Analysis of the association between mesiodens and SNP genotyping

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Abstract

A mesiodens is the most common supernumerary tooth present in the maxillary incisor area. There have been many reports regarding the prevalence and treatment for supernumerary teeth; however, the etiology of the condition is still unknown. This study aimed to identify genes associated with susceptibility to mesiodens formation. The study population comprised 24 patients with mesiodens and 24 controls. Genomic DNA was obtained from the soft tissue of extracted supernumerary teeth or saliva samples. Genotypes and allele frequencies of single nucleotide polymorphisms (SNPs) in genes associated with the initiation of odontogenesis, including *TP63, PITX2, LEF1, BMP2, BMP4, FGF9, FGF20, WNT10A, WNT10B, EDA, EDAR, MSX1, MSX2, PAX9, LHX6,* and *RUNX2* were compared between mesiodens group and controls using chi-squared tests. In addition, SNPs in genes associated with supernumerary incisor formation in rodents, including *SOSTDC1, LRP4, APC, PAX6*, and *CEBPB* were tested. No positive polymorphisms were found in the tested SNPs between the two groups. These results suggest that these SNPs might not be candidates of genetic marker for mesiodens formation.

Introduction

Supernumerary teeth are developmental anomaly characterized by the existence of teeth in addition to the normal dentition. The mesiodens, which is located in the upper central incisor area (1), is the most common type of supernumerary tooth, with a population prevalence of 0.15–1.9% and affecting males twice as frequently as females (2). In addition, permanent dentition is more frequently affected than primary dentition (3). Many complications can be associated with mesiodens, including displacement of neighboring teeth, failure of eruption, resorption of root , malocclusion, crowding, cystic lesions, eruption in nasal cavity, and abnormal root formation of permanent teeth (4). Therefore, early diagnosis and treatment are needed to help correct dental occlusion.

Several hypotheses have been suggested regarding the formation of supernumerary teeth, but the cause has not been fully explained. Dichotomy of the tooth germ, hyperactivity of the dental lamina, and molecular changes at the early stage of odontogenesis might be involved in the development of single supernumerary tooth (4). Genetic diseases such as cleidocranial dysplasia (5), Rubinstein-Taybi syndrome (6) and Gardner's syndrome (7) are associated with multiple supernumerary teeth, but the etiology of non-syndromic supernumerary teeth can be distinguished from these etiologies. Non-syndromic mesiodens occurs more frequently in patients with family history of the tooth than in general population (8). Cases of mesiodens in monozygotic twins have suggested the influence of genetic factor in its etiology (9). The inheritance patterns of mesiodens can be an autosomal dominant or autosomal recessive with incomplete penetrance or might be related to X-linked inheritance (1, 4).

Animal models provide insight into the complex mechanisms of tooth formation in human dentition. Recent analysis of mouse mutants has provided important insights into the mechanisms underlying tooth development including supernumerary tooth formation. A variety of secreted molecules including

wingless-type MMTV integration site family (WNT), sonic hedgehog (Shh), bone morphogenetic proteins (BMPs), and fibroblast growth factors (FGFs) orchestrate development to regulate tooth shape, size, and number (10). In previous studies, sclerostin domain-containing protein 1 (*Sostdc1*)-, *LDL receptor-related protein 4* (*Lrp4*)-, *CCAAT/enhancer-binding protein beta* (*CEBPB*)-, and adenomatous polyposis coli (*APC*)-deficient mice, as well as paired box 6 (*Pax6*)-mutant rats, presented with supernumerary teeth in the incisor area (11-15).

Tooth formation is a complex process involving many molecules and signaling pathways. Several studies have suggested that polymorphisms in genes might cause susceptibility for various diseases (16). Recent study has suggested that congenital lack of tooth in the general population are associated with gene polymorphisms in *WNT10A* (17). Therefore, gene polymorphisms might be a risk factor for supernumerary teeth. The aim of this study was to clarify the association between single nucleotide polymorphisms (SNPs) in genes contributing to early tooth development and non-syndromic mesiodens in a Japanese cohort consisting of patients with mesiodens and normal controls.

