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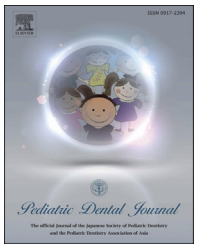

Original Article

Analysis of genetic factors influencing susceptibility to dental caries by using a chromosome 2 substitution mouse strain

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ABSTRACT

Objective: Dental caries, a major public health concern worldwide, is influenced by both environmental and genetic factors. The objective of this study was to identify genetic factors influencing susceptibility to dental caries using mouse strains that show differences in caries susceptibility.

Materials and methods: We investigated whether differences in phenotypic traits (salivary secretion volume, salivary buffering capacity, submandibular gland histology, and enamel hardness) are associated with different susceptibility to dental caries in the three mouse strains C3H/HeSlc, C57BL/6Slc, and B6-Chr.2^{C3H}. C3H/HeSlc is resistant to dental caries. Conversely, C57BL/6Slc is highly susceptible to dental caries. B6-Chr.2^{C3H} was constructed by substituting chromosome 2 of C3H/HeSlc into C57BL/6Slc. We also examined the expression level of two candidate genes, *Slc24a3* and *Slc9a8*, on chromosome 2, which are considered as regulatory factors of salivary secretion volume.

Results: The salivary secretion volume of B6-Chr.2^{C3H} was higher than that of C57BL/6Slc, while no clear differences were observed in any other traits. Further, we found that the expression levels of *Slc9a8* and *Slc24a3* in the submandibular gland were significantly different between C57BL/6Slc and C3H/HeSlc or B6-Chr.2^{C3H}.

Conclusion: We concluded that salivary secretion is one of the main factors affecting susceptibility to dental caries, and that the genes influencing salivary secretion volume are located on chromosome 2 in mice and play an important role in susceptibility to dental caries.

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1. Introduction

Dental caries is the most common, chronic, and multifactorial disease worldwide [1]. Susceptibility to dental caries is affected by both environmental and genetic factors [2]. Multiple studies have investigated various environmental factors influencing susceptibility, such as dietary sugar level or oral flora [3,4]; however, little is known about the host genetic factors.

Studies on twins and families have supported the role of genetic inheritance and estimated the genetic contribution to dental caries development at 40–60% [5–7]. In addition, previous studies have identified some genes involved in susceptibility to dental caries, including enamel formation genes such as *AMELX*, *ENAM*, *AMBN*, and *TUFTI* [8]; genes affecting amelogenesis ability such as *ACTN2* [9]; immunoresponse genes such as *CD14* [10] and *DEFB1* [11]; and genes influencing taste preference such as *TAS2R38* [12] and *TAS1R2* [12,13].

The importance of genetic factors in dental caries has also been supported by the existence of susceptibility loci for dental caries in animal models [14–16]. A previous study identified two inbred mouse strains, one resistant (C3H/HeSlc) and one highly susceptible (C57BL/6Slc), as indicated by the 20 times higher caries score, and revealed that genetic factors play a role in susceptibility [16,17]. Genetic analysis of these strains predicted a quantitative trait locus on chromosome 2 that was highly associated with dental caries [15]. To verify the effect of this quantitative trait locus, the chromosome substitution strain B6-Chr.2^{C3H} was constructed, in which chromosome 2 of C57BL/6Slc was replaced with that from the low-caries-susceptibility strain C3H/HeSlc [18]. B6-Chr.2^{C3H} showed approximately six times lower susceptibility to dental caries, as indicated by the caries score, suggesting the role of this quantitative trait locus in disease susceptibility [17].

