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Identification of chromosomal region(s) influencing initial dental caries susceptibility in mice

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Abstract Predicting the host genetic factors influencing dental caries susceptibility is important to developing preventive strategies in individuals. In this study, Quantitative Trait Locus (QTL) analysis was used to identify the candidate regions for gene(s) influencing initial dental caries susceptibility in mice, and that performed on genetic crosses of BALB/cJ and C3H/HeJ mice. Two significant QTLs on chromosomes 7 and 11 and five suggestive QTLs on chromosomes 3, 8, 16 and 17 were detected. Around the region 50 cM on Chromosome 7 and 46 cM on chromosome 11, the likelihood ratio statistic (LRS) scores showed higher than significant levels. Around 52.5 cM on chromosome 3, 38.4 cM on chromosome 8, 38.0 cM on chromosome 16, and 6.5 cM and 44.5 cM on chromosome 17, LRS scores showed higher than suggestive levels. Based on these results, it is suggested that the candidate gene(s) responsible for dental caries are located in the specified regions of six chromosomes, chromosomes 7 and 11 in particular are associated with initial dental caries.

Key words
Inbred mouse,
Initial dental caries,
QTL analysis,
Quantitative gene(s),
Streptococcus mutans

Introduction

Dental caries has a multifactorial etiology in which there is an interplay of three principal factors: the host, the microflora, and diet, plus a fourth factor: time. In the past century, the etiology of dental caries was investigated anatomically, physiologically, immunologically and bacteriologically, but little is known about the host genetic background. Predicting the host genetic factors influencing dental caries susceptibility will contribute to developing preventive strategies in individuals.

Studies in humans of the host genetic factors influencing the development of dental caries have been reported. Twin studies have shown that genetic factors contribute to caries susceptibility[1]. Individuals with either an inherited or acquired immune deficiency are subject to increased risks for and incidence of dental caries[2]. Altered immune response to the cariogenic bacteria may also increase susceptibility to dental caries[3]. However, no evidence has been found that dental caries is strongly controlled by genes. It is difficult to anticipate a principle gene influencing susceptibility to dental caries.

To determine the effect of a single factor on a multifactorial disease, it is advantageous to use animal models providing experimental conditions that can be controlled easily. Animal breeding[4] has indicated that dental caries has a genetic component. Previous research has shown that mouse models are powerful tools for studying the genetic contribution to dental caries. The notion that dental caries in animals is an infectious, transmissible disease was first demonstrated over sixty years ago[5]. It was suggested that mouse strain differences in susceptibility to dental caries are determined genetically[6]. It has also been demonstrated that the genetic factors associated with caries promoting ability might be located on chromosomes 2, 7 and 17[7], the H-2 region on chromosome 17[8], on chromosome 2[9], and on chromosomes 1, 2, 7 and 8[10]; the identification of genes controlling dental caries progress has not yet been performed.

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In this study, my focus was on caries susceptibility. I used Quantitative Trait Locus (QTL) analysis to identify the candidate regions for gene(s) influencing initial dental caries susceptibility in mice.

Materials and methods

Mice

BALB/cJ (caries-susceptible strain) and C3H/HeJ (caries-resistant strain) used in this study were purchased from CLEA Japan, Inc. (Tokyo, Japan). F2 intercross mice were obtained by mating F1 (BALB/cJ × C3H/HeJ) mice in our laboratory. All mice were kept in MK system-experimental animal vivariums (Meibunkan, Chiba) at room temperature, 25 ± 1°C, and a humidity of 55 ± 5%. Lighting was cycled in 12-hour intervals, and mice were fed "Diet 2000", containing 30% sucrose, and tap water *ad libitum*. The animal-use protocol in this study was reviewed and approved by the Tsurumi University Institutional Review Board.

Bacterial strain and culture conditions

S. mutans PS-14 (serotype c) was used in this study as a streptomycin-resistant (1.0 mg/m*l*) strain. Before inoculation, the bacterial strain was pre-cultured in 10 m*l* of brain heart infusion (BHI) broth (Difco, Becton Dickinson, Sparks, MD, USA) containing streptomycin (1 mg/m*l*) at 37°C for 6 hrs under aerobic conditions. After that, 100 μl of pre-culture was transported to 10 m*l* of BHI broth, and cultured at 37°C for 17 hrs under aerobic conditions.

