EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Streptococcus Mutans Adhesin Biotypes that Match and Predict Individual Caries Development

Anders Esberg [a,1], Nongfei Sheng [a,1], Lena Mårell [a], Rolf Claesson [a], Karina Persson [b], Thomas Borén [c], Nicklas Strömberg [a,*]

[a] Department of Odontology/cariology, Umeå University, SE-901 87 Umeå, Sweden
[b] Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden
[c] Department of Medical Biochemistry and Biophysics, Umeå University, SE-901 87 Umeå, Sweden

ARTICLE INFO

Article history:
Received 4 July 2017
Received in revised form 19 September 2017
Accepted 19 September 2017
Available online xxxx

Keywords:
Streptococcus mutans
Dental caries
Chronic infections
Virulence
SpaP
Adhesion

ABSTRACT

Dental caries, which affects billions of people, is a chronic infectious disease that involves *Streptococcus mutans*, which is nevertheless a poor predictor of individual caries development. We therefore investigated if adhesin types of *S.mutans* with sucrose-independent adhesion to host DMBT1 (*i.e.* SpaP A, B or C) and collagen (*i.e.* Cnm, Cbm) match and predict individual differences in caries development. The adhesin types were measured in whole saliva by qPCR in 452 12-year-old Swedish children and related to caries at baseline and prospectively at a 5-year follow-up. Strains isolated from the children were explored for genetic and phenotypic properties. The presence of SpaP B and Cnm subtypes coincided with increased 5-year caries increment, and their binding to DMBT1 and saliva correlated with individual caries scores. The SpaP B subtypes are enriched in amino acid substitutions that coincided with caries and binding and specify biotypes of *S. mutans* with increased acid tolerance. The findings reveal adhesin subtypes of *S. mutans* that match and predict individual differences in caries development and provide a rationale for individualized oral care.

1. Introduction

Dental caries, a persistent infectious disease, affects billions of people with large individual differences in numbers of caries lesions and activity (Selwitz et al., 2007; Nordlund et al., 2009; Kassebaum et al., 2015). At least half of school children and the vast majority of adults worldwide experience accordingly caries, and the economic burden of caries and dental diseases represents about 4.6% of global health expenditures (Listl et al., 2015). However, in Sweden and countries with a low caries prevalence, many children are either free of caries or have a low disease level while 15–20% have a high caries burden (Selwitz et al., 2007; Källestål, 2005). These high caries cases are poorly explained by life style-related variables, such as sugar consumption, oral hygiene, or fluoride use (*i.e.* relative risks 0.9–1.2) and seem to be largely unaffected by traditional prevention based on the same factors (Källestål, 2005). Accordingly, life style, saliva, and bacteria are poor predictors of caries development (Selwitz et al., 2007; Nordlund et al., 2009), and better etiological models and diagnostic and preventive tools are needed.

In spite of many advances in etiological and biochemical mechanisms related to caries disease during the last decades (Selwitz et al., 2007; Nobbs et al., 2009), dental caries is still generally considered a life style condition in which plaque acidification from sugar consumption shifts the oral ecology toward aciduric and acid-producing species; of these, *Streptococcus mutans* is the most well-known (Selwitz et al., 2007; de Soet et al., 2000; Cornejo et al., 2012; Palmer et al., 2013; Aas et al., 2008). The bacterium was early identified as the primary caries pathogen and vaccine candidate, but the inability of *S. mutans*, or any other species, to match or predict individual caries development has hampered its use in individualized oral care (Selwitz et al., 2007; Nordlund et al., 2009).

S. mutans (serotypes *c* > > *e* > *f* and *k*) infects the oral cavity of 40–80% of subjects depending on age, ethnicity and disease prevalence and colonize individuals with a dominant and largely unique genotype transmitted from parent to child through saliva (Lapirattanakul et al., 2008; Esberg et al., 2012). Cariogenic properties besides acid production that dissolves enamel are oxygen tolerance, bacteriocin production and adhesion and colonization at tooth surfaces (Cornejo et al., 2012; Palmer et al., 2013). Together, sucrose-independent adhesion of SpaP or Cnm adhesins to host salivary agglutinin/DMBT1 and collagen, respectively, and sucrose-dependent adhesion of glycosyltransferases to bacterial polysaccharides allow *S. mutans* to colonize naked and cavitated tooth surface and promote plaque growth (Nobbs et al., 2009).

