# Association of *MSX1* and *TGF-β1* genetic polymorphisms with hypodontia: meta-analysis

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**ABSTRACT.** We conducted a comprehensive meta-analysis of 4 case-control studies to explore the association between polymorphisms of Msh homeobox 1 (*MSX1*) and transforming growth factor-β1 (*TGF-β1*) genes and hypodontia. A total of 643 tooth agenesis cases and 733 healthy controls were included in this study. The meta-analysis results showed that the T allele and T carrier (CT + TT) of rs1095 in the *MSX1* gene were positively associated with hypodontia susceptibility. However, the T allele and T carrier (TC + CC) of rs12532 and rs8670 showed no association with cancer susceptibility. In addition, there were no strong associations between the T allele and T carrier (CT + TT) of C509T and the A allele and A carrier (AG + AA) of G800A in the *TGF-β1* gene polymorphisms between patients with tooth agenesis and healthy subjects. In conclusion, these meta-analysis results demonstrated that polymorphisms in the rs1095 region of the *MSX1* gene may influence the transcriptional activity of this gene and are associated with hypodontia in humans. However, the association between the *TGF-β1* gene and sporadic tooth agenesis is not well understood, and further studies are required to explore the correlation between

the *TGF-β1* gene and hypodontia.

## INTRODUCTION

Hypodontia, which includes some of the most common genetic dental abnormalities in humans, affects approximately 2-3% of the population (Escobar, 1990). It can occur as a familial (autonomic dominant, recessive, or cross-linked) or isolated sporadic trait (Vastardis, 2000). The most common permanent teeth missing are the third molars (20%), second premolars (3.4%), and maxillary lateral incisors (2.2%) (Simons et al., 1993). The absence of 6 or more teeth occurring in a patient, excluding the third molars, is known as oligodontia, and affects approximately 1.1% of the population. During the long process of dental development, complex interactions between genetic, epigenetic, and environmental factors can result in dental anomalies, as demonstrated by Brook (2009). Although hypodontia does not represent a serious public health problem, it may cause masticatory and speech dysfunctions, esthetic problems, and malocclusion. Over the past few years, several growth and transcription factors were shown to be expressed in developing teeth (Thesleff, 1996; Jernvall and Thesleff, 2000). However, the origin of sporadic or non-syndromic tooth agenesis, the most common form of isolated tooth agenesis in humans, remains unknown.

During early tooth development, transforming growth factor-β1 (TGF-β1) is a signaling molecule that takes part in the cascade of signaling events. The gene encoding TGF-β1 is expressed in both the mesenchyme and epithelia during all stages of odontogenesis, including the bud, cap, and bell stages (Vaahtokari et al., 1991). During the bud and cap stages, the dental mesenchyme proliferates rapidly, while the dental epithelium strongly expresses *TGF-β1* RNA. The mesenchyme also expresses *TGF-β1*, but at lower levels. As a result, cell proliferation may be regulated by local TGF-β1 expression in the dental epithelium in the underlying dental mesenchyme, which is also involved in tooth morphology (Mäkelä et al., 1987; Vaahtokari et al., 1991). Another potential function of TGF-β1 during tooth morphogenesis is the regulation of extracellular matrix deposition. TGF-β1 promotes synthesis of the extracellular matrix, modifies cell surface matrix receptors, and prevents degradation of the extracellular matrix (Rasmussen and Rapraeger, 1988; Rizzino, 1988).

Msh homeobox 1 (MSX1) is a transcription factor involved in the missing second premolars and third molars and is expressed in the dental mesenchyme during the initiation of tooth development. MSX1 belongs to the muscle segment homeobox family and is essential for the switch between the odontogenic potential in the epithelium to the mesenchyme (Ogawa et al., 2005, 2006). van den Boogaard et al. (2000) identified a nonsense mutation (Ser104Stop) in the *MSX1* gene in all affected individuals of a Dutch family with several combinations of dental agenesis (predominantly of the second premolars and molars), whose phenotypes were similar to those resulting from *MSX1* gene mutation in mice (Satokata and Maas, 1994). Nevertheless, a study performed in individuals of Brazilian origin with hypodontia failed to identify mutations in *MSX1* (Scarel et al., 2000). Lidral and Reising (2002) observed no mutations in *MSX1* in subjects with simple incisor and premolar agenesis, but they

did detect these mutations in families with multiple agenesis, indicating a different etiology for these cases (Mostowska, 2006). These cases showed a novel mutation in *MSX1* that may be responsible for oligodontia (lack of 14 permanent teeth in a proband), although with incomplete penetrance.