Experimental design

The mice were weaned at 21 days of age and fed on Diet 2000. They were orally infected with *S. mutans* for 7 days from 22 days to 28 days of age by inoculating 50 μl (1.0×10^8 CFU/m*l*) of a bacterial solution using a micropipette. At 29 days and 42 days of age, samples were obtained by sterile swab from the oral cavity and inoculated onto Mitis salivarius agar (Difco, Becton Dickinson, Sparks, MD, USA) containing streptomycin (0.2 mg/m*l*) to confirm colonization of *S. mutans*. At 42 days of age, each mouse was anesthetized with chloroform immediately before being killed. The mandible bones were removed; then the number of recovered colonies was counted and caries lesions were evaluated.

Calculation of caries score

After the soft tissues were removed, both sides of the mandible were immersed in murexide solution for 15 hrs for staining, washed with water, dried, and examined by microscope (× 30). Evaluation of the caries state was made according to the modified Keyes method applicable to mice[11–12].

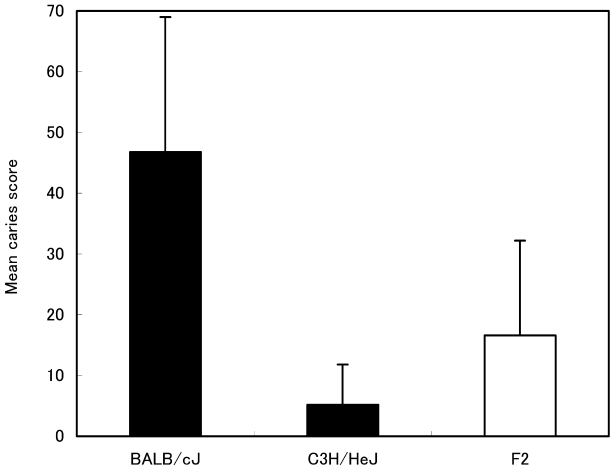

Fig. 1 The mean caries scores and standard deviations for BALB (n = 4), C3H (n = 5) and F2 mice (n = 100), respectively. The caries score for the F2 mice indicates the middle region of BALB and C3H as parent strains.

Extraction of DNA

Immediately after being killed, DNA was isolated from the spleen of the animal. The standard method was used to prepare high molecular weight DNA[13].

SSLP markers

A total of 53 MIT markers was purchased from Research Genetics Inc. (Boston, MA), and used to prime the polymerase chain reaction (PCR). These had previously been determined as informative markers, being polymorphisms between BALB/cJ and C3H/HeJ.

DNA amplification using PCR

A PCR thermal cycler PC806 (ASTEC, Tokyo, Japan) was used for DNA amplification. The reaction mixture for PCR was as follows: 2.0 μl (total 25 ng) genomic DNA was used for PCR amplification in a reaction mixture with 0.05 μl 1 unit of EX Taq polymerase (TaKaRa, Tokyo, Japan), 1.0 μl 10 × reaction buffer, 0.8 μl dNTPs mixture (2.5 mM each), 3.0 μl MIT primer mixture, and 3.15 μl sterilized water. DNA amplification was performed under following conditions: denaturation at 94°C for 3 min, followed by 25 cycles of denaturation at 94°C for

Table 1 Suggestive and significant quantitative trait loci (QTLs) detected by interval mapping

Chr.	Marker	[a]Location (cM)	Peak LRS	[b]Additive effect	[c]Trait variance (%)
3	D3Mit158	52.5	8.5**	−2.22	8
7	D7Mit126	50	14.4**	6.79	13
8	D8Mit78	38.4	13.8**	5.67	13
11	D11Mit262	46	17.3**	−8.28	16
16	D16Mit5	38	8.8**	−6.57	8
17	D17Mit113	6.5	8.7**	−6.47	8
17	D17Mit93	44.5	9**	−5.82	9

*: Suggestive QTLs, **: Significant QTLs
[a]: Location (cM) estimated at the position of the peak likelihood ratio statistics (LRS) scores.
[b]: The additive effect is half the difference between the C3H and BALB homozygotes.
[c]: The trait variance is the total phenotypic variance accounted for by each QTL.

15 s, annealing at 55°C for 2 min, and extension at 72°C for 2 min, then a final extension at 72°C for 7 min.

Genotyping

The PCR products were electrophoresed on a 7% polyacrylamide gel, using 1 × TBE buffer at 50 volts for 5 min, then at 120 volts for 1.6 hrs. The gels were stained with ethidium bromide for 5 min, and the polymorphisms were photographed under UV light using a digital camera (OLYMPUS, CAMEDIA, C-4100ZOOM), and the genotypes were examined on a computer screen.

