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Case Report

Novel heterozygous mutation in *TBX1* in an infant with hypocalcemic seizures

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Abstract. Patients with 22q11.2 deletion syndrome have characteristic facial appearance, palate abnormalities, hypoparathyroidism, thymic hypoplasia, and congenital heart disease. The 22q11.2 region includes *TBX1* and 30 other genes. Analysis of *Tbx1* transgenic mice showed that *TBX1* was associated with the 22q11.2 deletion syndrome. In humans, *TBX1* mutations have been reported in 22q11.2 deletion-negative patients with velocardiofacial syndrome or DiGeorge syndrome. Genotype-phenotype correlations are not fully understood in these patients. We report the case of an infant with a novel heterozygous *TBX1* mutation who experienced hypocalcemic seizures. This patient had no palate abnormalities, cardiac anomalies, or the typical facial appearance observed in 22q11.2 deletion syndrome. The presence of thymic hypoplasia prompted us to perform G-banding, fluorescent *in situ* hybridization, and subsequent *TBX1* analysis. We emphasize the importance of diagnosing thymic hypoplasia in hypocalcemic infants without 22q11.2 deletion for detecting *TBX1* mutations.

Key words: hypocalcemia, thymus gland, hypoparathyroidism

Introduction

The 22q11.2 deletion syndrome is a multiple gene deletion syndrome characterized by congenital heart disease, palate abnormalities such as cleft palate, sub-mucous cleft palate, and velopharyngeal insufficiency, hypoparathyroidism, immunodeficiency derived from thymus hypoplasia, and distinctive facial features (1). DiGeorge syndrome (OMIM: 188400), velocardiofacial syndrome (OMIM: 192430), and some cases of conotruncal anomaly face syndrome (OMIM: 217095) are also associated with this syndrome.

The 22q11.2 region covers approximately 30 genes, including *TBX1*, which was shown to be causative agent of the 22q11.2 deletion

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syndrome in *Tbx1* transgenic mice (2–4). *TBX1* encodes a transcription factor that regulates organogenesis. Three isoforms of the *TBX1* mRNA (*TBX1A*, *TBX1B*, and *TBX1C*) have been; although all three isoforms contain exons 1–8, they differ in the incorporation of subsequent exons, namely, exons 9A, 9B/10, and 9C (5). Of the three isoforms, *TBX1C* is the main transcript in humans. *TBX1* mutations were previously identified in patients with clinically diagnosed 22q11.2 deletion syndrome in whom deletion of the entire 22q11.2 region was not detected (5–9). However, the genotype-phenotype correlations have not yet been completely understood as only 10 *TBX1* mutations have been reported to date (8).

Here, we report the case of an infant with a novel heterozygous *TBX1* mutation who experienced hypocalcemic seizures from the neonatal period.

Case Presentation

A 2-mo-old boy was referred to the pediatric neurological department of Okayama University hospital because of repetitive seizures that started at 1 mo of age. He was born by vaginal delivery at 38 wk and 4 d and was the second child of non-consanguineous parents. His birth weight was 2,895 g (−0.48 SD), and he was solely breast-fed. Physiological examination at birth showed no abnormalities except for postaxial polydactyly of the right fifth toe (Fig. 1). His family history revealed that his uncle experienced febrile seizures during childhood (Fig. 2). The younger brother of his paternal grandfather also experienced seizures, although the etiology was unclear. There was no family history of polydactyly, congenital heart disease, and hypocalcemia.

At 1 mo of age, recurrent afebrile clonic left hand seizures were observed. Physiological examination did not reveal any new abnormalities, and palate abnormalities and characteristic facial features associated with the 22q11.2 deletion syndrome were not observed. Moreover, no abnormalities were detected by cerebrospinal fluid testing, head computed tomography, echocardiography, or electroencephalogram. Biochemical examination on admission (Table 1) revealed severe hypocalcemia (4.9 mg/dl), hyperphosphatemia (12.2 mg/dl), and low serum 25(OH) vitamin D levels (15 ng/ml). Urinary excretion of calcium was also low. Continuous intravenous calcium infusion and oral alfacalcidol (0.25 μg/d) corrected the hypocalcemia and hyperphosphatemia. A few days after admission,

Fig. 1. Appearance of postaxial polydactyly of the right fifth toe.

