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The WNT10A Gene in Ectodermal Dysplasias and Selective Tooth Agenesis

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Mutations in the *WNT10A* gene were first detected in the rare syndrome odonto-onycho-dermal dysplasia (OODD, OMIM257980) but have now also been found to cause about 35–50% of selective tooth agenesis (STHAG4, OMIM150400), a common disorder that mostly affects the permanent dentition. In our random sample of tooth agenesis patients, 40% had at least one mutation in the *WNT10A* gene. The *WNT10A* Phe228Ile variant alone reached an allele frequency of 0.21 in the tooth agenesis cohort, about 10 times higher than the allele frequency reported in large SNP databases for Caucasian populations. Patients with bi-allelic *WNT10A* mutations have severe tooth agenesis while heterozygous individuals are either unaffected or have a mild phenotype. Mutations in the coding areas of the *WNT10B* gene, which is co-expressed with *WNT10A* during odontogenesis, and the *WNT6* gene which is located at the same chromosomal locus as *WNT10A* in humans, do not contribute to the tooth agenesis phenotype. © 2014 Wiley Periodicals, Inc.

Key words: *WNT10A*; gene mutations; selective tooth agenesis; ectodermal dysplasia; *WNT10B*; *WNT6*

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INTRODUCTION

Adaimy et al. [2007] performed autozygosity mapping in three consanguineous Lebanese families with the rare odonto-onycho-dermal-dysplasia syndrome (OODD, OMIM 257980), which had been previously characterized phenotypically in the same population [Fadhil et al., 1983]. They found that all affected family members were homozygous for the same nonsense mutation in the *WNT10A* gene leading to the phenotypic features of severe hypodontia, onychodysplasia, smooth tongue as well as palmar and plantar hyperhidrosis and hyperkeratosis. The phenotype of heterozygous family members was not recorded. Two years later Bohring et al. [2009] reported that *WNT10A* mutations are not restricted to the rare OODD syndrome but also found in other ectodermal dysplasia entities like the Schöpf–Schulz–Passarge syndrome (OMIM 224750) which additionally features eyelid cysts and predisposition to adnexal skin tumors. Bohring et al. also described a high prevalence of apparently non-syndromic tooth agenesis among their homozygous patients as well as mild, predominantly dental symptoms in about half of the heterozygous family members. Three further reports about the high prevalence of *WNT10A* mutations in ectodermal dysplasia syndromes and in non-syndromic tooth agenesis followed in Cluzeau et al. [2011], Van den Boogaard et al. [2012], and Plaisancié et al. [2013].

The expression of Wnt10a along with Wnt10b, Shh, Bmps2 and 4 and other developmentally active gene products during mouse odontogenesis had been investigated as early as 1998 by Dassule and McMahon. They detected Wnt10a at around embryonic Day 12 (E12) by in situ hybridization in the inner epithelial/enamel knot

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area of the tooth bud, where it was co-expressed with Wnt10b. Since Wnt10b expression was recognizable a little earlier and more prominently than Wnt10a expression, further investigations in this study focused on the Wnt10b molecule. At later stages of tooth development (E14–E18), Wnt10a can also be found in the mesenchymal preodontoblast layer where it contributes to or initiates odontoblast differentiation, possibly through the up-regulation of dentin sialophosphoprotein (Dspp) expression [Yamashiro et al., 2007].

WNT10A, which is located adjacent to *WNT6* at 2q35 in humans, is also active during the development of hair follicles and limbs, and in hematopoiesis. In adult tissues it is expressed in lymph nodes, blood, adrenal gland, prostate, testis, ovary, retina, brain, lung, and kidney; and may also play a role in several neoplastic disorders, notably ameloblastomas, keratocystic odontogenic tumors, lymphomas, and leukemias but is also found up-regulated in several cancers. Functional studies showed that Wnt10a activates the canonical wnt pathway and regulates mesenchymal cell fate in that it inhibits adipogenesis and stimulates osteoblastogenesis [Cawthorn et al., 2012].

