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ORIGINAL RESEARCH

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Evaluation of *EDARADD*, *LPO* and *ACTN2* genes polymorphisms in children with dental caries compared to caries-free controls

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Abstract

Dental caries is a complex condition that results from a combination of genetic and environmental factors. Several genes have been found to play a role in teeth development and have been associated with various dental traits. In this study, our objective was to examine the potential correlation between the ectodysplasin a receptor (EDAR)-associated *via* death domain (EDARADD), Lactoperoxidase (LPO) and Actinin Alpha 2 (ACTN2) gene polymorphisms and susceptibility to dental caries. The study included a total of 600 participants, comprising 300 individuals with dental caries and 300 caries-free controls. The genotyping of the EDARADD (rs79233817), LPO (rs8178275) and ACTN2 (rs114880747) gene polymorphisms was performed using the tetra-primer amplification refractory mutation system Polymerase chain reaction (PCR) method. Individuals with dental caries were found to have a significantly higher frequency of the A allele (minor allele) for rs79233817 compared to controls. The specific single nucleotide polymorphism (SNP) (rs79233817) was associated with an increased risk of dental caries (DC) in both the co-dominant and dominant genetic models (p-value < 0.05). In addition, the study's findings revealed a significant association between the rs114880747 SNP and susceptibility to dental caries (p-value < 0.05). A higher frequency of the A allele (minor allele) of rs114880747 was observed in patients compared to the healthy controls. It is also worth mentioning that there was no association between rs8178275 susceptibility to dental caries (p-value > 0.05).It can be inferred that the EDARADD gene polymorphism (rs79233817) and ACTN2 gene polymorphism (rs114880747) potentially play a role in the genetic susceptibility to dental caries. To validate and delve deeper into these findings, it is necessary to conduct additional studies with larger sample sizes in diverse populations. This will help to establish the robustness of the results and further investigate the underlying mechanisms involved.

Keywords

Dental caries; EDARADD; ACTN2; LPO; Genetics; Diagnosis and treatment planning

1. Introduction

Dental caries (DC), commonly known as tooth decay, is a chronic multifactorial condition that affects a large proportion of the population worldwide [1]. It is caused by the interaction between oral bacteria, dietary carbohydrates, and host factors, such as saliva composition, tooth structure, and immune response [1]. DC develops in the crowns and roots of teeth and affects the primary teeth of infants and toddlers by destruction of tooth enamel, dentine and deeper structures [2, 3]. Among children, DC is widely recognized as the most prevalent chronic condition [4, 5], with around 500 million cases reported in children aged 0–14 years, specifically affecting their deciduous teeth [6]. DC is a widespread and multifactorial disease, despite the various preventive methods employed [7]. While behavioral and external influences have a significant function for developing DC, genetic factors are also known to contribute to individual susceptibility [8]. Numerous genetic variations have been recognized as potential contributors to the risk of developing DC. The advancement of molecular biology techniques, including DNA sequence analysis techniques has enabled more sophisticated and well-conducted and trustworthy studies, which have confirmed the importance of genetic traits in DC [9, 10]. Furthermore, the human genome project has facilitated the identification of specific genes located on chromosomes that contribute to an increased susceptibility to caries [11, 12]. Recent meta-analyses [13–15] have additionally demonstrated an association between poly-

morphisms and an increased susceptibility to DC. EDARADD is a protein made up of 208 amino acids. The N-terminus of the protein contains a Tnf receptor-associated factor (Traf)-binding consensus sequence, while the C-terminus features a death domain (DD). The Traf-binding consensus sequence serves as a docking site for Traf1, Traf2 and Traf3, which then recruits Traf members and activates nuclear factor kappa B (NF-κB) [16]. On the other hand, the DD helps EDARADD self-associate and interact with EDAR [16, 17]. Therefore, EDARADD plays a critical role in Edar signaling, where the N-terminal region is responsible for signal transduction, and the C-terminal death domain (DD) is necessary for receptor engagement. It has also been shown that mutations of the EDARADD gene can lead to Ectodermal Dysplasia Syndrome, Congenital Ectodermal Defect and Sweat Gland Diseases [18]. However, the current evidence on the association between SNPs within the EDARADD gene and DC susceptibility is limited. Additional studies are necessary to explore and investigate the functional effects of the EDARADD gene on tooth development and to replicate the association between polymorphisms and DC in diverse populations [19].

