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ORIGINAL ARTICLE

A novel *GJA1* mutation in oculodentodigital dysplasia with extensive loss of enamel

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OBJECTIVE: To characterize clinical features and identify genetic causes of a patient with oculodentodigital dysplasia (ODDD).
SUBJECTS AND METHODS: Clinical, dental, radiological features were obtained. DNA was collected from an affected Thai family. Whole-exome sequencing was employed to identify the disease-causing mutation causing ODDD. The presence of the identified variant was confirmed by Sanger sequencing.
RESULTS: The proband suffered with extensive enamel hypoplasia, polysyndactyly and clinodactyly of the 3rd–5th fingers, microphthalmia, and unique facial characteristics of ODDD. Mutation analysis revealed a novel missense mutation, c. 31C>A, p.L11I, in the *GJA1* gene which encodes gap junction channel protein connexin 43. Bioinformatics and structural modeling suggested the mutation to be pathogenic. The parents did not harbor the mutation.
CONCLUSIONS: This study identified a novel *de novo* mutation in the *GJA1* gene associated with severe tooth defects. These results expand the mutation spectrum and understanding of pathologic dental phenotypes related to ODDD.

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Keywords: GJA1; oculodentodigital; novel; mutation; enamel hypoplasia

Introduction

Oculodentodigital dysplasia (ODDD) (OMIM #164200) is a rare congenital autosomal dominant disorder characterized by abnormalities of the eyes, limbs, and teeth. Bilateral syndactyly of the 4th and 5th fingers or syndactyly type III is the main feature of the syndrome. Camptodactyly and a shortening of the 5th finger, and missing phalanges of the toes are common. The ophthalmologic findings include microphthalmia, microcornea, iris anomalies, glaucoma, and hypertelorism. Typical craniofacial features include a long and narrow nose with prominent nasal bridge and hypoplastic alae nasi, dry and sparse hair and eyebrows, and short palpebral fissures (Gorlin *et al*, 1963; Judisch *et al*, 1979). Some affected individuals also develop conductive hearing loss and neurological symptoms such as spastic paraplegia, ataxia, and occasionally delayed development (Judisch *et al*, 1979; Gutmann *et al*, 1991; Norton *et al*, 1995). Oral manifestations include generalized enamel hypoplasia, microdontia, and sporadically cleft lip and/or palate.

Oculodentodigital dysplasia is caused by mutations in the gap junction alpha1 gene (*GJA1*; OMIM*1210154) which is located on chromosome 6q22-q23 (Gladwin *et al*, 1997; Paznekas *et al*, 2003). It comprises two exons and one intron. The *GJA1* gene encodes the gap junction protein connexin 43 (Cx43), a member of human connexin protein family, comprising four transmembrane domains, two extracellular loop domains, and intracellular loop and domains consisting of the amino and carboxyl termini (Saez *et al*, 2003; Wei *et al*, 2004). The connexins hexamerise to form a hemichannel or connexon in the plasma membrane providing cell–cell communication, which plays a vital role during embryogenesis, cardiac development, and cellular homeostasis. There are 21 connexins found in humans (Sohl and Willecke, 2003), all of which share similar transmembrane structures and two or more of these proteins are co-expressed within the same cell type (Evans and Martin, 2002; Laird, 2006).

In this study, we identified ODDD in a Thai patient with extensive loss of tooth structure. Bioinformatics analysis and structural modeling were performed. Mutational analysis revealed a novel *de novo* heterozygous missense mutation, c.31C>A (p.L11I), in the *GJA1* gene. Our findings expand the dental characteristics and mutational spectrum associated with ODDD.

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Materials and methods

Recruitment of the family

A Thai patient and his parents were recruited for genetic studies. The study was exempted from review by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University. Clinical and radiographic examinations and blood collection were performed with the understanding and written consent of each participant according to the Declaration of Helsinki.

