



# A Novel *TBX19* Gene Mutation in a Case of Congenital Isolated Adrenocorticotropic Hormone Deficiency Presenting with Recurrent Respiratory Tract Infections

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Akcan N, Serakıncı N, Turkgenc B, Bundak R, Bahceciler N and Temel SG (2017) A Novel TBX19 Gene Mutation in a Case of Congenital Isolated Adrenocorticotropic Hormone Deficiency Presenting with Recurrent Respiratory Tract Infections. Front. Endocrinol. 8:64. doi: 10.3389/fendo.2017.00064 **Introduction:** Congenital isolated adrenocorticotropic hormone deficiency (CIAD) is a rare disease characterized by low adrenocorticotropic hormone (ACTH) and cortisol levels. To date, recurrent pulmonary infections in infancy have not been reported as an accompanying symptom of CIAD.

**Case presentation:** A 7-year-old boy was hospitalized nine times for recurrent lower respiratory tract infections. The results of all tests for the possible causes of wheezing were within the normal limits. His ACTH and cortisol levels were persistently low. All other pituitary hormone levels, and adrenal ultrasound and pituitary magnetic resonance imaging results, were normal. Molecular analyses confirmed the diagnosis of CIAD by identifying compound heterozygosity for two mutations in the *TBX19* gene. The first was a novel frameshift c.665delG variant in exon 4 of the *TBX19* gene, leading to premature termination that was predicted to result in a non-functional truncated protein. The second was a nonsense C-to-T transition in exon 6 of the *TBX19* gene, resulting in an arg286-to-ter mutation (dbSNP: rs74315376). Both parents were heterozygous for one of the mutations.

**Conclusion:** Here, we presented a new mutation in the *TBX19* gene in a patient with CIAD who presented with recurrent respiratory tract infections. This expands the mutation spectrum in this disorder. To conclude, adrenal insufficiency should be considered in patients with unexplained recurrent infections to prevent a delay in diagnosis.

Keywords: adrenal insufficiency, adrenocorticotropic hormone, cortisol, respiratory infections, TBX19 gene

# INTRODUCTION

Congenital isolated adrenocorticotropic hormone deficiency (CIAD) is a rare disease characterized by low plasma adrenocorticotropic hormone (ACTH) and cortisol levels while the other pituitary hormone levels remain normal. CIAD occurs as a result of homozygous or compound heterozygous mutations in the *T-box 19* (*TBX19*) gene, which is located on chromosome 1q24 (1, 2).

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Twenty-one different mutations were identified in the *TBX19* gene in the largest series (n = 91) of CIAD patients. Most CIAD cases present with severe hypoglycemia, seizures, or prolonged jaundice in the neonatal period (1). *TBX19* shares 94% amino acid identity with the mouse *Tpit* gene (2). Here, we report a CIAD case with a novel mutation presented with recurrent pulmonary infections. To our knowledge, this is the first reported case of CIAD presenting with recurrent pulmonary infection in infancy.

# CASE PRESENTATION

A 34-month-old boy was referred to our hospital with a prediagnosis of acquired ACTH deficiency. The patient was a term baby, delivered by cesarean section. He was intubated as a result of respiratory distress and supported by mechanical ventilation for 21 days in the neonatal intensive care unit. He was hospitalized nine times because of recurrent lower respiratory tract infections until the age of 10 months. In each period of hospitalization, he needed systemic steroid, and he also had continuously inhaled steroid during this period. Acquired ACTH deficiency secondary to glucocorticoid therapy or his medical conditions was decreed when the cortisol and ACTH levels were low at the age of 10 months. Hydrocortisone treatment (9 mg/m<sup>2</sup>/day) was started to prevent life-threatening complications. On admission to our hospital at the age of 34 months, he was on maintenance physiologic dose of hydrocortisone, his weight was 17.2 kg (+1.3 SDS), and his height was 97.5 cm (+0.5 SDS). He was normotensive and had natural skin and hair color, with normal male genitalia. Radiographic and biochemical analyses were normal. The cortisol response to a low-dose (1 µg) ACTH test was impaired (Table 1). The hydrocortisone treatment was continued with the same physiological replacement dose. Other causes of recurrent lung infection were ruled out by the Pediatric Allergy and Immunology Division. During follow-up, ACTH and cortisol levels were persistently low (ACTH < 5 pg/mL, cortisol < 1 ng/ mL), and he had never demonstrated hypoglycemia or electrolyte imbalance. Growth hormone tests were not performed because of the normal stature. Levels of the other pituitary hormones (thyroid hormones, prolactin), 17-hydroxyprogesterone, androstenedione, and adrenal ultrasound and pituitary magnetic resonance imaging results were all normal (Table 1). Based on the hormonal profile, CIAD was suspected. On final assessment at 7 years 2 months, the patient weighed 28.3 kg (+1.1 SDS), and his height was 123.7 cm (+0.3 SDS). He has experienced no further lung infections since the hydrocortisone treatment was started.

