Current Osteoporosis Reports (2022) 20:389–397
https://doi.org/10.1007/s11914-022-00761-8

CRANIOFACIAL SKELETON (TG CHU AND S AKINTOYE, SECTION EDITORS)

Rethinking the Genetic Etiology of Nonsyndromic Tooth Agenesis

Ariadne Letra [1]

Accepted: 1 September 2022 / Published online: 15 November 2022

Abstract

Purpose of Review Genetic studies in humans and animal models have improved our understanding of the role of numerous genes in the etiology of nonsyndromic tooth agenesis (TA). The purpose of this review is to discuss recently identified genes potentially contributing to TA.

Recent Findings Despite research progress, understanding the genetic factors underlying nonsyndromic TA has been challenging given the genetic heterogeneity, variable expressivity, and incomplete penetrance of putatively pathogenic variants often observed associated with the condition. Next-generation sequencing technologies have provided a platform for novel gene and variant discoveries and informed paradigm-shifting concepts in the etiology of TA.

Summary This review summarizes the current knowledge on genes and pathways related to nonsyndromic TA with a focus on recently identified genes/variants. Evidence suggesting possible multi-locus variation in TA is also presented.

Keywords Nonsyndromic tooth agenesis · Gene · Complex trait · Tooth development

Introduction

Tooth development is a genetically regulated process characterized by a series of sequential and reciprocal epithelial-mesenchymal interactions that drive tooth morphogenesis as well as differentiation of tooth-specific cell types [1]. Four major signaling pathways (BMP, FGF, SHH, WNT) and various transcription factors are key orchestrators of tooth development (Fig. 1). Disturbances at any stage of tooth development may lead to a plethora of dental anomalies, of which tooth agenesis (TA) is the most widely recognized [2].

TA is defined as the developmental failure of a tooth due to perturbations in the initiation stage of tooth development [2]. Agenesis of primary teeth is rare; however, there is a strong correlation between agenesis in the primary dentition and permanent dentition [3]. TA affects approximately 200 million individuals worldwide and can occur as a feature of many craniofacial syndromes, although it is more frequently found as a nonsyndromic, isolated trait segregating in families or as a new event. Autosomal dominant, autosomal recessive, or X-linked patterns of inheritance have been reported [4, 5]. Depending on the number of missing teeth, TA can be referred to as hypodontia (1 to 5 teeth missing), oligodontia (≥6 teeth missing) (Fig. 2), or anodontia (all teeth missing), the latter being primarily associated with syndromic forms [6].

The prevalence of TA, excluding third molars, has been reported to range between 1.6% and ~10%, depending on severity (hypodontia or oligodontia) and population studied. When third molars are included in the missing teeth count, the prevalence of TA reaches 25% [4]. Hypodontia is more common, whereas oligodontia is rare occurring in <1% of the population. The most commonly affected teeth are mandibular second premolars followed by maxillary lateral incisors, and maxillary second premolars. It is also generally accepted that the prevalence of TA varies by sex, with females being more affected than males in a 3:2 ratio [4].

TA imposes significant functional, esthetic, and financial burdens on affected individuals and families. Clinical management of TA is challenging and requires a multidisciplinary team to perform multiple orthodontic, rehabilitation, and/or surgical interventions to close the missing spaces and restore masticatory function and esthetics. TA is often accompanied by delays in permanent tooth development and additional dental anomalies including microdontia, taurodontism, transposition, and ectopic positioning of erupted teeth. The impact on oral health-related quality of life can be significant and depend

This article is part of the Topical Collection on *Craniofacial Skeleton*

✉ Ariadne Letra
AriadneLetra@pitt.edu

[1] Department of Oral and Craniofacial Sciences, and Center for Craniofacial and Dental Genetics, University of Pittsburgh School of Dental Medicine, Pittsburgh, PA 15219, USA

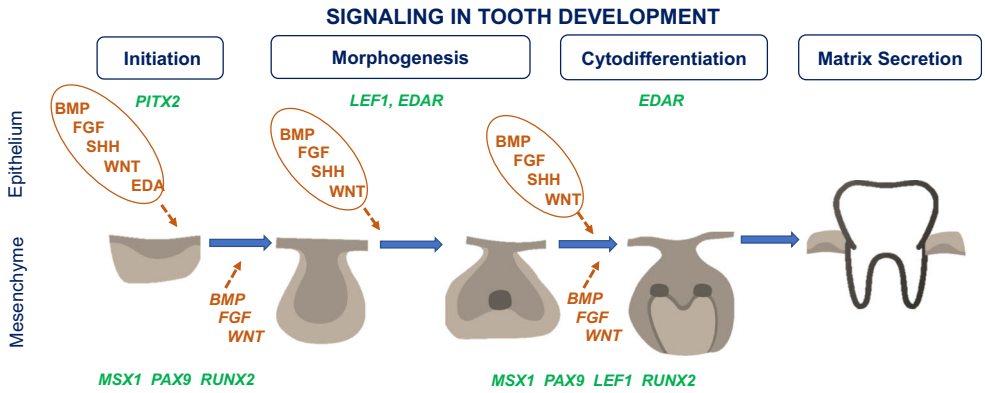

Fig. 1 Schematic representation of tooth development stages. Sequential and reciprocal interactions between epithelium and mesenchyme are tightly regulated by various signaling pathways (orange) and transcription factors (green). Disturbances in the expression of molecular players the initiation stage of tooth development results in tooth agenesis

on the affected individual's age, severity of TA, and the presence of additional dental anomalies. Treatment strategies to mitigate the functional and cosmetic consequences of TA vary and often include space management and maintenance, alignment of existing teeth, occlusal bite corrections, among other oral rehabilitation procedures that often come with a high cost [4].

Compelling evidence supports a predominantly genetic etiology for TA [5]. Studies in animal models have elucidated the role of numerous genes during tooth development thereby facilitating molecular diagnosis of TA in affected individuals [2, 3]. Despite research progress, understanding the genetic factors underlying nonsyndromic TA has been challenging. Genetic heterogeneity, variable expressivity, pleiotropic effects of genes, and incomplete penetrance may act as confounders complicating causal gene identification. Further, it has been proposed that the missing heritability of TA relies on mutations in genes yet unknown to tooth development, and/or on combined effects of multiple pathogenic variants [3, 7].

