Bone 123 (2019) 153–158

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Severe brachydactyly and short stature resulting from a novel pathogenic *TRPS1* variant within the GATA DNA-binding domain☆,☆☆

Anara Karaca[a,b], Monica Reyes[a], Lauren T. Shumate[a], Isilay Taskaldiran[b], Tulay Omma[b], Nese Ersoz Gulcelik[b], Murat Bastepe[a,*]

[a] Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[b] Department of Endocrinology and Metabolism, Ankara Training and Research Hospital, Ankara, Turkey

ARTICLE INFO

Keywords:
Brachydactyly type E
Tricho-rhino-phalangeal syndrome (TRPS)
TRPS1
GATA DNA-binding domain
Skeletal dysplasia

ABSTRACT

Brachydactyly type E, which can be an isolated finding or part of a syndrome in combination with other clinical anomalies, involves metacarpals and metatarsals with or without short phalanges. Herein we report two unrelated Turkish females who presented with brachydactyly type E and vitamin D deficiency in the absence of marked alterations in serum calcium, phosphate, and parathyroid hormone. After excluding disease-causing variants in two candidate genes, *PTHLH* and *PDE4D*, we identified different pathogenic variants in *TRPS1*, the gene mutated in patients with tricho-rhino-phalangeal syndrome (TRPS). In one of the patients, who displayed severe brachydactyly and short stature, we identified a novel heterozygous missense pathogenic variant in exon 6 (c.2783A > G, p.Tyr928Cys), located within the GATA DNA-binding domain. The second patient, who had relatively milder brachydactyly and was of normal height, carried a heterozygous nonsense pathogenic variant in exon 4 (c. 1870C > T, p.Arg624Ter), which has been previously described. Both pathogenic variants segregated in affected family members. The patients additionally showed sparse hair and a bulbous nose, consistent with the clinical features of TRPS. Our findings, in addition to identifying the genetic cause of brachydactyly in two unrelated kindreds, emphasize the role of pathogenic *TRPS1* variants in the development of brachydactyly type E and highlight the GATA DNA-binding region of TRPS1 protein with respect to phenotype-genotype correlation.

1. Introduction

Congenital malformations of the long bones of the hands or feet are seen in many skeletal dysplasias, including brachydactyly, which refers to disproportionate shortening of digits [1]. Brachydactyly type E (BDE) involves the shortening of one or more metacarpals with normal or short phalanges, combined occasionally with short metatarsals [MIM# 113300]. Some individuals with BDE may also be of moderately short stature. BDE is inherited as an autosomal dominant trait with variable expressivity and results from pathogenic variants of genes important during bone development [2]. *HOXD13* pathogenic variants are responsible for isolated BDE, but most patients display features overlapping with brachydactyly type D, characterized by shortness of the distal phalanx of the thumb; some patients can also display syndactyly or synpolydactylyl [3,4]. Pathogenic variants in *PTHLH* are associated with BDE with short stature [5,6], and pathogenic variants in *PDE3A* are responsible for BDE with hypertension (Bilginturan Disease) [7]. Deletions of chromosome 2q37 involving *HDAC4* lead to BDE with intellectual disability [8,9]. In some cases, the phenotype is additionally complicated by multihormone resistance, such as in Albright's hereditary osteodystrophy and pseudohypoparathyroidism, which are caused by pathogenic variants in Gsα-coding exons of the *GNAS* complex locus [10,11]. Acrodysostosis, a disorder of severe BDE, short stature, and facial dysmorphism, result from pathogenic variants in *PRKAR1A* [12], particularly when associated with hormone resistance, or *PDE4D* [13,14]. Pathogenic variants in *TRPS1* also lead to BDE, as seen in patients with Tricho-rhino-phalangeal syndrome (TRPS), a rare autosomal dominant disorder of skeletal abnormalities primarily affecting distal bones, abnormal facial features with a distinctive nose shape, and sparse scalp hair [15,16].

In this report, we present clinical and genetic characterization of two unrelated patients and their family members with BDE in whom we

☆ All authors declare no conflict of interest.
☆☆ Funding source: NIH/NIDDK RO1 DK073911.
* Corresponding author at: Endocrine Unit, Massachusetts General Hospital, 50 Bloosom St. Thier 10, Boston, MA 02114, USA.
E-mail address: bastepe@helix.mgh.harvard.edu (M. Bastepe).

https://doi.org/10.1016/j.bone.2019.03.028
Received 14 December 2018; Received in revised form 4 March 2019; Accepted 21 March 2019
Available online 23 March 2019
8756-3282/

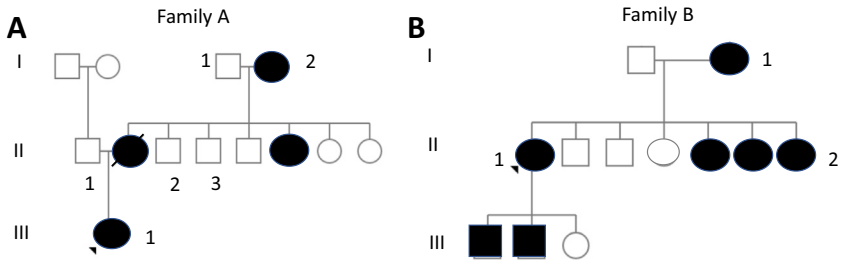


Fig. 1. Pedigrees of family A and B. Affected individuals are indicated by filled symbols and probands by arrows. Individuals whose DNA samples were available for the analysis are shown by Arabic numerals. In Family A, I-2 and III-1 were found to have a novel pathogenic missense variant in *TRPS1* exon 6; this variant was not present in individuals II-2 and II-3. In Family B, I-1, II-1, and II-2 were found to have a nonsense variant in *TRPS1* exon 4.

discovered one novel and one previously described pathogenic variants in *TRPS1*.

