

# Salivary protein polymorphisms and risk of dental caries: a systematic review

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**Abstract:** Dental caries is an oral pathology associated with both lifestyle and genetic factors. The caries process can be influenced by salivary composition, which includes ions and proteins. Studies have described associations between salivary protein polymorphisms and dental caries experience, while others have shown no association with salivary proteins genetic variability. The aim of this study is to assess the influence of salivary protein polymorphisms on the risk of dental caries by means of a systematic review of the current literature. An electronic search was performed in PubMed, Scopus, and Virtual Health Library. The following search terms were used: “dental caries susceptibility,” “dental caries,” “polymorphism, genetics,” “saliva,” “proteins,” and “peptides.” Related MeSH headings and free terms were included. The inclusion criteria comprised clinical investigations of subjects with and without caries. After application of these eligibility criteria, the selected articles were qualified by assessing their methodological quality. Initially, 338 articles were identified from the electronic databases after exclusion of duplicates. Exclusion criteria eliminated 322 articles, and 16 remained for evaluation. Eleven articles found a consistent association between salivary protein polymorphisms and risk of dental caries, for proteins related to antimicrobial activity (beta defensin 1 and lysozyme-like protein), pH control (carbonic anhydrase VI), and bacterial colonization/adhesion (lactotransferrin, mucin, and proline-rich protein Db). This systematic review demonstrated an association between genetic polymorphisms and risk of dental caries for most of the salivary proteins.

**Keywords:** Dental Caries; Polymorphism, Genetic; Saliva; Proteins.

## Introduction

Dental caries is the most prevalent human infectious oral disease and is linked to both lifestyle and socioeconomic and genetic factors,<sup>1</sup> as well as to characteristics of the oral environment.<sup>2</sup> Tooth surface colonization by cariogenic microorganisms is initiated by their interaction with proteins in the acquired pellicle,<sup>3</sup> and *in vitro* studies have shown that salivary proteins can interact with oral bacteria in different ways. Proteins such as lysozymes, interleukins, mucins, and lactotransferrin (LTF) can promote cell aggregation, inhibition, and/or bacterial adherence.<sup>4,5,6,7</sup> Other proteins, such as beta defensins, have



direct antibacterial effects.<sup>8</sup> Therefore, studies of salivary proteins and peptides indicate that these substances have diagnostic and interventional potential in several clinical situations,<sup>4</sup> which may allow the development of prevention programs or individualized treatment.<sup>9</sup>

Given the role of salivary proteins in caries pathophysiology, their related genes may also be strong candidates for explaining genetic variation in caries experience in human populations. However, the boom of genetic association studies in recent decades has given rise to controversy and conflicting or irreproducible results, mainly due to differences in study design, statistical analysis, and interpretation of findings.<sup>10,11</sup> Actually, in the context of salivary proteins and caries, while some results suggest an association between polymorphisms and caries susceptibility,<sup>12,13</sup> other studies have not identified such associations.<sup>14,15</sup> In order to shed some light on this issue, the present work aimed to systematically assess the available literature to report and discuss the main findings contributing to the focused question: are salivary protein polymorphisms a risk factor for dental caries?

## Methodology

### Search strategy

This systematic review was registered in PROSPERO database (CRD42016036030) and was conducted based on the guidelines of the Prisma Statement ([www.prismastatement.org](http://www.prismastatement.org)). An electronic search was conducted in the PubMed, Scopus and Virtual Health Library (BVS) databases. The following MeSH terms (Medical Subject Headings) ([www.nlm.nih.gov/mesh/meshhome.html](http://www.nlm.nih.gov/mesh/meshhome.html)) were used in the search: “dental caries susceptibility,” “dental caries,” “polymorphism, genetic,” “saliva,” “proteins,” and “peptides.” Furthermore, MeSH synonyms, related terms, and free terms were also used, including the main salivary proteins cited in the literature<sup>16</sup> (Figure 1). A broad search was conducted for papers published before March 18, 2016.

