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Original Research

Association study between polymorphisms within MPPED2 (rs12797813), MTR (rs61739582), ACTN2 (rs6656267) and LPO (rs7209537) genes and susceptibility to dental caries: a case-control study

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ABSTRACT

Introduction: The linkage between specific genetic variants and the susceptibility to dental caries remains a crucial area of investigation. This study aimed to examine the association of particular gene variants within *MPPED2* (rs12797813), *MTR* (rs61739582), *ACTN2* (rs6656267), and *LPO* (rs7209537) genes with the likelihood of developing dental caries. **Methods**: The study recruited a cohort of 600 participants, divided equally between 300 individuals diagnosed with dental caries and 300 healthy controls devoid of the condition. The focus was on the genotyping of the mentioned gene variants using the Tetra-Primer Amplification Refractory Mutation System PCR (Tetra-ARMS-PCR) technique. This method is noted for its efficacy in detecting genetic variations. Discussion: Analysis revealed significant associations between the occurrence of dental caries and the SNPs rs6656267 and rs7209537, with p-values less than 0.05 indicating statistical relevance. Notably, the presence of the C allele of rs6656267 and the A allele of rs7209537 was disproportionately higher amongst individuals with dental caries compared to the healthy control group. These associations were evident across both co-dominant and dominant genetic models, suggesting that these variants may increase the risk of developing dental caries. The discrepancy with the allele reference suggests a potential error in the citation of rs8178275, which likely should be rs7209537 based on the context provided. **Conclusion**: The findings of this study suggest that the rs6656267 and rs7209537 variants in the *ACTN2* and *LPO* genes, respectively, may serve as potential genetic markers for increased susceptibility to dental caries. However, the complex nature of genetic influences on dental caries risk necessitates further research to confirm these initial findings and to explore the mechanisms by which these SNPs influence caries development.

Key words: Dental caries, MPPED2 gene, MTR gene, ACTN2 gene, LPO gene, Polymorphism, Genetic, Case-control studies

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INTRODUCTION

Dental caries (DC), commonly known as tooth decay, is a chronic multifactorial condition that affects a large proportion of the population worldwide[1]. This condition arises from the interplay between oral bacteria, dietary carbohydrates, and various host factors, including saliva composition, tooth structure, and immune responses[1]. While behavioral and environmental factors play a crucial role in the development of DC, genetic influences have also been recognized as contributing to individual susceptibility[2]. Numerous genetic variations have been identified as potential contributors to a person's predisposition to developing DC. Among the genes studied in this context are metallophosphoesterase domain-containing 2 (*MPPED2*), methionine synthetase (*MTR*), alpha-actinin-2 (*ACTN2*), and lactoperoxidase (*LPO*). The *MPPED2* gene, located on chromosome 11 (11p14.1), codes for a metallophosphoesterase. Although the precise function of this gene is not yet fully understood, it appears to be involved in the development of the nervous system[3] and has been associated with aniridia 1, a congenital eye disorder[4]. Interestingly, while the *MPPED2* gene does not have a known direct role in DC, a study has shown that its expression was significantly reduced (by a factor of 5) in oral epithelial cell lines exposed to bacterial pathogens. This finding suggests a potential indirect link between the *MPPED2* gene and susceptibility to tooth decay[5].
The enzymes methionine synthase reductase (MTRR) and MTR play a crucial role in the metabolic pathways involving folic acid, homocysteine, and vitamin B12[6]. Previous studies have reported a relationship

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between vitamin B12 levels and the onset of DC[7]. Furthermore, cases with reduced concentrations of folic acid have been found to be more prone to caries development[8]. These findings collectively illustrate the prominent role of MTRR and MTR in the context of DC. It is known that gene polymorphisms can mediate the expression of MTRR and MTR, suggesting the potential involvement of *MTRR* and *MTR* gene polymorphisms in susceptibility to DC[9].

The ACTN2 gene is responsible for the production of the alpha-actinin-2 protein, a cytoskeletal protein involved in the organization and stabilization of actin filaments in muscle cells[10]. Interestingly, ACTN2 is also expressed in odontoblasts, the cells that synthesize and secrete dentin, which constitutes the majority of the tooth structure beneath the enamel[11]. *ACTN2* polymorphisms have been linked to various muscle-related disorders, such as hypertrophic cardiomyopathy and skeletal muscle myopathies[12,13]. However, the role of ACTN2 in susceptibility to DC is not well-established, and the mechanisms by which ACTN2 polymorphisms might affect tooth structure or function are not fully understood[14]. It has been hypothesized that variants within ACTN2 may 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[14]. Nevertheless, 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[15,16].

