk test. Since the Shapiro-Wilk test indicated a non-normal distribution of the data ($p < 0.05$), non-parametric tests were applied. Specifically, Kruskal-Wallis and Mann-Whitney U tests were used for group comparisons. Additionally, categorical data were compared using the Chi-square and Cramer's V tests. The factors predicting DMFT and DMFS scores were evaluated using binomial logistic regression analysis. Prior to conducting logistic regression, multicollinearity among predictors was assessed using the variance inflation factor (VIF), ensuring that no significant multicollinearity issues were present. For the logistic regression analysis, DMFT and DMFS scores were categorized using their median values (DMFT ≤ 5 and DMFS ≤ 8). This classification method was chosen to ensure balanced group sizes, allowing for a sufficient sample size in both comparison groups and increasing the statistical power of the regression model. By dichotomizing the data at the median, we aimed to achieve more reliable and interpretable statistical results while maintaining the robustness of the analysis. Statistical significance was considered at a value of $p < 0.05$.

Results

The study included a total of 154 participants, comprising 63% females ($n = 97$) and 37% males ($n = 57$). The mean age of the participants was 19.6 ± 1.4 years, with an age range spanning from 18 to 29 years. Our analysis did not reveal any significant differences in oral health or nutrition-related responses concerning AMY1 CNV, as detailed in Table 1. Similarly, no significant associations were observed between AMY1 CNV and any of the evaluated caries indices ($p > 0.05$). However, while all comparisons were statistically non-significant, the DMFT mandibular score ($p = 0.085$) approached significance, suggesting a potential trend that may warrant further investigation in larger sample sizes (Table 2; Fig. 1). Furthermore, correlation analysis showed no significant relationship between AMY1 CNV and either the DMFT or DMFS scores ($r = 0.06$, $p > 0.05$) (Fig. 2).

Table 3 explores the relationship between demographic, oral hygiene, and nutritional factors with dental caries indices (DMFT and DMFS). Among the variables examined, daily tooth brushing frequency showed the strongest association with DMFT (Cramer's V = 0.219, $p = 0.026$) and DMFS ($p = 0.008$), with participants brushing more than twice daily demonstrating lower caries indices. Similarly, preferred beverage consumption displayed a notable relationship with DMFS (Cramer's V = 0.219, $p = 0.007$), where higher consumption of cola/juice correlated with elevated caries scores. Other factors, including gender (Cramer's V = 0.086, $p > 0.05$), dental floss utilization (Cramer's V = 0.031, $p > 0.05$), mouthwash utilization (Cramer's V = 0.072, $p > 0.05$), tooth brushing techniques (Cramer's V = 0.042, $p > 0.05$), tongue brushing (Cramer's V = 0.070, $p > 0.05$), frequency of toothbrush replacement (Cramer's V = 0.084, $p > 0.05$), and frequently consumed foods (Cramer's V = 0.052, $p > 0.05$), exhibited weak or insignificant associations with DMFT or DMFS. Additionally, the relationship between AMY1 CNV and DMFT was weak (Cramer's V = 0.073, $p > 0.05$), indicating limited explanatory power of genetic variation in this context. These relationships are further visualized in the network graph in Fig. 3, which illustrates the strength of associations between variables using Cramer's V values, highlighting the dominant role of daily tooth brushing frequency and preferred beverage consumption in relation to caries indices.

The results of the binomial logistic regression analysis indicate that individuals with 4–6 ($OR_{adjusted}$=2.083, 95% CI 0.352–12.32), 7–9 ($OR_{adjusted}$=2.132, 95% CI 0.385–11.825), and 10–16 ($OR_{adjusted}$=1.897, 95% CI 0.336–10.708) CNVs exhibit approximately 2 times higher odds ratio for DMFT index compared to those with 2–3 CNVs, yet this fails to reach statistical significance ($p > 0.05$) (Table 4). Based on the DMFS index, individuals with 4–6 ($OR_{adjusted}$=2.519, 95% CI 0.335–18.959), 7–9 ($OR_{adjusted}$=5.223, 95% CI 0.737–36.984), and 10–16 ($OR_{adjusted}$=3.122, 95% CI 0.425–22.926) CNVs exhibit approximately 2, 5, and 3 times higher odds ratio for DMFS when compared to those with 2–3 CNVs, respectively. However, these relationships also failed to reach statistical significance ($p > 0.05$). Frequent tooth brushing (more than twice daily) significantly reduces the odds of elevated caries prevelances compared to less frequent brushing ($p = 0.026$ for DMFT, $p = 0.008$ for DMFS). (Table 4).

