Effects of the carbonic anhydrase VI gene polymorphisms on dental caries: A meta-analysis

Wpływ polimorfizmów genu anhidrazy węglanowej VI na próchnicę zębów – metaanaliza

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Abstract

Background. Carbonic anhydrase VI (CA VI) is considered to greatly participate in the buffering of saliva, ion transport, the regulation of pH, secretory processes, and saliva production. Various studies have been conducted to investigate the relationship between CA VI and dental caries.

Objectives. The goal of this study was to make a meta-analysis of studies that examined the effects of the CA VI gene polymorphisms on dental caries.

Material and methods. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guide was followed. Electronic databases (PubMed, Web of Science, Scopus, and Cochrane Library) were scanned by 2 independent researchers. The funnel plot, Egger's regression and Begg and Mazumdar's rank correlation test were used to determine publication bias. Cohen's *d* was used to measure the effect size.

Results. Four studies were included in the meta-analysis; a total of 3 polymorphisms (rs2274327, rs2274328, rs2274333) and a total of 13 polymorphism models were analyzed. According to Egger's regression and the Begg and Mazumdar's test, the meta-analysis had no significant publication bias ($p > 0.05$). The highest susceptibility effect was noticed in the rs2274328 (AA vs CC) model ($d = 0.18$; 95% CI (confidence interval): $-1.77, 2.13$), but this effect was not significant ($p = 0.237$), and the highest protective effect was observed in the rs2274328 (AA vs AC) model ($d = -0.13$, 95% CI: $-1.36, 1.11$), but this effect was not significant, either ($p = 0.195$). No association was found between any of the polymorphism models and dental caries ($p > 0.05$).

Conclusions. Even though CA VI plays an important role in the buffering of saliva, it was shown that polymorphisms in the CA VI gene did not affect the process of dental caries.

Key words: single-nucleotide polymorphism, meta-analysis, caries susceptibility, dental caries resistance, human genome

Słowa kluczowe: polimorfizm pojedynczego nukleotydu, metaanaliza, podatność na próchnicę, odporność na próchnicę zębów, ludzki genom

Introduction

Dental caries is one of the most common chronic diseases in the world and it can be affected by many factors, such as dietary habits, bacterial flora, fluoride intake, tooth position and morphological characteristics, oral hygiene, and amount and composition of saliva.^{1,2} Although there are many environmental factors affecting dental caries, some individuals seem to be more prone to the formation of dental caries. This suggests that genetic factors, in addition to environmental factors, play an important role in the pathogenesis of dental caries.

Many genetic studies in the literature have analyzed the effects of genetic variances, such as single-nucleotide polymorphisms (SNPs), on dental caries. The studies have focused on genes encoding proteins that are related to mineralization, the immune system, taste sensation, and saliva.^{3–6} Saliva is a complex body fluid containing essential protective components; it can regulate pH in the oral cavity due to its buffering capacity.^{7,8} With regard to the functions of saliva, various studies have investigated the effects of gene polymorphisms related to salivary proteins, such as C-type lysozyme, beta-defensin-1, lactotransferrin, carbonic anhydrase VI (CA VI), mucin, and proline-rich proteins (PRPs).⁹

Carbonic anhydrase (CA) is a zinc-containing metallo-enzyme which catalyzes the reversible hydration of carbon dioxide. Especially, CA plays an important role in the regulation of pH, and in the transport of liquids and ions.¹⁰ In mammals, 11 isozymes of CA have been identified. Four are cytosolic isozymes (I, II, III, and VII), 4 are membrane-bound (IV, IX, XII, and XIV), 2 are present in mitochondria (VA and VB), and 1 is a secretory isozyme (VI). Carbonic anhydrase VI is considered to greatly participate in the buffering of saliva, ion transport, the regulation of pH, secretory processes, and saliva production.

To date, according to our knowledge, there has been no meta-analysis to synthesize the effects of the CA VI gene polymorphisms on dental caries. This study aimed to perform a meta-analysis to synthesize the results of studies that investigated these effects.

Material and methods

Guidance and eligibility criteria

In this study, we abided by the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.¹¹ All studies that examined deciduous tooth caries (decayed, missing and filled teeth – DMFT) or permanent tooth caries (dmft) were included in the meta-analysis. In cases when there was no more than 1 study on a polymorphism, the relevant polymorphism was not included in the analysis. Additionally, studies that did not calculate the DMFT/dmft index as a caries index were excluded. Studies that did not present the frequencies

of genotype and phenotype polymorphisms clearly were also excluded. Studies published in English before 2019 were included. Letters to the editor, case reports, commentaries, and reviews were excluded from the meta-analysis.

