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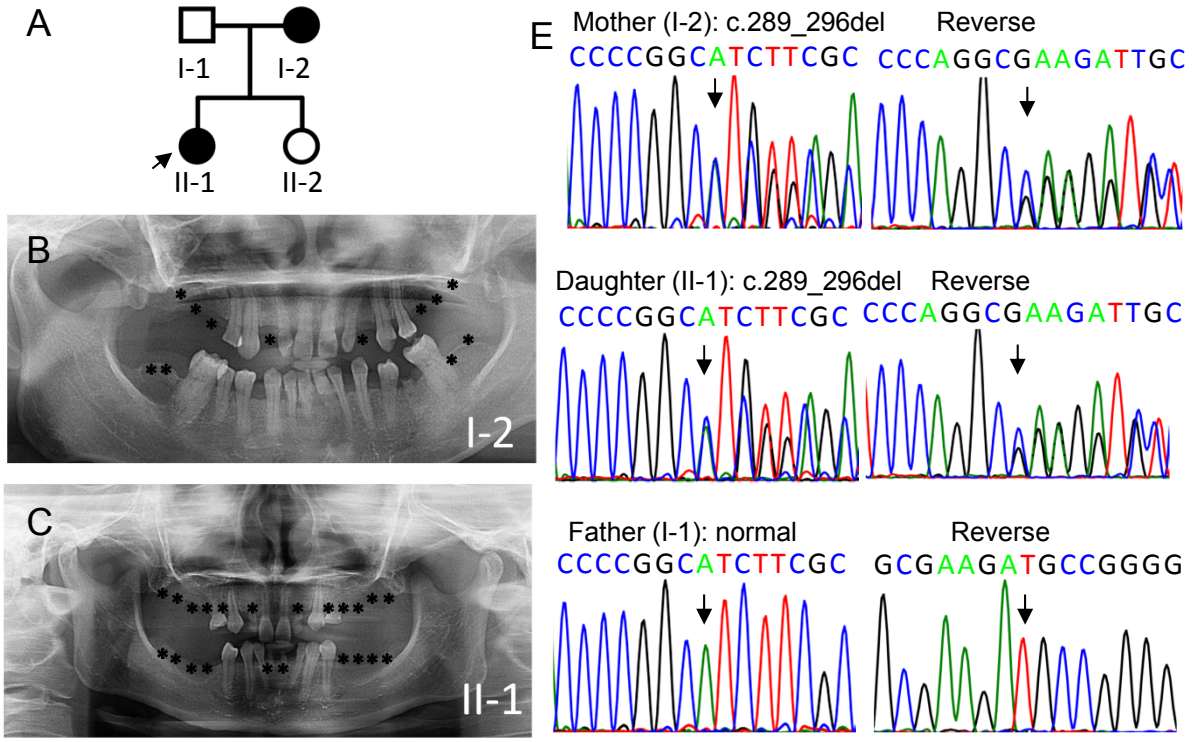
Supplemental Data

Mutations in *WNT10B* Are Identified in Individuals with Oligodontia

Ping Yu, Wenli Yang, Dong Han, Xi Wang, Sen Guo, Jinchen Li, Fang Li, Xiaoxia Zhang, Sing-Wai Wong, Baojing Bai, Yao Liu, Jie Du, Zhong Sheng Sun, Songtao Shi, Hailan Feng, and Tao Cai



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		Left								Right							
Subject	Teeth	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
I-2	Upper	*	*	*			*	▼			▼	*			*	*	*
	Lower	*	*													*	*
II-1	Upper	*	*	*	*	*		*			*		*	*	*	*	*
	Lower	*	*	*	*				*	*				*	*	*	*

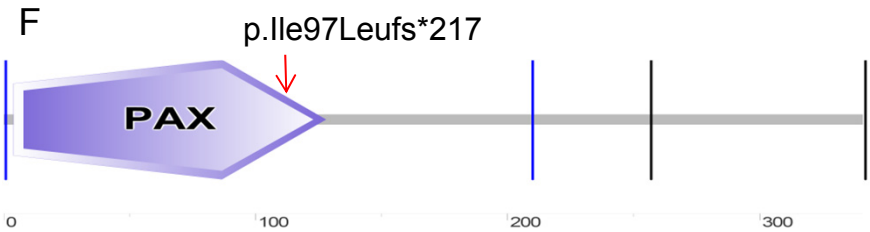

Figure S1. A frameshift mutation of *PAX9* identified in the individuals with inherited oligodontia (STHAG3).

