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Genetic mapping of fused root of the maxillary second molar in mice to chromosome 5

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Abstract Although studies have identified several genes that are involved in tooth root formation, little is known about the genetics of root fusion. The purpose of the present study was to identify the chromosomal region that includes the candidate gene causing root fusion, using SMXA recombinant inbred (RI) strains of mice. Fusion of the mesial and palatal roots of the upper second molars (M^2) was observed in 16 of 21 substrains of SMXA RI mice. The incidence of root fusion of the M^2 in substrains and parental strains showed a continuous spectrum of distribution between 0 and 85%. In a genome-wide linkage analysis, a high Lod score exceeding the suggestive threshold level was found between D5Mit97 and D5Mit31 on chromosome 5. These findings suggest that a polygenic system with incomplete penetrance is involved in the fusion of roots, and that one of the genes causing root fusion of the M^2 in mice is located in a distal region on chromosome 5.

Key words
Fused root,
Inbred mice,
Linkage analysis

Introduction

In humans, the frequency of fusion of molar roots varies among ethnic groups. In modern Europeans, fusion of roots occurs in 0.2% of maxillary first molars, 14.6% of maxillary second molars, 36.8% of maxillary third molars, 0.3% of mandibular first molars, 21.7% of mandibular second molars, and 19.2% of mandibular third molars[1)]. It is generally thought that the morphology of tooth roots is primarily determined by genetic rather than environmental factors[1)], but the genetics of specific fusion of roots are poorly understood. X-chromosomal aneuploidy has been found to be associated with taurodontism[2)], short root and root separation[3,4)]. Dentin dysplasia[5)] and dentinogenesis imperfecta[6)] also affect root morphology. However, there is no clear association between these syndromes and the fusion of molar roots.

Although fusion of roots is generally rare in mice, there have been reports of fusion of roots in inbred and mutant mice. In tabby mice, reports indicate a high frequency of fusion of the roots of the first and second molars, which involves reduction of root size[7)]. Mice with epilepsy-like disorder (EL) have a 60% incidence of fusion of roots of the upper first molar[8)], with an autosomal recessive pattern of inheritance. Asada[9)] reported that the C57L/J mouse strain is one of the most useful models for studying the cause of fusion of roots; their incidence of gutter-shaped root of the lower second molars is about 90 to 100%. A gene causing fusion of roots in C57L/J mice has been mapped to the distal region of chromosome 5[10)].

The SMXA recombinant inbred (RI) mouse strain set has been proven to be a powerful tool for analyzing multifactorial genetic traits[11,12)]. SMXA RI mouse strains were produced by systematic inbreeding from the F_2 generation of a cross between A/J and SM/J inbred strains[13)]. The SMXA RI strains show phenotypic difference in a variety of traits, including body weight, blood insulin and lipids levels[14)]. A detailed genetic profile of the

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SMXA RI strains has been reported[15]. We have previously observed that some substrains of SMXA RI mice have a high incidence of fusion of roots (mesial and palatal roots) of the upper second molars. The purpose of the present study was to identify the chromosomal region that contains the candidate gene causing fusion of roots, using SMXA RI mouse strains.

Materials and Methods

Mice

A total of 219 mice were used. The mice belonged to the parental strains A/J and SM/J and 21 of the 26 substrains of the SMXA RI set (n = 7–13 for each strain). Five SMXA RI strains (SMXA-3, -6, -11, -21 and -23) were excluded from the present study because of an insufficient number of samples. Mice were obtained from the Institute for Experimental Animals, Hamamatsu University School of Medicine (Hamamatsu, Japan). The mice were maintained under conventional conditions (25 ± 2°C, 55 ± 5% humidity, and 12-h light/dark cycle), and were fed a commercial diet (MR Breeder, Nihon Nohsan Co., Kanagawa, Japan) and tap water *ad libitum*.