### Eligibility criteria

The inclusion criteria outline articles written in any language according to the population, exposure, comparator, outcome, and study design (PECOS), where:

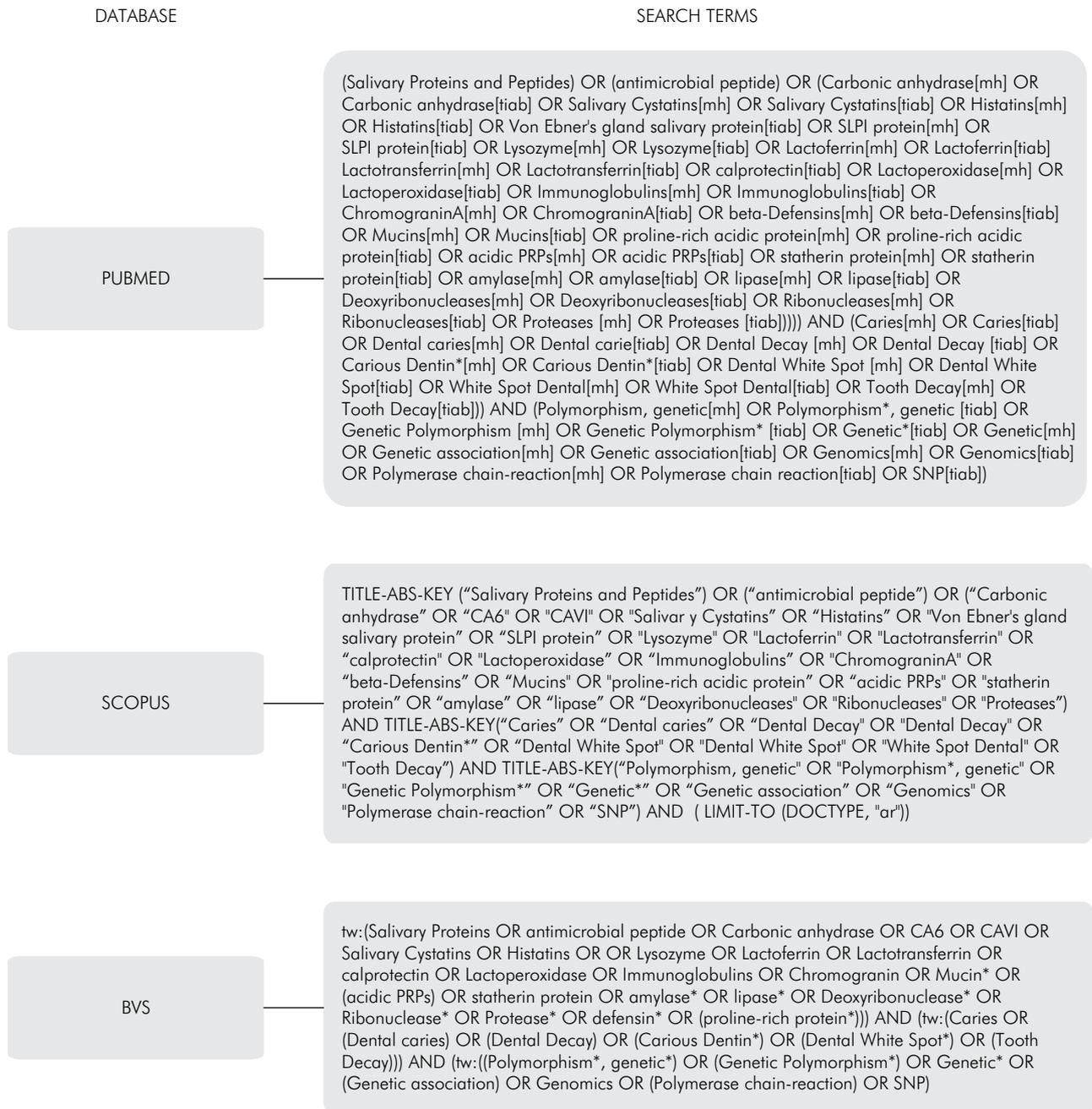
- a. Population (P): healthy humans who were not taking any medication that could affect salivary composition;
- b. Exposure (E): involvement of salivary protein polymorphisms in dental caries;
- c. Comparator (C): individuals with and without caries or individuals with high or low caries experience;
- d. Outcome (O): dental caries in primary or permanent dentition;
- e. Study design (S): Clinical trials, case-control studies, cross-sectional studies, or cohort studies published in scientific journals.

Exclusion criteria were as follows: case reports, review articles, book chapters, theses, guidelines, and *in vitro* studies. Articles that did not evaluate salivary protein polymorphisms through a molecular biology approach or did not evaluate caries experience by comparing groups with low and high caries experience were also excluded.

### Study selection and quality assessment

Two of the authors selected abstracts according to the above criteria, and the classification agreement was checked for the randomly selected 10% of the publications with a kappa statistic of 0.97. Any disagreement was discussed and solved by consensus or discussion with a third author. After selection of eligible abstracts, the respective full articles were read.