(A) The ZZWX-1 pedigree; (B-C) Panoramic radiographs of dentitions with tooth agenesis of two individuals. Missing teeth (23/32 molars and 6/24 incisors) are denoted with asterisks (*). (D) Schematic presentation of congenitally missing teeth of two individuals, which are filled by (*); cone-shaped tooth is represented by (▼); (E) Sanger sequencing shows the mutation c.289_296del. (F) The p.Ile97Leufs*217mutation is indicated on the schematic representation of the PAX9 protein.

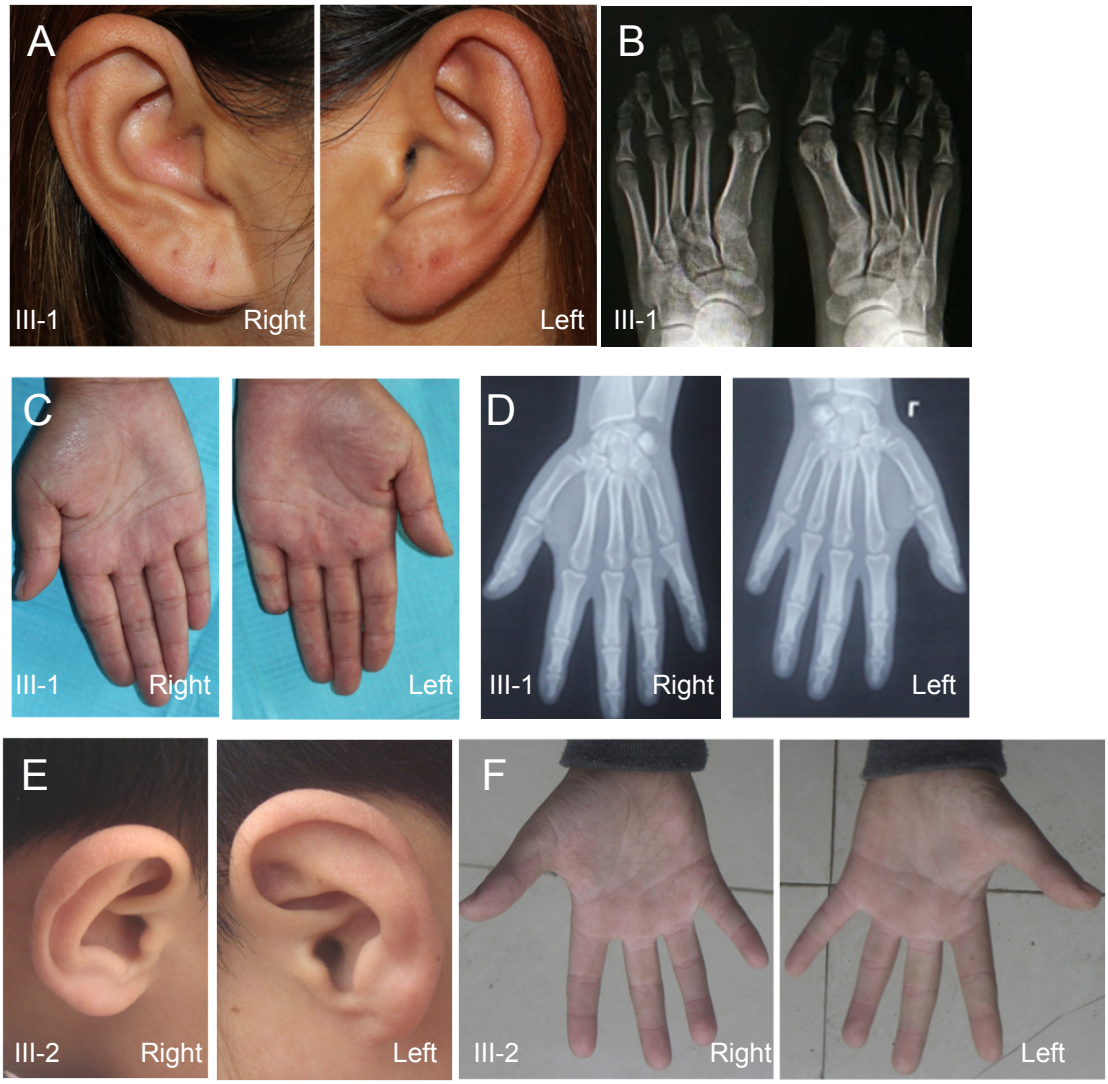

Figure S2. Additional clinical information of the affected individual III-1 and III-2. No obvious developmental abnormalities are seen in ear, hand, and foot. The 5th finger of left hand in individual III-1 (panel C and D) is shorter than normal due to an accident injury.