Whole-exome sequencing and bioinformatics

Genomic DNA was extracted from peripheral blood leukocytes and sent to Macrogen, Inc. (Seoul, Korea). The DNA sample was prepared as an Illumina sequencing library. The sequencing libraries were enriched by SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA, USA) and was sequenced onto Hiseq 4000 (Illumina, San Diago, CA, USA). The raw data per exome were mapped to the human reference genome hg19 using Burrows-Wheeler Aligner (BWA). Variant calling was performed using GATK with HaplotypeCaller. Finally, SNVs and Indels were annotated using SnpEff and annotation databases, dbpSNP 142, 1000Genome, ClinVar, and ESP. The variants were subsequently filtered out if they were present in our in-house database of 200 unrelated Thai exomes. The variants would be called novel if they were not listed in the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php) and the Exome Aggregation Consortium database (exac.broadinstitute.org). The amino acid sequence of human Cx43 (NP_000156.1) was compared with those of the rhesus monkey (NP_001247996), Bos taurus (NP_776493), Gallus gallus (NP_989917), Mus musculus (NP_034418.1), Xenopus tropicalis (NP_988856.1), and Danio rerio (NP_571113).

Sanger sequencing

The variants were confirmed by PCR and Sanger sequencing. DNA of his unaffected parents was also Sanger-sequenced to search for the mutation. The coding region of *GJA1* was amplified with primers: GJA1-E2F: TAC GTG AAA CCG TTG GTA GT and GJA1-E2R: GAA AGG CAG ACT GCT CAT CT. The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, OH) and sent for direct sequencing at Macrogen. Sequence data were analyzed using Sequencher (V.5.0; Gene Codes Corporation, Ann Arbor, MI, USA). The amino acid sequence of human GJA1 was compared with those of humans, monkeys, cattle, mice, chicken, frogs, and zebrafish. SIFT (http://sift.jcvi.org/), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org/) were used to predict the effect of the identified mutation.

Results

Clinical and radiographic investigations

The proband was a 3-year-old boy. He was delivered at 39 weeks of gestation by cesarean section. His birthweight was 3175 g (25th–50th centile); length 50 cm (25th–50th centile); and head circumference 36.5 cm (50th–75th centile). His parents were non-consanguineous and healthy (Figure 1a). Family history was unremarkable. At the age of 1 month, the proband was referred to our Genetics Clinic at the King Chulalongkorn Memorial Hospital. He had a flat face, sparse eyebrows and eyelashes, a long narrow nose, hypoplastic nasal alae, a depressed nasal bridge, and micrognathia. His hair was dry and lusterless (Figure 1b). Clinical and radiographic examinations revealed bilateral complete simple syndactyly of 3rd and 4th fingers, complete complex syndactyly of 4th and 5th fingers with camptodactyly, and complete simple syndactyly of 3rd and 4th toes (Figure 1c–i). Ophthalmic examinations showed bilateral microphthalmia, microcornea of 7 mm in both eyes, and short palpebral fissures. At 2 months of age, brain ultrasound demonstrated two well-defined cystic lesions, each in the right and left caudothalamic grooves (Figure 1j). The proband developed a cochlear lesion and hearing loss in his left ear at the age of 5 months. Surgical interventions under general anesthesia for syndactyly release and skin grafting of both hands and feet were performed at the ages of 12 and 17 months.

The proband presented for an initial oral examination at age 1 year. He had seven primary incisors with white hypoplastic patches on the enamel and high-arched palate (Figure 2a, b). At 27 months old, the proband revisited the dental facility due to extensive loss of tooth structure. At this visit, he had speech and language delay. His craniofacial appearance was characteristic for ODDD showing a mesocephalic facial feature, frontal bossing, dry and sparse eyebrows and eyelashes, retruded upper lip, and a long narrow nose with hypoplastic nasal alae. Ophthalmic examination showed microphthalmia, telecanthus, prominent epicanthal folds, short palpebral fissures, strabismus at left eye, and ptosis of both eyes. Palmar keratosis was observed.