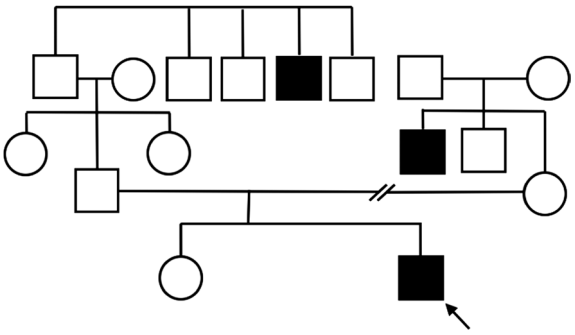

Fig. 2. Pedigree of the patient. The arrow indicates the proband and the black box indicates the history of seizure.

Table 1. Results of laboratory examination on admission

TP	5.4 g/dL	BUN	5.2 mg/dL
Alb	3.8 g/dL	CRTN	0.36 mg/dL
AST	46 U/L	fT4	1.0 ng/dL
ALT	18 U/L	TSH	3.75 μU/mL
ALP	2085 U/L	25(OH) vitamin D	15 ng/mL
LDH	651 U/L	$1,25(OH)_2$ vitamin D	62 pg/mL
CPK	1146 U/L	Intact PTH	42 pg/mL
Na	139 mmol/L	Ionized calcium	0.67 mmol/L
K	4.7 mmol/L	Urinary Ca	0.6 mg/dL
Cl	103 mmol/L	Urinary CRTN	22.7 mg/dL
Ca	4.7 mg/dL	Urinary Ca/CRTN	0.03
IP	12.2 mg/dL		
Mg	1.9 mg/dL		

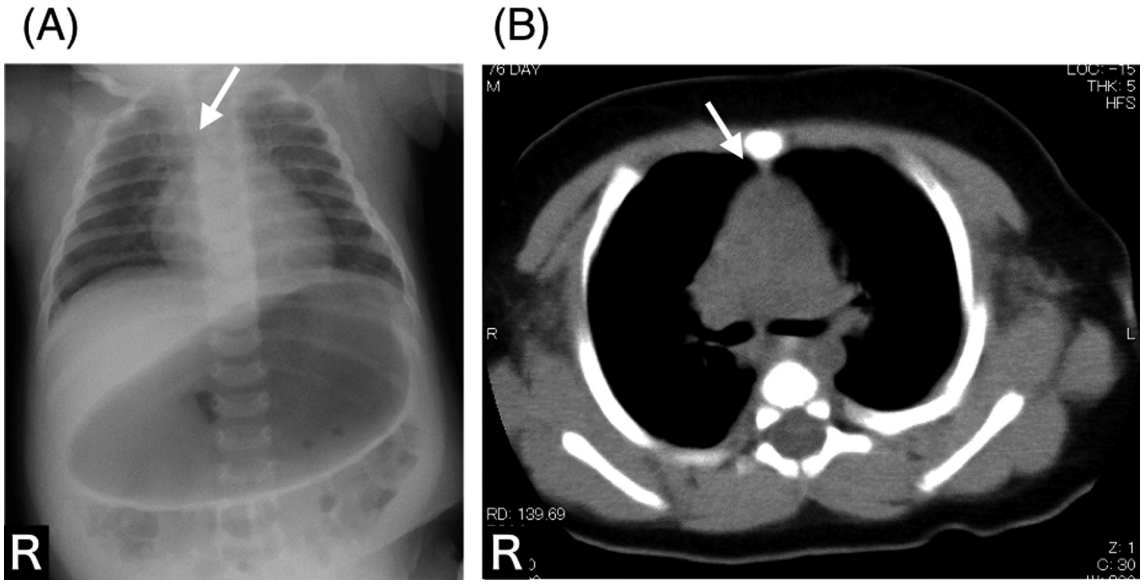

Fig. 3. Results of radiological examination on admission. Chest roentgenogram (A) and chest computed tomography analysis (B) revealed that the thymus (white arrows) is hypoplastic.

plasma PTH level was found to be normal (42 pg/ml) despite the hypocalcemia, suggesting that the patient had hypoparathyroidism. Radiological examination revealed thymic hypoplasia (Fig. 3), which is indicative of 22q11.2 deletion syndrome combined with hypoparathyroidism. No deletion of 22q11.2 was detected by G-banding and fluorescent *in situ* hybridization analysis.

