# A calcium channel *CACNA1S* responsible for teeth cusps and roots patterning

Orodental diseases
Autoimmune diseases
Maladies bucco-dentaires
Maladies auto-immunes
Autoimmunerkrankungen
Erkrankungen
im Mund- und Zahnbereich

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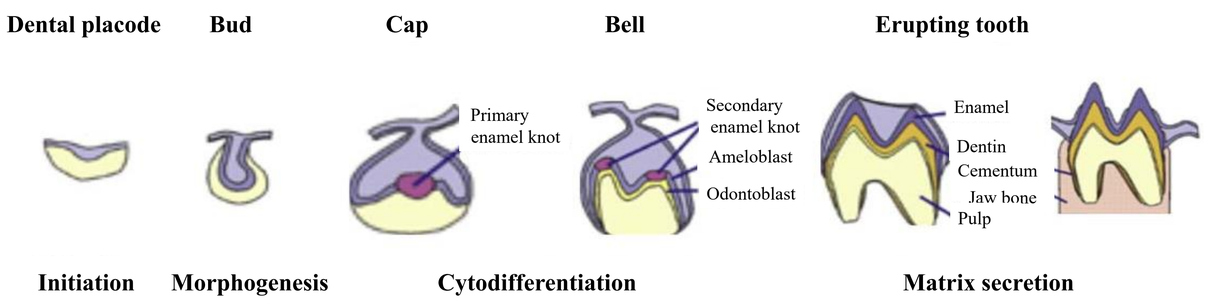
## Introduction

Odontogenesis occurs in sequential developmental stages initiated with dental epithelial placode induction, followed by the bud, cap, and bell morphogenetic stages, terminal differentiations of odontoblasts and ameloblasts, root formation and tooth eruption. The number of cusps is controlled by enamel knots, which are morphogen-expressing signalling centres. Indeed cusps form precisely at the location of secondary enamel knot signalling centres, which function as trophic regions inducing cusp growth and controlling the fine definition of cusp form.




**Human odontogenesis stages.** Bei, M. (2009). Molecular genetics of tooth development. *Curr. Opin. Genet. Dev.* 19, 504–510.

To date, heritable genetic alterations increasing cusp number are relatively rare in human populations. In this study, we explore a dominantly inherited supernumerary cusp phenotype in 5 Thai families.

## Methods

To identify the gene involved in this unique disorganised supernumary cusps and single root phenotype we used combined approaches:

**Patient examination** (Khon Kaen University, Thailand)

**Pathogenicity validation** (bioinformatics predictions, AA conservation)

**Identification of the gene involved**

**Sample collection** (Oragene® DNA OG-510 or OG-250)

**Segregation validation** (Sanger)

**Whole exome sequencing** (CNRGH, Evry France, HiSeq Illumina)

## Results

### 1) Patients show a striking multiple cusps single root phenotype.

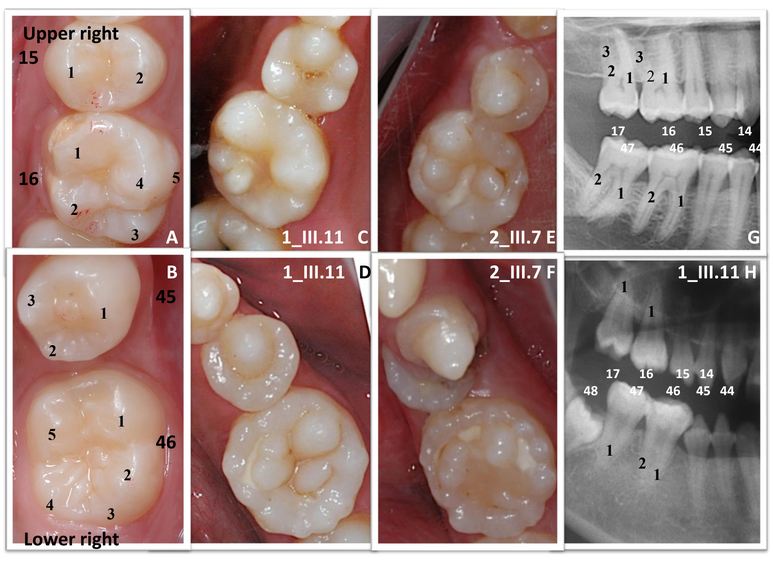



**Figure 1: Patients phenotype.** Normal crown morphology (A,B) of the right upper second premolar (15) and first permanent molar (16) and of the right lower premolar (45) and first permanent molar (46) could be compared to the multicusp pattern phenotype clearly visible both in the upper (C, E) and lower (D, F) jaws. On the close up of the right premolar-molar area on panoramic radiographs (G control, H affected) molars present a single root (1) pattern. Only the first lower permanent molar (46) shows a more apical root furcation leading to the taurodontic appearance of the tooth.