The Alpha-actinin-2 (ACTN2) gene is responsible for the production of alpha-actinin-2 protein, a cytoskeletal protein that is involved in the organization and stabilization of actin filaments in muscle cells [20]. ACTN2 is also expressed in odontoblasts, the cells that synthesize and secrete dentin, which composes the majority of the tooth structure beneath the enamel [21]. ACTN2 polymorphisms have been linked to several muscle-related disorders, such as hypertrophic cardiomyopathy and skeletal muscle myopathies [22, 23]. However, the role of ACTN2 in DC susceptibility is not well-established, and the mechanisms by which ACTN2 polymorphisms might affect tooth structure or function are not fully understood. The rs114880747 SNP in the ACTN2 gene is a rare variant that leads to the substitution of a proline amino acid residue with a serine residue at position 1740 of the protein sequence [24]. This variant has been hypothesized to affect the binding affinity of ACTN2 to actin filaments or other proteins involved in dentin formation, thereby altering tooth structure and increasing the risk of DC [24]. However, the available evidence on the association between ACTN2 gene polymorphisms and DC susceptibility is limited and conflicting, with some studies reporting positive associations and others finding no significant effects [25, 26].

Lactoperoxidase (LPO), which codes for bactericidal salivary enzyme, plays a crucial role in protecting the lactating mammary gland and the intestinal tract of newborn infants against pathogenic microorganisms [27, 28]. Besides its antimicrobial function, LPO has other reported functions, such as growth-promotion activity and anti-tumor activity [27, 29]. It has been shown that this gene plays an important role in xerostomia, absence of salivary glands, parotid aplasia or hypoplasia and congenital absence of salivary gland. The first genome-wide association study (GWAS) for dental caries focused on children aged 3 to 12 years and investigated the primary dentition [30]. This study identified several novel genes, including LPO. However, as GWAS is a hypothesis-generating method, the results require careful scrutiny and replication in independent samples to distinguish chance results from true associations.

As mentioned above, further studies are needed to clarify the potential role of the EDARADD, LPO and ACTN2 genes in DC susceptibility. These studies should investigate the functional effects of these genes on tooth development and replicate the association of polymorphisms in different populations [19]. Such studies could offer valuable insights into the genetic and molecular mechanisms underlying DC and inform the development of targeted prevention and treatment strategies for this prevalent oral disease. Therefore, the aim of this study was to investigate the potential association of the rs79233817 SNP of the EDARADD gene, the rs114880747 SNP of the ACTN2 gene and the rs8178275 SNP of the LPO gene with DC susceptibility in a population-based sample. Our findings could provide insights into the genetic basis of DC and contribute to the development of personalized prevention and treatment strategies for this common oral disease.

2. Material and methods

2.1 Patients

This case-control study was conducted on children referred to clinics supported by the Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. The study spanned a period of 24 months from 2021 to 2023. To determine the appropriate sample size, the researchers employed the formula $n = Z^2\ p(1 - p)/e^2$ where Z was assigned a value of 1.96 and the frequency was estimated to be 60%. Based on these parameters, the calculated sample size was determined to be 300 participants. The diagnostic criteria for dental caries typically involve a thorough examination of the teeth, including visual inspection, dental X-rays, and probing of the tooth surfaces. The number of examiners (6 dentists) involved in the study underwent training and standardization to ensure consistency in performing the clinical examination. A calibration workshop was conducted to establish uniformity in the assessment of dental caries. Dentists look for signs of cavities, such as visible holes or discolored spots on the tooth surface. They also assess the texture and integrity of the tooth enamel, as well as the presence of any soft or sticky areas indicating decay. The study had a control group consisting of age-matched individuals without DC. Inclusion criteria for the study comprised individuals of any gender, aged up to 15 years, who had dental caries. These individuals were assigned to the case group. Additionally, subjects who did not provide consent were excluded from the study. In order to ensure consistency in the population or ethnic group being studied, children from ethnic backgrounds other than Iranian were excluded from the association studies of polymorphisms. A total of 600 individuals were included in the study, comprising 300 patients diagnosed with DC and 300 healthy controls who had similar demographic characteristics and 5 mL of peripheral blood was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes.

