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Research Paper

Genetic- and Lifestyle-dependent Dental Caries Defined by the Acidic Proline-rich Protein Genes *PRH1* and *PRH2*

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ABSTRACT

Dental caries is a chronic infectious disease that affects billions of people with large individual differences in activity. We investigated whether *PRH1* and *PRH2* polymorphisms in saliva acidic proline-rich protein (PRP) receptors for indigenous bacteria match and predict individual differences in the development of caries. *PRH1* and *PRH2* variation and adhesion of indigenous and cariogenic (*Streptococcus mutans*) model bacteria were measured in 452 12-year-old Swedish children along with traditional risk factors and related to caries at baseline and after 5-years. The children grouped into low-to-moderate and high susceptibility phenotypes for caries based on allelic *PRH1*, *PRH2* variation. The low-to-moderate susceptibility children (P1 and P4a^{-}) experienced caries from eating sugar or bad oral hygiene or infection by *S. mutans*. The high susceptibility P4a (*Db*, *PIF*, *PRP1*$_2$) children had more caries despite receiving extra prevention and irrespective of eating sugar or bad oral hygiene or *S. mutans*-infection. They instead developed 3.9-fold more caries than P1 children from plaque accumulation in general when treated with orthodontic multibrackets; and had basic PRP polymorphisms and low DMBT1-mediated *S. mutans* adhesion as additional susceptibility traits. The present findings thus suggest genetic autoimmune-like (P4a) and traditional life style (P1) caries, providing a rationale for individualized oral care.

1. Introduction

Dental caries, a chronic infectious disease, affects billions of people with large individual differences in disease activity (Kassebaum et al., 2015; Selwitz et al., 2007). The global economic burden of dental diseases amounted to 442 billion USD in 2010 and was 4.6% of global health expenditure (Listl et al., 2015). The prevalence of caries has declined dramatically in Western countries, with ~80% of children being healthy or nearly caries-free and ~20% children having a high caries burden (Källestål, 2005). The high caries burden is poorly explained by eating and oral hygiene habits (relative risk 0.9–1.2) and is almost unaffected by prevention based on fluorides and life style (Källestål, 2005). Dental caries is detected when clinical symptoms arise but not by saliva, bacteria, or life style biomarkers, which do predict cross-sectional caries (Nordlund et al., 2009; Selwitz et al., 2007). Thus, new models to diagnose, prevent, and treat caries based on etiology are needed.

The traditional concept of dental caries is an imbalance in saliva defense, microbial load and life style habits (Nordlund et al., 2009; Selwitz et al., 2007). Saliva and salivary protein pellicles on teeth provide innate immunity clearance of bacteria, tooth homeostasis and adhesion-mediated colonization of both indigenous biofilms of *Actinomyces* and *Streptococcus* species and the caries pathogen *Streptococcus mutans* (Dawes, 2012; Gibbons, 1989; Esberg et al., 2017). Acidification of plaque from eating sugar emanates from *S. mutans* and other acid tolerant species, such as the abundant but moderate acid-producing non-mutans streptococci, and increases caries risk and shift ecology toward acid-producing species (Bradshaw et al., 1989; de Soet et al., 2000; Esberg et al., 2017). Bad oral hygiene, which increases the number of plaque bacteria, and eating sugar may act synergistically in plaque acidification. However, the presence of highly cariogenic adhesin biotypes of *S. mutans*, some with stroke and endocarditis risk (Nakano et al., 2011), points to specific disease mechanisms (Esberg et al., 2017). Despite such advances, dental caries is still grouped into different types based on affected surfaces (e.g. root, occlusal, approximal, coronal), dentition (i.e. primary, permanent) and age group (e.g. early childhood, adolescents, elderly) (Selwitz et al., 2007).