Salivary agglutinin, originally identified by its ability to agglutinate *S. mutans*, is identical to DMBT1 or gp340 (Prakobphol et al., 2000).

* Corresponding author.
E-mail address: nicklas.stromberg@umu.se (N. Strömberg).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.ebiom.2017.09.027

Salivary agglutinin or DMBT1 is a pattern-recognition receptor composed of multiple domains designed to bind *S. mutans* and a wide array of microbes along with many innate and adaptive immunity factors (Madsen et al., 2010; Loimaranta et al., 2005; Loimaranta et al., 2009). DMBT1 thus 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 by increased NF-κb mediated inflammation in human cases (Renner et al., 2007). The corresponding salivary size variant (designated gp340 or DMBT1 size variant I) coincides with increased levels of caries and *S. mutans* adhesion in children (Jonasson et al., 2007).

The AgI/II adhesin SpaP of *S. mutans* and AgI/II orthologs in various oral streptococci have been extensively explored for structural organization and interaction with its salivary receptor DMBT1 (Brady et al., 2010). AgI/II adhesins have an unusual tertiary structure where a central variable domain (V-domain) is presented at the tip of a stalk formed by intertwined, flanking alanine- and proline-rich regions (Larson et al., 2010). The carboxy-terminal domain (C-domain) to which a small N-terminal domain is bound is attached to the cell-wall via a cell-wall anchoring region (Heim et al., 2014). The SpaP binding sites for the DMBT1 agglutinin localize to the V-domain and the C-domain (Heim et al., 2013), and SpaP holds a supramolecular functional architecture at the cell surface (Heim et al., 2015). The SpaP adhesin harbors variants A, B and C (also referred to as A, B_1, and B_2) with clustered amino acid substitutions and different DMBT1 binding levels despite similar levels of SpaP expression (Esberg et al., 2012). The interaction of SpaP and AgI/II orthologs with DMBT1 depends on whether DMBT1 is in the fluid- or surface-bound form and also depending on the *S. mutans* strain (Loimaranta et al., 2005; Heim et al., 2013), suggesting that SpaP polymorphisms may modulate adhesion and aggregation by DMBT1 and consequently caries activity.

S. mutans also harbors collagen-binding Cnm and Cbm adhesins in 15% and 3% of clinical isolates, respectively, and more frequently in serotype *e*, *f* and *k* than in *c* isolates (Avilés-Reyes et al., 2017). Cnm/Cbm are highly homologous and consist, similar to collagen-binding proteins in *Staphylococcus aureus* and other bacteria (Kang et al., 2013; Xu et al., 2004), of an N-terminal collagen-binding domain presented on a stalk formed by several threonine-rich repeat domains and a cell wall anchoring region (Nomura et al., 2009, 2012). Whereas *S. mutans* (serotypes *c*, *e*, *f* and *k*) may cause infective endocarditis (Hoshino et al., 2005), serotypes *f* and *k* in which Cnm and Cbm are more frequent coincide with inflammatory bowel disease (Kojima et al., 2012) and Cnm phenotypes with hemorrhagic stroke (Nakano et al., 2011; Watanabe et al., 2016). The Cnm/Cbm phenotypes also increase strain virulence in endocarditis (Nomura et al., 2014). As potential virulence mechanisms in these so-called extra-oral infections, Cnm/Cbm mediate invasion of endothelial cells (Abranches et al., 2011, Review), formation of thrombus or heart valve vegetations or inhibition of platelet aggregation and wound healing (Nakano et al., 2011; Avilés-Reyes et al., 2017).

The aim of this study was to clarify the role of *S. mutans* as a caries pathogen by matching sucrose-independent adhesin types SpaP A, B, C and Cnm/Cbm with individual differences in caries development. We analyzed 452 Swedish children for the presence of *S. mutans* adhesin types and related them to baseline caries and 5-year increment and to cariogenic properties.

