Kantaputra Piranit Nik (Orcid ID: 0000-0001-9841-0881)
Panyarak Wannakamon (Orcid ID: 0000-0003-3557-586X)

Expanding genotypic and phenotypic spectrums of *LTBP3* variants in Dental Anomalies and Short Stature syndrome

Running title: Novel *LTBP3* mutations and novel findings

Piranit Kantaputra[1,2#], Yeliz Guven[3#], Tugba Kalayci[4], Pelin Karaca Özer[5], Wannakamon Panyarak[6]; Worrachet Intachai[1]; Bjorn Olsen, MD, PhD[7]; Bruce M. Carlson[8], Oranud Praditsap[9], Sissades Tongsima[10], Chumpol Ngamphiw[10], Peeranat Jatooratthawichot[11], Abigail S. Tucker[12], James R. Ketudat Cairns[11]

[1]Center of Excellence in Medical Genetics Research, Chiang Mai University, Thailand.

[2]Division of Pediatric Dentistry, Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Thailand.

[3]Department of Pedodontics, Faculty of Dentistry, Istanbul University, Turkey.

[4]Department of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, Turkey.

[5]Istanbul Medical Faculty, Department of Cardiology, Istanbul University, Turkey.

[6]Division of Oral and Maxillofacial Radiology, Faculty of Dentistry, Chiang Mai University, Thailand

[7]Department of Developmental Biology, Harvard School of Dental Medicine, Harvard University, Boston, MA. USA.

[8]Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, USA

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[9]Siriraj Genomics, Office of the Dean, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

[10]National Biobank of Thailand, National Science and Technology Development Agency, Pathum Thani 12120, Thailand.

[11]School of Chemistry, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand.

[12]Centre for Craniofacial and Regenerative Biology, King's College London, Guy's Hospital, London, UK

# These authors contributed equally

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***Correspondence To:**

Piranit Nik Kantaputra, D.D.S., M.S. Email: dentaland17@gmail.com

Abstract

Mutations in *LTBP3* are associated with Dental Anomalies and Short Stature syndrome (DASS; MIM 601216), which is characterized by hypoplastic type amelogenesis imperfecta, hypodontia, underdeveloped maxilla, short stature, brachyolmia, aneurysm and dissection of the thoracic aorta. Here we report a novel (p.Arg545ProfsTer22) and a recurrent (c.3107-2A>G) *LTBP3* variants, in a Turkish family affected with DASS. The proband, who carried compound heterozygous variant c.3107-2A>G, p.Arg545ProfsTer22, was most severely affected with DASS. The proband's father, who carried the heterozygous variant c.3107-2A>G had short stature and prognathic mandible. The mother and brother of the proband carried the heterozygous variant p.Arg545ProfsTer22, but only the mother showed any DASS characteristics. The c.3107-2A>G and the p.Arg545ProfsTer22 variants are expected to result in abnormal LTPB3 protein, failure of TGFβ-LAP-LTBP3 complex formation, and subsequent disruption of TGFβ secretion and activation. This is the first report of heterozygous carriers of *LTBP3* variants showing phenotypes. The new findings of DASS found in this family include taurodontism, single-rooted molars, abnormal dentin, calcified dental pulp blood vessels, prognathic mandible, failure of mandibular tooth eruption, interatrial septal aneurysm, secundum atrial septal defect, tricuspid valve prolapse, and a recurrent glenohumeral joint dislocation.

Keywords: TGFB; congenital heart diseases; tooth eruption; interatrial septal aneurysm; root defects; atrial septal defect

1. INTRODUCTION

Latent TGF-beta binding protein 3 (LTBP3; MIM 602090) is an extracellular matrix protein involved in bioavailability of TGFβ by regulating TGFβ secretion, folding, activation, and deposition into the extracellular matrix.[1] LTBP3 is required for TGFβ signaling, and mice lacking *LTBP3* have been shown to have long bone malformations resembling mice with impaired TGFβ signaling.[1]

Ltbp3 is expressed in developing teeth and bones, cardiac outflow tract, and the walls of major blood vessels.[2] Mutations in *LTBP3* have been associated with autosomal recessive Dental Anomalies and Short Stature syndrome (DASS; MIM 601216), which is characterized by hypoplastic type amelogenesis imperfecta, hypodontia, underdeveloped maxilla, short stature, brachyolmia, aneurysm and dissection of the thoracic aorta.[2-5]

Here we report on a Turkish family affected with DASS. One novel and one recurrent variants in *LTBP3* were identified. The newly recognized findings of DASS are reported.

