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ABSTRACT

Polymorphic, acidic proline-rich proteins (PRPs) in saliva influence the attachment of bacteria associated with caries. Our aims were to detect one of three acidic PRP alleles of the *PRH1* locus (*Db*) using polymerase chain-reaction (PCR) on genomic DNA, and to determine its association with caries. DNA was obtained from buccal swabs from Caucasian and African-American children, and their caries experience was recorded. PCR primers designed around exon 3 of the *PRH1* locus gave a 416-base product representing *Db* and a 353-base product representing the other two alleles (*Pa* or *Pif*). In Caucasians, *Db* gene frequency was 14%, similar to Db protein from parotid saliva. In African-Americans, however, it was 37%, 18% lower than Db from parotid saliva (reported previously). Compared with African-Americans, all Caucasians had significantly greater *Streptococcus mutans* colonization, but only *Db*-negative Caucasians had significantly more caries. Alleles linked to *Db* may explain racial differences in caries experience.

KEY WORDS: acidic PRPs, caries, saliva, genetics, polymerase chain-reaction, *Streptococcus mutans*.

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INTRODUCTION

Dental caries is caused by acidogenic and aciduric bacteria that colonize tooth-adherent biofilms and grow on dietary carbohydrate (Marsh, 2003). *Streptococcus mutans* is the best-characterized of these bacteria, but its association with the extent of dental caries is weak (Sullivan *et al.*, 1989). Other bacteria—*Lactobacillus* spp., *Actinomyces* spp., *Bifidobacterium* spp. and non-mutans streptococci—are increased in the biofilms from high-caries individuals, regardless of *S. mutans* colonization (Beighton, 2005).

Caries severity and *S. mutans* colonization have strong genetic components (Bretz *et al.*, 2005; Corby *et al.*, 2005), and, after adjustment for socio-nutritional factors, different racial groups significantly differ in caries experience (Zoitopoulos *et al.*, 1996). Much of these genetically determined differences in caries experience might be due to polymorphic, acidic proline-rich proteins (PRP) in saliva (Lenander-Lumikari and Loimaranta, 2000) encoded at two loci, *PRH1* and *PRH2*. Three alleles (*Db*, *Pa*, and *Pif*) provide polymorphisms at the *PRH1* locus, and 3 (*Pr1*, Pr1′, and *Pr2*) at the *PRH2* locus (Azen, 1993, 1998). The *Pr1′* allele is present in 16% of African-Americans, who can therefore express up to 36 combinations, whereas Caucasians express only up to 18 combinations (polymorphisms).

The acidic PRPs comprise 37% of the salivary proteins that adhere to freshly cleaned teeth (Bennick *et al.*, 1983). They attach to apatite by their acidic N-terminal domain, and this exposes their proline-rich C-terminal domain to bind to oral bacteria and initiate biofilm development (Gibbons *et al.*, 1991; Li *et al.*, 1999). A common *S. mutans* strain (serotype c) binds to an expressed allele of the *PRH2* locus (Pr1) more strongly than to any other tooth-attached salivary protein (Gibbons and Hay, 1989), but different mixtures of the acidic PRPs influence *S. mutans* binding independently. For example, *S. mutans* binding to parotid saliva-coated apatite is enhanced along with caries if the *Db* allele of *PRH1* is expressed as the *Db* protein in saliva, but *Actinomyces naeslundii* binding is enhanced if the Db protein is absent (Stenudd *et al.*, 2001). Thus, in the absence of *Db*, increased colonization by *A. naeslundii* may promote a high antibody response that associates with less caries in adults (Levine *et al.*, 2005). Different acidic PRP mixtures may therefore promote different mutualistic bacterial interactions (Yoshida *et al.*, 2006) that influence the extent of biofilm colonization by acidogenic and aciduric bacteria responsible for caries.