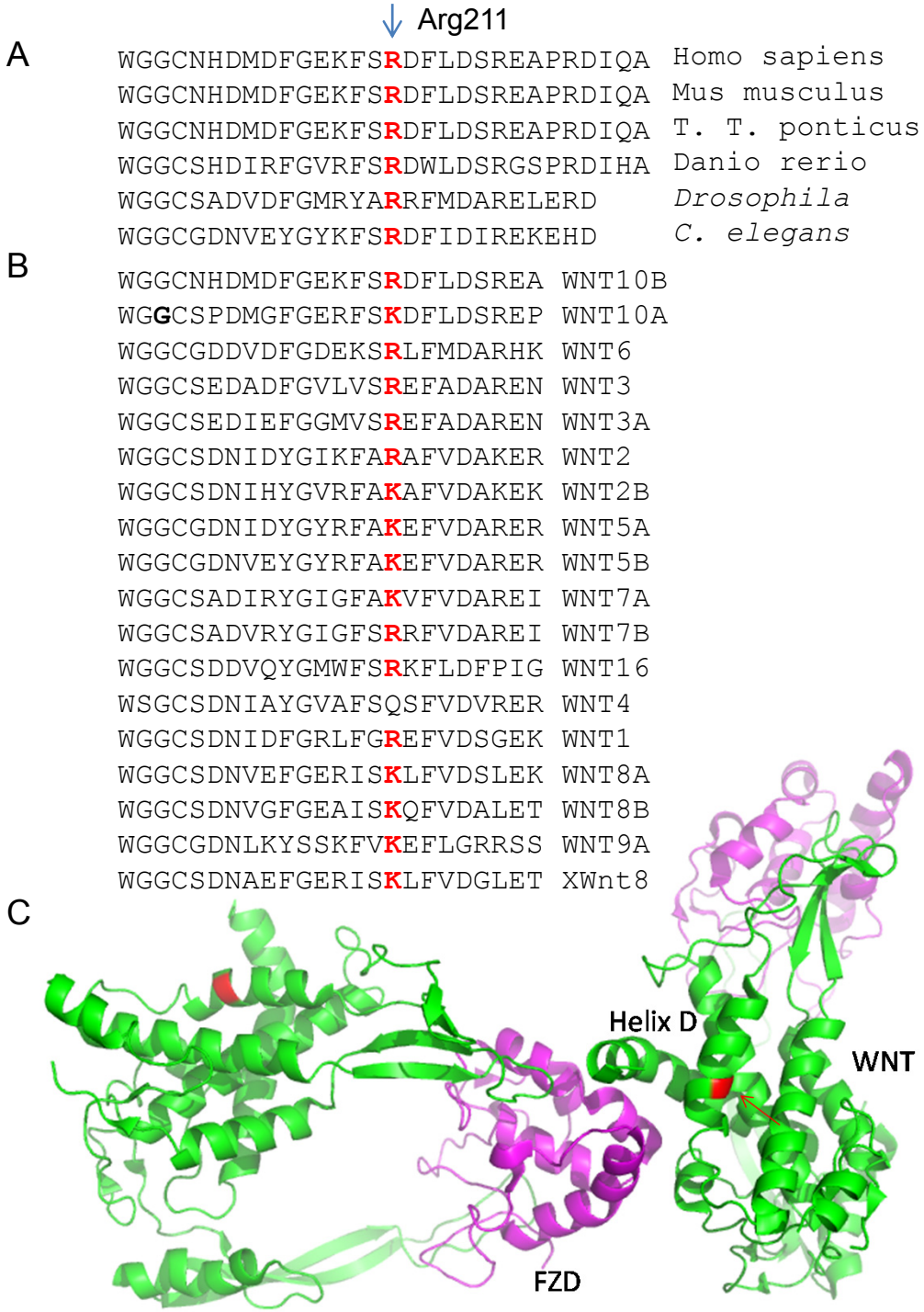

Figure S3. Multiple sequence alignment and 3D structural analysis.
(A) Sequence alignment of Arg211-containing region of WNT10B in different species.
(B) The Arg211-containing region in 18 different Wnt family members.
(C) 3D structural analysis of WNT10B paralog in Xenopus. The residue Arg211 (in red) is positioned in the helix D, interacting with cysteine-rich domain of Frizzled-8 (FZD). The model is established using PyMOL. PDB ID code, 4F0A (http://www.pdb.org).