2. Methods

2.1. Study Participants and Registration of Caries

A total of 452 12-year-old children were collected as two independent samples ($n = 218$, $n = 234$) from 13 clinics in the northern county of Västerbotten, Sweden (Fig. S1). Included in the first sample were children born in 1996 and caries cases (≥1 Decayed and Filled Surfaces, DFS, in the permanent dentition) and caries-free controls in a 1:1 ratio; and for the second sample, they were born in 1998 and caries cases (≥2 DFS) and controls in a 2.1 ratio, and receiving ordinary dental care at Public Dental Service Clinics. The exclusion criterion was unwillingness to participate in the study. Both samples were re-examined after 5 years, for a total of 390 examined children with 14% drop-out rate (62/452) for having moved out of the area (20 children) or repeatedly missed the examination (42 children). The children received operative treatment and prevention of caries between 12 and 17 years of age, and 15% of the children orthodontic treatment with multibrackets after 12 years of age (as established from dental records), according to ordinary routines and policies at the clinics. The study was approved by the Ethics Committee for Human Experiments at Umeå University, Sweden, and informed consent was obtained from the children and their parents before participation. All parents signed consent to participate.

Caries was recorded by three dentists (intra- and inter-examiner kappa ≥0.979) by a mirror, probe and two bitewing radiographs and mean number of Decayed (enamel caries included), Filled Surfaces in the permanent dentition (DeFS) was the primary caries outcome measure. The 5-year caries increment (ΔDeFS) was calculated by subtracting latest from earliest DeFS, dividing that value by the number of observed years and multiplying the result by 5. The 1:1 ratio (first sample) and increased 2:1 ratio (second sample) of caries cases *versus* controls and DeFS index generated a continuous gradient of discriminatory caries DeFS scores in the entire sample at baseline (Fig. S1, Table S1).

2.2. Genotyping of S. Mutans Adhesin Types in Whole Saliva

Whole saliva, collected by chewing on paraffin for 5 min that was stored frozen at −80 °C, was genotyped for *cnm*, *cbm* and *spaP A*, *B* and *C* status using bacterial DNA prepared with the GenElute™ bacterial genome DNA kit (Sigma-Aldrich, Sweden) and quantitative polymerase chain reaction (qPCR) using the KAPA SYBR Fast Universal qPCR kit (Tectum, Sweden). The primers for *cnm* and *cbm* were as described in Table S2 and did not cross-react in between or with other templates. The primers for *spaP A*, *B*, and *C* were from the *spaP* sequences (Table S2) and selected from prior testing and lack of cross-reactivity between *A*, *B*, and *C* or with DNA from oral streptococci with *spaP* analogs. The genotyping used internal standards and quantitative calibration curves based on dilutions of DNA purified from a reference genotype of each adhesin type, and cut-off values for *A* (3000 pg), *B* (3000 pg), *C* (6000 pg), *cnm* (3000 pg), and *cbm* (1000 pg).

2.3. Quantification of S. Mutans in Whole Saliva

S. mutans in whole saliva was quantified by culturing and qPCR (Yano et al., 2002). Serial dilutions of whole saliva were cultured on MSB agar plates and counted for colony-forming units of *S. mutans* (designated as ms). Plaque DNA purified from whole saliva samples was measured by qPCR using the KAPA SYBR FAST Universal qPCR kit (Sigma-Aldrich, Sweden) and Corbett Rotor-Gene 6000 and *S. mutans* specific primers (Table S2). Quantitative calibration curves from DNA prepared from serial dilutions of *S. mutans* strain Ingbritt (Esberg et al., 2012) were used to transform the qPCR responses into colony-forming units.

2.4. Isolation and Typing of S. Mutans Strains from Plaque

Plaque was collected from buccal surfaces of teeth 34–36 (premolars and the first molar of the left lower jaw) in caries-free children or from caries lesion in children with tooth decay (Esberg et al., 2012). Strains of *S. mutans* were isolated from the plaque samples by culturing on MSB agar plates and typed by Rapid ID 32 STREP Kit (Bio Merieux, La Balme les Grottes, France) metabolic tests (Hoshino et al., 2005). A total of 321 strains from 214 out of 217 infected children, one strain from each of the 214 children and additional isolates from 70 extreme

Please cite this article as: Esberg, A., et al., *Streptococcus Mutans* Adhesin Biotypes that Match and Predict Individual Caries Development, EBioMedicine (2017), https://doi.org/10.1016/j.ebiom.2017.09.027