A Novel TBX1 variant causing hypoparathyroidism and deafness.

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Abstract

**Background:** The *TBX1* gene encodes the T-box 1 protein that is a transcription factor involved in development. Haploinsufficiency of the *TBX1* gene is reported to cause features similar to DiGeorge syndrome. The *TBX1* gene is located within the DiGeorge syndrome region, and studies support that the *TBX1* gene is responsible for most of the features of the phenotype of hemizygous deletion of chromosome 22q11.2. In this study, we report a family of 4 (a father with 3 children) who presented with congenital hypoparathyroidism and hypocalcemia, facial asymmetry, deafness, normal intelligence, and no cardiac involvement.

**Methods:** We performed whole-genome sequencing, computational structural analysis of the mutants and gene expression studies for all affected family members.

**Results:** Whole-genome sequencing revealed a paternal inherited novel heterozygous variant c.1158_1159delinsT P.(Gly387Alafs*73) in the exon 9 isoform C *TBX1* gene causing a loss of nuclear localization sequence (NLS) and Transactivation Domain (TAD) with no change in gene expression and resulted in DiGeorge like phenotype.

**Conclusion:** A pathogenic variant in the *TBX1* gene exon 9 C that predicted to cause a loss in the NLS region and most of TAD leads to variable features of hypoparathyroidism, distinctive facial features, deafness, and no cardiac involvement. In addition, our report and previous reports indicate the presence of wide phenotypic spectrum of *TBX1* genetic variants and consistently absence of cardiac involvement in the case of pathogenic variants on exon 9 isoform C *TBX1* gene.

**Keywords:** DiGeorge syndrome, TBX1 gene, hypocalcemia, hypoparathyroidism, SNHL
Introduction

22q11.2 deletion syndrome is considered one of the most common microdeletional syndromes in humans, which presents with a wide spectrum of phenotypes ranging from mild adulthood onset to severe neonatal presentations \(^1^2\). The prevalence is up to 14/100,000 births, with a mean age of diagnosis at 5 years \(^3\). DiGeorge syndrome is a contiguous gene syndrome caused by a 1.5–3 Mb microdeletion of 22q11.2 \(^4\). DiGeorge syndrome (conotruncal anomaly face syndrome or velocardiofacial syndrome) presents with variable phenotypes, including variable cardiovascular manifestations, endocrine manifestations with hypoparathyroidism, hypothyroidism and thymic hypoplasia, cleft palate and nasal speech, and CNS features with delayed psychomotor development associated with characteristic facial features \(^1^2\). One of the 30-40 genes deleted in this condition is the \(TBX1\) gene, and there is accumulating evidence that this gene is responsible for most of the phenotype of the 22q11.2 deletion \(^5^6^7\). Moreover, several studies described the \(TBX1\) variants as a causative genetic defect for DiGeorge syndrome because these \(TBX1\) variants cause a similar phenotype without microdeletion \(^6^8^9^10^11\).

DiGeorge syndrome caused by a pathogenic variant in the \(TBX1\) gene is a rare autosomal dominant disease that is associated with variable degrees of pathological phenotypes. The \(TBX1\) protein encoded by the \(TBX1\) gene is a member of the conserved T-box transcription factor family and can act as a gene enhancer or repressor in transcriptional regulation of developmental processes \(^12\). It has been demonstrated that \(TBX1\) mutations resulted in one of five phenotypes of 22q11.2, namely, deletion including conotruncal anomaly face syndrome, cardiac defect, velopharyngeal insufficiency, thymic hypoplasia or hypoparathyroidism. Intellectual disability and psychomotor retardation are not consistent features of \(TBX1\) mutation \(^6^8^9^10^11\). Several \(TBX1\) variants have been described as disease-causing variants through both loss and gain-of-function mutations. \(^10\) (Table 1).
Further studies revealed that $TBX1$ has three isoforms that share exons 1-8 but differ in exons 9, 9A, 9B/10 and 9C. Of these three isoforms, TBX1C is the main transcript in mice, and it has been shown in humans that the mutation in TBX1C results in features similar to the 22q11.2 deletion$^{13-14-15}$.

Here, we report a family of four presenting with hearing loss, hypoparathyroidism, facial asymmetry, nasal speech and dysmorphic features with no other congenital cardiac anomaly and normal intellectual function. Whole-genome sequencing uncovered a novel $TBX1$, c.1158_1159delinsT p.(Gly387Alafs*73) variant, which is consistent with a genetic diagnosis of a TBX1-associated phenotype with an autosomal dominant mode of inheritance.