2. Patients and methods

2.1. Patients and healthy family members

We investigated two unrelated females presented at different times with varying severity of brachydactyly. The affected and unaffected family members were also studied via molecular genetic analyses. DNA samples, as well as clinical and biochemical information, were available for family A – two affected (I-2, III-1) and four unaffected individuals (I-1, II-1, II-2, II-3; Fig. 1A), and for family B –three affected family members (I-1, II-1, II-2; Fig.1B). All biochemical measurements related to clinical management were performed at Ankara Training and Research Hospital, Ankara, Turkey. The study involving molecular genetic analyses was approved by the Institutional Review Board of the Massachusetts General Hospital. Informed consent was obtained from each investigated individual.

2.2. DNA sequence analysis

Blood samples of two probands and their family members were collected, and genomic DNA was extracted from peripheral blood leukocytes using standard methods. The DNA sequence analysis was performed by PCR amplification of exons and flanking intronic regions, followed by direct Sanger sequencing of the purified PCR products. PCR primer sequences are listed in Table 1. Sequencing was performed at the Center for Computational and Integrative Biology DNA Core Facility, Massachusetts General Hospital. Sequencing results were analyzed by using Sequencher 5.4.6 (Gene Codes Corporation, USA). Multiple sequence alignment was obtained from ENSEMBL Compara (http://useast.ensembl.org/info/genome/compara/index.html). SWISS-MODEL [17] was utilized for the peptide modeling, using TRPS1 residues 887-945 (NP_054831) and the human transcription factor GATA-4 (SMTL ID: 2m9w.1) as template.

Table 1
Primers used in the amplification of *PTHLH*, *PDE4D*, and *TRPS1*.

Gene	Exon	Forward (5′-3′)	Reverse (5′-3′)
PTHLH	3	gctgagaggctccagagaaat	ggttagggagagggaccatc
	4	tacccatccagcttgtgtctta	ctgtcctccagcaccatagaga
PDE4D	4	acgctaaatgctttttgaaaatatg	gtgcactcaggttaaatctaccatt
	5	tttaatattgaacgacactgagcac	gcttgaatgaaccaagaatttgtat
	6	tctcttaacaaaccattttcaagtgt	ggctcaatcaagttgagaaaactta
	8	gctagcggacccgtgaag	ggggctatgggtaccagtg
	9	ggtgttttcccctcattgaa	ctcagaggcggatagtgtcg
	12	tcactagacatgtgagagtgcagtt	cattgaaaacccttcagataaaagt
	13	agactctcccttgacacattgtaac	aactttagggccaatacaactttct
	15	gcccccaaggagtagtaagg	gctgcttaagtggctggttc
	16	tatttttaagtgttgcagtcatcca	aagtaaccaaatgctaaagcggta
	17	ccatgatctaattaagcttttccat	cagatgacagtgaggtgtgacc
TRPS1	2	ctggggagggagaggatct	gcacgatgttttactgtgtgc
	3	tttttcctagaggaagcatttga	tggagtgtgtccatctcttctg
	4	tcacctgttactttggcctaca	ttccccaagtcattggaatc
	5	tcagaacgctgtctttgcag	tgcacagcacacacaaacac
	6	ctcctgggttgatttggtct	agccagggaatgggacttat
	7	gcagaaagcctatccctgct	tgttggataaggcaggctct

3. Clinical and laboratory findings

3.1. Patient 1 (Family A, III-1)

The first patient, a 17-year old female (Fig. 2A, B), presented to our endocrinology outpatient clinic with short stature an asymmetric brachydactyly. Her height was 152 cm (−2SD) [18], and her weight 55 kg (BMI: 23.8). She was single with no children. All her metacarpals were short, with the exception of the third one in her left hand. Additionally, proximal phalanges of both index fingers were short, and X-rays demonstrated cone-shaped epiphyses in the middle phalanges (Fig. 2A, B). All metatarsals of both feet were also short, except for the second metatarsals (Fig. 2C, D).

She was born small for gestational age (1700 g) at term to unrelated parents and stayed in the Neonatal Unit for a week. According to her father, she had normal development and her brachydactyly was noticed at adolescence. She also had normal puberty, and her periods were regular. Laboratory findings at the initial presentation showed normal levels of corrected calcium (9.55 mg/dl; normal: 8.8–10.6), phosphate (3.8 mg/dl; normal: 2.5–4.5) and PTH (68.6 pg/ml; normal: 14–72). TSH and fT4 were within normal limits, with negative TPO antibodies and normal liver and kidney functions. The 25(OH)vitamin D level was markedly low at 8.2 ng/ml (normal: 20–80, measured in winter), while 1,25(OH)2D and alkaline phosphatase measured within normal limits at 36 pg/ml (normal: 16–65) and 78 U/l (normal: 30–120), respectively. She had no evidence of pseudofractures or osteoporosis by X-rays and a dual-energy X-ray absorptiometry (DXA) scan. Regarding the etiology of vitamin D deficiency, all possible causes were ruled out, including coeliac disease, prolactinemia, and hepatic or renal diseases. She did not have history of medications that may affect vitamin D metabolism. However, she was wearing a hijab for several years and living indoors after graduation from high school, suggesting limited sunlight exposure as a cause of vitamin D deficiency in this patient. Vitamin D treatment according to the Endocrine Society guidelines [19] was commenced: 50,000 IU vitamin D3 once a week for the total of 8 weeks to achieve 25(OH)vitamin D levels greater than 30 ng/ml, followed by maintenance therapy of 600 IU/day.