In this review, we summarize the current knowledge on genes related to nonsyndromic TA and highlight the recent contributions of next-generation sequencing studies in advancing knowledge about the etiology of the condition.

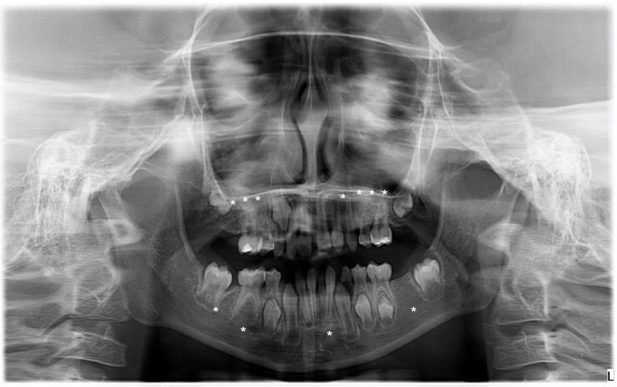

Fig. 2 Panoramic radiograph of 8-year old child presenting with severe tooth agenesis (oligodontia). Asterisks denote location of permanent teeth missing (asterisks)

Genes Involved in Nonsyndromic TA

Defects in genes involved in the process of odontogenesis have been reported as etiologic for nonsyndromic TA, often following clues from animal models or syndromic presentations (e.g., oral-facial cleft and ectodermal dysplasia syndromes) [5, 8]. While the scope of this review is limited to the genetic etiology of nonsyndromic TA, genes involved in syndromic TA and associated phenotypes have been extensively discussed [2, 3, 5]. Genes in which variations have been reported in nonsyndromic TA individuals are presented in Table 1.

"Classic" TA Genes

Some of the most widely known genes involved in nonsyndromic TA include the transcription factors *MSX1* (Msh homeobox 1) and *PAX9* (paired box 9) [2, 3, 9, 10]. Autosomal dominant mutations in these genes comprise the first reports of genetic variations in nonsyndromic TA [11]. A missense mutation in *MSX1* was found in a multigeneration family affected by TA in which affected individuals lacked second premolars, third molars, and upper lateral incisors [11]. *PAX9* was later identified as the second gene involved in TA, where a frameshift mutation was reported as etiologic in a family characterized by the absence of permanent molars [12]. Over the years, more than 50 mutations in *MSX1* and *PAX9* have been identified in nonsyndromic TA, affecting preferentially posterior teeth (premolars and molars), although a few reports of anterior tooth agenesis exist [3, 10, 13]. In mice, expression of both *MSX1* and *PAX9* in the dental mesenchyme is critical to initiate and regulate signaling during the transition from bud to cap stage of tooth development; further, both genes act synergistically to regulate the expression of *BMP4* (bone morphogenetic protein 4), a member of the transforming growth factor-β (TGF-β) family essential for bone, limb, and tooth development [14]. In addition to inducing TGF-β/BMP signaling, *PAX9* is required for activation of Wnt signaling during craniofacial morphogenesis [15].

Table 1 Genes in which mutations have been reported in nonsyndromic TA individuals

Gene	Location	Mode of Inheritance	Phenotype(s)		Missing teeth frequently associated	Gene discovery approach**
ANTRX1	2p13.3	AR	Oligodontia	----	mandibular incisors and canines	WES
ARHGAP15	2q22.2-q22.3	Unknown	----	Hypodontia	Not reported	GWAS
ASCL5	1q32.1	Unknown	----	Hypodontia	Not reported	GWAS
AXIN2	17q24.1	AD	Oligodontia	Hypodontia	maxillary and mandibular lateral incisors, premolars, and first molars	Positional cloning, sanger sequencing
ATF1	12q13.13	Unknown	Oligodontia	Hypodontia	maxillary and mandibular lateral incisors, premolars, and molars	SNP genotyping
BCOR	Xp11.4	Complex	Oligodontia	----	maxillary canines, second premolar and second molar, mandibular central and lateral incisor, second premolar	WES
CACNA1S	1q32.1	Unknown	----	Hypodontia	Not reported	GWAS
CASC8	8q24	Unknown	Oligodontia	Hypodontia	maxillary and mandibular lateral incisors, premolars, and molars	SNP genotyping
COL17A1	10q25.1	Complex	----	Hypodontia	maxillary lateral incisors, mandibular central and lateral incisors	WES
DKK1	10q21.1	Complex	----	Hypodontia	maxillary lateral incisors, mandibular central and lateral incisors	WES
DUSP10	1q41	Unknown	Oligodontia	Hypodontia	maxillary and mandibular lateral incisors, premolars, and molars	SNP genotyping
EDA	Xq13.1	XL	Oligodontia	Hypodontia	maxillary and mandibular central and lateral incisors	sanger sequencing
EDAR	2q13	AD	Oligodontia	Hypodontia	mandibular second premolars, maxillary lateral incisors	sanger sequencing
EDARADD	1q42.3-q43	AD	Oligodontia	Hypodontia	canines	sanger sequencing
FAM49A	2p24.2	Unknown	----	Hypodontia	maxillary lateral incisors	GWAS
FGF3	11q13.3	Unknown	----	Hypodontia	maxillary lateral incisors	SNP genotyping
FGF10	5p12	Unknown	----	Hypodontia	maxillary lateral incisors, premolars	SNP genotyping
FGFR1	8p11.23	AD	----	Hypodontia	maxillary and mandibular incisors and premolars	SNP genotyping
FGFR2	10q26.13	Unknown	----	Hypodontia	premolars	SNP genotyping
FOXI3	2p11.2	Unknown	----	Hypodontia	Not reported	GWAS
FOXP1	3p13	Unknown	----	Hypodontia	maxillary lateral incisors	GWAS
GLI2	2q14.2	Unknown	----	Hypodontia	mandibular incisors	SNP genotyping
GREM2	1q43	AD	Oligodontia	Hypodontia	maxillary and mandibular lateral incisors, premolars and molars	sanger sequencing
IRF6	1q32.2	AD	----	Hypodontia	maxillary and mandibular incisors, premolars and molars	SNP genotyping
LAMA3	18q11.2	Complex	Oligodontia	Hypodontia	maxillary lateral incisors and premolars, second molar, mandibular premolars	WES
LAMB3	1q32.2	AR	Oligodontia	----	maxillary lateral incisors and premolars, mandibular canines and first premolar	WES
LHX6	9q33.2	Unknown	----	Hypodontia	premolars	SNP genotyping
LRP6	12p13.2	AD	Oligodontia	Hypodontia	incisors and premolars	WES
MSX1	4p16.3-p16.1	AD	Oligodontia	Hypodontia	mandibular and maxillary premolars and molars, maxillary lateral incisors	sanger sequencing
NOL11	17q24.2	Unknown	----	Hypodontia	maxillary second premolars	GWAS
PAX9	14q12-q13	AD	Oligodontia	Hypodontia	mandibular and maxillary second molars and second premolars	sanger sequencing
SMOC2	6q27	AR	Oligodontia	----	mandibular and maxillary canines, second premolars and molars, and lateral incisors	sanger sequencing
TSPEAR	21q22.3	AD, AR	Oligodontia	----	mandibular and maxillary lateral incisors, canines, second premolars and second molars; maxillary first premolars, mandibular central incisors	WES
WNT10A	2q35	AD, AR, complex	Oligodontia	Hypodontia	mandibular and maxillary lateral incisors, canines, premolars and molars; mandibular central incisors, premolars and molars	sanger sequencing
WNT10B	12q13.12	AD	Oligodontia	Hypodontia	mandibular and maxillary incisors, canines, premolars and molars; mandibular incisors, premolars and molars	sanger sequencing
ZFHX4	8q21.13	Unknown	----	Hypodontia	Not reported	GWAS