Recently, various studies have investigated the associations between the *TGF-β1* and *MSX1* genes and hypodontia. Although mutations in the coding sequences of *TGF-β1* were shown to be causative factors in tooth agenesis, polymorphisms in this gene were not associated with sporadic tooth agenesis; in addition, the studies did not determine the exact role of *TGF-β1* and *MSX1* genetic polymorphisms in hypodontia, and conclusions regarding this link remain controversial. Therefore, we performed a meta-analysis that included recent and relevant publications to gather statistical evidence of these associations.

## 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 14, 2013. Search terms included ["msx1" or "MSX1" (Mesh)], ["tgf-b1" or "TGF-β1" (Mesh)], ["SNPs" or "SNP" or "polymorphism, genetic" (Mesh)], and ["hypodontia" or "oligodontia" or "tooth agenesis" (Mesh)]. The references in eligible studies and textbooks were also manually reviewed to identify potentially eligible studies.

### Inclusion and exclusion criteria

The studies included were required to meet the following criteria: 1) studies used a case-control design to examine the associations between *MSX1* and *TGF-β1* genetic polymorphisms and hypodontia risk; 2) all patients were diagnosed with hypodontia by panoramic radiographs and careful examination of their clinical charts; 3) the frequencies of alleles or genotypes in case and control groups could be extracted; and 4) the language of publication was English. Studies were excluded when they were 1) not case-control studies examining *MSX1* and *TGF-β1* genetic polymorphisms and hypodontia risk; 2) patients with other anomalies beyond agenesis; 3) based on incomplete data; 4) reporting irrelevant or overlapping data; and 5) meta-analyses, letters, reviews, or editorials.

### Data extraction

Using a standardized form, 2 reviewers (W.Z. and H.C.Q.) extracted data from published studies independently. The following information was extracted from each article included: 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 discussion with a third reviewer (Y.Z.).

## Quality assessment of included studies

Two reviewers (W. Zhang and H.C. Qu) 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 rated using 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. Zhang).

## 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 indicate 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 over a 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-effects model was used for meta-analysis; otherwise, the fixed-effects model was used. We tested whether genotype frequencies of controls were in HWE using the chi-squared test. Sensitivity analysis was mainly performed by sequential omission of individual studies.

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

## RESULTS

## Characteristics of included studies

The search strategy retrieved 96 potentially relevant studies. According to the inclusion criteria, 4 studies (Peres et al., 2004; Paixão-Côrtes et al., 2011; Antunes et al., 2012; Liu et al., 2012) were included in the meta-analysis and 92 were excluded. The flow chart of study selection is shown in Figure 1. The 4 case-control studies included 643 cases and 733 healthy controls and evaluated the relationship between *MSX1* and *TGF-β1* genetic polymorphisms and hypodontia. The publication year of the studies involved ranged from 2004-2012. All patients fulfilled the diagnostic criteria of hypodontia by panoramic radiographs and careful examination of their clinical charts. The controls included the healthy population. Three single nucleotide polymorphisms in the *MSX1* gene and 2 polymorphisms in the *TGF-β1* gene were examined. The HWE test was performed on the genotype distribution of the controls in all included studies and all were in HWE (P > 0.05). All quality scores of the studies included were

