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# DKK1 is a strong candidate for mesiodens and taurodontism

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## Graphical Abstract:

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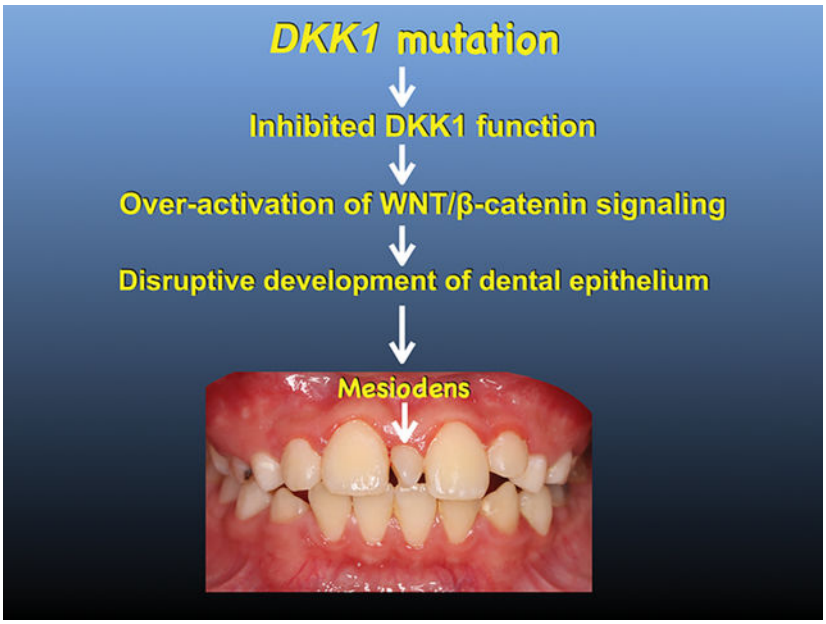
AUTHOR CONTRIBUTIONS

Piranit Kantaputra, Naomi Kottege, Robert P Anthonappa, Massupa Kaewgahya: patient care and evaluation. Sissades Tongsima, Chumpol Ngamphiw, Peeranat Jatooratthawichot, James R. Ketudat Cairns, Danilo Predes, Xi He; analysis and draft. All authors wrote and approved the final manuscript.

**CONFLICT OF INTEREST:** None




A mutation in *DKK1* gene leads to inhibitory DKK1 function, over-activation of WNT/β-catenin signaling, disruptive development of dental epithelium, and subsequent mesiodens formation

**Keywords**

Mesiodens; supernumerary tooth; WNT signaling; taurodontism

A mesiodens is a supernumerary tooth located in the premaxilla. Since genetic variants in WNT/β-catenin signaling, including *LRP5*, *LRP6*, and *WLS* were implicated in mesiodens,[1] we hypothesized that genetic variants in DKK1, an inhibitor of WNT/β-catenin signaling, might be associated with the mesiodens phenotype as well.

Whole exome sequencing (WES) was performed on the DNA of 95 patients affected with mesiodens. WES identified a rare heterozygous variant in *DKK1* (MIM 605189) chr10: g.54074690C>T; NM_012242.4:c.251C>T; NP_036374.1:p.Pro84Leu (rs201617558) in two male siblings (approximately 2%) affected with mesiodens. Taurodontism was also observed in patient 1 (Fig. 1a–c). The Wnt reporter assay (TOPFLASH)[2] showed that the p.Pro84Leu variant exhibits a significantly increased inhibitory function of DKK1 mutant protein on WNT/β-catenin signaling compared with the wildtype protein, suggestive of the p.Pro84Leu variant being a gain-of-function variant (Fig 1D).

This study involving human participants was approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University. It is hypothesized that mesiodens in patients 1 and 2 are associated with the *DKK1* variant, given that this variant is extremely rare in the general population with an allele frequency of 0.00008804 according to gnomAD. The amino acid residue Pro84 is highly conserved (Figure 1e) and thus its mutation is predicted to be damaging by MutationTaster and disease-causing by PolyPhen-2.