*Reflects first gene discovery study; *WES* whole-exome sequencing, *SNP* single nucleotide polymorphism, *AD* autosomal dominant, *AR* autosomal recessive

EDA (ectodysplasin-A) mutations result in anhidrotic ectodermal dysplasia and hypohidrotic ectodermal dysplasia, both which include oligodontia as a phenotypic feature [16, 17] *EDA* binds to its receptor *EDAR* to activate *EDA/EDAR/NF-κB* signaling pathway, which is required for normal skin, hair, and teeth formation [18]. Although these genes are more commonly associated with syndromic forms of TA, circa 30 *EDA* and/or *EDAR* mutations have been reported in individuals with TA and no other defects of ectodermal nature [16, 19–21]. In mice, loss of *Eda* confirms the ectodermal and dental phenotypes observed in humans.

WNT Pathway Genes

The Wnt/β-catenin signaling pathway regulates many aspects of embryonic development and is spatiotemporally activated throughout tooth development stages, thereby implying its critical role during odontogenesis [22] (Fig. 3).

Over the years, a number of WNT pathway genes have been implicated in the etiology of nonsyndromic TA. Mutations in *AXIN2* (axis inhibition protein 2), a negative regulator of Wnt signaling, were reported as etiologic for TA in a multigenerational family segregating with TA and colorectal cancer, thereby suggesting that TA could be a potential early marker for colorectal cancer in predisposed individuals [23]. In addition, this study showed for the first time, that *Axin2* was expressed in the developing mouse craniofacial and dental tissues, specifically the enamel knot and underlying mesenchyme during tooth formation [23]. Several studies have since investigated the association of genetic variation in *AXIN2* with sporadic and familial TA, with or without cancer history, although results are not conclusive. Therefore caution must be taken to avoid interpretation of such association findings as direct evidence of causation [24, 25].

LRP6 (LDL receptor-related protein 6) is a co-receptor in the Wnt/β-catenin pathway that can bind to various WNT ligands and inhibitors (e.g., *DKK, KREMEN*) to modulate signaling [26]. To date, circa 16 mutations in *LRP6* have been reported in individuals with nonsyndromic TA in different populations [27–33]. Most of the identified variants suggest