The proband's mother died a year after her delivery due to leukemia; unfortunately, only few details are known about her medical history, and her DNA was not available (Fig. 1A). However, she reportedly had short stature and abnormal-looking hands and feet. The proband's maternal grandmother was alive and 70 years old at the time of our evaluations. X-rays showed symmetrically short metacarpals and short proximal phalanx of index finger, with cone-shaped epiphyses at the proximal ends of middle phalanges (Fig. 2E). Her height was the same as her granddaughter, and her weight was 72 kg (BMI: 31.2). She reported that she had always been “tiny” in comparison with her siblings and peers, and was told that she took after her grandmother's looks.

The maternal grandfather of the proband was unaffected and unrelated to his wife (Fig. 1A). The proband's maternal three uncles also

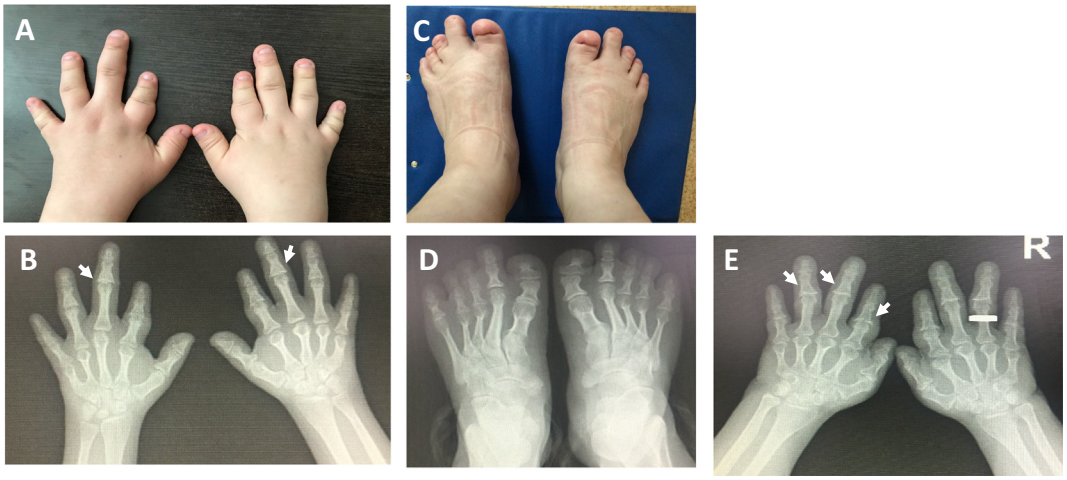


Fig. 2. Brachydactyly in Family A. A, B (Individual III-1). Asymmetric short metacarpals of the proband, with cone-shaped epiphyses at middle phalanges (arrows). C, D (Individual III-1). Short metatarsals in both feet, except for the 2nd metatarsals. E (Individual I-2). Symmetric short metacarpals of the maternal grandmother, with residual angulated deformity of proximal middle phalanges (arrows) related to fusion of prior cone-shaped epiphyses.

lacked any skeletal abnormalities. One of her maternal aunts was reportedly short and had a clinical phenotype similar to that of the proband; however, her DNA sample could not be obtained for our studies.

3.2. Patient 2 (Family B, II-1)

The second patient, a 40-year old female, had symmetric shortening of metacarpal bones, which was noticed by a physician to whom she went due to pain in her hand joints and back pain. Hand X-rays showed bilateral short fifth metacarpals and cone-shaped epiphyses in middle phalanges of all her fingers except for the thumb (Fig. 3A). She stood 156-cm tall and weighed 53.5 kg (BMI: 22). She was reportedly not short compared to her siblings. She was married and had three children at ages 10, 6, and 4 years.

The patient also suffered from pain in her back and thighs with generalized muscle pain, which disturbed her sleep at night and could be relieved with the use of analgesics. Her laboratory exam showed serum corrected calcium level of 8.74 mg/dl (normal: 8.8–10.6), phosphate level of 4 mg/dl (normal: 2.5–4.5), and PTH level of 74 pg/ml (normal: 14–72). The level of 25(OH)vitamin D was 19.3 ng/ml (normal: 20–80, measured in summer), 1,25(OH)2 vitamin D was 26 pg/ml (normal: 16–65), and alkaline phosphatase was 94 U/l (normal: 30–120). A DXA scan revealed decreased bone mineral density consistent with premenopausal osteoporosis (Lumbar spine, Z-score = −2.5 and T-score = −2.7; femoral neck, Z-score: −0.6 and T-score = −0.8). All possible other causes for vitamin D deficiency were ruled out as in the previous patient. The patient did not have any underlying pathology or medication history that could explain the low vitamin D level, except for spending most of her time indoors and wearing a hijab nearly all the time. Treatment for osteomalacia was initiated with 1000 mg/d calcium supplements and vitamin D in compliance with the Endocrine Society guidelines [19].