Observation of fusion of roots of the upper second molar

The mice used were 90 days old at sacrifice, and each was anesthetized with ether immediately before sacrifice. The skulls were defleshed and macerated in 1% potassium hydroxide at 42°C for 48 hours. The bilateral upper second molars (M^2) were extracted from maxilla. A total of 438 M^2 (n = 11–26 for each strain) from 219 mice were used. In normal mice, the maxillary second molars have 3 roots: mesial, palatal and distal. Fusion of roots of M^2 was observed using a stereoscopic microscope at × 32 magnification. The frequency of fusion of roots of M^2 was calculated for each SMXA RI and parental strain.

Linkage analysis

Linkage analysis was performed using Map Manager QTXb15[16] to detect the chromosomal region influencing fusion of roots of M^2. For genome-wide linkage analysis, we used 789 markers distributed through the genome that have informative strain distribution patterns[15], allowing the frequency of fusion of roots of M^2 in each SMXA RI strain to be analyzed as a quantitative trait[12]. The significance of each locus detected by interval mapping was represented as likelihood ratio statistic (LRS), and logarithm of odds (Lod) scores were then obtained by dividing the LRS by 4.605[14]. The significance threshold for the interval mapping was computed by permutation test[17]. The Lod scores calculated by 1,000 sets of permutation test were 2.3 for suggestive

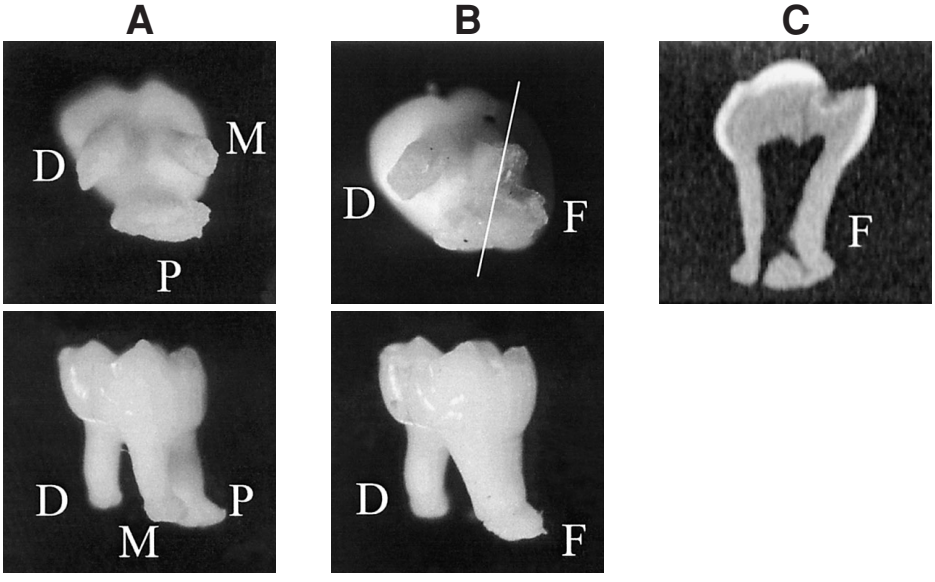

Fig. 1 Fusion of the mesial and palatal roots of the upper second molars in SMXA RI strains. **A**: normal roots; **B**: fused roots. White line indicates cross section of micro-CT image; **C**: Micro-CT image of fused roots. The mesial and palatal roots had a common pulp cavity, but root apexes were separate. D: distal root; M: mesial root; P: palatal root; F: fused roots

associations with the fusion of roots of M^2, and 4.3 for significant associations.

Results

Frequency of fusion of roots of M^2

Fusion of the mesial and palatal roots of M^2 (Fig. 1) was observed in the A/J strain and 17 of the 21 SMXA RI substrains. The mesial and palatal roots had a common pulp cavity, but had separate root apexes (Fig. 1-C). The frequencies of fusion of roots of M^2 in the SMXA RI and parental strains are shown in Fig. 2. SMXA-26 had the highest frequency of fusion of roots of M^2 (85%, n = 20). Fusion was not observed in SMXA-4 (n = 20), -12 (n = 11), -16 (n = 18), -17 (n = 22) or -27 (n = 16). The histogram of the frequency of fusion of roots showed a continuous distribution pattern. Both parental strains were positioned at the lower end in the histogram: SM/J, 0% (n = 22); A/J, 4% (n = 24).