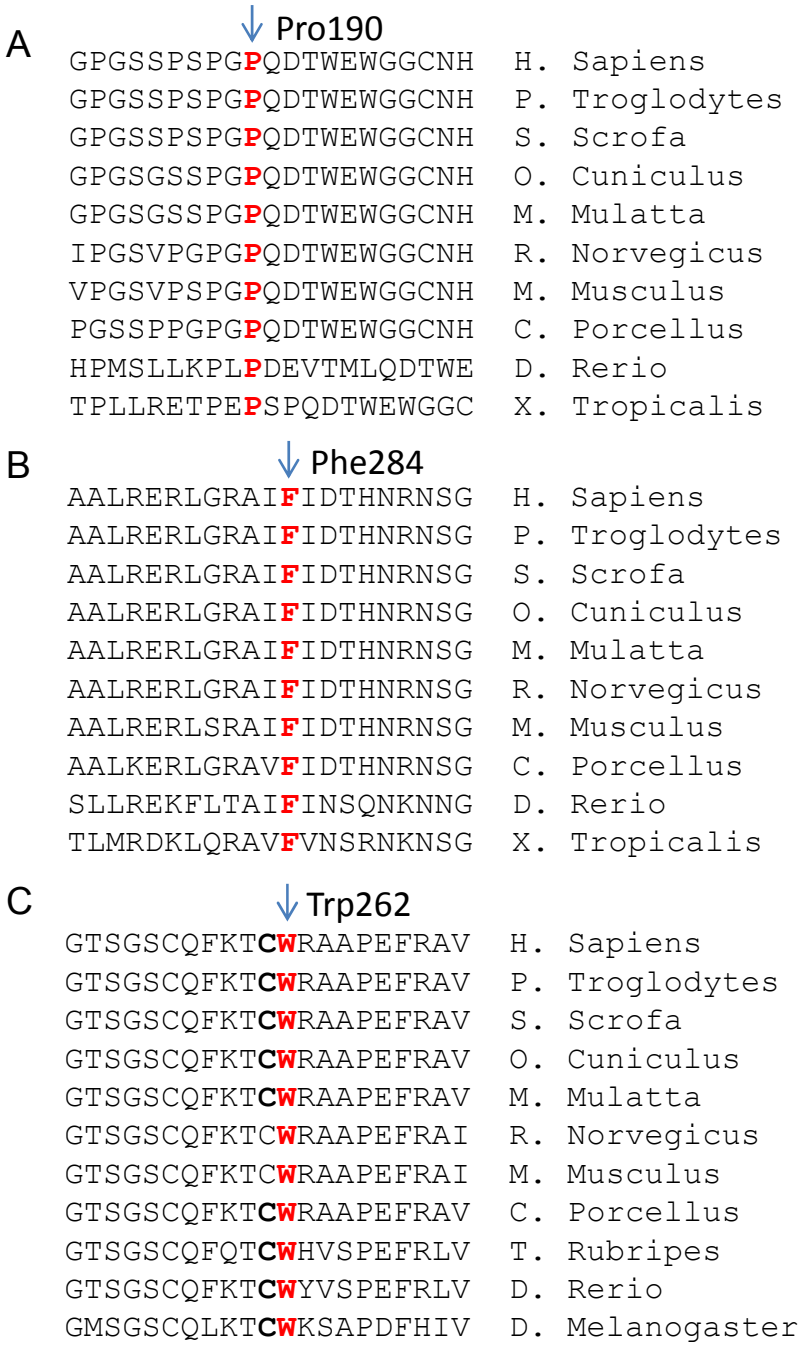

Figure S4. Multiple sequence alignment. The residue Pro190-, Phe284-, and Trp262-containing regions of WNT10B are compared in 10 different species as indicated.