The proband's parents were unrelated. She had four sisters three of whom had similar skeletal phenotypes consistent with BDE. Her two brothers were reportedly unaffected. X-rays of one of her sisters (II-2) showed short third, fourth, and fifth metacarpals and severe cone-shaped epiphyses of middle phalanges (Fig. 3B). Her biochemical parameters were normal with respect to calcium, phosphate, and PTH. She had the same height as the proband. Additional family members who displayed BDE included the proband's 68-year old mother and the proband's two sons (Fig. 1B).

4. Genetic analysis of the candidate genes

Since the patients and their affected family members did not have evidence of hormone resistance, hypertension, or intellectual disability, we considered *PTHLH*, *PDE4D*, and *TRPS1* as primary candidate genes. First, we screened exons 3 and 4 of *PTHLH* (NM_198965), because most disease-causing alterations have been identified in these exons, which comprise all of the coding sequence of the gene with the exception of the last eight nucleotides. We found no potentially disease-causing variants, but Patient 1 had a heterozygous single nucleotide polymorphism (SNP) in intron 2 (rs6242, Supplementary Table 1).

Next, we sequenced *PDE4D*, as described [20]. We found no variants that were potentially disease-causing in the exons and flanking intronic regions (NM_001165899). However, both patients shared the same previously identified SNPs in introns 7, 8, and 15, i.e. rs2279737, rs7724713, and rs10051847, respectively. Additionally, patient 1 had a heterozygous synonymous SNP (rs7736186) in exon 17 (Supplementary Table 1).

Finally, we examined all the coding exons and flanking intronic regions of *TRPS1* (NM_014112.4). In the patient 1 we found a novel

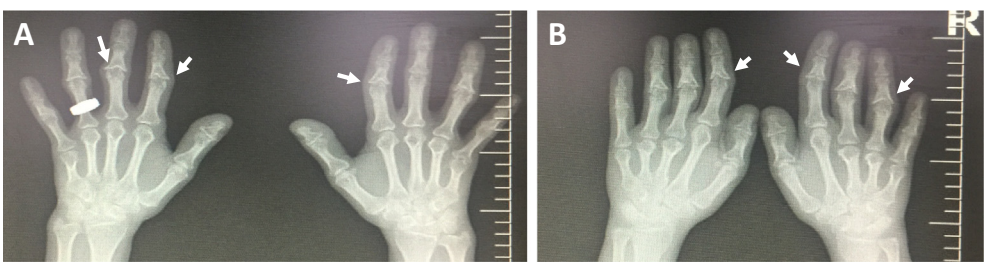


Fig. 3. Brachydactyly in Family B. A (Individual II-1). Short fifth metacarpals and cone-shaped epiphyses at proximal middle phalanges of the proband (arrows). B (Individual II-2). Short third, fourth, and fifth metacarpals of the proband's sister, with cone-shaped epiphyses at proximal phalanges (arrows).

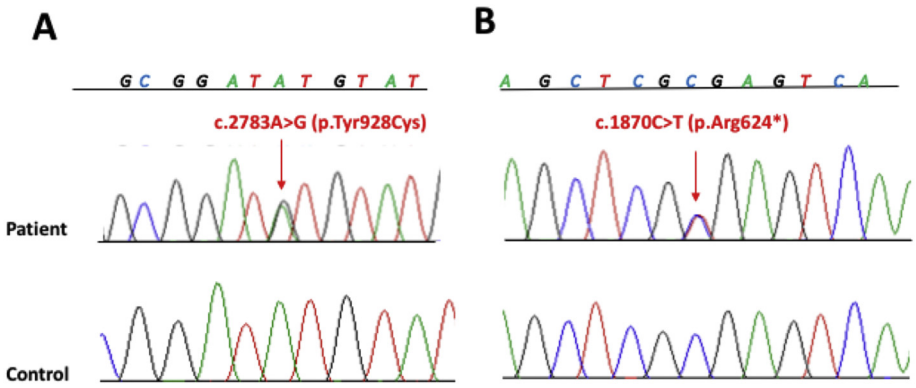


Fig. 4. Sequence traces showing the disease-causing nucleotide changes in patients compared to controls. A. Missense variant in exon 6, p.Tyr928Cys in patient 1. B. Nonsense variant in exon 4, p.Arg624* in patient 2.

Table 2
The pathogenic *TRPS1* variants identified in affected individuals of Family A and B.