QTL analysis

A total of 53 polymorphic markers were used in the QTL. Interval mapping was performed with the use of Map Manager QTXb19[14]. The permutation test was used to establish the significance of the likelihood ratio statistic (LRS) generated by interval mapping[15]. The obtained LRS introduced values for additive effect and trait variance. The threshold values labeled suggestive, significant, and highly significant were taken from the guidelines[16] and corresponded to the 37th, 95th, and 99.9th percentiles, respectively.

Results

Caries score

As shown in Fig. 1, the mean caries scores for BALB/cJ and C3H/HeJ were 46.8 ± 22.2 and 5.2 ± 6.6 (Mean ± S.D.), respectively. The mean caries score of F2 was 16.6 ± 15.6 (Mean ± S.D.), over an extensive range from 0 to 71. The colonization of *S. mutans* in infected mice was also confirmed at the end of the experimental period.

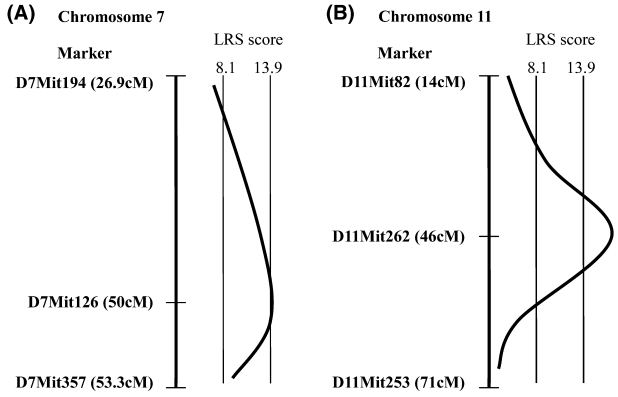

Fig. 2 LRS score curves from a genome scan with caries scores as phenotype of F2 mice (N = 100) on chromosome 7 (A) and chromosome 11 (B). The space between the thin vertical lines and that to the right of these lines represent the suggestive and significant levels, respectively, and correspond to 8.1 and 13.9. The curve indicates the LRS scores. The LRS scores in chromosomes 7 (A) and 11 (B) showed higher than significant levels. The numeric values in parentheses represent the distance between the centromere and the microsatellite marker.

QTL analysis

I performed quantitative trait locus (QTL) analysis. Genotyping was performed on a group of 100 F2 mice using MIT markers. As a result of the permutation test, the LRS scores used for detecting suggestive/significant associations with the caries score were 8.1/13.9. The results of the QTL analysis for caries score are shown in Table 1 and Figure 2.

Two significant QTLs and five suggestive QTLs were detected. The LRS from chromosomes 11 and 7 showed a higher than significant level ($P<0.05$). In chromosome 11, the peak LRS score was located at the region 46 cM from the centromere marker D11Mit262 (Fig. 2B), and the LRS score was 17.3 (Table 1: additive effect, −8.28, trait variance, 16%). In chromosome 7, the peak LRS score was located 50 cM from the centromere marker D7Mit126 (Fig. 2A), and the LRS score was 14.4 (Table 1: additive effect, 6.79, trait variance, 13%). These regions correspond to a 95% confidence interval.

In chromosomes 3, 8, 16 and 17, five suggestive QTLs were detected. Around the region 52.5 cM from the centromere of chromosome 3, the LRS score showed a higher than suggestive level (Table 1: LRS, 8.5, additive effect, −2.22, trait variance, 8%). Around the region 38.4 cM from the centromere of chromosome 8, the LRS score showed a higher than suggestive level (Table 1: LRS, 13.8, additive effect, 5.67, trait variance, 13%). Around the region 38.0 cM from the centromere of chromosome 16, the LRS score showed a higher than suggestive level (Table 1: LRS, 8.8, additive effect, −6.57, trait variance, 8%). Around the regions 6.5 cM and 44.5 cM from the centromere of chromosome 17, the LRS score showed a higher than suggestive level: respectively, (Table 1: LRS, 8.7, additive effect, −6.47, trait variance, 8%), and (Table 1: LRS, 9.0, additive effect, −5.82, trait variance, 9%). The 37% confidence interval of these regions were located around the markers D3Mit158 on chromosome 3, D8Mit78 on chromosome 8, D16Mit5 on chromosome 16 and D17Mit113 and D17Mit93 on chromosome 17.

Discussion

A century of research has provided a comprehensive description of caries development. Based on epidemiology observation, dental caries has been divided into two clinical phases of caries, early onset and progress. Factors responsible for variation in caries susceptibility between individuals are not clearly defined. Dental caries is affected by host genetic factors that may be related to the structure of dental enamel, immunologic response to cariogenic bacteria, and saliva[17]. Recently, molecular genetics studies of the genetic factors controlling dental caries progress have been performed. In this study, I focused on identifying the candidate regions influencing initial dental caries, rather than dental caries progress, by sacrificing the mice earlier than in the previous experiment.