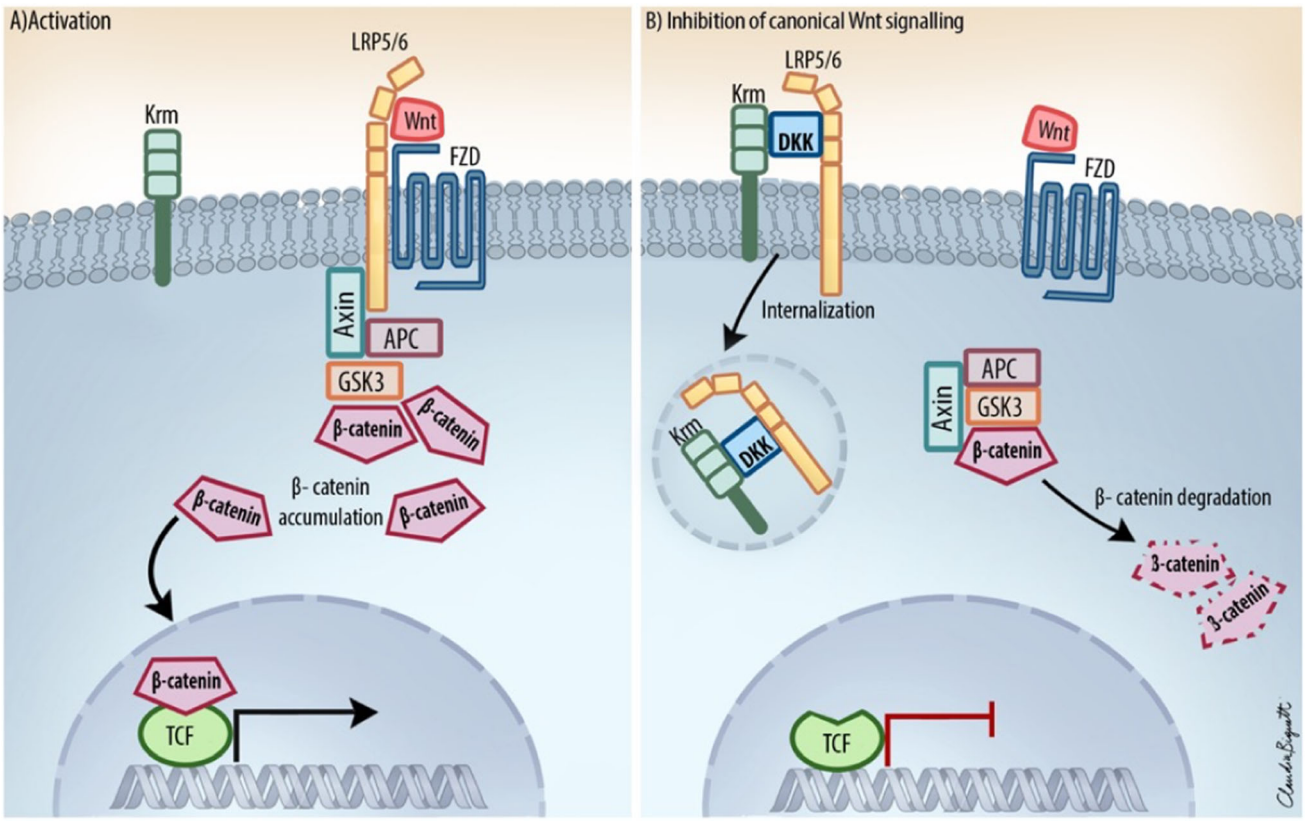

Fig. 3 The canonical WNT/β-catenin signaling pathway. **A** Wnt signaling is activated via Wnt ligands binding to cell surface receptors Frizzled (FZD) and LRP5/6. which then transduce the signals to a gene complex including *AXIN, APC* and *GSK3* to inhibit β-catenin degradation. Subsequently, β-catenin accumulates in the cytoplasm and translocates into the nucleus to interact with TCF and initiate transcription of target genes. **B** In the presence of *DKK*, a ternary complex formation between Kremen (*KRM*), *DKK* and *LRP6* results in depletion of *LRP5/6* from the membrane, blocking of Wnt signaling and triggering β-catenin degradation by the gene destruction complex and inhibition of gene transcription (From N. Dinckan, R. Du, L.E. Petty, Z. Coban-Akdemir, S.N. Jhangiani, I. Paine, E.H. Baugh, A.P. Erdem, H. Kayserili, H. Doddapaneni, J. Hu, D.M. Muzny, E. Boerwinkle, R.A. Gibbs, J.R. Lupski, Z.O. Uyguner, J.E. Below, and A. Letra. Whole-Exome Sequencing Identifies Novel Variants for Tooth Agenesis. Journal of Dental Research 97(1):49-59)

loss-of-function effects although functional characterization experiments are required to confirm their impact on gene/protein function. In one study, a splicing variant (c.3607+3_6del) in *LRP6* was identified segregating in autosomal dominant form in a multigenerational family with TA. Decreased mRNA expression levels were noted in peripheral blood samples of all affected individuals, indicating nonsense-mediated decay [28]. Intriguingly, the missense variant c.2570G > A (R857H), identified in a TA family and classified as potentially damaging due to its predicted effect on LRP6-Wnt binding did not result in changes in gene transcription, although maturation and phosphorylation levels of LRP6 were noted in the presence of mutant alleles suggesting perturbed Wnt signaling [34]. In mice, Lrp6 expression was noted in the tooth follicle and inner enamel epithelium [29], while homozygous deletion of *Lrp6* led to severe skeletal abnormalities and early lethality [35].

Mutations in *WNT10A* (wingless-type MMTV integration site family, member 10A) were first identified in individuals with the autosomal recessive disorders odontoonychodermal dysplasia (OODD) and Schopf-Schulz-Passarge syndrome (SSPS), and more recently reported to account for over 50% of the TA-associated genetic variation [36]. More than 60 rare, homozygous and heterozygous variants in *WNT10A* have been identified in association with nonsyndromic TA [3, 36]. Intriguingly, the same mutation (e.g., c.682T>A; F228I) has been associated with both autosomal recessive and autosomal dominant forms of nonsyndromic TA as well as with ectodermal dysplasia, and single heterozygous variants in *WNT10A* have been identified in approximately 2.5% of individuals without TA [3]. Consistent with several reports, compound heterozygous in *WNT10A* were associated with a larger number of missing teeth when compared to single heterozygous mutations. Further, the presence of *WNT10A* mutations was also frequently reported segregating with mutations in other genes [28, 37, 38] (see Multilocus variation section below). No preferential patterns of missing teeth were reported in individual studies [3, 28, 39–42], however, a recent meta-analysis suggested that the most frequently reported missing teeth patterns associated with *WNT10A* variation reflect third molars (78%), mandibular premolars (71%) and maxillary lateral incisors (60%) [43]. Although strong evidence implicates a role for *WNT10A* in nonsyndromic TA, its exact role in the pathogenesis of the condition remains unclear. *Wnt10a* mutant mice present defects in tooth morphogenesis but do not have missing teeth [44, 45]. In contrast, downregulation of *wnt10a* resulted in failure of tooth development in zebrafish embryos [40].

Additional WNT pathway genes in which variants were found in nonsyndromic TA patients include *WNT10B* (wingless-type MMTV integration site family, member 10B) and *DKK1* (Dickkopf WNT Signaling Pathway Inhibitor 1). Rare variants and single nucleotide polymorphisms in *WNT10B* were identified in three studies of nonsyndromic TA [46–48]. Two studies have suggested *DKK1* as a candidate gene for nonsyndromic TA, one of which highlighted a splicing variant in *DKK1* segregating with additional missense and splicing variants in *LAMA3* (Laminin Subunit Alpha 3) and *COL17A1* (Collagen Type XVII Alpha 1), respectively, in a family showing autosomal dominant inheritance of oligodontia phenotypes [28]. (see Multilocus variation section below).