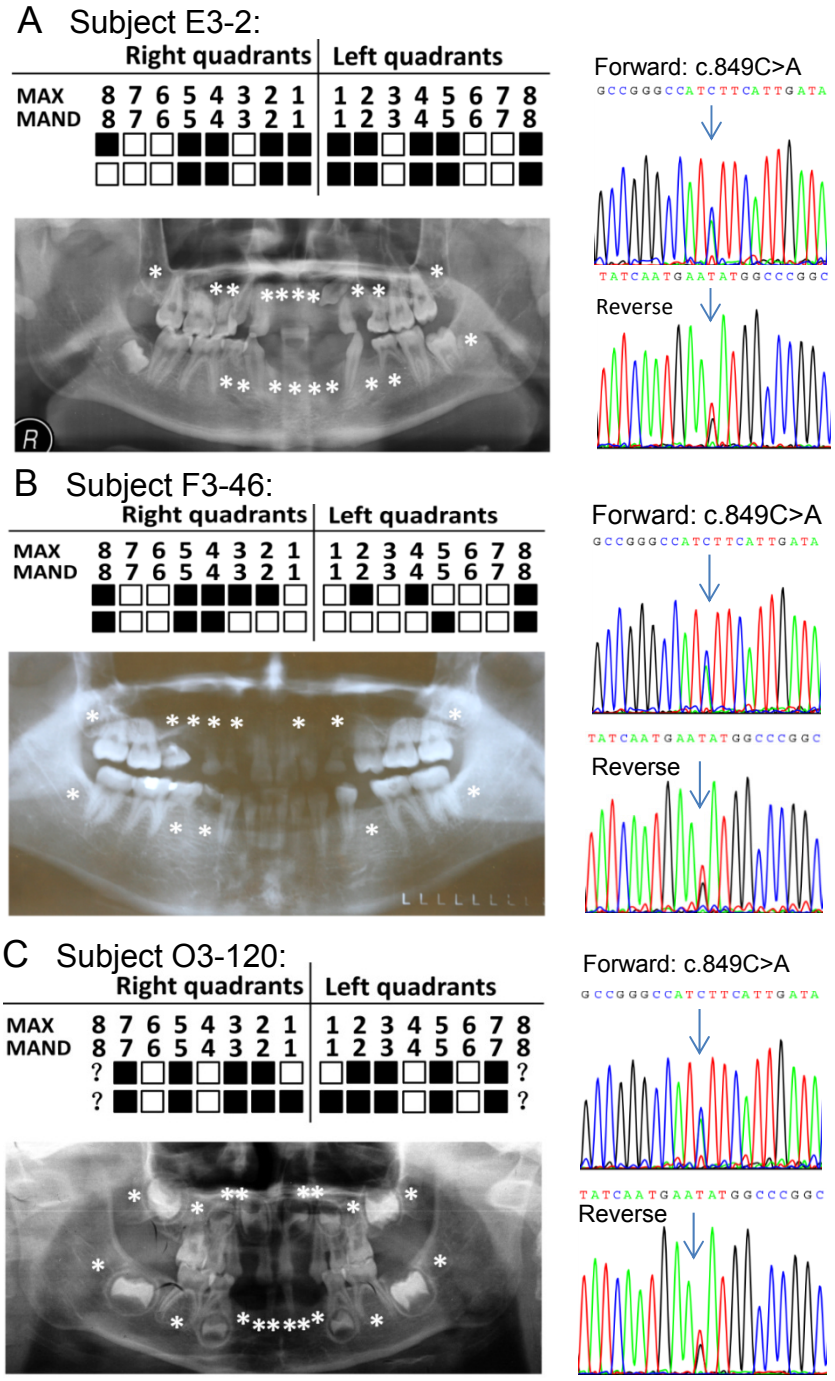

Figure S5. The c.849C>A variant of *WNT10B* is associated with oligodontia. Panoramic radiographs (left panels) and the variants in Sanger chromatograms (right panels) are shown for subject E3-2 (A), F3-46 (B), and O3-120 (C) respectively. Data of the deceased subject X0-401 with the same variant is not shown. Also, MAF of this variant in ExAc (49/119396 = 0.0004104) is markedly lower than the MAF (4/145 = 0.0275) in the cohort of affected individuals (P = 1.39569E-27). Positions of each of missing teeth are indicated by filled box in schematic maxillary (MAX) and lower mandible (MAND) locations.

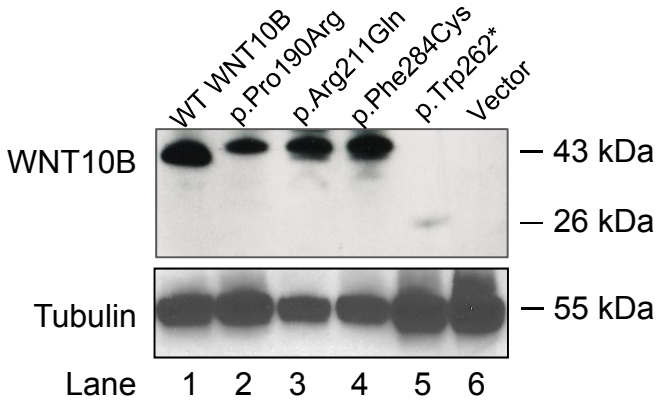

Figure S6. Expression of WT and mutant WNT10B in COS-7 cells.

COS-7 cells were transfected with each of the plasmids as indicated in the figure by Lipofectamine 2000 (Invitrogen). Stable transfectants were selected in medium containing G418 (500 μg/ml) for four weeks. Drug resistant cells were lysed with M-PER Mammalian Protein Extraction Reagent (Pierce Biotechnology) and then prepared (30 μg per lane) for Western blotting (ECL system, GE Healthcare Life Sciences) using anti-WNT10B antibody (H-70) (Santa Cruz Biotechnology) at 1:500 dilution. Tubulin level is also shown as a loading