Quality assessment and bias control were performed at the methodological level for genetic polymorphism studies, according to a previous work,<sup>17</sup> based on an 11-point scoring sheet (Figure 2). A final quality score was obtained by sum of all components, ranging from 0 to 11, and a decision was made about whether the methods were appropriate or not for producing useful information. Studies with at least seven items were considered to have “low risk of bias” and high level of evidence.



**Figure 1.** Terms for each database search strategy

## Data collection

The data were organized according to different categories, such as study population, sample size and age, caries index (DMFT – Decayed, Missing, Filled Teeth), and the values attributed to low and high caries experience, target proteins, genetic variations, molecular biology technique employed, and study outcome.

## Results

The electronic search identified 338 non-duplicated records (Figure 3). After applying the exclusion and inclusion criteria, 16 studies were selected for the systematic review. All articles were classified as having high level of evidence (Table 1).

Control Group	If there is a control group with dmft=0 or with low caries experience
Hardy-Weinberg equilibrium	Acceptable studies if their groups were in Hardy-Weinberg equilibrium
Case Group	Defined with sufficient details to allow its replication (inclusion and exclusion criteria)
Primer	Primer sequence published
Reproducibility	Described the genotyping method or provide a reference to allow replication
Blinding	Adequate studies performed the genotyping whilst blind to the clinical status of the patient
Power Calculation	The data were sufficient available to detect a significant difference in genomic frequency between case and control groups at the 0.05 level, and it was calculated for allelic and genomic associations.
Statistics	Present their major findings with described tests of significance including p values, confidence intervals or odds ratios
Corrected Statistics	If a study examined two or more SNPs it were acceptable the presence of Bonferroni's correction to avoid error type I (false-positive)
Independent replication	Studies that either performed as a confirmatory study or were specifically designed to confirm earlier polymorphism studies in different populations.
Compilation of reported associations and outcomes	Only results that pertained to dental caries in health subjects were included in the final analysis of reported polymorphism effects

**Figure 2.** Criteria for quality assessment of the selected articles, adapted from<sup>17</sup>.

The characteristics of the studies are described in Table 2. U.S. patients were studied in five articles, Brazilian patients in three, Polish individuals in two, and Turkish subjects in one. The sample sizes were notably variable, including from 30 to 920 individuals. About half of the selected studies analyzed children,<sup>13,14,18,19,20</sup> evaluating primary and permanent dentition, while the other half studied only adults.<sup>12,15,21,22,23,24</sup>

Dental caries prevalence was evaluated according to the number of decayed, missing, and filled teeth. There has been no distinction between primary (dmft) and permanent (DMFT) dentition for indexing low and high risk of caries. Pol<sup>17</sup> evaluated only adults and considered DMFT < 7 as low risk of caries, while Brancher et al.<sup>19</sup> classified individuals with DMFT/dmft = 0 as low risk and DMFT/dmft > 4 as high risk (Table 2), indicating an important methodological source of variability in results across different studies.

Eleven articles found an association between salivary protein polymorphism and risk of dental caries, while five articles did not (Table 2). It is important to note that association with caries experience was identified when these same target proteins with negative results (mucin, HBD1, LTF, and CA VI) were assessed for other polymorphisms, usually with larger sample sizes<sup>13,19,21,23</sup>. From the five conflicting pairs of studies (*i.e.*, one article reporting the existence and another the absence of association for the same protein), three included different age groups (children and adults), increasing the possible impact of methodological variability.