Genes Identified via Genome-Wide Association Study

To the best of our knowledge, a single well-powered genome-wide association study (GWAS) of TA has been conducted, which included 1,944 subjects with congenitally missing teeth and 338,554 controls of European ancestry [49]. Both rare and common genetic variants were identified in association with TA, five of which were located in/nearby genes involved in tooth development and/or additional ectodermal structures (*EDA, EDAR, FOXIE3, FOXP1,* and *LEF1*), whereas the remaining associated variants were located around loci with no previous evidence of a role in tooth development (*ASCL5/CACNA1S, ARHGAP15, NOL11,* and *FAM49A*) [49] (Table 1).

Genes Identified via Next-Generation Sequencing

The use of next-generation sequencing (NGS) and unbiased analysis approaches has made a significant and positive impact on our understanding of the genetic etiology of TA. Recent whole exome sequencing (WES) studies of individuals and families with TA have allowed the identification of novel genes (e.g., *ANTXR1, BCOR, COL17A1, DKK1, LAMA3, LAMB3, TSPEAR*), as well as novel variants in known genes as etiologic for TA [3].

ANTXR1 (anthrax toxin receptor 1), in which mutations have been shown to cause GAPO syndrome—characterized by delayed growth, alopecia, failure of tooth eruption, and optic atrophy—was recently identified as a novel TA gene [50]. A rare homozygous variant in *ANTXR1* (c.1312C>T; R438C) was identified by WES in a consanguineous Turkish family in which the affected individual showed agenesis of mandibular incisors and canines but no other structural abnormalities. Expression of Antxr1 was detected in the developing mouse craniofacies, particularly in the dental epithelium and mesenchyme at initial stages of tooth development, and later in the enamel organ and dental papilla [50].

Rare missense variants in *LAMA3* (c.1097G>A; R366H; and c.2798G>T; G933V), *LAMB3* (Laminin subunit beta-3; c.547C>T; R183C), and a splicing variant in *COL17A1* (c.3277+3G>C) were reported as likely pathogenic for nonsyndromic TA in two independent studies [28, 37]. Mutations in these genes cause non-Herlitz-type junctional

epidermolysis bullosa, an autosomal recessive skin disorder characterized by the appearance of blisters, erosions, dystrophic nails, enamel hypoplasia, and TA [51–53]. Carriers of heterozygous variants in *LAMA3, LAMB3,* and *COL17A1* have been well described with respect to their amelogenesis imperfecta phenotypes including hypoplastic enamel with the presence of grooves and pits [54]. In mice, deletion of *Lama3* resulted in abnormal enamel deposition and disorganized enamel epithelium, whereas deletion of *Lamb3* resulted in early lethality precluding analysis of enamel phenotype [54].

Mutations in *TSPEAR* (thrombospondin-type laminin G domain and EAR repeats) were identified to cause a form of ectodermal dysplasia characterized by facial dysmorphisms, scalp hypotrichosis, TA, and microdontia [55, 56]. These variants were predicted to completely abolish the EAR domains of the TSPEAR protein, or were located within conserved sites of those domains suggesting an important role for TSPEAR in normal development [56]. In two independent studies, a complex insertion/deletion variant (c.1726G>T; 1728delC) and a missense variant (c.1877C>T; P626S; and c) in *TSPEAR* were identified in families with nonsyndromic TA, although incomplete penetrance was observed in one family [37, 57]. Incomplete penetrance is a common finding in TA families and could be the result of a more complex genotype–phenotype relationship due to variation at other locus/loci, or mutational burden impacting expression of the disease trait associated with monoallelic variation [37].

Taken together, the results of these next-generation sequencing studies revealed the involvement of novel genes in tooth development as potentially etiologic for TA. Additional studies are warranted to confirm the exact mechanisms by which variations in these genes may impact gene/protein function leading to TA.

Multi-locus Variation in Nonsyndromic TA

Historically, the origin of both syndromic and nonsyndromic TA has been regarded as monogenic, in which a single putatively pathogenic mutation in a gene known to be involved in craniofacial and/or tooth development was deemed etiologic. This was likely due to the genetic research approaches (i.e., candidate gene sequencing) frequently utilized and the establishment of a molecular diagnosis once variation in a known gene was identified. While such efforts enabled molecular diagnosis for many TA individuals and families, variation in known genes account for ~50–55% of the estimated heritability of TA, indicating that additional genes remain to be identified [58].

As discussed above, the use of next-generation sequencing and unbiased analysis methods allowed the identification of novel genes, variants and variant combinations contributing to nonsyndromic TA [3]. Notably, the use of WES provided evidence that multi-locus variation and oligogenic inheritance is possible in TA, and these observations challenge the notion that a diagnostic investigation is complete after a single variant in a known or novel candidate gene has been obtained [3]. Through independent studies, rare variants in *WNT10A* were found co-segregating with variants in *GREM2*, *LAMA3*, *BCOR*, or *LRP6*, in individuals with nonsyndromic TA [28, 37, 38, 59]. Moreover, rare variants in *DKK1*, *LAMA3*, and *COL17A1* were also found segregating together and likely contributing to the variable TA phenotypes in familial TA [28]. In all these families, a single variant could not explain the TA phenotypes observed suggesting that additional mutational burden or segregation of rare variants at more than one locus could modulate phenotypic expression. Multi-locus variation has been reported as the most likely explanation for a number of Mendelian and complex conditions [60, 61]. TA is a clinically heterogeneous condition with nearly 100 associated loci, hence providing an excellent model for the investigation of mutational burden contributing to complex inheritance patterns. This approach provides a paradigm to maximize identification of the missing genetic liability and explanation for unusual inheritance patterns in TA families.

Challenges and Future Research Directions

TA is characterized by variable expressivity and genetic heterogeneity making genotype-phenotype correlations challenging. A few studies have addressed the patterns of missing teeth with their corresponding genetic variation although the results vary depending on the population, TA phenotypes (hypodontia versus oligodontia), and if sporadic or familial cases are included [43, 62]. Family-based study designs offer a more powerful approach in studies of complex disorders such as TA because they allow the detection of variant segregation in affected and unaffected family members and the ability to reveal parental-origin allelic effects meanwhile controlling for heterogeneity and population stratification [63].