The use of genomic DNA to detect *PRH1* and *PRH2* polymorphisms would overcome investigators' difficulties in obtaining parotid saliva from children and the need for the different protein alleles expressed by different individuals to be identified. The aims of this study were to detect the *Db* allele in whole, genomic DNA, to confirm that the allele's presence corresponds to Db protein expression in parotid saliva, and to determine how *Db* associates with caries in young children.

MATERIALS & METHODS

Db Gene Determination in Adult Caucasians

Prior to the study, a consent document was approved by the University of Oklahoma HSC Institutional Review Board. Thirty-nine local volunteers provided genomic DNA, of whom 10 also provided parotid saliva (5 mL) using a Lashley canula. DNA was collected by the gentle rubbing of sterile nylon bristle brushes on the inside of the cheek. A protocol and reagents for DNA purification from the bristle-adherent buccal cells were supplied with the brushes (Gentra Systems Inc., Minneapolis, MN, USA). Additional samples of DNA from 50 random US adult Caucasians were purchased from the Coriell Cell Repository (Camden, NJ, USA).

In allele *Db*, a 63-base duplication of part of the nucleotide sequence of exon 3 encodes an additional 21 amino acids not present in *Pa* and *Pif* (Azen *et al.*, 1987). *Db* is therefore 63 base pairs larger than *Pa* and *Pif* (Fig., A). Primers for amplifying this region were chosen after nucleotide BLAST analysis against the human genome (Altschul *et al.*, 1997) indicated significant similarity for only the *PRH1* locus. The primers were nucleotides 3247-3268 and the reverse complement of nucleotides 3577-3599 (bold regions in Fig., A). Each primer was synthesized, purified, and provided dry by Sigma-Genosys (St. Louis, MO, USA).