Linkage analysis

We detected strong linkage on the distal part of chromosome 5 exceeding the suggestive threshold level. The maximum Lod score, 3.6, was observed

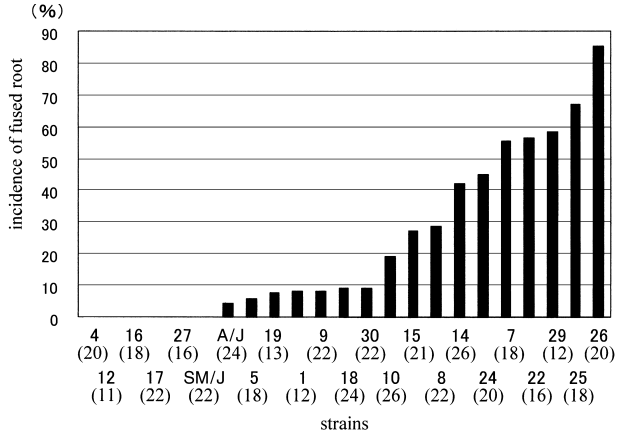

Fig. 2 Distribution of the incidence of fused root of the upper second molars in 21 SMXA RI substrains and 2 parental strains (SM/J and A/J). The histogram of the frequency of fused root showed a continuous distribution pattern suggesting that fusion of root is not controlled by simple Mendelian inheritance, and may be determined by multiple genes. Numbers in parentheses represent the numbers of teeth used.

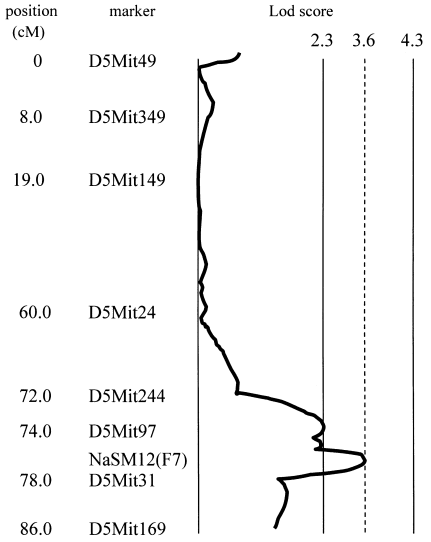

Fig. 3 Lod scores of chromosome 5. The thick solid line is the plot of the Lod scores. The vertical lines labeled with the numeric values 2.3 and 4.3 indicate the suggestive and significant level, respectively. The position 0 cM represents the centromere, and the position 86 cM represents the telomere. 1 suggestive locus is detected in the distal region of chromosome 5 suggesting the existence of a linkage between this locus and the locus controlling the growth of fused root. Map positions of the markers were obtained from the Mouse Genome Database.

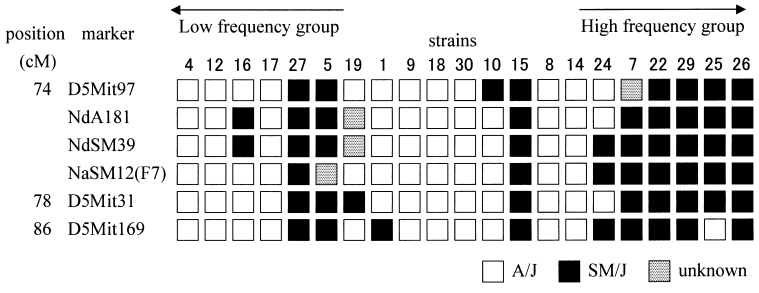

Fig. 4 Strain distribution pattern of chromosome 5 around the peak Lod scores. Genetic and microsatellite marker loci are listed at the left. Each column represents the genotype of a SMXA RI substrain reported by Mori *et al.*[15] The black boxes indicate the SM/J alleles; the white boxes indicate the A/J alleles; and the gray boxes indicate that the genotype was not determined.

between D5Mit97 and D5Mit31, which are located 74 and 78 cM from the centromere, respectively (Fig. 3). No other regions exceeding suggestive or significant Lod threshold scores were detected on any chromosomes. Figure 4 shows the strain distribution pattern of the loci with the peak Lod scores. SM/J alleles with the peak Lod scores were associated with high frequency of fusion of roots, whereas A/J alleles were associated with low frequency of fusion of roots.