2. MATERIALS AND METHODS

See supplementary information 1

Patient 1 (proband)

Patient 1 was a 19-year-old woman, born after an uncomplicated pregnancy (Figure 1A,B). Her psychomotor development and growth were normal [height 155 cm (-1.38 SD), weight: 57 kg (-0.17 SD), OFC: 55 cm (-0.89 SD)]. Her parathyroid hormone level

was unremarkable. Mitral valve prolapse was diagnosed at age 11 as an incidental finding.

Craniofacial manifestations included biparietal bossing, wide forehead, high frontal hairline, and short philtrum (Figure 1B). Oral examination showed generalized microdontia, yellow teeth, and irregular mandibular alveolar ridge (Figure 1C,D,F). Radiographic examination showed a prognathic mandible, absence of enamel, multiple unerupted teeth, calcifications in dental pulps, taurodontism, single rooted-molars, focal areas of dense bone, ankylosis of the primary molars and irregular mandibular alveolar bone level. The dental pulp spaces at age 19 were significantly narrower than those at age 5 (Figure 1E,G; Supplementary Figure S1). A recurrent left glenohumeral joint dislocation clinodactyly and mild brachydactyly of hands were noted (Supplementary Figure S2). The bone mineral density of L1-L4 vertebrae showed mild osteopenia (Z score: -1.2).

Echocardiography performed at age 19 revealed an 8-mm secundum atrial septal defect with a left-to-right shunt and an interatrial septal aneurysm (Figure 2A,B). Myxomatous degeneration was present in both atrioventricular valves (mitral and tricuspid), which prolapsed towards the atria in systole (Figure 2C). Mild mitral and mild tricuspid valve regurgitations were observed. There was a mild enlargement (37 mm) at the level of the sinus valsalva in the aortic root (Figure 2D).

Patient 2

Patient 2, a 44-year-old woman, was the mother of patient 1. She had normal growth [height: 156 cm (-1.21 SD), weight: 72 kg (1.9 SD)]. Craniofacial features

included mild maxillary hypoplasia, mandibular prognathism, and unremarkable teeth (Supplementary Figure S3). Echocardiography at age 44 revealed enlargement (39 mm) in the aortic root at the level of the sinus valsalva and aneurysm in the interatrial septum (Figure 2E,F).

Patient 3

Patient 3, a 52-year-old man, was the father of patient 1. He had proportionate short stature with a height of 159 cm (-2.79 S.D), a wide forehead, and mandibular prognathism. Teeth were unremarkable. Radiographs confirmed normal dentition and mandibular prognathism (Supplementary Figures S2,S3). Echocardiography of the patient was normal.

Patient 4

Patient 4, a 20-year-old man, was the elder brother of patient 1. His health was normal. Teeth appear unremarkable. He was normal in height (177 cm, 0.13 SD).

3. RESULTS AND DISCUSSION

A family carrying a novel (p.Arg545ProfsTer22) and a recurrent (c.3107-2A>G; rs112261752) *LTBP3* variants is reported (Supplementary Figure S4). The p.Arg545ProfsTer22 variant in *LTBP3* is predicted to result in nonsense-mediated decay. Any small amount of protein that is produced is predicted to be a truncated protein, lacking most of the essential functional domains (Supplementary Figure S5A-

C).[1,4,5] The absence of functional domains would result in failure of TGFβ-LAP-LABP3 complex formation, and subsequently disrupt TGFβ secretion and activation.

The most likely outcome of the splice site mutation c.3107-2A>G is predicted to be skipping of exon 23, resulting in deletion of the 46 amino acids that make-up EGF-CBD11, although more complex defects such as shortening of the exon, replacement of the exon with a part of a surrounding intron or skipping of multiple exons are possible.[6] Any of these outcomes would disrupt the protein functions involving CBD11 and some may result in frameshifts leading to disruption of the protein's C-terminal or nonsense mediated decay of the mRNA. The proband, who carried the p.Arg545ProfsTer22 and c.3107-2A>G variants, was most severely affected. Her father carried the heterozygous variant c.3107-2A>G was mildly affected with short stature and prognathic mandible. The heterozygous p.Arg545ProfsTer22 variant did not cause a disease in patient 4 but caused some DASS-associated phenotypes in the proband's mother. It appears that heterozygous carriers of this *LTBP3* variant may display aspects of the DASS phenotype, dependant on individual genetic background.