Case III-C

A 5-years-old boy diagnosed with hypoparathyroidism at the age of one month when he had neonatal seizures. He is also known for speech delay, and bilateral sensory neural hearing loss (SNHL). He was followed up at a peripheral hospital and was on one alpha calcidiol drop only. He presented to our emergency room (ER) with a history of vomiting, abdominal pain, and numbness of hands for one day after he visited our cochlear transplantation center. He was born at 40 weeks of gestation via normal spontaneous vaginal delivery to a 31-year-old healthy mother and 33-year-old father (see Figure 1). The antenatal course was normal, followed by an unremarkable perinatal course with a birth weight of 2700 g. His father and two of his siblings had the same presentation (cases II-C, III-A, III-B). Physical examination showed weight 17.6 kg (25th percentile), height 109.6 cm (50th percentile) and head circumference 51 cm (25th-50th percentile). He has mild facial dysmorphism in the form of a prominent nose with a bulbous tip, small mouth and eyes, ocular hypertelorism, low-set ears and long face (see Figure 1A). The chest, cardiac and abdominal examination findings were unremarkable. He had a positive Chvostek sign. Investigations upon presentation to the ER
showed serum total calcium concentration 1.28 mmol/L (normal range 2.20-2.70), corrected calcium 1.32 mmol/L, serum phosphate 2.31 mmol/L (normal 1.12-1.45 mmol/L), Mg 0.63 mmol/L, and ALP 185 unit/l. The serum PTH concentration was low at 3.8 pg/ml (normal range 15-65 pg/ml), 25(OH)D was 119 nmol/l (normal 75-125 nmol/l), he had normal urinary calcium:creatinine ratio. The following parameters were normal: blood absolute T lymphocyte subsets, serum thyroid stimulating hormone, and serum free thyroxin. The patient was admitted to the pediatric intensive care unit (PICU) due to a prolonged QT interval secondary to hypocalcemia and was managed with intravenous calcium gluconate. Ultrasound of the abdomen showed normal kidney size, shape, echogenicity and parenchymal thickness with no hydronephrosis or renal stones. An electrocardiogram performed during hypocalcemia showed a prolonged QT interval with a normal Holter monitor. Cardiac echography showed normal cardiac anatomy, and he had a normal skeletal survey. CT scan of the temporal bone showed congenital deformity of the cochlea and vestibules with cystic communication between the middle and apical cochlear turns and dilated vestibules with hypoplastic semicircular canal. MRI revealed bilateral underdevelopment of inner ear structures with normal cochlear nerve bilaterally. After controlling his calcium level, the patient underwent cochlear implants. Whole-genome sequencing (WGS) revealed a heterozygous likely pathogenic variant identified in the TBX1 gene, variant c.1158_1159delinsT p.(Gly387Alafs*73).

Currently, at the age of 7 years, he is on elemental calcium (57 mg/kg/day) and 1-alpha-calcidiol (one mcg daily). His latest investigations and his growth parameters were normal.

**Case III-B**

A 12-year-old boy was diagnosed with hypoparathyroidism incidentally. During the admission of his youngest brother (Case III-C) with acute hypocalcemia, we evaluated him
for hypoparathyroidism because he had bilateral SNHL and similar dysmorphic features.

Upon evaluation, the mother gave a history of two seizures in the context of fever that did not require treatment and one incidental hypocalcemia at the age of 2 years found during routine blood investigations, for which he was given calcium supplementation for less than one month then it was stopped. Since stopping the calcium supplements, he reports negative symptoms and signs for hypocalcemia. During admission, he was found to have a prolonged QT interval and severe hypocalcemia that was managed with intravenous calcium gluconate infusion.