Materials and Methods

Subject selection

The number of 48 individuals were evaluated, including 24 subjects from 16 families (19 males and 5 females) experienced mesiodens and 24 control subjects from 24 families (6 males and 18 females) in this analysis. The subjects were recruited at Nihon University Matsudo Hospital. Diagnosis of mesiodens was made by oral examination as well as periapical and panoramic radiographs or from interviews about past treatment for mesiodens. For the control subjects, the absence of supernumerary teeth was confirmed by panoramic radiographs and interviews confirming no family history of mesiodens. No subjects had any syndromic disease or other dental anomalies. This study was approved by Nihon University School of Dentistry at Matsudo, the ethical committee (approved number: EC16-15-012-1). Written, informed consent was obtained from all subjects who participate in this study

Sampling and DNA extraction

Genomic DNA from participants with mesiodens was obtained from the soft tissue of supernumerary teeth that were extracted for treatment using the DNeasy® Blood Tissue Kit (Qiagen, Tokyo, Japan) following instructions by the manufacturer. For participants whose supernumerary teeth had been extracted in the past and for control subjects, genomic DNA was obtained from 2 ml of saliva using the Oragene® DNA kit (DNA Genotek, Inc., Ottawa, Canada) following instructions by the manufacturer.

Genomic DNA amplification

Extracted genomic DNA was amplified by the Illustra™ GenomiPhi™ V2 DNA Amplification Kit (GE Healthcare, Chiltern, UK). One microliter of template (10 ng) was diluted tenfold with sample buffer and

kept at 94 °C for 3 min to denature the DNA. Then, the sample was cooled on ice for 3 min and mixed with 9 µl of reaction buffer and 1 µl of Phi29 DNA polymerase. After incubation at 30 °C overnight, the enzyme was inactivated at 65 °C for 10 minutes.

SNP markers

Sixteen SNPs in 16 genes associated with the initiation of odontogenesis, including rs11915751, rs3796902, rs10022956, rs1005464, rs2071047, rs4770190, rs10106536, rs2385199, rs833843, rs6625561, rs3827760, rs3775261, rs4868442, rs17176643, rs10818649, and rs1321081 were examined. Five SNPs in 5 genes associated with supernumerary incisor formation in rodents, including rs12699798, rs2306037, rs2431512, rs2239789, and rs6095811 were also tested. SNP probes for the genotyping were purchased from Applied Biosystems (Foster City, CA, USA). These SNPs were selected based on their allele frequencies of more than 20% in the Japanese population using data from the International HapMap Project (www.ncbi.nlm.nih.gov/probe/docs/projhapmap/).

SNP genotyping analysis

Extracted and amplified genomic DNA from participants was quantified by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Genotyping was carried out using Taqman® Genotyping assays (Applied Biosystems) and QuantStudio™ 6 Flex (Applied Biosystems). Reaction mixtures of PCR were as follows: 10 µl TaqMan® Genotyping Master Mix (Applied Biosystems), 1 µl of DNA (10 ng), 1 µl of TaqMan® probe, and 8 µl of distilled water to a final volume of 20 µl. Amplification and detection were performed using QuantStudio™ 6 Flex. Amplification conditions were as follows: an initial cycle at 94 °C for 5 min, and then 40 cycles at 95 °C for 15 sec and 1 min at 60 °C. Genotype calling was performed by QuantStudio™ Real-Time PCR Software v1.3 (Applied Biosystems).

Statistical Analyses

Statistical analysis of genotyping data was performed by IBM SPSS Statistics (ver. 19.0, Armonk, NY, USA). To test the relationship between mesiodens and SNP genotype and allele frequencies, a Pearson chi-squared test was performed. The level of two-sided significance was set at 0.01.

Results

SNPs in genes associated with the initiation of odontogenesis

Genotype frequencies of Sixteen SNPs in 16 genes, which are associated with early tooth development, are shown in Table 1, and allele frequencies of them are shown in Table 2. There were no significant differences in the 16 SNPs between mesiodens group and controls. Rs4868442 showed suggestive differences ($p = 0.04$) in allele frequencies.

SNPs in genes associated with supernumerary incisor formation in rodents

Genotype frequencies of five SNPs in 5 genes, which relate to supernumerary incisor formation in rodents, are shown in Table 3, and allele frequencies of them are shown in Table 4. No positive polymorphisms were detected in the five SNPs between mesiodens group and controls. Rs2239789 showed suggestive differences ($p = 0.04$) in allele frequencies.