A post-hoc power analysis was conducted using the comparison values of DMFT total scores with AMY1

Table 1 Comparison of demographic, oral hygiene, and nutritional habits across AMY1 CNV groups

	AMY1 Copy Number Variation					
	2–3 (*N* = 7)	**5–6 (*N* = 33)**	**7–9 (*N* = 63)**	**10–16 (*N* = 51)**	**Total (*N* = 154)**	***p* value**
Gender						0.594[1]
Female	3.0 (42.9%)	22.0 (66.7%)	38.0 (60.3%)	34.0 (66.7%)	97.0 (63.0%)	
Male	4.0 (57.1%)	11.0 (33.3%)	25.0 (39.7%)	17.0 (33.3%)	57.0 (37.0%)	
Age						0.644[2]
Mean (SD)	20.1 (0.9)	19.8 (1.5)	19.5 (1.6)	19.6 (1.2)	19.6 (1.4)	
Range	19.0–21.0	18.0–25.0	18.0–29.0	18.0–24.0	18.0–29.0	
Daily tooth brushing frequency						0.439[1]
Daily > 2	5.0 (71.4%)	16.0 (48.5%)	35.0 (56.5%)	32.0 (62.7%)	88.0 (57.5%)	
Daily 1	1.0 (14.3%)	13.0 (39.4%)	23.0 (37.1%)	18.0 (35.3%)	55.0 (35.9%)	
Weekly 2–3	1.0 (14.3%)	4.0 (12.1%)	4.0 (6.5%)	1.0 (2.0%)	10.0 (6.5%)	
Dental floss utilization						0.971[1]
Daily > 2	0.0 (0.0%)	0.0 (0.0%)	2.0 (3.2%)	1.0 (2.0%)	3.0 (1.9%)	
Daily 1	1.0 (14.3%)	4.0 (12.1%)	7.0 (11.1%)	4.0 (7.8%)	16.0 (10.4%)	
Weekly 2–3	1.0 (14.3%)	9.0 (27.3%)	19.0 (30.2%)	14.0 (27.5%)	43.0 (27.9%)	
None	5.0 (71.4%)	20.0 (60.6%)	35.0 (55.6%)	32.0 (62.7%)	92.0 (59.7%)	
Mouthwash utilization						0.369[1]
Daily > 2	0.0 (0.0%)	0.0 (0.0%)	4.0 (6.3%)	4.0 (7.8%)	8.0 (5.2%)	
Daily 1	0.0 (0.0%)	5.0 (15.2%)	6.0 (9.5%)	2.0 (3.9%)	13.0 (8.4%)	
Weekly 2–3	2.0 (28.6%)	7.0 (21.2%)	8.0 (12.7%)	13.0 (25.5%)	30.0 (19.5%)	
None	5.0 (71.4%)	21.0 (63.6%)	45.0 (71.4%)	32.0 (62.7%)	103.0 (66.9%)	
Tooth brushing techniques						0.862[1]
Horizontal	1.0 (14.3%)	2.0 (6.1%)	4.0 (6.3%)	3.0 (5.9%)	10.0 (6.5%)	
Vertical	2.0 (28.6%)	12.0 (36.4%)	16.0 (25.4%)	18.0 (35.3%)	48.0 (31.2%)	
Both of them	4.0 (57.1%)	19.0 (57.6%)	43.0 (68.3%)	30.0 (58.8%)	96.0 (62.3%)	
Tongue brushing						0.584[1]
Yes	5.0 (71.4%)	25.0 (75.8%)	39.0 (61.9%)	34.0 (66.7%)	103.0 (66.9%)	
No	2.0 (28.6%)	8.0 (24.2%)	24.0 (38.1%)	17.0 (33.3%)	51.0 (33.1%)	
Frequency of toothbrush replacement						0.610[1]
Monthly	0.0 (0.0%)	1.0 (3.0%)	1.0 (1.6%)	3.0 (5.9%)	5.0 (3.2%)	
1 in 3 months	2.0 (28.6%)	12.0 (36.4%)	31.0 (49.2%)	16.0 (31.4%)	61.0 (39.6%)	
1 in 6 months	4.0 (57.1%)	18.0 (54.5%)	27.0 (42.9%)	30.0 (58.8%)	79.0 (51.3%)	
1 in 12 months	1.0 (14.3%)	2.0 (6.1%)	4.0 (6.3%)	2.0 (3.9%)	9.0 (5.8%)	
Preferred beverages						0.465[1]
Ayran (A traditional Turkish yogurt drink)	4.0 (57.1%)	15.0 (45.5%)	28.0 (44.4%)	16.0 (31.4%)	63.0 (40.9%)	
Water	0.0 (0.0%)	2.0 (6.1%)	2.0 (3.2%)	1.0 (2.0%)	5.0 (3.2%)	
Cola/Juice	2.0 (28.6%)	14.0 (42.4%)	25.0 (39.7%)	31.0 (60.8%)	72.0 (46.8%)	
Tea/Coffee	1.0 (14.3%)	2.0 (6.1%)	8.0 (12.7%)	3.0 (5.9%)	14.0 (9.1%)	
Frequently consumed foods						0.661[1]
Carbohydrate	5.0 (71.4%)	15.0 (45.5%)	32.0 (50.8%)	25.0 (49.0%)	77.0 (50.0%)	
Protein	2.0 (28.6%)	18.0 (54.5%)	31.0 (49.2%)	26.0 (51.0%)	77.0 (50.0%)	