2.2 DNA genotyping

The standard salting out method was used to extract genomic DNA from EDTA anti-coagulated blood samples, which

were then stored at −20 °C until the genetic study. For the genotyping of SNP rs79233817, SNP rs114880747 and SNP rs8178275, the tetra-primer amplification refractory mutation system PCR (Tetra-ARMS-PCR) method was employed, and the primers were designed using the Primer 1 online tool found at http://primer1.soton.ac.uk/primer1.html. The accuracy of genotyping was monitored by utilizing negative control samples lacking genomic DNA and positive controls with known genotypes, which were compared with respective sequencing results.

The amplification temperature protocol for the EDARADD SNP rs79233817 included an initial denaturation step at 95 °C for 5 minutes, followed by 30 cycles of 95 °C for 30 seconds, 63 °C for 30 seconds, and 72 °C for 2 minutes, and a final extension at 72 °C for 5 minutes. The amplification temperature conditions for the ACTN2 SNP rs114880747 PCR reaction were as follows: an initial denaturation step at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 64.5 °C for 30 seconds, and extension at 72 °C for 2 minutes. A final extension step was performed at 72 °C for 5 minutes. The temperature conditions for amplification of LPO SNP rs8178275 sequence consisted of initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of 95 °C for 30 seconds, 61.5 °C for 30 seconds, and 72 °C for 2 minutes, and a final extension at 72 °C for 5 minutes. For each PCR reaction, 1 μL of each inner primer (10 PM) and 1 μL of each outer primer (5 PM), 1 μL of Mastermix (amplicon®Mastermix containing Magnesium chloride 2 ($MgCl_2$), Taq PCR buffer, Taq DNA polymerase and dNTPs), and 2 μL of DNA (50 ng/μL) adjusted to 20 μL by double-distilled water (ddH_2O) were used for both SNPs. The primer sequences are displayed in Table 1.

2.3 Statistical analysis

We utilized the SNPStats web tool (https://www.snpstats.net/start.htm) to evaluate the selected SNP's conformity to Hardy-Weinberg equilibrium (HWE) and to investigate the associations between SNPs and DC using various models (co-dominant, dominant, recessive and overdominant). The impact of each variant was quantified using an odds ratio (OR) accompanied by its respective 95% confidence interval, while statistical significance was determined by a p-value of 0.05 or lower.

3. Results

Our study enrolled 300 patients with DC and 300 healthy samples. The average age of participants in the case group was 15 ± 3.4 years, while in the control group it was 16 ± 3.1 years. The case group consisted of 98 males (32.7%) and 202 females (67.3%), while the control group had 115 males (38.3%) and 185 females (61.7%) (Table 2). Comparison of the mean age between the case and control groups and sex between the case and control groups showed no statistically significant difference (p-value = 0.21 and p-value = 0.16, respectively). The severity of DC was assessed by calculating the Decayed, Missing, and Filled (DMFT) score, which quantified the presence of decayed, missing and filled teeth for each individual participant. We found an association between the rs114880747 SNP in the ACTN2 gene and DMFT score in DC cases (p = 0.030), and an association between the rs79233817 SNP in the EDARADD gene and DMFT score in DC cases (p = 0.021), as presented in Table 3. In addition, our findings, presented in Table 3, have shown that there is no association between the rs8178275 SNP in the LPO gene and DMFT score in dental caries cases (p = 0.09).

TABLE 1. The primer sequences and product size.

