PEDIATRIC DENTAL JOURNAL XXX (2015) 1–8

Available online at www.sciencedirect.com

Pediatric Dental Journal

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

Original Article

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

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ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form
15 October 2015
Accepted 31 October 2015
Available online xxx

Keywords:
Consomic mouse
Dental caries
Susceptibility gene

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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http://dx.doi.org/10.1016/j.pdj.2015.10.002

Please cite this article in press as: Hiraki A, Shimizu K, Analysis of genetic factors influencing susceptibility to dental caries by using a chromosome 2 substitution mouse strain, Pediatric Dental Journal (2015), http://dx.doi.org/10.1016/j.pdj.2015.10.002

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

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Received 21 August 2015, Received in revised form 15 October 2015, Accepted 31 October 2015

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Running article title: Genetic factors and susceptibility to dental caries

Running author names: A. Hiraki et al.

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*, which are located on chromosome 2 and 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.

Keywords: consomic mouse, dental caries, susceptibility gene

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, C3H/HeSlc, C57BL/6Slc 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 hours. 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 (C3H/HeSlc, C57BL/6Slc 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 minutes, they were intraperitoneally administered isoproterenol (0.20 μg 100g 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-minute intervals following the administration of isoproterenol and pilocarpine (0–10 minutes, 10–20 minutes, and 20–30 minutes) 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 (C3H/HeSlc, C57BL/6Slc 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 seconds 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 (C3H/HeSlc, C57BL/6Slc 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 (6.75 μm × 5 μm, 8.95×10^6 pixcel) 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 (Figure 1).

2.5 Measurement of enamel hardness

Enamel hardness was measured as described previously [26]. At 49 days of age, mandibles were extracted from three male mice per strain (C3H/HeSlc, C57BL/6Slc and

B6-Chr.2^{C3H}), immersed in 2% KOH, and incubated at 42°C for 24 hours. The soft tissue was removed, and the remaining mandible was fixed to a glass slide with composite resin. The enamel hardness of the lingual side of the left first molar was measured with a Dynamic Ultra Micro Hardness Tester (Shimadzu, Kyoto, Japan). This instrument measures dynamic indentation depths using Berkovich indenters [26,28]. In one mouse per strain, enamel hardness was measured at three sites on the left first molar, and the mean was calculated.

2.6 Reverse transcription polymerase chain reaction

At 49 days of age, the submandibular glands were extracted from three male mice per strain (C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H}). Extracted tissues were immersed in RNA*later*® solution and stored at 4°C until RNA extraction, which was carried out using RNeasy Mini Kit (Qiagen, Venlo, The Netherlands). We designed primers for reverse transcription polymerase chain reaction (RT-PCR) of *Slc9a8* and *Slc24a3* (Table 1)based on Ensembl (http://asia.ensembl.org/index.html). A glyceraldehyde-3-phosphate dehydrogenase gene (*Gapdh*) was used as an endogenous control. RT-PCR products spanned at least one intron, allowing cDNA products to be distinguished from potential genomic DNA products. Total RNA (100 ng) was reverse-transcribed into complementary DNA (cDNA) with a PrimeScript High Fidelity RT-PCR kit (Takara, Tokyo, Japan). The PCR conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds in the GeneAmp® PCR System 9700 (Applied Biosystems, Tokyo, Japan). The products were electrophored using 2%

agarose gel and analyzed.

2.7 Reverse transcription quantitative polymerase chain reaction

At 49 days of age, the submandibular glands were extracted from three male mice per strain (C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H}). Extracted tissues were immersed in RNA*later*® solution and stored at 4°C until RNA extraction, which was carried out using RNeasy Mini Kit (Qiagen, Venlo, The Netherlands). The sequences of primers for reverse transcription quantitative polymerase chain reaction (RT-qPCR) were the same as those used for RT-PCR (Table 1). *Gapdh* was used as an endogenous control. Total RNA (100 ng) was reverse-transcribed into cDNA with Takara Thermal Cycler Dice mini (Takara, Shiga, Japan). RT-qPCR conditions were as follows: DNA denaturation at 94°C, for 5 minutes, followed by 45 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. Thermo Scientific DyNAmo SYBR Green qPCR kits (Thermo Fisher Scientific, Kanagawa, Japan) and DNA Engine Opticon (Bio-Rad, Hercules, CA, USA) was used for RT-qPCR. The levels relative to *Gapdh* mRNA in each sample were calculated from standard curves obtained by sequential dilution of total RNA extracted from the submandibular gland.