In addition, the triple screening test of the mother during her second pregnancy showed low estriol (E3) and human chorionic gonadotropin (hCG) levels, which may be related to glucocorticoid deficiency. Genetic counseling was given to the pregnant woman. It was explained that early disease diagnosis allows for immediate commencement of glucocorticoid therapy, and proper instructions for stress management were provided. Although this can prevent unnecessary neonatal deaths from

#### TABLE 1 | Clinical findings and laboratory results.

	On admission	Final examination
Age	34 months	7.2 years
Weight (kg)/weight-SDS	17.2/1.3	28.3/1.1
Height (cm)/height-SDS	97.5/0.5	123.7/0.3
BMI (kg/m²)/BMI-SDS	18/1.30	18.5/1.4
Tanner stage (pubic hair development)	1	1
Testes volumes	Testes 2/2 mL	Testes 2/2 mL
Laboratory tests (reference ranges)		
FBG (60–100 mg/dL)	65	90
Na (135–145 mmol/L)	142	140
K (3.5–5 mmol/L)	4.6	4.9
ACTH (6–48 pg/mL)	<5	-
Cortisol (3–21 µg/dL)	0.9	-
TSH (0.6–5.5 μIU/mL)	1.2	2.6
fT4 (0.85–1.75 ng/dL)	1.2	1
PRL (3–18 ng/mL)	16.1	16.3
DHEAS (13–83 µg/dL)	21	
A4 (10–17 ng/dL)	13	-
17α-OHP (<91 ng/dL)	11	_
Peak cortisol response to low-dose (1 $\mu$ g) ACTH stimulation test ( $\geq$ 18 $\mu$ g/dL)	0.1	-
Karyotype	46, XY	
Bone age (years)	2	7
Adrenal USG	Normal	
Cranial and pituitary MRI	Normal	

ACTH, adrenocorticotropic hormone; A4, androstenedione; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; FBG, fasting blood glucose; fT4, free thyroxine; K, potassium; Na, sodium; MRI, magnetic resonance imaging; PRL, prolactin; SDS, standard deviation score; TSH, thyroid-stimulating hormone; USG, ultrasonography; 17 $\alpha$ -OHP, 17 $\alpha$ -hydroxyprogesterone (abnormal findings are shown in bold).

an easily treatable disease, in this case the family decided to terminate the pregnancy.

# MOLECULAR ANALYSES

Written informed consent was obtained from the patient's legal guardians. Molecular analysis was performed when he was 6 years old during the mother's pregnancy with her second baby. Further molecular genetic testing of the parents was performed to assess whether both variants were on the same (*cis*) or a different (*trans*) *TBX19* allele and the abortion material was also analyzed molecularly. The pedigree was shown in **Figure 1A**.

In the molecular analysis of the index case, a novel heterozygous c.665delG (p.Arg222Lys\*4) variation in exon 4 (**Figures 1B,C**) and pathologic heterozygous c.856C>T (p.Arg286Ter) variation in exon 6 (**Figure 1D**) of the *TBX19* gene (NM\_005149.2, NP\_005140.1) were detected in the compound heterozygous state. The initial genetic test suggested a possible splice site variation (c.665 + 1delG) in intron 4 of the *TBX19* gene instead of a c.665delG variant, because of the consecutive GG repeat at the exon–intron boundary. Therefore, further molecular analyses were performed at the mRNA level to gather information about the location of the variation (the last nucleotide of exon 4 or the first nucleotide of intron 4) and to identify a possible splicing effect. The mRNA analyses revealed



of heterozygous c.665delG (p.Arg222Lysfs\*4) deletion in exon 4, which was inherited from the mother. The study was performed from the genomic DNA material. (C) The image of the same frameshift p.Arg222Lysfs\*4 deletion studied from the RNA material of the index case and his carrier mother. (D) The image of known heterozygous c.856C>T mutation inherited from the father.

that the real variation was c.665delG (p.Arg222Lysfs\*4) and that it was a novel exonic frameshift mutation in spite of the splice site mutation (**Figure 1C**). As a consequence of the deletion of guanine 665 at the cDNA level, the first affected amino acid arginine was replaced with lysine at residue 222, and the new reading frame ended with a stop codon at position 4.