Twin (Boraas et al., 1988), human experimental (Krasse, 2001) and association studies, including genome wide association studies (GWAS) (Werneck et al., 2010), have suggested that genetic factors play a role in caries. Salivary acidic and basic proline-rich proteins (PRPs) (Ayad et al., 2000; Stenudd et al., 2001), agglutinin/DMBT1

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(Jonasson et al., 2007) and HLA (Lehner et al., 1981) involved in innate and adaptive immunity, and proteins involved in enamel formation (Vieira et al., 2008), have been associated with susceptibility to caries and may be used to resolve the etiological types of disease.

Acidic and basic PRPs, encoded by *PRH1-2* and *PRB1-4* respectively, are major salivary innate immunity polypeptides (Bennick, 1987; Hay et al., 1994). Whereas basic PRPs survey and neutralize microbial pathogens in saliva (Burgener et al., 2012), acidic PRPs are pellicle receptors for adhesion of indigenous *Actinomyces* (e.g. *A. oris*). and *Streptococcus* (e.g. *S. gordonii*) species (Gibbons, 1989), which co-operate (mutualism) in biofilm formation (Kolenbrander et al., 2002). Acidic PRPs also modulate adhesion of *S. mutans* (Esberg et al., 2012), provide a barrier against bacterial acids (Bennick, 1987), and are degraded into bioactive peptides that modulate biofilm properties (Drobni et al., 2006). Allelic acidic PRP variants PRP1, PRP2 (*PRH2*) and Pa, Db, PIF (*PRH1*) and their post-translational variants generate many mixed PRP phenotypes in saliva (Azen, 1993; Hay et al., 1994). Db-positive phenotypes have been implicated in the susceptibility to caries and *S. mutans* adhesion and Db-negative phenotypes in resistance to caries and indigenous adhesion (Stenudd et al., 2001; Jonasson et al., 2007).

Salivary agglutinin, which aggregates *S. mutans*, is also known as DMBT1 or gp340 (Prakobphol et al., 2000). The DMBT1 pattern-recognition molecule binds to *S. mutans* and a wide array of microbes, as well as innate and adaptive immunity factors, through its multiple domains (Madsen et al., 2010; Loimaranta et al., 2005). Thus, DMBT1 modulates innate and adaptive immunity, including complement activation, NF-κb signaling via Toll receptors and cellular proliferation (Madsen et al., 2010). A 6.2 kb *dmbt1* deletion variant has been associated with cancer (Madsen et al., 2010) and inflammatory bowel disease via increased NF-κB mediated inflammation in humans (Renner et al., 2007). The corresponding salivary size variant (designated gp340 or DMBT1 size variant I) coincides with increased caries and *S. mutans* adhesion in children (Jonasson et al., 2007).

The present study was performed to further explore the etiology of caries. The primary aim was to replicate and further explore the role of acid PRP polymorphisms and adhesion of indigenous (*A. oris* LY7) and cariogenic (*S. mutans* Ingbritt) model bacteria in caries development. We therefore matched *PRH1*, *PRH2* genetic variation with individual differences in baseline caries and 5-year development of caries in Swedish children measured also for traditional variables and bacterial adhesion.

2. Materials and Methods

2.1. Study Participants

A total of 452 12-year-old children were enrolled as two independent samples ($n = 218$, $n = 234$) from 13 Public Dental Service Clinics in the county of Västerbotten, Sweden. The first sample collected in 2008 included children born in 1996 with caries cases (≥1 decayed, filled surfaces [DFS] in the permanent dentition) and controls (DFS = 0 in 2007) in a 1:1 ratio. The second sample collected in 2010 included children born in 1998 and caries cases (≥ 2 DFS lesions) and controls (DFS = 0 in 2009) in a 2:1 ratio to increase the portion of caries cases in the total sample of 452 children (Table S1). All children in the study received ordinary dental care at the clinics. The exclusion criterion was unwillingness to participate in the study. Both samples were re-examined after 5 years ($n = 390$ children, 14% drop-out). Twenty children had moved out of the area and 42 had repeatedly missed the examination so they were not included in the follow-up. The children received operative treatment and caries prevention between 12 and 17 years of age, and 15% of the children underwent orthodontic treatment with multibrackets after 12 years of age (as established from dental records) according to ordinary routines and policies at the clinics. The Ethics Committee for Human Experiments at Umeå University, Sweden, approved the study, and informed consent was obtained from the children and their parents prior to participation. All parents signed consent to participate.