Oral examination, at age 2 years and 6 months, revealed sixteen primary teeth. All teeth were affected with severe enamel hypoplasia and destructive tooth structure (Figure 2c, d). Dental radiographs revealed extensive loss of enamel on all erupted teeth (Figure 2e–g). Notably, the mandibular left second molar was partially erupted, but the enamel was partially destroyed on the occlusal surface suggesting pre-eruptive loss of tooth structure (Figure 2h). Oral soft tissues including gingiva, tongue, buccal mucosa, oropharynx, and frenum were unremarkable. Dental treatment was performed under general anesthesia. Four maxillary incisors were extracted due to pulp necrosis and unrestorable tooth structure. The maxillary right canine was treated with pulpectomy and stainless steel crown (SSC). Eleven remaining teeth were restored with SSC. Partially erupted second molars were sealed with glass ionomer cement (Figure 2i, j). Regular recall and special monitoring of the proband's oral health was implemented.

Genetic investigation

Chromosomal analysis revealed a normal male karyotype. Mutation analysis was performed by whole-exome sequencing (WES). Total yield of 6 009 467 680 bp was achieved. The capture efficiency varied across the target with 92% more than 10× and the mean read depth of target regions was 69×. Sanger sequencing of the candidate variants was performed in the proband and his parents. It was found that the proband harbored a heterozygous substitution (c.31C>A) in the *GJA1* gene (NM_000165.4), resulting in a missense mutation changing leucine to isoleucine at codon 11 (p.L11I) at the N-terminal of the Cx43 (Figure 3a). This position is highly conserved among several species (humans, monkeys, cattle, mice, chicken, frogs, and zebrafish) (Figure 3b). The p.L11I was predicted to be probably damaging by PolyPhen2 and SIFT. Neither his parents harbored the p.L11I mutation. In addition, it was absent in our in-house database of 200 unrelated Thai exomes.