N (%),[1] Chi-squared test, [2] Kruskal-Wallis Test, Bold characters indice significance

CNV, yielding an effect size of 0.145 and a power of 28.9%. These findings suggest that while the study met the minimum sample size criteria, the achieved power for certain analyses may have been limited, potentially impacting the ability to detect smaller effects.

Discussion

Numerous studies have established a strong correlation between genetic variations and the prevalence of dental caries. These variations can impact the composition of saliva [23, 24], the sensitivity of taste receptors [25, 26], the mineralization of teeth [27, 28], and the immune system [29–32], all of which play a significant role in the development and progression of dental caries. Therefore, it is essential to acknowledge the role of genetic factors in the etiology of dental caries and leverage this knowledge to develop effective prevention and treatment strategies. These insights can pave the way for a more personalized approach to dentistry, where genetic testing provide

Table 2 Relationship of DMFT and DMFS caries indices with AMY1 copy number variations

	2–3 (*N* = 7)	5–6 (*N* = 33)	7–9 (*N* = 63)	10–16 (*N* = 51)	*p*-value
DMFT mandibular	1(0–3)	2(0–8)	2(0–6)	3(0–7)	0.085[1]
DMFT maxilla	1(0–6)	2(0–7)	2(0–11)	2(0–11)	0.684[1]
DMFT anterior	0(0–2)	0(0–4)	0(0–6)	0(0–5)	0.771[1]
DMFT posterior	2(0–6)	4(0–12)	5(0–11)	4(0–13)	0.299[1]
DMFT total	2(0–8)	5(0–13)	5(0–15)	5(0–18)	0.350[1]
DMFS mandibular	1(0–11)	4(0–21)	4(0–26)	4(0–15)	0.241[1]
DMFS maxilla	1(0–9)	2(0–15)	3(0–22)	4(0–16)	0.693[1]
DMFS anterior	0(0–2)	0(0–8)	0(0–6)	0(0–7)	0.782[1]
DMFS posterior	5(0–16)	6(0–26)	7(0–36)	7(0–24)	0.482[1]
DMFS total	5(0–18)	6(0–28)	9(0–38)	7(0–30)	0.419[1]

Median (Min-Max), [1] Kruskal-Wallis

valuable information to optimize treatment plans and improve patient outcomes.

Salivary alpha-amylase enzyme is a highly versatile molecule that serves several biological functions. It participates in carbohydrate digestion through its enzymatic activity, binds to oral streptococci for bacterial clearance and nourishment, and plays a crucial role in the adhesion of alpha-amylase-binding bacteria [33]. The binding of alpha-amylase to bacteria and teeth can have significant implications for dental plaque formation, leading to tooth demineralization and caries.

Several studies have examined the link between salivary alpha amylase and dental caries. However, the findings have been inconsistent, despite the theoretical notion that amylase activity can increase the risk of dental caries. Some studies have reported an inverse correlation between salivary alpha-amylase level and dental caries [34–36], while others have found a positive relationship [10, 37], and some have failed to detect any association

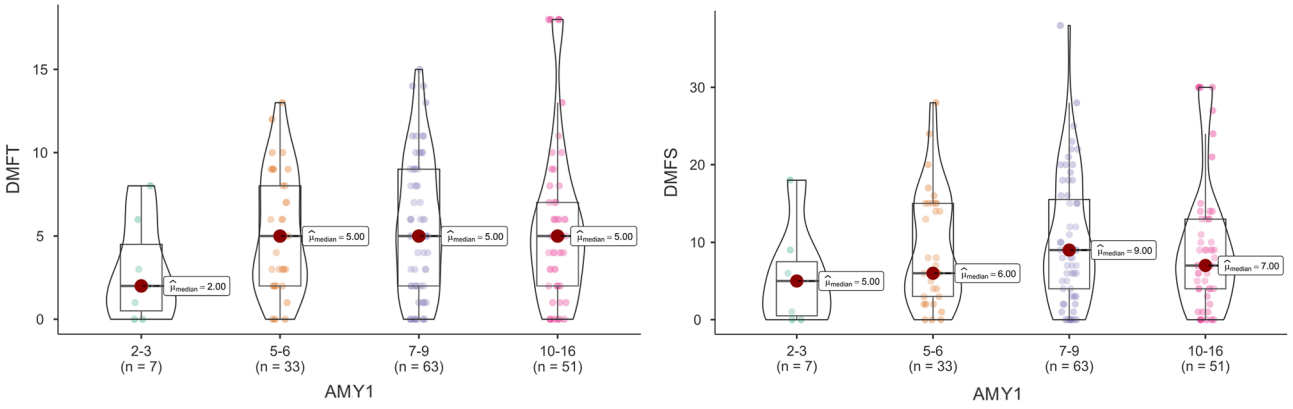

Fig. 1 Box-Violin Plots presenting the comparison between AMY1 copy number variation and dental caries experience. Statistical comparisons were performed using the Kruskal-Wallis test