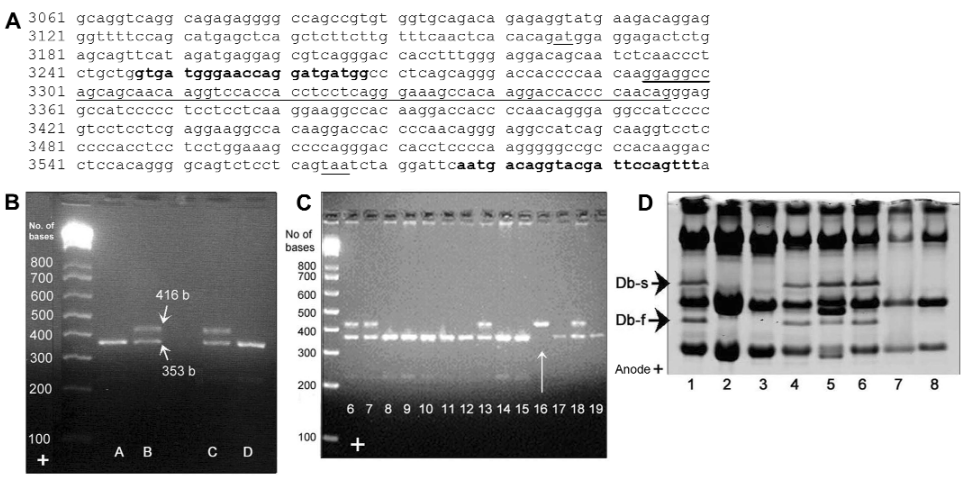

Figure. PCR detection of *Db*. **(A)** Region surrounding exon3 of the *PRH1* locus. Exon3 begins with the last 2 nucleotides of a codon (at) and ends with a release codon (taa), indicated by short underlines. The underlined region indicates the 63-base-pair segment of alleles *Pif* and *Pa* that are repeated in *Db* (not shown). The bold regions indicate the sequences of the forward and reverse PCR primers. **(B)** Bands detected following PCR. The larger band (416 bases) contains the 63-base insert corresponding to *Db*. The lower band corresponds to either *PIF* or *Pa*. Persons A and D do not encode *Db*; persons B and C encode *Db* with either *PIF* or *Pa*. **(C)** Demonstration of a homozygous *Db* result. The presence of a single upper band only (arrow) indicates a person who is homozygous for *Db* (*PIF* and *Pa* both absent). The DNA was from random Caucasians, #6 through #19, from the Coriell collection (see METHODS). **(D)** Detection of *Db* in parotid saliva. Db_s is the intact protein, and Db_f is a large N-terminal fragment derived from cleavage of Db_s at residue 127 (Hay *et al.*, 1994). Db_f migrates to the anode more than Db_s (Azen and Yu, 1984). Genomic DNA from persons who had yielded only the 353-base product after PCR did not display either Db protein (lanes #2, 3, 7, and 8). Genomic DNA from persons who had also yielded the 416-base products after PCR (#1, 4, 5, and 6) showed the 2 bands Db_s and Db_f in the expected positions. A fifth *Db*-positive and a fifth *Db*-negative individual gave the same, respective results [not shown in (D)].

Polymerase chain-reaction (PCR) was performed on purified DNA with a PTC-100 Programmable Thermal Controller with Peltier Effect Cycling (Levine *et al.*, 2001). A PCR reaction (total volume 50 µL) contained 20 ng genomic DNA, 2.5 mM deoxyribonucleotide triphosphates, 20 units/mL Taq polymerase, 5 µL Taq Reaction Buffer (Taq Mastermix; Continental Lab Products, San Diego, CA, USA), and 10 pmol of each primer. A cycle of heating to 94°C, annealing at 62°C, and extension at 72°C (each 1 min) was repeated 38 times, followed by a final extension for 10 min and cooling to 4°C. Products were visualized with ethidium bromide on a 2.5% agarose gel in tris-borate-buffer, pH 8.3 (tris base, 0.1 M; boric acid, 0.1 M; and EDTA, 2 mM).

To ensure that the correct nucleotide sequences were obtained, we cut selected PCR products from the gel and purified them using a QIAquick Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA); products were taken up in 50 µL of tris-HCl buffer, pH 8.5. The samples were reacted with BigDye® Terminator v1.1 Sequencing Reagents and sequenced with an AB3731 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

The presence or absence of the *Db* allele (gene) was determined by PCR and compared with whether the corresponding protein (Db) was present in parotid saliva from local volunteers. The saliva (5 mL) was dialyzed for 3 hrs against 6 changes of a hundred-fold excess volume of 0.1 M ammonium carbonate, freeze-dried overnight, and dissolved in 1.0 mL of 4.0 M urea. Approximately 20 µL was applied to a non-denaturing alkaline Mini-gel (BioRad Labs Inc., Hercules, CA, USA) and stained with Coomassie blue in 20% v/v trichloroacetic acid (Azen and Denniston, 1981).

Db Gene and Caries Measurements in Children

Eligible children had to be enrolled in the US Head Start Program for low-income (low socio-economic status) families and to reside in Iowa in communities with optimally fluoridated water. Prior to entry, consent for a dental examination and collection of DNA from buccal oral mucosa was obtained from at least one parent, according to University of Iowa Institutional Review Board (IRB) guidelines (Slayton *et al.*, 2005). Participants were 89 children of Caucasian parentage, 96 of African-American parentage, and 23 of mixed parentage (one Caucasian and one African-American). They were examined once for caries by means of a flashlight and mouth mirror. We determined the extent of caries by recording the decayed, missing, and filled surfaces of primary teeth in each child and calculating the dmft and dmfs. The Child-Care Centers were racially mixed, and all children received two meals a day, which controlled for differences in diet that could affect caries development as far as practicable. 'Outside' dietary habits, *i.e.*, between-meals habits, were not evaluated.

