



Genetic analysis: Wnt and other pathways in nonsyndromic tooth agenesis

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Abstract

Tooth agenesis (TA) is one of the most common developmental anomalies that affects the number of teeth. An extensive analysis of publicly accessible databases revealed 15 causative genes responsible for nonsyndromic TA, along with their signaling pathways in Wnt/ β -catenin, TGF- β /BMP, and Eda/Edar/NF- κ B. However, genotype-phenotype correlation analysis showed that most of the causal genes are also responsible for syndromic TA or other conditions. In a total of 198 different mutations of the 15 genes responsible for nonsyndromic TA, 182 mutations (91.9%) are derived from seven genes (*AXIN2*, *EDA*, *LRP6*, *MSX1*, *PAX9*, *WNT10A*, and *WNT10B*) compared with the remaining 16 mutations (8.1%) identified in the remaining eight genes (*BMP4*, *DKK1*, *EDAR*, *EDARADD*, *GREM2*, *KREMEN1*, *LTBP3*, and *SMOC2*). Furthermore, specificity analysis in terms of the ratio of nonsyndromic TA mutations versus syndromic mutations in each of the aforementioned seven genes showed a 98.2% specificity rate in *PAX9*, 58.9% in *WNT10A*, 56.6% in *MSX1*, 41.2% in *WNT10B*, 31.4% in *LRP6*, 23.8% in *AXIN2*, and 8.4% in *EDA*. These findings underscore an important role of the Wnt and Wnt-associated pathways in the genetic etiology of this heterozygous disease and shed new lights on the discovery of novel molecular mechanisms associated with tooth agenesis.

KEYWORDS

craniofacial genetics, hypodontia, oligodontia, tooth agenesis, Wnt pathway

1 | INTRODUCTION

Tooth agenesis (TA), the absence of teeth due to developmental failure, is one of the most common developmental malformations. TA may present in a syndromic form with the involvement of other organs or tissues, or in a nonsyndromic form that only affects the dentition. Epidemiological studies indicate that the prevalence of nonsyndromic TA ranges from 1.6% to 9.6% in different geographical areas and races (Galluccio, Castellano, & Monaca, 2012; Zhang et al.,

2015). Based on the number of missing teeth in permanent dentition, TA can be further classified into three categories: hypodontia (less than six missing teeth), oligodontia (six or more missing teeth), and anodontia (complete agenesis of the dentition). The congenital loss of teeth consequently leads to masticatory, speech, esthetic, and psychological problems, thus placing a heavy burden on the affected individuals and associated societies.

Although many factors may contribute to the etiology of TA (e.g., epigenetic and environmental effects), there is compelling evidence

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proving that genetic factors play a predominant role in the pathogenesis of the disease (Ye & Attai, 2016). As a mutation of *MSX1* was first discovered in affected individuals with selective TA (Vastardis, Karimbox, Guthua, Seidman, & Seidman, 1996), various mutations in several dozens of genes have been identified in affected individuals with syndromic and nonsyndromic TA, thus reflecting the allelic and genetic heterogeneities of these conditions. Notably, mutated genes encoding the components in the canonical Wnt/ β -catenin pathway and Wnt-associated genes account for the highest genetic risk of isolated TA compared with mutated genes involved in several other pathways (van den Boogaard et al., 2012, Yin & Bian, 2015).

In this review, we aim to summarize all causal genes responsible for nonsyndromic TA (Supporting information Table S1), which are either curated in publicly accessible databases or recently discovered by whole-exome sequencing (WES) analysis. We also illustrate the pathogenic effects of Wnt and other associated signaling pathways on TA and related dental anomalies. In addition, potential molecular and cellular mechanisms associated with the alteration of Wnt and other pathways in nonsyndromic TA are briefly discussed.