2.8 Statistical analysis

The results are presented as the mean ± standard deviation. One-way factorial analysis of variance and Tukey's test were used to compare the values between strains, and differences were considered significant at $p < 0.05$.

3. Results

3.1 Time-based measurement of stimulated salivary secretion volume

As shown in Figure 2, the salivary secretion volumes of C3H/HeSlc (0.24 ± 0.04 g) and B6-Chr.2^{C3H} (0.22 ± 0.02 g) were significantly higher than that of C57BL/6Slc (0.07 ± 0.02 g) at 0–10 minutes after the administration of isoproterenol and pilocarpine. At 10–20 minutes and 20–30 minutes after administration, the salivary secretion volumes of C3H/HeSlc (0.18 ± 0.02 g and 0.12 ± 0.03 g, respectively) were significantly higher than those of C57BL/6Slc (0.04 ± 0.02 g and 0.03 ± 0.01 g, respectively) and B6-Chr.2^{C3H} (0.14 ± 0.06 g and 0.06 ± 0.03 g, respectively); however, no significant differences were found between C57BL/6Slc and B6-Chr.2^{C3H}. The total salivary secretion volumes of C3H/HeSlc (0.55 ± 0.05 g) and B6-Chr.2^{C3H} (0.42 ± 0.10 g) were significantly higher than that of C57BL/6Slc (0.14 ± 0.04 g).

3.2 Salivary buffering capacity

The pH before and after the addition of buffer solution, and the buffering capacity of the saliva for the different strains are presented in Table 2. The salivary buffering capacity of C57BL/6Slc was lower than those of C3H/HeSlc and B6-Chr.2^{C3H}; however, the

differences were not significant.

3.3 Histological comparison of the submandibular gland

The proportions of the duct area and the acinar area in the submandibular gland in C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H} are shown in Figure 3. The proportions of the duct area were 37.9 ± 6.0% in C3H/HeSlc, 44.5 ± 3.0% in C57BL/6Slc and 47.6 ± 6.2% in B6-Chr.2^{C3H}. Accordingly, the proportions of the acinar area were 62.1 ± 6.0% in C3H/HeSlc, 55.5 ± 3.0% in C57BL/6Slc and 52.4 ± 6.2% in B6-Chr.2^{C3H}. The proportions differed significantly between C3H/HeSlc and C57BL/6Slc or B6-Chr.2^{C3H}, while the difference between C57BL/6Slc and B6-Chr.2^{C3H} was not significant.

3.4 Enamel hardness

As shown in Figure 4, the enamel hardness of the left first molar was 73.23 ± 6.13 GPa in C3H/HeSlc, 41.42 ± 17.17 GPa in C57BL/6Slc and 42.76 ± 11.52 GPa in B6-Chr.2^{C3H}. The means differed significantly between C3H/HeSlc and C57BL/6Slc or B6-Chr.2^{C3H}; however, the difference between C57BL/6Slc and B6-Chr.2^{C3H} was not significant.

3.5 Expression levels of Slc9a8 and Slc24a3 in the submandibular gland

We examined the expression level of two candidate genes, *Slc24a3* and *Slc9a8,* which are located on chromosome 2 and considered as ion channels of salivation process.

The expression levels of these candidate genes in the submandibular gland in C57BL/6Slc were significantly lower than those of C3H/HeSlc and B6-Chr.2^{C3H}; however, no significant differences were detected between C3H/HeSlc and B6-Chr.2^{C3H} (Figures 5A–D).