Information sources and search strategy

Electronic databases (PubMed, Web of Science, Scopus, and Cochrane Library) were scanned by 2 independent researchers. Related Medical Subject Headings (MeSH) terms and text words ('gene' OR 'genome' OR 'genetic' OR 'polymorphism' OR 'carbonic anhydrase' AND 'dental' OR 'tooth' OR 'decay' OR 'caries' OR 'saliva') were used in our search strategy. The reference list of each of the studies obtained was carefully reviewed to access subject-related studies. Additionally, recent articles citing the obtained studies were found.

Study selection and data collection process

The titles and abstracts of the obtained studies were evaluated by 2 different researchers. The same studies retrieved from multiple databases were defined as single studies. Additionally, we contacted the authors to provide their articles in case the full-text access was not allowed. Polymorphisms investigated in no more than 1 study in the literature were excluded from the meta-analysis. Studies to be included in the meta-analysis were determined by a unanimous decision of the researchers.

Publication bias and quality assessment

Egger's regression and Begg and Mazumdar's rank correlation test were used in assessing publication bias. The quality assessment was performed by 2 researchers based on the modified Newcastle–Ottawa Scale (NOS), employed in previous meta-analyses related to polymorphisms.¹² The scores on the scale range from 0 (the worst) to 10 (the best) points. In case the score of a study was ≥ 5 , it was accepted to have a low risk of bias, whereas if the score was < 5 , it was accepted to have a high risk of bias (Table 1).

Summary measures and the synthesis of the results

The David B. Wilson Meta-Essentials software, v. 1.2 (<http://mason.gmu.edu/~dwilsonb/ma.html>) was used for the statistical analysis of the data. Weight calculation was performed separately for each group. Cohen's *d*-value was preferred in measuring the effect size. The DMFT/dmft caries index was used as a variable. The fixed effect model (FEM) with 95% confidence intervals (CIs) was used as the meta-analysis model. Heterogeneity was evaluated with Cochran's *Q* and *I*² test. The significance level of the effect size was determined based on the two-tailed test. In all tests, the level of significance was set at $p < 0.05$.

Table 1. Modified Newcastle–Ottawa Scale (NOS) for the quality assessment

Criteria	Score
Representativeness of cases	
Consecutive/randomly selected cases with a clearly defined sampling frame	2
Not consecutive/randomly selected cases or without a clearly defined sampling frame	1
Not described	0
Source of controls	
Population-based controls	2
Hospital-based controls and/or healthy controls	1
Not described	0
Hardy–Weinberg equilibrium in controls	
Hardy–Weinberg equilibrium	2
Hardy–Weinberg disequilibrium	1
Not available	0
Genotyping examination	
Genotyping done under blinded conditions and repeated again	2
Genotyping done under blinded conditions or repeated again	1
Genotyping done under unblinded conditions or not mentioned, and unrepeated	0
Association assessment	
Assessed the association between genotypes and caries with appropriate statistics and an adjustment for confounders	2
Assessed the association between genotypes and caries with appropriate statistics and without an adjustment for confounders	1
Inappropriate statistics used	0

Results

Study selection and characteristics

The literature review was performed between February 7, 2018 and May 10, 2019. The databases were scanned with the aforementioned queries and 5,112 papers were found (PubMed: 1,337; Web of Science: 1,903; Scopus: 1,826; and Cochrane Library: 46). This number was reduced to 2,372 after eliminating repetitive studies. The abstracts of the studies were examined and 2,244 studies that did not comply with the criteria were excluded. After thoroughly reviewing the full texts of the remaining 117 studies, 113 more studies that did not meet the meta-analysis criteria were excluded, and finally 4 studies were included in the meta-analysis (Fig. 1). The characteristics of the 4 studies published before 2019 are presented in Table 2.

Publication bias and quality assessment

According to Egger's regression and Begg and Mazumdar's test, the studies had no significant bias ($p > 0.05$) (Table 3). After the assessment of the modified NOS, all studies were found to have a low-risk of bias (Table 4).