We obtained microbiological data by touching the dorsal surface of each child's tongue with a wooden blade until wet. We inoculated a growth medium selective for either *Streptococcus*

Table 1. *Db* Allele Occurrence and Gene Frequency

Measurement	Caucasians	Mixed-race[a]	African-Americans
Number[b]	178	23	116
Db present (%)	26.4	52.2	72.4
Gene frequency (%)	13.5	26.1	37.5
Observed homozygous	1	0	3
Expected homozygous[c]	3	1.5	16

[a] Mixed-race: One parent was Caucasian and the other was African-American.
[b] Number of unrelated individuals: African-Americans included 96 caries cases and control individuals, plus 20 who were not categorized. Caucasians included 89 caries cases and control individuals, plus another 89 for whom caries was not determined (50 from the Coriell repository and 39 local individuals).
[c] Expected number of homozygous individuals calculated assuming Hardy-Weinberg allelic equilibrium (statistics given in the RESULTS).

mutans or *Lactobacillus* sp. (CRT Bacteria Kit, Ivoclar Vivadent, Schaan, Liechtenstein) by placing the blade onto the agar surface. Samples were incubated at 37°C for 48 hrs and then compared with visual standards (Edelstein and Tinanoff, 1989). Although low, medium, or high bacterial growth was noted, the plates were simply recorded as positive or negative for the respective bacteria for this study.

Each Caucasian and African-American adult and child participant has 2 *PRH1* loci in their genome. Allele *Db* may be absent (no copies), present at one locus (heterozygous), or present at both loci (homozygous). We therefore determined the gene frequency by totaling all heterozygous individuals, adding each homozygous individual twice, and dividing by twice the total number of individuals in the population. The population was divided into control individuals (dmfs 0) and cases (dmfs > 3), and the frequencies of *Db* and race were compared by chi-square. Caries experience (dmfs or dmft) was not normally distributed, and persons with dmfs 1, 2, and 3 were excluded. Differences in dmfs and dmft by *Db* and race were examined by the non-parametric Kruskal-Wallis test, followed by Dunn's procedure for pair-wise comparisons.

Table 2. Control Individuals, Cases, and Bacterial Colonization by *Db* and Racial Group

Category[a]	Cntls[b]	Cases[b]	Cases (%)[c]	Number[d]	%SM[e]	%(SM+L)[f]
AA (*Db* absent)	21	9	30.0	4	25.0	25.0
AA (*Db* present)	46	20	30.3	13	46.2	30.8
Cauc (*Db* absent)	29	37	56.1	28	82.6	50.0
Cauc (*Db* present)	12	11	47.8	8	75.0	60.7

[a] African-American (AA) or Caucasian (Cauc), and *Db* present or absent.
[b] Cntls (Control individuals), dmfs = 0; Cases, dmfs > 3.
[c] Cases as percent of total cases plus control individuals.
[d] Number of cases used to detect *S. mutans* and lactobacilli.
[e] Percent of cases with *S. mutans*.
[f] Percent of cases with lactobacilli.

RESULTS

Db Detection, Occurrence, and Relationship to Expressed Protein Db

The 416-base segment representing *Db* and the 353-base segment representing *Pa* or *Pif* were detected as strong, ethidium-bromide-staining bands after PCR of genomic DNA (Fig., B). Most individuals had only the 353-base lower band (no *Db*), or both bands (heterozygous; *Db* present with either *Pa* or *Pif*). One Caucasian (Fig., C) and three African-American children showed only the 416-base upper band (homozygous for *Db*). DNA from all local and Coriell individuals, and 10 randomly selected children from Iowa, was tested at least twice, with identical results. The duplicates included two individuals whose DNA produced only the 416-bp upper band after PCR. In addition, the products, 11 upper bands from six Coriell and five local individuals, and a lower band from one Coriell and one local individual, all had the expected sequence (Fig., A).

The *Db* gene frequency in Caucasian local adult volunteers and Coriell adults was, respectively, 10.3% and 17%. The overall gene frequency of this combined Caucasian population was 14.0%, similar to that in 89 Caucasian children from Iowa (12.9%). Compared with Caucasians, the mixed-race group had a two-fold and African-Americans a three-fold greater *Db* gene frequency (Table 1). The differences in gene frequency between Caucasians and African-Americans ($X^2 = 60.2$, $p < 0.0001$) and among all three racial groups (Caucasians, mixed-race, and African-Americans; $X^2 = 6.5$, $p < 0.03$) were significant. The difference between mixed-race and African-Americans approached significance ($X^2 = 3.68$, $p = 0.055$). A deviation from Hardy-Weinberg equilibrium (fewer than expected homozygous *Db* individuals) was significant in African-Americans ($X^2 = 27.8$; $p < 0.001$), but a similar deviation in Caucasian and mixed-parentage populations was not significant, because of the small numbers.

