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A case–control study of the association between tooth-development gene polymorphisms and non-syndromic hypodontia in the Chinese Han population

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Hypodontia is one of the most common anomalies of human dentition. Recent genetic studies provide information on a number of genes related to both syndromic and non-syndromic forms of hypodontia. Fifty putative single nucleotide polymorphisms (SNPs) in 20 genes that play important roles in tooth development were selected, and a case–control study was conducted in 273 subjects with hypodontia (cases) and 200 subjects without hypodontia (controls). DNA was obtained from samples of whole blood or saliva. Genotyping was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). A significant difference was observed, between subjects with non-syndromic hypodontia and controls, in the allele and genotype frequencies of two markers [rs929387 of GLI family zinc finger 3 (*GLI3*) and rs11001553 of Dickkopf-related protein 1 (*DKK1*)]. Similar results were observed in a subgroup analysis of test subjects (stratified by gender or missing tooth position). However, this analysis showed no significant difference in the haplotype distribution between the controls and the affected subjects. These data demonstrate an association between some SNPs in tooth development-associated genes and sporadic non-syndromic hypodontia in Chinese Han individuals. This information may provide further understanding of the molecular mechanisms of tooth agenesis. Furthermore, these genes can be regarded as candidates for mutation detection in individuals with tooth agenesis.

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Tooth agenesis, or hypodontia, which is the congenital absence of one or more teeth, is the most common developmental anomaly in human dentition. More than 20% of humans fail to develop at least one-third of molars (wisdom teeth) and one or more (3–10%) other permanent teeth (1). Although tooth agenesis does not represent a serious health problem, it may contribute to masticatory dysfunction, speech alteration, esthetic problems, and malocclusion (2). Tooth agenesis may present as part of a syndrome, such as anhidrotic ectodermal dysplasia (EDA) (3, 4), Rieger syndrome (5), or Witkop syndrome (6). However, the isolated, non-syndromic form is more common. Isolated, non-syndromic agenesis can be sporadic or familial and may be inherited in a Mendelian-dominant or Mendelian-recessive autonomic mode, or may be X-linked (7).

With advances in molecular genetics, the genetic causes of tooth agenesis are becoming clearer. To date, mutations in Msh homeobox 1 (*MSX1*) (8), paired box 9 (*PAX9*) (9), axis inhibition protein 2 (*AXIN2*) (10), and *EDA* (11, 12) have been determined to be associated with non-syndromic tooth agenesis. However, tooth agenesis in the absence of identified mutations in the *MSX1*, *PAX9*, *AXIN2*, or *EDA* genes is also common. Furthermore, the origin of sporadic non-syndromic tooth agenesis, the most common form of isolated tooth agenesis in humans, remains to be elucidated.

Dental development is a complicated process involving many genes and signaling pathways (13). Molecular studies have shown that tooth development requires a sequential array of epithelial–mesenchymal interactions involving many signaling molecules such as growth factors and their receptors, transcription factors, and other modifier proteins (14, 15). Alterations in one or more of the many genes encoding these signaling molecules

may cause the most common congenital anomaly in humans – tooth agenesis.

Gene polymorphism underlies the mechanism by which individuals exhibit variations within the extent of what is considered biologically normal. There is a close relationship between gene polymorphisms and disease susceptibility. Single nucleotide changes, which occur at a high frequency in the human genome, are the most common polymorphisms and may affect the function of genes. Such variants are regarded as hypomorphic (reduced activity but not complete loss of function) or as 'risk alleles' and can occur either in the coding or the non-coding regions and affect the amount of protein that is produced, rather than the protein function (1). In recent years, studies have focused on investigations of single nucleotide polymorphisms (SNPs) associated with disorders such as hypertension, cancer, and diabetes. However, studies on SNPs associated with tooth agenesis are rare, with only two conducted in the Chinese population (16, 17).

In this study, a large-sample-size and polygene parallel study was conducted to investigate the association between gene polymorphisms and sporadic non-syndromic hypodontia in the Chinese Han population.

Material and methods

Subject selection and sampling

A total of 273 subjects (125 men and 148 women) diagnosed with sporadic non-syndromic hypodontia (excluding the third molar) and 200 healthy control subjects (100 men and 100 women) were recruited from the Peking University School and Hospital of Stomatology during the period July 2006 to May 2009. All individuals participating in this study were genetically unrelated ethnic Han Chinese from Beijing or the surrounding regions.