Discussion

Aberrant morphological features of the molar roots constitute one of the most common developmental tooth anomalies in humans. Findings by Ackerman *et al.*[1] suggest that occurrence of taurodont, pyramidal and fused molar roots is influenced by genetic factors. Shimizu *et al.*[18] performed genetic crosses using 2 strains of mice: C57L/J with fused roots of the lower second molars (M_2), and C57BL/6J with normal roots. They found that the fusion of roots of M_2 was controlled by genetic factor on chromosome 5 with an autosomal recessive pattern of inheritance[10]. Ohta *et al.*[19] found that an autosomal recessive factor affected fusion of the mesial and palatal roots of the upper first molar (M^1) in EL mice, in an experiment in which EL mice were mated with DDY mice with normal roots. However, the loci for fused root of M^1 in EL mice are still unmapped.

In present study, the continuous spectrum of distribution of the incidence of fusion of roots in M^2 of parental strains and 21 SMXA RI strains suggests the involvement of a polygenic system. In the histogram of the incidence of fusion of roots, the parental strains, SM/J and A/J, are clearly located at the lower end, indicating that some SM/J- and A/J-derived alleles have strong suppressive effects on the fusion of roots. However, the histogram shows that many substrains have a higher frequency of fusion of roots than the parental strains, suggesting that other SM/J- and A/J-derived alleles promote fusion of roots.

In linkage analysis, a Lod score exceeding the suggestive threshold level was found between D5Mit97 and D5Mit31 on chromosome 5. This suggests that one of the genes causing fusion of roots of M^2 in mice is located in that region. The genotypes of mice with a high frequency of fusion of roots included SM/J-derived alleles around the peak Lod scores on chromosome 5. This finding indicates that the SM/J alleles on chromosome 5 are associated with promotion of fusion of roots. However, the SM/J strain had the lowest frequency (0%) of fusion of roots among all substrains and parental strains. Therefore, it is possible that there remain undetected loci that are responsible for fusion of roots, and that their effects are suppressed by the SM/J alleles.

In a previous study, an allele controlling fusion of roots of M_2 in C57L/J mice was found at the distal region of chromosome 5[10], which is very close (approximate distance, 2 cM) to the loci detected in the present study. Thus, although fusion of roots of M_2 was not observed in SMXA RI or parental strains in the present study, the available evidence suggests that previous loci controlling fused root of M_2 in C57L/J mice and the loci detected in this study may be common. We searched the Mouse Genome Database for candidate genes that map within the interval exceeding the suggestive level. There are about 50 genes including novel genes between D5Mit97 and D5Mit31. However, we found no potential candidate genes involved in tooth development within the candidate region, suggesting that novel genes are involved in the regulation of specific processes affecting fusion of roots.

Confirmation of the candidate loci and further definition of chromosomal location requires a fine mapping study using a F_2 (SM/J × A/J) intercross. The results of the present study and subsequent fine mapping will help clarify the underlying mechanisms of dental root growth in man, because mouse and human genes are highly syntenic, contributing to the actualization of the tooth regeneration.