Saliva was obtained from five local individuals whose DNA gave the 416-bp PCR product and five others whose DNA gave only the 353-bp PCR product. The Db proteins were present in saliva from only those individuals whose DNA gave the 416-bp product on PCR (Fig., D). The proteins appeared on Coomassie-blue-stained gels of parotid saliva as a double band (Db) that served as a unique identifier (see DISCUSSION).

Db and Dental Caries

Males and females were present in approximately equal numbers. They displayed similar caries experience (mean dmfs or dmft) regardless of age, and also similar frequencies of cases, control individuals, and *Db* (data not shown). African-Americans had significantly fewer cases compared with control individuals than did Caucasians, regardless of *Db* (Table 2; $X^2 = 10.7$, $p < 0.001$). The reduced caries experience of African-Americans was mirrored by fewer African-American cases being positive for *S. mutans* alone (Table 2, %SM in rows 1 and 4; $X^2 = 8.2$, $p <$

0.01), or for both *S. mutans* and lactobacilli [Table 2, %(SM+L) in rows 1 and 4; $X^2 = 3.9$, $p < 0.05$].

The racial difference between cases and control individuals was not significant for *Db*-positive individuals ($X^2 = 2.3$, n.s.), despite being significant for *Db*-negative individuals ($X^2 = 5.6$, $p < 0.03$). The Kruskal-Wallis (KW) test confirmed racial and *Db* differences (observed KW statistic = 15.0, critical KW = 7.8, degrees of freedom = 2, $p < 0.002$). The Dunn Multiple Comparisons Test indicated that the median dmfs of African-Americans, regardless of *Db*, was significantly less than the median dmfs of *Db*-negative Caucasians, whereas that for *Db*-positive Caucasians did not differ significantly from that of either *Db*-negative Caucasians or African-Americans, regardless of *Db* (Table 3). Similar findings were obtained for dmft.

Table 3. Caries Experience by *Db* and Racial Group

Category[a]	Total	Median	Groupings[b]
AA (*Db*-positive)	66	80.1	A
AA (*Db*-negative)	30	80.5	A
Cauc (*Db*-positive)	23	99.0	A & B[c]
Cauc (*Db*-negative)	66	109.5	B

[a] African-American (AA) or Caucasian (Cauc), and *Db*-positive or -negative.
[b] Groupings determined by multiple pair-wise comparisons using Dunn's procedure (two-tailed test). Bonferroni corrected significance level: < 0.01. Different capital letters indicate significantly different medians.
[c] Not significantly different from the other 3 means.

DISCUSSION

The results of this study indicate that, in young children of low socio-economic status, African-Americans are protected from caries compared with Caucasians; they had more control individuals, fewer cases, and less caries experience. This agrees with The *U.S. Surgeon General's 2000 Report on Oral Health*: 41% of African-Americans aged 12 to 17 yrs are caries-free, compared with 32% of Caucasians. Indeed, in London, UK, less caries and a lower frequency of *S. mutans* isolation were previously reported in three- to four-year-old African-Caribbean children, compared with Caucasian children of similar socio-economic status (Zoitopoulos *et al.*, 1996).