He was born at 38 weeks by spontaneous vaginal delivery with a birth weight of 2.7 kg. The developmental milestones were reported to be delayed, and he is currently attending special needs school. Physical examination showed hypertelorism, downward slanting palpebral fissures, bulbous nose, and low-set ears (See Figure 1B). Height was 161.4 cm (31.44 percentile), weight was 57 kg (67.66 percentile), and BMI was 21.88 kg/m2 (79.32 percentile). He had a positive Chvostek sign. Other systemic examinations were normal. Laboratory investigations revealed serum total calcium concentration 1.48 mmol/L, corrected calcium 1.58 mmol/L, phosphate 3.32 mmol/L, magnesium 0.76 mmol/L, 25(OH)D 65.1 nmol/l, and PTH: 0.53 pg/ml. Blood absolute T lymphocyte subsets, serum thyroid stimulating hormone (TSH), serum free thyroxin (FT4). Brain MRI showed severe malformation of the cochlea. Currently, at the age of 14 years, he is on 42 mg/kg/day of elemental calcium and calcitriol 1.25 mcg twice daily. His current serum total calcium concentration is 2.09 mmol/L, corrected calcium 2.13, phosphate 2.56 mmol/L, magnesium 0.7 mmol/L, and 25(OH)D: 71.63 nmol/l.
Case III-A

A 14-year, 6-month-old sister of cases III-B and III-C was evaluated for bilateral SNHL and hypocalcaemia following the hospitalization of case III-C. Unfortunately, at age of 1 year, she was treated for recurrent otitis media as a presumed cause of hearing impairment until the age of 3 years. She had history of seizure at age of 3 years and at age of 12 years; at that time hypoparathyroidism was not diagnosed. Evaluation of inner ear pathology by MRI revealed bilateral congenital deformity of the inner ear structures, absent left cochlear nerve and the right is significantly small. Temporal bone computed tomography revealed slightly hypoplastic internal auditory canals. During the preoperative assessment of cochlear implant, she was found to have prolong QT interval secondary to hypocalcemia. Holter monitoring was performed for 24 hours and was normal then she was referred to an adult endocrinology for follow up.

She was born at term via spontaneous vaginal delivery with normal antenatal and perinatal courses. Her birth weight was 3 kg. Physical examination showed low-set ears with delayed eruption of adult teeth, and normal development. Weight was 51.1 kg (5th percentile), height was 146.2 cm (< 3rd percentile) and head circumference was 54.5 cm (50th percentile). She had negative Chvostek sign and Trousseau signs. The chest, cardiac and abdominal examination findings were all unremarkable. The serum total calcium concentration was low at 1.82 mmol/L, corrected calcium was at 1.86 mmol/L, serum phosphate 1.96 mmol/L, 25(OH)D 62.52 nmol/L, and PTH 0.679 pg/ml. She had a normal thyroid function test. Renal ultrasound showed that both kidneys were normal in size, shape and position, normal cortical thickness and echogenicity with no evidence of stones, hydronephrosis or renal masses. Audiology screening showed bilateral severe profound sensory neural hearing loss. A skeletal survey showed no deformity. Currently, at the age of 16 years, she is on 1-alpha-calcidiol, one mcg twice per day and calcium carbonate 600 mg twice a day. Her last evaluation revealed a
total corrected calcium of 2.21 mmol/L, phosphorus of 1.9 mmol/L, and her growth parameters; her weight was 44.6 (< 25<sup>th</sup> percentile) and her height was 152 cm (5<sup>th</sup> percentile).

**Case II-C**

The father is 39 years old and was diagnosed with hypoparathyroidism at 4 years old. He presented to a peripheral hospital with a low calcium level leading to seizure. Since then, he had been having recurrent symptoms of perioral numbness, blacking out, and seizures. His symptoms were relieved with intermittent calcium infusion during emergency department visits. He had no regular follow-up for his hypocalcemia and had not been on regular calcium or vitamin D replacement since the age of 4 years. He had been complaining of delayed eruption of adult teeth. He is not known to have SNHL, but he has conductive hearing impairment.

He is married to a non-consanguineous healthy female (see Figure 1) His parents, siblings, and 2<sup>nd</sup> degree relatives did not have a history of SNHL, hypocalcemia, sudden death or cardiac diseases. He was referred to an adult endocrinology clinic during hospitalization for Case III-C. At the first visit in our adult endocrinology clinic, his calcium level was 1.98 mmol/L, corrected calcium was 2.00 mmol/L, phosphorus level was 1.62 mmol/L, and 25(OH)D was 32.2 nmol/L. He has a normal thyroid function test and immunological studies. He was seen at the otolaryngology clinic because he was complaining of left ear pain and nasal obstruction for a long time, and diagnosed with left ear central perforation and nasal deformity. He had a normal hearing assessment with no mental or developmental delay.