Naturally missing teeth within the adult dentition were confirmed by X-ray examination and no other dental anomalies were observed in any subjects. The subject population with hypodontia was 15–38 yr of age and the control population was 17–30 yr of age. Details of the study population are presented in Table 1. All samples were obtained with informed consent, and blood samples and oral swabs were coded to maintain confidentiality. Genomic DNA was extracted from peripheral blood lymphocytes using a standard high-salt method or from buccal epithelial cells using a salt/ethanol-precipitation method (18). The extracted DNA samples were stored at −20°C before analysis. This study was approved by the Institutional Review Board of Peking University School and Hospital of Stomatology.

SNP selection

Twenty genes, linked to tooth agenesis in human or animal studies, were selected. Fifty putative SNPs were selected based on their location in these genes according to the dbSNP (www.ncbi.nlm.nih.gov/SNP). The majority of these SNPs were located in coding and regulatory regions with minor allele frequencies of >10%. Details of the selected SNPs are presented in Table 2.

Table 1

Characteristics of subjects with naturally missing teeth

Characteristic	Value *n* (%)
Gender distribution	
Male	125 (45.9)
Female	148 (54.2)
Number of teeth missing per subject	
1	137 (50.2)
2	92 (33.7)
3 or more	44 (16.1)
Type of teeth more often missing (total teeth missing = 585)	
Maxillary central incisors	2 (0.3)
Mandibular central incisors	101 (17.3)
Maxillary lateral incisors	52 (8.9)
Mandibular lateral incisors	119 (20.3)
Maxillary canines	32 (5.5)
Mandibular canines	19 (3.2)
Maxillary first premolars	31 (5.3)
Mandibular first premolars	19 (3.2)
Maxillary second premolars	72 (12.3)
Mandibular second premolars	114 (19.5)
Maxillary first molars	3 (0.5)
Mandibular first molars	4 (0.7)
Maxillary second molars	12 (2.1)
Mandibular second molars	5 (0.9)
Number of patients with missing incisors	91 (33.3)
Number of patients with missing canines	29 (10.6)
Number of patients with missing premolars	184 (67.4)
Number of patients with missing molars	8 (2.9)

Polymorphism genotyping

All genotyping experiments were performed by Shanghai Benegene Biotechnology (Shanghai, China; www.benegene.com.cn/). Primers for PCR and single base extensions were designed using the Assay Designer software package (Sequenom, San Diego, CA, USA). Genotyping of SNPs was performed using the MASSARRAY system (Sequenom) by means of the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) method, according to the instructions provided by the manufacturer. Completed genotyping reactions were spotted onto a 384-well spectroCHIP (Sequenom) using the MASSARRAY system (Sequenom) and analyzed using MALDI-TOF-MS (19). Genotype calling was performed in real time using MASSARRAY RT software, version 3.0.0.4, and analyzed using the MASSARRAY TYPER software, version 3.4 (Sequenom). Confirmation of method used (MALDI-TOF MS) by direct sequencing were showed in Additional Supporting Information (Fig S1).

Statistical analysis

Hardy–Weinberg equilibrium was tested using a goodness-of-fit chi-square test to compare the observed genotype frequencies with the expected genotype frequencies among the control subjects. Clinical information and gender were compared across genotypes, using chi-square tests. $P < 0.05$ was considered statistically significant. The associations between genotypes and the risk of sporadic non-syndromic hypodontia were estimated by computing the ORs and their 95% CI from logistic regression analyses. The 2LD software was used to calculate the D' value for linkage disequilibrium among the 50 SNPs, and the

Table 2

Analysis of single nucleotide polymorphisms (SNPs)