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References

1) Ackerman, J.L., Ackerman, A.L. and Ackerman,

A.B.: Taurodont, pyramidal and fused molar roots associated with other anomalies in a kindred. *Am J Phys Anthropol* **38**: 681–694, 1973.
2) Jaspers, M.T. and Witkop, C.J. Jr.: Taurodontism, an isolated trait associated with syndromes and X-chromosomal aneuploidy. *Am J Hum Genet* **32**: 396–413, 1980.
3) Midtbo, M. and Halse, A.: Root length, crown height, and root morphology in Turner syndrome. *Acta Odontol Scand* **52**: 303–314, 1994.
4) Varrela, J.: Root morphology of mandibular premolars in human 45,X females. *Arch Oral Biol* **35**:109–112, 1990.
5) Seow, W.K. and Shusterman, S.: Spectrum of dentin dysplasia in a family: case report and literature review. *Pediatr Dent* **16**: 437–442, 1994.
6) Pettiette, M.T., Wright, J.T. and Trope, M.: Dentinogenesis imperfecta: endodontic implications. Case report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **86**: 733–737, 1998.
7) Gruneberg, H.: The molars of the tabby mouse, and a test of the 'single-active X-chromosome' hypothesis. *J Embryol Exp Morphol* **15**: 223–244, 1966.
8) Asada, Y., Shimizu, T., Matsune, K., Shimizu, K., Suzuki, Y., Takamori, K. and Maeda, T.: Absence of the third molars in strain EL mice. *Ped Dent J* **10**(1): 19–22, 2000.
9) Asada, Y.: Discovery and evaluation of the gutter-shaped root (GSR) in inbred mice. *Jpn J Ped Dent* **33**: 774–784, 1995. (in Japanese)
10) Shimizu, T.: Mapping of a gene causing mouse gutter-shaped tooth root to chromosome 5. *Arch Oral Biol* **44**: 917–924, 1999.
11) Kobayashi, M., Ohno, T., Tsuji, A., Nishimura, M. and Horio, F.: Combinations of nondiabetic parental genomes elicit impaired glucose tolerance in mouse SMXA recombinant inbred strains. *Diabetes* **52**: 180–186, 2003.
12) Ishih, A., Ohno, T., Nishimura, M. and Terada, M.: Genetic analysis of mortality in murine angiostrongyliasis costaricensis using SMXA recombinant inbred mouse strains. *Parasitol Int* **49**: 335–338, 2000.
13) Nishimura, M., Hirayama, N., Serikawa, T., Kanehira, K., Matsushima, Y., Katoh, H. Wakana, S. Kojima, A. and Hiai, H.: The SMXA: a new set of recombinant inbred strain of mice consisting of 26 substrains and their genetic profile. *Mamm Genome* **6**: 850–857, 1995.
14) Anunciado, R.V., Ohno, T., Mori, M., Ishikawa, A., Tanaka, S., Horio, F., Nishimura, M. and Namikawa, T.: Distribution of body weight, blood insulin and lipid levels in the SMXA recombinant inbred strains and the QTL analysis. *Exp Anim* **49**: 217–224, 2000.
15) Mori, M., Akiyoshi, S., Mizuno, Y., Okuizumi, H., Okazaki, Y., Hayashizaki, Y. and Nishimura, M.: Genetic profile of the SMXA recombinant inbred mouse strains revealed with restriction landmark genomic scanning. *Mamm Genome* **9**: 695–709, 1998.
16) Manly, K.F., Cudmore, R.H. Jr. and Meer, J.M.: Map Manager QTX, cross-platform software for genetic mapping. *Mamm Genome* **12**: 930–932, 2001.
17) Doerge, R.W. and Churchill, G.A.: Permutation tests for multiple loci affecting a quantitative character. *Genetics* **142**: 285–294, 1996.
18) Shimizu, T., Maruyama, H., Matsune, K., Shimizu, K., Asada, Y and Maeda, T.: Molecular genetic study of the gutter shaped root (GSR) in inbred mice. *Ped Dent J* **8**(1): 93–97, 1998.
19) Ohta, M., Nomura, R., Matsune, K., Shimizu, T., Maeda, T. and Asada, Y.: Genetic study of the fused upper molar roots in inbred mice. *Jpn J Ped Dent* **41**(1): 189–193, 2003. (in Japanese)