Until recently, the field had focused predominantly on protein-coding variation affecting genes involved in craniofacial and/or tooth development, whereas the role of non-coding variantion in TA has been less explored. The use of WES facilitated the discovery of novel coding and non-coding variants often located in regulatory regions of the genome as likely pathogenic for TA [28, 37, 49, 50]. Further, ample evidence now exists to support instances of multilocus variation and potential oligogenic inheritance in TA [3]. While there may be private events happening in private families, as technologies for rare variant detection continue to improve and new TA genes are identified, instances of multi-locus variation are likely to increase. Therefore, excluding known genes

and/or focusing on candidate gene identification in7 NGS studies of TA might preclude proper molecular diagnosis [3].

Importantly, as new TA variants are identified, functional characterization experiments *in vitro* and *in vivo* will be required to determine the mechanisms by which they may disrupt gene/protein function and affect tooth development leading to TA. For many TA genes, the biological effects of variants reported as pathogenic for TA remain poorly understood. One such example is *WNT10A*, in which most variants associated with TA are predicted to affect protein folding and/or destabilization, although these predictions often did not translate into altered gene/protein functions in cell or animal model systems of TA [40, 64]. For those *WNT10A* variants with functional significance, the effects were allele-specific, and shown to impact WNT signaling or expression of additional relevant genes [64].

Conclusions

TA is a common abnormality of tooth development with significant functional and esthetic consequences requiring extensive and costly oral rehabilitation treatments. While TA etiology may be monogenic in some cases, evidence supporting multi-locus variation is increasing given the wider utilization of NGS in genetic studies of TA. These observations further highlight the importance of employing unbiased filtering and prioritization strategies for NGS data to maximize gene/variant discovery. Importantly, thorough clinical phenotyping to exclude syndromic forms of TA with subtle manifestations are critical for proper interpretation of genetic findings and improved recurrence risk estimates and genetic counseling.

Funding information This work was supported by grants from the National Institute for Dental and Craniofacial Research R56DE028302 (AL).

Declarations

Conflict of Interest The author declares no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Thesleff I. The genetic basis of tooth development and dental defects. Am J Med Genet A. 2006;140(23):2530–5.
2. Yin W, Bian Z. The gene network underlying hypodontia. J Dent Res. 2015;94(7):878–85.
3. Williams MA, Letra A. The changing landscape in the genetic etiology of human tooth agenesis. Genes (Basel). 2018;9(5).
4. Polder BJ, van't Hof MA, van der Linden FPGM, Kuijpers-Jagtman AM. A meta-analysis of the prevalence of dental agenesis of permanent teeth. Community Dent Oral Epidemiol. 2004;32(3):217–26.
5. Nieminen P. Genetic basis of tooth agenesis. J Exp Zool B Mol Dev Evol. 2009;312B(4):320–42.
6. Hennekam, R, Allanson J, Krantz I. Gorlin's syndromes of the head and neck. 5th ed. New York: Oxford University Press, 2010.
7. Williams MA, Biguetti C, Romero-Bustillos M, Maheshwari K, Dinckan N, Cavalla F, Liu X, Silva R, Akyalcin S, Uyguner ZO, Vieira AR, Amendt BA, Fakhouri WD, Letra A. Colorectal cancer-associated genes are associated with tooth agenesis and may have a role in tooth development. Sci Rep. 2018;8(1):2979.
8. Vieira AR. Oral clefts and syndromic forms of tooth agenesis as models for genetics of isolated tooth agenesis. J Dent Res. 2003;82(3):162–5.
9. Matalova E, Fleischmannova J, Sharpe PT, Tucker AS. Tooth agenesis: from molecular genetics to molecular dentistry. J Dent Res. 2008;87(7):617–23.
10. Ye X, Attaie AB. Genetic basis of nonsyndromic and syndromic tooth agenesis. J Pediatr Genet. 2016;5(4):198–208.
11. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. Nat Genet. 1996;13(4):417–21.
12. Stockton DW, das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of PAX9 is associated with oligodontia. Nat Genet. 2000;24(1):18–9.
13. Brook AH, Elcock C, Aggarwal M, Lath DL, Russell JM, Patel PI, Smith RN. Tooth dimensions in hypodontia with a known PAX9 mutation. Arch Oral Biol. 2009;54(Suppl 1):S57–62.
14. Ogawa T, Kapadia H, Feng JQ, Raghow R, Peters H, D'Souza RN. Functional consequences of interactions between Pax9 and Msx1 genes in normal and abnormal tooth development. J Biol Chem. 2006;281(27):18363–9.
15. Jia S, Zhou J, Fanelli C, Wee Y, Bonds J, Schneider P, Mues G, D'Souza RN. Small-molecule Wnt agonists correct cleft palates in Pax9 mutant mice in utero. Development. 2017;144(20):3819–28.
16. Arte S, Parmanen S, Pirinen S, Alaluusua S, Nieminen P. Candidate gene analysis of tooth agenesis identifies novel mutations in six genes and suggests significant role for WNT and EDA signaling and Allele Combinations. PLoS ONE. 2013;8(8):e73705.
17. Fan H, Ye X, Shi L, Yin W, Hua B, Song G, Shi B, Bian Z. Mutations in the EDA gene are responsible for X-linked hypohidrotic ectodermal dysplasia and hypodontia in Chinese kindreds. Eur J Oral Sci. 2008;116(5):412–7.
18. Deshmukh S, Prashanth S. Ectodermal dysplasia: a genetic review. Int J Clin Pediatr Dent. 2012;5(3):197–202.
19. Song S, Han D, Qu H, Gong Y, Wu H, Zhang X, Zhong N, Feng H. EDA gene mutations underlie non-syndromic oligodontia. J Dent Res. 2009;88(2):126–31.
20. Mues G, Tardivel A, Willen L, Kapadia H, Seaman R, Frazier-Bowers S, Schneider P, D'Souza RN. Functional analysis of Ectodysplasin-A mutations causing selective tooth agenesis. Eur J Hum Genet. 2010;18(1):19–25.
21. Han D, Gong Y, Wu H, Zhang X, Yan M, Wang X, Qu H, Feng H, Song S. Novel EDA mutation resulting in X-linked non-syndromic hypodontia and the pattern of EDA-associated isolated tooth agenesis. Eur J Med Genet. 2008;51(6):536–46.
22. Thesleff I. Current understanding of the process of tooth formation: transfer from the laboratory to the clinic. Aust Dent J. 2014;59(Suppl 1):48–54.
23. Lammi L, Arte S, Somer M, Järvinen H, Lahermo P, Thesleff I, Pirinen S, Nieminen P. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. Am J Hum Genet. 2004;74(5):1043–50.

