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ORIGINAL

Evaluation of EDARADD (rs79233817) Gene Polymorphism in Children with Dental Caries Compared to Caries-Free Controls

Evaluación del polimorfismo del gen EDARADD (rs79233817) en niños con caries dental comparados con controles libres de caries

MohammadHassan Lotfalizadeh[1], Forough Taheri[2], Sepehr Kobravi[3], Aida Jafari[4], Zahra Pirmoradi[5], Kasra Nazari[5], Mehri Safari[6], Atefe Ali Moradi[7], Akram Goharifar[8], Farzaneh lal Alizadeh[9]

1. North Khorasan University of Medical Sciences, Bojnurd, Iran. 2. Metabolic Disorder Research Centre, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran. 3. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tehran Azad University, Tehran, Iran. 4. Faculty of Dentistry (Khorasgan), Isfahan Azad University, Isfahan, Iran. 5. School of International Education, Faculty of Stomatology, Zhengzhou University, Zhengzhou, China. 6. Operative Dentistry Specialist, Department of Operative Dentistry, Dental School, Islamic Azad University of Isfahan branch, Isfahan, Iran. 7. Tabriz University of Medical Sciences, Faculty of Medicine, Tabriz, Iran 8. Department of Prosthodontics, School of Dentistry, Islamic Azad University, Isfahan Branch (Khorasgan), Isfahan, Iran. 9. Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Corresponding author
Farzaneh lal Alizadeh
E-mail: alizadehfarzaneh13@gmail.com

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Abstract

Objective: Dental caries is a complex condition that results from a combination of genetic and environmental factors. The EDARADD gene has been found to play a role in the teeth development and has been associated with various dental traits. In this study, our objective was to examine the potential correlation between the EDARADD gene polymorphism (rs79233817) and susceptibility to dental carie.
Methods: The study included a total of 400 participants, comprising 200 individuals with dental caries and 200 caries-free controls. The genotyping of the EDARADD gene polymorphism was performed using the tetra-primer amplification refractory mutation system PCR metho.
Results: Individuals with dental caries were found to have a meaningfully higher frequency of the A allele (minor allele) for rs79233817 compared to controls. The odds ratio (95% confidence interval) was determined to be 0.745 (0.624-0.751), with a p-value of 0.002. The specific SNP (rs79233817) was associated with an elevated risk of dental caries (DC) in both the co-dominant and dominant genetic models. The odds ratios were calculated as 0.45 (0.27-0.75) and 0.41 (0.26-0.72) for the respective models, with corresponding p-values of 0.012 and 0.02.
Conclusions: It can be inferred that the EDARADD gene polymorphism (rs79233817) potentially plays 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.

Key words: Dental caries, EDARADD gene, Genetics, Diagnosis and treatment planning.

Resumen

Objetivo: La caries dental es una enfermedad compleja que resulta de una combinación de factores genéticos y ambientales. Se ha descubierto que el gen EDARADD desempeña un papel en el desarrollo de los dientes y se ha asociado a diversos rasgos dentales. En este estudio, nuestro objetivo fue examinar la posible correlación entre el polimorfismo del gen EDARADD (rs79233817) y la susceptibilidad a la caries dental.
Métodos: El estudio incluyó un total de 400 participantes, de los cuales 200 eran individuos con caries dental y 200 controles libres de caries. El genotipado del polimorfismo del gen EDARADD se realizó mediante el método PCR del sistema de mutación refractaria por amplificación de tetraprimer.
Resultados: Se observó que los individuos con caries dental presentaban una frecuencia significativamente mayor del alelo A (alelo menor) para rs79233817 en comparación con los controles. Se determinó que la odds ratio (intervalo de confianza del 95%) era de 0,745 (0,624-0,751), con un valor p de 0,002. El SNP específico (rs79233817) se asoció con un riesgo elevado de caries dental (DC) tanto en el modelo genético codominante como en el dominante. Las odds ratio se calcularon en 0,45 (0,27-0,75) y 0,41 (0,26-0,72) para los respectivos modelos, con valores p correspondientes de 0,012 y 0,02.
Conclusiones: Se puede inferir que el polimorfismo del gen EDARADD (rs79233817) desempeña potencialmente un papel en la susceptibilidad genética a la caries dental. Para validar y profundizar en estos hallazgos, es necesario realizar estudios adicionales con muestras de mayor tamaño en poblaciones diversas. Esto ayudará a establecer la solidez de los resultados y a investigar más a fondo los mecanismos subyacentes implicados.