The general role of canonical wnt signaling during tooth development has been explored in more detail by stabilization of β-catenin or depletion of apc, a positive and a negative regulator of canonical wnt signaling respectively. Both procedures constitutively activate wnt signaling in tooth bud epithelium leading to the formation of many accessory tooth buds sprouting from the original tooth anlage. The resulting supernumerary teeth are often small but otherwise completely normal [Järvinen et al., 2006; Wang et al., 2009]. Interestingly, the expression of Pax9 and Msx1, two normally essential transcription factors in tooth bud mesenchyme, are not required for the formation of these supernumerary teeth. Furthermore, the inactivation of the Wnt secretion facilitator Wls was recently shown to prevent intraepithelial Wnt signaling leading to tooth developmental arrest [Zhu et al., 2013].

When we sequenced the *WNT10A* gene (in 2012) in the random collection of tooth agenesis patients who participate in our "missing tooth" study, we also found a large number of WNT10A mutations in our samples confirming the importance of WNT10A in tooth development.

MATERIALS AND METHODS

Patient Recruitment

Tooth agenesis study participants were recruited via website and through collaboration with Drs. Alexandre Vieira (University of Pittsburgh) and Ophir Klein (University of California at San Francisco), following IRB approved protocols. People of all ages with any number of missing teeth except third molars were included, and only patients with overt ectodermal dysplasia symptoms were excluded. The final cohort consisted of 90 unrelated samples; half of them were from Caucasian Americans and the other half from patients from Turkey which are considered to be mostly of Mediterranean–European ancestry. Cheek swab or saliva samples were collected for the isolation of genomic DNA. The wild type sequence and allele frequencies of common variants in control populations were obtained from the NCBI SNP databases as well as the NHLBI Exome sequencing project (ESP).

DNA Extraction From Buccal Swabs

DNA extraction was performed with the Puregene Buccal Cell Kit (Qiagen, Germantown, MD). Since buccal swabs do not yield sufficient material for the analysis of multiple genes, the samples were amplified by Whole Genome Amplification (WGA) with the REPLI-g WGA system (Qiagen). Successful genome amplification was verified by gel electrophoresis of amplified samples together with a quantitation marker. DNA samples received from our collaborators were also amplified by WGA.

Polymerase Chain Reaction and Sequencing of Products

Exons of the *WNT10a*, *WNT10b*, and *WNT6* genes were PCR amplified with GoTaq reagents (Promega, Madison, WI) using a 96-well plate format for the 90 samples and the controls. Quality and quantity of PCR products was confirmed by gel-electrophoresis, followed by treatment with ExoSapIt (USB) and addition of the sequencing primers. Automated dideoxy chain terminator sequencing was done by GenScript, Piscataway, NJ.

Analysis of Sequencing Results

All sequences were visually inspected for heterozygous base changes and compared to the corresponding wild type sequences using the "BLAST" program. Once a nucleotide change was found, the SNP (single nucleotide polymorphism) database was consulted to determine if the SNP is a common polymorphism. For appropriate SNPs, the allele frequencies were compared between the experimental groups consisting of Caucasian and Turkish samples; and between the experimental and the Caucasian population control groups reported in NCBI and NHLBI databases.

RESULTS

Wnt10A But Neither *WNT6* Nor *WNT10B* Contribute to Tooth Agenesis

About 40% of our random group of tooth agenesis patients had at least one missense, nonsense or frameshift mutation in the *WNT10A* gene. The different mutations encountered in our patient samples are shown in Figure 1. Most common was the mono- or bi-allelic Phe228Ile mutation with a prevalence of about 31% and an allele frequency of 0.21 compared to an allele frequency of about 0.02 in large Caucasian control populations and only 0.007 in African American controls (Table I). The allele frequency of Phe228Ile was also calculated separately for our Caucasian (0.216) and Turkish participants (0.20) to exclude any influence that ethnic background differences could have had on the allele frequency.