The LPO gene codes for a bactericidal salivary enzyme that plays a crucial role in protecting the lactating mammary gland and the intestinal tract of newborn infants against pathogenic microorganisms[17,18]. In addition to its antimicrobial function, LPO has been reported to have other functions, such as growth-promotion activity and anti-tumor activity[19,20]. Interestingly, a genome-wide association study (GWAS) focused on DC in children aged 3 to 12 years, examining their primary dentition, identified several novel genes, including LPO[21]. However, it is important to note that GWAS is a hypothesis-generating method, and the results require careful scrutiny and replication in independent samples to distinguish chance results from true associations.

To further elucidate the potential role of the *MPPED2*, *MTR*, *ACTN2*, and *LPO* genes in susceptibility to DC, additional studies are needed to investigate their functional effects on tooth development and to replicate the associations of their polymorphisms in different populations[22]. Such investigations could provide valuable insights into the genetic and molecular mechanisms underlying DC, which could inform the development of targeted prevention and treatment strategies for this common oral disease. In the present study, we aimed to investigate the potential association of specific single nucleotide polymorphisms (SNPs) within the *MPPED2* (rs12797813), *MTR* (rs61739582), *ACTN2* (rs6656267), and *LPO* (rs7209537) genes with susceptibility to DC in a population-based sample. Our findings could offer important insights into the genetic basis of DC and contribute to the development of personalized prevention and treatment approaches for this widespread oral health condition.

METHODS

Subjects

We carefully calculated the necessary sample size for the investigation using the statistical formula: $n = Z^2p(1\text{-}p)/e^2$, where the value of Z was set at 1.96, and the estimated frequency was 60%. Based on these parameters, the researchers determined that the study would need to include 300 participants. To ensure a robust comparison, the study design incorporated a control group consisting of age-matched individuals who did not have dental caries (DC). Individuals who did not consent to participate were excluded from the study. In total, the final study sample comprised 600 individuals - 300 participants diagnosed with DC and 300 healthy controls who shared similar demographic characteristics. A dentist conducted a comprehensive oral examination of all the study participants. The DC (cavities) experience in the participants' teeth was assessed using the dmfs index, which measures the number of tooth surfaces that are decayed, missing due to decay, or filled/restored. Importantly, the study was conducted in full compliance with the ethical principles outlined in the Declaration of Helsinki. Each participant provided written informed consent, and 5 ml of peripheral blood was collected in EDTA-containing tubes for further analysis as part of the research protocol.

SNPs Genotyping

The genetic samples used in this study were obtained by extracting genomic DNA from EDTA-treated blood samples through the standard salting-out method. The extracted DNA was then stored at -20°C until the genetic analysis was conducted. To genotype the specified SNPs (rs12797813, rs61739582, rs6656267, and rs7209537), the tetra-primer amplification refractory mutation system PCR (Tetra-ARMS-PCR) approach was employed. The primer

Table 1: The primer sequences and Product size

Gene (SNP)	Primers	Sequence (3'-5')	Product size
ACTN2 (rs114880747)	Forward inner primer (G allele)	AGGTTTGCTATTTGTAAAAAATTTCATG	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')	TTAAAAGGGACAAATGTGCTCAGGGCA'	Two outer primers: 340
	Reverse outer primer (5' - 3')	TCTTACCTGCCCAGTGCCTTGTCTTTTC	

Table 2: Association of ACTN2 (rs114880747) and LPO (rs8178275 genotypes with decayed missing filled teeth score (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	-	-	-	-	< 0.05
	AG	65	40	15	10	4	2	1	
	GG	113	15	2	4	3	1	1	
LPO (rs8178275)	AA	20	19	5	3	3	1	1	< 0.05
	AG	49	29	12	9	2	2	1	
	GG	121	17	2	2	2	-	-	

design for this method was facilitated by the Primer 1 online tool available at http://primer1.soton.ac.uk/primer1.html. The accuracy of the genotyping process was ensured by including negative control samples without genomic DNA and positive controls with known genotypes, which were compared to the corresponding sequencing results. The thermal cycling conditions for the PCR amplification of the individual SNPs were as follows:

- ***MPPED2* SNP rs12797813**: Initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 62.5°C for 30 seconds, and extension at 72°C for 2 minutes, with a final extension step at 72°C for 5 minutes.
- ***MTR* SNP rs61739582**: Initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 30 seconds, and extension at 72°C for 2 minutes, with a final extension step at 72°C for 5 minutes.
- ***ACTN2* SNP rs6656267**: Initial denaturation 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, with a final extension at 72°C for 5 minutes.
- ***LPO* SNP rs7209537**: Initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 61.5°C for 30 seconds, and extension at 72°C for 2 minutes, with a final extension at 72°C for 5 minutes.

For each PCR reaction, the following components were used: 1 μl of each inner primer (10 PM), 1 μl of each outer primer (5 PM), 1 μl of Mastermix (Amplicon® Mastermix containing $MgCl_2$, Taq PCR buffer, Taq DNA polymerase, and dNTPs), and 2 μl of DNA (50 ng/μl), adjusted to a total volume of 20

μl with ddH_2O. The primer sequences are provided in **Table 1**.

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 over-dominant). 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.

RESULTS

Our study enrolled 300 patients with DC and 300 healthy individuals, with the patients having a mean age of 17 years and the controls having a mean age of 20.5 years. The case group consisted of 98 males (32.7%) and 202 females (67.3%), while the control group comprised 115 males (38.3%) and 185 females (61.7%). **Table 2** presents the responses to questionnaires that children and their parents completed. The severity of DC was assessed by calculating the *DMFT* score, which quantified the presence of decayed, missing, and filled teeth for each individual participant. We found an association between the rs6656267 SNP in the *ACTN2* gene and the *DMFT* score in DC cases (p = 0.025), as presented in Table 2. Additionally, our findings, shown in **Table 3**, demonstrate an association between the rs7209537 SNP in the *LPO* gene and the *DMFT* score in DC cases (p = 0.001).

In accordance with the Hardy-Weinberg equilibrium, the genotype distributions of the analyzed SNPs were consistent with expectations (p > 0.05). The results of the exact tests conducted for the various SNPs are as follows: for rs12797813, the p-values were 0.10 for cases and 0.17 for controls; for rs61739582, the p-values were 0.08 for cases and 0.13 for controls; for rs6656267, the p-values were 0.11 for cases and 0.18 for controls; and for rs7209537, the p-values were 0.08 for cases and 0.16 for controls, as presented in **Table 4**. The DNA band positions on the agarose gel are depicted in **Figure 1**.

A statistically significant increase in the frequency of the C allele (minor allele) for rs6656267 was observed in individuals with DC compared to healthy controls (OR (95% CI) = 0.596 (0.432-0.811), p = 0.013; as shown in **Table 4**). This SNP was associated with the risk of DC in both co-dominant and dominant models (OR (95% CI) = 0.58 (0.38-0.86), p-value = 0.03; OR (95% CI) = 0.55 (0.37-0.81), p-value = 0.029, respectively). Additionally, the frequency of the A allele (minor allele) for rs7209537 was found to be significantly higher in individuals with DC compared to healthy controls, with an odds ratio (95% confidence interval) of 0.543 (0.411-0.809) and a p-value of 0.045. These particular SNPs were linked to an increased risk of DC in both co-dominant and dominant genetic models, with odds ratios (95% confidence intervals) of 1.71 (1.02-2.86) and 2.06 (1.08-4.38) and p-values of 0.014 and 0.019, respectively. It is also worth mentioning that there was no association between rs12797813 and rs61739582 and susceptibility to DC in any of the models (p-value > 0.05).