24. Callahan N, Modesto A, Meira R, Seymen F, Patir A, Vieira AR. Axis inhibition protein 2 (AXIN2) polymorphisms and tooth agenesis. Arch Oral Biol. 2009;54(1):45–9.
25. Letra A, Menezes R, Granjeiro JM, Vieira AR. AXIN2 and CDH1 polymorphisms, tooth agenesis, and oral clefts. Birth Defects Res A Clin Mol Teratol. 2009;85(2):169–73.
26. Davidson G. LRPs in WNT Signalling. Handb Exp Pharmacol. 2021;269:45–73.
27. Yu M, Fan Z, Wong SW, Sun K, Zhang L, Liu H, Feng H, Liu Y, Han D. Lrp6 dynamic expression in tooth development and mutations in oligodontia. J Dent Res. 2021;100(4):415–22.
28. Dinckan N, du R, Petty LE, Coban-Akdemir Z, Jhangiani SN, Paine I, Baugh EH, Erdem AP, Kayserili H, Doddapaneni H, Hu J, Muzny DM, Boerwinkle E, Gibbs RA, Lupski JR, Uyguner ZO, Below JE, Letra A. Whole-exome sequencing identifies novel variants for tooth agenesis. J Dent Res. 2018;97(1):49–59.
29. Ockeloen CW, Khandelwal KD, Dreesen K, Ludwig KU, Sullivan R, van Rooij IALM, Thonissen M, Swinnen S, Phan M, Conte F, Ishorst N, Gilissen C, Roa Fuentes L, van de Vorst M, Henkes A, Steehouwer M, van Beusekom E, Bloemen M, Vankeirsbilck B, et al. Novel mutations in LRP6 highlight the role of WNT signaling in tooth agenesis. Genet Med. 2016;18(11):1158–62.
30. Wang H, Liu Y, Zheng Y, Zhao X, Lin S, Zhang Q, Zhang X. A novel missense mutation of LRP6 identified by whole-exome sequencing in a Chinese family with non-syndromic tooth agenesis. Orthod Craniofac Res. 2021;24(2):233–40.
31. Massink MP, et al. Loss-of-Function Mutations in the WNT Co-receptor LRP6 Cause Autosomal-Dominant Oligodontia. Am J Hum Genet. 2015;97(4):621–6.
32. Goto H, Kimura M, Machida J, Ota A, Nakashima M, Tsuchida N, Adachi J, Aoki Y, Tatematsu T, Takahashi K, Sana M, Nakayama A, Suzuki S, Nagao T, Matsumoto N, Tokita Y. A novel LRP6 variant in a Japanese family with oligodontia. Hum Genome Var. 2021;8(1):30.
33. Li S, et al. Variants in the Wnt co-receptor LRP6 are associated with familial exudative vitreoretinopathy. J Genet Genomics. 2021.
34. Huang YX, Gao CY, Zheng CY, Chen X, Yan YS, Sun YQ, Dong XY, Yang K, Zhang DL. Investigation of a novel LRP6 variant causing autosomal-dominant tooth agenesis. Front Genet. 2021;12:688241.
35. Pinson KI, Brennan J, Monkley S, Avery BJ, Skarnes WC. An LDL-receptor-related protein mediates Wnt signalling in mice. Nature. 2000;407(6803):535–8.
36. Yu M, Wong SW, Han D, Cai T. Genetic analysis: Wnt and other pathways in nonsyndromic tooth agenesis. Oral Dis. 2019;25(3):646–51.
37. Du R, et al. Identification of likely pathogenic and known variants in TSPEAR, LAMB3, BCOR, and WNT10A in four Turkish families with tooth agenesis. Hum Genet. 2018;137(9):689–703.
38. Chu KY, et al. Synergistic mutations of LRP6 and WNT10A in familial tooth agenesis. J Pers Med. 2021:11(11).
39. Song S, Zhao R, He H, Zhang J, Feng H, Lin L. WNT10A variants are associated with non-syndromic tooth agenesis in the general population. Hum Genet. 2014;133(1):117–24.
40. Yuan Q, Zhao M, Tandon B, Maili L, Liu X, Zhang A, Baugh EH, Tran T, Silva RM, Hecht JT, Swindell EC, Wagner DS, Letra A. Role of WNT10A in failure of tooth development in humans and zebrafish. Mol Genet Genomic Med. 2017;5(6):730–41.
41. van den Boogaard MJ, Créton M, Bronkhorst Y, van der Hout A, Hennekam E, Lindhout D, Cune M, Ploos van Amstel HK. Mutations in WNT10A are present in more than half of isolated hypodontia cases. J Med Genet. 2012;49(5):327–31.
42. Arzoo PS, Klar J, Bergendal B, Norderyd J, Dahl N. WNT10A mutations account for (1/4) of population-based isolated oligodontia and show phenotypic correlations. Am J Med Genet A. 2014;164A(2):353–9.
43. Fournier BP, Bruneau MH, Toupenay S, Kerner S, Berdal A, Cormier-Daire V, Hadj-Rabia S, Coudert AE, de la Dure-Molla M. Patterns of dental agenesis highlight the nature of the causative mutated genes. J Dent Res. 2018;97(12):1306–16.
44. Xu M, Horrell J, Snitow M, Cui J, Gochnauer H, Syrett CM, Kallish S, Seykora JT, Liu F, Gaillard D, Katz JP, Kaestner KH, Levin B, Mansfield C, Douglas JE, Cowart BJ, Tordoff M, Liu F, Zhu X, et al. WNT10A mutation causes ectodermal dysplasia by impairing progenitor cell proliferation and KLF4-mediated differentiation. Nat Commun. 2017;8:15397.
45. Yang J, Wang SK, Choi M, Reid BM, Hu Y, Lee YL, Herzog CR, Kim-Berman H, Lee M, Benke PJ, Kent Lloyd KC, Simmer JP, Hu JCC. Taurodontism, variations in tooth number, and misshapened crowns in Wnt10a null mice and human kindreds. Mol Genet Genomic Med. 2015;3(1):40–58.
46. Yu P, Yang W, Han D, Wang X, Guo S, Li J, Li F, Zhang X, Wong SW, Bai B, Liu Y, du J, Sun ZS, Shi S, Feng H, Cai T. Mutations in WNT10B are identified in individuals with oligodontia. Am J Hum Genet. 2016;99(1):195–201.
47. Magruder S, Carter E, Williams MA, English J, Akyalcin S, Letra A. Further evidence for the role of WNT10A, WNT10B and GREM2 as candidate genes for isolated tooth agenesis. Orthod Craniofac Res. 2018;21(4):258–63.
48. Kantaputra PN, Hutsadaloi A, Kaewgahya M, Intachai W, German R, Koparal M, Leethanakul C, Tolun A, Ketudat Cairns JR. WNT10B mutations associated with isolated dental anomalies. Clin Genet. 2018;93(5):992–9.
49. Jonsson L, Magnusson TE, Thordarson A, Jonsson T, Geller F, Feenstra B, Melbye M, Nohr EA, Vucic S, Dhamo B, Rivadeneira F, Ongkosuwito EM, Wolvius EB, Leslie EJ, Marazita ML, Howe BJ, Moreno Uribe LM, Alonso I, Santos M, et al. Rare and common variants conferring risk of tooth agenesis. J Dent Res. 2018;97(5):515–22.
50. Dinckan N, du R, Akdemir ZC, Bayram Y, Jhangiani SN, Doddapaneni H, Hu J, Muzny DM, Guven Y, Aktoren O, Kayserili H, Boerwinkle E, Gibbs RA, Posey JE, Lupski JR, Uyguner ZO, Letra A. A biallelic ANTXR1 variant expands the anthrax toxin receptor associated phenotype to tooth agenesis. Am J Med Genet A. 2018;176(4):1015–22.
51. McGrath JA, Pulkkinen L, Christiano AM, Leigh IM, Eady RAJ, Uitto J. Altered laminin 5 expression due to mutations in the gene encoding the beta 3 chain (LAMB3) in generalized atrophic benign epidermolysis bullosa. J Invest Dermatol. 1995;104(4):467–74.
52. Kivirikko S, McGrath JA, Baudoin C, Aberdam D, Ciatti S, Dunnill MGS, McMillan JR, Eady RAJ, Ortonne JP, Meneguzzi G, Ultto J, Christiano AM. A homozygous nonsense mutation in the alpha 3 chain gene of laminin 5 (LAMA3) in lethal (Herlitz) junctional epidermolysis bullosa. Hum Mol Genet. 1995;4(5):959–62.
53. Nakano A, Chao SC, Pulkkinen L, Murrell D, Bruckner-Tuderman L, Pfendner E, Uitto J. Laminin 5 mutations in junctional epidermolysis bullosa: molecular basis of Herlitz vs. non-Herlitz phenotypes. Hum Genet. 2002;110(1):41–51.
54. Smith CEL, Poulter JA, Antanaviciute A, Kirkham J, Brookes SJ, Inglehearn CF, Mighell AJ. Amelogenesis imperfecta; genes, proteins, and pathways. Front Physiol. 2017;8:435.
55. Delmaghani S, Aghaie A, Michalski N, Bonnet C, Weil D, Petit C. Defect in the gene encoding the EAR/EPTP domain-containing protein TSPEAR causes DFNB98 profound deafness. Hum Mol Genet. 2012;21(17):3835–44.
56. Peled A, Sarig O, Samuelov L, Bertolini M, Ziv L, Weissglas-Volkov D, Eskin-Schwartz M, Adase CA, Malchin N, Bochner R, Fainberg G, Goldberg I, Sugawara K, Baniel A, Tsuruta D, Luxenburg C, Adir N, Duverger O, Morasso M, et al. Mutations in TSPEAR, encoding a regulator of notch signaling, affect tooth and hair follicle morphogenesis. PLoS Genet. 2016;12(10):e1006369.