4. Discussion

In this study, we aimed to identify phenotypic differences influencing susceptibility to dental caries using C3H/HeSlc, C57BL/6Slc, and B6-Chr.2^{C3H}. The traits measured and analyzed in this study were salivary secretion volume, salivary buffering capacity, histology of the submandibular gland, and enamel hardness.

Flushing and neutralization of microbial effects are probably the most important dental caries-preventive functions of saliva [29]. Patients who suffer from reduced salivary output are much more susceptible to dental caries than healthy individuals [30]. A previous study in a homogeneous group of children showed that salivary flow is important in determining dental caries experience [31]. Our study showed that the rate of salivary flow was significantly higher in C3H/HeSlc than in C57BL/6Slc, suggesting that this trait might affect susceptibility to dental caries. We also found that the salivary flow rate was significantly higher in B6-Chr.2^{C3H} than in C57BL/6Slc, suggesting that the amount of salivary secretion was strongly affected by genes located on C3H/HeSlc-derived chromosome 2. Therefore, the low susceptibility of B6-Chr.2^{C3H} to dental caries may be due to an increased salivary self-cleaning action caused by relatively higher salivation.

The major and minor salivary glands secrete saliva, and the submandibular gland,

one of the major salivary glands, produces the most. Previous studies have shown that the acinar area in the submandibular gland, which secretes primary saliva (a NaCl-rich plasma-like isotonic fluid), is narrow in mice with dry mouth [26,32]. In this study, C57BL/6Slc had a significantly smaller acinar cell area than C3H/HeSlc. However, the areas of acinar and duct cells were not significantly different between C57BL/6Slc and B6-Chr.2^{C3H}. Therefore, the significant difference in the amount of salivary secretion between C57BL/6Slc and B6-Chr.2^{C3H} might be due to differences in acinar cell function and not in the ratio of acinar to duct cells.

The salivary buffering capacity is strongly associated with susceptibility to dental caries. In children, the physicochemical properties of saliva such as salivary flow rate, pH, buffering capacity, and viscosity are related to and markers of dental caries activity [27]. Among the elderly, a negative correlation between salivary buffering capacity and the potential activity of caries lesions has been identified [33-35]. In our experiments, the salivary pH and buffering capacity of C57BL/6Slc tended to be lower than those of the two other strains. Since no significant differences were found, it could be concluded that genes located on chromosome 2 might not be strongly involved in the salivary buffering capacity.

Dental caries mostly affect the tooth enamel first. A previous study showed that sucrose challenge caused a standard pH depression and a change in microhardness in bovine enamel evaluated by intraoral cariogenicity test, and that the enamel is a major component of tooth resistance to dental caries [36]. Moreover, genetic variations in the *AMELX*, *TUFT1*, *AMBN*, and *ENAM* genes, which are involved in enamel formation, have been reported to be associated with susceptibility to dental caries [8]. This study showed that enamel hardness was significantly higher in C3H/HeSlc than in

C57BL/6Slc; however, the difference between C57BL/6Slc and B6-Chr.2^{C3H} was not significant, suggesting that genes located on chromosome 2 might not be involved in dental enamel hardness.

Our results suggested that the low susceptibility of B6-Chr.2^{C3H} to dental caries was strongly related to the amount of salivary secretion. Therefore, we determined the expression levels of *Slc24a3* and *Slc9a8*, two genes located on chromosome 2 and influencing the amount of salivary secretion in the submandibular gland. We found significant differences between C57BL/6Slc and C3H/HeSlc or B6-Chr.2^{C3H}. One of the two genes, *Slc24a3*, encodes NCKX3, and NCKX3 is localized in various tissues that are rich in smooth muscle cells [21,22,37,38]. Although a previous study showed that *Slc24a3* is expressed in the submandibular gland, it is not clear where the gene products localize [28]. *Slc24a1* encodes NCKX1, which is localized in duct cells and salivary myoepithelial cells [23], where NCKX3 may also be present. Salivary myoepithelial activity speeds up the outflow of saliva, reduces the luminal volume, contributes to the secretory pressure, supports the underlying parenchyma, and helps salivary flow to overcome increases in peripheral resistance [39,40]. If *Slc24a3* is expressed in duct cells, NCKX3 may be indirectly involved in salivary pH through excretion of Ca^{2+} ions into the saliva and through Na^{+} reabsorption. However, if *Slc24a3* is expressed in myoepithelial cells, NCKX3 may be involved in the functions of myoepithelial cells that control the rate of salivary secretion.