### 2) Families show a missense heterozygous mutation in *CACNA1S*.

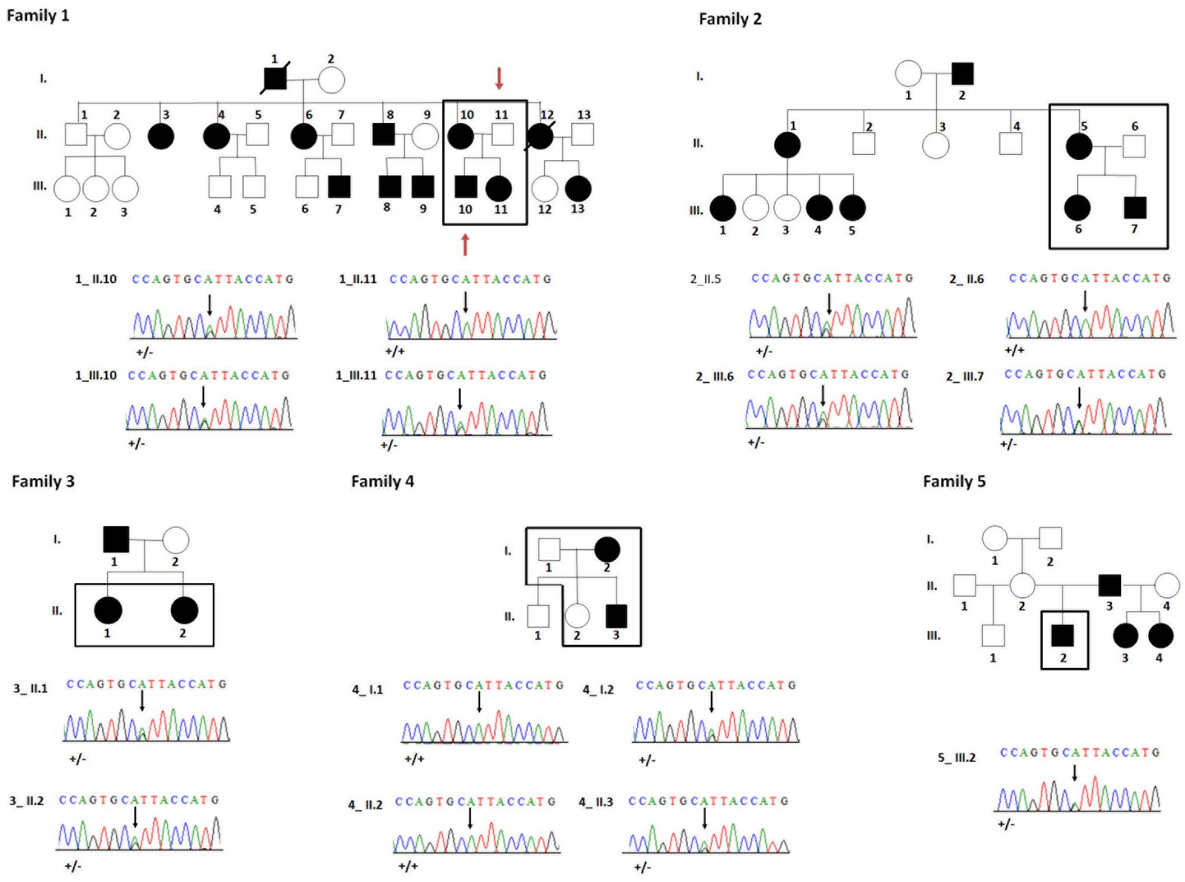



**Figure 2: Pedigrees and sequences analysis of families 1 to 5.** Black squares indicate the patients analysed in this study. In affected patients, electropherograms reveal a heterozygous missense mutation in exon 6 of *CACNA1S* (Cav1.1) (NM_000069.2: c.[865A>G];[=] p.[Ile289Val];[=]), the Calcium Channel, Voltage-Dependent, L Type, Alpha-1s Subunit, OMIM #114208), encoding a highly conserved amino-acid isoleucine residue within the pore forming subdomain of CACNA1S protein. (1_II.10, 1_III.10, 1_III.11, 2_II.5, 2_III.6, 2_III.7, 3_II.1, 3_II.2, 4_I.2, 4_II.3, 5_III.2).