The other c.856C>T substitution was a nonsense variant predicted to result in the substitution of an arginine by a premature stop codon at position 286 in the protein (p.Arg286Ter) (**Figure 1D**). The c.856C>T variant was previously described in the Human Genome Mutation Database; it is associated with isolated deficiency of the pituitary and ACTH (HGMD #CM014746) (2). Also, it is listed as a pathogenic allele in the dbSNP (rs74315376) and ClinVar databases,<sup>1</sup> with a lower minor allele frequency of 0.00005 (6 of 121,334 alleles).<sup>2</sup>

Molecular analysis revealed that the mother was a carrier of the c.665delG variation (**Figures 1B,C**) and the father was a carrier of the p.R286X mutation (**Figure 1D**). The abortion material also showed the same pathological variations in the *TBX19* gene.

### DISCUSSION

Recurrent wheezing or pulmonary infections in infancy can be related to asthma, immune deficiency, congenital anatomic causes, and cystic fibrosis (3). However, recurrent pulmonary infections in infancy have not been reported as an accompanying symptom of any kind of GC deficiency. Although there is no relevant information regarding the relationship between cortisol insufficiency and recurrent infections, GCs play a critical role in restraining, shaping, and maintaining the homeostasis of the immune response (4, 5). Moreover, many reviews have focused on the bidirectional communication between the immune system and the HPA axis (4, 5). The ability of steroids to inhibit pro-inflammatory cytokines by switching off inflammatory genes through interactions with transcription factors but to enhance the anti-inflammatory cytokine may be the major effects of GCs, which may contribute to reduce the bronchial hyperresponsiveness (4-6).

The *TBX19* gene, previously known as *TPIT*, is a member of a phylogenetically conserved family of genes, which share a common DNA-binding domain: the T-box. *T-box* genes encode the transcription factors involved in regulating embryonic development. *TBX19* is a transcriptional regulator that interacts with target genes through its T-box domain

<sup>&</sup>lt;sup>1</sup>https://www.ncbi.nlm.nih.gov/clinvar/variation/5440/.

<sup>&</sup>lt;sup>2</sup>http://exac.broadinstitute.org/gene/ENSG00000143178.