Gene	SNP site	Position	Gene	SNP site	Position
AXIN2	rs2240308	Exon 2 (Pro→Ser)	*GLI3*	rs846266	Exon 5 (Thr→Ala)
BMP2	rs15705	3′-UTR		rs929387	Exon 15 (Pro→Leu)
	rs235768	Exon 3 (Arg→Ser)		rs35280470	Exon 15 (Gly→Glu)
	rs3178250	3′-UTR		rs35364414	Exon 15 (Arg→Cys)
BMP4	rs17563	Exon 2 (Val→Ala)	*IRF6*	rs742215	Near 3′-UTR
DKK1	rs11001553	Near 5′-UTR		rs861019	5′-UTR
DLX1	rs788172	3′-UTR		rs2235371	Exon 7 (Val→Ile)
	rs788173	3′-UTR	*LEF1*	rs4245927	3′-UTR
	rs813720	Near 3′-UTR	*MSX1*	rs12532	3′-UTR
DLX2	rs743605	5′-UTR		rs3821947	Near 5′-UTR
EDAR	rs3749096	3′-UTR		rs3821949	Near 5′-UTR
	rs3749110	5′-UTR	*PAX9*	rs2073244	Near 5′-UTR
	rs3827760	Exon 12 (Val→Ala)		rs2073247	Near 5′-UTR
	rs6749207	Near 5′-UTR		rs4904155	5′-UTR
EDARADD	rs966365	Exon 12 (Met→Ile)		rs4904210	Exon 4 (Ala→Pro)
	rs3916983	3′-UTR		rs10141087	Near 3′-UTR
	rs6428955	3′-UTR	*PVRL1*	rs3829260	Intron
	rs7513402	3′-UTR		rs7940667	Exon 6 (Val→Gly)
FGFR1	rs13317	3′-UTR	*SHH*	rs288746	Near 5′-UTR
	rs881310	Intron		rs9333594	5′-UTR
	rs3213849	5′-UTR	*TGFA*	rs503314	3′-UTR
FST	rs722910	3′-UTR		rs1058213	3′-UTR
GLI2	rs2278741	3′-UTR	*WNT10A*	rs1057306	Exon 4 (Pro→Thr)
	rs3738880	Exon 14 (Ala→Ser)		rs6744926	Exon 2 (Ile→Val)
	rs12711538	Exon 14 (Asp→Asn)		rs34972707	Exon 4 (Asn→His)

Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; *AXIN2*, axis inhibition protein 2; *BMP2*, bone morphogenetic protein 2; *BMP4*, bone morphogenetic protein 4; Cys, cysteine; *DKK1*, Dickkopf-related protein 1; *DLX1*, distal-less homeobox 1; *DLX2*, distal-less homeobox 2; *EDAR*, ectodysplasin A receptor; *EDARADD*, EDAR-associated death domain; *FGFR1*, fibroblast growth factor receptor 1; *FST*, follistatin; *GLI2*, GLI family zinc finger 2; *GLI3*, GLI family zinc finger 3; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; *IRF6*, interferon regulatory factor 6; *LEF1*, lymphoid enhancer-binding factor 1; Leu, leucine; Met, methionine; *MSX1*, Msh homeobox 1; *PAX9*, paired box 9; Pro, proline; *PVRL1*, poliovirus receptor-related 1; Ser, serine; *SHH*, sonic hedgehog; *TGFA*, transforming growth factor alpha; Thr, threonine; Val, valine; *WNT10A*, wingless-type MMTV integration site family member 10A; UTR, untranslated region.

PHASE 2.1 software program was used to analyze the haplotypes. All statistical tests for this analysis were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).

Results

Five markers (rs929387, rs11001553, rs3916983, rs7888172, and rs813720) in four genes [GLI family zinc finger 3 (*GLI3*), Dickkopf-related protein 1 (*DKK1*), ectodysplasin A receptor-associated death domain (*EDARADD*), and distal-less homeobox 1 (*DLX1*)] exhibited significant differences in allele and/or genotype frequencies in comparisons of the case group (individuals with hypodontia) with the control group (normal individuals) (Table 3). The C allele was identified in the marker rs929387 of *GLI3* in 22.4% of the case group and in 15.1% of the control group ($P = 0.0082$). The CC and TT genotype frequencies were 6.7% and 61.9%, respectively, in the case group and were 2.9% and 72.6%, respectively, in the control group ($P = 0.0395$). Regarding the marker rs11001553 of *DKK1*, the C allele was observed at a frequency of 91.2% in the case group and at a frequency of 86.1% in the control group ($P = 0.133$). The genotype CC was observed in 82.5% of the case group but in only 74.2% of the control group ($P = 0.0130$). A significant difference was observed in the distribution of alleles in rs3916983 of *EDARADD* ($P = 0.0446$) and in rs7888172 of *DLX1* ($P = 0.0476$). A significant difference was observed in the genotype distribution in rs813720 of *DLX1* ($P = 0.0415$). Four blocks of haplotypes were identified as a result of linkage disequilibrium (LD) analyses, but no relevant haplotypes were identified in further analyses.

Four markers (rs11001553 of *DKK1*, rs4904155 of *PAX9*, and rs7888172 and rs813720 of *DLX1*) showed significant differences in allele and/or genotype frequencies in comparisons of the female case group (female individuals with hypodontia) with the female control group (normal female individuals) (Table 4). Comparison of the case group with the control group revealed greater significant differences in the two markers (rs7888172 and rs813720) of *DLX1*. In rs7888172, allele "A" seemed to be a risk factor for female individuals (OR = 2.346; 95% CI = 1.483–3.712; $P = 0.0002$) and in rs813720, allele "C" seemed to be a risk factor for female individuals (OR = 2.194; 95% CI = 1.423–3.384; $P = 0.0003$).