2 | WNT IS A MAJOR PATHWAY RESPONSIBLE FOR TA

Tooth development is a dynamic process that includes the bud, cap, and bell stages; root development; and tooth eruption (Huang & Chai,

2012). The Wnt/ β -catenin signaling pathway is involved in many aspects of embryonic development and is spatiotemporally activated in tooth-forming regions at all stages of tooth development, thereby implying its essential role in the process of odontogenesis (Thesleff & Sharpe, 1997). The machinery of the Wnt pathway comprises extracellular secreted glycoproteins (19 Wnt ligands at human level), seven-transmembrane-spanning receptors (Frizzled and LRP5/6), cytoplasmic proteins (DVL, APC, AXIN, GSK3 β , and β -catenin), nuclear transcription factors (TCF/LEF), and several Wnt-associated molecules (*MSX1*, *DKK1*, *KREMEN1*, and *ANTXR1*) (Figure 1, left panel).

The genetic link between TA and the Wnt pathway was first evidenced by the identification of a mutation of the *AXIN2* gene in an oligodontia family (OMIM: 608,615). To date, nine different mutations in *AXIN2* have been identified in nonsyndromic TA, while 12 mutations were identified in syndromic TA and other conditions (Supporting information Table S1). *AXIN2* encodes an intracellular inhibitor of Wnt/ β -catenin signal and is highly expressed in the enamel knot and mesenchymal odontoblasts during tooth formation. *AXIN2* missense mutants were found to enhance β -catenin degradation and reduced Wnt activation, whereas the truncated mutants seemed to heighten the activation of Wnt/ β -catenin (Yue, Liang, Yang, Hua, & Bian, 2016).

A large amount of mutations in genes encoding Wnt ligands (e.g., *WNT10A* and *WNT10B*) and associated receptors (*LRP6* and *KREMEN1*) were recently discovered by whole exome and Sanger