57. Song JS, Bae M, Kim JW. Novel TSPEAR mutations in non-syndromic oligodontia. Oral Dis. 2020;26(4):847–9.
58. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet. 2011;12(3):167–78.
59. Kantaputra PN, Kaewgahya M, Hatsadaloi A, Vogel P, Kawasaki K, Ohazama A, Ketudat Cairns JR. GREMLIN 2 Mutations and Dental Anomalies. J Dent Res. 2015;94(12):1646–52.
60. Karaca E, Posey JE, Coban Akdemir Z, Pehlivan D, Harel T, Jhangiani SN, Bayram Y, Song X, Bahrambeigi V, Yuregir OO, Bozdogan S, Yesil G, Isikay S, Muzny D, Gibbs RA, Lupski JR. Phenotypic expansion illuminates multilocus pathogenic variation. Genet Med. 2018;20(12):1528–37.
61. Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, Coban Akdemir ZH, Walkiewicz M, Bi W, Xiao R, Ding Y, Xia F, Beaudet AL, Muzny DM, Gibbs RA, Boerwinkle E, Eng CM, Sutton VR, Shaw CA, Plon SE, et al. Resolution of disease phenotypes resulting from multilocus genomic variation. N Engl J Med. 2017;376(1):21–31.
62. Zhou M, Zhang H, Camhi H, Seymen F, Koruyucu M, Kasimoglu Y, Kim JW, Kim-Berman H, Yuson NMR, Benke PJ, Wu Y, Wang F, Zhu Y, Simmer JP, Hu JCC. Analyses of oligodontia phenotypes and genetic etiologies. Int J Oral Sci. 2021;13(1):32.
63. Ott J, Kamatani Y, Lathrop M. Family-based designs for genome-wide association studies. Nat Rev Genet. 2011;12(7):465–74.
64. Zeng Y, Baugh E, Akyalcin S, Letra A. Functional effects of WNT10A rare variants associated with tooth agenesis. J Dent Res. 2021;100(3):302–9.