Fig. 1. Flow diagram of the studies involved in the meta-analysis

Table 2. Features of the studies included in the meta-analysis

No.	Study	Year	Population	Age [years]	Caries index (low; high)	Investigated genes (genetic variations)
1	Peres et al. ²¹	2010	Brazilian 245 children	7–9	dmft/DMFT (0; ≥1)	CA6 (rs2274333, rs2274328, rs2274327)
2	Li et al. ²⁴	2015	Chinese 355 individuals	51.16 ± 9.48 (mean ± SD)	DMFT (≤2; ≥3)	CA6 (rs2274328, rs17032907, rs11576766, rs2274333, rs10864376, rs3765964, rs6680186)
3	Sengul et al. ²²	2016	Turkish 178 children (M = 81, F = 97)	6–16	dmft/DMFT (0; ≥1)	CA6 (rs2274327)
4	Yildiz et al. ²³	2016	Turkish 154 individuals	20–60	DMFT (≤5; ≥14)	CA6 (rs2274327)

M – males; F – females; SD – standard deviation; dmft/DMFT – the decayed, missing and filled teeth index, related to permanent/deciduous tooth caries.

Table 3. Meta-analysis model, the heterogeneity assessment and publication bias

Genetic marker (model)	Meta-analysis model	n	Q	pQ	I ²	τ ²	τ	EG	BG
rs2274327 (CC vs TT)	FEM	3	1.87	0.392	<0.01	<0.01	<0.01	0.341	0.059
rs2274327 (CC vs CT)	FEM	3	2.22	0.330	0.10	<0.01	0.07	0.752	0.301
rs2274327 (C vs T)	FEM	2	0.13	0.718	<0.01	<0.01	<0.01	NC	0.159
rs2274327 (CC vs CT+TT)	FEM	3	2.19	0.335	0.09	<0.01	0.06	0.567	0.301
rs2274327 (CC+CT vs TT)	FEM	3	2.37	0.306	0.16	0.01	0.10	0.543	0.301
rs2274328 (AA vs CC)	FEM	2	0.14	0.712	<0.01	<0.01	<0.01	NC	0.159
rs2274328 (AA vs AC)	FEM	2	<0.01	0.999	<0.01	<0.01	<0.01	NC	0.159
rs2274328 (AA vs AC+CC)	FEM	2	2.50	0.114	0.60	0.03	0.17	NC	0.159
rs2274328 (AA+AC vs CC)	FEM	2	0.10	0.754	<0.01	<0.01	<0.01	NC	0.159
rs2274333 (AA vs GG)	FEM	2	0.15	0.699	<0.01	<0.01	<0.01	NC	0.159
rs2274333 (AA vs AG)	FEM	2	0.45	0.500	<0.01	<0.01	<0.01	NC	0.159
rs2274333 (AA vs AG+GG)	FEM	2	0.19	0.662	<0.01	<0.01	<0.01	NC	0.159
rs2274333 (AA+AG vs GG)	FEM	2	0.42	0.519	<0.01	<0.01	<0.01	NC	0.159

FEM – fixed effect model; n – number of included studies; EG – Egger's regression (*p*-value); BG – Begg and Mazumdar's rank correlation test (*p*-value); NC – not calculated.

Table 4. Quality assessment of the studies with the modified Newcastle–Ottawa Scale (NOS)

No.	Study	Representativeness of cases	Source of controls	Hardy–Weinberg equilibrium in controls	Genotyping examination	Association assessment	Score
1	Peres et al. ²¹	1	2	0	1	2	6
2	Li et al. ²⁴	1	1	2	1	2	7
3	Sengul et al. ²²	1	2	2	1	2	8
4	Yildiz et al. ²³	1	1	0	1	2	5

Results of individual studies and the synthesis of the results

A total of 3 polymorphisms were included in the study and a total of 13 polymorphism models were analyzed. The number of studies in any analysis ranged from 2 to 3. No significant heterogeneity existed in any of the analyses ($p_Q > 0.05$) (Table 3). Therefore, in all analyses, FEM was employed.

In rs2274327, the highest susceptibility effect was noted for the rs2274327 (C vs T) model ($d = 0.08$; 95% CI: –1.03, 1.19) and the protective effect was observed only in the rs2274327 (CC+CT vs TT) model ($d = -0.01$;

95% CI: –0.56, 0.54), but this effect was too low and non-significant ($p = 0.944$). In rs2274328, the highest susceptibility effect was noticed in the rs2274328 (AA vs CC) model ($d = 0.18$; 95% CI: –1.77, 2.13), but this effect was not significant ($p = 0.237$), and the highest protective effect was observed in the rs2274328 (AA vs AC) model ($d = -0.13$; 95% CI: –1.36, 1.11), but this effect was not significant, either ($p = 0.195$). In rs2274333, all of the effects were protective and the highest protective effect was reported for the rs2274333 (AA vs AG) model ($d = -0.10$; 95% CI: –1.34, 1.15), but this effect was also non-significant ($p = 0.330$). No association was found between any of the polymorphism models and dental caries ($p > 0.05$) (Fig. 2).