Amelogenesis imperfecta, hypoplastic type observed in the patient recapitulates the enamel defect of *Ltbp3* mutant mice.[2,7] Interestingly, abnormally large dental pulps and root canal spaces of all teeth of the proband at age 5 indicated impaired dentin formation. Abnormal dentin has not previously been reported in patients with *LTBP3* mutations[2,4,5], however, *Ltbp3* mutant mice show abnormal dentin tubules.[7] The speckled appearance of the walls of the dentinal tubules found in the proband is unique. Of note, in previous reports wide dental pulp spaces have been illustrated on panoramic radiographs, but impaired dental formation was not mentioned.[2]

Abnormal mineralization found in the proband included vertebral osteopenia, focal areas of sclerotic alveolar bone in the mandible, ankylosis of the primary teeth, irregular mandibular alveolar bone, dental pulp stones, and calcification of blood vessels in the dental pulps. The calcium content in the calcified pulpal bood vessels, which was comparable to that of root dentin as identified by SEM/EDX, indicated dysregulation of mineralization (Supplementary information 2). TGFβ signaling is required for bone formation with disrupted TGFβ signaling leading to severe skeletal malformations.[8] *Ltbp3* mutant mice have dysregulation of bone mineralization, premature ossification of cranial synchondroses, and an underdeveloped maxilla, with subsequent mandibular prognathism.[1,2] The mineralization phenotypes presented here are, therefore, likely to be the result of the *LTBP3* mutation.

Failure of tooth eruption in the maxilla has not been reported in patients with DASS, however, decreased TGFβ signaling results in decreased expression of *Parathyroid hormone 1 receptor* gene (*PTH1R*; MIM 168468),[1] and mutations in *PTH1R* cause primary failure of tooth eruption.[9] Together, this suggests that failure of tooth eruption and osteosclerosis in the proband are related to a disruption in the LTBP3-TGFβ-PTH1R signaling pathway. Defects in root development, such as taurodontism and single-rooted molars observed in the proband, have not previously been reported in patients with *LTBP3* mutations, but as TGFβ signaling has important roles in root development,[10] a role for LTBP3 in this process is likely.

The proband and her mother shared phenotypes of aortic root dilatation, and interatrial septal aneurysm. However, the proband who carried two *LTPB3* variants also had a secundum atrial septal defect and atrioventricular valve prolapses. We believe

that these anomalies were the consequences of abnormal TGFβ signaling. Firstly, TGFβ signaling is required for the differentiation of smooth muscle cells into quiescent cells.[11] Secondly, TGFβ signaling is required for development of the atrioventricular endocardial cushions into atrioventricular valves, including mitral and tricuspid valves,[12] and mitral valve prolapse has been reported in patients with *LTBP2* and *LTBP3* mutations[3,5], supporting the association of mitral valve prolapse and aberrant TGFβ signaling.[13] Thirdly, disrupted TGFβ signaling drives aneurysm progression in a number of diseases including Marfan and Loeys-Dietz syndromes.[13]

The secundum atrial septal defect and atrial septal aneurysm, were likely caused by the *LTBP3* variants as TGFβ-BMP signaling has been shown to be important for development of the atrioventricular septal complex and septum primum, with disruptions resulting in atrial septal defects.[14]

In conclusion, a novel (p.Arg545ProfsTer22) and a recurrent (c.3107-2A>G; rs112261752) *LTBP3* variants are reported in a patient with DASS. Our study shows that heterozygous carriers can display minor phenotypes associated with DASS, but without the characteristic features of significant short stature with brachyolmia and dental anomalies.[2-5] To the best of our knowledge, this is the first report showing that heterozygous carriers of *LTBP3* variants could have phenotypes.The patient who carried two *LTPB3* variants was more severely affected than those with a variant. The newly recognized findings of DASS found in this family include abnormal dentin, calcified dental pulp blood vessels, taurodontism, single-rooted molars, failure of mandibular tooth eruption, irregular alveolar bone, prognathic mandible, secundum atrial septal defect, interatrial septal aneurysm, and a recurrent glenohumeral joint

dislocation. These findings should be investigated in DASS patients in the future to determine whether they are key, but unappreciated features of the syndrome.