Since Phe228Ile is so much more common in Caucasian tooth agenesis patients, it either is the causative factor or is closely linked to the causative mutation. Since *WNT10A* is located only a few kb telomeric of *WNT6* on chromosome 2q35 we included the latter in our analysis but did not find any mutations or polymorphisms that

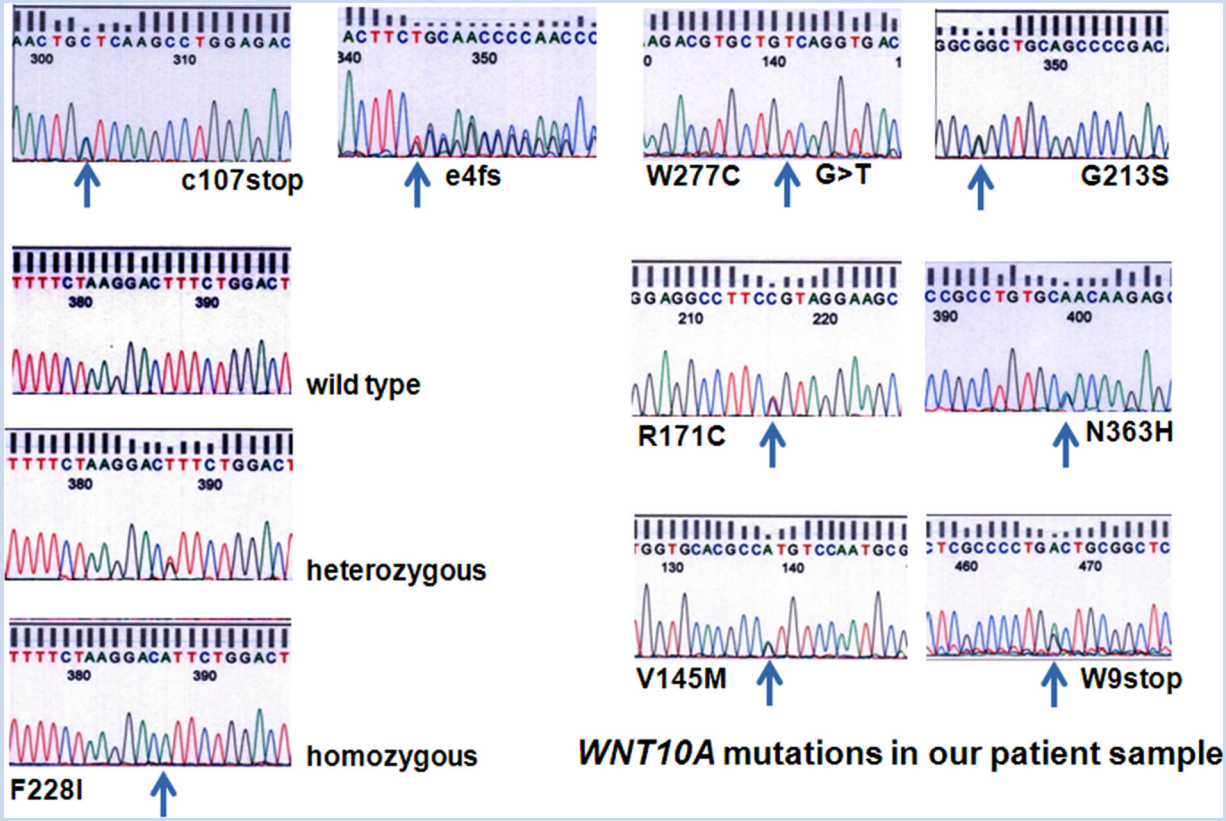

FIG. 1. ***WNT10A*** **mutations found in our tooth agenesis patient cohort. e4, exon4 of WNT10A; fs, frame shift; F, phenylalanine; I, isoleucine; W, tryptophan; N, asparagine; R, arginine; V, valine; M, methionine; H, histidine; C, cysteine; G, glycine; S, serine.**

were syntenic with the nucleotide change leading to *WNT10A* Phe228Ile. The common WNT6 variant Pro155Arg occurred in our tooth agenesis population at a frequency similar to normal control populations and only one of the 10 patients who had the *WNT6* Pro155Arg variant also had *WNT10A* Phe228Ile.

Since the WNT10A and WNT10B proteins are co-expressed in the inner dental epithelium of developing teeth and share 62 percent identity, it was conceivable that *WNT10B* mutations may also cause missing teeth. But sequencing of the whole coding area of the *WNT10B* gene did not reveal any nucleotide changes that could possibly be implicated in the tooth agenesis phenotype.