Discussion

Research in recent years helped to understand many of the molecular mechanisms underlying tooth morphogenesis and differentiation (18). Individuals with different polymorphic alleles might express slight and specific phenotypic variations in tooth formation (16). Therefore, there could be associations between gene polymorphisms and mesiodens; accordingly, this study focused on such associations.

The tooth germ develops through sequential and reciprocal interactions between the epithelium and mesenchyme which are common in various ectodermal organs. The ectodermal organs start to develop from a placode, and the dental lamina was formed by thickening of the oral placode, then develops through the bud, cap, and bell stages before hard tissue mineralization (19). Morphological initiation of the supernumerary tooth germ is required for mesiodens formation. Therefore, in this study, SNPs in genes associated with the initiation of odontogenesis were tested in a population with mesiodens.

The transcription factor *p63* is one of the essential genes regulating placode formation and expressed on the surface ectoderm (20). In *p63*-deficient mice, tooth development stops before epithelial budding (20). Other transcription factors in the dental lamina include *Pitx2* (21) and *Lef1* (22), which are linked to the acquisition of odontogenic potential in the oral epithelium. Epithelial-mesenchymal interactions on tooth morphogenesis are mediated by conserved signaling pathways including the sonic hedgehog (Shh), bone morphogenic protein (BMP), fibroblast growth factor (FGF), WNT, and ectodysplasin A (EDA) pathways (23). The dental lamina expresses the signaling molecules such as *Shh*, *Bmp2*, *Bmp4*, *Fgf9*, *Fgf20*, *Wnt10a*, and *Wnt10b*, which likely function as mediators of odontogenic potential from the epithelium to the mesenchyme (21, 23-25). The tumor necrosis factor (TNF) family ligand EDA and its receptor EDAR are locally expressed in the placodes of all ectodermal organs as well as in the primary enamel knot, and inactivating mutations in *EDA* or *EDAR* cause hypohidrotic ectodermal dysplasia (26). Mice with overexpression of Eda in the epithelium under control of the keratin-14 promotor develop supernumerary teeth in front of the molars (27). Therefore, SNPs in *TP63*, *PITX2*, *LEF1*, *BMP2*, *BMP4*, *FGF9*, *FGF20*, *WNT10A*, *WNT10B*, *EDA*, and *EDAR* involved in the initiation of odontogenesis were investigated as genetic markers in this study. However, they showed no significant differences. *Shh* was not tested owing to the lack of a good marker with appropriate allele frequencies.

Transcription factors induced in the mesenchyme between the placode and dental lamina stages including *Msx1, Msx2, Pax9, Lhx6*, and *Runx2* were also tested in this study. These genes are responsible for the acquisition of odontogenic potential in the mesenchyme and are critical for early tooth

development, as demonstrated in the knockout mice (20, 23). Genetic mutations in *MSX1* (28) and *PAX9* (29) cause tooth agenesis, and mutations in *RUNX2* are associated with cleidocranial dysplasia, that is characterized by multiple supernumerary teeth, including those in the upper incisor region (5). The results of this study suggested that SNPs in *MSX1, MSX2, PAX9, LHX6*, and *RUNX2* might be not associated with genetic markers of mesiodens. Rs4868442 in *MSX2* showed suggestive differences in allele frequencies between the mesiodens group and controls ($p = 0.04$); however, this might be because allele frequencies in the controls were different from those in the general population based on the Hapmap database, suggesting that these SNPs are candidates of genetic marker for mesiodens formation.