Multiple sequence alignment of c.665delG region				
OUERY	SEPETOFIAVTAYONEEITALKIKYNPEAKAELDAKE	R N-HLRDVPEAISESQHVTYSHLGG-WIFSNPDGVCTAG		
sp G1LCE8#1	SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HLKDVPEAVSESQHVAYSHLGG-WIFSNPDGVCAAG		
SD G1T7M7#1		R N-HLKDVPEAISESQHVTYSHLGG-WIFSNPEGMCTAG		
sp UPI000184DA39#1		R N-HLKDIPEAVSEGQRVAYSHLGG-WIFSNPDGVCTAG		
sp G3T736#1	SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HLKDVPEGVSEGKHVAYSHLGG-WIFSNPDGVCTAG		
sp G1TR27#1		R N-HLKDVPEAISESQHVTYSHLGG-WIFSNPEGMCT		
sp D4A592#1	SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HLKEIPEAVSESQHVTYSHLGG-WILSNPDGMCTTG		
sp G5AQ59#1	SFPETQFIAVTAYQNEEITALKIK VNPFAKAFLDAKE SLVHSCDCLSERGALKIK VNPFAKAFLDAKE	R N-HLKDIPEAVAEGQHVAYSHLGG-WIFSNPDGVCTAG		
sp Q0V8E2#1	SF <mark>PETOFIAVTAYONEE</mark> ITALKIKYNPFAKAFLDAKE	R N-HLKDIPEAMSESQHVTYSHLGG-WIFSNPDGVCTAG		
sp G3X3X0#1	SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HPKDAPEVISEGQHITYSHLGG-WLISNPDAVCTSG		
sp F6PMR4#1	SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HPKDVPEVISEGQHMTYSHLGG-WLISNPDAVCTSG		
sp G1PJE7#1	SEPETOFIAVTAYONEEIITALKIKYNPEAKAELDAKE	R N-HLKDIPEAISESOHLACPHLGG-WIFSNP-GVCSAG		
sp F1NSW8#1	SEPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HPKDAPEAASEGOHMTYSHLGG-WLISNPDTVCASG		
sp P79778#1	SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HPKDAPEAASEGQHMTYSH		
sp F1NR88#1	SF <mark>PETOFIAVTAYONEE</mark> ITALKIKYNPFAKAFLDAKE	R N-HPKDAPEAASEGQHMTYSH		
sp UPI000194BA35#1	SFPETOFIAVTAYONEE ITALKIKYNPFAKAFLDAKE	R N-HSKDAPETVSEGQHMTYSH		
sp G1KKG0#1		R N-HTKDASDIVSESQHMTYSHLGG-WLISNPDAMCAAG		
sp F7BFD0#1		R N-HLRDVPEALSESQHVTCSHL		
sp UPI0002039131#1	SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HTKDASDIVSESQHMTYSHLGG-WLISNPDAMCAAG		
sp F1PKS0#1	SFPETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R N-HLKDIPEAVSESQHVAYSHL		
sp F1Q7B7#1	SFTDTOFIAVTAYONEEITALKIKHNPFAKAFLDAKE	R S-HPKNHLEPPVENQHMGIPHSGG-WFISNPDSLCSAS		
sp UPI0000546136#1	SFTDTOFIAVTAYONEEITALKIKHNPFAKAFLDAKE	S-HPKNHLEPPVENQHMGIPHC-G-WFISNPDSLCSAS		
sp F8W5N0#1	SFIDTOFIAVTAYONEEITALKIKHNPFAKAFLDAKE	S-HPKNHLEPPVENQHMGIPHC-G-WFISNPDSLCSAS		
sp G3HE57#1	SFPETOFIAVIAYONEEITALKIKYNPFAKAFLDAKE	R M-RLQMMPYSKDSLQHSAFLYI		
sp UPI00017B37CC#1	SFKEAQFIAVIAYONEEIIALKIKYNPFAKAFLDAKE	R ECHAGIQPLSVLNNESKTYVCAGGDWALCSPGG		
sp C4B8I5#1	AFPETRFIAVIAYONEEVISLKILHNPFAKGILDAKE	S-HTMKDSN-VSDCPQSPYSHASHGWWMGKPQ		
sp UPI00022B318C#1	SFKETQF1AVTAYONEE1TALK1KYNPFAKAFLDAKE	RIPGGRSLPESSESRVGIQSC-WSLCSAGG		
sp Q6Y5S0#1		R S-HTMKDSN-VSDCPQSPYSHASHGWWMGKPQ		
sp UPI00016E6AEE#1	SFKETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	SH-DRNTGVRGLPESQECHAGIQPY-WSMCATGG		
sp F6PMM1#1	SFPETOFIAVTAYONEEITALKIK NPFAKAFLDAKE SFKEAOFIAVTAYONEEITALKIK NPFAKAFLDAKE	R N-HPKDVPEVISEGQHMTYSHL		
sp Q4SUA5#1	SFKEAOFIAVTAYONEEITALKIKYNPFAKAFLDAKE SFKETOFIAVTAYONEEITALKIKYNPFAKAFLDAKE	R Q		
sp UPI00016E6AED#1	PFPETOFIAVTAYONEETTALKIKYNPFAKAFLDAKE	R		
sp Q8T8E3#1	PFPETOFIAVTAYONEEVTALKIKYNPFAKAFLDAKE	R		
sp Q08KF4#1 sp E0VR50#1	PF <mark>PETOFIAVTAYONEEVTSLKIKYNP</mark> FAKAFLDAKE PF <mark>PETOFIAVTAYONEEVTSLKIKYNP</mark> FAKAFLDAKE			
sp P55965#1				
shle22202#1	PFPETOFIAVTAYONEEVTSLKIKYNPFAKAFLDAKE			