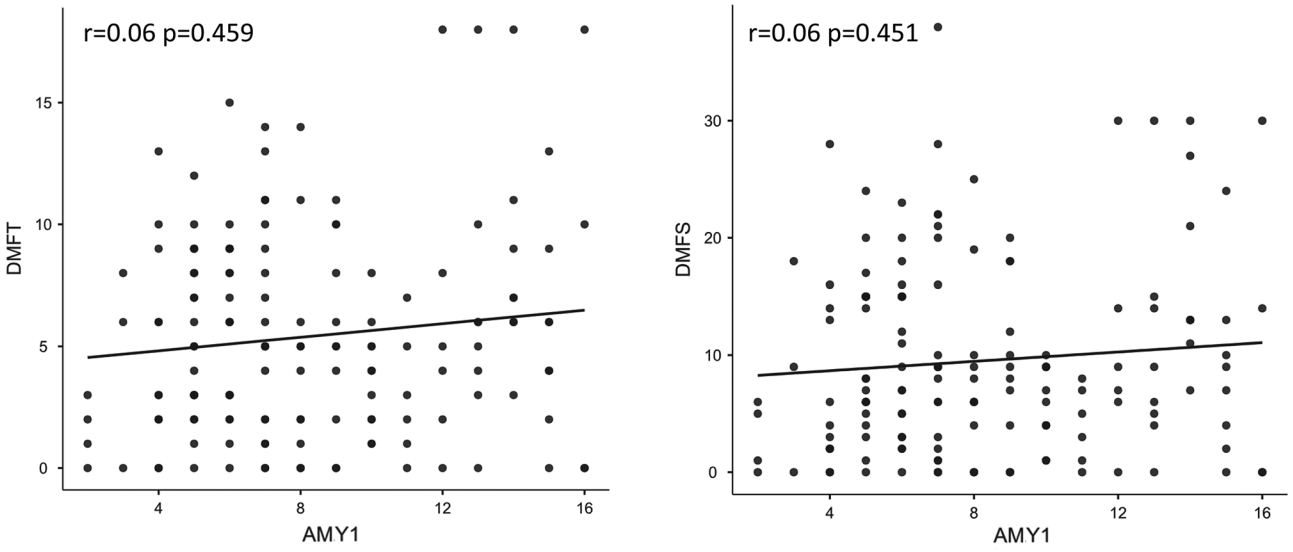

Fig. 2 Scatter plot presenting the correlation between AMY1 copy number variation and dental caries experience. The correlation was assessed using Spearman's correlation analysis

Table 3 Relationship of demographic, oral hygiene and nutritional factors with dental caries indices