In this study, SNPs in *SOSTDC1, LRP4, APC, PAX6,* and *CEBPB*, which are associated with supernumerary incisor formation in rodents, were tested. Sostdc1 (ectodin) is a Bmp antagonist that plays key roles as a local regulator of Bmp activity (30) and as a context-dependent activator or inhibitor of Wnt signaling (31). Both Bmp and Wnt are important molecules controlling tooth morphogenesis (32-34). Analysis of teeth in Sostdc1-null mice showed eruption of supernumerary maxillary incisors (11). LRP4 is a member of the low-density lipoprotein (LDL) receptor family, which is a large conserved group of transmembrane proteins. Lrp4 modulates Bmp and Wnt signaling during tooth development by binding the secreted Bmp antagonist, Sostdc1 (12). In *Lrp4*-deficient mice, eruption of supernumerary incisors was seen in both the maxilla and mandible (12). *APC* (adenomatous polyposis coli), a tumor suppressor gene, inhibits the activity of the WNT/β-catenin signaling pathway, which plays an important role in the maintenance of homeostasis and during development (13). Conditional *APC* knockout mice exhibited supernumerary tooth formation from the incisor and molar epithelium (13). These previous reports suggest that activation of Wnt signaling by loss of function of its inhibitors leads to supernumerary teeth (20). Pax6, a paired homeobox protein transcription factor, is involved in neuronal and craniofacial development, including that of the optic and olfactory systems (14). In *Pax6 rSey2/rSey2* mutant rats, histological analysis at E13.0 showed two dental placodes that were far apart, which was as opposed to those of wild-type embryos, and showed supernumerary incisors near the original upper incisor at E20 (14). CEBPB (CCAAT/enhancer-binding protein beta) is a transcription factor that binds to consensus sequences and regulates the transcription of various genes involved in proliferation and differentiation; it is also suggested to be an important factor in osteogenesis and odontogenesis (35). CEBPB is purportedly involved in odontogenesis through interactions with DSPP (dentine sialophosphoprotein) and RUNX2 (runt-related transcription factor 2) (35, 36). In adult *Cebpb*-deficient mice, supernumerary teeth and complex odontomas close by the root of the maxillary incisor were reported (37). Whereas loss-of-function of *SOSTDC1*, *LRP4*, *APC*, *PAX6,* and *CEBPB* are associated with supernumerary incisor formation in rodents, the findings of present study suggested that SNPs tested in these genes were not associated with mesiodens in humans. Rs2239789 in *PAX6* showed suggestive differences ($p = 0.04$) in allele frequencies between the mesiodens group and controls; however, this might be because allele frequencies in controls were different from those in the general Japanese population based on data from

the International HapMap Project. These results suggested that the SNPs in tested can be ruled out as candidates for mesiodens etiology.

No positive polymorphisms were detected between the mesiodens group and controls in the intron SNPs tested in this study, althought the SNPs were the highest frequency in Japanese. However, the entire region of each gene was not analyzed; therefore, SNPs as genetic markers might still be present in these genes. Alternatively, novel genes might be involved in the control of mesiodens formation. Detection of candidate loci and further definition of genes responsible for mesiodens could require a genome-wide survey based on a large number of markers and whole sequencing of candidate genes. Future studies should clarify the underlying mechanisms of mesiodens formation in humans and focus on the translation of those findings into clinical tools for early diagnosis and treatment.

References

1. Marya CM, Kumar BR: Familial occurrence of mesiodentes with unusual findings: case reports. Quintessence Int, 29: 49-51, 1998.

2. Gallas MM, García A: Retention of permanent incisors by mesiodens: a family affair. Br Dent J, 188: 63-64, 2000.

3. Batra P, Duggal R, Parkash H: Non-syndromic multiple supernumerary teeth transmitted as an autosomal dominant trait. J Oral Pathol Med, 34: 621-625, 2005.

4. Subasioglu A, Savas S, Kucukyilmaz E, Kesim S, Yagci A, Dundar M: Genetic background of supernumerary teeth. Eur J Dent, 9: 153-158, 2015.

5. Lu H, Zeng B, Yu D, Jing X, Hu B, Zhao W, Wang Y: Complex dental anomalies in a belatedly diagnosed cleidocranial dysplasia patient. Imaging Sci Dent, 45: 187-192, 2015.

6. Tirali RE, Sar C, Cehreli SB: Oro-facio-dental findings of rubinstein-taybi syndrome as a useful diagnostic feature. J Clin Diagn Res, 8: 276-278, 2014.

7. Ramaglia L, Morgese F, Filippella M, Colao A: Oral and maxillofacial manifestations of Gardner's syndrome associated with growth hormone deficiency: case report and literature review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 103: e30-34, 2007.

8. Kawashima A, Nomura Y, Aoyagi Y, Asada Y: Heredity may be one of the etiologies of supernumerary teeth. Pediatric Dent J, 16: 115-117, 2006.