Gene (SNP)	Primers	Sequence	Product size
ACTN2 (rs114880747)			
	Forward inner primer (G allele)	AGGTTTGCTATTTGTAAAAAATTTCATGTG	For G allele: 191
	Reverse inner primer (A allele)	GGGGCAATCACATAAGCATATTAGATAT	For A allele: 258
	Forward outer primer (5′-3′)	GCGCTTCATAAATAGGTTTATTTCTGAG	Two outer primers: 391
	Reverse outer primer (5′-3′)	CCAAAAATCTTTTGGGTAGTCTTTTTAA	
LPO (rs8178275)			
	Forward inner primer (G allele)	GCTATTGCATCAACCAATCCCTGACG	For G allele: 229
	Reverse inner primer (A allele)	GGCAGATACACCAGGAAACTGCAGCAT	For A allele: 164
	Forward outer primer (5′-3′)	TTAAAAGGGACAAATGTGCTCAGGGCAT	Two outer primers: 340
	Reverse outer primer (5′-3′)	TCTTACCTGCCCAGTGCCTTGTCTTTTC	
EDARADD (rs79233817)			
	Forward inner primer (G allele)	CAGAGAATTAAGAAGCCAAACTCAACAGCG	For G allele: 156
	Reverse inner primer (A allele)	CTGTTTAGTCGTCCTGAGGCCCATTGT	For A allele: 207
	Forward outer primer (5′-3′)	AAATTTCCCTTCCTATCCGAAGGCAGAC	Two outer primers: 306
	Reverse outer primer (5′-3′)	AGCAACCTCTGGCTAAAAACTCAGCTCTG	

SNP: Single nucleotide polymorphism; ACTN2: Actinin Alpha 2; LPO: Lactoperoxidase; EDARADD: ectodysplasin A receptor (EDAR)-associated via death domain.

TABLE 2. Demographic characteristics of group of participants studied.

Variable	Case	Control	*p-value
Sex n (%)			
Male	98 (32.7%)	115 (38.3%)	0.21
Female	202 (67.3%)	185 (61.7%)	
Age mean (yr)	15 yr	16 yr	0.16

**chi-squared test.*

TABLE 3. The correlation between the EDARADD (rs79233817), ACTN2 (rs114880747) and LPO (rs8178275) genotypes and the decayed missing filled teeth (DMFT) score in DC cases.

Gene (SNP)	Genotype in DMFT Score n = 300	Decayed Missing Filled Teeth Score (DMFT score)							*p-value
		1 DMFT 190 (63.35%)	2 DMFT 65 (21.6%)	3 DMFT 19 (6.3%)	4 DMFT 14 (4.6%)	5 DMFT 7 (2.33%)	6 DMFT 3 (1%)	7 DMFT 2 (0.66%)	
ACTN2 (rs114880747)									
	AA	12	10	2	-	-	-	-	
	AG	65	40	15	10	4	2	1	0.03
	GG	113	15	2	4	3	1	1	
LPO (rs8178275)									
	AA	20	19	5	3	3	1	1	
	AG	49	29	12	9	2	2	1	0.09
	GG	121	17	2	2	2	-	-	
EDARADD (rs79233817)									
	AA	110	41	15	9	5	2	-	
	AG	44	25	6	1	1	-	-	0.021
	GG	20	10	4	4	2	-	1	

**SNPStats; SNP: Single nucleotide polymorphism; ACTN2: Actinin Alpha 2; LPO: Lactoperoxidase; EDARADD: EDAR-associated via death domain.*

3.1 Accordance with Hardy-Weinberg equilibrium

The genotype distributions of the analyzed SNPs were found to be consistent with Hardy-Weinberg equilibrium ($p > 0.05$). When conducting an exact test for rs114880747, the resulting *p*-values were 0.14 for cases and 0.29 for controls. When conducting an exact test for rs79233817, the resulting *p*-values were 0.16 for cases and 0.11 for controls. When conducting an exact test for rs8178275, the resulting *p*-values were 0.12 for cases and 0.17 for controls, as shown in Table 4. DNA bands and their positions on the agarose gel are shown in Fig. 1.

