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DATA REPORT

WNT10A variants isolated from Japanese patients with congenital tooth agenesis

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It has been reported that dozens of WNT10A variants are associated with human isolated tooth agenesis, however, little is known about the precise phenotypes. In 50 Japanese patients with severe congenital tooth agenesis, we identified 11 patients with WNT10A variants. Comparing phenotypes between the tooth agenesis patients carrying the wild-type and variants of *WNT10A*, we revealed that the development of lateral incisors is relatively susceptive to insufficiency of WNT/β-catenin signaling.

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Congenital tooth agenesis is one of the most common anomalies in human development, which is highly heritable and observed in the general population with racial/ethnic difference in prevalence of 0.08–0.16%.[1–3] It has been identified some homeodomain transcription factor cording genes as genetic causes of isolated tooth agenesis, such as *MSX1* [OMIM 142983],[4] and *PAX9* [OMIM 167416].[5] In addition to these homeoprotein transcription factors, haploinsufficiency of WNT signaling-related molecules including wingless-type MMTV integration site family, member 10A (*WNT10A*) [OMIM150400],[6–8] the axis inhibitor 2 (*AXIN2*) [OMIM 608015],[9] and low-density lipoprotein receptor-related protein 6 (*LRP6*) [OMIM 616724] cause human tooth agenesis.[10] AXIN2 is a negative regulator of the WNT signaling, which plays a pivotal role in the canonical WNT signal transduction pathway in the absence of a WNT ligands.[11] LRP6 can interact with the WNT receptor Frizzled, and function as a component of WNT receptor complex.[12] These findings indicate that WNT signal, which inhibits the β-catenin degradation and activates nuclear transcription factors LEF/TCF, is crucial for human tooth development.

Recent studies revealed that heterozygous WNT10A variants are causative candidates of autosomal-dominant selective tooth agenesis (STHAG4) [OMIM150400] including maxillary lateral incisor agenesis,[6,13,14] as well as other autosomal recessive ectodermal dysplasia syndromes.[15] However, little is known about the mechanism underlying the broad spectrum in phenotypes of haploinsufficiency in WNT10A. Here we report allelic frequency of WNT10A variants in Japanese tooth agenesis patients, and describe phenotypic features peculiar to tooth agenesis related to WNT10A gene variants.

Fifty unrelated subjects with apparent isolated tooth agenesis of four or more permanent teeth visited the Department of Maxillofacial Surgery, Aichi-Gakuin University School of Dentistry, the Department of Oral and Maxillofacial Surgery, Toyota Memorial Hospital, Toyota, Japan, and the Department of Dentistry and Oral Surgery, Aichi Children's Health and Medical Center, Obu, Japan. Clinical examination by the dentist, dental panoramic radiographs of the patients, intraoral photographs were used to assess missing tooth phenotypes (summarized in Figure 1a). Possible symptoms originating from ectodermal structures, including sweat glands, skin, hair and nails, were assessed using a standardized questionnaire. The control group consisted of 50 healthy individuals without dental agenesis or any dysmorphic features or evident abnormalities of teeth, nails, skin, hair and sweat glands, who was recruited for the purpose of the current study. All study participants were Japanese origin.

A blood sample was obtained with informed consent following Institutional Review Board (IRB) approval at Aichi-Gakuin University, Toyota Memorial Hospital, Aichi Children's Health and Medical Center, and the Institute for Developmental Research. Genomic DNA was extracted from saliva, using Oragene Saliva Collection Kits (DNA Genotek, Inc., Kanata, ON, Canada). Mutational analysis was performed with fragments covering the entire coding region of the *WNT10A* gene, which was amplified from the genomic DNA of the patients using specific primers.

Direct sequence analysis of *WNT10A* (NM_025216.2) coding regions in 50 Japanese patients with tooth agenesis identified four different missense mutations in 11 unrelated patients (Table 1). Thus the frequency of WNT10A variant in unrelated probands was 22% (11/50). All the missense variants with the predicted protein change (c.143G>A, p.Arg48His; c.511C>T, p.Arg171Cys; c.637G>A, p.Gly213Ser and: c.889G>A p.Ala297Thr), were identified in the heterozygous state in our study. The variants, p.Arg48His has not been reported as causative or predisposing variant of tooth agenesis to date. We detected no WNT10A variants in the Japanese normal population, which consists of carefully selected 50 individuals without any missing tooth including wisdom teeth as the negative control for this study.