	Family A	Family B
Variant	c.2783A > G, p.Tyr928Cys	c.1870C > T, p.Arg624*
Genomic position (hg19)	chr8:116,430,599	Chr8: 116616326
Exon (according to NM_014112)	6	4
Protein domain	Zinc finger GATA-DNA binding	Not applicable
dbSNP	–	rs121908431
MAF (gnomAD)	–	0.000008027
Previous report	No	Momeni P et al. [23] Maas SM et al. [24] (2017)
MutationTaster	Disease causing	Disease causing
SIFT	Damaging	Not available
PolyPhen-2	Probably damaging	Not available

MAF, minor allele frequency as reported in Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/).
MutationTaster: http://www.mutationtaster.org/
SIFT: http://sift.bii.a-star.edu.sg/
PolyPhen-2 http://genetics.bwh.harvard.edu/pph2/

heterozygous missense variant in exon 6 c.2783A > G (p.Tyr928Cys) (Fig. 4A, Table 2). The substitution was located in the region giving rise to the putative GATA DNA-binding zinc-finger domain, which is indispensable for the transcription repression activity of TRPS1 [21]. This pathogenic variant was not present in large population databases, including Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org). In silico tools, including Mutation Taster, SIFT, and Polyphen-2, predicted this missense variant to have an adverse effect on protein function (Table 2). DNA sequence analysis of family members revealed that the same single nucleotide change was also present in the proband's affected maternal grandmother (I-2), but not in the unaffected maternal grandfather or the two unaffected uncles of the proband (I-1, II-2 and II-3). Thus, according to the current guidelines of the American College of Medical Genetics and Genomics [22], the p.Tyr928Cys pathogenic variant can be classified as "likely pathogenic".

In patient 2 we identified a heterozygous nonsense pathogenic variant in exon 4, c.1870C > T (p.Arg624*) (Fig. 4B, Table 2). We subsequently performed sequence analysis of genomic DNA from the proband's affected mother (I-1) and one of her affected sisters (II-2), thus confirming the presence of the same nucleotide substitution in those individuals. The p.Arg624* variant has been previously described in multiple patients with TRPS [23,24]. This variant is in ClinVar and considered to be pathogenic in multiple entries (https://www.ncbi.nlm.nih.gov/clinvar/variation/5570). The *TRPS1* transcript (NM_014112.4) has seven exons. As the p.Arg624* variant is located in exon 4, it is predicted to lead to nonsense-mediated decay of the transcript and, thereby, loss of protein.

Both patient 1 and patient 2, as well as their affected family members, had additional polymorphisms located in *TRPS1* exon 2 corresponding to the 5′-untranslated regions (Supplementary Table 1).

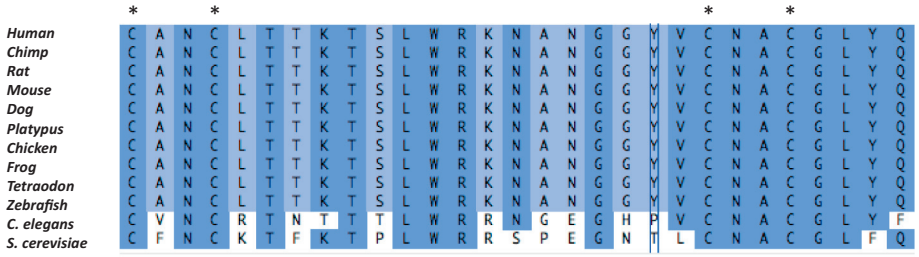
5. Discussion

In this study, we report two families with severe BDE, in whom we identified heterozygous pathogenic variants in the *TRPS1* gene. These pathogenic variants likely explain the brachydactyly phenotype observed in the probands and their affected family members. The skeletal abnormalities associated with pathogenic *TRPS1* variants are quite variable, and the most typical radiographic findings are cone-shaped epiphyses, which predominantly involve the middle phalanges, but can also be found at other tubular bones [25,26]. The X-rays of our patients clearly demonstrated cone-shaped epiphyses, particularly in the middle phalanges. In addition, consistent with the variable expressivity, the involvement of the metacarpal bones and the severity of shortening varied significantly, even among affected individuals of the same family.

Patients with *TRPS1* pathogenic variants have typical facial features including a pear-shaped nose, underdeveloped alae nasi, and long flat philtrum [15,24,26]. These patients also have sparse scalp hair and thin lateral eye brows. Our probands were not originally recognized to have those features. On further evaluation, however, each patient, as well as her affected family members, appeared to have a bulbous nose with a broad ridge and tip, including underdeveloped alae nasi and broad septum (Fig. 5). These features were more prominent in patient 1, who also showed a long flat philtrum, and thin lateral eyebrows (Fig. 5A, B). In addition, both our patients had thin sparse and slow-growing hair, more prominently at temporal regions of the head, consistent with ectodermal dysplasia. Thus, combined with the severe BDE, the phenotypes of our patients match TRPS.

Vitamin D deficiency is the most common cause of osteomalacia, which is usually characterized by bone pain and reduced bone mineral density. The low vitamin D levels could be with or without hypocalcemia and hyperphosphatemia. Patient 1 had strikingly low levels of vitamin D, but her serum calcium, phosphate, and PTH levels were within the normal range. She also lacked pseudofractures or reduced bone mineral density. Patient 2 had modestly low vitamin D levels but also showed a mild decrease of serum calcium coupled with a mild increase of serum PTH. These biochemical changes were associated with symptoms and findings of osteomalacia in Patient 2. These differences in presentation between the two probands may reflect differences in age and gravidity-parity. In the literature, biochemical

Fig. 5. Facial features of the probands with *TRPS1*-associated BDE. Photographs of individuals III-1 (A, B) from Family A (Patient 1) and II-1 (C) from Family B (Patient 2). Each patient had a bulbous nose, long flat philtrum, and thin upper lip, although the latter two features were more pronounced in Patient 1.