DISCUSSION

Dental caries (DC) is influenced by both external environmental factors and genetic predisposition[23]. High-sugar diets, particularly those rich in candies and chocolates, are a common cause of DC[24]. Additionally, inadequate oral hygiene practices, insufficient consumption of fluoride, and prolonged bottle-feeding are other contributing factors to the onset of DC[25]. Implementing preventive measures early on can help mitigate the occurrence of DC at an early stage. Numerous studies have explored the genetic predisposition to tooth decay, and various genes have been identified that may contribute to the development of this condition[26–29]. The genetic susceptibility to tooth decay 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 for the creation of novel preventive and therapeutic approaches. The current study aimed to enhance the understanding of the genetic foundation of caries susceptibility. To achieve this, samples were collected from a population in Iran, both with and without DC, and genotyped for specific polymorphisms (rs12797813 SNP of the *MPPED2* gene, SNP rs61739582 of the *MTR* gene, rs6656267 SNP of the *ACTN2* gene, and rs7209537 SNP of the *LPO* gene). These genes were identified as candidate genes in previous genome-wide association studies (GWAS) for DC[15,16]. The current study found a positive association between the rs6656267 and rs7209537 polymorphisms and susceptibility to DC. This observation aligns with the findings from a previous study conducted on a cohort of 65 Greek children aged 5 to 12 years, which showed a positive association between the *ACTN2* (rs6656267) gene polymorphism and DC[16]. Furthermore, another GWAS has provided significant evidence of an association between

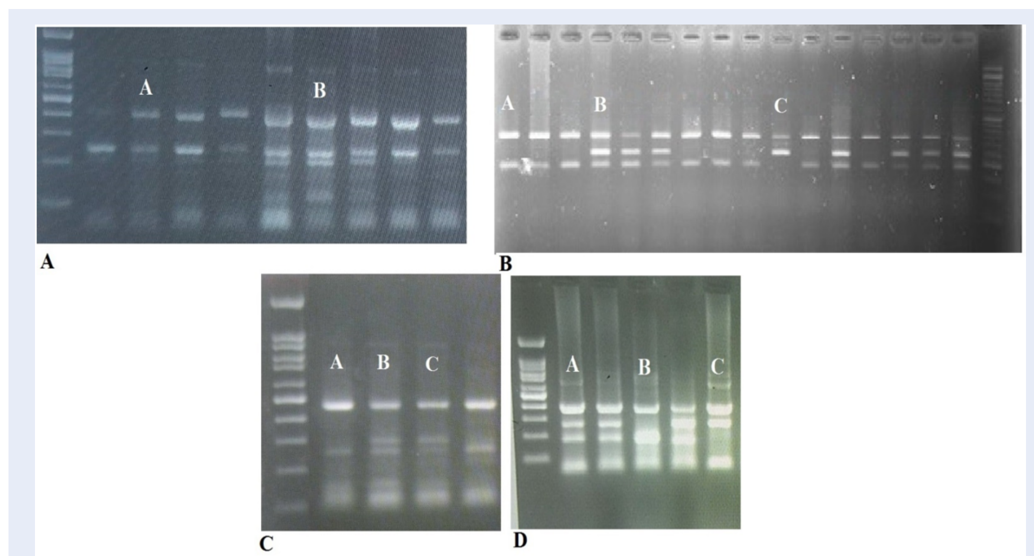

Figure 1: **Gel electrophoresis of the T-ARMS PCR products from of *ACTN2* (rs6656267), LPO (rs7209537), *MPPED2* (rs12797813) and *MTR* gene (rs61739582) genes on 2.5% agarose gel electrophoresis**. (**A**) ACTN2 gene (rs6656267) (Ladder 100bp); Lane A: AA genotype (271 and 415 bp); Lane B: GG genotype (204, 271 and 415 bp), (**B**) LPO gene (rs7209537) (Ladder: 50bp); Lane A: GG genotype (187 and 377 bp); Lane B: AG genotype (187, 245 and 377 bp), Lane C: AG genotype (187, 245 and 377 bp), (**C**) MPPED2 gene (rs12797813) (Ladder 100bp); Lane A: GG genotype (240 and 477 bp); Lane B: AG genotype (240, 293 and 477 bp), and (**D**) MTR gene (rs61739582) (Ladder 100bp); Lane A: AG genotype (190. 258 and 394 bp); Lane B: GG genotype (190 and 394 bp), Lane C: AA genotype (258 and 394 bp).