3.2 Case-control study

A statistically significant increase in the frequency of the A allele (minor allele) for rs114880747 was observed in individuals with DC compared to healthy controls (OR (95% CI) = 1.596 (1.043–1.811), $p = 0.005$; as shown in Table 5. This SNP exhibited an association with the risk of DC in both the co-

TABLE 4. Exact test for Hardy-Weinberg equilibrium.

SNP	Patients	Healthy controls	*p-value
rs114880747			
A/A	24	19	
A/G	137	120	0.11
G/G	139	161	
rs8178275			
A/A	52	30	
A/G	104	124	0.13
G/G	144	146	
rs79233817			
A/A	182	141	
A/G	77	95	0.09
G/G	41	64	

**SNPStats. SNP: Single nucleotide polymorphism.*

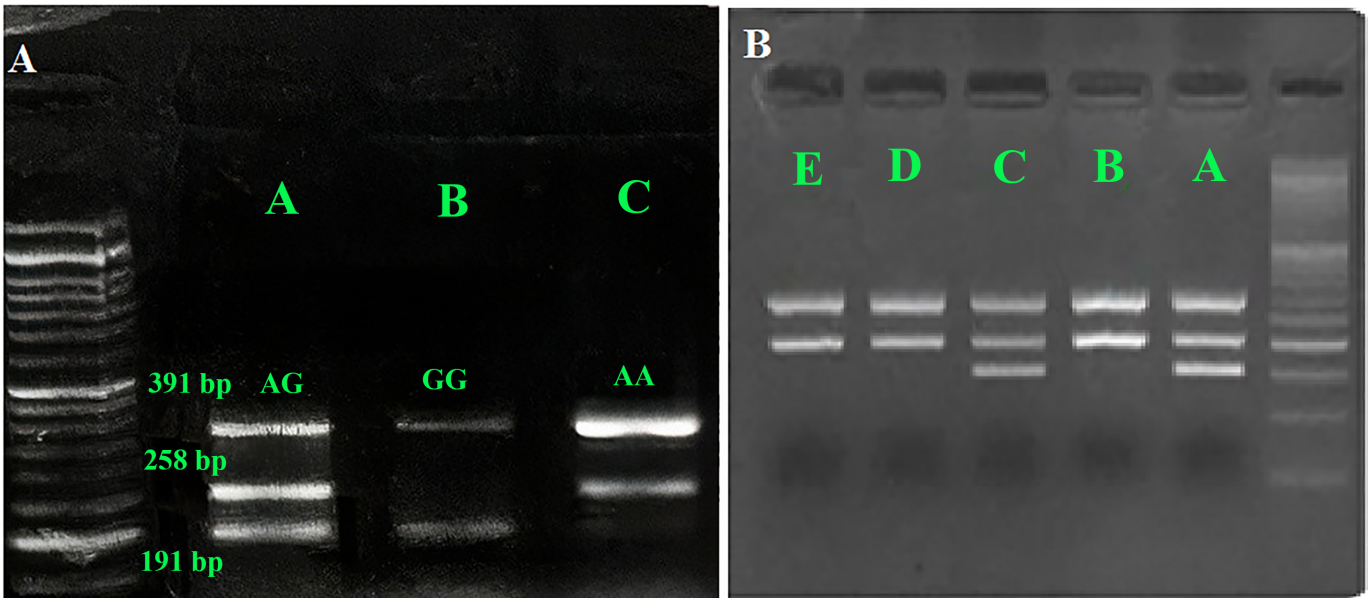

FIGURE 1. Gel Electrophoresis. (A) Gel electrophoresis of the T-ARMS PCR products from the ACTN2 (rs114880747) gene on a 2.5% agarose gel. Lane A: AG genotype (391, 258 and 191 bp); Lane B: GG genotype (391 and 191 bp); Lane C: AA genotype (391 and 258 bp). (B) Gel electrophoresis of the T-ARMS PCR products from of the EDARADD (rs79233817) gene on a 2.5% agarose gel. Lane A and C: AG genotype (306, 207 and 156 bp); Lanes B, D and E: AA genotype (306 and 207 bp).