In this study, the *Db* gene frequency in Caucasians was 14%, in agreement with a 15 to 17% expression frequency reported previously (Hay *et al.*, 1994). The correspondence between Db protein expression and PCR-determined *Db* gene presence has been confirmed by the detection of Db in polyacrylamide gels of parotid saliva (Azen and Yu, 1984). The present study also confirmed a greater frequency of *Db* in African-Americans, but the gene frequency of 37.5% was 18.5% lower than that reported previously (Azen and Maeda, 1988). One possibility is that the Pr1′ protein (Azen, 1998), which is absent from Caucasians, may have been confused with *Db* in parotid saliva. Antibody detection is impractical, because the additional amino acid sequence that characterizes *Db* duplicates part of the *Pa* and *Pif* sequences (Azen *et al.*, 1987). The findings of this study call into question the reported association of *Db* gene expression with greater caries experience in African-American adults (Friedman *et al.*, 1980).

Compared with African-Americans, the caries experience of *Db*-positive Caucasians was less in magnitude and not significant. In Caucasian young adults, factors that enhance antibody responses to actinomyces, presumably by increasing the extent of actinomyces colonization, minimize caries development (Levine *et al.*, 2005). This study suggests that acidic PRP alleles in linkage disequilibrium with *Db* could enhance the mutualistic growth of actinomyces in biofilms, and promote the observed antibodies and reduced caries experience observed in young adult Caucasians.

Thus, the complete genotype of an individual at both acidic PRP loci (*PRH1* and *PRH2*) may yet account for genetic differences in caries susceptibility. Results from a study in which 100 African-Americans and 206 Caucasians, aged 5 to 15 yrs, were typed for *PRH1* and *PRH2* alleles (Yu *et al.*, 1986) suggested a greater increase in caries with age in children whose saliva was homozygous for Pr2 and expressed Pa. Unfortunately, the *Db* frequency in African-Americans may have been over-estimated, and the Pr1′ protein was not examined. The present study suggests that the *PRH1* and *PRH2* alleles in an individual's parotid saliva may be determined more accurately from genomic DNA than by the identification of expressed proteins. A powerful modification of the procedure in this paper would be the use of the dye-labeled oligonucleotide ligation (DOL) procedure (Chen *et al.*, 1998).

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REFERENCES

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, *et al.* (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389-3402.

Azen EA (1993). Genetics of salivary protein polymorphisms. *Crit Rev Oral Biol Med* 4:479-485.

Azen EA (1998). A frequent mutation in the acidic proline-rich protein gene, PRH2, causing a Q147K change closely adjacent to the bacterial binding domain of the cognate salivary PRP (Pr1′) in Afro-Americans. Mutations in brief no. 154. Online. *Hum Mutat* 12:72.

Azen EA, Denniston C (1981). Genetic polymorphism of PIF (parotid isoelectric focusing variant) proteins with linkage to the PPP (parotid proline-rich protein) gene complex. *Biochem Genet* 19:475-485.

Azen EA, Maeda N (1988). Molecular genetics of human salivary proteins and their polymorphisms. *Adv Hum Genet* 17:141-199.

Azen EA, Yu PL (1984). Genetic polymorphisms of Pe and Po salivary proteins with probable linkage of their genes to the salivary protein

gene complex (SPC). *Biochem Genet* 22:1065-1080.

Azen EA, Kim HS, Goodman P, Flynn S, Maeda N (1987). Alleles at the PRH1 locus coding for the human salivary-acidic proline-rich proteins Pa, Db, and PIF. *Am J Hum Genet* 41:1035-1047.

Beighton D (2005). The complex oral microflora of high-risk individuals and groups and its role in the caries process. *Community Dent Oral Epidemiol* 33:248-255.

Bennick A, Chau G, Goodlin R, Abrams S, Tustian D, Madapallimattam G (1983). The role of human salivary acidic proline-rich proteins in the formation of acquired dental pellicle *in vivo* and their fate after adsorption to the human enamel surface. *Arch Oral Biol* 28:19-27.

Bretz WA, Corby PM, Schork NJ, Robinson MT, Coelho M, Costa S, *et al.* (2005). Longitudinal analysis of heritability for dental caries traits. *J Dent Res* 84:1047-1051.