The other candidate gene, *Slc9a8*, encodes NHE8 [22]. Although a previous study showed that *Slc9a8* is expressed in the submandibular gland, it is not clear where the gene products localize [40]. If *Slc9a8* is expressed in acinar cells, NHE8 may be involved in salivary fluid secretion. The initial responses of acinar cells to a fluid

secretion stimulus are acidification of the cytosol resulting from the efflux of HCO_3^- into the basolateral membrane and generation of acid equivalents via metabolic pathways linked to increased membrane transporter activity [41,42]. This intracellular acid load is buffered by an increase in NHE activity, which, by alkalinizing the cytosol, promotes both the secretion of HCO_3^- via the apical anion channel and the uptake of Cl^- mediated by the basolateral Cl^-/HCO_3^- exchanger [31]. Thus, upregulation of Na^+/H^+ exchanger activity plays a key role in the secretory process by enhancing HCO_3^- and transepithelial Cl^- movement [43]. However, if *Slc9a8* is expressed in duct cells, NHE8 may be involved in HCO_3^- secretion and H^+ ion transport [44].

Mouse chromosome 2 has homologous regions on human chromosomes 10, 9, 2, 11, 15, and 20 [45]. *Slc24a3* and *Slc9a8* are located on human chromosome 20, and are expressed in the salivary gland [46,47]. Although the detailed gene functions are unclear, these genes are considered to be determining factors of saliva volume in humans too. Although localization of NCKX3 and NHE8 needs further study, the significantly different expression levels of *Slc24a3* and *Slc9a8* in C57BL/6Slc and B6-Chr.2^{C3H} might be associated with the mechanisms of salivary secretion in salivary acinar cells or duct cells, and might affect the rate of salivary secretion.

In conclusion, our results suggested that the salivary secretion volume, proportions of duct area and acinar area in the submandibular gland, and enamel hardness were important factors related to dental caries susceptibility in C57BL/6Slc when compared with C3H/HeSlc. By contrast, only the salivary secretion volume of B6-Chr.2^{C3H} was higher than that of C57BL/6Slc, while no significant differences were observed in the other traits. Hence, salivary secretion volume was the main factor related to dental caries susceptibility in C57BL/6Slc when compared with B6-Chr.2^{C3H}. Overall, the

results suggested that genes influencing the volume of salivation might be located on chromosome 2 and play an important role in dental caries susceptibility. Furthermore, *Slc24a3* and *Slc9a8* might differentiate C57BL/6Slc from B6-Chr.2^{C3H} with regard to saliva production and salivary secretion. However, more research is needed to identify the mechanisms underlying salivation and immune function, and to elucidate the host defense mechanism influencing dental caries development. These studies would be useful for developing new prophylaxis for patients who are highly susceptible to dental caries because of reduced salivary secretion.

Conflicts of interest

Authors have no conflicts of interest to declare.

Acknowledgments

We would like to thank Drs Takehiko Shimizu, Chika Endo and Yasuhiro Tanimoto for their invaluable assistance. We would like to thank various teachers of the Department of Pediatric Dentistry, Nihon University School of Dentistry at Matsudo. This study was funded by a grant from the Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Captions

Figure 1. Representative image of the submandibular gland histology.

The area of a given region on the image of the submandibular gland is designated as (*a*) and (*b*) represents the duct area. Scale bar indicates 20 μm.