The development and growth of the patient is normal at 9 yr of age, and no seizures have been observed since the correction of hypocalcemia. His facial appearance is also normal. The dosage of oral alfacalcidol has been increased to 1.0 μg/d from 0.25 μg/d at 2 mo of age because mild hypocalcemia was detected during his growth.

We obtained informed consent from his parents to conduct *TBX1* analysis. Genomic DNA from peripheral leukocytes was extracted using the QIAamp DNA blood mini kit (Qiagen Inc., Tokyo, Japan). The genetic analysis was approved by the ethical committee of the Okayama University Hospital. All exons and

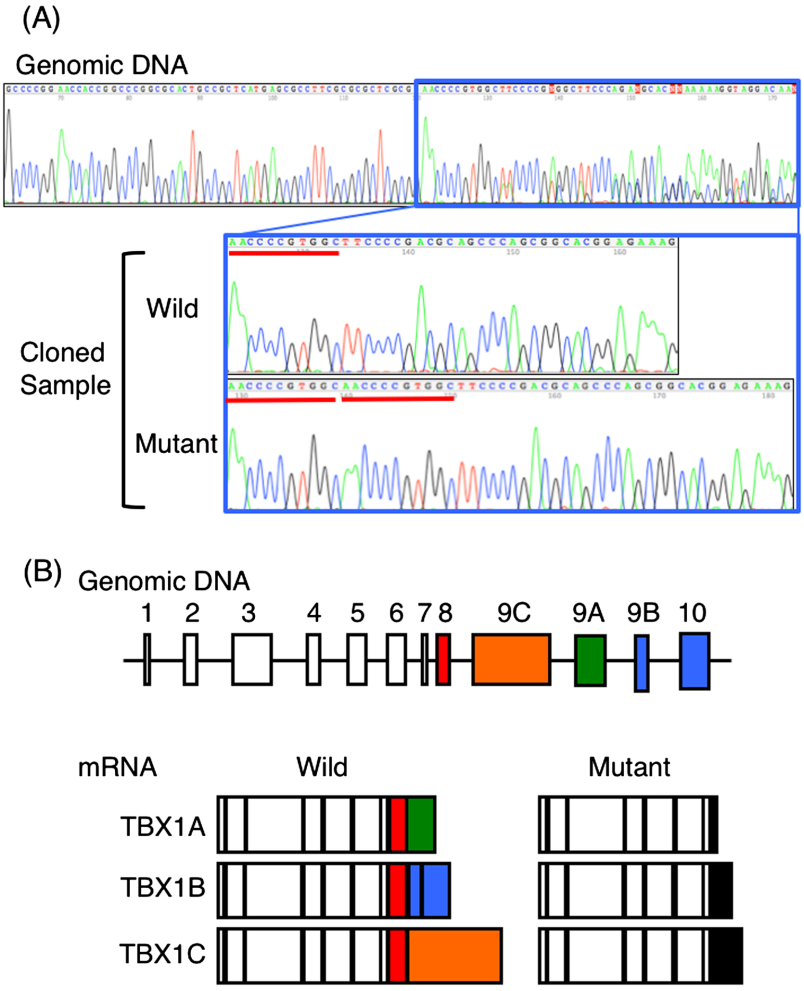

Fig. 4. Results of *TBX1* analysis. A: A frameshift mutation was identified in *TBX1* exon 8. An 11 bp duplication (red bar) of the wild-type allele was detected in the mutant allele (c.967_977dupAACCCCGTGGC). B: Schematic representation of *TBX1* genomic DNA and *TBX1* mRNA. Premature termination occurs because of the 11 bp duplication in exon 8 (red box), which shortens all mutant isoforms of *TBX1* mRNA: p.Ala326AlafsX20 in *TBX1A* mRNA (NM_080646.1), p.Ala326AlafsX33 in *TBX1B* (NM_005992.1), and Ala326AlafsX48 in *TBX1C* mRNA (NM_080647.1).