dominant and dominant models (OR (95% CI) = 1.58 (1.38–1.86), *p*-value = 0.022; OR (95% CI) = 1.55 (1.37–1.81), *p*-value = 0.011, respectively). The frequency of the A allele (minor allele) for rs79233817 was observed to be significantly higher in DC patients compared to controls. The odds ratio (95% confidence interval) for this association was calculated as 1.812 (1.715–2.02) and a *p*-value of 0.014 (refer to Table 4). In both co-dominant and dominant genetic models, this specific SNP (rs79233817) was found to be associated with an elevated risk of DC. The odds ratios (95% confidence intervals) were determined as 1.341 (1.23–1.81) and 1.48 (1.31–1.84) for the respective models, with corresponding *p*-values of 0.022 and 0.015. However, the frequency of the A allele (minor allele) for rs8178275 was not found to be statistically significant between individuals with DC and healthy controls (*p*-value = 0.13).

4. Discussion

The current study aimed to enhance our understanding of the genetic foundation of caries susceptibility. To achieve this, samples were collected from a population in Iran, both with and without dental caries, and genotyped for specific polymorphisms (rs79233817 SNP of the EDARADD, rs114880747 SNP of the ACTN2 gene and rs8178275 SNP of the LPO gene). These genes were identified as candidate genes in a GWAS for DC [25]. Our results have shown an association between specific SNPs in the ACTN2 and EDARADD genes and the DMFT score in DC cases. The rs114880747 SNP in the ACTN2 gene demonstrated a statistically significant association with the DMFT score in DC cases. Similarly, the rs79233817 SNP in the EDARADD gene showed an association with the DMFT score in DC cases, although the *p*-value (0.09) was not statistically significant. Furthermore, we observed a statistically significant increase in the frequency of the A allele (minor allele) for the rs114880747 SNP in individuals with DC compared to healthy controls. This SNP exhibited an association with the risk of DC in both the co-dominant and dominant genetic models. Similarly, the frequency of the A allele (minor allele) for the rs79233817 SNP was significantly higher in DC patients compared to controls. This specific SNP (rs79233817) was found to be associated with an elevated risk of DC in both the co-dominant and dominant genetic models.

DC is influenced by both external and genetic causes [31]. High sugar diets, particularly those containing candies and chocolates, are a common cause of DC [32]. Inadequate oral hygiene practices, insufficient consumption of fluoride, and prolonged bottle-feeding are additional factors that contribute to the onset of DC [33]. Taking precautionary measures early on can help prevent the occurrence of DC at an early stage. Genetic predisposition to tooth decay has been the subject of several studies, and various genes have been identified that may contribute to the development of this condition [34–37]. Overall, genetic predisposition to tooth decay is a complex issue that involves multiple genes and environmental factors. Identifying the genes involved in the development of tooth decay could provide valuable insights into the etiology of this condition and pave the way to facilitate the creation of novel preventive and therapeutic approaches. The differences in association studies could be attributed to genetic differences. Genetic variations can vary among populations due to factors like ancestry, geographical location, and ethnic diversity. If the genetic makeup of the trait or disease differs between populations, association studies conducted in different populations may produce dissimilar outcomes. It is crucial to take into account population-specific genetic factors when interpreting the results of association studies. In this study, we have shown a positive association between the rs114880747

TABLE 5. The frequencies of allele and genotype distributions of SNPs in both patients and healthy controls.