On physical examination, he has low-set prominent ears, facial asymmetry, absent adult teeth, nasal speech and a bulbous nose (See Figure 1C). His height was 156 cm (<3th centile), and his weight was 72 kg with a BMI of 29.24. He was started on regular calcium carbonate 1200
mg three times daily and one mcg twice per day and 1-alpha-calcidiol one mcg daily. Since then, he has normal calcium levels and no further symptoms of hypocalcemia. During his last visit, his total calcium level was 2.34 mmol/L, and his corrected calcium level was 2.36 mmol/L phosphorus 1.69 mmol/L.

**Materials and methods:**

**Whole genome sequencing :**

Genomic DNA was fragmented by sonication, and Illumina adaptors were ligated to generated fragments for subsequent sequencing on the HiSeqX platform (Illumina) to yield an average coverage depth of ~30X. An end-to-end in-house bioinformatics pipeline including base calling, primary filtering of low quality reads and probable artifacts, and annotation of variants was applied. CNV calling is based on the HAS pipeline. All disease-causing variants reported in HGMD®, in ClinVar or in CentoMD® in addition to all variants with minor allele frequency (MAF) of less than 1% in the gnomAD database were considered. Evaluation is focused on coding exons along with flanking +/-20 intronic bases; however, extended to the complete gene region for candidate genes or in search for a second previously described variant in AR inheritance pattern. All pertinent inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate eventually identified variants. All identified variants were evaluated with respect to their pathogenicity and causality. All variants related to the phenotype of the patient, except benign or likely benign variants, are reported. CNVs of unknown significance are not reported. Reported CNVs are confirmed with another method, such as MLPA and qPCR. Variants of relevance identified by NGS are continuously and individually validated in-house for quality aspects; those variants that meet our internal QC criteria (based on extensive
validation processes) are not validated by Sanger. To study mutations indicated by exome sequencing, Sanger sequencing was performed for all family members.

**Computational structural analysis of mutants**

MUSCLE (www.ebi.ac.uk/Tools/msa/muscle) was used for sequence alignments. RaptorX was used to produce homology models and to predict secondary structure and disorder. The eukaryotic linear motif (ELM) resource was used for the identification of functional sites in proteins.

**Gene expression studies**

RNA was extracted from PBMC of all family members. The isolated RNA was reverse-transcribed into cDNA using the M-MLV reverse transcriptase assay (Promega Corporation, Maddison, WI, USA). Real-time PCR amplification of the obtained cDNA targets was performed using the Power SYBR Green PCR Kit (Applied Biosystems). Specific cDNA primers (Integrated DNA Technologies, Leuven, Belgium) were used to determine the expression of TBX1. The expression was determined using the 7900HT fast real-time quantitative PCR system (Applied Biosystems, CA, USA). The relative expression of all genes of interest were normalized to GAPDH and determined by the ΔΔCt method.

**Results**

**TBX1 gene mutations**

A whole genome sequencing was performed on III-C and a Gly387Ala_fs* heterozygous variant was identified in TBX1 gene. This variant was also found in II-C, III-A, and III-B but not in II-D and III-D by sanger sequencing. The mutation c.1158_1159delinsT was
mapped to the *TBX1* gene and was predicted to be of high impact variation resulting in a frameshift by *in-silico* protein prediction algorithms and predicted to be present on protein TBX1-*isoform* C with a length of 495 aa. A mutation change from Glycine to Alanine was found at position 387 and with a stop codon at position 73, changing the protein length to 459 aa. No other mutations causing hypoparathyroidism were identified.

**Predicted effect**

The TBX1-*iso* C protein is 495 amino acids long, and the DNA-binding T-box domain is formed by residues 109-206 \(^{18}\) (Figure 2). The frameshift mutation results in a protein that deviates from TBX1-*iso* C after residue 386, substituting the normal C-terminal 109-residue sequence with an unrelated sequence of 72 amino acids (Figure 2A). The affected protein region is predicted to be unstructured, and hence, the mutation would not affect an existing 3D protein fold. The function of the replaced residues in TBX1 is unknown. However, the deleted region contains several sequence motifs that are predicted to serve as sites for protein interactions or posttranslational modifications (PTM). Importantly, the deleted region contains a nuclear localization sequence (NLS, residues 430 – 441) and a transactivation domain (TAD; localized within residues 409-495 \(^{13}\) (Figure 2). Combined deletion of these two functional regions has already been observed in DiGeorge syndrome patients and is likely also causing the phenotype observed here.