## Discussion

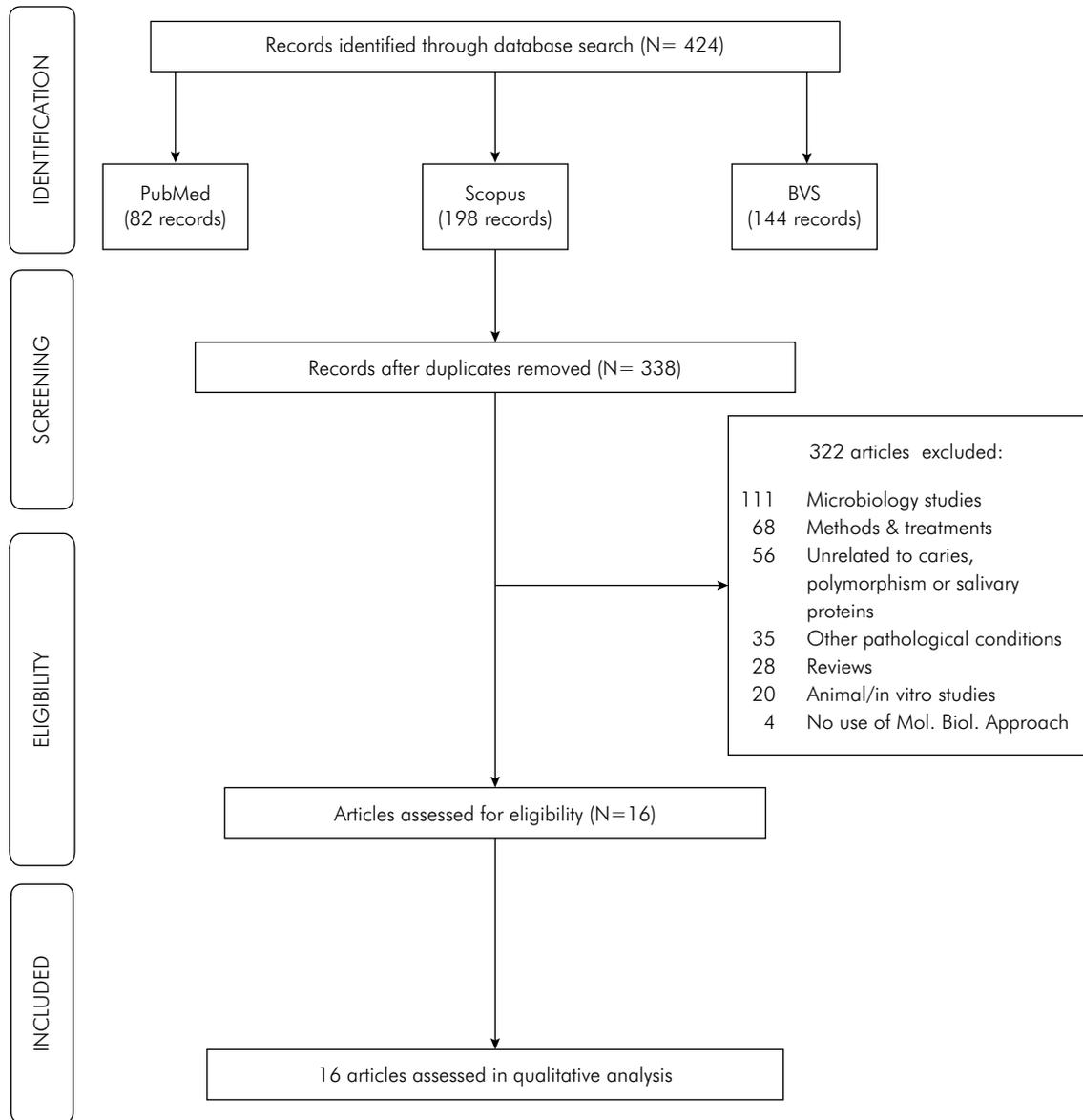
It is difficult to establish a single genetic variable as predictive for dental caries severity due to its multifactorial etiology.<sup>25</sup> Nevertheless, the present study reviewed the scientific literature, showing that genetic variants of salivary proteins, in general, affect dental caries experience.

The molecular techniques employed varied considerably, but all of them are commonly used in genetic studies, going from RFLP, which involves gene identification by digestion with restriction enzymes, gel electrophoresis, and southern hybridization,<sup>26</sup> to DNA-sequencing performed with beadchip microarray for genome-wide genotyping.<sup>24</sup> All the selected articles were qualified methodologically as having high levels of evidence. With such techniques, for each salivary protein investigated, at least one study identified genetic components as important factors for caries susceptibility, comprising the main groups of salivary function related to caries pathophysiology (Figure 4).

**Table 1.** Methodological scoring protocol based on quality assessment for selected studies.

Criteria evaluated	Articles															
	Tao et al., 2005	Zakhsary et al., 2007	Azevedo et al., 2010	Ozturk et al., 2010	Peres et al., 2010	Bancher et al., 2011	Pol et al., 2011	Yarat et al., 2011	Buczowska-Radlinska et al., 2012	Fine et al., 2013	Shaffer et al., 2013	Volckva et al., 2014	Abbasoglu et al., 2015	Doetzer et al., 2015	Li et al., 2015	Yildiz et al., 2016
Control group	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hardy-Weinberg equilibrium	0	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0
Case group	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Primer	0	0	1	0	1	1	0	1	1	1	0	1	0	1	1	1
Reproducibility	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Blinding	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Power calculation	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Statistics	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Corrected statistics	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Independent replication	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Compilation of reported associations and outcomes	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Score	8	10	9	9	9	9	8	9	9	9	9	10	10	10	10	9

For the quantification of criteria: "1" means present and "0" means absent.