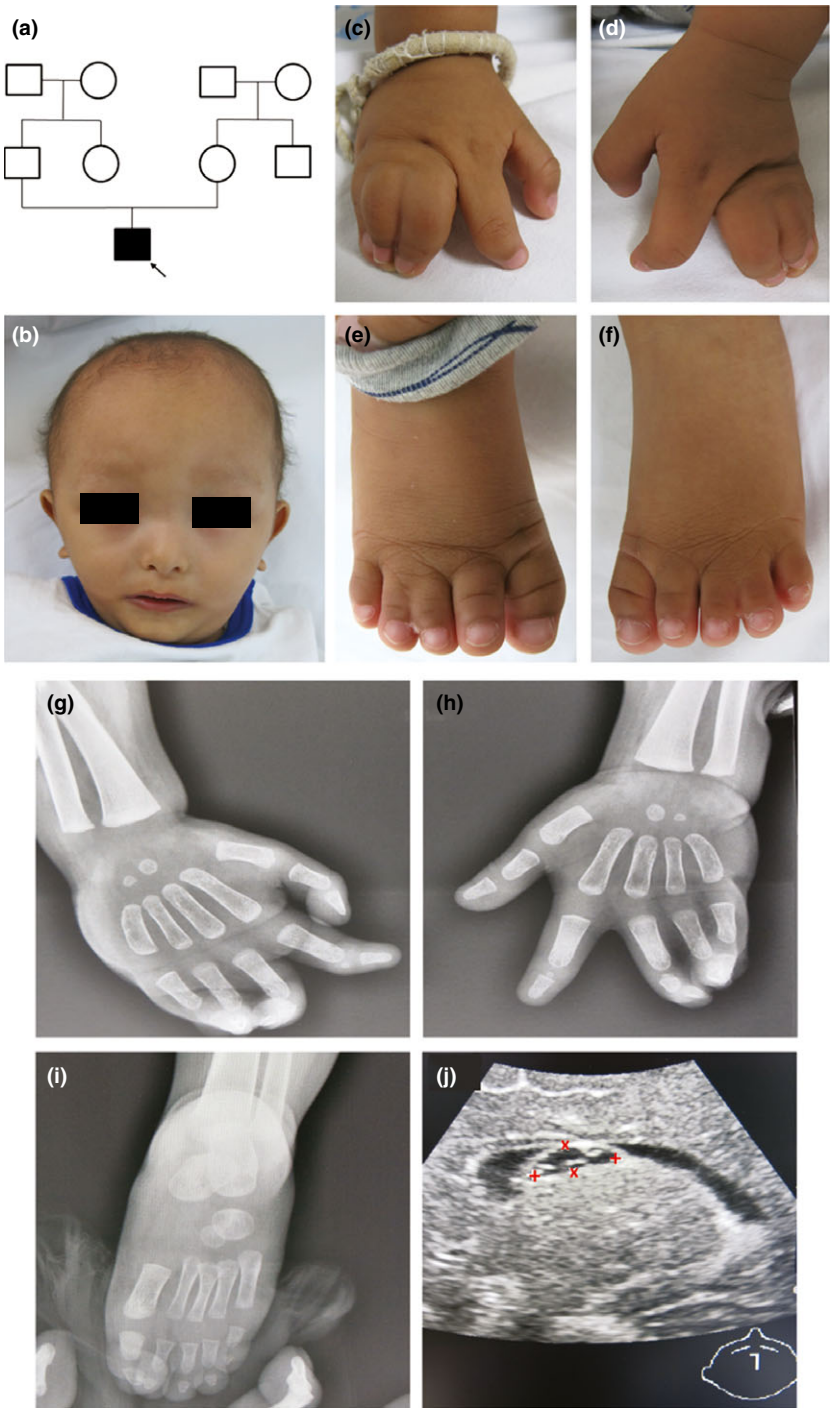

Figure 1 Pedigree, clinical photographs, and radiographs of the family and proband at age 1. (**a**) Pedigree of the family. The proband is indicated with a black arrow. (**b**) Frontal clinical photograph of the proband showed facial characteristics of ODDD. (**c–i**) Clinical photographs and radiographs revealed bilateral syndactyly and camptodactyly of the 3rd–5th fingers and syndactyly of the 3rd and 4th toes. **(j)** Ultrasonography of the brain demonstrated a well-defined cystic lesion in the right caudothalamic groove

Discussion

In this study, we identified a patient with ODDD, who possessed unique craniofacial characteristics, ocular anomalies, and severe enamel hypoplasia. Until now, dental phenotypes related to ODDD have not been well characterized. Our proband presented with enamel hypoplasia in his primary dentition. The enamel and dentin were rapidly destroyed soon after tooth eruption. These suggest that the *GJA1* is vital in the development of the enamel, and possibly, the dentin. This corresponds with the gene expression. The *GJA1* gene encodes Cx43 protein which is a vital component of gap junctions important for direct cell–cell communication, propagation of electric signals in cardiac tissues, and regulation of cell growth and differentiation (Harris, 2001; Laird, 2006). Cx43 is the most widely observed connexin in human beings found in more than 35 different cells and tissues (Laird, 2006). In human tooth germs, Cx43 was observed in epithelial cells including pre-ameloblasts, stratum intermedium, stellate

