PAX-9 polymorphism may be a risk factor for hypodontia: a meta-analysis

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ABSTRACT. To evaluate the association between paired box 9 (*PAX9*) gene polymorphisms and tooth agenesis in isolated humans, we performed a comprehensive meta-analysis. We examined 6 case-control studies, with a total of 855 hypodontia cases and 1201 healthy controls. The G allele and G carrier (AG + GG) of A1031G were positively associated with hypodontia susceptibility. Similarly, the T allele and T carrier (CT + TT) of C912T and rs12881240 in the *PAX9* gene also indicated an increased risk of hypodontia. In addition, the C allele and C carrier (CG + CC) of 718C, IVS2-109, rs4904210, and rs7143727 showed no significant association with oligodontia. The G allele and G carrier (AG + GG) of IVS2-41 in the *PAX9* gene were not related factors. Interestingly, the genotype (AG + GG) of IVS2-54 in the *PAX9* gene may be a protective factor for oligodontia (odds ratio = 0.21, 95% confidence interval = 0.07-0.63, P = 0.005). However, no significant differences were found in the allele frequency of IVS2-54 in the *PAX9* polymorphism between controls and subjects with sporadic tooth agenesis. In conclusion, our meta-analysis results revealed 4 genetic sites of the *PAX9* gene involved in hypodontia cases, of which 3 sites may be risk factors and 1 may have a protective role.

Key words: Genetic polymorphism; Hypodontia; Meta-analysis; *PAX9*

INTRODUCTION

Hypodontia, or tooth agenesis, which is the congenital absence of one or a few teeth, is a common condition in human dentition. It affects up to 20% of people worldwide and includes 2 types, syndromic or non-syndromic, and can occur as a familial (autosomal-dominant, autosomal-recessive, or cross-linked) or isolated sporadic trait (Vastardis, 2000). During the long process of dental development, complex interactions between genetic, epigenetic, and environmental factors cause dental anomalies (Brook, 2009). These factors are variable, and completed factors occurring over time are multi-factorial, multilevel, multidimensional, and progressive.

The paired box 9 (*PAX9*) gene encodes for transcription factors that are expressed in the dental mesenchyme at all stages of odontogenesis, including the initiation, bud, cap, and bell stages (Vainio et al., 1993; Peters et al., 1998). Protein products of this gene serve as transcription factors responsible for the cross-talk between epithelial and mesenchymal tissues. In addition, they are essential for establishing the odontogenic potential of the mesenchyme (Peters and Balling, 1999; Tucker and Sharpe, 1999; Jernvall and Thesfleff, 2000; Scarel-Caminaga et al., 2003). Pax9-deficient mice were shown to lack pharyngeal pouch derivates and teeth and exhibited severe craniofacial and limb abnormalities (Peters et al., 1998).

Polymorphisms are a mechanism by which individuals may exhibit variations within the range of what is considered to be biologically normal. A strong association exists between gene polymorphisms and disease susceptibility. Most polymorphisms are single-nucleotide exchanges that occur at a high frequency in the human genome and may affect gene function. These types of variants are regarded as hypomorphic and accompany clinical symptoms showing reduced activity but not a complete loss in activity; these 'risk alleles' can occur either in coding or non-coding regions. The mechanism affects the amount of protein produced rather than the protein function (Nieminen, 2009). Single-nucleotide polymorphisms (SNPs) are the most common form of human genetic variation and may contribute to individual susceptibility to hypodontia.

Over the past few years, several growth and transcription factors have been shown to be expressed in developing teeth (Thesleff, 1996; Jernvall and Thesleff, 2000). For example, mutations or polymorphisms in the *PAX9* gene are responsible for congenital or familial forms of tooth agenesis. However, why most cases of non-syndromic or sporadic forms of tooth agenesis occur in humans remains unknown. We performed a meta-analysis to determine the mechanisms of tooth angenesis.