9. Vecchione Gurgel C, Soares Cota AL, Yuriko Kobayashi T, Moura Bonifácio Silva S, Aparecida Andrade Moreira Machado M, Rios D, Garib DG, Marchini Oliveira T: Bilateral mesiodens in monozygotic twins: 3D diagnostic and management. Case Rep Dent, 193614, 2013.

10. Nakamura T, Fukumoto S: Genetics of supernumerary tooth formation. J Oral Biosci, 55: 180-183, 2013.

11. Murashima-Suginami A, Takahashi K, Kawabata T, Sakata T, Tsukamoto H, Sugai M, Yanagita M,

Shimizu A, Sakurai T, Slavkin HC, Bessho K: Rudiment incisors survive and erupt as supernumerary teeth as a result of USAG-1 abrogation. Biochem Biophys Res Commun, 359: 549-555, 2007.
12. Ohazama A, Johnson EB, Ota MS, Choi HY, Porntaveetus T, Oommen S, Itoh N, Eto K, Gritli-Linde A, Herz J, Sharpe PT: Lrp4 modulates extracellular integration of cell signaling pathways in development. PLoS One, 3: e4092, 2008.
13. Wang XP, O'Connell DJ, Lund JJ, Saadi I, Kuraguchi M, Turbe-Doan A, Cavallesco R, Kim H, Park PJ, Harada H, Kucherlapati R, Maas RL: Apc inhibition of Wnt signaling regulates supernumerary tooth formation during embryogenesis and throughout adulthood. Development, 136: 1939-1949, 2009.
14. Kriangkrai R, Chareonvit S, Yahagi K, Fujiwara M, Eto K, Iseki S: Study of Pax6 mutant rat revealed the association between upper incisor formation and midface formation. Dev Dyn, 235: 2134-2143, 2006.
15. Huang B, Takahashi K, Sakata-Goto T, Kiso H, Togo Y, Saito K, Tsukamoto H, Sugai M, Akira S, Shimizu A, Bessho K: Phenotypes of CCAAT/enhancer-binding protein beta deficiency: hyperdontia and elongated coronoid process. Oral Dis, 19: 144-150, 2013.
16. Liu H, Han D, Wong S, Nan X, Zhao H, Feng H: rs929387 of GLI3 is involved in tooth agenesis in Chinese Han population. PLoS One, 8: e80860, 2013.
17. Song S, Zhao R, He H, Zhang J, Feng H, Lin L: WNT10A variants are associated with non-syndromic tooth agenesis in the general population. Hum Genet, 133: 117-124, 2014.
18. Khambete N, Kumar R: Genetics and presence of non-syndromic supernumerary teeth: A mystery case report and review of literature. Contemp Clin Dent, 3: 499-502, 2012.
19. Ahtiainen L, Uski I, Thesleff I, Mikkola ML: Early epithelial signaling center governs tooth budding morphogenesis. J Cell Biol, 214: 753-767, 2016.
20. Bei M: Molecular genetics of tooth development. Curr Opin Genet Dev, 19: 504-510, 2009.
21. St Amand TR, Zhang Y, Semina EV, Zhao X, Hu Y, Nguyen L, Murray JC, Chen Y: Antagonistic signals between BMP4 and FGF8 define the expression of Pitx1 and Pitx2 in mouse tooth-forming anlage. Dev Biol, 217: 323-332, 2000.
22. Kratochwil K, Galceran J, Tontsch S, Roth W, Grosschedl R: FGF4, a direct target of LEF1 and Wnt signaling, can rescue the arrest of tooth organogenesis in Lef1(-/-) mice. Genes Dev, 16: 3173-3185, 2002.
23. Balic A, Thesleff I: Tissue interactions regulating tooth development and renewal. Curr Top Dev Biol, 115: 157-186, 2015.
24. Dassule HR, McMahon AP: Analysis of epithelial-mesenchymal interactions in the initial morphogenesis of the mammalian tooth. Dev Biol, 202: 215-227, 1998.
25. Häärä O, Harjunmaa E, Lindfors PH, Huh SH, Fliniaux I, Åberg T, Jernvall J, Ornitz DM, Mikkola ML, Thesleff I: Ectodysplasin regulates activator-inhibitor balance in murine tooth development through Fgf20 signaling. Development, 139: 3189-3199, 2012.
26. Podzus J, Kowalczyk-Quintas C, Schuepbach-Mallepell S, Willen L, Staehlin G, Vigolo M, Tardivel