>20 (moderate to high quality). The characteristics and methodological quality of the included studies are summarized in Table 1. The genotype distributions of the *MSX1* and *TGF-β1* genetic polymorphisms in the case and control groups are shown in Figures 2 and 3.
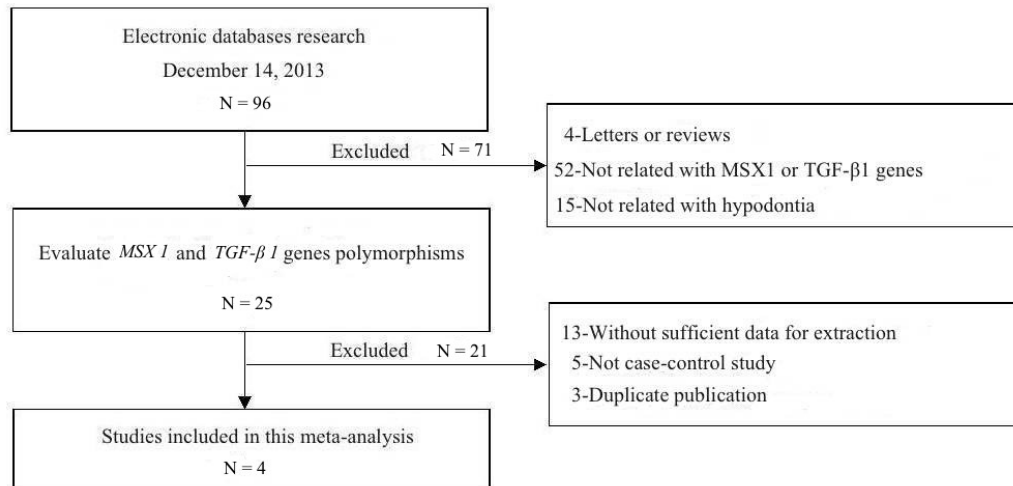


**Figure 1.** Flow chart showing study selection procedure.

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

| Author | Year | Case (number) | Control (number) | Gene site | Quality score |
|---|---|---|---|---|---|
| Peres et al. | 2004 | 51 | 48 | *TGF-β1* (C-509T) (G-800A) | 22 |
| Antunes et al. | 2012 | 48 | 125 | *TGF-β1* (C-509T) (G-800A) | 23 |
| | | 46 | 88 | | |
| Paixão-Côrtes et al. | 2011 | 100 | 100 | *MSX1* (rs8670) (rs1095) (rs12532) | 25 |
| Liu et al. | 2012 | 147 | 124 | *MSX1* (rs12532) | 22 |