MATERIAL AND METHODS

Literature search

We performed an extensive electronic search of the PubMed, Cochrane Library, Embase, Web of Science, Springerlink, China National Knowledge Infrastructure, and the Chinese Biomedical Database to identify relevant studies published until December 6, 2013. Search terms included ["pax9" or "*PAX9*" (Mesh)] or ["SNPs" or "SNP" or "polymorphism, genetic" (Mesh)], and ["hypodontia" or "oligodontia" or "tooth agenesis" (Mesh)]. References in the eligible studies and textbooks were also manually reviewed to identify other potentially eligible studies.

Inclusion and exclusion criteria

Studies included were required to meet the following criteria: 1) the type of study should be a case-control study; 2) these case-control studies should focus on the association between *PAX9* genetic polymorphisms and hypodontia risk; 3) all patients were diagnosed with hypodontia based on panoramic radiographs and careful examinations of their clinical charts; 4) the frequencies of alleles or genotypes in case and control groups could be extracted; and 5) the language of publication was English. Studies were excluded when they were: 1) not case-control studies examining *PAX9* genetic polymorphisms and hypodontia risk; 2) included patients with other anomalies beyond agenesis; 3) based on incomplete or irrelevant data; 4) reported irrelevant or overlapping data; or 5) if they were meta-analyses, letters, reviews, or editorials.

Data extraction

Using a standardized form, 2 reviewers (W.Z. and H.C.Q.) extracted data from studies published independently. The following information was extracted from each article, including: first author, year of publication, country, language, ethnicity, study design, diagnostic criteria, source of cases and controls, number of cases and controls, mean age, sample, number of missing teeth, genotype methods, polymorphism genotype frequency, and evidence of Hardy-Weinberg equilibrium (HWE) in controls. In cases of conflicting evaluations, an agreement was reached after a discussion with a third reviewer (Y.Z.).

Quality assessment of studies included

Two reviewers (W.Z. and H.C.Q.) independently assessed the quality of selected papers according to a modified Strengthening the Reporting of Observational Studies in Epidemiology quality score system (von Elm et al., 2007; Zhang et al., 2011). Forty assessment items related to quality appraisal were used in this meta-analysis, with a scale ranging from 0-40. Scores of 0-20, 20-30, and 30-40 were defined as low, moderate, and high quality, respectively. Disagreement was resolved through discussion with a third reviewer (Y.Z.).

Statistical analysis

The odds ratio (OR) and 95% confidence interval (95%CI) were calculated using Review Manager Version 5.1.6 [provided by Cochrane Collaboration; http://ims.cochrane.org/revman/download (accessed 9 August 2013)] and STATA Version 12.0 (Stata Corp; College Station, TX, USA). Between-study variations and heterogeneities were estimated using Cochran's Q statistic (Higgins and Thompson, 2002; Zintzaras and Ioannidis, 2005). A P value of ≤0.05 was considered to be a manifestation of statistically significant heterogeneity.

We also quantified the effect of heterogeneity using the I^2 test. I^2 represents the proportion of inter-study variability that can be attributed to heterogeneity rather than to chance with the range of 0-100%. I^2 values of 25, 50, and 75% were defined as low, moderate, and high estimates, respectively. When a significant Q test ($P < 0.10$) or an I^2 of >50% indicated heterogeneity across studies, the random-effect model was used for meta-analysis; otherwise,

the fixed-effect model was used. We tested whether genotype frequencies of controls were in HWE using the chi-squared test. Sensitivity analysis was mainly performed through sequential omission of individual studies.

Publication bias was investigated using Begger's funnel plot, and funnel plot asymmetry was assessed with the Egger linear regression test (Peters et al., 2006). Statistical significance was reached when the P value of the Egger test was <0.05. All P values were 2-sided. To ensure the reliability and accuracy of the results, 2 reviewers (W.Z. and H.C.Q.) populated the data in the statistical software programs independently and obtained the same results.