**Gene expression studies:**

Our data revealed an unchanged gene expression by RT-PCR between the affected and non-affected individuals, the predicted effect is a protein dysfunction that lacks NLS and TAD (Figure 3).
Discussion

Craniofacial features and hypoparathyroidism were the presenting features in all affected family members, but deafness was variable within the family that suggested an autosomal dominant inheritance. The lack of renal involvement in all four cases made HDR syndrome (hypoparathyroidism, sensorineural deafness and renal disease) less likely, and the lack of concotruncal cardiac involvement and recurrent infections made DiGeorge syndrome less likely at the time of evaluation. Other causes of hypoparathyroidism are not typically associated with hearing loss, such as Kenny-Caffey type 1 caused by mutation in TBCE gene and type 2 caused by mutation in FAM111A, Kearns-Sayre syndrome due to deletion in mitochondrial DNA, familial hypercalciuric hypocalcemia with mutation in CASR gene, the GMC2 gene mutation and isolated PTH gene defects will cause autosomal dominant and recessive hypothyroidism. Therefore, we assumed that we are dealing with a novel syndrome or a known syndrome with novel presentation.

Whole-genome sequencing identifies a high impact novel variation resulting in a frameshift in the exon 9 TBX1 gene (Gly387Ala_fs*) that was co-segregated within the family. This report is the third to describe a variant affecting isoform C in the TBX1 gene that resulted in a 22q11.2 deletion-like phenotype. This mutation causes a shift of the reading frame, rearranging the PTM sites (S394 and R401), and most likely disturbing the T-box protein conformation, stability and/or function. Additionally, both nuclear localization signal (NLS) region (430-441 aa) and transactivation domain (409-495) are two regions proceeding the frameshift that are likely to be abolished (Figure 4), resulting in the inability of the protein to localize to the nucleus. Given that our data revealed an unchanged gene expression by RT-PCR between the affected and non-affected individuals (Figure 3), the predicted effect is a protein dysfunction that lacks NLS and TAD.
Ogata et al. (2014) described a Japanese family with presentation similar to our family; the family had craniofacial changes and hypocalcemia with variable deafness, and the TBX1 variant was very close to the variant in our family (c.1253delA, p. Y418fsX459), producing a nonfunctional protein lacking NLS and most TAD. Additionally, Li et al. (2018) reported a mutation that disrupted the NLS and TAD region and presented mostly with hypoparathyroidism, and deafness presentation varied in these patients, similar to ours. Both of these families have no cardiovascular involvement, similar to ours. It seems that as long as T-box is intact, there is no cardiac involvement. Yagi et al. (2003) described a link between the genotype and the phenotype by examining the type of mutated TBX1 transcripts (TBX1A, TBX1B, and TBX1C), as reported in the family with the TBX1C mutation, which had a lower score of the 22q11.2 deletion phenotype. Our family is the third family that has a mutation affecting TBX1C transcripts and resulted in a 22q11.1 similar phenotype but without cardiac involvement.

Most likely, the TBX1 isoform C is the major functioning transcript isoform. In addition to this characteristic feature, the TBX1 gene has other unique features including: (1) the TBX1 gene acts as a dosage-sensitive transcription factor either as a gene enhancer or repressor and is thus involved in the transcriptional regulation of developmental processes, (2) A specific genotype resulted in variable phenotype with significant intra- and interfamilial variability even among monozygotic twins ranging from an asymptomatic heterozygous carrier, isolated non-syndromic deafness or isolated hypoparathyroidism to a DiGeorge-like phenotype which indicates variable expressivity or reduced penetrance, (3) autosomal dominant disorder with gain-of-function and loss-of-function variants disrupt gene function and result in a similar phenotype, and (4) the TBX1 gene is considered responsible for most clinical features in 22q11.2 deletion syndrome.
Few theoretical suggestions could explain the phenotypic variability in TBX1 gene (Figure 5), including the genotype and phenotype correlation, e.g., loss of NLS and TAD domains is presented as a similar phenotype in our family and 2 other families, and the minor differences could be attributed to the level of disruption of these domains, variable expressivity and reduced penetrance as well as environmental factors. Additionally, epigenetic changes might be considered in the future.

One of the limitations in our study is the absence of a functional study of the variants; hence, we are unable to prove the defect in nuclear localization.