**Figure 3.** Summary of systematic steps according to Prisma Statement 2009.

One work<sup>24</sup> suggested significant associations in a genome wide association study (GWAS), but did not identify caries experience using global caries phenotypes (DMFT and dmft). Instead, that work was innovative in suggesting novel caries phenotypes, developed by hierarchical clustering analysis on tooth-surface-level data. Previously, the authors have shown that relevant categories were obtained by grouping surfaces based on caries co-occurrence.<sup>27</sup> This suggests that new models of phenotypes may capture more biologically informative patterns of

tooth decay. This method indicated that bacteriolytic *LYZL2* polymorphism was implicated in reducing caries on mandibular anterior tooth surfaces (incisors, canines, and first premolars).<sup>24</sup> Unfortunately, it is still unclear by which mechanisms *LYZL2* affects dental caries. Nevertheless, the protein related to this gene belongs to the family of c-type lysozymes, well-recognized bacteriolytic host defense factors.<sup>28</sup> Its putative bacterial function and the genetic association findings suggest this protein is a potential dental caries biomarker.<sup>24</sup>

**Table 2.** Description of the selected studies.

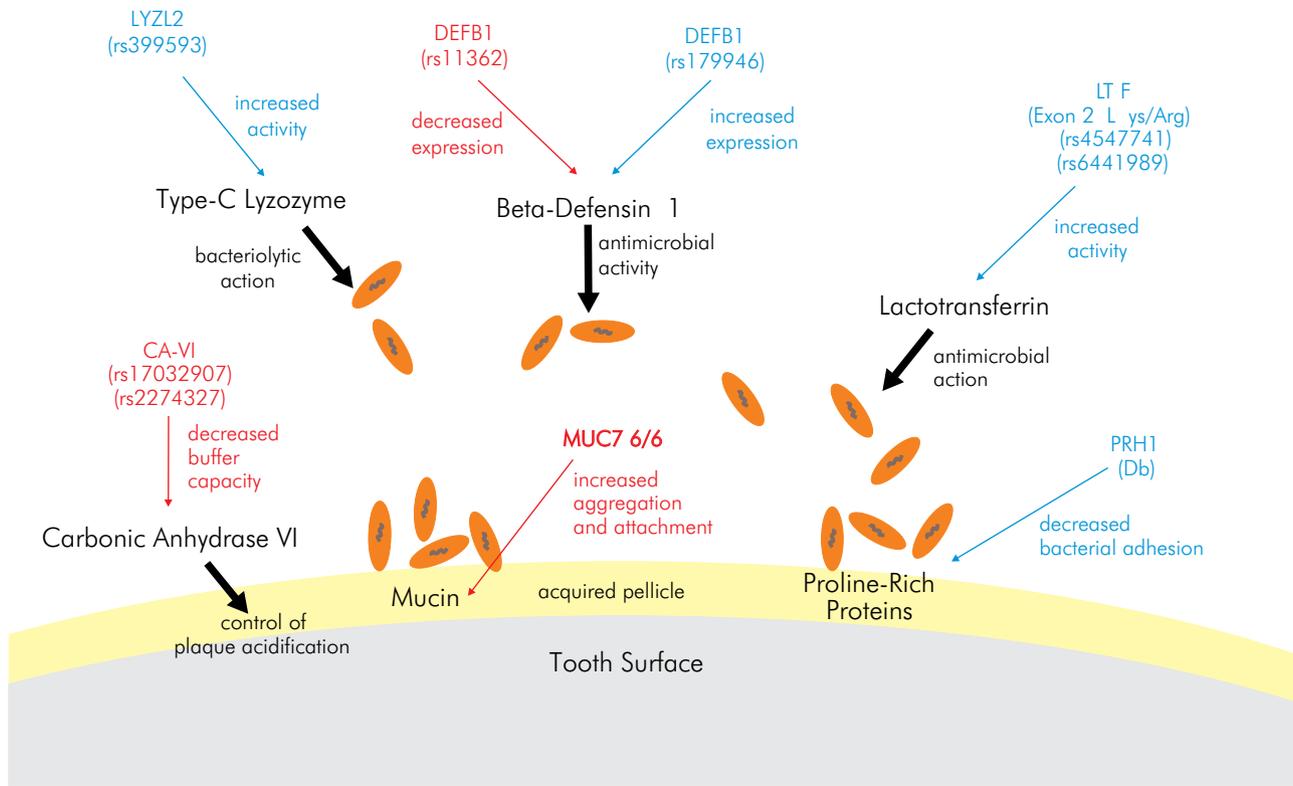
Authors	Population	n	Age range (years)	Carries index (low ; high)	Protein	Gene	Location	Genetic variation	Function	Molecular biology technique	Results
Too et al., 2005	USA Hispanic; Caucasian	149 children	11-15	DMFT/dmft (0 ; ≥ 1)	Human beta defensin 1 (HBD1)	DEFB1	Chromosome 8 (23.1)	rs11362, rs1800972	Antimicrobial	PCR- TaqMan assay	No significant association
Zakhary et al., 2007	USA African- descent; Caucasian and Mixed	208 children; 50 adults	\	DMFT/dmft (0 ; ≥ 4)	Acidic Proline-rich Protein Db	PRH1	Chromosome 12 (13.2)	Db absent, Db present	bacterial attached to biofilm	PCR-RFLP	No significant association
