Search for candidate chromosomes that specify mesiodistal tooth crown length of the mandibular first molar using MSM, C57BL/6J and their consomic mice

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Abstract
Tooth size directly affects abnormalities in dental occlusion, and is decided by genetic and environmental factors. Malalignment of teeth occurs as a result of discrepancies in jaw bone and tooth size. In this study, F 2 mice acquired by crossing C57BL/6J (B6) strain having large mesiodistal tooth crowns and MSM strain having small mesiodistal crowns were used to conduct quantitative trait locus (QTL) analysis and to identify chromosomes involved in determining mesiodistal tooth size. Analysis revealed that the QTL responsible for tooth size could be mapped chromosomes 3 and 17 in mice. Therefore, we measured mesiodistal crown length of the first molar of the mandible, and analyzed candidate chromosomes that decide the size of teeth in vivo using consomic mice between B6 and MSM strains. Mesiodistal crown length was largest in consomic mice of B6-Chr.3MSM, thus some genes for mesiodistal crown development may be located on chromosome 3.

Introduction
Predicting occlusion in adulthood is important for pediatric dentists; however, future occlusion it is difficult to predict. Most dentists depend on their experience, as well as information on occlusion and maxillofacial traits in the patient’s parents. Jaw bone and tooth size are factors that directly affect abnormalities in dental occlusion, and these are decided by genetic and environmental factors

Alternative growth of the mandible in the down and forward directions showed dominant inheritance in an investigation of the Hapsburg family. This indicates that growth of the mandible is strongly influenced by genetic factors. Breeding experiments have mated mice having large mandibles (MRL/n, RF/J, and A/J strain) and mice having small mandibles (C57BL/6J and SM/J strain). Line segment measurements of the mandible size clearly demonstrate dominant inheritance, but multiple genes are also apparently involved, as simple modes of Mendelian inheritance are not seen. Such traits are called quantitative traits (QT). Quantitative trait locus (QTL) analysis has been very successful in identifying chromosomal regions for polygenic effects, such as body weight and alcoholism susceptibility. Mandible size can be considered a QT, and thus genetic analysis can be performed using the QTL method.

Furthermore, from QTL analysis using of 21 strains of SMXA recombinant inbred (RI) mice derived from SM/J and A/J mice with differing mandible sizes, it became clear that the QTL related to mandible size was present on chromosomes 10 and 11. In addition, it has been reported that an independent gene cluster affects the determination of tooth and mandible size in SMXA RI mice.

Several QTL analyses have focused on molar size in mice, and the results have indicated that tooth size is strongly affected by genetic factors in F2 progeny from a cross between the LG/J and SM/J

Key words
Chromosome 3, Chromosome 17, Consomic mice, Quantitative trait locus, Tooth size

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mice\textsuperscript{10}. It has been reported that tooth crowns in mice decrease in size due to mutations in the tabby gene (X chromosome), crinkled gene (chromosome 13)\textsuperscript{11}, and crooked gene (chromosome 6)\textsuperscript{12}, but mice with these mutations have phenotypes that resemble anhidrotic ectodermal dysplasia; thus, the gene clusters are differentiated from those involved in the size of tooth crowns in inbred mice.

Consomic mice have recently been established, and used in QTL analyses\textsuperscript{13}. Consomic strains (or chromosome substitution strains) are those in which paired chromosomes of different types have been introduced into an existing inbred strain. In this study, we used consomic mice derived from C57BL/6J (B6) and MSM. This consomic strain was produced by mating B6 as the host strain with MSM as the donor strain. It carries a copy of an MSM-derived chromosome and the other chromosomes are derived from B6 (for example, the chromosome 1 substitution strain, represented as B6-Chr.1\textsuperscript{MSM}, has chromosome 1 derived from MSM and chromosomes 2 to 19, as well as the sex chromosome, derived from B6). F\textsubscript{2} mice that were acquired by crossing B6 mice having large mesiodistal crowns and MSM mice having small mesiodistal crowns were used to analyze QTL and identify chromosomes involved in teeth size. In addition to QTL analysis, we analyzed candidate chromosomes involved in determining tooth size in vivo using the consomic mice.

**Materials and Methods**

**Mice**

B6 (large tooth size) mice were purchased from Sankyo Lab Service Corporation (Tokyo, Japan) and MSM (small tooth size) mice were obtained from the National Institute of Genetics (Shizuoka, Japan).