SNP	Model		DC patients n (%)	Controls n (%)	OR (95% CI)	*p-value
rs114880747						
	Allele	A *vs.* G	185 (30.8%) 415 (69.2%)	158 (26.3%) 442 (73.7%)	1.596 (1.043–1.811)	0.005
	Co-dominant	A/A	24 (8%)	19 (6.3%)	1.00	0.022
		A/G	137 (45.7%)	120 (40%)	1.58 (1.38–1.86)	
		G/G	139 (46.3%)	161 (53.7%)	1.50 (0.23–1.08)	
	Dominant	A/A	24 (8%)	19 (6.3%)	1.00	0.011
		A/G-G/G	276 (92%)	281 (93.7%)	1.55 (1.37–1.81)	
	Recessive	A/A-A/G	161 (53.7%)	139 (46.3%)	1.00	0.16
		G/G	139 (46.3%)	161 (53.7%)	1.59 (1.28–1.76)	
	Overdominant	A/A-G/G	163 (54.3%)	180 (60%)	1.00	0.059
		A/G	137 (45.7%)	120 (40%)	1.72 (1.01–1.91)	
rs8178275						
	Allele	A *vs.* G	208 (34.7%) 392 (65.3%)	184 (30.7%) 416 (69.3%)	1.54 (1.411–1.809)	0.13
	Co-dominant	A/A	52 (17.3%)	30 (10%)	1.00	0.14
		A/G	104 (34.7%)	124 (41.3%)	1.71 (1.02–2.86)	
		G/G	144 (48%)	146 (48.7%)	1.00	
	Dominant	A/A	52 (17.3%)	30 (10%)	1.00	0.35
		A/G-G/G	248 (82.7%)	270 (90%)	2.06 (1.08–4.38)	
	Recessive	A/A-A/G	156 (52%)	154 (51.3%)	1.00	0.12
		G/G	144 (48%)	146 (48.7%)	1.87 (1.76–4.62)	
	Overdominant	A/A-G/G	196 (65.3%)	176 (58.7%)	1.00	0.09
		A/G	104 (34.7%)	124 (41.3%)	1.45 (1.61–2.30)	
rs79233817						
	Allele	A *vs.* G	441 (73.5%) 159 (26.5%)	377 (62.8%) 223 (37.2%)	1.812 (1.715–2.02)	0.014
	Co-dominant	A/A	182 (60.7%)	141 (47%)	1.00	0.022
		A/G	77 (25.7%)	95 (31.7%)	1.341 (1.23–1.81)	
		G/G	41 (13.6%)	64 (21.3%)	1.00	
	Dominant	A/A	182 (60.7%)	141 (47%)	1.45 (1.31–1.51)	0.015
		A/G-G/G	118 (39.3%)	159 (53%)	1.48 (1.31–1.84)	
	Recessive	A/A-A/G	259 (86.4%)	236 (78.7%)	1.61 (1.39–1.68)	0.21
		G/G	41 (13.6%)	64 (21.3%)	1.53 (1.37–1.59)	
	Overdominant	A/A-G/G	223 (74.3%)	205 (68.3%)	1.00	0.071
		A/G	77 (25.7%)	95 (31.7%)	1.43 (1.61–1.66)	

**SNPStats. SNP: Single nucleotide polymorphism; DC: Dental Caries; OR: Ods Ratio; CI: Confidence interval.*

polymorphism and susceptibility DC. This observation aligns with the findings from a study conducted on a cohort of 65 Greek children, all within the specified age range of 5 to 12 years [26]. That study has shown the ACTN2 (rs6656267) gene polymorphism is positively associated with DC. In addition, another GWAS study has shown that there is significant evidence of an association between ACTN2 SNPs and DC [25]. The precise mechanism that explains the connection between ACTN2 (located on 1q42–q43; MIM# 102573) and DC remains incompletely understood. However, one possible explanation is that the ACTN2 gene appears to be involved in regulating and organizing ameloblasts, which are cells responsible for tooth enamel formation [38, 39]. The ACTN2 gene is situated on chromosome 1 (1q43) and is responsible for encoding a protein involved in the formation of the cytoskeleton that is a member of the superfamily of spectrin genes. It has been associated with several disorders, such as cardiomyopathy [38, 39].