RESULTS

Characteristics of studies included

The search strategy retrieved 102 potentially relevant studies. According to the inclusion criteria, 6 studies (Peres et al., 2005; Pawlowska et al., 2010; Pinho et al., 2010; Paixão-Côrtes et al., 2011; Liu et al., 2012; Wang et al., 2013) were included in the meta-analysis and 96 were excluded. The flow chart of study selection is shown in Figure 1. The 6 case-control studies selected included 855 cases and 1201 healthy controls evaluating the relationship between *PAX9* genetic polymorphisms and hypodontia. The publication year of involved studies ranged from 2005-2013. All patients fulfilled the diagnostic criteria of hypodontia by panoramic radiographs and careful examination of their clinical charts. The source of controls was a healthy population. Nine SNPs in the *PAX9* gene were addressed. The HWE test was performed on the genotype distribution of the controls in all studies included and all were found to be in HWE (P > 0.05). All quality scores of studies included were >20 (moderate to high quality). The characteristics and methodological quality of the studies included are summarized in Table 1. The genotype distributions of *PAX9* genetic polymorphisms in case and control groups are shown in Figures 2-4.
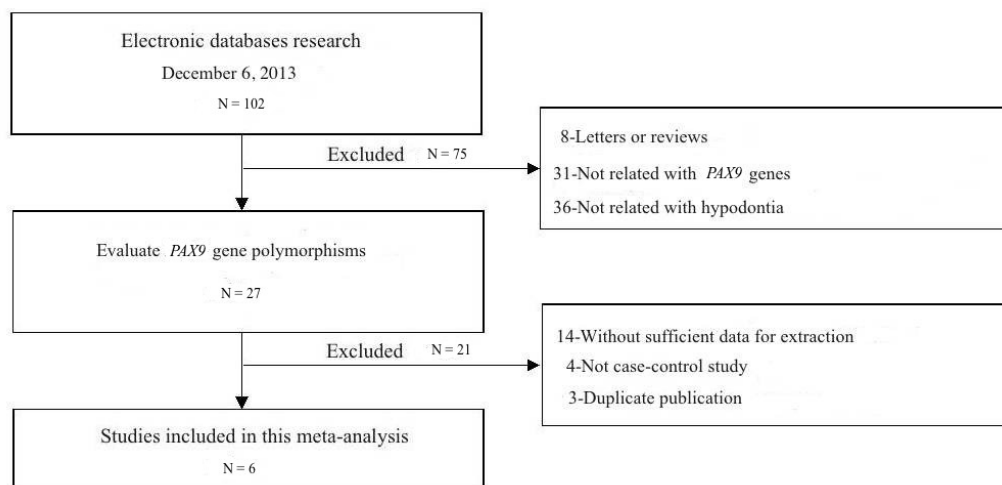

Figure 1. Flow chart showing study selection procedure.

Table 1. Characteristics of individual studies in this meta-analysis.