#### sequence alianment of a 665delG reai Multiple

# Multiple sequence alignment of p.Arg286Ter region

uc001gfl.2_hg19	SNPDGVCTAGNSNYOYAAPLPLPAPHTHHGCEHYSGL RIGHROAPYPSAYMHRNHSPSVNLTESSSNNLOVFSGPDS
uc001gfl.2_equCab2	SNPDGVCTAGNANYOYATPLPLSAPHTHQGCEHYPGL RIGHROAPYPSAYMHRNHSPSVNLTESSSNNLOVFSGADS
uc001gfl.2_canFam2	SNPDGVCAAGNANYOYATPLPLSAPHTHHGCEPYPGL RIGHROAPYPSAYMHRNHSPSVNLTESSSNNLOVFSGPDS
uc001gfl.2_turTru1	SNPDGVCAAGNANVOVATPLPLSAPHTHHGCEPYPGL R GHROAP PSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNANVOVATPLPLSAPHTHHGCEHYSGL R GHROAPYPSAYMHRNHSPSVNLTESPSNNLOVFSGADS SNPBGTCTAGNTSYOYAAPLPLPAPHTHPGCEHYPGL R GHROAPYPSAVVHRNHSPSVNLTESSSNNLOVFSGPDS
uc001gfl.2_oryCun2	SNPEGMCTAGNTSYOYAAPLPLPAPHTHPGCEHYPGL R GHROAPYPSAYVHRNHSPSVNLTESSSNNLOVFSGPDS
uc001gfl.2_mm9	SNPDGVCTAANSNYQYATPLPLPAPHTHHGCEHYAGL R GHROAPYPSAYMHRNHSPSVNLIESSSNNLOVFSGPDS
uc001gfl.2_micMur1	ENPDGVCPAGNTNYPYAAPLPLPAPHP-HGCEHYSGL R GHROAPYPSAYMHRNHAPSVNLTESSSNNLOVFSGPDS
uc001gfl.2_cavPor3	SNPOGVCTAAGNANVOYATPLPLSAPTHOGGENPGLR GROAP PSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPOGVCTAGNANVOYATPLPLSAPTHHGGENYGLR GROAP PSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNANVOYATPLPLSAPTHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNSNOVATPLPLPAPTHPGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNSNOVATPLPLPAPHTHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNSNOVATPLPLPAPHTHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNGSVOVAAPLPLPAPHTHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNGSVOVAAPLPLPAPHTHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNGSVOVAAPLPLPAPHTHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNGSVOVAAPLPLSAPTHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNNNOVAAPLSLPAPHSHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SNPDGVCTAGNNNOVAAPLSLPAPHSHHGGENYGLR GROAP YSAVMHRNHSPSVNLTESSSNNLOVFSGADS
uc001gfl.2_felCat3	SNELIGA AAGNASYUWATELEI SATETEEGYEENEGTIK GERUAEYESAYOERNESESYNT ESSSNNL WESGAUS
uc001gfl.2_loxAfr3	
uc001gfl.2_bosTau4	SN POGVCTAGNTNVOVTTPLPLSAPHTHHGCEHYPGI R GHROAP PSAVMHRNHSPSVNLTESSSNNLOVFSGADN SN POGVCTAGNSNVOVAAPLPLPAPHTHHGCEHYSGL R GHROAP YPSAVMHRNHSPSVNLTESSSNNLOVFSGPDS SN PDG7CTTGSANVOVATPLPLPAPHTRHGCEHYAGL R GHROAP YPSAVMHRNHSPSVNLTESSSNNLOVFSGPES