### 3) A putative change in the selectivity or the gatting of the channel.

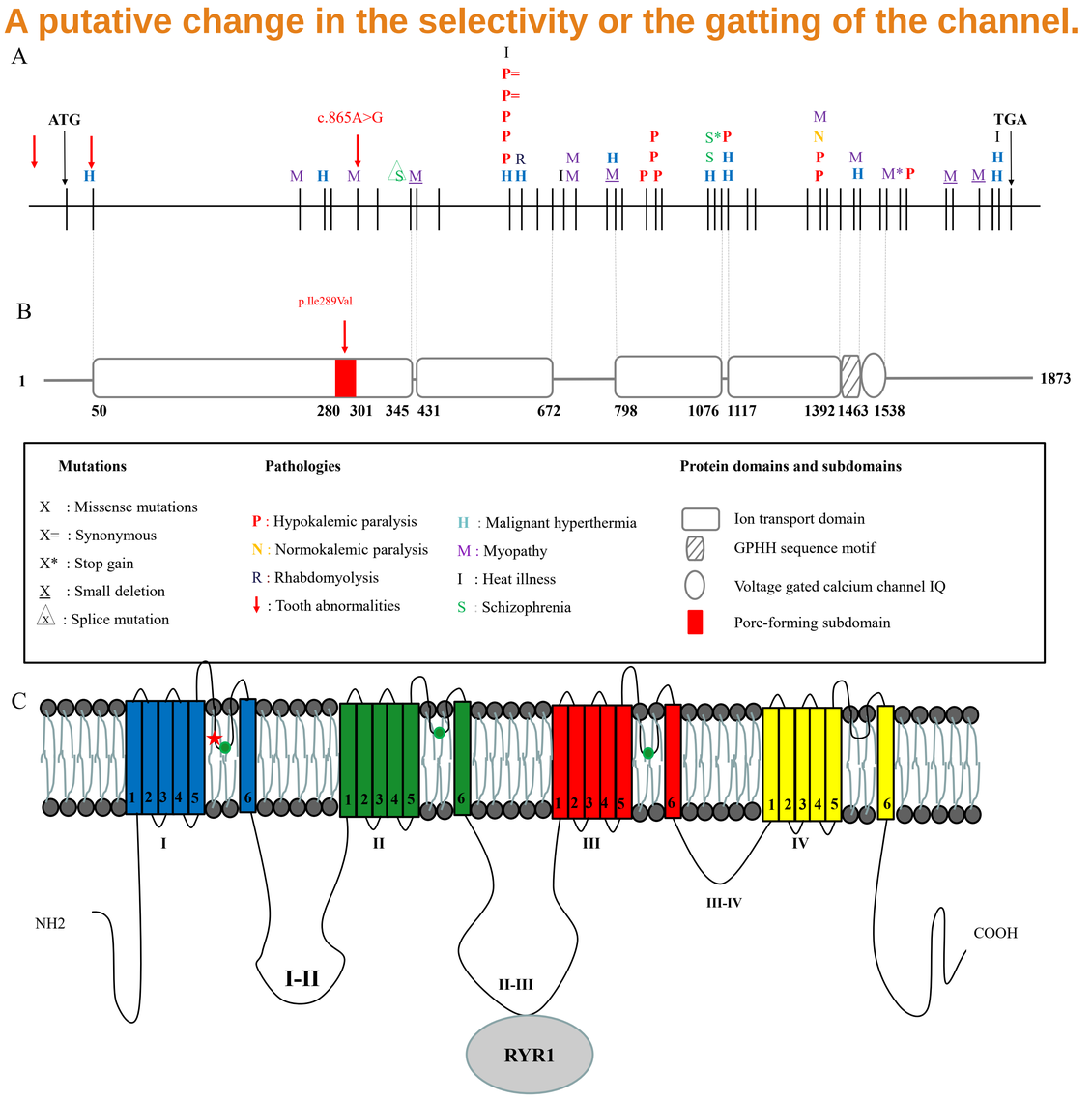



**Figure 3: *CACNAS1* mutations and corresponding protein domains.** (A) The *CACNA1S* human gene contains 44 exons (vertical black line). The mutation is highlighted by a red arrow. Previously described mutations in the gene are symbolized by a letter above the corresponding exon. (B) The protein domains are represented according to the PFAM database. (C) Schematic view of the alpha1 subunit of DHPR. The subunit has four transmembrane domains (I, II, III, IV) composed by six segments (1–6) and three intracellular loop domains (loop I-II, loop II-III, and loop III-IV). The mutated highly conserved isoleucine (red star) is located in the first pore-forming intramembrane domain close to one of the 3 amino-acids involved in calcium selectivity (green circles). The II_III loop interacts with RYR1 to allow excitation/contraction in muscle.

## Conclusion & Discussion

We report an unique dominantly inherited disorganised supernumerary cusps and single root phenotype presented by 11 affected individuals belonging to 5 north-eastern Thai families. Using WES, we identified a missense mutation in *CACNA1S* that segregates with the phenotype. This is a direct genetic indication that a voltage-dependent calcium ion channel can alter tooth morphogenesis and patterning. We suggest that the mutation in our patients, changes the selectivity or the gating of the channel rather than totally abolishing the calcium binding and this different mechanism results in a different pathophysiology and phenotype than those already reported in this gene.

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