## Association between *MSX1* gene polymorphisms and hypodontia

A summary of the meta-analysis findings of the association between *MSX1* gene polymorphisms and hypodontia is shown in Figure 2. The T allele and T carrier (CT + TT) of rs1095 in the *MSX1* gene were positively associated with hypodontia susceptibility (OR = 25.83, 95%CI = 1.50-444.58, P = 0.03; OR = 48.09, 95%CI = 2.86-808.68, P = 0.007, respectively), suggesting that the C allele and C carrier (TC + CC) of rs1049216 may increase the risk of hypodontia (Figure 2). However, the T allele and T carrier (TC + CC) of rs12532 and rs8670 showed no significant association with hypodontia susceptibility (OR = 0.64, 95%CI = 0.11-3.69, P = 0.61; OR = 0.66, 95%CI = 0.11-3.96, P = 0.65; OR = 1.54, 95%CI = 0.77-3.09, P = 0.22; OR = 1.66, 95%CI = 0.94-2.95, P = 0.08). Sensitivity analysis was conducted by omitting single studies, and the significance of the pooled MD was not influenced.
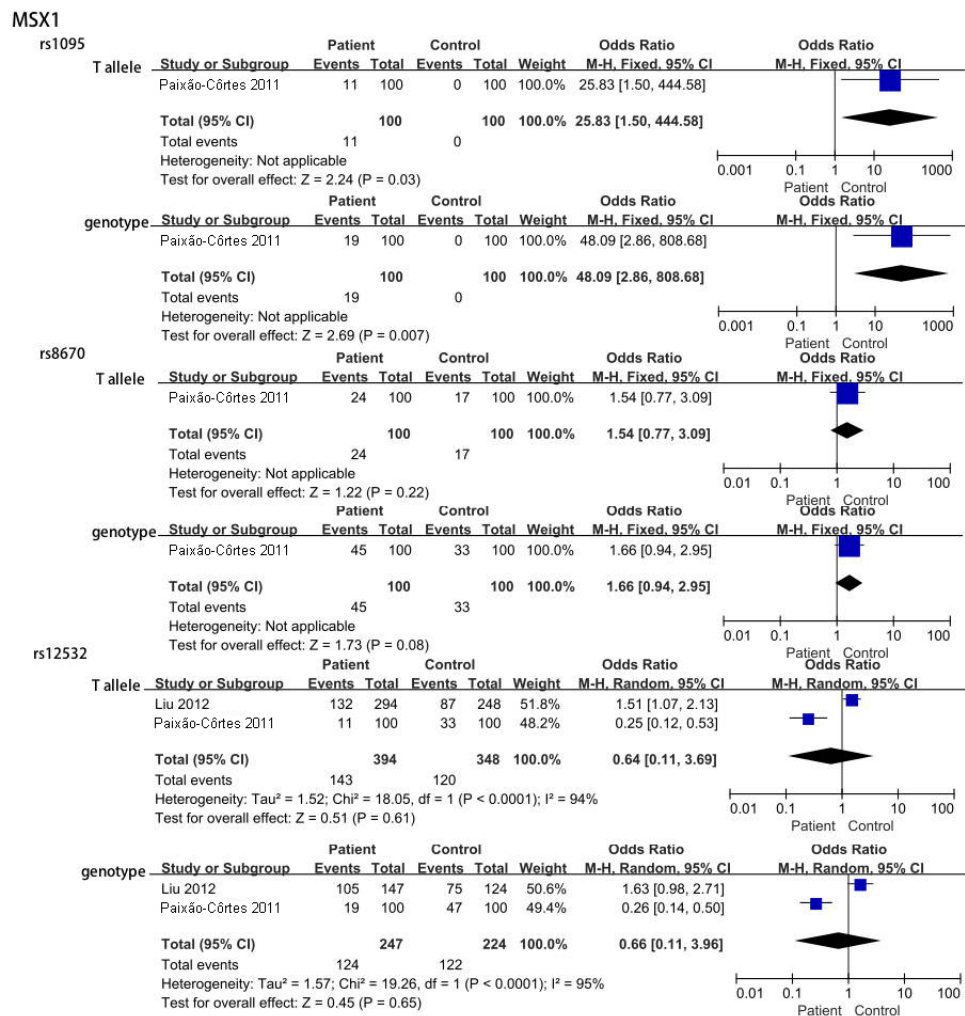
**Figure 2.** The rs1095, rs8670, and rs12532 genotype distributions of *MSX1* genetic polymorphisms in case and control groups.

## Association between *TGF-β1* gene polymorphisms and hypodontia

Associations between *TGF-β1* gene polymorphisms and hypodontia are shown in Figure 3. Pooled analysis revealed no significant associations between the T allele and T carrier (CT + TT) of C509T and A allele and A carrier (AG + AA) of G800A in *TGF-β1* gene polymorphisms between patients with hypodontia and healthy subjects, with all P > 0.05 (OR = 0.79, 95%CI = 0.44-1.43, P = 0.44; OR =1.43, 95%CI = 0.63-3.24, P = 0.39; OR = 0.75, 95%CI = 0.48-1.17, P = 0.20; OR = 0.54, 95%CI = 0.17-1.69, P = 0.29, respectively). Sensitivity analysis was conducted by omitting single studies, and no influence was found in the significance of the pooled OR.
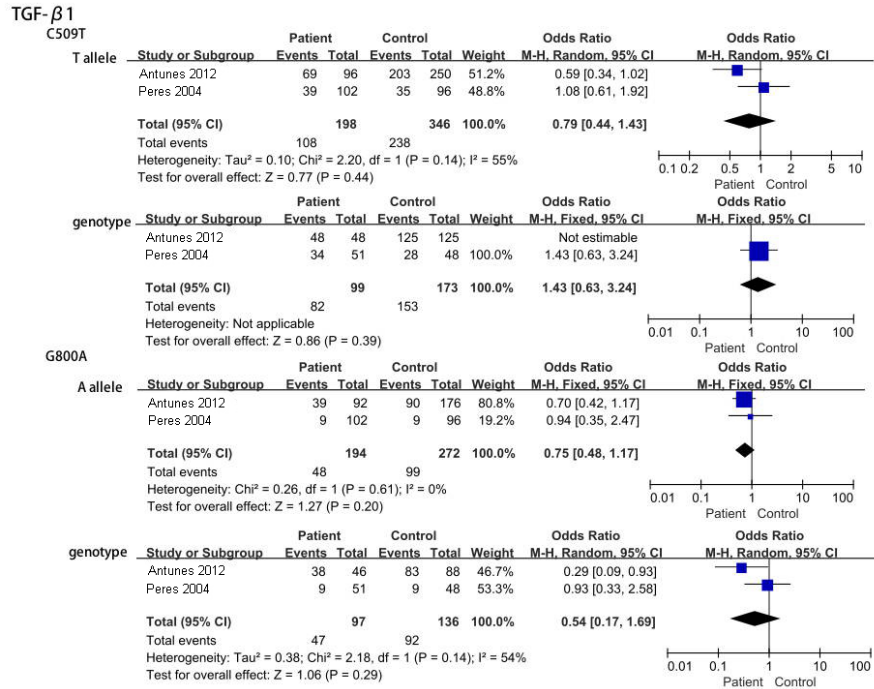
**Figure 3.** The C509T and G800A genotype distributions of *TGF-β1* genetic polymorphisms in case and control groups.