For QTL analysis, 85 F\textsubscript{2} mice (F\textsubscript{1}×F\textsubscript{1}) were obtained by mating F\textsubscript{1} (B6×MSM) mice in our laboratory. The consomic mice used in this study were B6-Chr.1\textsuperscript{MSM}, B6-Chr.3\textsuperscript{MSM}, B6-Chr.6\textsuperscript{MSM}, B6-Chr.7\textsuperscript{MSM}, B6-Chr.8\textsuperscript{MSM}, B6-Chr.9\textsuperscript{MSM}, B6-Chr.15\textsuperscript{MSM}, B6-Chr.17\textsuperscript{MSM} and B6-Chr.19\textsuperscript{MSM}, which are now available from the National Institute of Genetics, and the genotypes of these consomic mice were confirmed by polymerase chain reaction using microsatellite markers in our laboratory. Each of the strains was crossed to obtain 10 male and 10 female mice (total of 180 mice). All mice were maintained under conventional conditions: 25±1°C, 55±5% humidity, and 12-hour light/dark cycle. Mice were fed standard solid mouse feed and were provided with distilled water *ad libitum*.

**Tooth preparation**

When mice were 49 days of age, they were euthanized under CO\textsubscript{2} and their heads were removed. The heads were immersed for 48 hours in 1% KOH at 42°C in order to remove soft tissue, and were then washed with water and dried for skeletal preparations. The right mandible first molar was subsequently removed from all mice.

Finally 200 first molars having no crown fracture of right mandible were used in this study.

All procedures were approved by the Institutional Animal Care Committee, and were performed according to Nihon University School of Dentistry at Matsudo Guidelines for the Care and Use of Laboratory Animals (No. 03-0035).

**Measurement of tooth crown mesiodistal length of first molar of F\textsubscript{2} mice and consomic mice**

The right mandibular first molars were set up the distance as the maximum crown contours diameter and were lined up, grid sheets with 1 mm scales were placed on the left and right sides of those teeth, and their images were enlarged with an EPSON scanner for measurement (×20 magnifications). Mesiodistal crown length was measured the greatest distance using a slide gauge, as described by Kristenova-Cermakova\textsuperscript{14}. Statistical analysis was performed using SPSS, and included Least Significant Difference (LSD) of Multiple Comparisons. Measuring tooth crown length in the consomic mice was performed as described for above.

**QTL analysis**

QTL analysis was performed as described elsewhere\textsuperscript{5,6}. Briefly, the kidneys were removed from each F\textsubscript{2} mouse and DNA was extracted using a DNeasy Tissue kit (QIAGEN) according the manufacturer’s instructions. Analyses were conducted using the tooth sizes from MSM and B6 mice, and polymorphism information of markers was set in the chromosomes every 20 cM (Fig. 1). Likelihood Ratio Statistic (LRS) values were calculated in QTL analyses and LRS values were determined at the 5% significance standard (suggestive) and the 1% significance standard (significant).

Interval mapping, which is one of the methods in QTL analysis, was performed in a genome-wide scan using Map Manager QTXb15 software\textsuperscript{15,16}. 
**Results**

**Measurement of tooth crown mesiodistal length of first molar of F_2 mice**

For QTL analysis, we measured tooth crown mesiodistal length of first molar of F_2 mice. The minimum value was 1.67 mm, the maximum value was 2.02 mm and the average value ± S.D. was 1.83 ± 0.35 mm. B6 mice showed lengths of 1.87 ± 0.02 mm and MSM mice showed lengths of 1.67 ± 0.02 mm.

**QTL analysis**

The results of the QTL analysis of tooth size are shown in Fig. 1. The permutation tests found that the criteria for QTL detection are suggestive when LRS score is between 10.1 and 17.8, and significant when.
it is over 17.8. QTL analysis was conducted for all chromosomes and found that suggestive values were indicated for marker D3Mit73 on chromosome 3 and that significant values were indicated for marker D17Mit108 on chromosome 17. No significant values were seen for the other chromosomes.

**Table 1** Number of right mandible first molars subjected to measurement

<table>
<thead>
<tr>
<th></th>
<th>MSM</th>
<th>B6</th>
<th>Chr.1</th>
<th>Chr.3</th>
<th>Chr.6c</th>
<th>Chr.7</th>
<th>Chr.8</th>
<th>Chr.9</th>
<th>Chr.15</th>
<th>Chr.17</th>
<th>Chr.19</th>
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<td>10</td>
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<td>10</td>
<td>8</td>
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</table>

Fig. 2 Comparative graph of mesiodistal crown length of right mandibular first molars

The value for the B6 strain with large tooth crowns was $1.87 \pm 0.02$ mm and the value for the MSM strain with small tooth crowns was $1.67 \pm 0.02$ mm. In the consomic mice, B6-Chr.3<sup>MSM</sup> had the highest value of $1.90 \pm 0.02$ mm and B6-Chr.6c<sup>MSM</sup> (6c means that centromere side and telomere side are derived by MSM and B6, respectively) had the lowest value of $1.69 \pm 0.03$ mm. In addition, consomic mice with chromosome 17, which had a high value on QTL analysis, showed an intermediate value of $1.75 \pm 0.03$ mm. *: c means centromere

**Table 2** LSD values obtained by statistical analysis

<table>
<thead>
<tr>
<th></th>
<th>chr.1</th>
<th>chr.3</th>
<th>chr.6c</th>
<th>chr.7</th>
<th>chr.8</th>
<th>chr.9</th>
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<th>chr.19</th>
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<td>—</td>
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<tr>
<td>chr.7</td>
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<td>—</td>
</tr>
</tbody>
</table>

**:<0.01, #:<0.05, Blank space: no significant, 0.000 means: 0<*<0.01
The significance for Chr.3 against the other chromosomes was 0.000, which means that Chr.3 was significant vs. other chromosomes.