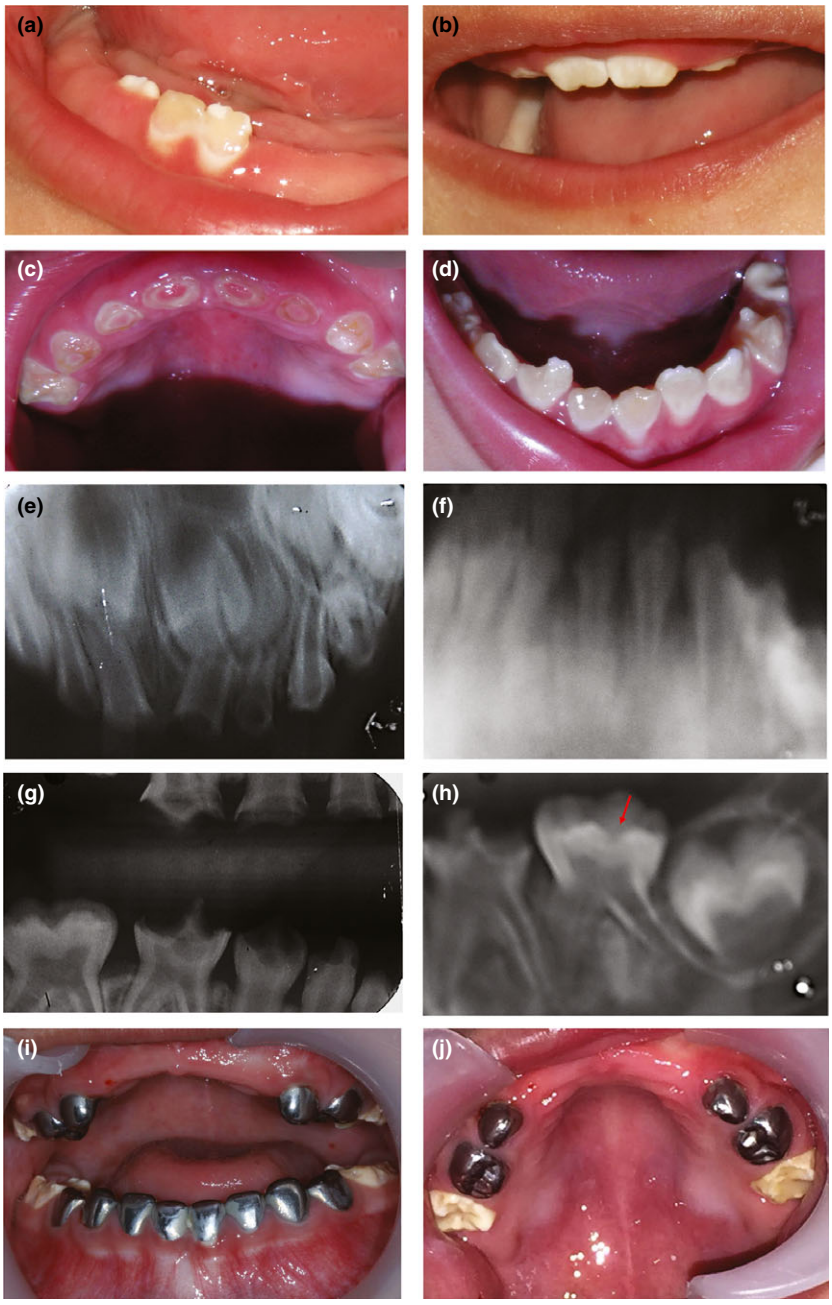

Figure 2 Clinical photographs and radiographs of primary teeth and oral cavity of the proband. (**a, b**) Frontal clinical photographs at age 1. White hypoplastic patches were noticed on the enamel. (**c, d**) Clinical photographs of the maxillary and mandibular teeth at age 2 years 6 months showed extensive tooth deterioration. (**e–h**) Dental radiographs of the maxillary incisors, mandibular incisors, right molars, and mandibular left molar, respectively. Loss of enamel was noticed on all erupted teeth and erupting molar (a red arrow). (**i, j**) Four maxillary incisors were extracted. The remaining teeth were restored with stainless steel crowns. Partially erupted second molars were protected with glass ionomer cement

reticulum, and differentiating odontoblasts at bell stage (About *et al*, 2002). In rats, Cx43 was expressed during differentiation of both ameloblasts and odontoblasts (Fried *et al*, 1996; Inai *et al*, 1997; João and Arana-Chavez, 2003). The mouse model of ODDD that harbored the G60S Cx34 mutant exhibited disorganized ameloblast layer and abnormal expression of amelogenin. In the mutant, the enamel integrity and thickness were compromised leading to rapid erosion whereas the dentin was thicker indicating a mechanical stress response to the eroded enamel (Toth *et al*, 2010).