Comparison of the male case group (male individuals with hypodontia) with the male control group (normal male individuals) revealed that one marker of *GLI3*

Table 3

Distribution of genotypes and alleles for five single nucleotide polymorphisms (SNPs) in the case and control groups

Gene	SNP site	Samples	*n*	Allele *n* (%)		*P*	OR (95% CI)	Genotype *n* (%)			*P*
GLI3	rs929387			C	T			CC	CT	TT	
		Case	252	113 (22.4)	391 (77.6)	**0.0082**	1.620 (1.130–2.320)	17 (6.7)	79 (31.3)	156 (61.9)	**0.0395**
		Control	175	53 (15.1)	297 (84.9)			5 (2.9)	43 (24.6)	127 (72.6)	
DKK1	rs11001553			C	T			CC	CT	TT	
		Case	268	489 (91.2)	47 (8.8)	**0.0133**	1.686 (1.111–2.559)	221 (82.5)	47 (17.5)	0 (0)	**0.0130**
		Control	190	327 (86.1)	53 (13.9)			141 (74.2)	45 (23.7)	4 (2.1)	
EDARADD	rs3916983			G	C			CC	CG	GG	
		Case	271	492 (90.8)	50 (9.2)	**0.0446**	1.525 (1.008–2.308)	2 (0.7)	46 (17.0)	223 (82.3)	0.116
		Control	190	329 (86.6)	51 (13.4)			2 (1.1)	47 (24.7)	141 (47.2)	
DLX 1	rs7888172			A	G			AA	AG	GG	
		Case	270	143 (27.4)	379 (72.6)	**0.0476**	1.365 (1.003–1.860)	22 (8.4)	99 (37.9)	140 (53.6)	0.1378
		Control	194	84 (21.6)	304 (78.4)			12 (6.2)	60 (30.9)	122 (62.9)	
DLX 1	rs813720			C	G			CC	CG	GG	
		Case	260	154 (29.6)	366 (70.4)	0.0586	1.355 (0.989–1.801)	20 (7.7)	114 (43.8)	126 (48.5)	**0.0415**
		Control	194	93 (24.0)	295 (76.0)			15 (7.7)	63 (32.5)	116 (59.8)	

DLX1, distal-less homeobox 1; *DKK1*, Dickkopf-related protein 1; *EDARADD*, EDAR-associated death domain; *GLI3*, GLI family zinc finger 3; A, Adenine; C, Cytosine; G, Guanine; T, Thymine; AA, Adenine/Adenine; AG, Adenine/Guanine; CC, Cytosine/Cytosine; CG, Cytosine/Guanine; CT, Cytosine/Thymine; GG, Guanine/Guanine; TT, Thymine/Thymine; *P*-values lower than 0.05 were written bold.

Table 4

Distribution of genotypes and alleles for four single nucleotide polymorphisms (SNPs) in the female case and control groups

Gene	SNP site	Samples	*n*	Allele *n* (%)		*P*	OR (95% CI)	Genotype *n* (%)			*P*
DKK 1	rs11001553			C	T			CC	CT	TT	
		Case (female)	145	268 (92.4)	22 (7.6)	**0.0074**	2.215 (1.225–4.006)	123 (84.8)	22 (15.2)	0 (0)	**0.0160**
		Control (female)	91	154 (84.6)	28 (15.4)			66 (72.5)	22 (24)	3 (3.3)	
PAX9	rs4904155			C	G			CC	CG	GG	
		Case (female)	144	166 (57.6)	122 (42.4)	**0.0206**	1.542 (1.068–2.227)	51 (35.4)	64 (44.4)	29 (20.1)	**0.0327**
		Control (female)	96	90 (46.9)	102 (53.1)			19 (19.8)	52 (54.2)	25 (26.0)	
DLX1	rs7888172			A	G			AA	AG	GG	
		Case (female)	143	89 (31.1)	197 (68.9)	**0.0002**	2.346 (1.483–3.712)	12 (8.4)	65 (45.5)	66 (46.2)	**0.0002**
		Control (female)	96	31 (16.1)	161 (83.9)			5 (5.2)	21 (21.9)	70 (72.9)	
DLX1	rs813720			C	G			CC	CG	GG	
		Case (female)	144	99 (34.4)	189 (65.6)	**0.0003**	2.194 (1.423–3.384)	13 (9.0)	73 (50.7)	58 (40.3)	**< 0.0001**
		Control (female)	96	37 (19.3)	155 (80.7)			8 (8.3)	21 (21.9)	67 (69.8)	

DKK1, Dickkopf-related protein 1; *DLX1*, distal-less homeobox 1; *PAX9*, paired box 9; A, Adenine; C, Cytosine; G, Guanine; T, Thymine; AA, Adenine/Adenine; AG, Adenine/Guanine; CC, Cytosine/Cytosine; CG, Cytosine/Guanine; CT, Cytosine/Thymine; GG, Guanine/Guanine; TT, Thymine/Thymine.