The objective of this study was to identify differences in phenotypic traits (saliva volume, salivary buffering capacity, submandibular gland histology, and enamel hardness) influencing susceptibility to dental caries using C57BL/6Slc, C3H/HeSlc, and B6-Chr.2^{C3H}. Further, we examined the expression levels of two candidate susceptibility genes, *Slc9a8* and *Slc24a3*, on chromosome 2 [19,20]. *Slc24a3* encodes NCKX3, a K^+-dependent Na^+/Ca^{2+}exchanger that plays a critical role in the transport of one Ca^{2+} and one K^+ ion in exchange for four extracellular Na^+ ions [21]. *Slc9a8* encodes NHE8, a member of the Na^+/H^+ exchanger family of integral membrane transporter proteins [22]. These genes are expressed in the submandibular gland, where they are considered to control the salivary secretion volume [23–25].

2. Materials and methods

2.1. Animals

Three mouse strains, C57BL/6Slc, C3H/HeSlc, and B6-Chr.2^{C3H}, were used in this study. Twenty-day-old mice were obtained from Sankyo Lab Service Co. (Tokyo, Japan) and maintained in clean racks at room temperature (25 ± 1 °C) with a relative humidity of 55 ± 5% and a light cycle of 12 h. Mice were allowed access *ad libitum* to a commercial diet (MR Breeder; Nihon Nohsan Co, Kagawa, Japan) and pure bottled water. B6-Chr.2^{C3H} mice were developed in our laboratory and maintained at Sankyo Lab Service Co. All the animal-use protocols of this study were reviewed and approved by the Nihon University Institutional Review Board (Chiba, Japan; AP13MD017).

2.2. Time-based measurement of stimulated salivary secretion volume

The stimulated salivary secretion volume was measured as described previously [26]. At 49 days of age, five male mice per strain (C57BL/6Slc, C3H/HeSlc, and B6-Chr.2^{C3H}) were intraperitoneally administered thiamylal sodium diluted 2:3 with phosphate buffer solution (1.50 mg/100 g body weight). After 5 min, they were intraperitoneally administered isoproterenol (0.20 μg 100 g body weight) and pilocarpine (0.05 μg 100 g body weight). Saliva was collected from the oral cavity of each mouse with a pipette at 10-min intervals following the administration of isoproterenol and pilocarpine (0–10 min, 10–20 min, and 20–30 min) and weighed.

2.3. Examination of pH and buffering capacity

The pH and buffering capacity of the saliva were measured using a manual pH meter as described previously [27]. Saliva was collected from five male mice of each strain (C57BL/6Slc, C3H/HeSlc, and B6-Chr.2^{C3H}) at 49 days of age, and the pH was measured using a Checkbuff pH meter (Horiba, Kyoto, Japan). Then, a 50 μL saliva sample was added to 50 μL of acid solution (pH 4.0; Horiba). The mixture was shaken and allowed to stand for 30 s before the final pH was measured using Checkbuff. The buffering capacity was calculated from the difference in pH before and after the addition of buffer solution.

2.4. Histological analysis of the submandibular gland

Histological analysis of the submandibular gland was performed as described previously [26]. At 49 days of age, three male mice of each strain (C57BL/6Slc, C3H/HeSlc, and B6-Chr.2^{C3H}) were anesthetized with pentobarbital diluted 1:10 with normal saline (40 mg/mL), and fixed by perfusion with phosphate-buffered saline and 4% paraformaldehyde. The left submandibular gland was extracted, and paraffin-embedded blocks were prepared. Blocks were sliced into 4 μm sections with a microtome and stained with hematoxylin–eosin. The slices were examined and photographed with an all-in-one fluorescence microscope (Biorevo BZ-9000 Generation II; Keyence, Osaka, Japan). The duct area was traced on histological images. A scanner was used to redigitize tracings with an image analysis resolution of 300 dpi, and the ImageJ version 1.33u (NIH, Bethesda, MD, USA) image analysis software was used for measurements. The area of a given region on the image was measured and designated as (a), and the duct area in (a) was measured and designated as (b). The proportion of the duct area in (a) was calculated as (b/a). The proportion of the acinar area in (a) was calculated as $[(a - b)/a]$. In one mouse per strain, the area was measured at six sites on histological images, and the mean was calculated (Fig. 1).