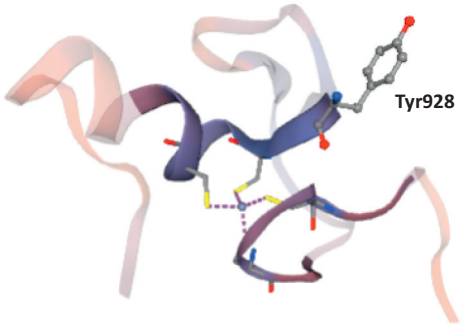


Fig. 6. Protein sequence conservation at the TRPS1 GATA DNA-binding domain and the modeling of the region comprising Tyr928. The multiple alignment of the primary amino acid sequence with orthologs (top) indicate the evolutionary conservation; asterisks, cysteine residues predicted to interact with Zn^{2+}. The tyrosine residue at position 928 is delineated on both sides. The peptide modeling (bottom) was performed by using SWISS-MODEL with residues 887–945 (template: human GATA-4). The Zn^{2+} ion and the four interacting cysteine residues are highlighted as ball-and-stick figures, as well as the native tyrosine residue at position 928.

alterations, particularly in vitamin D levels, have not been commonly addressed in TRPS patients. We found reports of only two unrelated TRPS patients who displayed vitamin D deficiency, which, in both cases, was accompanied by hyperparathyroidism [27,28]. It remains currently unknown whether this disorder specifically affects vitamin D metabolism. In contrast, TRPS1 protein has been investigated with respect to its role in bone remodeling and mineralization. Molecular studies have indicated that it modulates mineralized bone matrix formation in proliferating and differentiating osteoblast cells [29]. And polymorphisms in *TRPS1* have been associated with bone mineral density in mice and humans [30–32]. Moreover, osteopenia and osteoporosis have been reported in several patients with TRPS [33–35]. Hence, it is possible that TRPS pathophysiology may include defective bone mineralization and/or reduced bone mass, which could further be complicated by vitamin D deficiency in some cases.

Three TRPS subtypes have been described: TRPS I [MIM# 190350], caused by pathogenic variants in the *TRPS1* on chromosome 8; TRPS II [MIM# 150230], a contiguous gene deletion syndrome affecting the *TRPS1*, *RAD21*, and *EXT1* genes; and TRPS III [MIM# 190351], a form with severe BDE and severe short stature. Lüdecke et al. [26] reported that TRPS III is the severe end of the TRPS spectrum and can be caused by a specific class of pathogenic variants in *TRPS1*. The TRPS1 protein is a zinc-finger transcription factor consisting of 1294 amino acids (NP_054831). It contains an unusual combination of nine zinc-finger motifs of eight different types. Thus far, the function of only the last three motifs has been identified, including the GATA DNA-binding zinc-finger, which is flanked by two nuclear localization signals. This region, harboring the novel p.Tyr928Cys variant identified in Patient 1 and her affected family members, shows strong evolutionary conservation (Fig. 6) and is critical for the transcription repressor activity of TRPS1 [21]. All identified missense pathogenic variants in this region have been reported to cause a severe phenotype including short-stature (i.e. TRPS III). The severe phenotype is proposed to result from a dominant-negative effect induced by those missense pathogenic variants as opposed to loss-of-function variants, such as nonsense or frameshift, which are likely to yield no more than a 50% reduction in activity, i.e. haploinsufficiency [26,35]. These missense *TRPS1* variants likely impair DNA-binding, and this is probably the case for the tyrosine-to-cysteine substitution at position 928, which could disrupt zinc ion-binding and/or the folding of the entire domain (Fig. 6). The ability of TRPS1 to form complexes with other proteins, however, may be preserved. Given that TRPS1 form homodimers for its activity [36], it is tempting to speculate that the transcriptionally inactive TRPS1 mutant dimerizes with and hinders the activity of the wild-type TRPS1 protein, thereby leading to a severe functional impairment. Consistent with this phenotype-genotype correlation, the phenotype in our patients carrying the p.Tyr928Cys variant was more severe than the phenotype in those carrying the p.Arg624* variant. Nonetheless, it seems that some loss-of-

function pathogenic variants can also present with a severe phenotype, as a frameshift variant in exon 4 has been reported to cause a mild TRPS I-like phenotype in one kindred and, unexpectedly, a severe TRPS III-like phenotype in another [37]. Thus, the genetic and molecular mechanisms governing the effects of pathogenic *TRPS1* variants remain incompletely understood.

In conclusion, we identified a novel missense pathogenic variant within the GATA DNA-binding region and a previously described nonsense pathogenic variant in *TRPS1* as the genetic causes of BDE in two families. Our findings explain the clinical phenotypes of the affected individuals and contribute further to the phenotype-genotype correlation in TRPS.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2019.03.028.

Conflict of interests

The authors have no conflict of interest

Acknowledgments

This work was supported in part by a research grant from the National Institutes of Health/the National Institute of Diabetes and Digestive and Kidney Diseases (RO1 DK073911 to M.B.). Karaca A was supported by a grant from The Society of Endocrinology and Metabolism of Turkey.