A GWAS study has reported that the gene EDARADD, which is situated on the 1q42–q43 region (MIM# 606603), is responsible for producing the EDARADD protein, and it has been found to be linked with DC [30]. The objective of the present study was to examine the relationship between a SNP, namely rs79233817, located within the EDARADD gene, and its association with DC in the Iranian population. The findings of this investigation have contributed to our knowledge regarding the genetic factors implicated in the onset and progression of DC, including interactions between genes and the environment. These findings have significant implications, potentially leading to advancements in early detection, risk assessment, dental treatment, and more effective public health interventions. Our results indicate a statistically significant elevation of rs79233817 in individuals with DC compared to the control group (p-value < 0.05). Our findings align with a study conducted by Shaffer *et al.* [30], which also reported an association between SNPs within the EDARADD gene and DC in children aged 3 to 12 years in the United States. The significance of our study lies in the recognition that association studies investigating the relationship between SNPs and complex diseases should consider ethnic and population factors [40]. By conducting our study in the Iranian population, we provide further confirmation of the role of the EDARADD gene in DC susceptibility, particularly among patients in the United States. Therefore, our research adds to the growing body of evidence supporting the involvement of the EDARADD gene in DC across different populations and ethnicities. In 2001, Headon and colleagues [41] made a significant discovery regarding the EDARADD and EDAR genes. They found that these genes are co-expressed in epithelial cells during the developmental stages of hair follicles and teeth. Additionally, they observed that EDARADD possesses a self-associating property, which is a characteristic often seen in death domain proteins. The researchers conducted experiments in which they overexpressed EDARADD in HEK293T cells and observed the activation of an NF-κB reporter gene, with the degree of activation correlating with the dosage of EDARADD. Through their investigations, they also determined that the activation of EDAR is triggered by EDA, and that EDARADD functions as an adaptor molecule, forming an intracellular signal-transducing complex. This linear pathway is responsible for the similar phenotypic traits observed in Tabby, downless, and crinkled mutant mice, as well as the genetic diversity observed in hypohidrotic ectodermal dysplasia in humans [41].

In the context of oral health, the LPO gene (17q22, MIM# 150205) plays a crucial role in defending against DC [42]. It also helps regulate the microbial composition in the oral cavity, preventing the growth of pathogenic microorganisms associated with periodontitis [43]. The physiological properties of LPO have been harnessed in the development of oral hygiene products enriched with the LPO protein to prevent these diseases [44]. Our results have shown that rs8178275 SNP of the LPO gene was not associated with DC susceptibility. This observation is aligned with the findings of Stanley *et al.*'s [25] findings. They have shown, *via* meta-analysis study, that SNPs within the LPO gene are not associated with DC susceptibility. This study did not consider certain factors that might also affect the occurrence and progression of DC, for example hygiene habits or fluoride exposure, which could be viewed as a limitation of the current research. However, the present study offers a thorough investigation into the Single Nucleotide Polymorphisms within genes responsible for encoding enamel formation proteins, exploring their potential association with DC. Additionally, our extensive review of the literature makes a significant contribution to the field of DC research.

5. Conclusions

In summary, our study provides evidence that the rs114880747 variant in the ACTN2 gene and the rs79233817 variant in the EDARADD gene are promising candidate genes associated with susceptibility to dental caries in primary teeth among children from Iran. These SNPs may serve as potential predictors for diagnosing dental caries in deciduous dentition. However, it is important to note that further replication of our analysis using a larger sample size is necessary to strengthen this hypothesis. Additionally, conducting comparisons with children from different ethnic and population groups, including diverse races, would provide valuable insights in this field.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

MHL—designed the research study and performed the research; SS—wrote the manuscript; SK—wrote the manuscript and performed the data analysis; AS—wrote the manuscript; YN—performed the research; ZP—wrote the manuscript; KN—performed the research; MS—performed the research; FT, FLA—designed the research study and performed the research and data analysis. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in adherence to the ethical principles outlined in the Declaration of Helsinki and was approved by the Ethical Committee of Mashhad University of Medical Sciences (MUMS-1399-73). Each participant's parent or legal guardian provided written informed consent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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