(rs929387), three markers of *PAX9* (rs4904155, rs4904210, and rs2073247), and two markers of *MSX1* (rs3821947 and rs3821949) exhibited significant differences in allele and/or genotype frequencies (Table 5). Although these differences were not highly significant, these data indicate that polymorphisms in *PAX9* and *MSX1* are associated with non-syndromic hypodontia in men.

rs2073247 of *PAX9*, rs12532 and rs3821949 of *MSX1*, and rs7888172 and rs813720 of *DLX1* showed significant differences in the alleles and/or genotype frequencies between the female case group (female individuals with hypodontia) and the male case group (male individuals with hypodontia) (Table 6), indicating that different SNPs may be responsible for hypodontia in men and women.

Comparison of groups in whom teeth are missing at specific locations with the control group (all normal individuals) are shown in Tables 7 and 8. Significant differences in rs3821947 of *MSX1* were identified in a comparison performed of genotypes in the control group with genotypes in the group with missing maxillary teeth ($P = 0.01$), while the other two markers showed very modest differences in similar comparisons ($P = 0.04$ for rs813720 and $P = 0.043$ for rs13317). Eleven positive results were identified in comparisons of allele distribution. Interestingly, differences in the allele distribution of the marker rs929387 of *GLI3* were identified in comparisons of the group with missing maxillary teeth and the group with missing mandibular teeth with the control group (group with missing maxillary teeth vs. the control group, $P = 0.04$; group with missing mandibular teeth vs. the control group, $P = 0.037$). However, for rs11001553 of *DKK1*, the distribution of alleles was almost identical (group with missing maxillary teeth vs. the control group, $P = 0.017$; group with missing mandibular teeth vs. the control group, $P = 0.018$). Distribution of genotypes and alleles for all SNPs in the case

Table 5

Distribution of genotypes and alleles for six single nucleotide polymorphisms (SNPs) in the male case and control groups

Gene	SNP site	Samples	*n*	Allele *n* (%)		*P*	Odds ratio (95% CI)	Genotype *n* (%)			*P*
GLI3	rs929387			C	T			CC	CT	TT	
		Case (male)	115	60 (26.1)	170 (73.9)	**0.0067**	2.009 (1.206–3.346)	10 (8.7)	40 (34.8)	65 (56.5)	**0.0327**
		Control (male)	87	26 (14.9)	148 (85.1)			2 (2.3)	22 (25.3)	63 (72.4)	
PAX9	rs4904155			G	C			CC	CG	GG	
		Case (male)	116	118 (50.9)	114 (49.1)	**0.0488**	1.147 (1.001–2.156)	32 (27.6)	50 (43.1)	34 (29.3)	0.0522
		Control (male)	98	81 (41.3)	115 (58.7)			32 (32.7)	51 (52.0)	15 (15.3)	
PAX9	rs4904210			C	G			CC	CG	GG	
		Case (male)	117	119 (50.9)	115 (49.1)	**0.0288**	1.533 (1.044–2.249)	36 (30.8)	47 (40.2)	34 (29.1)	**0.0082**
		Control (male)	98	79 (40.3)	117 (59.7)			13 (13.3)	53 (54.1)	32 (32.7)	
PAX9	rs2073247			T	C			CC	CT	TT	
		Case (male)	124	127 (51.2)	121 (48.8)	**0.0129**	1.651 (1.106–2.358)	32 (25.8)	57 (46.0)	35 (28.2)	**0.0432**
		Control (male)	99	78 (39.4)	120 (60.6)			36 (36.4)	48 (48.5)	15 (15.2)	
MSX1	rs3821947			A	G			AA	AG	GG	
		Case (male)	122	109 (44.7)	135 (55.3)	0.9524	1.012 (0.573–1.220)	19 (15.6)	71 (58.2)	32 (26.2)	**0.0098**
		Control (male)	98	87 (44.4)	109 (55.6)			25 (25.5)	37 (37.8)	36 (36.7)	
MSX1	rs3821949			A	G			AA	AG	GG	
		Case (male)	124	92 (37.1)	156 (62.9)	0.7037	1.078 (0.731–1.591)	11 (8.9)	70 (56.5)	43 (34.7)	**0.0478**
		Control (male)	99	70 (35.4)	128 (64.6)			15 (15.2)	40 (40.4)	44 (44.4)	

GLI3, GLI family zinc finger 3; *MSX1*, Msh homeobox 1; *PAX9*, paired box 9; A, Adenine; C, Cytosine; G, Guanine; T, Thymine; AA, Adenine/Adenine; AG, Adenine/Guanine; CC, Cytosine/Cytosine; CG, Cytosine/Guanine; CT, Cytosine/Thymine; GG, Guanine/Guanine; TT, Thymine/Thymine.