Figure 2. Comparison of time-based measurement of the stimulated salivary secretion volumes among C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H}.

Bars indicate time-based stimulated saliva secretion volume in each strain.

* Significant differences at $p < 0.05$, as determined by Tukey's test.

Figure 3. Comparison of duct area ratios among C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H}.

Bars indicate the ratio of the duct area to the total area in a given area of the mandibular gland in each strain.

* Significant differences at $p < 0.05$, as determined by Tukey's test.

Figure 4. Comparison of enamel hardness among C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H}.

Bars indicate the enamel hardness in each strain.

* Significant differences at $p < 0.05$, as determined by Tukey's test.

Figure 5. Comparison of *Slc24a3* and *Slc9a8* expression levels in the submandibular gland among C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H}.

(A) Confirmation of *Slc24a3* expression in the submandibular gland. *Gapdh* was used as an endogenous control.

(B) Differences in the expression of *Slc24a3* between C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H} as indicated by RT-qPCR.

(C) Confirmation of *Slc9a8* expression in the submandibular gland. *Gapdh* was used as an endogenous control.

(D) Differences in the expression of *Slc9a8* between C3H/HeSlc, C57BL/6Slc and B6-Chr.2^{C3H} as indicated by RT-qPCR.

* Significant differences at $p < 0.05$, as determined by Tukey's test.

Gapdh = glyceraldehyde-3-phosphate dehydrogenase; RT-qPCR = reverse transcription quantitative polymerase chain reaction.

Table 1. Primers used in this study.

Gene		Sequence	Product size (bp)
Slc24a3	Sense:	AGAGCAGCAAGAACTGCACA	155
	Antisense:	GACGAAGAAGTCATCGCACA	
Slc9a8	Sense:	CCAGTCACCCAGATCCTCAT	204
	Antisense:	TCGGAAGAAGTTCAGCAGGT	
Gapdh	Sense:	GGAAGCCCATCACCATCTTC	203
	Antisense:	CGTGGTTCACACCCATCACA	

Gapdh = glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Saliva buffer capacity of C57BL/6Slc, C3H/HeSlc, and B6-Chr.2^{C3H}.

Strains (n = 5)	First pH	Last pH	Difference (buffer capacity)
C3H/HeSlc	9.16 ± 0.38	8.44± 0.33	0.72 ± 0.34
C57BL/6Slc	8.94 ± 0.18	7.82± 0.45	1.12 ± 0.54
B6-Chr.2^{C3H}	9.58 ± 0.04	8.80 ± 0.35	0.78 ± 0.37

Fig.1.

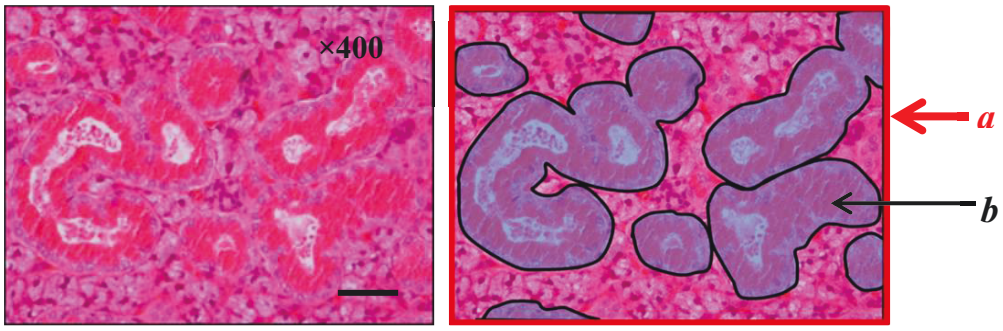


Fig.2.