uc001gfl.2_gorGor1	SNPDGVCTAGNSNYOYAAPLPLPAPHTHHGCEHYSGL R GHROAPYPSAYMHRNHSPSVNLIESSSNNLOVFSGPDS
uc001gfl.2_rn4	SNPDGMettcsAnyoyAtplplpAphtrhgcehyAgl R GhroapypsAymhrnhspsvnltesssnnlovfsgpes
uc001gfl.2_dasNov2	SNPEGMCTAGNANYOYATPLPLPPPHSH0GCEHCPGL R GHR0APYPSAYMORNHAPAVNLLESSSNNLOVFSGPES
uc001gfl.2_dipOrd1	SN POGVCTAGNSNYOVAPLELPAPHTHHGCEHVSGL R GHROAPY PSAVHRNHSPSVNLIESSSNNLOVFSGPDS SN POGVCTAGNSNYOVAPLELPAPHTHHGCEHVSGL R GHROAPY PSAVHRNHSPSVNLIESSSNNLOVFSGPES SN PEGVCTAGNANVOVATPLELPAPHTHHGCEHVSGL R GHROAPY PSAVHRNHSPSVNLIESSSNNLOVFSGPES SN PEGVCTAGNANVOVATPLELPAPHTHHGCEHVSGL R GHROAPY PSAVMRNHSPSVNLIESSSNNLOVFSGPES SN PGQLAASNTNYOVATPLELPAPHTHHGCEHVSGL R GHROAPY PSAVMRNHSPSVNLIESSSNNLOVFSGPES SN PGQLAASNTNYOVATPLELPAPHTHHGCEHVSGL R GHROAPY PSAVMRNHSPSVNLIESSSNNLOVFSGPES
uc001gfl.2 ochPri2	SNPEGVCTTGNTNYOYAAPLPLPAPHTHPGCEHESGL R GHRDAPYPSAYMHRNHSPSVNLIESSSNNLOVFSGPDT
uc001gfl.2 vicPac1	S-RUGV-LAGNINMOMALE PLSAPE LEPIGTE MP.G. IN GEROAPMESAWVERNES PSVNL LEISSNN LOVESGAUS
uc001gfl.2 choHof1	SSPEGVCTACSASYOYATPLSLPAPHSHOGCERCPGL R SPROAPYPSAYVHRNHSPTVN-IESSSNNLOVESGPES
uc001g+1.2_tarSyr1	SINP DOV CAALONT NY NID (#SSSINN COVERSCEP).S
uc001gfl.2_monDom5	SNP DAV CAAGNINY SNP DAV CTSGNSNYOY PAPLPLAAPHSHHGCEHYSGL SNP DAV CTSGNSNYOY PAPLPLAAPHSHHGCEHYSGL R GHRQAP Y PSAWHRNHSPSTVNL IDESSNNLOVFSGNS SNP EGV GAANTNYOY AAPLPLSAPHTHHGCERYAGL R GHRQAP Y PSAWHRNHSPSTVNL IDESSNSLOVFSGNS SNP TAMETSGNSNYOY PAPLPLAAPHSHHGCEHYSGL H GHRQAP Y PSAWHRNHSPSTVNL IDESSNSLOVFSGNS SNP TAMETSGNSNYOY PAPLPLAAPHSHHGCEHYSGL H GHRQAP Y PSAWHRNHSPSTVNL IDESSNNLOVFSGPDG SNP TAMETSGSNYOY CAPLSLPAPHTHHGCERYSGL R GHRQAP Y PSAWHRNHSPSTVNL IDESSNNLOVFSGPDG SNP TAMETSGSNYOY CAPLSLPAPHTHHGCERYSGL R GHRQAP Y PSAWHRNHSPSTVNL IDESSNNLOVFSGPDG SNP TAMETSGSSNYOY TAPLPLAAPHSHHGCERYSAL R GHRQAP Y PSAWHRNHSPSTVNL IDESSNNLOVFSGPDG SNP TAMETSGSSNYOY TAPLPLAAPHSHHGCERYSAL R GHRQAP Y PSAWHRNHSPTVNLIESSSNNLOVFSGPDG SNP DAMETSGSSNYOY TAPLPLAAPHSHHGCERYSAL R GHRQAP Y PSAWHRNHSPTVNLIESSSNNLOVFSGPDS SNP DGWTAATTNYOY ATPLSLPAPHSHHGCERYSAL R GHRQAP Y PSAWHRNHSPTVNLIESSSNNLOVFSGPDS
uc001gfl.2_pteVam1	SNPDGVCGAGNTNYOYAAPLPLSAPHTHHGCERYPGL R GHRQAPYPSAYVHRNHSPSVNLIDTASNNLQVFPGADG
uc001gfl.2_myoLuc1	SNPEGVCSAGNANFOYAPPLPLSAPHTHHGCERYAGL R GHRDAPYPSAYMHRTHSPS
uc001gfl.2 macEug1	SNPDAMOTSGNSNYOYPAPLPLAAPHSHHGCEHYSGL H GHRAAPYPPPYMHRNHSSTVNLIDSSSNSLOVFSGNNS