Palabras clave: Caries dental, Gen EDARADD, Genética, Diagnóstico y planificación del tratamiento.

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Introduction

Ensuring proper oral health is vital for the overall well-being and development of children[1]. Tooth decay, also known as dental caries, is a chronic condition that affects the tooth enamel[2] and is prevalent worldwide[2,3]. Among children, dental caries (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]. The development of caries is strongly influenced by a combination of genetic and environmental factors, with their interplay affecting the disease's progression. Identifying these factors and understanding their mechanisms is crucial to fully comprehend the nature of caries[8,9]. Several environmental factors can contribute to DC in children, including oral bacteria[10,11], dietary habits such as high sugar intake[12], oral health behavior like using fluoridated toothpaste and regular brushing[13], feeding practices including breastfeeding and night bottle-feeding[13,14], geographic location[15], and socioeconomic status such as income, education, and social class. Additionally, in the past century, scientists have investigated the impact of hereditary factors on caries risk through various studies. 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[16,17]. Furthermore, the human genome project has facilitated the identification of specific genes located on chromosomes that contribute to an increased susceptibility to caries[18,19]. Recent meta-analyses[20-22] have additionally demonstrated an association between polymorphisms and an elevated 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 recruit Traf members and activate NF-κB[23]. On the other hand, the DD helps EDARADD self-associate and interact with EDAR[23,24]. 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.

The current evidence on the association between SNPs within EDARADD gene and DC susceptibility is limited. Additional studies are necessary to explore and investigate the functional effects of EDARADD gene on tooth development and to replicate the association between polymorphisms and DC in diverse populations 25. Such studies can help uncover the molecular and genetic mechanisms underlying DC and inform the development of targeted prevention and treatment strategies for this widespread oral disease. The objective of this study is to explore the possible association between rs79233817 and the susceptibility to DC in a population-based sample. The findings from our study have the potential to provide insights into the genetic basis of DC. In addition, these findings may contribute to the development of personalized prevention strategies for DC and treatment approaches for this prevalent oral condition.

Material and methods

Patients

To determine the sample size, the researchers used the formula $n = Z^2 p(1-p)/e^2$, where Z was set to 1.96 and the prevalence was assumed to be 60%. According to the parameters, the sample size was determined to be 300 patients. The study included a control group consisting of age-matched individuals who did not have DC. Non-consenting individuals were not included in the study and were excluded from the analysis. A total of 400 individuals, including 200 individuals with a definite clinical diagnosis of DC and 200 healthy controls with similar demographic characteristics, were included in the study. The study was conducted following the guidelines set forth in the Declaration of Helsinki, and it received approval from the relevant ethics committee. Written informed consent was obtained from all participants, and 5 ml of peripheral blood was collected in EDTA-containing tubes.

DNA Genotyping

The standard salting-out method was employed to extract genomic DNA from the blood samples collected in EDTA anti-coagulated tubes. SNP rs79233817 genotyping, with primers designed using the Primer 1 online tool (http://primer1.soton.ac.uk/primer1.html), was performed using Tetra-ARMS-PCR method. **Table I** displays the primer sequences used in this study. Negative control samples lacking genomic DNA and positive controls with

Table I: The primer sequences and Product size.

Primers	Sequence	Product size
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	

known genotypes were included to ensure genotyping accuracy. These control samples were subsequently compared to the sequencing results. The amplification temperature protocol 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. Additionally, for each PCR reaction, 1.5µl of each inner primer (10 PM) and 1.5µl of each outer primer (5PM), 2µl of Mastermix (amplicon® Mastermix containing MgCl2, Taq PCR buffer, Taq DNA polymerase, and dNTPs), and 3µl of DNA (50ng/ul) adjusted to 20 µl with ddH2O were used.