Azevedo et al., 2010	Brazil Caucasian	110 children	12	DMFT/dmft (0 ; ≥ 1)	Lactotransferrin	PRH1	Chromosome 3 (21)	Second Exon (AA, AG, GG)	Antibacterial	PCR-SSCP	Association between LTF A/G and low dental caries experience
Ozturk et al.	USA Caucasian, African- American	296 adults	17-84	DMFT/dmft [(< 14; > 14) up 30 years; (< 9; > 9) under 30 years]	HBD1	HBD1	Chromosome 8 (23.1)	rs11362, rs1800972	Antimicrobial	RT-PCR	Association of rs179946 with low DMFT score, and rs11362 with high DMFT
Peres et al., 2010	Brazil Caucasian, Black	245 children	7-9	DMFT/dmft (= 0 ; ≥ 1)	Carbonic anhydrase VI	CA VI	Chromosome 1(36.2)	rs2274327, rs2274328,rs2274333	pH regulation	PCR-RFLP	Association between rs2274327 and buffer capacity
Brancher et al., 2011	Brazil Caucasian	50 children	12	DMFT/dmft (= 0 , ≥ 4)	Lactotransferrin	LTF	Chromosome 3 (21)	Biomarkers in promoter region	Antibacterial	PCR-SSCP	No significant association
Pol J, 2011	Poland Caucasian	158 adults	20-21	DMFT (≤ 7 ; ≥ 15)	Mucin	MUC7	Chromosome 4 (13.21)	MUC7 5/5, MUC 7 5/6., MUC7 6/6	Formation of dental pellicle	PCR-RFLP	Association between MUC7 6/6 genotype and high dental caries experience
Yarat et al., 2011	Turkey	44 adults	18-26	DMFT (< 6 ; ≥ 6)	Carbonic anhydrase VI	CA VI	Chromosome 1(36.2)	Rs2274327, Rs227428	pH regulation	DNA sequencing	No association between biomarker and low caries experience High experience group absent in the study
Buczowska-Radlinska et al., 2012	Poland Caucasian	158 adults	20-21	DMFT (≤ 7 ; ≥ 15)	Mucin	MUC7	Chromosome 4 (13.21)	MUC7 5/5, MUC 7 5/6., MUC7 6/6	Formation of dental pellicle	PCR-RFLP	No significant association
Fine et al., 2013	USA	30 adults	29-38	DMFT	Lactotransferrin	LTF	Chromosome 3 (21)	KK, KR, RR	Antibacterial	PCR-RFLP	LTF/K variance can influence caries susceptibility