exon–intron boundaries of *TBX1* (accession number: NC_000022.11) were amplified using standard polymerase chain reaction (PCR). Primer sequences are available on demand. Amplified products were purified by the QIAquick PCR purification kit (Qiagen Inc.) and sequenced using the BigDye® Terminator v3.1 cycle sequencing kit and the ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Next, the amplicon was cloned into PCR® 4-TOPO® vector using the TOPO® TA cloning kit for sequencing (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and each transformant was sequenced. As a result, we identified a frameshift mutation caused by the heterozygous duplication of 11 bases (c.967_977dupAACCCCGTGGC) in exon 8 (Fig. 4A). This duplication was not found in databases such as dbSNP (https://www.ncbi.nlm.nih.gov/SNP) and the Human Genetic Variation database (http://www.hgvd.genome.med.kyoto-u.ac.jp). The mutation was predicted to cause a premature termination in the three

isoforms of the *TBX1* mRNA (Fig. 4B). Familial analysis was not performed because his parents were divorced. Therefore, we could not determine whether this mutation was *de novo* or inherited from his parents.

Discussion

We identified a novel heterozygous *TBX1* mutation in a 2-mo-old boy with hypocalcemic seizures. To our knowledge, this is the second youngest patient in whom a *TBX1* mutation has been identified (8).

Tbx1 is a key gene involved in the development of the normal parathyroid gland, and abnormal *Tbx1* mRNA expression has been shown to cause hypoparathyroidism in *Tbx1* transgenic or knockout mice (10). Here, we report that thymic hypoplasia is also caused by dosage change of *Tbx1*. On the contrary, thymic hypoplasia is not reported in patients with *CRKL* and *MAPK1* deletions and in heterozygous or homozygous *Crkl* knock out mouse (11–13). Furthermore, chromosomal analysis and FISH did not identify 22q11.2 deletion in our patient. Therefore, we analyzed *TBX1* for the presence of mutations.

Both gain-of-function and loss-of-function mutations were reported in patients with clinical features of the 22q11.2 deletion syndrome (7). Increased or decreased dose of *Tbx1* in mouse leads to hypoplasia of the thymus and parathyroid glands, which is also observed in human patients with the 22q11.2 deletion syndrome (10). The *TBX1* mutation identified in our case is predicted to cause premature termination of all *TBX1* mRNA isoforms, although we could not determine whether this mutation causes nonsense-mediated RNA decay. Further experiments are required to clarify the pathogenicity of this mutation in our patient.

We initially suspected vitamin D-deficient hypocalcemia because his serum 25(OH) vitamin D levels were low and other symptoms suggestive of 22q11.2 deletion syndrome, such as cardiac anomaly, palate abnormalities, and characteristic facial features, were not observed except for postaxial polydactyly of the toe which is observed occasionally among patients with 22q11.2 deletion (14). Thymic hypoplasia identified by radiological examination was the key finding that prompted us to consider the 22q11.2 deletion syndrome, and to conduct chromosomal analysis and subsequent *TBX1* analysis in this patient. A previous study of *TBX1* mutation-positive patients showed that all the phenotypes of the 22q11.2 deletion syndrome are not present in patients, resulting in the absence of a genotype-phenotype correlation (8). These results indicate the existence of a broad spectrum of phenotypes of patients with *TBX1* mutations, ranging from all symptoms observed in cases with the 22q11.2 deletion syndrome, such as cardiac anomaly, thymic hypoplasia, palate abnormalities, characteristic facial features, and hypoparathyroidism, to combinations of some of these symptoms as observed in our patient and in previously reported cases (5–9).

In conclusion, we identified a novel heterozygous *TBX1* mutation that resulted in hypoparathyroidism and thymic hypoplasia in our patient. We propose that 22q11.2 deletion-negative neonates and infants, who present with only hypoparathyroidism, should be examined for thymic hypoplasia, which might lead to the detection of *TBX1* mutations.

Conflict of Interest: The authors declare that they have no conflict of interest.

Acknowledgements

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