The group of 50 unrelated Japanese patients (male, 21; female, 29) had an average number of 11.0 missing teeth (8.9; excluding wisdom tooth loss; Figure 1a) without any overt ectodermal

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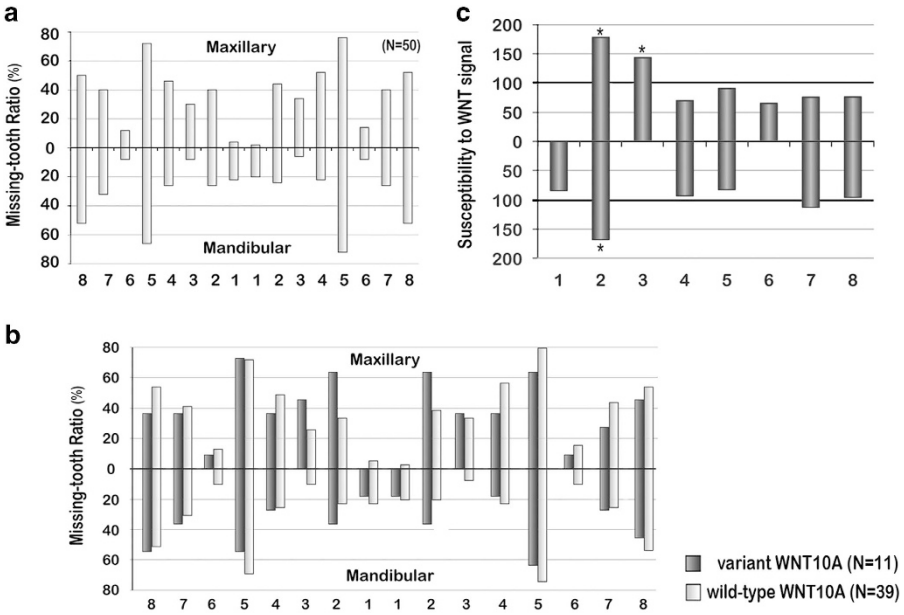

Figure 1. (**a**) The ratio of missing teeth in each maxillary and mandibular position of all the 50 patients with tooth agenesis in this study (male, $N=21$; female, $N=29$). (**b**) The ratio of missing teeth in each maxillary and mandibular position of the tooth agenesis patients with (dark gray columns; $N=11$), and without miss sense variant of WNT10A gene (light gray columns; $N=39$). (**c**) The susceptibility of each tooth to the WNT signal defect. These values are defined as the quotient of the each missing tooth ratio of the patients with WNT10A variant by that without WNT10A variant. Lateral incisors of maxilla and mandible, and maxillary canine are sensitive to WNT signaling. We combined the left and right teeth, since no statistically significant difference between the left and right sides.

dysplasia symptoms. The group of 11 individuals with WNT10A variants consisted of 4 males and 7 females (Table 1). They had a total of 112 missing teeth (92 excluding wisdom tooth) resulting in a mean of 10.2 missing teeth (8.4; excluding wisdom tooth loss). Dentograms on all 11 patients with WNT10A mutations showed a total of 64 absent teeth (55 excluding wisdom tooth loss) in the upper jaw, and 48 (37 excluding wisdom tooth loss) absent teeth in the lower jaw, resulting in a mean of 5.8 and 4.4 (5.0 and 3.4 excluding wisdom tooth loss), respectively (Table 1). The comparison of missing tooth phenotypes with and without WNT10A variants were summarized in the Figure 1b.

Mutations in the *WNT10A* gene are the most frequently found mutations in patients with nonsyndromic tooth agenesis in several populations studied to date. However, in our current study, the prevalence of *WNT10A* variants in the Japanese tooth agenesis patients with more than three-tooth loss was lower than that of other reports studying in other ethnic population.[6,7,13,14,16–18] Most of the prevalences of WNT10A variants were ranged about 30–50% in patients among different ethnic groups. In the East Asia, WNT10A variants were detected in 51.6% (16/31) of the Chinese patients with four or more missing teeth, while 15.8% of patients with one-three missing teeth.[6] In the current study, we identified 22% (11/50) of patients with missense mutation in *WNT10A* gene in 50 Japanese patients lacking at least 4 teeth excluding wisdom teeth, and 0% in the healthy 50 Japanese control.