## Publication bias

Publication bias of the literature was assessed by 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 studies included appeared to be symmetrical (Figures 4 and 5). The Egger test also revealed no publication bias.
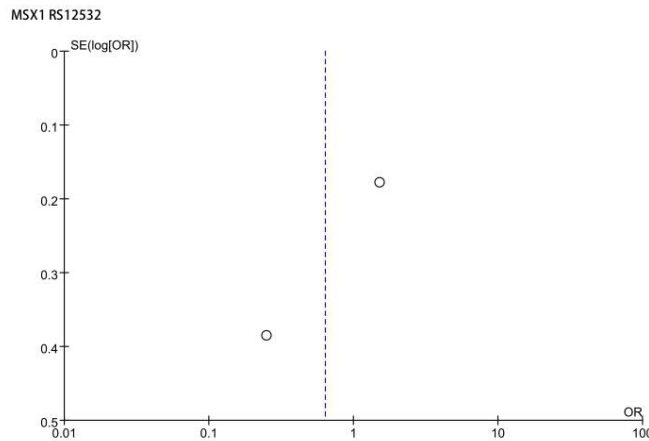


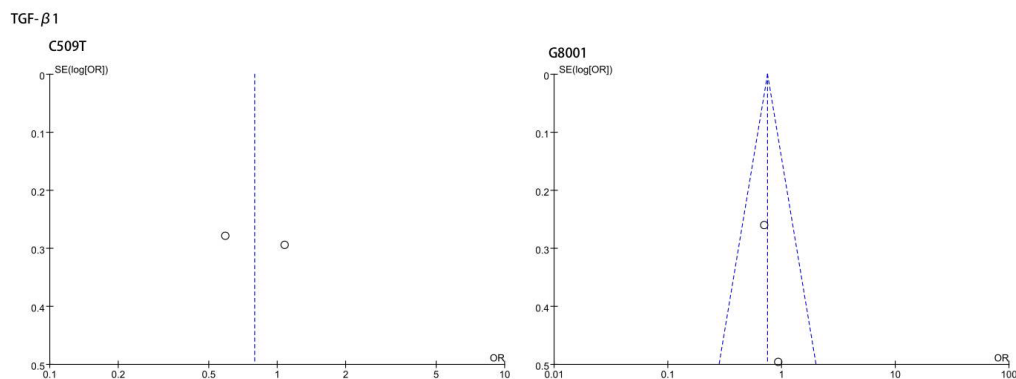**Figure 4.** Begger's funnel plot of publication bias for *MSX1*.