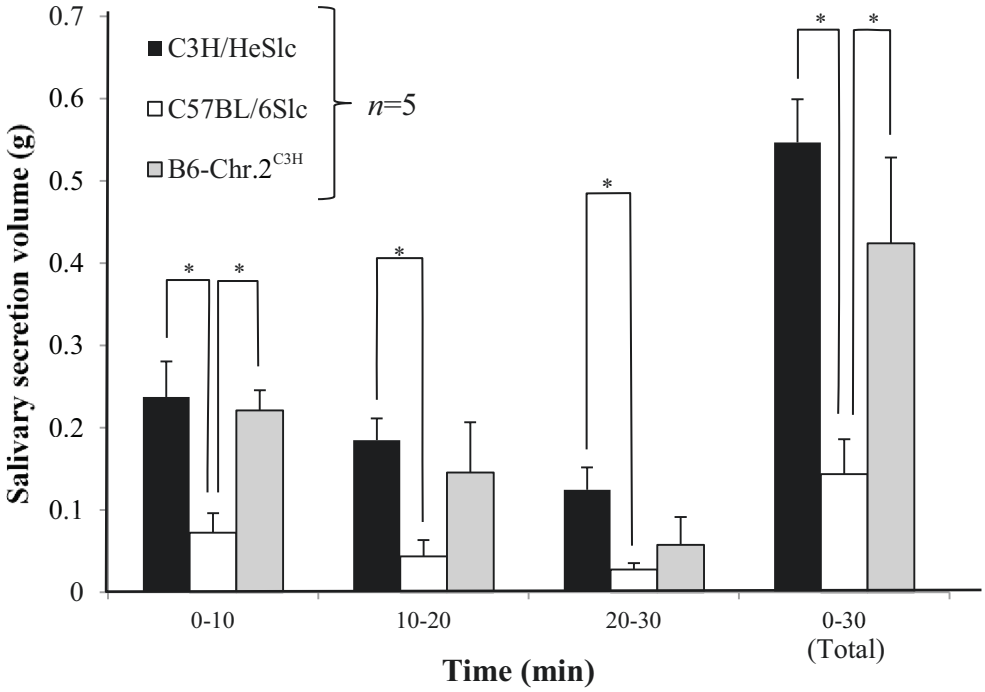

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Salivary secretion volume (g)
C3H/HeSlc
C57BL/6Slc
B6-Chr.2C3H
n=5
*
*
*
*
*
*
0-10
10-20
20-30
0-30
(Total)
Time (min)

Fig.3.

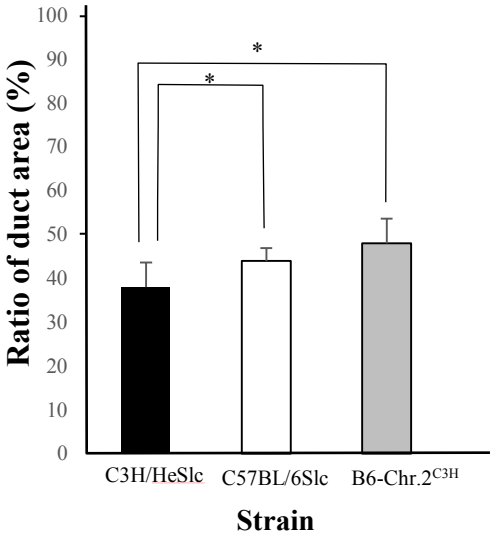

Ratio of duct area (%)
100
90
80
70
60
50
40
30
20
10
0
*
*
C3H/HeSlc
C57BL/6Slc
B6-Chr.2C3H
Strain

Fig.4.