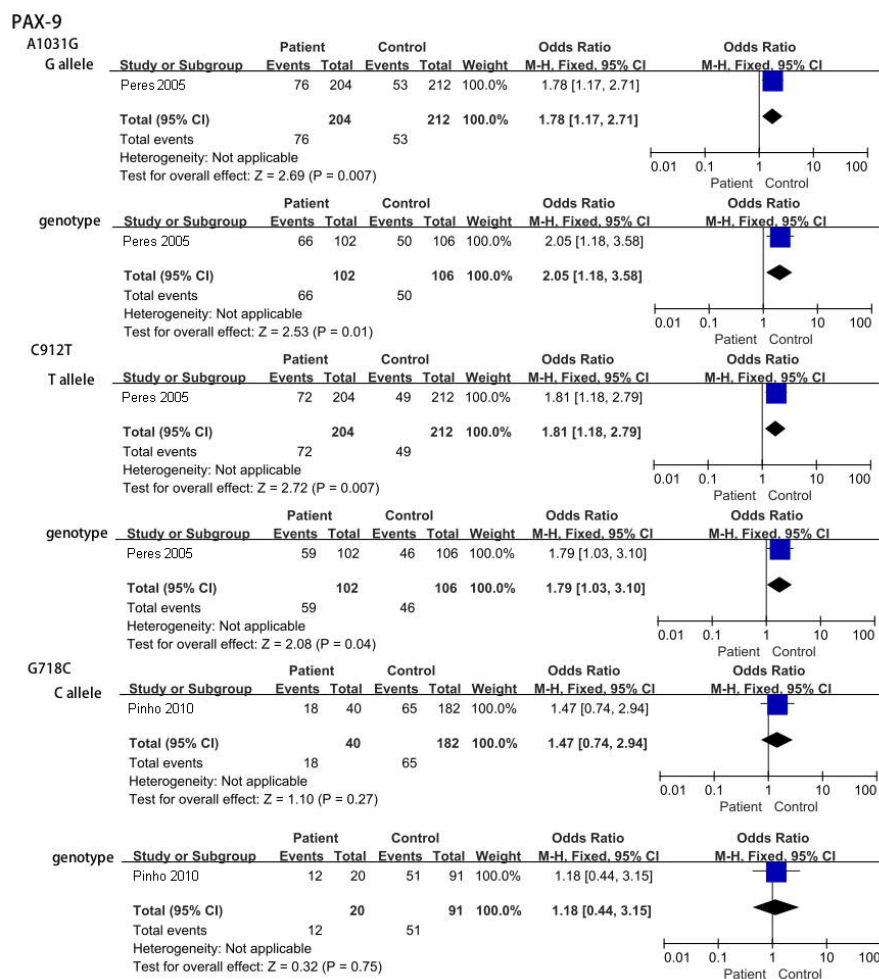| Author	Year	Case (number)	Control (number)	Gene site	Quality score
Peres et al.	2005	102	106	G-1031A C-912T	23
Pinho et al.	2010	20	91	G718C	23
Pawlowska et al.	2010	38	100	IVS2-54 IVS2-109 IVS2-41	24
Paixão-Côrtes et al.	2011	100	100	rs714373727 rs12881240 rs4904210	26
Liu et al.	2012	117	98	rs4904210	22
Wang et al.	2013	50	100	rs12881240 rs4904210	25

Figure 2. A1031G, C912T, and G718C genotype distributions of *PAX9* genetic polymorphisms in case and control groups.
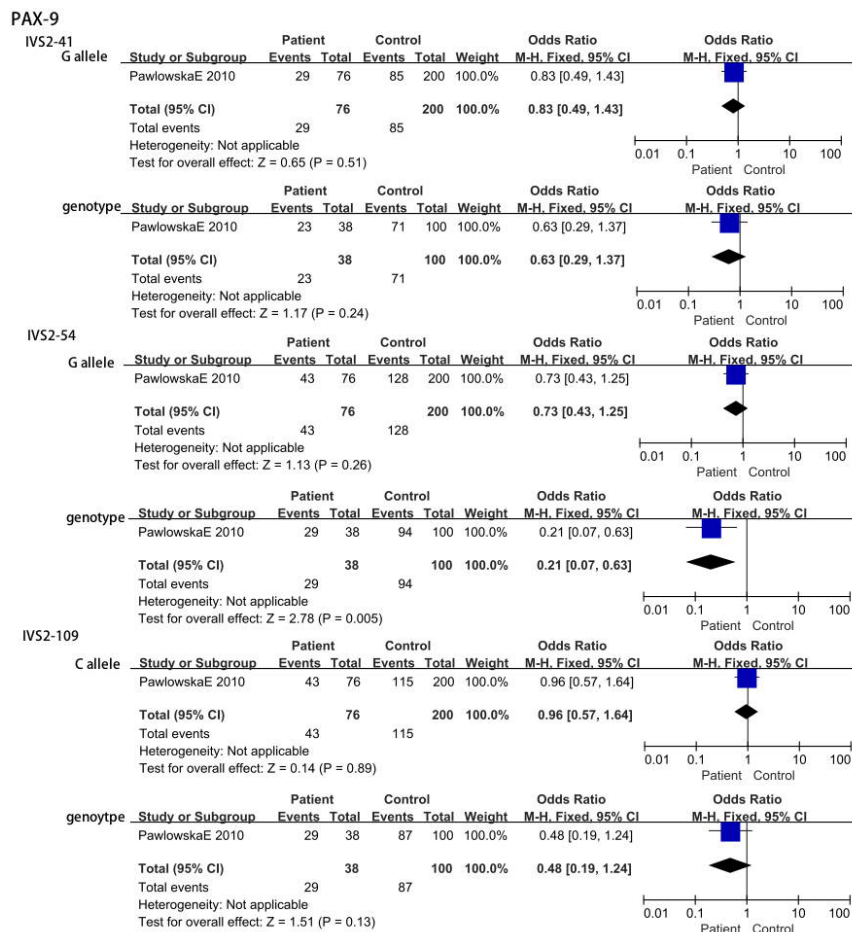
Figure 3. IVS2-41, IVS2-54, and IVS2-109 genotype distributions of *PAX9* genetic polymorphisms in case and control groups.