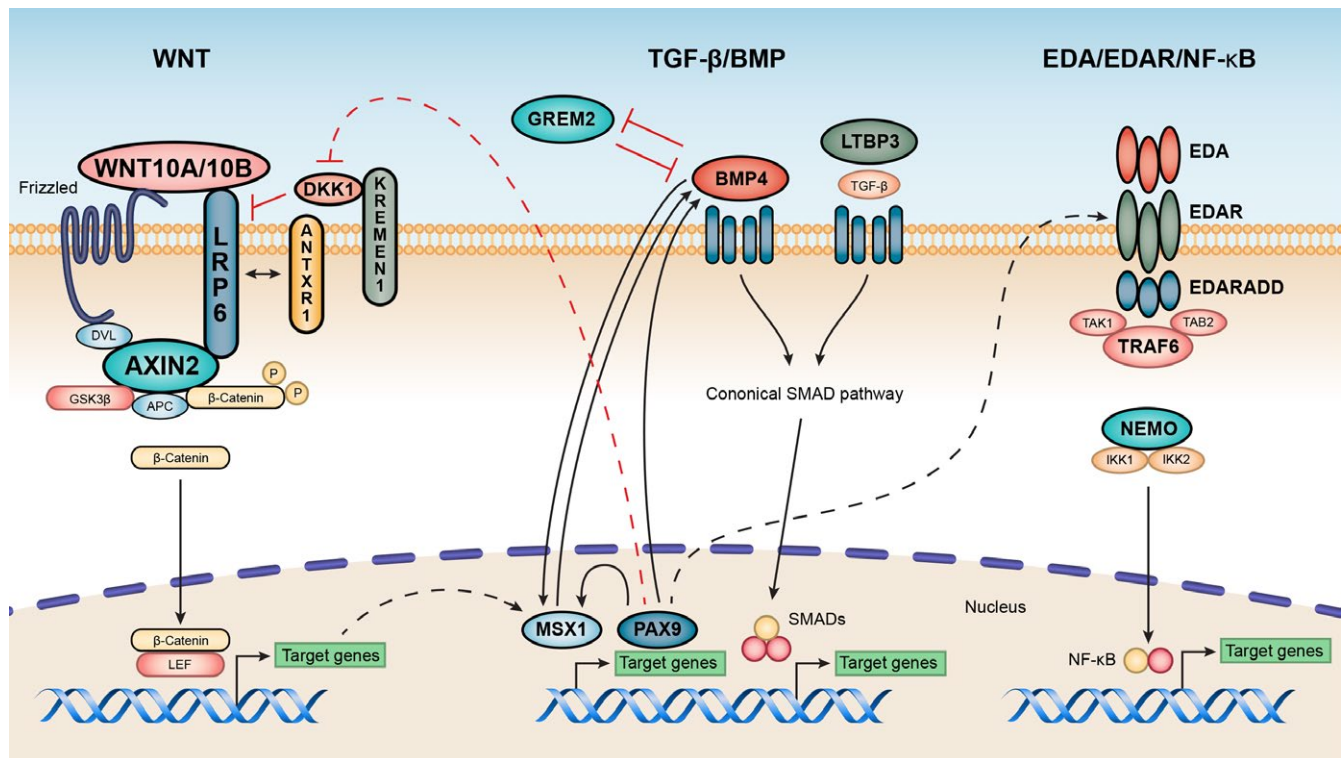


FIGURE 1 Three tooth agenesis-associated signaling pathways. Mutated components of these pathways that underlie nonsyndromic tooth agenesis in affected human subjects are shown in bolded gene symbols. Genetic interactions between different pathways are briefly discussed in the context. Left panel: the Wnt/ β -catenin signaling pathway. Middle panel: the TGF- β /BMP pathway. Right panel: the Eda/Edar/NF- κ B pathway. The *MSX1* encoded transcriptional repressor is involved in the Wnt (www.genecards.org) as well as the BMP4 pathway

sequencing. *WNT10A* is mapped on chromosome 2q35 and is preferentially expressed in dental epithelium and enamel knots during tooth development (He et al., 2013). As the first recessive mutations in *WNT10A* were identified in individuals with odonto-onycho-dermal dysplasia (OMIM 257,980), 73 different mutations have been identified to be responsible for 14 different diseases/phenotypes based on data obtained from the Human Gene Mutation Database (HGMD). Forty-three of the mutations (58.9%, Supporting information Table S1) resulted in the nonsyndromic TA (HGMS), which are categorized into six conditions (tooth agenesis, nonsyndromic hypodontia, hypodontia, maxillary lateral incisor agenesis, oligodontia, and nonsyndromic selective tooth agenesis) (van den Boogaard et al., 2012). This makes *WNT10A* as the second most frequently mutated gene in individuals with nonsyndromic TA (Figure 2). Clinical analysis showed a more severe TA condition in affected individuals carried with biallelic *WNT10A* mutations compared to those with monoallelic mutations (Ye & Attaie, 2016).

WNT10B is mapped to chromosome 12q13.12 and, at protein level, shares 60% identity and 72% similarity to *WNT10A*. During tooth development in mice, *Wnt10b* is also expressed in the dental epithelium at the bud and cap stages. Using the WES technique, four deleterious mutations in *WNT10B* were identified in an affected family, as well as three unrelated individuals with nonsyndromic oligodontia (Yu et al., 2016), which is designated as the autosomal dominant tooth agenesis, selective 8, *STHAG8* (OMIM 617,073). In a separate study, two additional novel mutations in *WNT10B* were

identified in five families with isolated dental anomalies, including oligodontia and several other types of isolated TA (Kantaputra et al., 2018). Functional studies showed that *WNT10B* mutants are insufficient at activating Wnt signaling, thus resulting in an impaired odontoblastic differentiation and vasculogenesis of dental pulp stem cells (DPSCs) (Yu et al., 2016). This finding provides evidence that odontoblasts as well as vascular endothelial cells play a critical role in success of dental pulp tissue engineering. Further research on how the Wnt pathway regulates the decision between odontoblastic fate and vasculogenic fate of DPSCs may shed light on TA pathogenesis and tooth regeneration.

LRP6 encodes a key component of the coreceptor complex for the transmission of Wnt/ β -catenin signaling cascade for cell differentiation and proliferation. Although *LRP6* mutations are believed to be associated with coronary artery disease (OMIM: 610,947) and several other conditions, 11 different mutations (Supporting information Table S1) were identified in affected families and individuals with nonsyndromic TA (Massink et al., 2015). The transfected *LRP6* mutants in mammalian cells failed to activate β -catenin due to their abnormal glycosylation and immature high-mannose form in the endoplasmic reticulum, thereby leading to the abolishment of Wnt activation (Massink et al., 2015). In the recent past, *LRP6* in the Wnt signaling was also shown to be necessary for the vasculogenic differentiation of human DPSCs (Silva et al., 2017).