A, Headon D, Kirby N, Mikkola ML, Schneider H, Schneider P: Ectodysplasin A in biological fluids and diagnosis of ectodermal dysplasia. J Dent Res, 96: 217-224, 2017.
27. Mustonen T, Pispa J, Mikkola ML, Pummila M, Kangas AT, Pakkasjärvi L, Jaatinen R, Thesleff I: Stimulation of ectodermal organ development by Ectodysplasin-A1. Dev Biol, 259: 123-136, 2003.
28. van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK: MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. Nat Genet, 24: 342-343. No abstract available, 2000.
29. Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI: Mutation of PAX9 is associated with oligodontia. Nat Genet, 24: 18-19, 2000.
30. Laurikkala J, Kassai Y, Pakkasjärvi L, Thesleff I, Itoh N: Identification of a secreted BMP antagonist, ectodin, integrating BMP, FGF, and SHH signals from the tooth enamel knot. Dev Biol, 264: 91-105, 2003.
31. Itasaki N, Jones CM, Mercurio S, Rowe A, Domingos PM, Smith JC, Krumlauf R: Wise, a context-dependent activator and inhibitor of Wnt signalling. Development, 130: 4295-4305, 2003.
32. Dong X, Shen B, Ruan N, Guan Z, Zhang Y, Chen Y, Hu X: Expression patterns of genes critical for BMP signaling pathway in developing human primary tooth germs. Histochem Cell Biol, 142: 657-665, 2014.
33. Nascimento MA, Nonaka CF, Barboza CA, Freitas RA, Pereira Pinto L, Souza LB: Immunoexpression of BMP-2 and BMP-4 and their receptors, BMPR-IA and BMPR-II, in ameloblastomas and adenomatoid odontogenic tumors. Arch Oral Biol, 73: 223-229, 2017.
34. Chen J, Lan Y, Baek JA, Gao Y, Jiang R: Wnt/beta-catenin signaling plays an essential role in activation of odontogenic mesenchyme during early tooth development. Dev Biol, 334: 174-185, 2009.
35. Wiper-Bergeron N, St-Louis C, Lee JM: CCAAT/Enhancer binding protein beta abrogates retinoic acid-induced osteoblast differentiation via repression of Runx2 transcription. Mol Endocrinol, 21: 2124-2135, 2007.
36. Narayanan K, Ramachandran A, Peterson MC, Hao J, Kolstø AB, Friedman AD, George A: The CCAAT enhancer-binding protein (C/EBP) beta and Nrf1 interact to regulate dentin sialophosphoprotein (DSPP) gene expression during odontoblast differentiation. J Biol Chem, 279: 45423-45432, 2004.
37. Huang B, Takahashi K, Sakata-Goto T, Kiso H, Togo Y, Saito K, Tsukamoto H, Sugai M, Akira S, Shimizu A, Bessho K: Phenotypes of CCAAT/enhancer-binding protein beta deficiency: hyperdontia and elongated coronoid process. Oral Dis, 19: 144-150, 2012.

Table 1. Comparison of genotype frequencies of genes associated with the initiation of odontogenesis between the mesiodens groups and controls