Table 3: Exact test for Hardy-Weinberg equilibrium

		Patients	Healthy controls	P-value
rs114880747	A/A	24	19	> 0.05
	A/G	137	120	
	G/G	139	161	
rs8178275	A/A	52	30	> 0.05
	A/G	104	124	
	G/G	144	146	

ACTN2 single nucleotide polymorphisms (SNPs) and DC [15]. While the precise mechanism linking *ACTN2* to DC remains incompletely understood, one possible explanation is that the *ACTN2* gene appears to be involved in regulating and organizing ameloblasts, the cells responsible for tooth enamel formation [30,31]. The *ACTN2* gene is located on chromosome 1 (1q43) and encodes a protein that is a member of the spectrin gene superfamily and is involved in the formation of the cytoskeleton. *ACTN2* has been associated with several disorders, including cardiomyopathy [30,31]. The LPO protein plays a crucial role in defending against DC in the context of oral health [32]. It also helps regulate the microbial composition in the oral cavity, preventing the growth of pathogenic microorganisms associated with periodontitis [33]. The physiological properties of LPO have been exploited in the development of oral hygiene products enriched with the LPO protein to prevent these oral diseases [34]. Salivary LPO has the ability to convert thiocyanate ions (SCN-) and hydrogen peroxide into hypothiocyanite ions (OSCN-). These OSCN- ions possess the capacity to kill bacteria by binding to their proteins. As a result, the presence of LPO in saliva promotes oral health and helps prevent the development of periodontitis [35]. The available research indicates that the concentration of LPO in the saliva is higher in patients with periodontitis compared to

Table 4: Distribution of allele and genotype frequencies of SNPs in patients and healthy controls

Model			DC patients Number (%)	Controls Number (%)	OR (95% CI)	P-value
rs114880747	Allele	A vs. G	185 (30.8%) 415 (69.2%)	158 (26.3%) 442 (73.7%)	0.596 (0.432-0.811)	0.005
	Co-dominant	A/A	24 (8%)	19 (6.3%)	1.00	0.022
		A/G	137 (45.7%)	120 (40%)	0.58 (0.38-0.86)	
		G/G	139 (46.3%)	161 (53.7%)	0.50 (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%)	0.55 (0.37-0.81)	
	Recessive	A/A-A/G	161 (53.7%)	139 (46.3%)	1.00	0.16
		G/G	139 (46.3%)	161 (53.7%)	0.59 (0.28-1.26)	
	Overdominant	A/A-G/G	163 (54.3%)	180 (60%)	1.00	0.059
		A/G	137 (45.7%)	120 (40%)	0.72 (0.51-1.01)	
rs8178275	Allele	A vs. G	208 (34.7%) 392(65.3%)	184 (30.7%) 416(69.3%)	0.543 (0.411-0.809)	0.013
	Co-dominant	A/A	52 (17.3%)	30 (10%)	1.00	0.014
		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	0.035
		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 (0.76-4.62)	
	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 (0.91-2.30)	