As the result of the QTL analysis, I detected two significant QTLs on chromosomes 7 and 11 and five suggestive QTLs on chromosomes 3, 8, 16 and 17. Around the region D7Mit126 (50 cM) on chromosome 7 and D11Mit262 (46 cM) on chromosome 11, LRS scores showed higher than significant levels. Around D3Mit158 (52.5 cM) on chromosome 3, D8Mit78 (38.4 cM) on chromosome 8, D16Mit5 (38.0 cM) on chromosome 16, and D17Mit113 (6.5 cM) and D17Mit93 (44.5 cM) on chromosome 17, LRS scores showed higher than suggestive levels. From these results, I suggest that the candidate gene(s) responsible for initial dental caries are located in the specified regions of six chromosomes: 3, 7, 8, 11, 16 and 17.

The linkage between MHC haplotype and dental caries was first reported[18]. They reported that HLA-DR antigen, a Human MHC class antigen, is associated with helper T cell activity in the control of dental caries. On the other hand, another study reported that dental caries was not correlated with HLA haplotypes[19]. Molecular genetics studies of dental caries using mouse models have been promoted, because the relationship between the HLA gene and dental caries is unclear. The caries-promoting experiments were carried out using H-2 congenic mice (BALB.K/Ola) generated by introducing the MHC region derived from a caries-resistant strain (C3H/HeJ) into a caries-prone strain (BALB/cJ)[8]. They demonstrated that the H-2 region on chromosome 17 was one of the genetic factors in the susceptibility to dental caries. It was reported that there are genetic factor for dental caries promoting ability, which was located on chromosome 2, 7 and 17[7]. The candidate gene(s) responsible for caries susceptibility were confirmed on chromosome 2[9]. On the heels of their result, it was suggested that dental caries susceptibility might be located on chromosome 1, 2, 7 and 8[10].

Based on my result and previous reports, I can hypothesize that chromosome 11 is strongly related to initial dental caries, chromosome 2 and 8 are related to dental caries progress, and chromosome 7 is related to both initiation and progression of dental caries. Around two regions centromere (6.5 cM) and telomere (44.5 cM) in chromosome 17, the LRS scores showed higher than suggestive levels. However, the H-2 region on chromosome 17 was not detected as a QTL. I can suppose that this is because

the H-2 region is related to dental caries progress, while the two regions centromere and telomere in chromosome 17 are related to initial dental caries. In this study, QTLs were not detected on chromosomes 1 and 2. This result is explained by differences of the mouse strain employed in the studies.

The Mouse Genome Database and Map viewer (National Center for Biotechnology Information) were searched for candidate genes according to regions that were significant. I found seven and thirteen genes as candidate gene(s) on chromosome 7 (kcnj11, pgia21, Garp, Tria4, Hbb, H1 and Pth) and chromosome 11 (IL-13, Irf1, IL-4, Canx, Pgia7, Foxn1, Ccl18, Crlf3, Cacnb1, Cacna1g, Cacng1, Cacng4 and Cacng5), respectively. I found three important trends among these genes: (1) H1, Tria4, Foxn1, Ccl8, Canx, Irf1, Crlf3, IL-13 and IL-4 are related to the immune system. Tria4 controls T cell proliferative response[20]. Foxn1 promotes T cell development[21]. Irf1 plays a role in the regulation of the IFN system[22]. (2) Cacnb1, Cacna1g, Cacng1, Cacng4 and Cacng5 are related to calcium ion binding or transport, and kcnj11 is related to ion transport of potassium, which is one of the salivary components. (3) I paid attention to proteoglycan as the other candidate gene family. It is suggested that dentin proteoglycan induces calcium/phosphate-containing mineral, and that it is important for the remineralization of dentin in conjunction with the caries process[23]. I have found gene(s) relating to proteoglycan within the candidate regions of five chromosomes: 3, 7, 8, 11 and 17.

To sum up the result of this study, I suggest that potential candidate gene(s) influencing dental caries exist on chromosomes 3, 7, 8, 11, 16 and 17—chromosomes 7 and 11 in particular are associated with initial dental caries. The mouse genome has a high homology with human genes. If candidate genes were identified by future study, the genes would be useful in the investigation of human caries. In the near future, given the rapidly expanding understanding of the human genome and relevant medical applications, the identification of host genes controlling dental caries in mice may contribute to a preventive approach for human dental caries by identifying patients at risk.

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