Gene	SNP	Genotype	Mesiodens (n=24)	Controls (n=24)	*p* -value	Japanese * frequency
PITX2	rs3796902	CC	10	16	0.22	43%
		CT	10	6		48%
		TT	4	2		9%
LEF1	rs10022956	CC	4	6	0.58	15%
		CT	12	10		43%
		TT	8	8		42%
EDAR	rs3827760	CC	14	18	0.27	64%
		CT	9	6		33%
		TT	1	0		3%
MSX1	rs3775261	AA	7	6	0.87	35%
		AC	15	15		38%
		CC	2	3		27%
RUNX2	rs1321081	AA	11	9	0.46	42%
		AG	11	10		45%
		GG	2	5		13%
FGF9	rs4770190	AA	6	4	0.29	14%
		AC	13	10		50%
		CC	5	10		36%
FGF20	rs10106536	CC	9	3	0.06	24%
		CT	10	18		45%
		TT	5	3		31%
WNT10A	rs2385199	AA	16	16	0.19	53%
		AG	8	6		42%
		GG	0	2		5%
BMP4	rs2071047	CC	0	2	0.05	24%
		CT	18	11		49%
		TT	6	11		27%
BMP2	rs1005464	AA	8	6	0.27	21%
		AG	12	9		45%
		GG	4	9		34%
PAX9	rs17176643	AA	2	6	0.16	19%
		AC	20	14		57%
		CC	2	4		24%
LHX6	rs10818649	CC	2	4	0.68	20%
		CT	13	12		49%
		TT	9	8		31%
TP63	rs11915751	CC	5	4	0.93	22%
		CT	8	8		44%
		TT	11	12		34%
MSX2	rs4868442	AA	4	2	0.16	6%
		AG	6	3		40%
		GG	14	19		54%
WNT10B	rs833843	CC	8	7	0.94	17%
		CT	9	9		47%
		TT	7	8		36%
EDA	rs6625561	CC	14	9	0.12	60%
		CT	3	9		19%
		TT	7	6		21%

* International HapMap Project

Table 2. Comparison of allele frequencies of genes associated with the initiation of odontogenesis between the mesiodens groups and controls

Gene	SNP	Allele	Mesiodens (n=24)	Controls (n=24)	*p* -value	Japanese * frequency
PITX2	rs3796902	C	30	38	0.09	67%
		T	18	10		33%
LEF1	rs10022956	C	20	22	0.68	37%
		T	28	26		63%
EDAR	rs3827760	C	37	42	0.18	80%
		T	11	6		20%
MSX1	rs3775261	A	29	27	0.68	55%
		C	19	21		45%
RUNX2	rs1321081	G	15	20	0.29	35%
		A	33	28		65%
FGF9	rs4770190	C	23	30	0.15	61%
		A	25	18		39%
FGF20	rs10106536	C	28	24	0.41	47%
		T	20	24		53%
WNT10A	rs2385199	G	8	10	0.60	26%
		A	40	38		74%
BMP4	rs2071047	C	18	15	0.52	49%
		T	30	33		51%
BMP2	rs1005464	G	20	27	0.15	56%
		A	28	21		44%
PAX9	rs17176643	A	24	26	0.68	47%
		C	24	22		53%
LHX6	rs10818649	C	17	20	0.53	44%
		T	31	28		56%
TP63	rs11915751	C	18	16	0.67	44%
		T	30	32		56%
MSX2	rs4868442	G	34	42	0.04	74%
		A	14	6		26%
WNT10B	rs833843	C	25	23	0.68	41%
		T	23	25		59%
EDA	rs6625561	C	31	27	0.40	70%
		T	17	21		30%

* International HapMap Project

Table 3. Comparison of genotype frequencies of genes associated with supernumerary incisor formation in rodents between the mesiodens groups and controls

Gene	SNP	Genotype	Mesiodens (n=24)	Controls (n=24)	*p* -value	Japanese * frequency
CEBPB	rs6095811	TT	6	9	0.49	36%
		CT	11	11		44%
		CC	7	4		20%
PAX6	rs2239789	AA	9	5	0.20	-
		AT	8	6		-
		TT	7	13		-
SOSTDC1	rs12699798	AA	5	8	0.58	19%
		AG	13	10		56%
		GG	6	6		25%
APC	rs2431512	TT	15	11	0.32	50%
		CT	7	12		42%
		CC	2	1		8%
LRP4	rs2306037	GG	5	2	0.46	25%
		CG	10	11		43%
		CC	9	11		32%

* International HapMap Project

Table 4. Comparison of allele frequencies of genes associated with supernumerary incisor formation in rodents between the mesiodens groups and controls

Gene	SNP	Allele	Mesiodens (n=24)	Controls (n=24)	*p* -value	Japanese * frequency
CEBPB	rs6095811	C	25	19	0.22	58%
		T	23	29		42%
PAX6	rs2239789	A	26	16	0.04	49% **
		T	22	32		51%
SOSTDC1	rs12699798	A	23	26	0.54	46%
		G	25	22		54%
APC	rs2431512	C	11	14	0.49	29%
		T	37	34		71%
LRP4	rs2306037	C	28	33	0.29	53%
		G	20	15		47%

* International HapMap Project

** Chinese frequency