**Figure 5.** Begger's funnel plot of publication bias for *TGF-β1*.

## DISCUSSION

Teeth are among the most important models in the field of evolutionary developmental biology that connect molecular strategies controlling morphological evolution and organ development (Jernvall, 2000; Polly, 2000; Line, 2003). Hypodontia of the human dentition may be an oligogenetic trait caused by the simultaneous mutation of different genes. Polymorphic genetic loci have been directly associated with morphological variation in non-vertebrates. It is important to understand how genetic variations affect development and are translated into morphological changes. It is well-established that morphological changes among and within species are ultimately determined by genetic interference during the developmental process because small changes during development can produce large changes in adult morphology (Kim et al., 2000; Raff, 2000). During the long process of dental development, complex interactions between genetic, epigenetic, and environmental factors cause dental anomalies, as demonstrated by Brook (2009). These varied and completed factors are multi-factorial, multilevel, multidimensional, and progressive over time. Affected individuals in the same family express different phenotypes, often revealing variations as a result of the multi-directional role of genetic, epigenetic, and environmental factors over time.

Polymorphisms are a mechanism by which individuals can exhibit variations within the range of what is considered to be biologically normal. Gene polymorphisms are also associated with disease susceptibility. Most polymorphisms are single-nucleotide exchanges that occur at a high frequency in the human genome and may affect gene function.

A polymorphism in the first intron of the *MSX1* gene was previously associated with hypodontia in humans (Vieira et al., 2004). In humans, a heterozygous mutation in *MSX1* was reported to be sufficient to cause agenesis of premolars or molars. Although the case-control results showed no significant differences, some interesting findings were reported. For instance, the *MSX1* rs1095-derived allele appeared only in agenesis-affected patients (no mutant allele C was observed in controls). The other 2 *MSX1* polymorphisms (rs8670 and rs12532) had previously been associated with dental agenesis (Pawlowska et al., 2009).

TGF-β1 is a pleiotropic cytokine that plays a role in inflammation, tissue repair, and embryonic growth and development (Clark and Coker, 1998). Although the role of TGF-β1 in tooth development has not been extensively studied, the expression of its mRNA detected through *in situ* hybridization experiments indicates a role during the diverse phases of odon-

togenesis. During the bud and cap stages of mouse molar odontogenesis, TGF-β1 is expressed in the dental epithelium and mesenchyme, indicating a role in the growth of tooth primordia. Histologic analysis of odontogenesis in TGF-β1 knockout mice showed that morphogenesis, cytodifferentiation, and histogenesis were unaffected. Although no *TGF-β1* mRNA was detected by *in situ* hybridization, TGF-β1 immunoreactive material was found in the tissues of knockout mice. D'Souza and Litz (1995) suggested that maternally derived *TGF-β1* is responsible for the rescue of developmental events. Unlike in mice, the development of most human permanent teeth occurs months or years after birth.

Overall, our fixed model analysis showed that the T allele and T carrier (CT + TT) of rs1095 in the *MSX1* gene may increase the risk of hypodontia, and may be risk factors for the susceptibility to hypodontia. The T allele and T carrier (TC + CC) of rs12532 and rs8670 showed no association with hypodontia susceptibility. In addition, there were no strong links between the T allele and T carrier (CT + TT) of C509T and the A allele and A carrier (AG + AA) of G800A in the *TGF-β1* gene polymorphisms between patients with tooth agenesis and healthy subjects. In conclusion, our meta-analysis results demonstrated that polymorphisms in the rs1095 region of the *MSX1* gene may influence the transcriptional activity of this gene and are associated with hypodontia in humans. However, the connection between the *TGF-β1* gene and sporadic tooth agenesis is not well understood, and further studies are required to determine the correlation between the *TGF-β1* gene and hypodontia.

Similar to other meta-analyses, there were some limitations to our study. First, given that 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 that were included in the meta-analysis was limited, and some relevant studies were excluded from our analysis because of incomplete raw data. Furthermore, we could not address the sources of heterogeneity among all studies. Fourth, meta-analysis studies are retrospective and 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. Most 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 4 case-control studies demonstrated that *MSX1* and *TGF-β1* genetic polymorphisms are involved in the pathogenesis of tooth agenesis. Because few studies have been conducted, current evidence remains limited. Therefore, large studies with adequate methodological quality and proper control of confounding factors are required.

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