Phenotypes Associated With *WNT10A* Mutations

We did not receive any reports about missing primary teeth although some patients remembered having relatively small deciduous teeth. The number of missing teeth in the permanent dentition depended strongly on whether the affected individual was heterozygous or homozygous/compound heterozygous for WNT10A

TABLE I. The WNT10A Mutation Phe228Ile Is Unlikely to Be a Common Polymorphism Since Its Allele Frequency is About 10 Times Higher in the Tooth Agenesis Group Than It Is in Several Large Control Groups

Source	Ethnicity/study group	Number of detected alleles	F228I allele frequency
Our Tooth Agenesis (TA) study	Caucasians with TA	127 Phe and 33 Ile	0.206
NCBI SNP database	Caucasians	3,348 Phe and 67 Ile	0.02
NHLBI Exome Sequencing Project (ESP)	Caucasians	8,389 Phe and 209 Ile	0.024
NHLBI Exome Sequencing Project (ESP)	African Americans	4,370 Phe and 32 Ile	0.007
Bohring et al., 2009 Am J Hum Genet 85: 97	Caucasians without TA	396 Phe and 2 Ile	0.005

mutations. Heterozygous patients were missing up to 6 permanent teeth while homozygous patients were generally missing from 6 to 26, most often 16 teeth.

While all patients with bi-allelic mutations had oligodontia, many heterozygous relatives of study participants were not affected suggesting incomplete penetrance (Fig. 2). Syndromic ectodermal dysplasia manifestations were not encountered in our study population because they constituted exclusion criteria for participation in the study. One study participant however had a history of benign skin tumors, possibly bearing some resemblance to the Schöpf–Schulz–Passarge syndrome and another one reported mild heat intolerance.

The tooth agenesis pattern in heterozygous patients parallels that of common mild tooth agenesis with a predominant absence of lower second premolars and upper lateral incisors; but mandibular incisors are also frequently absent, occasionally even a canine or a first premolar. The pattern is similar to EDA pathway associated selective tooth agenesis in that the anterior teeth are more often affected [Tarpey et al., 2007; Mues et al., 2009, 2010]. Patients with homozygous *WNT10a* mutations have also posterior tooth agenesis similar to syndromic EDA pathway mutation phenotypes [Lexner et al., 2006].

DISCUSSION

Traditionally we distinguished between syndromic and non-syndromic tooth agenesis. Syndromic tooth agenesis was most often encountered as part of an ectodermal dysplasia phenotype while nonsyndromic tooth agenesis, also called isolated or selective tooth agenesis/hypodontia (STHAG1-6 and X1 in OMIM), should only affect the dentition without any systemic manifestations. Inherent in the syndromic versus nonsyndromic classification was the assumption that the two disorders had fundamentally different genetic causations.

However, the more we learn about the pathogenesis of tooth agenesis the more we realize that there is an extensive overlap between the genetic basis of syndromic and nonsyndromic forms of tooth agenesis [Nieminen, 2009] and the phenotypic distinction may not have been helpful for the search of additional TA genes. From a genetic point of view, nonsyndromic and syndromic tooth agenesis are often caused by the same genes, but the development of some teeth seems to be more sensitive to gene dosage and thus are the first organs to be affected, while other ectodermal appendices may still form normally [Mues et al., 2010].

The distinction between syndromic and nonsyndromic TA may also be problematic with respect to the new, biologically based diagnostic and therapeutic approaches for which dental professionals who are traditionally the only health care providers for nonsyndromic TA patients, may still be little prepared. Classification of the STHAGs as ectodermal dysplasia entities may therefore be desirable from a clinical point of view.

