Archives of Oral Biology 80 (2017) 75–81

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Research Paper

The association of Enamelin, Lactoferrin, and Tumour necrosis factor alpha gene polymorphisms with high caries susceptibility in Chinese children under 4 years old

Mengchen Wang, Man Qin, Bin Xia*

Department of Pediatric Dentistry, Peking University School and Hospital of Stomatology, 22 Zhongguancun Avenue South, Haidian District, Beijing 100081, PR China

ARTICLE INFO

Article history:
Received 4 November 2016
Received in revised form 15 March 2017
Accepted 29 March 2017

Keywords:
Caries
Gene polymorphisms
Oral behavioural habits

ABSTRACT

Objective: The aim of this study was to assess the role of *ENAM* rs3796703, *LTF* rs1126478, and *TNF-α* rs1800629 in high caries susceptibility.
Design: The present case–control study included 1005 unrelated children under 4 years old: 505 with severe caries (dmft index ≥4) and 500 who were caries-free (dmft index = 0 and without white-spot lesions). Questionnaires were obtained from parents and gardians about the children's diet and oral behavioural habits. All the children received dental examinations and oral swabbing for human genomic DNA collection. *ENAM* rs3796703, *LTF* rs1126478, and *TNF-α* rs1800629 were genotyped by Sanger sequencing.
Results: The frequency of the *ENAM* rs3796703 T allele (6.7% in the caries group and 4.2% in the caries-free group), CT genotype (12.7% in the caries group and 8.4% in the caries-free group), *TNF-α* rs1800629 A allele (4.8% in the caries group and 6.8% in the caries-free group), and AG genotype (8.7% in the caries group and 13.2% in the caries-free group) were significantly different between the caries and caries-free groups ($p < 0.05$). No significant difference was found in the *LTF* rs1126478 allele frequency and genotype distribution between the two groups. The *ENAM* rs3796703 CT genotype increased caries susceptibility by 60.9% compared to the CC genotype ($\beta = 0.746$, OR = 1.609), and the *TNF-α* rs1800629 AG genotype reduced caries susceptibility by 47.4% compared to the GG genotype ($\beta = -0.642$, OR = 0.526). In terms of habits covariates, prolongation of night feeding time by 1 month increased caries susceptibility by 3.3% ($\beta = 0.033$, OR = 1.033); additionally, sweets and acidic drinks consumption 1–2 times per day increased caries susceptibility by 218.2% ($\beta = 1.158$, OR = 3.182), and consumption 3 or more times pe/r day increased susceptibility by 883.5% ($\beta = 2.286$, OR = 9.835) compared to non-consumption. Topical fluoride application decreased caries susceptibility by 43.0% ($\beta = -0.562$, OR = 0.570).
Conclusions: The *ENAM* and *TNF-α* genes are likely associated with caries experience in Chinese children. The *ENAM* rs3796703 CT genotype might be involved in caries susceptibility, while *TNF-α* rs1800629 AG genotype might be involved in caries protection.

1. Introduction

In China, caries remains the biggest threat for the oral health of children. Caries prevalence for children aged 1 to 6 years was 0.3%, 17.3%, 40.2%, 54.4%, 66.1%, and 70.7%, respectively (Zhang et al., 2016). If left untreated, caries decay may lead to intense pain and occlusion disorder, decrease masticatory performance, incur focal infection and affect general health. However, the prevention of this lifestyle-related disease is not an easy matter because of the complexity of its aetiology. Dietary habits, hygiene practices, and fluoride intake were all relevant factors for this disease (Kuriakose, Prasannan, Remya, Kurian, & Sreejith, 2015). Individual susceptibility also played a role. The higher occurrence of caries in specific individuals (termed polarisation) had been widely discussed (Tanner et al., 2013). As early as the 20th century, scholars had already noticed the phenomenon of family aggregation and

Abbreviations: ENAM, enamlin; LTF, lactoferrin; TNF-α, tumor necrosis factor alpha; SNP, single nucleotide polymorphism; dmft, decayed, missing due to caries, filled teeth; MAF, minor allele frequency.

* Corresponding author at: Department of Pediatric Dentistry, Peking University School and Hospital of Stomatology, 22 Zhongguancun Avenue South, Haidian District, Beijing 100081, PR China.
E-mail addresses: wangdoudou_bjmu@126.com (M. Wang), qinman@gmail.com (M. Qin), summerinbeijing@vip.sina.com (B. Xia).

http://dx.doi.org/10.1016/j.archoralbio.2017.03.023
0003-9969/

heritability of caries (Klein & Bethesda, 1946). Twin studies found high concurrence of caries status in both dizygotic and monozygotic twins, further verifying the influence of heredity on caries (Boraas, Messer, & Till, 1988). These findings all raised possibilities for associations between genes and caries. In recent years, thanks to progresses in molecular genetics research, genetic association studies were applied to detect the hereditary characteristics of caries, and gradually revealed how various genes might affect caries. Generally, four kinds of genes were involved in caries susceptibility: enamel formation genes, immune response genes, saliva protein genes, and taste genes (Werneck, Mira, & Trevilatto, 2010).