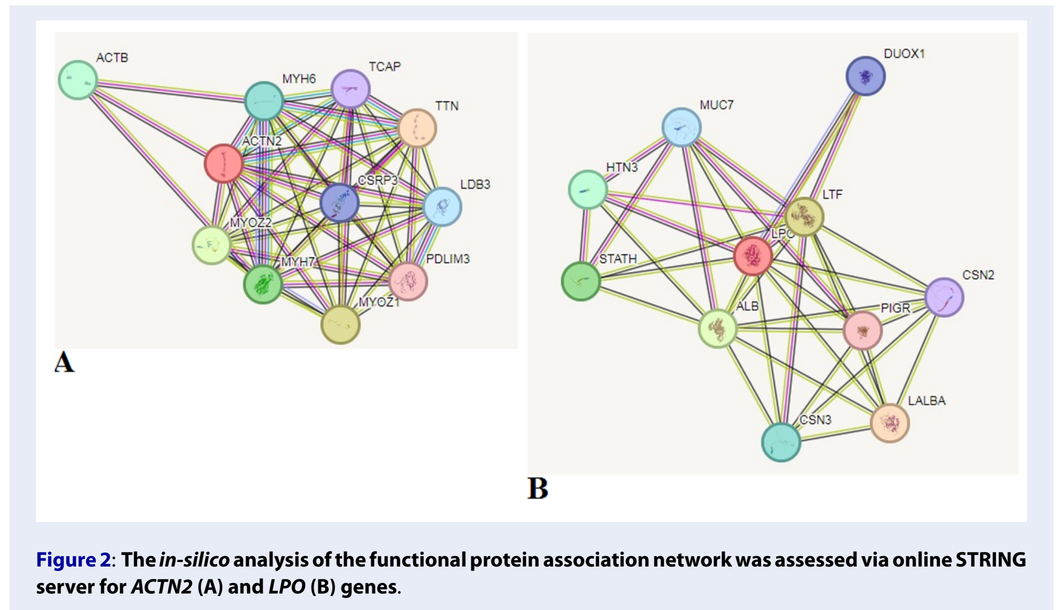

Figure 2: **The *in-silico* analysis of the functional protein association network was assessed via online STRING server for *ACTN2* (A) and *LPO* (B) genes**.

healthy individuals[36–38]. As early as 1995, the LPO concentration was proposed as a potential marker to evaluate the degree of periodontal tissue destruction[39]. Subsequently, later studies have shifted their focus to evaluating the product of LPO activity, malondialdehyde (MDA), which is a lipid peroxidation biomarker[40]. Dakovic's study found a relationship between the concentration of MDA in the saliva and the degree of inflammation in the periodontium, concluding that the levels of this marker in saliva indicate the presence of inflammatory activity at that particular time[41]. Other researchers have corroborated these findings[42,43], and the most recent study by Veljovic *et al.* in 2022 obtained statistically significant results demonstrating a positive association between periodontitis and the levels of MDA and LPO in both blood and saliva[44]. In contrast, the current study has found that the rs7209537 SNP of the *LPO* gene is significantly associated with susceptibility to DC. This observation is not in alignment with the findings of Stanley *et al.*, who, through a meta-analysis, reported that SNPs within the *LPO* gene are not associated with DC susceptibility[15]. These discrepancies in results may be attributable to genetic differences between the study populations. Given the diverse functions of *ACTN2* and *LPO* genes, it is plausible that complex interactions between them could modulate an individual's risk of developing DC. Exploring these potential gene-gene interactions may provide valuable insights into the multifactorial nature of DC susceptibility.

It is also worth mentioning that our results have shown that there is no association of polymorphisms within the *MTR* (rs61739582) and *MPPED2* (rs12797813) genes with DC in studied participants. The *MTR* gene, responsible for methionine and homocysteine production, has not been previously implicated in DC, although maternal MTR may be associated with non-syndromic cleft lip and palate[45], which in turn is associated with DC[46–48]. The findings from the current study are notable in the context of previous research. Unlike our study population, Weilian Du *et al.*[49] have shown that the risk of DC is associated with the *MTR* gene rs1805087 polymorphism in Chinese children. Notably, Antunes *et al.*[50] reported that the *MTRR* gene rs1801394 polymorphism was identified as a genetic risk factor for early childhood caries (ECC) susceptibility in Rio de Janeiro, while the *MTR* gene rs1805087 polymorphism was not associated with ECC. This is consistent with the results of the present study. The reason for this discrepancy is not entirely clear, but the researchers believe that the *MTR* gene rs1805087 polymorphism is worthy of further examination. Additionally, in line with the current study, Katifelis *et al.*[16]have shown that polymorphisms within the *MPPED2* gene are not associated with the risk of DC. However, another study has revealed that *MPPED2* polymorphisms are significantly associated with childhood caries. These mixed findings highlight the complexity of the genetic factors underlying susceptibility to oral diseases, such as DC. The discrepancies between studies may be attributable to factors like differences in study populations, methodologies, and genetic backgrounds.

DC is a multifactorial disease, and the interplay between various genetic factors may significantly influence an individual's risk of developing the condition. By focusing solely on the selected SNPs, our study may have overlooked the potential synergistic or antagonistic effects of genetic variants across different genes. The findings from our study, which identified significant associations between the rs6656267 and rs7209537 variants and susceptibility to DC, have the potential to contribute to the evolving field of personalized dentistry and public health interventions. The identification of genetic risk factors for DC can have significant public health implications. DC is a highly prevalent and chronic oral health condition, presenting a substantial burden on healthcare systems globally. By understanding the genetic underpinnings of DC susceptibility, we can explore strategies for early identification, targeted prevention, and personalized management of this condition. In addition, the incorporation of genetic screening for DC risk factors into routine dental care practices could provide valuable insights for both clinicians and patients. By assessing an individual's genetic profile, healthcare providers can gain a better understanding of their predisposition to developing DC. This information can then be used to tailor preventive strategies, optimize oral hygiene regimens, and monitor high-risk individuals more closely. Building on the genetic risk profiles obtained through screening, healthcare providers can develop personalized preventive measures and treatment approaches for patients.