Table 6

Distribution of genotypes and alleles for five single nucleotide polymorphisms (SNPs) in the female and the male case groups

Gene	SNP site	Samples	*n*	Allele *n* (%)		*P*	Odds ratio (95% CI)	Genotype *n* (%)			*P*
PAX9	rs2073247			C	T			CC	CT	TT	
		Case (female)	147	171 (58.2)	123 (41.8)	**0.0292**	1.459 (1.038–2.050)	53 (36.1)	65 (44.2)	29 (19.7)	0.1133
		Case (male)	124	121 (48.8)	127 (51.2)			32 (25.8)	57 (46.0)	35 (28.2)	
MSX1	rs12532			A	G			AA	AG	GG	
		Case (female)	147	132 (44.9)	162 (55.1)	**0.0203**	1.508 (1.065–2.135)	27 (18.4)	78 (53.1)	42 (28.6)	**0.0499**
		Case (male)	124	87 (35.1)	161 (64.9)			12 (9.7)	63 (50.8)	49 (39.5)	
MSX1	rs3821949			G	A			AA	AG	GG	
		Case (female)	147	188 (63.1)	106 (36.1)	0.8018	1.046 (0.703–1.485)	22 (15.0)	62 (42.2)	62 (42.9)	**0.0494**
		Case (male)	124	156 (62.9)	92 (37.1)			11 (8.9)	70 (56.5)	43 (34.7)	
DLX1	rs7888172			A	G			AA	AG	GG	
		Case (female)	143	89 (31.1)	197 (68.9)	**0.0357**	1.523 (1.027–2.257)	12 (8.4)	65 (45.5)	66 (46.2)	**0.0181**
		Case (male)	118	54 (22.9)	182 (77.1)			10 (8.5)	34 (28.8)	74 (62.7)	
DLX1	rs813720			C	G			CC	CG	GG	
		Case (female)	144	99 (34.4)	189 (65.6)	**0.0081**	1.686 (1.143–2.485)	13 (9.0)	73 (50.7)	58 (40.3)	**0.0132**
		Case (male)	116	55 (23.7)	177 (76.3)			7 (6.0)	41 (35.3)	68 (58.6)	

DLX1, distal-less homeobox 1; *MSX1*, Msh homeobox 1; *PAX9*, paired box 9; A, Adenine; C, Cytosine; G, Guanine; T, Thymine; AA, Adenine/Adenine; AG, Adenine/Guanine; CC, Cytosine/Cytosine; CG, Cytosine/Guanine; CT, Cytosine/Thymine; GG, Guanine/Guanine; TT, Thymine/Thymine.

group and the control group were showed in Additional Supporting Information (Table S1).

Discussion

Various factors, including genetic and environmental factors, multireagent chemotherapy, and radiotherapy, contribute to tooth agenesis (7). Although the exact mechanism has not been fully elucidated, genetic factors are believed to play a central role in tooth agenesis.

The findings of this study provide some insight into the prevalence of tooth agenesis observed in recent years (20). The significant disparity between the high incidence of tooth agenesis and the relative lack of information regarding the mechanism of its development suggests that tooth agenesis is a highly heterogeneous trait caused by several independent defective genes, acting alone or in combination with other genes and leading to specific phenotypes. Individuals with distinct polymorphic alleles may exhibit subtle and specific phenotypic variations in dental patterning. Consequently, it can be speculated that association studies between gene polymorphisms and hypodontia, as well as other mild malformations, could reflect qualitative defects of embryogenesis (21). Moreover, such

Table 7

Distribution of genotypes in the control group and missing teeth groups

Gene	SNP site	Samples	Missing teeth position*	*n*	Genotype *n* (%)			*P*
MSX1	rs3821947		Maxillary		GG	GA	AA	
		Case		93	19 (20.4)	56 (60.2)	18 (19.4)	**0.0101**
		Control		189	68 (36.0)	80 (42.3)	41 (21.7)	
DLX1	rs813720		Maxillary		CC	CG	GG	
		Case		85	8 (9.4)	40 (47.1)	37 (43.5)	**0.0396**
		Control		194	15 (7.7)	63 (32.5)	116 (59.8)	
FGFR1	rs13317		Premolar		CC	CT	TT	
		Case		83	16 (19.3)	29 (34.9)	38 (45.8)	**0.0428**
		Control		186	18 (9.7)	88 (47.3)	80 (43.0)	