In summary, DiGeorge syndrome caused by a pathogenic variant in the TBX1 gene is a rare autosomal dominant disease that is associated with variable phenotype. We report a family of 4 members who presented with congenital hypoparathyroidism, facial dysmorphism, normal intelligence, deafness and no cardiac involvement. Whole-genome sequencing revealed a paternally inherited novel heterozygous variant c.1158_1159delinsT p.(Gly387Alafs*73) in the exon 9 isoform C TBX1 gene, which we think is responsible for the phenotype within the family.
Declaration of Interest

The authors have no conflict of interest to declare.

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Informed consent: Written informed consent was obtained from the family in Arabic language.
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Figure legends:

Figure 1. Family pedigree and the distinctive facial features of the affected individuals.

Figure 2. Effect of the p.(Gly387Alafs*73) mutation in the protein. A) Color-coded sequence alignment of the TBX1 isoform C and its mutant sequence. The DNA-binding T-box domain is underlined. The position where wild-type and mutant deviate is indicated by an arrow. The NLS is indicated with a box. B) Structural context of the mutation. The 3D structure of the TBX1 T-box dimer (dark and light gray) bound to DNA is shown (taken from PDB 4a04). For one molecule of the dimer, the flexible regions are illustrated by dashed (N-terminal) and dotted (C-terminal) lines. The arrow and boxed region show the approximal location of the mutation and NLS, respectively.

Figure 3. Relative expression of TBX1 mRNA.

Figure 4. TBX1 gene from genomic to protein landscape displaying the mature protein length. Protein Domain Modeling is based on mature protein with length of 495 amino acids. Exhibited mutation change Glycine to Alanine on position 387 and with a stop-codon at position 73, changing protein length to 459 amino acid. Both Nuclear Localization Signal (NLS) region (430-441aa) and Transactivation Domain (TAD) (409-495) are two regions proceeding the frameshift that likely to be abolished.

Figure 5: A correlation between 22q11 deletion site / TBX1 gene mutation site and phenotypic expression. The bars below depicted the site and the size of 22q11 deletion and
the associated clinical phenotype. The bar on the top depicted the location of \( \text{TBX1} \) gene pathogenic variant and the associated phenotype\(^1\).
Table 1: Reported TBX1 gene related phenotypes

| References                  | Conotruncal anomaly | Endocrine | Immunology | Craniofacial | Velopharyngeal | Deafness | Psychodevelopmental | Others | Molecular genetic |
|-----------------------------|---------------------|-----------|------------|--------------|----------------|----------|---------------------|--------|-------------------|
| **Yagi, et al. (2003)**     |                     |           |            |              |                |          |                     |        |                   |
| F-1                         | TOF/PA              | -ve       | -ve        | +ve          | +ve            | -ve      | Normal              | F148Y  | GoF               |
| F-2                         | IAA/VSD             | +ve       | +ve        | +ve          | +ve            | +ve      | Normal              | G310S  | GoF               |
| F-3-1                       | TOF/RAA             | -ve       | +ve        | +ve          | -ve            | -ve      | Normal              | 1223delC | LoF            |
| II                          |                     | -ve       | -ve        | +ve          | +ve            | -ve      |                     |        |                   |
| III                         |                     | +ve       | -ve        | +ve          | +ve            | -ve      |                     |        |                   |
| **Paylor, et al. (2006)**   |                     | NA        | NA         | Characteristic facial appearance of VCFS and hypernasal speech | NA | NA | Depression/normal IQ |        |                   |
| F-4-1                       | Negative            | NA        | NA         | NA           | NA             | DD       | Normal              | H194Q  | GoF               |
| II                          | TOF                 | -ve       | -ve        | +ve          | -ve            | DD       | Normal              |        |                   |
| III                         | Pulmonary stenosis  | -ve       | -ve        | +ve          | -ve            | DD       | Normal              |        |                   |
| **Zweier, et al. (2007)**   |                     | -ve       | -ve        | +ve          | -ve            | -ve      | DD                  |        |                   |
| F-5-I                       |                     | -ve       | -ve        | +ve          | -ve            | DD       | DD                  |        |                   |
| -II                         |                     | +ve       | -ve        | +ve          | -ve            | DD       | DD                  |        |                   |
| **Rauch, et al. (2010)**    |                     | TOF/VSD/absent Pulmonary veins | Facial asymmetry | Normal | Scoliosis | Frequent infections |        |                   |
| F-6-I                       |                     | +ve       | -ve        | NA           | +ve            | +ve      | DD                  | c.1399-1428dup30 | LoF |
| II                          |                     | -ve       | -ve        | NA           | +ve            | +ve      | DD                  |        |                   |
| III                         |                     | +ve       | -ve        | NA           | +ve            | +ve      | DD                  |        |                   |
| IV                          |                     | +ve       | -ve        | NA           | +ve            | +ve      | DD                  |        |                   |
| **Ogata, et al. (2014)**    |                     | -ve       | +ve        | -ve          | +ve            | +ve      | DD                  | c.1253delA, p.Y418fsX459 | Loss of NLS |
| F-7-1                       |                     | -ve       | -ve        | NA           | +ve            | +ve      | DD                  |        |                   |
| II                          |                     | -ve       | +ve        | NA           | +ve            | +ve      | DD                  |        |                   |
| III                         |                     | -ve       | -ve        | NA           | +ve            | +ve      | DD                  |        |                   |
| IV                          |                     | -ve       | +ve        | NA           | +ve            | +ve      | DD                  |        |                   |
| V                           |                     | +ve       | -ve        | -ve          | -ve            | DD       | Graves disease      |        |                   |
| **Hasegawa, et al. (2018)** |                     | -ve       | +ve        | Thymic hypoplasia | -ve | -ve | Normal | Postaxial polydactyly of |        |                   |
| F-8                         |                     | -ve       | +ve        | -ve          | -ve            | DD       | DD                  | c.967_977dupAACCCCGTGGC | LoF |
|                           |                     |           |            |              |                |          |                     |        |                   |