uc001gfl.2_eriEur1	SNPVCAAGSANYOYGPPLSLPAPHTHHGCEHYSGL R GHROTPYPSAYVHRSHSPSVNLIESSSNNLOVFSGPDS
uc001gfl.2_otoGar1	SNPDGVCPAGNANYOYAAPLSLPAPHPHHSCEHYSGL R GHRQAPYPSAYMHRNHSPSVNLIESSSNNLOVFSGPDG
uc001gfl.2_anoCar1	SNPDAMCAAGGSGYOYSGTESLPASHSHHGCEHYSAL R GHRAVPYPPPYMCRNPSPSVNLMDSTNNELOVSSGLEG
uc001gfl.2_galGal3	SNPDTVCASCSSNYOYTAPLPLPAPQAPHGCERYSAL R GHRAAPYPPSYMORNHSPTVNEFESSSNNLOVFSGHDS
uc001gfl.2_echTel1	SNP DAVCISSINSNOV PAPLE LAAP IS HHGCERYSGL HIGHRAAFY PPD YHRNHSS IVNLIESSSINSLOVFSGNDS SNP DGVCGAGNTNYOY AAPLE LSAP IT HHGCERYSGL HIGHRAAFY PSAVYHRNHSS IVNLIESSSINSLOVFSGNDS SNP EGVCSAGNANFOYAPPLP LSAP IT HHGCERYSGL HIGHRAAFY PDS YHRNHSS IVNLIESSSINSLOVFSGNDS SNP DAMCTSGNSNYOY PAPLP LAAP IS HHGCERYSGL HIGHRAAFY PDS YHRNHSS IVNLIESSSINSLOVFSGNDS SNP DAMCTSGNSNYOY PAPLP LAAP IS HHGCERYSGL HIGHRAAFY PDS YHRNHSS IVNLIESSSINSLOVFSGNDS SNP DGVCPAGNANYOY AAPLSLPAP IT HHGCERYSGL HIGHRAAFY PDS YHRNHSS IVNLIESSSINSLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IT HHGCERYSGL HIGHRAAFY PDS YHRNHSD SVINLIESSSINLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IT HHGCERYSGL HIGHRAAFY PDS YHRNHSD SVINLIESSSINLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IS HHGCERYSAL HIGHRAAFY PDS YMCRNDS DS VINLIESSSINLOVFSGPDS SNP DTVCASCSSNYOY TAPLP LPAPSHHGCERYSAL HIGHRAAFY PDS YMCRNDS DS VINLIESSSINLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IS HHGCERYSAL HIGHRAAFY PDS YMCRNDS DS VINLIESSSINLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IS HHGCERYSAL HIGHRAAFY PDS YMCRNDS DS VINLIESSSINLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IS HHGCERYSAL HIGHRAAFY PDS YMCRNDS DS VINLIESSSINLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IS HHGCERYSAL HIGHRAAFY PDS YMCRNDS DS VINLIESSSINLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IS HHGCERYSAL HIGHRAAFY PDS SMONTHSPT VNLIESSSINLOVFSGPDS SNP DGVCPAGNANYOY AAPLSLPAP IS HHGCERYSAL HIGHRAAFY PDS YMCRNDS DS VINLIESSSINLOVFSGPDS
uc001gfl.2_proCap1	
uc001gfl.2_ornAna1	SNPDTVGPAGNSNYOYLGPLPLOVPHSHHGCERYGGL R GARASPYPAPYLPRNDAOTVNFMENSSHNLOVFPGHDS
uc001gfl.2 tupBel1	SNPDTVGPAGNSNVOVLGPLPLOVPLSHHGGERVGGL SNPDGNTSVQVTAPLPLPAPLTHHGGERVSGL GHRQAPVPPAVVHRNHSPTLQVFSGPDS
uc001gfl.2_danRer6	
uc001gfl.2_xenTro2	
uc001gfl.2_gasAcu1	
uc001gfl.2_tetNig2	
uc001gfl.2_fr2	
	L L