Enamelin (ENAM) is the largest enamel matrix protein. The protein was coded by the *ENAM* gene and was exclusively expressed by ameloblasts at the secretory and early maturation stages. It concentrated near the mineralisation front by the C-terminus and facilitates crystal elongation and organisation through self-assembling nanostructures (Hu et al., 2014). *ENAM* mutations could cause hereditary enamel hypoplasia. Polymorphisms of *ENAM* gene probably influenced enamel thickness (Daubert et al., 2016) and were also associated with magnesium and calcium concentrations in teeth (Halusic et al., 2014), further facilitated the progression of the carious process. *ENAM* rs3796704, rs12640848 and rs7671281 were reported to be associated with caries susceptibility (Gerreth, Zaorska, Zabel, Borysewicz-Lewicka, & Nowicki, 2016; Abbasoğlu et al., 2015). *ENAM* rs12640848 was an intron variant and had no effect on gene expression. The functional locus within exon sequence including rs3796703 deserve more attention.

Lactoferrin (LTF) is an iron-binding glycoprotein that has a broad spectrum of antimicrobial activity and is associated with immunoreaction and inflammation processes. High concentrations of this multifunctional protein in saliva might enable oral tissues to withstand the attack of bacteria, viruses, and fungi. It also displayed bioactivities against other acid-producing microbes (Velliyagounder et al., 2003) and regulated the aggregation and development of dental plaque biofilm, thus influenced caries susceptibility. An *in vitro* function study suggested that lactoferrin could exert anti-*Streptococcus mutans* effect in saliva (Fine et al., 2013) by killing Gram-positive bacteria, both directly (through direct ion-binding interactions with bacteria by its strongly basic N-terminal region) and indirectly (through sequestering the iron that bacteria require for growth). *LTF* rs1126478 exhibited stronger antibacterial effect and was involved with some oral infectious diseases, such as localized juvenile periodontitis and caries, by applying modifications of inflammatory response (Azevedo, Pecharki, Brancher, & Cordeiro, 2010; Velliyagounder et al., 2003). The association between rs1126478 and sever caries in Chinese children is still in need of verification.

Tumour necrosis factor alpha (TNF-α) is a pleiotropic cytokine produced mainly by monocytes and macrophages. Caries was a chronic infectious disease, which could elicit immune responses (Gómez, Jaramillo, Moreno, Roa, & Rodríguez, 2015). Elevated TNF-α had been detected in unstimulated whole saliva of caries patients as one of the initial responses of the host immune system to pathological insults (Gornowicz et al., 2012). Under the actions of cariogenic bacteria, TNF-α was highly expressed in caries-affected dental pulp and/or odontoblasts (Gornowicz et al., 2012). *TNF-α* rs1800629 was thought to be involved in systemic inflammation and autoimmune diseases. Associations were found between *TNF-α* rs1800629 allele A frequency and aggressive periodontitis patients with clinical attachment level ≥4 mm in Turkish population (Özer Yücel et al., 2015). But it is still unknown whether *TNF-α* rs1800629 affects caries susceptibility.

In this study, we investigated the distributional difference of *ENAM* rs3796703, *LTF* rs1126478, and *TNF-α* rs1800629 polymorphic locus in severe caries and caries-free children, with the aim of assessing the role of these genetic factors in high caries susceptibility.

2. Materials and methods

2.1. Sample size calculation

Caries prevalence was 47% for 3-year-old children living in Beijing (Li, Miao, & Zhang, 2012). When the alpha level set as 0.05 and expected odds ratio at 1.6, a sample size approaching 470 children in each group was required in order to achieve an enough statistics power (β = 0.8). Sample size calculation was carried out using Quanto software (http://biostats.usc.edu/Quanto.html).

2.2. Participants

All the children in the caries group were selected from the outpatients who came to the Pediatric Department in the Peking University Hospital of Stomatology during the period of August 2015 to September 2016. The caries-free children came from 16 kindergarderns in Haidian District, Beijing, at the same time.

The inclusion criteria were: child under 4 years old; the child's mother had lived in Beijing during the whole pregnancy and the child was brought up in Beijing; mother had no pregnancy-related disease; child was born by normal full-term delivery (range 37 to 42 weeks in pregnancy); child without systemic diseases; child's teeth without enamel hypoplasia or dentin hypoplasia; child's dmft index (number of decayed, missing, filled teeth) ≥4 in the caries group; child's dmft index = 0 in the caries-free group. Written informed consents were obtained from the parents or guardians of all the participants prior to the enrolment. Questionnaires were filled by guardians about the children's basic information, diet, oral behavioural habits and application of topical fluoride.

Finally, a total of 505 children with severe caries (dmft index ≥4) and 500 caries-free children were incorporated.

The study design, protocol and informed consent details were approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-201628050).

2.3. Dental examination

Teeth that were decayed, missing due to caries, or filled were recorded according to the modified World Health Organization [1997] caries diagnostic criteria. At the beginning of the oral examination, possible food debris was removed by a piece of sterilized cotton, and the teeth surfaces were gently dried by air scavenging before taking record of the caries condition. In the caries group, white-spot lesions were also recorded but not calculated in the dmft index. No radiographs were taken. Caries examination and diagnosis was performed by a single examiner and the examination consistency was ensured by examining 20 children before the initiation of the study. The κ value for intra-examiner agreement was 0.861.

2.4. DNA collection

Sterile buccal swabs were used to obtain DNA by swabbing the buccal mucosa, and were stored at room temperature before transporting to laboratory within one day. Genomic DNA was extracted with a TIANamp Swab DNA Kit (TIANGEN BIOTECH, BEIJING, China), according to the manufacturer's instructions. The isolated DNA was stored at −20 °C until use.