Association between *PAX9* gene polymorphisms and hypodontia

As shown in Figure 3, the meta-analysis revealed a significant difference in the G allele and G carrier (AG + GG) of IVS-41 and IVS2-54 in the *PAX9* gene between patients with tooth agenesis and healthy subjects (OR = 0.83, 95%CI = 0.49-1.43, P = 0.51; OR = 0.63, 95%CI = 0.29-1.37, P = 0.24; OR = 0.73, 95%CI = 0.43-1.25, P = 0.26; OR = 0.21, 95%CI = 0.07-0.63, P = 0.005, respectively), indicating that those with the G allele and G carrier (AG + GG) of IVS-41 and IVS2-54 in the *PAX9* gene polymorphism may have an increased risk of hypodontia. For the C allele and C carrier (CG + CC) of 718C, IVS2-109, rs4904210, and rs7143727 in the *PAX9* gene, no significant difference was observed (OR = 1.47, 95%CI = 0.74-2.94, P = 0.27; OR = 1.18, 95%CI = 0.44-3.15, P = 0.75; OR = 0.96, 95%CI = 0.57-1.64, P = 0.89; OR = 0.48, 95%CI = 0.19-1.24, P = 0.13; OR = 1.07, 95%CI = 0.68-1.69, P = 0.76; OR = 1.17, 95%CI = 0.81-1.70, P = 0.40; OR = 7.22, 95%CI = 0.37-141.52, P = 0.19; OR =