Whole-exome sequencing revealed that the patient had a novel *de novo* heterozygous missense mutation, c. 31C>A, p.L11I, in the *GJA1* gene. To date, more than 60Cx43 mutations have been identified which are usually inherited in an autosomal dominant manner comprising nonsense, frameshift, and mainly missense mutations (Fenwick *et al*, 2008; Paznekas *et al*, 2009). Autosomal recessive inheritance of ODDD has also been reported with severe phenotypes (Richardson *et al*, 2006). Our proband harbored a novel *de novo* missense mutation c.31C>A resulting in p.L11I substitution in the N-terminal domain of Cx43 (Figure 3c). It has been suggested that the N-terminal domain is critical for proper channel assembly (McLachlan *et al*, 2005; Roscoe *et al*, 2005), transjunctional voltage-dependent gating of gap junction channels, and regulating ion permeability (Harris, 2001). The leucine at the amino acid residue 11 of Cx43 was highly

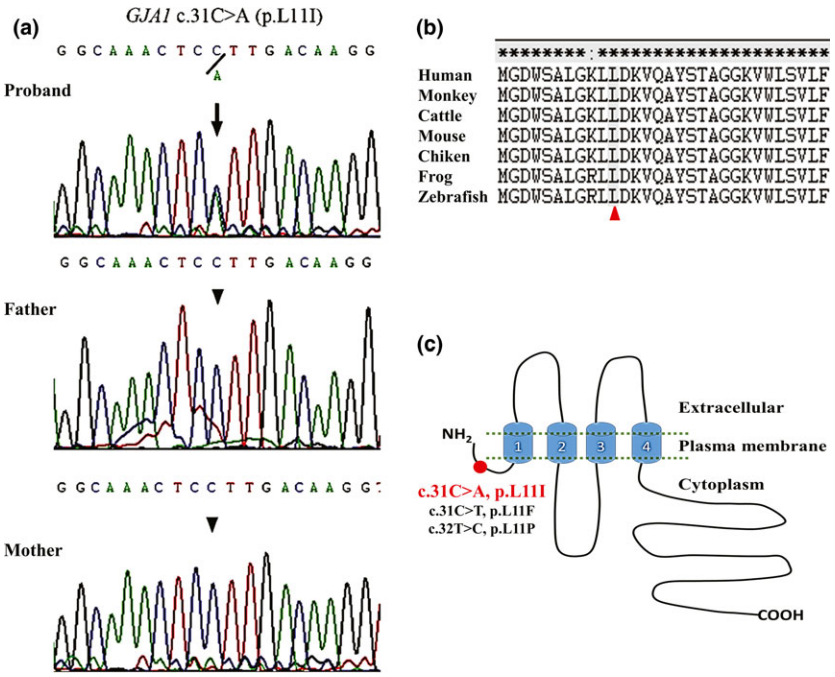

Figure 3 Genetic analysis of the proband. (**a**) Chromatograms of the proband and parents. The position of the mutation is indicated by an arrow. (**b**) Sequence alignment of partial amino acid sequence of Cx43. Conservation of the P.L11 across species is indicated by a red arrowhead. (**c**) Domain structure of Cx43. The p.L11I occurred in the N-terminal domain. The p.L11F and p.L11P were previously reported (Liu *et al*, 2001; Jamsheer *et al*, 2009; Gabriel *et al*, 2011; Shao *et al*, 2012)