	DMFT			DMFS		
	≤ 5 (*N* = 86)	**> 5 (*N* = 68)**	***p* value**	**≤ 8 (*N* = 82)**	**> 8 (*N* = 72)**	***p* value**
Gender			0.287[1]			0.432[1]
Female	51.0 (59.3%)	46.0 (67.6%)		54.0 (65.9%)	43.0 (59.7%)	
Male	35.0 (40.7%)	22.0 (32.4%)		28.0 (34.1%)	29.0 (40.3%)	
Age			0.463[2]			0.570[2]
Mean (SD)	19.5 (1.3)	19.7 (1.6)		19.5 (1.2)	19.7 (1.7)	
Range	18.0–25.0	18.0–29.0		18.0–25.0	18.0–29.0	
Daily tooth brushing frequency			**0.026**[1]			**0.008**[1]
Daily > 2	55.0 (64.7%)	33.0 (48.5%)		53.0 (65.4%)	35.0 (48.6%)	
Daily 1	28.0 (32.9%)	27.0 (39.7%)		25.0 (30.9%)	30.0 (41.7%)	
Weekly 2–3	2.0 (2.4%)	8.0 (11.8%)		3.0 (3.7%)	7.0 (9.7%)	
Dental floss utilization			0.985[1]			0.883[1]
Daily > 2	2.0 (2.3%)	1.0 (1.5%)		1.0 (1.2%)	2.0 (2.8%)	
Daily 1	9.0 (10.5%)	7.0 (10.3%)		8.0 (9.8%)	8.0 (11.1%)	
Weekly 2–3	24.0 (27.9%)	19.0 (27.9%)		24.0 (29.3%)	19.0 (26.4%)	
None	51.0 (59.3%)	41.0 (60.3%)		49.0 (59.8%)	43.0 (59.7%)	
Mouthwash utilization			0.849[1]			0.267[1]
Daily > 2	5.0 (5.8%)	3.0 (4.4%)		3.0 (3.7%)	5.0 (6.9%)	
Daily 1	6.0 (7.0%)	7.0 (10.3%)		7.0 (8.5%)	6.0 (8.3%)	
Weekly 2–3	16.0 (18.6%)	14.0 (20.6%)		12.0 (14.6%)	18.0 (25.0%)	
None	59.0 (68.6%)	44.0 (64.7%)		60.0 (73.2%)	43.0 (59.7%)	
Tooth brushing techniques			0.872[1]			0.487[1]
Horizontal	5.0 (5.8%)	5.0 (7.4%)		5.0 (6.1%)	5.0 (6.9%)	
Vertical	26.0 (30.2%)	22.0 (32.4%)		29.0 (35.4%)	19.0 (26.4%)	
Both of them	55.0 (64.0%)	41.0 (60.3%)		48.0 (58.5%)	48.0 (66.7%)	
Tongue brushing			0.385[1]			0.527[1]
Yes	55.0 (64.0%)	48.0 (70.6%)		53.0 (64.6%)	50.0 (69.4%)	
No	31.0 (36.0%)	20.0 (29.4%)		29.0 (35.4%)	22.0 (30.6%)	
Frequency of toothbrush replacement			0.780[1]			0.901[1]
Monthly	3.0 (3.5%)	2.0 (2.9%)		3.0 (3.7%)	2.0 (2.8%)	
1 in 3 months	31.0 (36.0%)	30.0 (44.1%)		34.0 (41.5%)	27.0 (37.5%)	
1 in 6 months	47.0 (54.7%)	32.0 (47.1%)		41.0 (50.0%)	38.0 (52.8%)	
1 in 12 months	5.0 (5.8%)	4.0 (5.9%)		4.0 (4.9%)	5.0 (6.9%)	
Preferred beverages			0.061[1]			**0.007**[1]
Ayran (A traditional Turkish yogurt drink)	41.0 (47.7%)	22.0 (32.4%)		38.0 (46.3%)	25.0 (34.7%)	
Water	3.0 (3.5%)	2.0 (2.9%)		3.0 (3.7%)	2.0 (2.8%)	
Cola/Juice	32.0 (37.2%)	40.0 (58.8%)		29.0 (35.4%)	43.0 (59.7%)	
Tea/Coffee	10.0 (11.6%)	4.0 (5.9%)		12.0 (14.6%)	2.0 (2.8%)	
Frequently consumed foods			0.516[1]			0.333[1]
Carbohydrate	41.0 (47.7%)	36.0 (52.9%)		38.0 (46.3%)	39.0 (54.2%)	
Protein	45.0 (52.3%)	32.0 (47.1%)		44.0 (53.7%)	33.0 (45.8%)	

N (%), [1] Chi-squared test, [2] Mann-Whitney U test

[38–41]. In a meta-analysis study, where the results were pooled to provide an overall estimate, it was concluded that there is no significant association between salivary alpha-amylase level and dental caries [42]. The findings suggest that salivary alpha-amylase level alone may not be a reliable predictor of dental caries risk.

Salivary alpha-amylase is a protein enzyme that is highly abundant in human saliva, accounting for approximately 40–50% of the total protein content [43]. The AMY1 CNV has been found to range from two to twenty [44]. It is worth noting that the AMY1 CNV has been shown to be positively correlated with the amount of starchy foods in the diet of individuals. Recent studies have shown a strong link between lower levels of AMY1 CNV and high body mass index [45, 46], diabetes [47, 48], and metabolic syndrome [48]. Recent meta-analyses have demonstrated that individuals with diabetes [49] and obesity [50] are at a significantly greater risk of developing dental caries, possibly due to decreased salivary flow rate [51, 52]. In this context, it is possible that low