Chen X, Livak KJ, Kwok PY (1998). A homogeneous, ligase-mediated DNA diagnostic test. *Genome Res* 8:549-556.

Corby PM, Bretz WA, Hart TC, Filho MM, Oliveira B, Vanyukov M (2005). Mutans streptococci in preschool twins. *Arch Oral Biol* 50:347-351.

Edelstein B, Tinanoff N (1989). Screening preschool children for dental caries using a microbial test. *Pediatr Dent* 11:129-132.

Friedman RD, Azen EA, Yu PL, Green PA, Karn RC, Merritt AD (1980). Heritable salivary proteins and dental disease. *Hum Hered* 30:372-375.

Gibbons RJ, Hay DI (1989). Adsorbed salivary acidic proline-rich proteins contribute to the adhesion of *Streptococcus mutans* JBP to apatitic surfaces. *J Dent Res* 68:1303-1307.

Gibbons RJ, Hay DI, Schlesinger DH (1991). Delineation of a segment of adsorbed salivary acidic proline-rich proteins which promotes adhesion of *Streptococcus gordonii* to apatitic surfaces. *Infect Immun* 59:2948-2954.

Hay DI, Ahern JM, Schluckebier SK, Schlesinger DH (1994). Human salivary acidic proline-rich protein polymorphisms and biosynthesis studied by high-performance liquid chromatography. *J Dent Res* 73:1717-1726.

Lenander-Lumikari M, Loimaranta V (2000). Saliva and dental caries. *Adv Dent Res* 14:40-47.

Levine M, Progulske-Fox A, Denslow ND, Farmerie WG, Smith DM, Swearingen WT, *et al.* (2001). Identification of lysine decarboxylase as a mammalian cell growth inhibitor in *Eikenella corrodens*: possible role in periodontal disease. *Microb Pathog* 30:179-192.

Levine M, Owen WL, Avery KT (2005). Antibody response to actinomyces antigen and dental caries experience: implications for caries susceptibility. *Clin Diagn Lab Immunol* 12:764-769.

Li T, Johansson I, Hay DI, Stromberg N (1999). Strains of *Actinomyces naeslundii* and *Actinomyces viscosus* exhibit structurally variant fimbrial subunit proteins and bind to different peptide motifs in salivary proteins. *Infect Immun* 67:2053-2059.

Marsh PD (2003). Are dental diseases examples of ecological catastrophes? *Microbiology* 149:279-294.

Satcher D (2005). Oral Health in America: A Report of the Surgeon General. La Vergne, TN, USA: University Press of the Pacific, Ingram Book Group Distributors.

Slayton RL, Cooper ME, Marazita ML (2005). Tuftelin, mutans streptococci, and dental caries susceptibility. *J Dent Res* 84:711-714.

Stenudd C, Nordlund A, Ryberg M, Johansson I, Källestål C, Strömberg N (2001). The association of bacterial adhesion with dental caries. *J Dent Res* 80:2005-2010.

Sullivan A, Granath L, Widenheim J (1989). Correlation between child caries incidence and *S. mutans*/lactobacilli in saliva after correction for confounding factors. *Community Dent Oral Epidemiol* 17:240-244.

Yoshida Y, Palmer RJ, Yang J, Kolenbrander PE, Cisar JO (2006). Streptococcal receptor polysaccharides: recognition molecules for oral biofilm formation. *BMC Oral Health* 6(Suppl 1):S12 (http://www.biomedcentral.com/content/pdf/1472-6831-6-S1-S12.pdf).

Yu PL, Bixler D, Goodman PA, Azen EA, Karn RC (1986). Human parotid proline-rich proteins: correlation of genetic polymorphisms to dental caries. *Genet Epidemiol* 3:147-152.

Zoitopoulos L, Brailsford SR, Gelbier S, Ludford RW, Marchant SH, Beighton D (1996). Dental caries and caries-associated micro-organisms in the saliva and plaque of 3- and 4-year-old Afro-Caribbean and Caucasian children in south London. *Arch Oral Biol* 41:1011-1018.