*Missing teeth position includes subjects in whom one or more teeth are missing in specific locations and does not exclude missing teeth in other locations.
DLX1, distal-less homeobox 1; *FGFR1*, fibroblast growth factor receptor 1; *MSX1*, Msh homeobox 1; SNP, single nucleotide polymorphism; AA, Adenine/Adenine; CC, Cytosine/Cytosine; CG, Cytosine/Guanine; CT, Cytosine/Thymine; GA, Guanine/Adenine; GG, Guanine/Guanine; TT, Thymine/Thymine.

Table 8

Distributions of alleles in the control group and missing teeth groups

Gene	SNP site	Samples	Missing teeth position	*n*	Allele *n* (%)		*P*	OR (95% CI)
DKK1	rs11001553		Maxillary		C	T		
		Case		92	171 (92.9)	13 (7.1)	**0.017**	2.132 (1.131–4.020)
		Control		190	327 (86.1)	53 (13.9)		
DLX1	rs7888172		Maxillary		A	G		
		Case		87	54 (31.0)	120 (69.0)	**0.017**	1.629 (1.090–2.434)
		Control		194	84 (21.6)	304 (78.4)		
DLX1	rs813720		Maxillary		C	G		
		Case		85	56 (32.9)	114 (67.1)	**0.027**	1.558 (1.049–2.315)
		Control		194	93 (24.0)	295 (76.0)		
PVRL1	rs7940667		Maxillary		A	C		
		Case		85	16 (9.4)	154 (90.6)	**0.023**	2.231 (1.099–4.529)
		Control		191	17 (4.5)	365 (95.5)		
GLI3	rs929387		Maxillary		C	T		
		Case		86	44 (25.6)	128 (74.4)	**0.004**	1.926 (1.228–3.022)
		Control		175	53 (15.1)	297 (84.9)		
DKK1	rs11001553		Mandibular		C	T		
		Case		221	407 (91.3)	39 (8.7)	**0.018**	1.691 (1.091–2.662)
		Control		190	327 (86.1)	53 (13.9)		
EDARADD	rs3916983		Mandibular		G	C		
		Case		225	410 (91.1)	40 (8.9)	**0.037**	1.589 (1.025–2.464)
		Control		190	329 (86.6)	51 (13.4)		
GLI3	rs929387		Mandibular		C	T		
		Case		207	87 (21.0)	327 (79.0)	**0.037**	1.491 (1.024–2.171)
		Control		175	53 (15.1)	297 (84.9)		
DKK1	rs11001553		Incisor		C	T		
		Case		180	329 (91.4)	31 (8.6)	**0.022**	1.894 (1.184–3.031)
		Control		175	297 (84.9)	53 (13.9)		
DKK1	rs11001553		Premolar		C	T		
		Case		90	167 (92.8)	13 (7.2)	**0.021**	2.292 (1.214–4.328)
		Control		175	297 (84.9)	53 (15.1)		
GLI3	rs929387		Premolar		C	T		
		Case		81	40 (24.7)	122 (75.3)	**0.009**	1.837 (1.158–2.915)
		Control		175	53 (15.1)	297 (84.9)		

Missing teeth position include subjects that miss one or more teeth in specific locations and do not exclude missing teeth in other locations.
DKK1, Dickkopf-related protein 1; *DLX1*, distal-less homeobox 1; *EDARADD*, EDAR-associated death domain; *GLI3*, GLI family zinc finger 3; *PVRL1*, poliovirus receptor-related 1; SNP, single nucleotide polymorphism.

studies are important for the detection and prioritization of candidate genes for mutation detection.

In this hospital-based case–control study, the associations of 50 SNPs in 20 tooth development-associated genes with risk of sporadic isolated tooth agenesis were investigated in an eastern Chinese Han population.

The data obtained in this case–control study indicated that five SNPs in four genes (*GLI3*, *EDARADD*,

DKK1, and *DLX1*) were associated with sporadic isolated tooth agenesis and that polymorphisms in these genes may be risk factors for tooth agenesis. In these five SNPs, statistical analyses of rs929287 and rs11001553 showed more significant differences.