Statistical Analysis

The selected SNP underwent a test for Hardy-Weinberg equilibrium (HWE). Using SNPStats (https://www.snpstats.net/start.htm), associations between rs79233817 and DC were examined under various models, including co-dominant, dominant, recessive, and over dominant. The odds ratio (OR) and its corresponding 95% confidence interval were used to determine the effect size of each variant. A significance level of 0.05 or lower was considered statistically significant.

Results

The study involved 200 patients with DC and 200 healthy controls, with the former having a mean age of 15 years and the latter of 17 years. The case group included 53 males (26.5%) and 147 females (73.5%), while the control group comprised 65 males (32.5%) and 135 females (67.5%). The severity of DC was evaluated using the DMFT score, which tallies the number of decayed, missing, and filled teeth in each participant. Our findings, presented in **table II**, demonstrate a correlation between the rs79233817 SNP in the EDARADD gene and DMFT score in DC cases (p=0.015).

Accordance with Hardy-Weinberg equilibrium

The distribution of genotypes for the tested SNP was found to be consistent with Hardy-Weinberg equilibrium ($p > 0.05$). In cases and controls, the exact test for rs79233817 yielded P-values of 0.13 and 0.10, respectively (**Table III**).

Concordance with sequencing results

To verify the precision of T-ARMS genotyping, we selected a few samples that were previously genotyped and subjected them to genetic sequencing analysis. As depicted in **figure 1**, the Gel electrophoresis of the Tetra-ARMS PCR products from of EDARADD (rs79233817) gene on 2.5% agarose has been shown.

Case-control study

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 0.745 (0.624-0.751) and a p-value of 0.002 (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 0.45 (0.27-0.75) and 0.41 (0.26-0.72) for the respective models, with corresponding p-values of 0.012 and 0.02.

Table II: The correlation between the EDARADD (rs79233817) genotype and the decayed missing filled teeth (DMFT) score in DC cases.

Genotype in	Decayed Missing Filled Teeth Score (DMFT score)							p-value
DMFT Score n=200	1 DMFT 130 (65%)	2 DMFT 40(20%)	3 DMFT 15 (7.5%)	4 DMFT 8(4%)	5 DMFT 4(2%)	6 DMFT 2(1%)	7 DMFT 1(0.5%)	<0.05
AA	89	25	9	6	3	2	-	
AG	30	11	4	-	-	-	-	
GG	11	4	2	2	1	-	1	

Table III: Exact test for Hardy-Weinberg equilibrium.

SNP	rs79233817			P-value
	A/A	A/G	G/G	
Patients	134	45	21	>0.05
Healthy controls	111	54	35	>0.05

Table IV: The frequencies of allele and genotype distributions of SNPs in both patients and healthy controls.

SNP	Model		DC patients Number (%)	Controls Number (%)	OR (95% CI)	P-value
rs79233817	Allele	A vs. G	313(78.25%) 87(21.75%)	276(69%) 124(31%)	**0.745 (0.624-0.751)**	**0.002**
	Co-dominant	A/A A/G G/G	134 (67%) 45 (22.5%) 21 (10.5%)	111 (55.5%) 54 (27%) 35 (17.5%)	1.00 **0.45 (0.27-0.75)** 1.00	**0.012**
	Dominant	A/A A/G-G/G	134 (67%) 66 (33%)	111 (55.5%) 89 (44.5%)	0.48 (0.26-1.25) **0.41 (0.26-0.72)**	**0.02**
	Recessive	A/A-A/G G/G	179 (89.5%) 21 (10.5%)	165 (82.5%) 35 (17.5%)	0.53 (0.45-1.15) 0.42 (0.25-1.06)	0.16
	Overdominant	A/A-G/G A/G	155 (77.5%) 45 (22.5%)	146 (73%) 54 (27%)	1.00 0.49 (0.55-1.03)	0.059

Figure 1: Gel electrophoresis of the T-ARMS PCR products from of EDARADD (rs79233817) gene on 2.5% agarose gel electrophoresis. Lane A: AG genotype (306, 207 and 156 bp); Lanes B, C and D: AA genotype (306 and 207 bp).