References

[1] S.A. Temtamy, M.S. Aglan, Brachydactyly, Orphanet J. Rare Dis. 3 (2008) 15, , https://doi.org/10.1186/1750-1172-3-15.
[2] A. Pereda, et al., Brachydactyly E: isolated or as a feature of a syndrome, Orphanet J. Rare Dis. 8 (2013 Sep 12) 141, https://doi.org/10.1186/1750-1172-8-141.
[3] D. Johnson, S.H. Kan, M. Oldridge, et al., Missense mutations in the homeodomain of HOXD13 are associated with brachydactyly types D and E, Am. J. Hum. Genet. 72 (4) (2003) 984–997. Apr.
[4] N. Brison, P. Tylzanowski, P. Debeer, Limb skeletal malformations - what the HOX is going on? Eur. J. Med. Genet. 55 (1) (2012) 1–7, https://doi.org/10.1016/j.ejmg.2011.06.003 Jan.
[5] P.G. Maass, J. Wirth, A. Aydin, et al., A cis-regulatory site downregulates PTHLH in translocation t(8;12)(q13;p11.2) and leads to Brachydactyly type E, Hum. Mol. Genet. 19 (5) (2010) 848–860, https://doi.org/10.1093/hmg/ddp553 Mar 1.
[6] E. Klopocki, B.P. Hennig, K. Dathe, et al., Deletion and point mutations of PTHLH cause brachydactyly type E, Am. J. Hum. Genet. 86 (3) (2010) 434–439, https://doi.org/10.1016/j.ajhg.2010.01.023 Mar 12.
[7] P.G. Maass, A. Aydin, F.C. Luft, et al., PDE3A mutations cause autosomal dominant hypertension with brachydactyly, Nat. Genet. 47 (6) (2015) 647–653, https://doi.org/10.1038/ng.3302 Jun.
[8] M. Chaabouni, M. Le Merrer, O. Raoul, et al., Molecular cytogenetic analysis of five 2q37 deletions: refining the brachydactyly candidate region, Eur. J. Med. Genet. 49 (3) (2006) 255–263 May-Jun.
[9] S.R. Williams, M.A. Aldred, V.M. Der Kaloustian, et al., Haploinsufficiency of HDAC4 causes brachydactyly mental retardation syndrome, with brachydactyly type E, developmental delays, and behavioral problems, Am. J. Hum. Genet. 87 (2) (2010) 219–228, https://doi.org/10.1016/j.ajhg.2010.07.011 Aug 13.
[10] L.S1. Weinstein, P.V. Gejman, E. Friedman, et al., Mutations of the Gs alpha-subunit gene in Albright hereditary osteodystrophy detected by denaturing gradient gel electrophoresis, Proc. Natl. Acad. Sci. U. S. A. 87 (21) (1990 Nov) 8287–8290.
[11] J.L. Patten, D.R. Johns, D. Valle, et al., Mutation in the gene encoding the stimulatory G protein of adenylate cyclase in Albright's hereditary osteodystrophy, N. Engl. J. Med. 322 (20) (1990) 1412–1419 May 17.
[12] A. Linglart, C. Menguy, A. Couvineau, et al., Recurrent PRKAR1A mutation in acrodysostosis with hormone resistance, N. Engl. J. Med. 364 (23) (2011) 2218–2226, https://doi.org/10.1056/NEJMoa1012717 Jun 9.
[13] C. Michot, C. Le Goff, A. Goldenberg, et al., Exome sequencing identifies PDE4D mutations as another cause of acrodysostosis, Am. J. Hum. Genet. 90 (4) (2012) 740–745, https://doi.org/10.1016/j.ajhg.2012.03.003 Apr 6.
[14] H. Lee, J.M. Graham Jr., D.L. Rimoin, et al., Exome sequencing identifies PDE4D mutations in acrodysostosis, Am. J. Hum. Genet. 90 (4) (2012) 746–751, https://doi.org/10.1016/j.ajhg.2012.03.004 Apr 6.
[15] R. Böni, R. Huch Böni, D. Tsambaos, Trichorhinophalangeal syndrome, Dermatology 190 (1995) 152–155, https://doi.org/10.1159/000246667.
[16] S. Maas, A. Shaw, H. Bikker, R.C.M. Hennekam, Trichorhinophalangeal syndrome, in: M.P. Adam, H.H. Ardinger, R.A. Pagon, S.E. Wallace, L.J.H. Bean, K. Stephens, A. Amemiya (Eds.), GeneReviews®[Internet], University of Washington, Seattle, Seattle (WA), 1993–2018.
[17] A. Waterhouse, M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, F.T. Heer, T.A.P. de Beer, C. Rempfer, L. Bordoli, R. Lepore, T. Schwede, SWISS-MODEL: homology modelling of protein structures and complexes, Nucleic Acids Res. 46 (W1) (2018) W296–W303.
[18] H. Günöz, R. Bundak, A. Furman, et al., Z-score reference values for height in Turkish children aged 6 to 18 years, J. Clin. Res. Pediatr. Endocrinol. 6 (1) (2014) 28–33, https://doi.org/10.4274/Jcrpe.1260.
[19] Michael F. Holick, Neil C. Binkley, A. Heike, et al., Weaver evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline, J. Clin. Endocrinol. Metab. 96 (7) (1 July 2011) 1911–1930, https://doi.org/10.1210/jc.2011-0385.