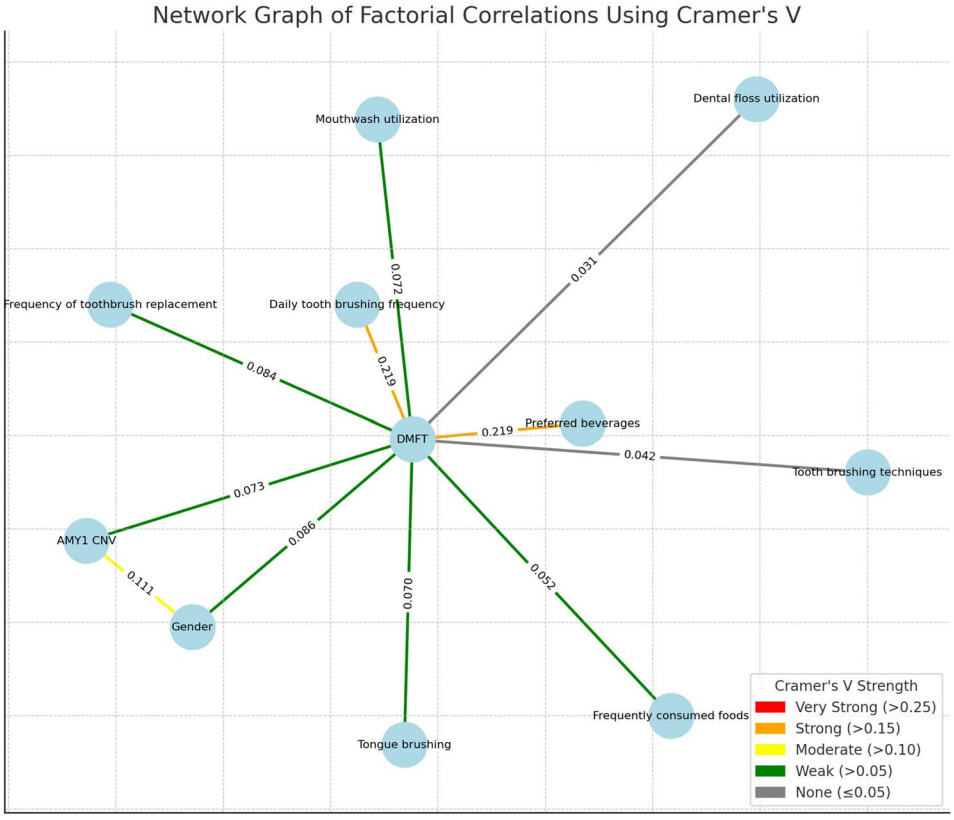

Fig. 3 Network graph of factorial correlations using Cramer's V

AMY1 CNV and consequently low salivary amylase level may be linked to high caries risk. However, our study did not find any significant relationship between AMY1 CNV and dental caries.

The relationship between AMY1 CNV and dental caries has been studied in only one research paper to date. The study by Stangvaltaite-Mouhat, et al. [21] found that smooth-surface caries experience was significantly higher in individuals with other copy number ranges compared to those with 2–3 CNVs. Specifically, their research showed that people with 4–5 CNVs had approximately 13 odds ratio higher smooth-surface caries than those with 2–3 CNVs. In contrast, our study utilized DMFT and DMFS indices to explore this relationship. Although we observed that individuals with other copy numbers had approximately 2 odds ratio more dental caries than those with 2–3 copy numbers, we did not find a significant relationship.

One possible explanation for this discrepancy is the difference in caries assessment methods. Stangvaltaite-Mouhat, et al. [21] specifically focused on smooth-surface caries, which primarily occur in areas with less mechanical plaque removal and are more influenced by biological factors such as salivary composition and host genetics. In contrast, DMFT and DMFS indices include all types of carious lesions, including occlusal and proximal surfaces, which are also significantly affected by external factors such as oral hygiene practices and fluoride exposure. Therefore, while AMY1 CNV may have a more direct impact on smooth-surface caries due to its role in starch digestion and salivary amylase activity, its association with overall caries experience (as measured by DMFT/DMFS) may be diluted by other behavioral and environmental influences.

Additionally, differences in population characteristics may contribute to the contrasting findings. Our study focused on individuals aged between 18 and 30 years to minimize confounding factors related to periodontal and prosthetic interventions at older ages. However, other environmental factors such as diet, fluoride exposure, and socioeconomic status could also play a role. Lithuania, where the previous study was conducted, has different dietary habits compared to our study population, particularly in terms of carbohydrate consumption and fluoride exposure from drinking water and oral care products. If the Lithuanian population had higher exposure to fluoride, for example, this could have influenced caries susceptibility and potentially modified the impact of AMY1 CNV on smooth-surface caries. Furthermore, socioeconomic disparities may affect oral hygiene practices, access to dental care, and overall caries risk, further

Table 4 Evaluation of factors predicting DMFT and DMFS index with binomial logistic regression analysis