Genetic variations can differ significantly among populations due to factors like ancestry, geographical location, and ethnic diversity. These population-specific genetic differences can lead to discrepancies in the results of association studies conducted across diverse populations. It is crucial to account for these population-specific genetic factors when interpreting the findings of such studies. Furthermore, the extensive review of the relevant literature conducted as part of this study makes a significant contribution to the field of DC research. This comprehensive review of existing knowledge provides a valuable foundation for understanding the complex genetic underpinnings of this widespread oral health condition. Continued research is necessary to fully elucidate the genetic determinants of oral health conditions and their potential utility as diagnostic or prognostic markers.

CONCLUSIONS

The present study investigated the potential association between specific genetic variants within the *MPPED2* (rs12797813), *MTR* (rs61739582), *ACTN2* (rs6656267), and *LPO* (rs7209537) genes and susceptibility to DC. Using the reliable T-ARMS-PCR method for genotyping, the researchers found a significant association between the rs6656267 single nucleotide polymorphism (SNP) in the ACTN2 gene and the rs7209537 SNP in the *LPO* gene with an increased risk of developing DC. These findings suggest that the rs6656267 variant of the ACTN2 gene and the rs7209537 variant of the *LPO* gene may serve as potential genetic risk factors for DC. The observed associations were noted in both the co-dominant and dominant genetic models, further strengthening the relevance of these gene variants in DC susceptibility. While the study provides valuable insights into the potential role of the rs6656267 and rs7209537 variants in DC, the researchers acknowledge the need for additional investigations to confirm and expand upon these findings. Further studies with larger sample sizes, diverse population cohorts, and functional analyses are warranted to validate the associations and elucidate the underlying mechanisms by which these gene variants influence DC susceptibility. Unraveling the genetic basis of DC can have significant implications for disease prevention, early detection, and personalized treatment approaches. By understanding the genetic determinants of DC, researchers and clinicians can potentially develop targeted interventions and strategies to mitigate the impact of this prevalent oral health condition. In conclusion, the present study presents evidence supporting the association of the rs6656267 variant within the *ACTN2* gene and the rs7209537 variant within the *LPO* gene with increased susceptibility to DC. These findings contribute to the growing body of knowledge regarding the genetic factors underlying this widespread oral disease and highlight the importance of continued research in this field. In future studies, it will be crucial to investigate the interactions between the *ACTN2, LPO, MPPED2*, and MTR genes, as well as other relevant genes, while performing functional studies to better understand the complex genetic landscape underlying DC susceptibility.

ABBREVIATIONS

ACTN2 - Alpha-actinin-2, **CI** - Confidence Interval, **DC** - Dental Caries, **DMFT** - Decayed, Missing, and Filled Teeth, **ECC** - Early Childhood Caries, **EDTA** - Ethylenediaminetetraacetic acid, **GWAS** - Genome-Wide Association Study, **HWE** - Hardy-Weinberg Equilibrium, **LPO** - Lactoperoxidase, **MDA** - Malondialdehyde, **MPPED2** - Metallophosphoesterase domain-containing 2, **MTR** - Methionine synthase, **MTRR** - Methionine Synthase Reductase, **OR** - Odds

Ratio, **PCR** - Polymerase Chain Reaction, **SNPs** - Single Nucleotide Polymorphisms, **Tetra-ARMS-PCR** - Tetra-Primer Amplification Refractory Mutation System PCR

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AUTHOR'S CONTRIBUTIONS

SA: designed the research study and performed the research, SD: wrote the manuscript and performed the research, YN: wrote the manuscript and performed the research, SK: wrote the manuscript and performed the data analysis, AF: wrote the manuscript and collaboration in data analysis, MRA: performed the research, BB: performed the research, FA: designed the research study and performed the research and data analysis. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in adherence to the ethical principles outlined in the Declaration of Helsinki and approved by ethical committee of Mashhad University of Medical Sciences.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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