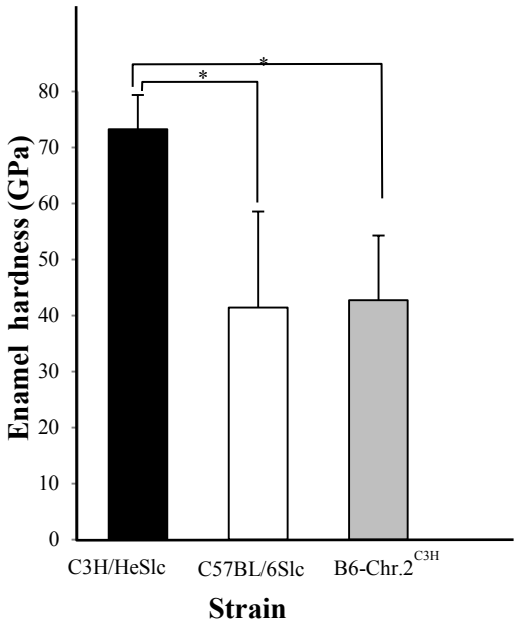
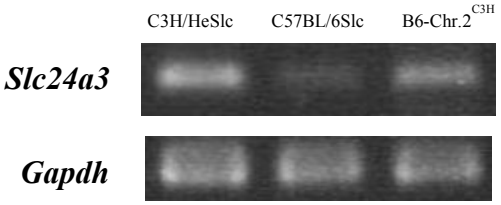
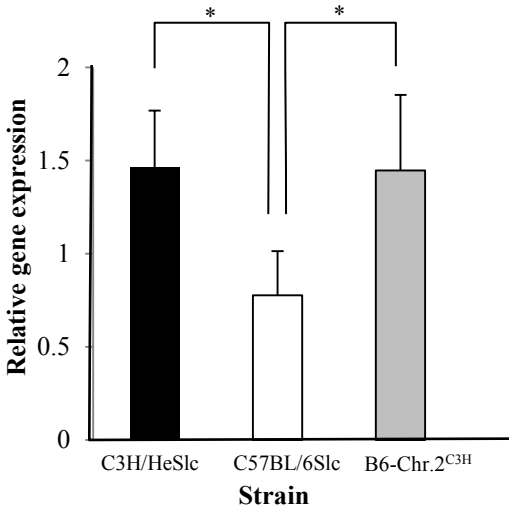
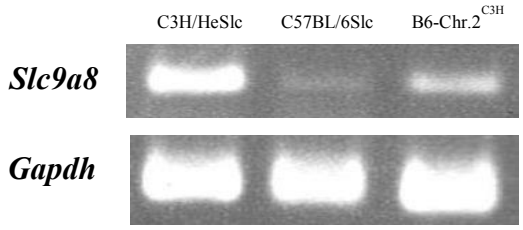

Enamel hardness (GPa)
0
10
20
30
40
50
60
70
80
*
*
C3H/HeSlc
C57BL/6Slc
B6-Chr.2C3H
Strain

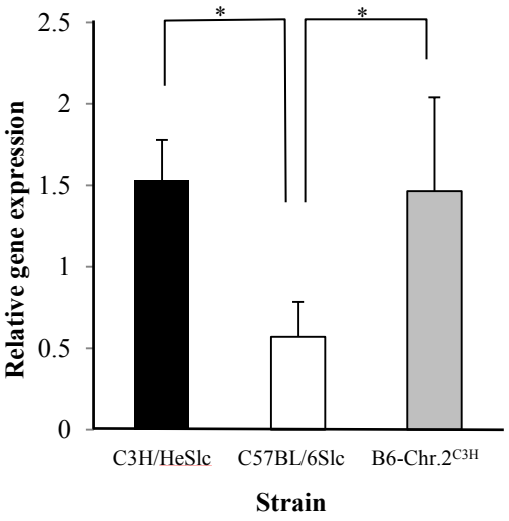
Fig.5.


C3H/HeSlc
C57BL/6Slc
B6-Chr.2C3H
Slc24a3
Gapdh


*
*
2
1.5
1
0.5
0
Relative gene expression
C3H/HeSlc
C57BL/6Slc
B6-Chr.2C3H
Strain

(A) (B)


C3H/HeSlc
C57BL/6Slc
B6-Chr.2C3H
Slc9a8
Gapdh


*
*
2.5
2
1.5
1
0.5
0
Relative gene expression
C3H/HeSlc
C57BL/6Slc
B6-Chr.2C3H
Strain

(C) (D)