control. A 43-kDa of main band is detected as predicted in the WT and each of the three missense mutation constructs transfected cells. In contrast, the cells transfected with p.Trp262*-containing plasmid only produced a small amount of protein with lower molecular weight, suggesting that the expression of the nonsense mutant is unstable.

Subject	II-1	III-1	III-2	N2-107	H3-63	O3-113
Family history	Yes	Yes	Yes	No	No	No
Gender	Female	Female	Male	Female	Female	Male
Age at first visit	~40	21	11	21	20	25
Sparse hair	No	Yes	Yes	No	No	No
Hair pigment	Black	Light brown	Light brown	Black	Brown	Black
Eyebrows	Sparse	Sparse	Sparse	Normal	Normal	Normal
Eyelid cysts	No	No	No	No	No	No
Vision	N.E.	Astigmatism (right eye)	Myopia (right eye)	Myopia (both)	Myopia and astigmatism (both eyes)	Normal
Lacrimal duct defect	No	No	No	No	No	No
Hearing ability	No	No	Right ear reduced	No	No	No
Shape of tooth	4 cone-shaped	Small	Small, cone-shaped		11, 21 shovel-shaped	11, 21 shovel-shaped; 12 22 cone-shaped
Missing teeth	24	12	15	16	9	10
Dry skin	No	Yes	Yes	No	No	No
Hyperhidrosis of plantar hands/feet	No	No	No	No	No	No
Dystrophic fingernails	No	No	No	No	No	Yes
Hyperkeratosis of plantar hands/feet	No	No	No	No	No	No
Mutation	p.Arg211Gln	p.Arg211Gln	p.Arg211Gln	p.Pro190Arg	p.Trp262*	p.Phe284Cys