In an interesting manner, mutations in *MSX1*, encoding a transcriptional repressor in the loop of both Wnt and BMP4 pathways, have been repeatedly found to cause nonsyndromic TA (Wong et al., 2014). Among 53 different mutations in *MSX1* (HGMD), 30 of them (56.6%, Supporting information Table S1) are associated with isolated TA, often missing second premolars and third molars. In contrast, loss-of-function mutations of *MSX1* were reported to cause more severe conditions, such as Witkop syndrome, Wolf-Hirschhorn syndrome, and oligodontia with cleft lip/palate (HGMD). Deletion of *Msx1* in mice was found to cause an arrested tooth development, deficiency of alveolar bones, and cleft palate (Jumlongras et al., 2001).

It is worthwhile to mention that rare mutations of *DKK1* and *KREMEN1* (Supporting information Table S1) were recently identified by WES analysis to be implicated in isolated TA or oligodontia accompanied with ectodermal dysplasia (Dinckan, Du, Petty, et al., 2018; Issa et al., 2016). *DKK1* encodes the Dickkopf Wnt signaling pathway inhibitor 1, which binds to its transmembrane receptor *KREMEN1* as well as *LRP6* coreceptor to inhibit Wnt/ β -catenin signaling in embryonic and vascular development. *KREMEN1* encodes a transmembrane receptor that functionally cooperates with *DKK1* to block Wnt/ β -catenin signaling. Further studies are required to demonstrate the functional significance of *DKK1* and *KREMEN1* variants in TA (Dinckan, Du, Petty, et al., 2018). In addition, recessive mutations of the *ANTXR1* gene (OMIM: 230,740) have been identified in one case with syndromic TA (Dinckan, Du, Akdemir, et al., 2018) and 11 cases with GAPO syndrome and vascular anomalies (HGMD). Of note, *ANTXR1* encodes a transmembrane protein directly interacting with *LRP6* (Figure 1, left panel) for beta-catenin stabilization and modulating Wnt signaling during normal vascular development.

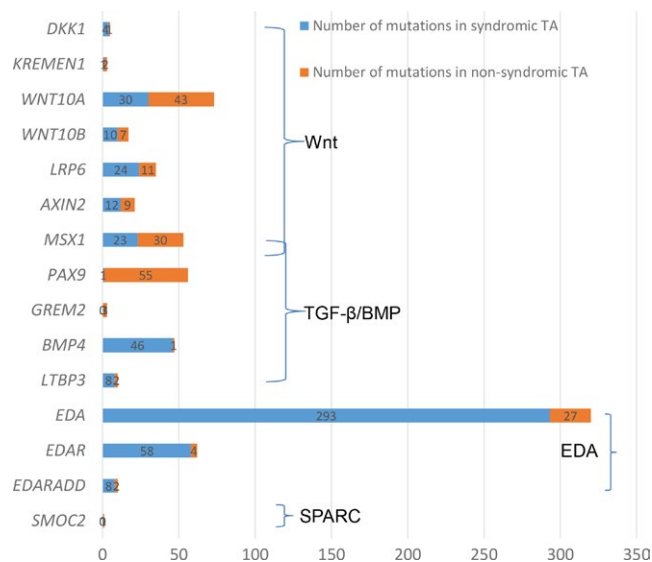


FIGURE 2 Spectrum of causal mutations in 15 genes responsible for nonsyndromic TA. Numbers of mutations in each of the causal genes in affected individuals with nonsyndromic TA (in red bar) versus syndromic TA or other conditions (in blue bar) are shown based on the data curated in Human Gene Mutation Database (HGMD), which also are summarized in more details in Supplementary Table 1. More specific genes for nonsyndromic TA are clustered, including four Wnt genes (*WNT10A*, *WNT10B*, *LRP6*, and *AXIN2*) and two genes (*MSX1* and *PAX9*) in the loop of Wnt and BMP4 pathways

3 | GENE MUTATIONS IN THE TGF- β /BMP PATHWAY CONTRIBUTE TO TA

The TGF- β /BMP pathway plays an important role in embryonic development (Figure 1, middle panel). Mutations in *BMP4*, which encodes a secreted ligand of the TGF- β family of proteins, result in more than 20 different conditions, including autosomal dominant inherited syndromic microphthalmia (OMIM 607,932), multiple oral and craniofacial development-related disorders, and also nonsyndromic TA in one case (HGMD).