13.83, 95%CI = 0.77-248.79, P = 0.07, respectively). In addition, the T allele and T carrier (CT + TT) of rs12881240 also showed no association with hypodontia susceptibility (OR = 1.81, 95%CI = 1.18-2.79, P = 0.007; OR = 1.79, 95%CI = 1.03-3.10, P = 0.04). However, the G allele and G carrier (AG + GG) of A1031G and the T allele and T carrier (CT + TT) of C912T in the *PAX9* gene were positively associated with hypodontia (OR = 1.78, 95%CI = 1.17-2.71, P = 0.007; OR = 2.05, 95%CI = 1.18-3.58, P = 0.01; OR = 1.81, CI = 1.18-2.79, P = 0.007; OR = 1.79, 95%CI = 1.03-3.10, P = 0.04; respectively). The significance of the pooled OR in all individual analyses was not influenced excessively by omitting any single study.
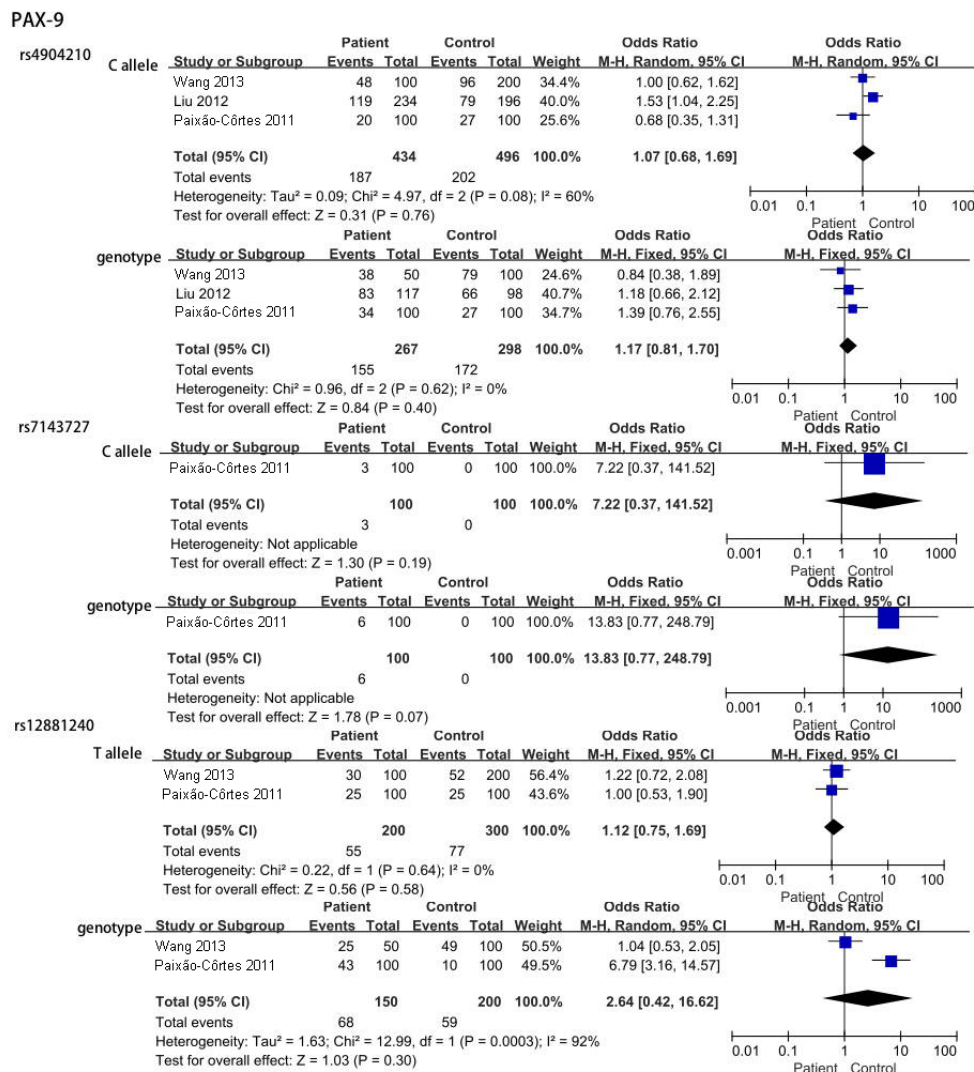

Figure 4. rs4904210, rs7143727, and rs12881240 genotype distributions of *PAX9* genetic polymorphisms in case and control groups.

Publication bias

Publication bias of the literature was assessed using Begger's funnel plot and the Egger linear regression test. The Egger linear regression test was used to measure the asymmetry of the funnel plot. All graphical funnel plots of the included studies were symmetrical (Figures 5). The Egger test also showed that there was no publication bias.
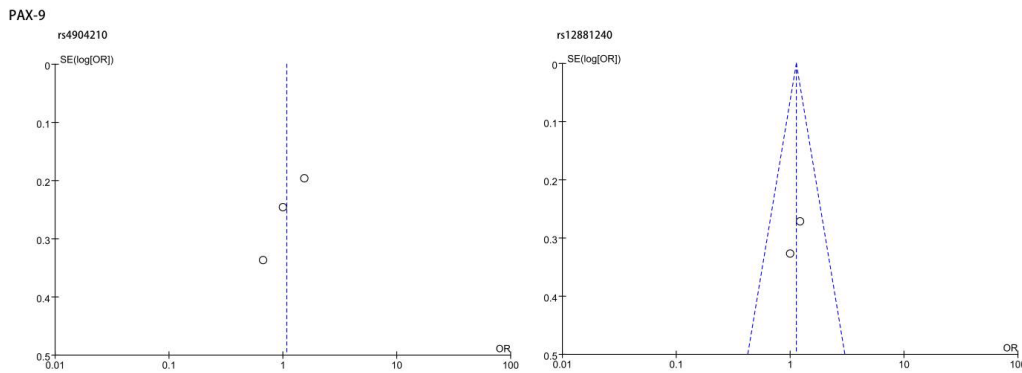

Figure 5. Begger's funnel plot of publication bias.