The high prevalence of *WNT10A* mutations is truly astounding, especially since this gene is hardly ever mentioned in the extensive literature about the molecular genetics of tooth development. Even

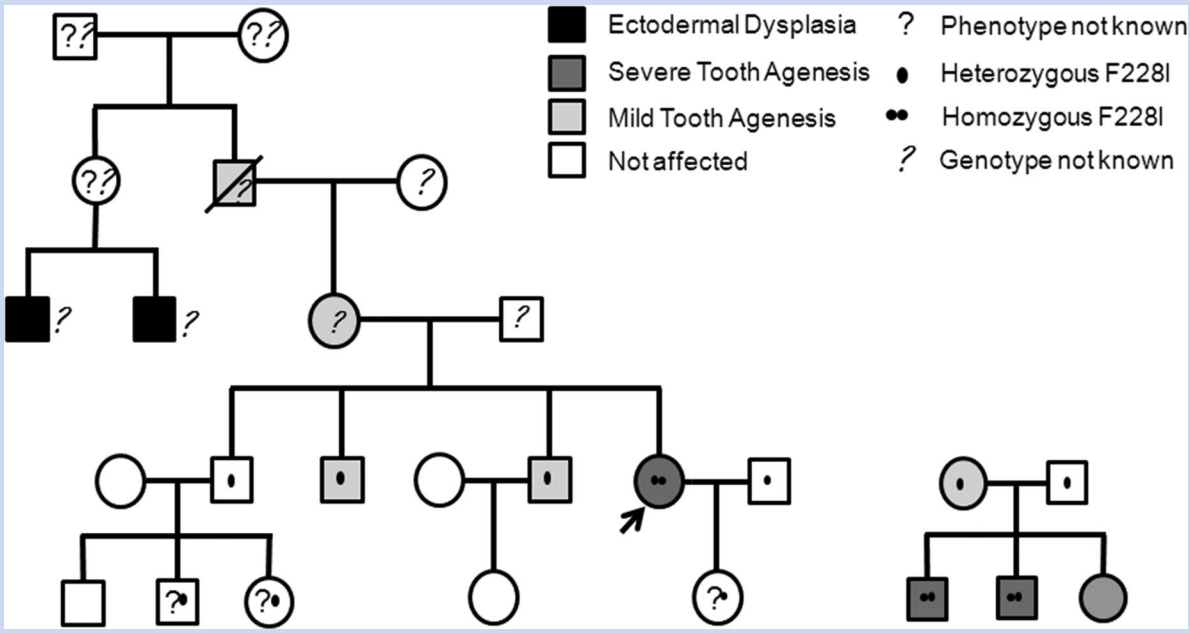

FIG. 2. Two pedigrees showing incomplete penetrance and variability of the phenotype depending on the number of WNT10A alleles affected. The fifth generation of the pedigree on the left is still too young for phenotype evaluation. An additional feature in this family is that the husband of the homozygous index patient (arrow) also carries the F228I variant, a 1 in 50 chance. The pedigree on the right also shows that two different tooth agenesis genes may contribute to the phenotypes, because the daughter has several missing teeth but no F228I mutation while her two homozygous brothers have severe tooth agenesis. F, phenylalanine; I, isoleucine.

more surprising is the large number of Caucasian tooth agenesis patients with one particular mutation, WNT10A Phe228Ile. Pathogenic mutations are usually lost from the gene pool of a population unless they have some kind of survival advantage like for example heterozygous mutations in the hemoglobin genes, which are of benefit in areas with high malaria incidence. The survival advantage is often lost in individuals with homozygous mutations like in sickle cell disease, but since homozygously affected individuals are quite rare, the mutation has an overall positive effect on population growth. It will certainly be interesting to find a cause for the high prevalence of *WNT10A* Phe228Ile mutations.

Interesting is also that the tooth agenesis pattern of patients with *WNT10A* mutations resembles that of *EDA* pathway associated hypodontia. Both WNT and EDA pathways are known to operate predominantly in the epithelial layer of the developing tooth and repeated interactions between the two pathways have been observed during ectodermal appendage formation and in vitro [Laurikkala et al., 2001; Durmowicz et al., 2002; Zhang et al., 2009]. It is therefore possible that the new, biological replacement therapies for EDA pathway mutation associated disorders may also be of value in the much more common WNT10A disorder. It would certainly be worth testing this possibility because EDA replacement therapies have shown great efficacy in ameliorating disease symptoms in animals and are currently tested in humans, while the generation of WNT10A specific therapeutics would be quite complicated not only from a chemical engineering point of view but also because of the lack of a one to one correspondence of ligands and receptors in the WNT pathway, potentially leading to severe adverse effects, and even more importantly, a lack of a wnt10a deficient, diphyodont animal model for the testing of these therapeutics.

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