The *LTBP3* gene encodes a ligand that forms a complex with TGF- β , which is involved in the assembly, secretion and targeting of TGF- β molecules. Mutations in *LTBP3* were shown to cause inherited dental anomalies and short stature as well as geleophysic dysplasia 3 (OMIM: 602,090). In 10 different reported mutations, two were found to cause isolated oligodontia (Supporting information Table S1). Experimentally, *Ltbp3*^{-/-} mice exhibit abnormal enamel and root morphogenesis (Huckert et al., 2015).

GREM2 encodes an antagonist protein to *BMP4*, which participates in the regulation of TGF- β signaling in tooth development. Three mutations in *GREM2* identified so far (OMIM: 608,832) are associated with isolated TA, microdontia, short tooth roots, and taurodontism (Kantaputra et al., 2015). In similar manner, *Grem2*^{-/-} mice manifested small deformed incisors (Kantaputra et al., 2015).

At last, it is worthwhile to emphasize that almost all 56 mutations of the *PAX9* gene are specific (Supporting information Table S1), making *PAX9* the most prevalent gene for nonsyndromic TA (Figure 2). Only one allele (640A > G, S214G, rs375436662) in the exon 3 of *PAX9* was detected in two siblings with cleft lip and their phenotypically normal mother (HGMD). However, this SNP is not an extremely rare deleterious mutation (MAF = 0.001084 in East Asian, ExAC database), as predicted by MutationTaster.

PAX9 encodes a member of the paired box family of transcription factors and is expressed in the presumptive dental mesenchyme to induce odontogenic signals and initiate dental development (Wong et al., 2018). Most of the mutations are clustered in the exon 2, which encodes the paired box DNA binding domain involving protein-DNA interactions, often affecting the second molars (Wong et al., 2018). *Pax9*-deficient mice exhibit arrests of tooth and taste bud development, and cleft palate (Peters, Neubuser, Kratochwil, & Balling, 1998). In an interesting manner, the deletion of *Pax9* in mice was found to upregulate the expression of Wnt pathway genes including two Wnt signaling antagonists *DKK1* and *DKK2* (Jia et al., 2017).

4 | GENE MUTATIONS IN EDA/EDAR/NF- κ B PATHWAY UNDERLIE TA

The *EDA* gene-encoded ligand, Ectodysplasin A, functions to bind to its receptor *EDAR* to activate the *IKBK*G-NF- κ B signaling for the development of ectodermal organs and teeth (Figure 1, right panel). In the past two decades, 293 different mutations in *EDA* were identified

to cause the X-linked hypohidrotic ectodermal dysplasia compared with 27 additional mutations in nonsyndromic TA (Han et al., 2008), reaching 8.4% of the specificity (Supporting information Table S1). The effects of *EDA* on ectodermal dysplasia and tooth development were confirmed in the *Eda* mutant mice (OMIM: 300,451).

In a similar manner, a majority of *EDAR* mutations were found to result in ectodermal dysplasia, compared to four cases with nonsyndromic TA (6.5%, Supplementary Table S1). Of 10 different mutations in *EDARADD*, two were associated with nonsyndromic TA and eight with ectodermal dysplasia (Supporting information Table S1). In 149 different mutations of *IKBK*G (aka *NEMO*), most of them are responsible for incontinentia pigmenti and ectodermal dysplasia (HGMD). In addition, two mutations in the *TRAF6* gene, which encodes a TNF receptor-associated factor (TRAF) protein (OMIM: 602,355), were identified in a family with ectodermal dysplasia and TA (HGMD). Mutations in the above-mentioned genes are expected to affect the NF- κ B activity, thereby resulting in ectodermal dysplasia, nonsyndromic TA, and other conditions.