In the Chinese patients with at least 4 missing tooth, the variant c.511C>T (p.R171C) and c.637G>A (p.G213S) *WNT10A* was frequently detected (22.6%; 7/31 and 25.8%; 8/31).[6] In our current Japanese case study, while the variant, c.511C>T was detected only in one patient, the other variant, c.637G>A, was also dominantly present in the tooth agenesis patients with *WNT10A* gene variants (16%; 8/50). In addition, although the c.511C>T and c.637G>A *WNT10A* variants were detected in normal Chinese control (2.0% and 2.7%, respectively),[6] none of the healthy Japanese carrying these variants were identified in our control samples. According to the Japanese genetic variation database, HGDV, the population ratios of c.511C>T and c.637G>A variant are 3.1% (72/2340) and 3.0% (70/2318), and the allelic frequencies are 0.0147, and 0.0149, respectively, indicating that the population frequency of p.Gly213Ser variant is concentrated in the tooth agenesis patients (16.0%; 8/50) than general Japanese population (3.0%; 70/2318) as the pervious report with the Chinese population.6 Interestingly, compare to other regions, the frequency of p.Gly213Ser in the East Asia is 373.8 times. Beside these two variants, the p.Arg48His and p.Ala297Thr variants are rare (Supplementary Table S1). The p.Arg48His, p.Arg171Cys and p.Gly213Ser variants, but not p.Ala297Thr variant, are listed in the Japanese genetic variation database HGDV (http://www.hgvd.genome.med.kyoto-u.ac.jp/) and iJGVD (https://ijgvd.megabank.tohoku.ac.jp/about/), however, no information is attached in the database about tooth phenotypes of these individuals with the WNT10A variant. Generally, there is concern that tooth agenesis phenotypes were overlooked in database samples, since nonsyndromic tooth agenesis is rather mild anomaly than other serious congenital disorders.

Though there are minor differences, which may arise from ethnic background, our findings support the notion that the common variants of *WNT10A* such as c.511C>T (p.Arg171Cys) and c.637G>A (p.Gly213Ser) are predisposing genetic factors for causation,[6] and other unknown genetic or environmental factors are needed to express developmental tooth anomaly in human.

The missing teeth species in Japanese severe tooth agenesis patients (at least 4 missing-tooth) with *WNT10A* gene variants varies from those in Chinese patients.[6] Especially, the ratio of the missing in the maxillary lateral incisor is higher in Japanese patients (63.6%, Figure 1b) than those in Chinese with severe tooth agenesis (31.3%). By contrast with the missing tooth of Japanese patients with wild-type WNT10A and those with WNT10A variant, lateral incisors of the mandibular and maxilla, and the canine in maxilla are relatively sensitive to insufficiency of WNT/β-catenin signaling (Figure 1c). This finding is partly supported by a previous study on Polish patients with the maxillary lateral incisor agenesis.[8] However, the phenotype may

Table 1. Tooth phynotypes of Japanese patients with WNT10A variant (OMIN #150400, STHAG4; tooth agenesis, selective, 4)

Case ID	Hered.		Right Tooth phenotype Left																Gen.	# miss. (- w. teeth)	Variant ID	Mutation	Base change Protein alteration	ClinVar	Polyphen 2	SIFT
		U	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8								
		L	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8								
190	de novo	U				●									●	●		●	F	6 (5)	rs375577530	NC_000002.11:g.219746912G>A	NM_025216.2:c.143G>A NP_079492.2:p.Arg48His	NA	0.102	delet.
		L				●									●											
31	inherited	U				●		●								●			M	7 (5)	rs116998555	NC_000002.11:g.219754840C>T	NM_025216.2:c.511C>T, NP_079492.2:p.Arg171Cys	Conflict	0.999	delet.
		L	●			●									●			●								
1	de novo	U				●		●	●			●	●		●				F	9 (8)						
		L	●						●			●														
61	de novo	U	●			●		●	●			●	●	●	●			●	M	16 (12)						
		L	●			●		●							●			●								
79	inherited	U	●		●				●			●						●	M	9 (5)						
		L	●		●													●								
90	inherited	U				●		●	●			●	●		●				F	9 (9)						
		L								●	●															
142	de novo	U	●			●		●							●			●	F	12 (8)	rs147680216	NC_000002.11:g.219754966G>A	NM_025216.2:c.637G>A NP_079492.2:p.Gly213Ser	Conflict	1	delet.
		L	●			●		●							●			●								
189	de novo	U	●		●												●	●	F	9 (5)						
		L	●		●												●	●								
195	inherited	U		●		●			●			●			●				F	12 (12)						
		L		●				●	●			●			●		●									
205	inherited	U		●		●		●	●			●	●	●	●		●		F	15 (15)						
		L		●					●			●			●		●									
172	de novo	U							●			●							M	8 (8)	rs530717943	NC_000002.11:g.219757628G>A	NM_025216.2:c.889G>A NP_079492.2:p.Ala297Thr	NA	1	delet.
		L				●			●	●	●	●			●											

●–Missing tooth. Abbreviations: delet, deleterious; F, female; Gen, gender; Hered, hereditary; L, lower jaw; M, male; NA, Not assigned; U, upper jaw; –w. teeth, number of missing teeth except for wisdom teeth; # miss., Number of missing teeth.

be swayed by ethnic backgrounds. In addition, it has been demonstrated that about one half of individuals with heterozygous null mutation of WNT10A show a mild ectodermal dysplasia phenotypes with a significantly higher proportion of tooth anomalies in males than in females.[15] However, in the current study, female patients with tooth agenesis were higher proportion than male (63.6%, 7/11). This may arise from difference in biological activity of WNT10A variants. The common variants such as p.R171C and p.G213S are detected in a healthy population, suggesting that these variants may have biological function at least in part.