Table S1. **Clinical Manifestations in Individuals with Oligodontia.**

Number of missing teeth (the third molar tooth is not included); N.E., not examined;

WNT10B	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product (bp)
Mutation screening primers:			
Exon 2 & 3	CCTGAACCCGCATCAAGTCT	GCCGCGAAACCATCCCTT	607
Exon 4	CTCAGCTGCCTGTCAACCTTA	TGACTTGCTGATGGTGAGTGT	547
Exon 5	ACTGCAATGTCCTTTCTGTTCTG	GCTTCCAGGGACCAAGAGTG	714
Primers for mutagenesis:			
c.569C>A	GGCTCAAGCCCCAGCCCTGGCCGCC AGGACACATGGGAATGGGG	CCCCATTCCCATGTGTCCTGGCGG CCAGGGCTGGGGCTTGAGCC	Mutated
c.632G>A	GACTTTGGAGAGAAGTTCTCTCAGG ATTTCTTGGATTCCAGGGAA	TTCCCTGGAATCCAAGAAATCCTG AGAGAACTTCTCTCCAAAGTC	Mutated
c.786G>A	CAGCTGCCAGTTCAAGACATGCTGA AGGGCGGCCCCAGAGTTCCG	CGGAACTCTGGGGCCGCCCTTCAG CATGTCTTGAACTGGCAGCTG	Mutated
c.851T>G	GAGCGGCTGGGCCGGGCCATCTGCA TTGATACCCACAACCGCAAT	ATTGCGGTTGTGGGTATCAATGCA GATGGCCCGGCCCAGCCGCTC	Mutated

Table S2. Primers used for PCR amplification of human *WNT10B* and mutagenesis.

Note: The exon 1 of *WNT10B* is a non-coding exon, which was not included in the screening.

Subject	Genetic form	Mutation coding seq	Mutation in protein	Protein domain	Phenotypes	Ref.
	Heterozygous	-607G>C	No	Promoter	Obesity	[1]
	Heterozygous	c.767G>A	p.Cys256Tyr	WNT domain	Obesity	[2]
1985223	Heterozygous	c.868C>T	p.Arg290Cys	WNT domain	No split-hand/foot; No teeth agenesis	This study
HCM914	Heterozygous	c.901C>T	p.Pro301Ser	WNT domain	No split-hand/foot; No teeth agenesis	This study
	Homozygous recessive	c.986C>G	p.Thr329Arg	WNT domain	Split-hand/foot; teeth abnormalities not observed	[3]
1872988	Heterozygous	c.995G>A	p.Arg332Gln	WNT domain	No split-hand/foot; No teeth agenesis	This study
	Homozygous recessive	c.994C>T	p.Arg332Trp	WNT domain	Split-hand/foot; Teeth not mentioned	[4]
	Homozygous recessive	c.1165_1168 delAAGT	p.Lys388Glufs *36	Influencing binding with Fzd8	Split-hand/foot; teeth abnormalities not observed	[5]
	Homozygous recessive	c.300_306 dupAGGGCGG	ND	Predicted to be LoF	Split-hand/foot; teeth abnormalities not observed	[5]
	Homozygous recessive	c.458_461 dupAGCA	ND	Predicted to be LoF	Split-hand/foot; teeth abnormalities not observed	[6]

Table S3. Summary of reported variants in *WNT10B* related to individuals with disorders.

Note: Mutations of *WNT10B* in individuals with split-hand/foot (SHFM6, OMIM 225300). LoF, loss-of-function; ND, not determined. Also, heterozygous missense mutations or polymorphic variants in *WNT10B* were linked to obesity. However, this phenotype was not observed in the present study. Furthermore, three individuals in our inhouse database, who did not show teeth agenesis (ID #1985223, HCM914, and 1872988), were found to carry heterozygous rare missense variants in *WNT10B*, suggesting the *C*-terminal variants of the gene are not associated with teeth agenesis.

References

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