conserved among several species. Interestingly, there were two mutations previously reported which involved this amino acid residue of Cx43, p.L11P, and p.L11F. The N-terminal domain p.L11P mutant was generated and expressed in Cx43-positive normal rat kidney (NRK) cells. It was found that the p.L11P mutant failed to form functional gap junction channel and exhibited dominant-negative effects on co-expressed Cx43 (Shao *et al*, 2012). It is possible that the p.L11I reported in this study could exert a dominant-negative effect causing the protein not capable to assemble into gap junction-like plaques, thus reducing the efficiency of intercellular channels. The p.L11F (c.31C>T) has been previously reported associated with ODDD and deafness (Liu *et al*, 2001; Jamsheer *et al*, 2009; Gabriel *et al*, 2011). We provide strong evidence supporting the importance of this highly conserved amino acid residue on the normal function of *GJA1*.

The major phenotypes of ODDD include ocular, digital, and tooth anomalies. The spectrum of neurological manifestations was reported in ODDD including subcortical white matter lesions, basal ganglia calcification, and cerebral atrophy (Loddenkemper *et al*, 2002). Cystic lesions were observed in bilateral caudothalamic grooves in our proband. Our proband had more severe phenotypes compared to ODDD cases with the p.L11F mutation. Remarkably, bilateral microcornea (7 mm in diameter), bilateral syndactyly and camptodactyly of the 3rd–5th fingers, syndactyly of the 3rd and 4th toes, and severe enamel hypoplasia were observed in our proband while cornea with 8–9 mm in diameter, syndactyly and camptodactyly of the 4th and 5th fingers, and normal teeth were observed in p.L11F cases (Jamsheer *et al*, 2009; Gabriel *et al*, 2011). In addition, the homozygous p.L11F mutation has been reported in the patients suffered from bilateral congenital sensorineural hearing loss (Liu *et al*, 2001). Our proband had the lesion in the cochlea which could disturb the function of sensory cells suggesting sensorineural hearing loss. Connexins were shown to facilitate the recycling of potassium ions during auditory transduction (Krutovskikh and Yamasaki, 2000). The Leu residue at position 11 is located in the cytoplasmic N-terminal domain which is involved in the voltage gating of gap junction channels and insertion of connexins into the membranes. These therefore suggest that the mutation of this locus in the *GJA1* gene could disturb the function of cochlear gap junction which is critical for normal hearing. Leucine, isoleucine, and phenylalanine were hydrophobic amino acids. The variation in phenotypes emphasizes the clinical heterogeneity of the disease even when the mutations occur at the same position and same category of amino acids. In addition, Cx32 and Cx26 have also been found in the developing enamel organ and odontoblasts, respectively (Fried *et al*, 1996). It is also possible that diverse pathophysiological changes in ODDD could be related to other modifier genes or epigenetic involvement.

The understanding of how similar Cx43 mutations could cause such a diverse range of physical and dental phenotypes continues to be challenging. Our findings raise concern among dental practitioners to pay special attention to tooth anomalies and rapid deterioration of the dentition associated with ODDD. We provided evidence that determination of genetic causes of the disorder would be highly beneficial and proposed that the mutation analysis could be a prime tool to provide the best management of orodental disease.

In conclusion, we identified a novel heterozygous missense mutation, c.31C>A, p.L11I, in the *GJA1* gene associated with ODDD in a Thai patient. The proband had extensive enamel hypoplasia in the primary dentition. This study expands the mutation spectrum of the *GJA1* gene and the understanding of dental characteristics associated with ODDD.

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Author contributions

T. Porntaveetus examined the patients, collected the samples, and drafted the manuscript. V. Shotelersuk designed the study and revised the manuscript. C. Srichomthong performed mutation analysis. A. Ohazama and K. Suphapeetiporn revised the manuscript.

Conflict of interests

The author declared no conflict of interests.

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