2.2. Measurement of Caries

Caries were recorded by three dentists (intra- and inter-examiner kappa ≥ 0.98) using a mirror, probe and two bitewing radiographs. The mean number of decayed (enamel caries included), filled surfaces in the permanent dentition (DeFS) were the primary caries outcome measure (Källestål, 2005; Nordlund et al., 2009). The 5-year change in caries (ΔDeFS, increment), a good estimate of incidence rate in this age group and population (Källestål and Stenlund, 2003), was calculated by subtracting latest DeFS measure from earliest DeFS measure, dividing it by the number of observed years, and then multiplying by 5. The 1:1 ratio in the first sample and increased 2:1 ratio in the second sample and DeFS index generated a continuous gradient of discriminatory caries DeFS scores in the entire sample at baseline (Table S1) (Esberg et al., 2017).

2.3. Collection of Biological Samples

Parotid and whole saliva (PS and WS, respectively) secretions were measured (mL/min), collected and stored frozen at −80 °C (Nordlund et al., 2009; Stenudd et al., 2001). All saliva analyses used baseline samples. Plaque was collected by pelleting from the buccal surfaces of premolars and the first molar of the left lower jaw (teeth 34–36). Fresh WS and plaque (pl) sampled at baseline were cultured on selective substrates to establish whether children infected with *S. mutans* (ms) or lactobacilli (lbc), as well as the proportion of ms out of oral streptococci (strept) in saliva (% ms) and plaque (% ms pl) (Nordlund et al., 2009).

2.4. Sociodemographic and Life Style Data

Sociodemographic data (sex and ethnicity) were collected from parents via pre-posted questionnaires, and oral behavior data, such as oral hygiene, intake frequency of sweets (e.g., cookies, biscuits, ice cream, or dried fruit) and sugary drinks (never, once per month, once per week, several times per week, once per day, several times per day), and the use of extra fluoride were collected from the children at the clinic using a questionnaire that was previously utilized in a cohort of 3400 12-year-old children (Källestål, 2005). Strict Swedish ethnicity was defined when both parents reported Swedish ethnicity. Oral hygiene was measured as how often the child brushes his/her teeth: irregularly, once per day, twice per day, more than twice per day. Extra fluoride treatment in addition to fluoride in toothpaste was recorded as: none, fluoride mouth rinse more than once per month, fluoride-containing chewing gum 1–3 pieces per day, or 1–3 fluoride tablets per day.

2.5. Typing Acidic PRP Phenotypes in Saliva

Acidic PRPs in PS were typed using native alkaline electrophoresis as described (Azen and Yu, 1984; Esberg et al., 2012). Saliva was lyophilized and re-dissolved to the same volume using distilled water containing 1% glycine, 10% glycerol and 0,025% bromphenol blue. Roughly 40 test and reference saliva samples (each 25 μL) were separated in a single run under native conditions on 7.0% (w/w) polyacrylamide (27:1 bisacrylamide w/w) gels using a 1.5 mM TRIS, 38 mM glycine electrophoresis buffer (pH 8.4) and a PROTEAN IIxi apparatus (BioRad). The gels were stained with 0.1% Commassie Brilliant Blue (CBB-R250) in 20% trichloroacetic acid for 1 h and destained over night in 2% acetic acid. A total of 218 samples were analyzed and typed in a double-blind fashion, with the vast majority typable ($n = 213$), and quantified via densitometry (optical density x mm^2) using Molecular Analyst 1.5 software (BioRad). Single representatives of each PRP phenotype were validated using high-performance liquid chromatography on a Bischoff LC-caDI22-14 HPLC system (Bischoff, Germany) and a Gen-Pak™ FAX

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