**Variant**
- F148Y: Frameshift
- G310S: Missense
- 1223delC:frameshift deletion (1320-1342del23bp)
- H194Q: Missense
- c.1399-1428dup30: frameshift deletion
- c.1253delA, p.Y418fsX459: frameshift deletion
- c.967_977dupAACCCCGTGGC: frameshift deletion

**Predicted effect**
- GoF: Gain of function
- LoF: Loss of function
- Normal: Normal phenotype
- DD: Developmental delay
- Short stature: Short stature
- Graves disease: Graves disease
- Thymic hypoplasia: Thymic hypoplasia
- Hypernasal speech: Hypernasal speech
- Characteristic facial appearance of VCFS: Charateristic facial appearance of VCFS
- Normal IQ: Normal IQ
| Our family | III-C | -ve | +ve | -ve | +ve | -ve | Normal | c.1158_1159delinsT p.(Gly387Ala fs*73) | Loss of NLS |
| III-B | -ve | +ve | -ve | +ve | -ve | +ve | DD | c.1158_1159delinsT p.(Gly387Ala fs*73) | Loss of NLS |
| III-A | -ve | +ve | -ve | +ve | -ve | +ve | Normal | c.1158_1159delinsT p.(Gly387Ala fs*73) | Loss of NLS |
| II-C | -ve | +ve | -ve | +ve | -ve | -ve | Normal | c.1158_1159delinsT p.(Gly387Ala fs*73) | Loss of NLS |

DD: Developmental Delay

GoF: Gain of function

LoF: Loss of function

Loss of NLS (Nuclear Localization Signal)
Figure 1

[Genetic diagram showing family tree with labeled ages: I, II, III, A, B, C, D, E, F]

A 17-year old 14-year old 7-year old 6-month old

III-C

B

III-B

C

II-C
Figure 3: No significant modification of relative TBX1 gene expression in patients. The relative expression of TBX1 mRNAs obtained from PBMC cells was determined using the ΔΔCt method. No significant change in the relative expression of TBX1 was noted.
Figure 4

- New amino-acid sequence in the mutant protein
- DNA binding region
- Frameshift
- TAD region
- NLS

Genomic DNA

Ex 1 2 3 4 5 6 7 8 9C

Mature protein

Mutant protein

T-box transcription factor T-box DNA binding site

Ex 9
c.1158_1159delinsT
p.(Gly387Alafs*73)
Figure 5

Cardiac anomalies
- T-box transcription factor
- T-box DNA binding site
- Velopharyngeal insufficiency
- Facial anomalies
- Parathyroid dysfunction

TBX1

3 Mb deletion
2 Mb deletion
1.5 Mb deletion

Cent A B C D E F G H

B-D nested deletion Distal deletion

Phenotype
1-Facial anomaly
2-Hypoplastic thymus
3-Parathyroid dysfunction
4-Cardiac ventricular defects
5-Velopharyngeal insufficiency

1-Severe learning disability
2-Mental retardation
3-Psychiatric illness

1-Choanal atresia
2-CHARGE like