DISCUSSION

Hypodontia of the human dentition, caused by simultaneous mutations in different genes, may be an oligogenetic trait. According to our results, 33% of subjects who received orthodontic treatment had agenesis of one or more teeth. The tooth with the highest agenesis frequency was the third molar, followed by the lower premolars and upper lateral incisors. In fact, teeth are among the most important models in the field of evolutionary developmental biology linking the molecular strategies controlling organ development and morphological evolution (Line, 2003). They also link development with the generation of novelty in mammalian teeth (Polly, 2000). As with most evolutionary changes, tooth agenesis results from the loss of 1 or 2 elements. In most cases, oligodontia of the human dentition may be an oligogenetic trait resulting from simultaneous mutation of different type of genes. In addition, polymorphic genetic loci have been directly associated with morphological variation in non-vertebrates. It is well-established that morphological changes among and within species are ultimately determined by genetic interference during development because small changes during the developmental process can produce large changes in adult morphology (Kim et al., 2000; Raff, 2000).

Recent studies have shown that polymorphisms at specific sites of the *PAX9* gene were associated with hypodontia in humans, supporting the hypothesis that the mutation causes oligodontia through a pathogenic mechanism. Kangas et al. (2004) reported that dental anomalies, which generally include size and shape alterations of teeth, tooth agenesis, or supernumerary teeth, were determined based on a set of genes in tooth development. However, 2 years later, *PAX9* gene mutations were ruled out in a number of instances with severe oligodontia (Gerits et al., 2006). These mutations, however, only partially account for the development of tooth agenesis (Chranowska et al., 1990; Fryns et al., 1990; Vieira et al., 2004). Although the

case-control results showed no significant differences and the exact mechanism has not been fully elucidated, genetic factors are thought to play a central role in tooth agenesis.

Our meta-analysis included 6 independent case-control studies to examine the association between *PAX9* gene polymorphisms and hypodontia. Our fixed model analysis showed that the G allele and G carrier (AG + GG) of A1031G in the *PAX9* gene may increase the risk of hypodontia and may be risk factors for the susceptibility to hypodontia. Similarly, the T allele and T carrier (CT + TT) of C912T and rs12881240 in the *PAX9* gene were positively associated with hypodontia susceptibility. In addition, the C allele and C carrier (CG + CC) of 718C, IVS2-109, rs4904210, and rs7143727 showed no significant association with oligodontia. The G allele and G carrier (AG + GG) of IVS2-41 were identified in the *PAX9* gene but were not related to oligodontia. Interestingly, we identified protective roles for the genotype (AG + GG) of IVS2-54 in the development of teeth, which is a protective factor in the formation of hypodontia (OR = 0.21, 95%CI = 0.07-0.63, P = 0.005). However, no significant differences were found in the allele frequency of IVS2-54 in the *PAX9* polymorphism between controls and subjects with sporadic tooth agenesis.

Similar to other meta-analyses, there are some limitations to our study. First, because the eligible number of studies in this meta-analysis was small, these results require further investigation. In addition, the number of studies and subjects according to the references included in the meta-analysis was limited, and some relevant studies were excluded from our analysis because incomplete raw data were included. Furthermore, we could not address the sources of heterogeneity among all studies. Fourth, a meta-analysis is a retrospective study that is subject to methodological limitations. Fifth, although the cases and controls of each study were well defined with similar inclusion criteria, factors that were not taken into account may have influenced our results. Importantly, our meta-analysis was based on unadjusted OR estimates because not all published studies presented adjusted ORs or presented ORs adjusted by the same potential confounders such as age, gender, ethnicity, and exposure. In conclusion, this meta-analysis of 6 case-control studies demonstrated that *PAX9* genetic polymorphisms are involved in the pathogenesis of tooth agenesis. Because of the limited number of studies published in this field, the current available evidence remains limited. Therefore, we emphasize the necessity of conducting large studies with adequate methodological quality and proper control of confounding factors to obtain valid results.

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