Characteristics	DMFT		DMFS	
	OR	Adjusted OR	OR	Adjusted OR
AMY1 Copy Number Variation				
2–3	1	1	1	1
4–6	2.083 (0.352–12.32)	2.32 (0.347–15.519)	1.625 (0.273–9.658)	2.519 (0.335–18.959)
7–9	2.132 (0.385–11.825)	2.639 (0.422–16.504)	2.75 (0.496–15.246)	5.223 (0.737–36.984)
10–16	1.897 (0.336–10.708)	1.992 (0.304–13.068)	2.222 (0.394–12.529)	3.122 (0.425–22.926)
Gender				
Female	1	1	1	1
Male	0.697 (0.358–1.356)	0.929 (0.423–2.041)	1.301 (0.675–2.506)	1.705 (0.752–3.864)
Age	1.088 (0.868–1.363)	1.042 (0.803–1.353)	1.067 (0.853–1.335)	1.085 (0.821–1.434)
Daily tooth brushing frequency				
Daily > 2	1	1	1	1
Daily 1	1.607 (0.812–3.180)	1.833 (0.813–4.112)	1.817 (0.920–3.591)	1.978 (0.855–4.573)
Less than daily 1	6.667 (1.335–33.301)	7.258 (1.104–47.733)	3.533 (0.856–14.593)	4.753 (0.804–28.093)
Dental floss utilization				
Daily > 2	1	1	1	1
Daily 1	1.556 (0.116–20.854)	1.194 (0.04–35.8)	0.5 (0.037–6.683)	0.459 (0.016–13.577)
Weekly 2–3	1.583 (0.133–18.808)	1.377 (0.041–46.078)	0.396 (0.033–4.702)	0.279 (0.008–9.508)
None	1.608 (0.141–18.362)	1.533 (0.048–48.615)	0.439 (0.038–5.01)	0.349 (0.011–11.23)
Mouthwash utilization				
Daily > 2	1	1	1	1
Daily 1	1.944 (0.322–11.756)	2.319 (0.178–30.257)	0.514 (0.085–3.109)	1.31 (0.101–16.956)
Weekly 2–3	1.458 (0.294–7.231)	1.631 (0.153–17.436)	0.9 (0.18–4.489)	2.644 (0.241–29.007)
None	1.243 (0.282–5.48)	1.698 (0.174–16.524)	0.43 (0.097–1.896)	1.4 (0.142–13.754)
Tooth brushing techniques				
Horizontal	1	1	1	1
Vertical	0.846 (0.216–3.308)	0.67 (0.136–3.306)	0.655 (0.167–2.573)	0.608 (0.121–3.069)
Both of them	0.745 (0.202–2.746)	0.674 (0.147–3.096)	1 (0.272–3.679)	1.018 (0.219–4.741)
Tongue brushing				
Yes	1	1	1	1
No	1.353 (0.684–2.677)	0.786 (0.355–1.74)	0.804 (0.409–1.58)	0.757 (0.335–1.714)
Frequency of toothbrush replacement				
Monthly	1	1	1	1
1 in 3 months	1.452 (0.226–9.309)	0.924 (0.109–7.852)	1.191 (0.186–7.644)	1.479 (0.152–14.384)
1 in 6 months	1.021 (0.161–6.461)	0.764 (0.088–6.604)	1.39 (0.22–8.777)	2.107 (0.215–20.657)
1 in 12 months	1.2 (0.13-11.052)	1.36 (0.093–19.874)	1.875 (0.204–17.268)	4.366 (0.257–74.108)
Preferred beverages				
Ayran (A traditional Turkish yogurt drink)	1	1	1	1
Water	1.242 (0.193–8.002)	1.236 (0.142–10.728)	1.013 (0.158–6.503)	1.128 (0.125–10.148)
Cola/Juice	2.33 (1.161–4.672)	2.345 (1.087–5.058)	2.254 (1.13–4.495)	2.44 (1.112–5.353)
Tea/Coffee	0.745 (0.209–2.654)	0.67 (0.178–2.53)	0.253 (0.052–1.23)	0.232 (0.043–1.247)
Frequently consumed foods				
Carbohydrate	1	1	1	1
Protein	1.235 (0.653–2.335)	0.773 (0.376–1.588)	0.731 (0.387–1.379)	0.646 (0.306–1.365)

Estimates represent the log odds of "DMFT > 5" vs. " DMFT ≤5" and "DMFS > 8" vs. "DMFS ≤ 8"

contributing to the differences between our findings and those of the Lithuanian study.

Another important consideration is the potential impact of sample size and measurement variability on our findings. In both studies, individuals with 2–3 CNVs constituted only 5% of the total sample. The small number of participants in this group may have increased the margin of error in these comparisons. Additionally, while the scatter plot suggested a slight trend of increasing dental caries with higher AMY1 CNV, the correlation coefficient was very low ($r = 0.06$). This suggests that even if there was an effect, it was too weak to be statistically significant within the sample size and study design constraints. Future studies with larger and more diverse

populations, as well as refined methodologies for measuring AMY1 CNV and salivary amylase activity, may be necessary to further explore this relationship.