**Measurement of mesiodistal crown length of right mandibular first molar in the consomic mice**

First molars that could not be measured due to damage, such as fractured tooth crowns during tooth extraction, were eliminated (Table 1).
The value for the B6 strain with large tooth crowns was $1.87 \pm 0.02 \text{ mm}$ and the value for the MSM strain with small tooth crowns was $1.67 \pm 0.02 \text{ mm}$. In the consomic mice, B6-Chr.3\text{MSM} had the highest value of $1.90 \pm 0.02 \text{ mm}$ and B6-Chr.6\text{cMSM} (6c means that centromere side and telomere side are derived by MSM and B6, respectively) had the lowest value of $1.69 \pm 0.03 \text{ mm}$. In addition, consomic mice with chromosome 17, which had a high value on QTL analysis, showed an intermediate value of $1.75 \pm 0.03 \text{ mm}$ (Fig. 2).

The results of statistical analysis are shown in Table 2. B6-Chr.3\text{MSM} showed significantly larger tooth size when compared with other consomic mice, and B6 and MSM strains.

**Discussion**

Mammalian teeth represent structures of considerable taxonomic, anthropological and evolutionary significance\(^{17,18}\); therefore, it is not surprising that they have been the focus of numerous genetic studies. Particularly in recent years, developmental geneticists have discovered a number of genes that regulate specific processes leading to tooth formation\(^{19}\). Tooth size is one factor that directly affects abnormalities in dental occlusion, and it is known to involve genetic and environmental factors. Animal models, particularly mice, have contributed to our understanding of tooth formation\(^{20,21}\). The mouse has a high homology with humans in both gene and chromosomal segments, and so isolating the genes responsible for tooth size in mice may provide us with candidate genes for teeth size in humans.

Studies using twin pairs or different ethnic groups have shown that variations in crown size include a strong genetic component\(^{22,23}\). Studies of individuals with sex chromosome aneuploidies or anomalies have also found that sex-linked genes on both the X and Y chromosomes modulate tooth size\(^{24}\). Certain autosomal syndromes, including Down’s syndrome, are associated with reductions in the size of permanent teeth, indicating involvement of autosomal factors in dental crown growth\(^{25}\).

A recent study reported that determination of mesiodistal diameter and crown height is governed by cellular apoptosis in the enamel knot during molar development, based on tooth germ cells from mice\(^{25}\). Apoptosis in the enamel knot are necessary for the proper formation of molar teeth, particularly for appropriate shape and size. Apoptosis in the enamel knot may be a key factor in determining tooth size.

The results of the QTL analysis revealed that the candidate chromosomes determining tooth size are chromosome 3 and chromosome 17, as suggestive LRS value in chromosome 3 and significant one in chromosome 17 were detected. Therefore, we believe that candidate chromosomes involved in tooth size can be clarified in vivo by measuring the first molar tooth crown lengths of consomic mice of B6-Chr.3\text{MSM} and B6-Chr.17\text{MSM}. Consomic mice with B6-Chr.3\text{MSM} showed crown length greater values than B6 strains. This suggests that a gene factor that increases tooth size exists on chromosome 3 in both MSM and B6. In addition, this candidate region was corresponded to human 4q27-31 as mice D3Mit73 and human 6q21 as mice D17Mit108, respectively. RIKKEN cDNA 9930021J17 putative gene (Mouse Genome Informatics) related to maxilla and mandible containing endochondral, membraneous bone, formed joints, tendon, ligaments, dermis, epidermis, muscle, and teeth with newly forming dentin and enamel was mapped near the D3Mit73 on chromosome 3, that distance was only 158 kbp. Namely this gene is close position marker D3Mit73. Therefore it suggests that the key genes for tooth development are located on mice chromosome 3. On the other hand, QTL analysis indicated that the LRS for chromosome 17 was significant, however B6-Chr.17\text{MSM} had intermediate crown length when compared with B6-Chr.3\text{MSM} and the other consomic mice. The gene factors on chromosome 17 may act in concert with those located in the other chromosome(s), and thus B6-Chr.17\text{MSM} exhibited intermediate tooth size. Mesiodistal crown length, which varied in all 9 strains used in this research, suggests that tooth size is not determined by monogenic Mendelian heredity and that it might be affected by a number of genes involved in tooth development, such as \textit{Shh}, \textit{Bmp}, and \textit{Fgf}\(^{26}\).

Finally, we believe that a number of main genes determining factor of tooth size map on chromosome 3 and chromosome 17.

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References