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CONFLICT OF INTEREST All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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Figure legends

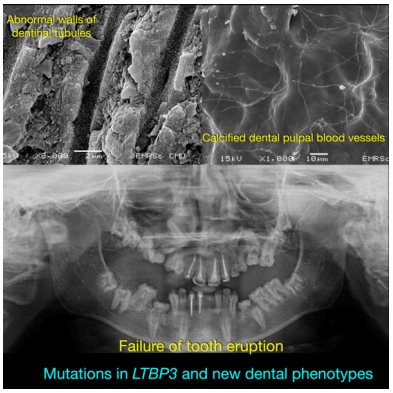
Figure 1. A) Pedigree and *LTBP3* variants. B) Patient 1 at age 19 years. C,D,F) Amelogenesis imperfecta, hypoplastic type, micodontia, irregular mandibular alveolar ridge (arrows), and mandibular prognathism. E) Panoramic radiograph at age 5 shows generalized large pulp chambers and root canal spaces, generalized enamel hypoplasia of the erupted and unerupted teeth. Ankylosis of the mandibular second primary molars is associated with unerupted mandibular second premolars. G) Panoramic radiograph at age 19 shows generalized absence of enamel, multiple unerupted teeth and focal areas of sclerotic bone. Taurodontism and single-rooted molars (asterisks). Mandibular alveolar bone defects (arrows), ankylosed teeth, and unerupted mandibular second premolars. Pulp stones are observed.

Figure 2. Echocardiographs. Patient 1 at age 19 years. A) 8-mm secundum atrial septal defect with a left-to-right shunt. B) An interatrial septal aneurysm. C) Mitral valve prolapses towards the atrium in systole. D) Aortic root enlargement at the level of sinus valsalva. **Patient 2. At age 44 years.** E) A mild aortic root enlargement at the level of sinus valsalva. F) An interatrial septal aneurysm.

Figure 3. An exfoliated primary tooth of patient 1. SEM of coronal dentin A) Speckled appearance of the wall of dentinal tubules. B) **Normal tooth as control.** Collagen bundles are evident in normal dentin. **SEM of root dentin. C-F.** Numerous areas of lacunae and network of mineralized blood vessels at the wall of lacunae.

Graphical abstract text

The newly recognized findings of DASS include abnormal dentin, calcified dental pulp blood vessels, taurodontism, single-rooted molars, failure of mandibular tooth eruption, irregular alveolar bone, prognathic mandible, secundum atrial septal defect, interatrial septal aneurysm, and a recurrent glenohumeral joint dislocation.



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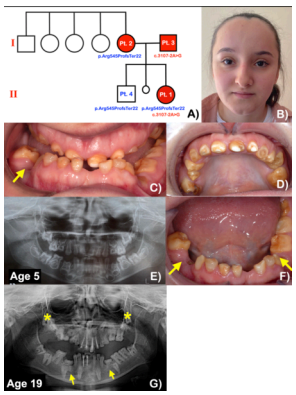

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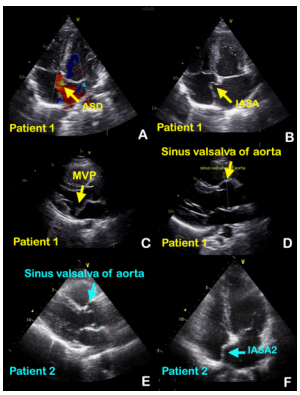

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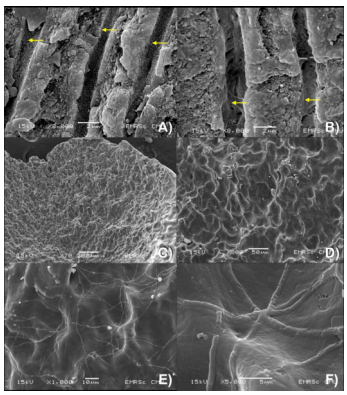

CGE_14134_LTBP3 revision Figure 3.tiff