The significant relationship observed between daily tooth brushing frequency and caries indices underscores the critical role of oral hygiene in caries prevention. Our findings indicate that individuals who brushed their teeth more than twice daily exhibited significantly lower DMFT and DMFS scores compared to those who brushed less frequently. This aligns with previous studies emphasizing that regular and effective tooth brushing reduces plaque accumulation, limits bacterial activity, and enhances fluoride exposure, thereby mitigating caries risk [53, 54]. From a public health perspective, these results reinforce the necessity of promoting frequent and proper brushing habits.

The present study was subject to certain limitations. Foremost, dental caries is a multifactorial disease influenced by numerous factors, and it was impracticable to entirely eliminate the effect of all such factors. Furthermore, despite attempts to standardize the assessment of dental caries experience using dental caries indices, these indices do not provide information regarding the severity and activity of dental caries. Additionally, the cross-sectional evaluation of dental caries during a specific time period is not an accurate assessment of a patient's risk of dental caries, as the most accurate evaluation requires a longitudinal assessment. Another limitation of this study was the relatively low statistical power (28.9%) achieved in the post-hoc power analysis for certain analyses. This lower power may have limited our ability to detect smaller but potentially meaningful effects, emphasizing the need for cautious interpretation of non-significant findings and larger, adequately powered studies in the future. The present study was subject to certain limitations. Foremost, dental caries is a multifactorial disease influenced by numerous factors, and it was impracticable to entirely eliminate the effect of all such factors. Furthermore, despite attempts to standardize the assessment of dental caries experience using dental caries indices, these indices do not provide information regarding the severity and activity of dental caries. Additionally, the cross-sectional evaluation of dental caries during a specific time period is not an accurate assessment of a patient's risk of dental caries, as the most accurate evaluation requires a longitudinal assessment. Another limitation of this study was the relatively low statistical power (28.9%) achieved in the post-hoc power analysis for certain analyses. This lower power may have limited our ability to detect smaller but potentially meaningful effects, emphasizing the need for cautious interpretation of non-significant findings and larger, adequately powered studies in the future.

Furthermore, certain potential confounders, such as socioeconomic status and fluoride exposure, were not explicitly controlled for in this study. Socioeconomic disparities can influence oral health behaviors, access to dental care, and dietary habits, all of which are critical determinants of dental caries risk. Similarly, variations in fluoride exposure—whether from drinking water, toothpaste, or professional applications—may significantly modulate caries susceptibility and potentially obscure genetic associations. The lack of data on these variables represents a limitation in our ability to fully isolate the effect of AMY1 CNV on dental caries. Future research should aim to address the limitations of this study by employing a longitudinal study design, which would allow for a more accurate assessment of the dynamic nature of dental caries development and its relationship with AMY1 CNV over time. Prospective cohort studies following individuals across different life stages could help determine whether AMY1 CNV influences not only current caries status but also future caries progression.

Conclusion

This study sheds light on the potential role of AMY1 gene CNV in dental caries susceptibility within a Turkish population. While the findings did not reveal statistically significant associations between AMY1 CNVs and dental caries indices (DMFT and DMFS), the data suggest that genetic variation in salivary amylase production could be further explored in future studies with larger sample sizes and diverse populations. Among the analyzed factors, oral hygiene practices—particularly daily tooth brushing frequency—and dietary behaviors, such as preferred beverage consumption, were strongly associated with caries risk, emphasizing their importance in caries prevention and management.

The absence of significant correlations between AMY1 CNVs and caries indices underscores the multifactorial nature of dental caries, where genetic predispositions may interplay with environmental and behavioral factors. This study highlights the need for larger, more diverse population studies to validate these findings and unravel the complex genetic and environmental interactions underlying caries development. Regardless of genetic predisposition, promoting consistent oral hygiene habits, such as twice-daily tooth brushing with fluoride toothpaste, and reducing the consumption of sugary beverages remain essential and immediate strategies for mitigating caries risk. Public health initiatives should focus on raising awareness about these preventive measures, as they offer a universally applicable approach to improving oral health outcomes. By bridging genetics with personalized oral health strategies, future research could pave the way for targeted interventions that integrate genetic susceptibility, oral hygiene behaviors, and dietary patterns.

Author contributions
Ö.H, F.S contributed in study design. Ö.H performed data collection, data analysis, writing the main manuscript. F.S performed genetical analysis and reviewed the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets generated and/or analyzed during the current study are not publicly available considering that we have not required consents to publish this data, but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All participants provided an online informed consent to participate in the study and were assured that their information would be kept confdential. All study procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki. Ethical approval was obtained by Clinical Research Ethics Committee of the Faculty of Medicine at Sutcu Imam University, during its meeting held on 02.10.2019. The study received approval, as it was deemed suitable with the committee's decision, numbered 2019/254.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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