Continue

Continuation	Author, Year	Population	n	Age	DMFT/dmft	Gene	Polymorphisms	Chromosome	SNPs	Bacteriolytic factor	DNA sequencing	Association with mandibular anterior surfaces
Shaffer et al., 2013	USA Caucasian	920 adults	18-75	DMFT and DMFTS	LYZL2	LYZL2	Chromosome 10 (11.23)	rs399593	Bacteriolytic factor	DNA sequencing	Association with mandibular anterior surfaces	
Volckova et al., 2014	Czech, Caucasian	637 children	2-5	DMFT/dmft (= 0; ≥ 1)	Lactotransferrin	LTF	Chromosome 3 (21)	rs1126478	Antibacterial	PCR-RFLP	No significant association	
Abbasoglu et al., 2015	Turkish	259 children	2-5	DMFT/dmft (0 ; ≥ 1)	Lactotransferrin, HBD1	LTF, defb1	Chromosome 3 (21), Chromosome 8 (23.1)	rs2269436, rs743658, rs4547741, rs17078878, rs4547741	Antibacterial, Antimicrobial	RT-PCR	CT in LTF (rs4547741) protective for caries	
Doetzer et al., 2015	Brazil, Caucasian	677 children	12	DMFT/dmft (0 ; ≥ 1)	Lactotransferrin	LTF	Chromosome 3 (21)	rs6441989, rs2073495, rs11716497	Antimicrobial	PCR-SSCP	Allele A for rs6441989 protected for caries	
Li et al., 2015	Chinese	355 adults	mean age = 48	DMFT (≤ 2; > 3)	Carbonic anhydrase VI	CA VI	Chromosome 1(36.2)	Several SNPs in intron regions	pH regulation	DNA sequencing	rs17032907 and haplotype(ACA) associated with caries susceptibility	
Yildiz et al., 2016	Turkish	15 adults	not provided	DMFT (≤ 5; ≥ 14)	Carbonic anhydrase VI	CA VI	Chromosome 1(36.2)	rs2274327	Antibacterial	PCR-RFLP	rs11362 positively associated with caries experience	
					HBD1	DEFB1	Chromosome 8 (23.1)	rs11362	Antimicrobial			

Mucin, another important protein within the oral cavity, can be of two types: high-molecular-weight mucin (MG1), encoded by the MUC5B gene, and low-molecular-weight mucin (MG2), encoded by the MUC7 gene.<sup>29</sup> Both types of mucins are involved in dental plaque formation and in bacterial adhesion. Two Polish studies have added to this knowledge by considering the role of their polymorphisms in caries experience, albeit with conflicting results. While Pol<sup>12</sup> has found an association between the MUC7 6/6 genotype and high dental caries experience (p=0.09), in a study with 158 young adults, Buczkowska-Radlinska et al.<sup>22</sup> have reported that the distribution of the value of the DMFT index was similar to that of the control group. Vieira et al.<sup>10</sup> suggested that these conflicting results might be related to study design issues such as population heterogeneity and statistical power, therefore not yet refuting the results of the original study.

Lactotransferrin (LTF), a specific glycoprotein that can affect dental biofilm aggregation by inhibiting *S. mutans* adhesion and present antimicrobial activity through activation of the immune system, had polymorphisms associated with low caries experience in Turkish children<sup>30</sup>, Brazilian 12-year-old students,<sup>13,31</sup> and North American adults.<sup>25</sup> Whole saliva from American individuals, homozygous for a lysine variant, presented increased antimicrobial activity against *S. mutans* as compared to “wild-type” arginine from LTF,<sup>23</sup> supported by a reduction in caries prevalence in the lysine group (p = 0.001; relative risk: 3.6; 95%CI:1.5–11.13). Furthermore, the creation of a lysine variant peptide demonstrated the broad spectrum of antimicrobial activity that this variant can have on gram-positive, acid-producing/caries-associated bacteria. Two Brazilian studies<sup>13,31</sup> also have shown that lactotransferrin A/G (exon 2, Lys/Arg, rs 1126478) and a tag SNP located outside and downstream of the LTF gene (rs6441989) were associated with dental caries susceptibility, especially in the presence of gingivitis (p = 0.020) or plaque (p = 0.035),<sup>31</sup> confirming the influence of LTF lysine variants on the caries process. More recently, a study<sup>30</sup> observed an association (p = 0.036, OR:0.47 95%CI:0.23–0.95) for the CT genotype in rs4547741 with reduced caries in the primary dentition of 2-to-5-year-old Turkish children,



**Figure 4.** Salivary proteins and functions (black) that present polymorphisms associated positively (red) or negatively (blue) with dental caries experience.

confirming the protective role of this polymorphism in Early Childhood Caries (ECC).