The marker rs929387 (c.2993C→T) is located in exon 15 of *GLI3* and includes a C→T transversion, resulting in the change Pro998Leu. *GLI3* encodes a protein belonging to the subclass of C2H2-type zinc finger proteins of the Gli family, which are characterized as DNA-binding transcription factors and are mediators of Sonic hedgehog (Shh) signaling (22). This pathway plays important roles during embryogenesis. Sonic hedgehog is involved in both lateral (epithelial–mesenchymal) and planar (epithelial–epithelial) signaling in early tooth development, and GLI3 is expressed in both the epithelial and mesenchymal layers. The failure of tooth development to progress beyond a rudimentary bud stage in $Gli2^{-/-}$ and $Gli3^{-/-}$ embryos supports the notion that these two genes are functionally redundant for tooth development (23). The data obtained in this study demonstrated an association between rs929387 of *GLI3* and sporadic isolated tooth agenesis in the Han population and implicated the C allele as its risk factor (OR = 1.620, 95% CI = 1.130–2.320).

rs11001553 (C/T) is located near the 5′-untranslated region (5′-UTR) of the *DKK1* gene, which encodes a member of the Dickkopf family. This secreted protein contains two cysteine-rich regions and is involved in embryonic development via its inhibition of the wingless-type (WNT) signaling pathway (24). Recently, wingless-type MMTV integration site family member 10A (*WNT10A*) mutations have been reported in patients with syndromic tooth agenesis (25–28) and isolated tooth agenesis (29). It can be speculated that DKK1 functions as an antagonist of the WNT signaling pathway, inhibiting the expression of WNT10A and therefore that DKK1 plays a significant role in tooth development. Furthermore, the data indicate that variation in *DKK1* expression is associated with poradic isolated tooth agenesis and implicate the T allele as its risk factor (OR = 1.686, 95% CI = 1.111–2.559).

Interestingly, following stratification of the case and control groups on the basis of gender, comparisons revealed marked differences in the SNPs between the gender groups rather than between all case–control groups. Furthermore, differences in prevalence were observed between the female and male case groups. rs929387 of *GLI3* exhibited differences between male case and male control groups, but no differences between the female case and female control groups. In contrast, rs11001553 of *DKK1* exhibited differences only in women. Moreover, the markers of *DLX1* (rs7888172 and rs813720) exhibited differences only between the female case and female control groups and the markers of *MSX1* (rs3821947 and rs3821949) exhibited differences only between the male case and male control groups. In most reports, differences in the prevalence of dental agenesis are observed only between men and women. The results of this study indicate that the association of some SNPs with tooth agenesis susceptibility differs between men and women. Furthermore, it is hypothesized that this deduction explains a commonly observed clinical phenomenon in which a son has a different dentition from his mother or a daughter has a different dentition from her father.

Interestingly, it was observed that genotype and allele frequencies of some SNPs varied among missing teeth position. There were no differences between the case and control groups for some SNPs, although differences were detected between the specific missing teeth position group and the control group, and these differences were more pronounced for some SNPs in such comparisons. Tooth development is known to be a complicated process in which different genes are involved in the development of each tooth. The results of this study are consistent with this point.

Previous studies of polymorphisms and tooth agenesis are very rare, with most conducted using a small sample size and focusing on a few SNPs in a single gene. This study was performed on a larger sample size than that of previous studies and detected 50 SNPs in 20 genes, including some SNPs that have been analyzed previously. Callahan *et al.* (30) demonstrated that a significant association of rs2240308 in *AXIN2* alone in Brazilian subjects involved the absence of at least one incisor, although this association was not detected in the present study, probably because of the ethnic differences of the population under investigation. In a study of the Chinese Han population, Pan and coworkers (17) reported that no statistically significant difference was observed between the two markers (rs2073244 and rs2073247) of *PAX9*. However, the investigation of these two SNPs in the present study revealed significant differences in rs2073247 between the male case and male control groups. This discrepancy might be attributed to the differences in sample size between the two studies.

In summary, the discovery of associations between some SNPs in tooth development-associated genes and sporadic non-syndromic hypodontia in Chinese Han people will greatly enhance our understanding of the genetic and molecular mechanisms involved in normal and abnormal tooth development. Moreover, the genes analyzed in this study could be regarded as candidates for mutation detection in individuals with tooth agenesis. However, interpretation of the results is limited by the sample size. Analyses involving a larger sample size and advanced methods, such as genome-wide association studies (GWAS), are required for further elucidation of these observations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Distribution of genotypes and alleles for all SNPs in the case group and the control group.

Fig. S1. Confirmation by direct sequencing of the single nucleotide polymorphisms (SNPs) screening method used (MALDI-TOF MS).

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