5 | NETWORK OF TOOTH AGENESIS-ASSOCIATED SIGNALING PATHWAYS

Tooth morphogenesis is orchestrated by a complex development signaling network, which mainly includes the Wnt, TGF- β /BMP, and *Eda/Edar/NF- κ B* pathways. Extensive studies have uncovered cross-link loops among these pathways, which are interconnected and mutually dependent (Figure 1).

PAX9 appears to be at the top hierarchy of above-mentioned odontogenic pathways, as it has been shown to induce activation of both Wnt and TGF- β /BMP signaling for organogenesis (Jia et al., 2017). Conversely, *Pax9* deficiency resulted in a decreased activation of Wnt/ β -catenin *in vivo*, whereas small-molecule Wnt agonists were able to rescue the craniofacial defect in *Pax9* knockout mice (Jia et al., 2017). In an interesting manner, the expression level of *Edar* was also observed to be significantly decreased in *Pax9*^{-/-} mice, suggesting potential interactions between *PAX9* and the *Eda/Edar/NF- κ B* pathway.

As a direct target of Wnt/ β -catenin, increased expression of *MSX1* will then upregulate *Bmp4* expression and also activates TGF- β /BMP signaling for odontogenesis (Yin & Bian, 2015). Furthermore, *MSX1* can physically interact with *PAX9* to synergistically augment its own and *Bmp4* expression.

Wnt/ β -catenin and *Eda/Edar/NF- κ B* pathways also display reciprocal controls, as evidenced by their spatiotemporal coexpression in developing tooth. It has been shown that Wnt/ β -catenin activates the *Eda/Edar/NF- κ B* pathway by inducing the expression of *Eda*, which in turn is required for maintaining of the sustained Wnt/ β -catenin activities. Clinical evidence showed that digenic mutations in *EDA* and *WNT10A* modified the phenotypic severity in affected individuals with TA (He et al., 2013).

In addition, a causal mutation in the *SMOC2* gene, which seems not linked to above signaling pathways, has also been identified in two cousins with severe nonsyndromic TA (Bloch-Zupan et al.,

2011). This gene encodes a secreted protein, which promotes matrix assembly and stimulates endothelial cell proliferation and migration, as well as angiogenic activity. Knockdown of *Smoc2* in zebrafish showed pharyngeal teeth that had abnormalities reminiscent of the human phenotype (Bloch-Zupan et al., 2011).

6 | SUMMARY AND FUTURE DIRECTION

Enormous advancements have been made to decode the genetic complexity and heterogeneity of TA over the last few decades, especially during the past several years. Majority of the mutations (91.9%) responsible for affected individuals with nonsyndromic TA are identified only from seven genes (i.e., *PAX9*, *WNT10A*, *MSX1*, *EDA*, *LRP6*, *WNT10B*, and *AXIN2*). Specificity analysis in terms of the ratio of nonsyndromic TA mutations versus syndromic mutations of these genes showed a dramatic difference from 98.2% to 8.4% (as listed in descending order: *PAX9*, *WNT10A*, *MSX1*, *WNT10B*, *LRP6*, *AXIN2*, and *EDA*). These findings demonstrate a major role of the Wnt and Wnt-associated pathways in the molecular pathogenesis of the nonsyndromic TA. It is conceivable that high-throughput sequencing techniques, such as WES and postgenomic bioinformatics, will markedly enhance the detection of extremely rare mutations in known and novel causal genes.

For affected individuals with more complicated nonsyndromic and syndromic TA, renewed efforts shall be made to establish large databases for in-depth analysis of genotype–phenotype correlation, single- or multiple-allele traits, epigenetic effectors, and genetic modifiers. Gene function studies should be performed in mammalian cells and animal models to uncover molecular and cellular mechanisms of TA, for instance, whether the above-mentioned Wnt-associated components play a crucial role in determining of odontoblastic and vasculogenic fate of DPSCs in affected individuals with nonsyndromic TA during tooth development. Based on the understanding of genetic defects, ongoing studies utilizing small-molecular inhibitors in the Wnt pathway have shown a great potential in the treatment of developmental disturbances in experimental animals and ultimately in clinical trials.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

MY, SWW, and DH participated in the writing. SWW and TC made the figures and tables. TC supervised the work and edited the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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