Beta defensins are small cationic antimicrobial peptides with an important role in the innate immune system. Salivary levels of human beta defensin 1 (HBD1) have been correlated with the risk for periodontal disease or caries.<sup>32</sup> One study,<sup>21</sup> after adjusting results by age, gender, ethnicity, and smoking status, found two polymorphisms in the promoter region of the gene responsible for HBD1 (*DEFB1*) associated with caries experience: rs11362 (G-20A) ( $p = 0.007$ ; OR = 5.28 95%CI:1.99-14.05), which increased DMFT up to 5 times, and rs1799946 (G-52A), which was associated with lower caries experience (OR = 0.32,  $p = 0.014$ ). Another recent study confirmed rs11362 as a risk factor for caries in Turkish adults ( $p = 0.000$ ) with contributing environmental factors such as high level of dental plaque, age, and saliva buffer capacity.<sup>33</sup> Literature findings actually indicate that HBD1 is reduced in the saliva of patients with *DEFB-1* polymorphisms.<sup>34</sup> However, while the

antibiotic action of defensins comes from unspecific pore formation from electrostatic interactions with microbial membranes, leading to cell leakage and death, HBD1 presented the lowest *in vitro* antimicrobial activity against cariogenic bacteria when compared with other beta defensins.<sup>35</sup> Nevertheless, a study<sup>36</sup> provided an explanation to this paradoxical production of an “ineffective molecule,” showing that, after reduction of disulfide bridges, HBD1 becomes a potent antimicrobial peptide. This is even more relevant if we consider that the oral cavity may provide anaerobic niches that are favorable to such reduction.<sup>37</sup>

Acidic proline-rich proteins (PRPs) represent 37% of salivary proteins adhered to freshly cleaned teeth and may be responsible for differences in bacterial interactions with the tooth surface, having an impact on biofilm colonization.<sup>38</sup> Acidic PRPs are encoded by two genes, PRH1 and PRH2, with three different alleles at PRH1 (Db, Pa, and Pif).<sup>39</sup> When the allele Db was studied,<sup>20</sup> it was present in 72% of African Americans and in 26% of Caucasians. Curiously

enough, large groups of African American children, regardless of the presence of *Db*, had significantly less caries experience than Caucasian subjects ( $p < 0.01$ ). The racial difference between subjects with high caries experience compared to those with low caries experience was significant for *Db*-negative individuals ( $p < 0.01$ ) but not significant for *Db*-positive individuals, suggesting that alleles linked to *Db* may explain racial differences in caries experience.<sup>20</sup>

Four studies on Carbonic Anhydrase VI (CA VI) have assessed the impact of polymorphisms on caries experience. The study reported on two SNPs (rs2274327 [C/T], rs2274328 [A/C]) in exon 2 of CA VI<sup>15</sup> has found no association between these SNPs and dental caries, salivary pH, or buffering capacity in children and adults. However, Peres et al.<sup>22</sup> studied the same SNPs and reported that the variant rs2274327 was associated with a decreased activity of the enzyme in saliva buffer capacity of Brazilian children ( $p=0.046$ ). Similar results were found later with Brazilian adults, and polymorphism rs2274333 was also associated with decreased CA VI concentration.<sup>40</sup> Recently, a study with a northwestern Chinese population indicated yet another genotype (TT, rs17032907) with an increased risk of dental caries (OR = 2.144, 95%CI:1.096–4.195).<sup>41</sup> Intriguingly, CA VI concentration in the saliva has been previously shown to not regulate alone salivary pH or buffer capacity, and this enzyme may have a different role or may participate in these processes

together with other CA types.<sup>42,43</sup> It is known that bicarbonate in the saliva diffuses into dental plaque and combines with  $H^+$  to form carbonic acid. CA VI contributes to the neutralization of plaque acid, whose buffering is mainly provided by bicarbonate, which may contribute to dental caries development.<sup>44</sup>

Saliva as a diagnostic medium for various biochemical tests provides a noninvasive and accessible tool, with advantages over other body fluids, such as blood and urine, and readily available appropriate technologies enable the use of saliva in the diagnosis and monitoring of disease progression.<sup>45</sup> Consequently, the search for salivary disease biomarkers has motivated the cataloguing of the human salivary proteome.<sup>3,46</sup> In the present systematic review, even though it is clear that high methodological variability (age, sample size, and caries identification procedures) still makes it difficult to compare results from different studies, it is shown that scientific literature strongly indicates a series of different salivary protein polymorphisms that impact on caries experience, for all proteins (even though not for all polymorphic loci) investigated.

## Conclusion

Scientific evidence confirms the general pattern of association between risk of dental caries and salivary protein polymorphisms.

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