DKK proteins share two highly conserved cysteine-rich domains (CRD), the N-terminal CRD1, which is unique to the DKK proteins, and the C-terminal CRD2, which has a pattern of 10 cysteines that is related to the colipase fold.[3] The amino acid residue Pro84 is located at the end of the loop leading into the N-terminal CRD1, the domain unique for DKK proteins (Fig. 1f). The Pro84 residue confers rigidity to this loop and is conserved in DKK1 and DKK2 proteins, although not in DKK4 (MIM 605417). As such, it is likely to be essential for the specific functions of DKK1 and DKK2. Having Leu or Gln in this position in DKK4 may lead to a more DKK4-like function. Therefore, the DKK1 p.Pro84Leu variant may result in a malfunction. Since the CRD1 is not necessary for LRP5/6 binding, we speculate that the gain-of-function effect of p.Pro84Leu might be due to a decreased affinity to other protein partners, such as CKAP4,[4] known to interact with the CRD1 domain, or to improved DKK1 secretion.

It is hypothesized that the increased inhibition by the DKK1 mutant protein, as shown by TOPFLASH reporter assay, disrupted WNT-β-catenin signaling and resulted in mesiodens and taurodontism. However, the p.Pro84Leu variant identified in patients with bone density phenotypes was reported not to affect DKK1 inhibitory function in a luciferase reporter assay.[2] The discrepancy in the results might have been due to the difference in sensitivity and experimental design.

Expression of *Dkk1* blocks signaling in epithelial and underlying mesenchymal cells and subsequently downregulates key morphogenetic regulators of tooth development, *Bmp4*, *Msx1*, and *Msx2*. Fine-tuning of WNT/β-catenin signaling is necessary and modulated by several evolutionarily conserved activators and inhibitors, including DKK1 and DKK2. The presence of taurodontism in patient 1 and its absence in patient 2 might have been due to the variation in their genetic backgrounds. In conclusion, abnormal WNT/β-catenin signaling as a result of *DKK1* variant might disrupt development of dental epithelium involved in tooth initiation and invagination of the Hertwig epithelial root sheath, resulting in mesiodens and taurodontism, respectively.

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## DATA AVAILABILITY STATEMENT

Data are available upon request (P.K).

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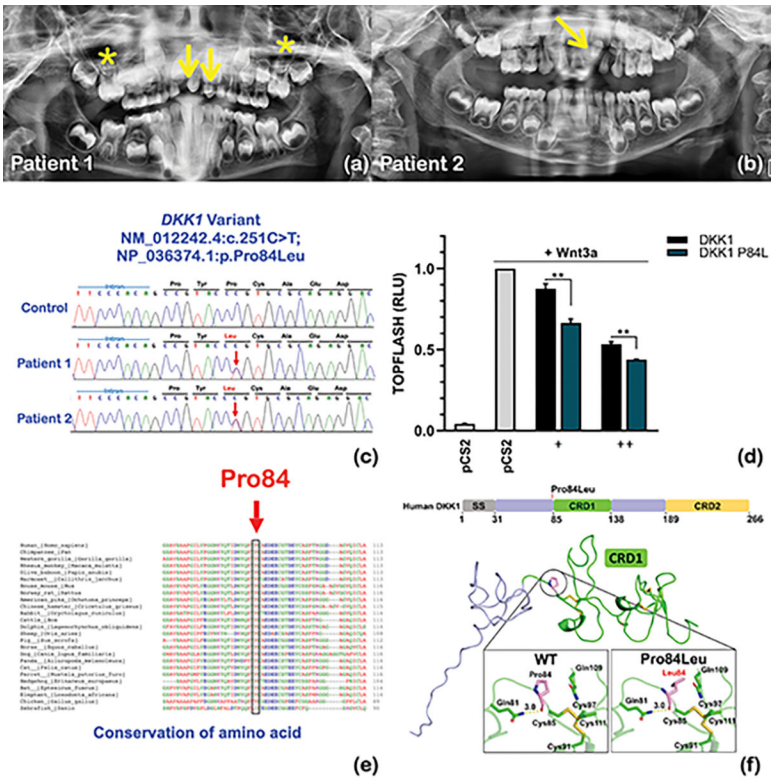


**Figure 1.**

(a) Patient 1. Double mesiodens (arrows) and taurodontism (asterisks). (b) Patient 2. Mesiodens (arrow). (c) *DKK1* sequence chromatograms. (d) TOPFLASH reporter assay (*Renilla* luciferase as internal control) of HEK293T cells transfected with 1 or 3 ng pCS2 DKK1 wt or p.Pro84Leu. Mutant Dkk1 shows increased inhibitory function. Wnt signaling was activated with Wnt3a plasmid. (RLU: Relative Luciferase Units). (e) Amino acid residue Pro84 is highly conserved across species. (f) Human DKK1 (NCBI: NP_036374.1) domain diagram and structure around Pro84Leu. Pro84 is located at the end of the loop leading into CRD1.