Finally, it seems to be more direct contribution of the rare variants c.143G>A (p.Arg48His) and c.889G>A (p.Ala297Thr) *WNT10A* to human tooth agenesis, but biochemical and cell biological studies are needed to elucidate whether these WNT10A variants cause developmental anomaly in human tooth formation.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.1651; http://dx.doi.org/10.6084/m9.figshare.hgv.1654; http://dx.doi.org/10.6084/m9.figshare.hgv.1657; http://dx.doi.org/10.6084/m9.figshare.hgv.1660.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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REFERENCES

1 Polder BJ, Van't Hof MA, Van der Linden FP, Kuijpers-Jagtman AM. A meta-analysis of the prevalence of dental agenesis of permanent teeth. *Community Dent Oral Epidemiol* 2004; **32**: 217–226.
2 Sarnas KV, Rune B. The facial profile in advanced hypodontia: a mixed longitudinal study of 141 children. *Eur J Orthod* 1983; **5**: 133–143.
3 Machida J, Nishiyama T, Kishino H, Yamaguchi S, Kimura M, Shibata A *et al.* Genetic epidemiology of tooth agenesis in Japan: a population- and family-based study. *Clin Genet* 2014; **88**: 167–171.
4 Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. *Nat Genet* 1996; **13**: 417–421.
5 Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of PAX9 is associated with oligodontia. *Nat Genet* 2000; **24**: 18–19.
6 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**: 117–124.
7 Mues G, Bonds J, Xiang L, Vieira AR, Seymen F, Klein O *et al.* The WNT10A gene in ectodermal dysplasias and selective tooth agenesis. *Am J Med Genet A* 2014; **164A**: 2455–2460.
8 Mostowska A, Biedziak B, Zadurska M, Matuszewska-Trojan S, Jagodzinski PP. WNT10A coding variants and maxillary lateral incisor agenesis with associated dental anomalies. *Eur J Oral Sci* 2015; **123**: 1–8.
9 Lammi L, Arte S, Somer M, Jarvinen H, Lahermo P, Thesleff I *et al.* Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am J Hum Genet* 2004; **74**: 1043–1050.
10 Massink MP, Creton MA, Spanevello F, Fennis WM, Cune MS, Savelberg SM *et al.* Loss-of-function mutations in the WNT co-receptor LRP6 cause autosomal-dominant oligodontia. *Am J Hum Genet* 2015; **97**: 621–626.
11 Jho EH, Zhang T, Domon C, Joo CK, Freund JN, Costantini F. Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. *Mol Cell Biol* 2002; **22**: 1172–1183.

12 Schweizer L, Varmus H. Wnt/Wingless signaling through beta-catenin requires the function of both LRP/Arrow and frizzled classes of receptors. *BMC Cell Biol* 2003; **4**: 4.
13 van den Boogaard MJ, Creton M, Bronkhorst Y, van der Hout A, Hennekam E, Lindhout D *et al*. Mutations in WNT10A are present in more than half of isolated hypodontia cases. *J Med Genet* 2012; **49**: 327–331.
14 Mostowska A, Biedziak B, Zadurska M, Dunin-Wilczynska I, Lianeri M, Jagodzinski PP. Nucleotide variants of genes encoding components of the Wnt signalling pathway and the risk of non-syndromic tooth agenesis. *Clin Genet* 2013; **84**: 429–440.
15 Bohring A, Stamm T, Spaich C, Haase C, Spree K, Hehr U *et al*. WNT10A mutations are a frequent cause of a broad spectrum of ectodermal dysplasias with sex-biased manifestation pattern in heterozygotes. *Am J Hum Genet* 2009; **85**: 97–105.
16 Kantaputra P, Sripathomsawat W. WNT10A and isolated hypodontia. *Am J Med Genet A* 2011; **155A**: 1119–1122.
17 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**: 353–359.
18 Tardieu C, Jung S, Niederreither K, Prasad M, Hadj-Rabia S, Philip N *et al*. Dental and extra-oral clinical features in 41 patients with WNT10A gene mutations: A multicentric genotype-phenotype study. *Clin Genet* 2017; **92**: 477–486.

Supplementary Information for this article can be found on the Human Genome Variation website (http://www.nature.com/hgv).