#### FIGURE 2 | Multiple sequence alignments of TBX19 variants across different species due to PolyPhen-2 data.



when compared to wild type.

(2, 7). *TBX19*-knockout mice had an almost complete lack of proopiomelanocortin-expressing cells, which resulted in severe ACTH and glucocorticoid deficiencies (8). Most of the mutations reported to date are clustered in the *T-box* region in the DNA-binding region of the gene, and as such might lead to loss of function. The T-box domain stretches between amino acids 45 and 218 and is essential for DNA binding.<sup>3</sup> However, many of the mutant transcripts, such as R179X, R286X, and 782delA, which would be predicted to result in the premature truncation of the protein are reportedly destroyed by nonsense-mediated mRNA decay (9).

The *R286X* mutant transcript was one of the variations found in our case, in a heterozygous manner outside the *T-box* region. The mRNA transcripts resulting from the 573del4 mutant and the 5.2 kb deletion may be similarly destroyed or lead to severely truncated proteins (10). In contrast, the T58A, S128F, and I171T mutant transcripts produce defective proteins because these variations are within the T-box region. Therefore, the S128F and I171T mutant proteins exhibit no transcriptional activity or DNA-binding capacity, and the T58A mutant protein had greatly reduced levels of both transcription and DNA binding (9). This variant may lead to a truncated protein or reduce mRNA levels due to mRNA decay.

In our case, c.665delG (Arg222Lysfs\*4) was a novel heterozygous mutation that may not disrupt DNA binding, since this residue is outside the T-box region. Mutations in splice regions may lead to the retention of large segments of intronic DNA by the mRNA, or to entire exons being spliced from the mRNA. This led to a premature stop codon, shortening the protein. Conservation of the involved residues was examined across different species using the UCSC Genome Browser<sup>4</sup> and PolyPhen-2.5 Multiple sequence alignments of different species showed that arginine-222 is highly conserved, whereas arginine-286 is relatively conserved (Figure 2). RaptorX<sup>6</sup> and PSIPRED<sup>7</sup> were used for in silico analysis of the effects of TBX19 variants on the secondary structures of proteins. Comparison of the secondary and three-dimensional structures of the proteins revealed that helix, beta sheet, and loop structures were altered in both of the mutant proteins, which led to premature termination compared with the wild-type protein (Figures 3A,B).

In a series of 91 CIAD patients (the largest neonatal CIAD case series reported to date), among the 69 patients with neonatal-onset CIAD (37 and 32 patients with and without the *TPIT* mutation, respectively), 57 had complete ACTH deficiency and 12 had partial ACTH deficiency. In neonatal-onset cases with *TPIT* mutations, neonatal hypoglycemia was reported in all patients (37/37, 100%) whereas the frequency of other clinical signs, such as prolonged cholestatic jaundice and seizures, was 62% (21/34) and 53% (16/30), respectively. No recurrent respiratory infections were reported (1). However, the current case

<sup>6</sup>http://raptorx.uchicago.edu/StructurePrediction.

presented with recurrent respiratory infections. Interestingly, he had no hypoglycemia, seizures, or prolonged neonatal jaundice. It may be speculated that intermittent use of systemic steroids during infection may also prevent hypoglycemia and electrolyte imbalance, which could delay diagnosis in the early months. In addition, the novel and previously reported compound heterozygous mutations were both located outside of the DNAbinding domain and may be associated with the phenotype of the current case.

During the second pregnancy of the mother, low levels of E3 and hCG were detected. Low E3 levels in the context of normal fetal sonography and growth are reported in placental sulfatase deficiency and Smith–Lemli–Opitz syndrome where fetal steroidogenesis is defective. Low maternal E3 levels during pregnancy should raise suspicion of deficient fetal steroidogenesis (11, 12). Therefore, prompt evaluation of the infant for glucocorticoid deficiency in the first postnatal days will allow for early diagnosis.

In conclusion, adrenal insufficiency should be taken into account to prevent fatality and/or complications in infants with recurrent lung infections. Genetic analysis confirmed the diagnosis, and a new mutation was identified in our patient. We postulated that this novel and previously reported compound heterozygous mutations may be associated with recurrent lung infections in infants with CIAD. In addition, knowledge of the molecular mechanisms underlying this clinical entity could allow prenatal or early neonatal diagnosis in families at risk, and significantly prevent lethality.

# PATIENT CONFIDENTIALITY

The patient's guardian provided informed consent for publication of the submitted article, and the results of the accompanying genetic analyses, after a full explanation of the purpose and nature of all the procedures used.

# AUTHOR CONTRIBUTIONS

NA: following-up the patient, clinical diagnosis, collecting all data, summarizing and writing main draft of the article. NS, BT, and ST: genetic analyses; writing and checking all the data in the article. RB: following-up the patient, clinical diagnosis, and writing and checking all the data in the article. NB: differential diagnosis for recurrent infections; writing and checking all the data in the article.

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<sup>&</sup>lt;sup>3</sup>http://www.uniprot.org/uniprot/O60806.

<sup>&</sup>lt;sup>4</sup>https://genome.ucsc.edu/cgi-bin/hgBlat.

<sup>&</sup>lt;sup>5</sup>http://genetics.bwh.harvard.edu/pph2/.

<sup>7</sup> http://bioinf.cs.ucl.ac.uk/psipred/.

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**Conflict of Interest Statement**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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