[20] F.M. Elli, P. Bordogna, L. de Sanctis, et al., Screening of PRKAR1A and PDE4D in a large Italian series of patients clinically diagnosed with Albright hereditary Osteodystrophy and/or pseudohypoparathyroidism, J. Bone Miner. Res. 31 (6) (2016) 1215–1224, https://doi.org/10.1002/jbmr.2785 Jun.
[21] T.H. Malik, S.A. Shoichet, P. Latham, et al., Transcriptional repression and developmental functions of the atypical vertebrate GATA protein TRPS1, EMBO J. 20 (2001) 1715–1725, https://doi.org/10.1093/emboj/20.7.1715.
[22] S. Richards, N. Aziz, S. Bale, et al., of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet. Med. 17 (2015) 405–423.
[23] P1. Momeni, G. Glöckner, O. Schmidt, et al., Mutations in a new gene, encoding a zinc-finger protein, cause tricho-rhino-phalangeal syndrome type I, Nat. Genet. 24 (1) (2000 Jan) 71–74, https://doi.org/10.1038/71717.
[24] S.M. Maas, A.C. Shaw, H. Bikker, et al., Phenotype and genotype in 103 patients with tricho-rhino-phalangeal syndrome, Eur. J. Med. Genet. 58 (5) (2015) 279–292, https://doi.org/10.1016/j.ejmg.2015.03.002 May.
[25] Naselli A, Vignolo M, Di Battista E et al. Trichorhinophalangeal syndrome type I in monozygotic twins discordant for hip pathology: report on the morphological evolution of cone-shaped epiphyses and the unusual pattern of skeletal maturation. Pediatr. Radiol. 28:851–855. doi: https://doi.org/10.1007/s002470050481.
[26] H.J. Lüdecke, J. Schaper, P. Meinecke, et al., Genotypic and phenotypic spectrum in tricho-rhino-phalangeal syndrome types I and III, Am. J. Hum. Genet. 68 (1) (2000) 81–91.
[27] D. Ye, Y. Fei, Y.-E. Sheng et a. Analysis of a Chinese pedigree with trichorhino-phalangeal syndrome derived from a missense mutation in the TRPS1 gene, Clin. Exp. Dermatol. (2017), https://doi.org/10.1111/ced.13082.
[28] M. Crippa, I. Bestetti, M. Perotti, et al., New case of trichorinophalangeal syndrome-like phenotype with a de novo t(2;8)(p16.1;q23.3) translocation which does not disrupt the TRPS1 gene, BMC Med. Genet. 15 (2014) 52, https://doi.org/10.1186/1471-2350-15-52 May 2.
[29] D.M. Piscopo, E.B. Johansen, R. Derynck, et al., Identification of the GATA factor TRPS1 as a repressor of the osteocalcin promoter, J. Biol. Chem. 284 (46) (2009) 31690–31703, https://doi.org/10.1074/jbc.M109.052316.
[30] Ackert-Bicknel CL1, Demissie S, Tsaih SW et al. Genetic variation in TRPS1 may regulate hip geometry as well as bone mineral density. Bone. 2012 May;50(5):1188–95. doi: https://doi.org/10.1016/j.bone.2012.01.011
[31] N. Ishimori, I.M. Stylianou, R. Korstanje, et al., Quantitative trait loci for BMD in an SM/J by NZB/BlNJ intercross population and identification of Trps1 as a probable candidate gene, J. Bone Miner. Res. 23 (9) (2008) 1529–1537, https://doi.org/10.1359/jbmr.080414 Sep.
[32] L. Wang, Lu W2, Zhang L et al. Trps1 differentially modulates the bone mineral density between male and female mice and its polymorphism associates with BMD differently between women and men, PLoS One 9 (1) (2014) e84485, https://doi.org/10.1371/journal.pone.0084485 Jan 8.
[33] S. Stagi, G. Bindi, F. Galluzzi, et al., Partial growth hormone deficiency and changed bone quality and mass in type I trichorhinophalangeal syndrome, Am. J. Med. Genet. A 146A (12) (2008) 1598–1604, https://doi.org/10.1002/ajmg.a.32348 Jun 15.
[34] C. Shao, J. Tian, D.H. Shi, et al., A novel mutation in TPRS1 gene caused tricho-rhino-phalangeal syndrome in a Chinese patient with severe osteoporosis, Chin. Med. J. 124 (10) (2011) 1583–1585 May.
[35] M. Macchiaiolo, M. Mennini, M.C. Digilio, et al., Thricho-rhino-phalangeal syndrome and severe osteoporosis: a rare association or a feature? An effective therapeutic approach with bisphosphonates, Am. J. Med. Genet. A 164A (3) (2014 Mar) 760–763, https://doi.org/10.1002/ajmg.a.36327.
[36] McCarty AS1, Kleiger G, Eisenberg D, Smale ST. Selective dimerization of a C2H2 zinc finger subfamily. Mol. Cell. 2003 Feb;11(2):459–70.
[37] M. Itoh, Y. Kittaka, Y. Niida, Y. Saikawa, et al., A novel frameshift mutation in the TRPS1 gene caused Tricho-rhino-phalangeal syndrome type I and III in a Japanese family, Clin. Pediatr. Endocrinol. 25 (3) (2016) 115–118, https://doi.org/10.1297/cpe.25.115.