Fig. 2. Effect sizes of the polymorphisms of the carbonic anhydrase (CA VI) gene compared to the wild-type and forest plot presentation

Discussion

Several measures of the effect size have been recommended for meta-analyses, such as Pearson's correlation coefficient r , Cohen's d or the odds ratio (OR). We preferred Cohen's d in this meta-analysis. While the value of 0.00 indicates no association, (+) indicates that the related polymorphism is associated with susceptibility to dental caries and (–) indicates that the related polymorphism is associated with protection against dental caries. According to Cohen's statistic, $d = 0.2$ (OR ≈ 2.3), $d = 0.5$ (OR ≈ 8.07) and $d = 0.8$ (OR ≈ 28.25) indicate that the effect size is small, medium and large, respectively.¹³ The value of $p_Q < 0.05$ and high I^2 values indicate that the meta-analysis has significant heterogeneity, and when heterogeneity is high in studies, the random effect model (REM) ought to be preferred. However, in this meta-analysis, none of the analyses showed heterogeneity, so we preferred FEM. Furthermore, deciduous and permanent tooth caries was not evaluated in subgroups due to the small numbers of studies examining the same polymorphisms.

Saliva plays an important role in oral homeostasis, and alterations in salivary secretion may cause oral infections and caries. Saliva contains inorganic compounds and various proteins that influence conditions in the oral cavity. The salivary buffering capacity has its share in protecting the tooth surface from caries.⁷ Carbonic anhydrase VI is part of the defense system of saliva, increasing the buffering capacity by catalyzing the reaction of carbon dioxide.¹⁴ In the literature, various studies have investigated the effect of CA VI on dental caries. Öztürk et al. performed a study on young adults, and they found no association between dental caries and CA VI concentration.¹⁵ However, Kivelä et al., Frassetto et al. and Szabó reported that there was an association between low CA VI concentration and a higher caries index.^{16–18} Esberg et al. performed a study on Swedish adolescents to determine the relationship between dental caries and variations in the CA VI gene.¹⁹ In their study, it was found that the CA VI gene polymorphisms, such as rs10864376 (T), rs3737665 (T) and rs12138897 (G), and the TTG haploblock of CA VI are associated with *Streptococcus mutans* colonization, overall microbiota composition and dental caries.¹⁹

In the literature to date, only 5 studies have examined the relationship between the CA VI exon 2 polymorphisms and dental caries. In the study of Yarat et al., which was performed on 44 Turkish subjects, no significant negative correlation was obtained between DMFT and CA activity, and no correlation was found between SNPs and the salivary buffering capacity.²⁰ Similarly, Peres et al. investigated 3 different polymorphisms (rs2274333 A>G, rs2274328 A>C and rs2274327 C>T) in 245 Brazilian schoolchildren, and no association was found between the rs2274333 A>G, rs2274328 A>C and rs2274327 C>T polymorphisms and dental caries.²¹ However, they found that the rs2274327 C>T polymorphism decreased the salivary buffering capacity.²¹ In another study, performed on 178 Turkish children by Sengul et al., no correlation was found between rs2274327 and dental caries.²² In the same year, Yildiz et al. performed their study on Turkish adults, and no association was found between rs2274327 and dental caries,²³ similarly to other studies. Li et al. investigated in their study 7 different SNPs (rs2274328, rs17032907, rs11576766, rs2274333, rs10864376, rs3765964, and rs6680186) related to CA VI and they found an association only in the rs17032907 genetic variant, which suggested that the rs17032907 polymorphism might be associated with dental caries susceptibility.²⁴

In the present meta-analysis, 4 different studies were included. The study of Yarat et al. was not included due to insufficient knowledge about the frequencies of genotypes.²⁰ In the literature, there were 3 studies related to the rs2274327 polymorphism, and 2 studies related to the rs2274328 and rs2274333 polymorphisms. In any polymorphism model, no association could be obtained between the rs2274327, rs2274328 and rs2274333 polymorphisms and dental caries.

Conclusions

Although CA VI plays an important role in the buffering of saliva, it was found that polymorphisms in the CA VI gene do not affect the process of dental caries. Due to an inadequate number of studies in the literature, we could only examine the rs2274327, rs2274328 and rs2274333 polymorphisms. Furthermore, due to an inadequate number of studies, the power of the meta-analysis was lower. In further studies, we recommend that researchers investigate also other polymorphisms related to the CA VI gene. Additionally, we recommend the separation of the dmft and DMFT variables as subgroups as the number of studies increases.

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