Discussion

Advancements in the field of molecular biology and DNA sequencing techniques have enabled researchers to determine the significance of hereditary factors in DC[26]. Indeed, our current understanding of the precise contribution of genetic factors to the risk of DC remains limited[21]. Previous research investigating the role of genetic factors in DC has predominantly concentrated on four main categories of genes. These include genes associated with enamel development, saliva formation and composition, immune response, and carbohydrate metabolism. These genetic factors have been explored due to their potential influence on the susceptibility to DC[27]. However, recent studies have shown that other genes, which were not previously thought to have an impact on this disease, may also play a role. EDARADD (located on 1q42–q43; MIM# 606603) is the gene responsible for encoding the protein EDAR-associated death domain (EDARADD) and has been reported to associated with DC in a GWAS study[26]. Current study aimed to investigate the association of a SNP (rs79233817) within this EDARADD with DC in Iranian population. The findings of this study have expanded our understanding of the genetic factors involved in the development and progression of DC, including gene-environment interactions. These insights could potentially pave the way for improved early detection, risk assessment, dental treatment, and more effective public health interventions.

Our results have shown that rs79233817 is meaningfully higher in cases compared to controls (P-value: 0.002). Our data is consistent with another study performed by Shaffer et al.,[26] and they have found that SNPs within EDARADD are associated with DC in US children aged 3 to 12 yrs. Our study is important from the point of view that association studies of SNPs with complex diseases should be based on ethnic and population[28], and the present study in the Iranian population confirms the role of EDARADD gene in susceptibility to DC among US patients.

In 2001, Headon and colleagues[29] discovered that the EDARADD and EDAR genes are co-expressed in epithelial cells during the development of hair follicles and teeth. They also found that EDARADD has a self-associating property, which is typical of many death domain proteins. Overexpression of EDARADD in HEK293T cells led to the activation of an NF-kappa-B reporter gene, with activation levels correlated to the dose. The researchers also determined that the activation of EDAR is triggered by EDA, and that EDARADD serves as an adaptor to create an intracellular signal-transducing complex. This linear pathway is responsible for the similar phenotypes observed in Tabby, downless, and crinkled mutant mice, as well as the genetic heterogeneity of hypohidrotic ectodermal dysplasia in humans[29].

The study did not take into account environmental risk factors such as the duration and frequency of tooth brushing, frequency of sugar intake, use of fluoridated toothpaste, and dental flossing. Additionally, other single nucleotide polymorphisms of EDARADD should be assessed to confirm the important role of this gene in susceptibility of DC.

Conclusion

Our study presents evidence that supports an association between the polymorphism (rs79233817) in the EDARADD gene and susceptibility to DC. In our study, we identified a statistically significant difference in the frequency of the minor allele (A allele) between individuals with DC and caries-free controls, with a higher frequency observed in the DC group. The observed association between this specific genetic variant and a higher frequency of DC suggests that this variant may indeed contribute to an increased risk of developing DC. Furthermore, our findings indicate that the rs79233817 polymorphism is associated with DC susceptibility in both co-dominant and dominant genetic models. These results strengthen the significance of this genetic variant in influencing the development of dental caries. Nevertheless, it is important to acknowledge that our findings require further validation and replication through additional research. Larger sample sizes and studies involving diverse populations would help to confirm the association between the EDARADD gene polymorphism and DC susceptibility. Additionally, investigating the underlying mechanisms by which this genetic variant influences DC risk would provide valuable insights into the pathogenesis of the disease. The identification of genetic risk factors, such as the EDARADD gene polymorphism, holds promise for the development of personalized preventive strategies for DC. By understanding the genetic predisposition to DC, healthcare professionals can tailor preventive interventions and treatment approaches to individuals based on their genetic profiles. This approach may lead to more effective and targeted strategies in the prevention and management of DC.

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Conflict of interest

All authors have no conflict of interest to report.

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