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The Pitt-Hopkins Syndrome: Report of 5 Patients and Literature Comparison

Pitt-Hopkins Sendromu: 5 Vaka Sunumu ve Literatür Karşılaştırması

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ABSTRACT

Pitt-Hopkins syndrome (PTHS) is characterized by developmental delay, intellectual disability and behavioral changes, distinctive facial gestalt, and breathing abnormalities. PTHS is caused by deletions or pathological variants in the TCF4 gene located at 18q21.2. In this report, we aimed to describe the clinical and genetic findings of patients diagnosed with PTHS and compare our patients with the literature. Patients who were followed up with severe intellectual disability and a variable association of features previously described as characteristic of the PTHS phenotype in the pediatric neurology clinic of Antalya Training and Research Hospital were screened for TCF4 mutations using next-generation sequencing (NGS)-based tests, between 2017 and 2020. A genetic mutation associated with PTHS was detected in five patients. This paper emphasis on mutational and clinical spectrum of PTHS and its significant part in the differential diagnosis of severe mental retardation

Keywords: Angelman syndrome; breath-holding episode; intellectual disability; Pitt-Hopkins syndrome; TCF4

ÖZ

Pitt-Hopkins sendromu (PTHS) gelişimsel gecikme, mental retardasyon ve davranış değişiklikleri, belirgin yüz görünümü ve solunum anormallikleri ile karakterizedir. PTHS, 18q21.2'de bulunan TCF4 genindeki delesyonlardan veya varyantlardan kaynaklanır. Bu yazıda PTHS tanısı alan hastaların klinik ve genetik bulgularını tanımlamayı ve bulgularımızı literatür ile karşılaştırmayı amaçladık. Antalya Eğitim ve Araştırma Hastanesi pediatrik nöroloji kliniğinde 2017 ve 2020 arasında takip edilen, ağır mental retardasyon ve daha önce PTHS fenotipinin karakteristiği olarak tanımlanan özellikleri taşıyan hastalar, yeni nesil dizileme (NGS) tabanlı testler ile TCF4 mutasyonları açısından tarandı. 5 hastada PTHS ile ilişkili bir genetik mutasyon tespit edildi. Bu yazıda, PTHS'nin mutasyonel ve klinik spektrumuna ve ciddi zihinsel geriliğin ayırıcı tanısındaki önemli kısmına vurgulandı.

Anahtar kelimeler: Angelman sendromu, nefes tutma nöbetleri, Pitt-hopkins sendromu, TCF4, zeka geriliği

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INTRODUCTION

Pitt-Hopkins syndrome (PTHS; MIM #610954) is a rare autosomal dominant disease caused by the haploinsufficiency of the transcription factor 4 (TCF4) gene on chromosome 18q21 [1]. Until now, approximately 500 PTHS patients were reported worldwide[2]. PTHS is characterized by severe developmental delay (DD) with moderate-to-severe intellectual disability (ID) and behavioral disturbances, loss of speech, characteristic facial features, tendency to epilepsy, constipation, high myopia, and episodic hyperventilation and/or breath-holding while awake. PTHS is phenotypically similar to several neurodevelopmental disorders such as Angelman Syndrome (AS) (MIM #105830), Rett Syndrome (MIM #312750), Mowat-Wilson Syndrome (MWS) (MIM #235730), and Alpha-thalassemia/mental retardation, X-linked (ATR-X) Syndrome (MIM #301040) [3].

PTHS diagnosis is based on molecular confirmation of characteristic clinical features. The diagnosis is suspected on clinical findings and confirmed by identification on molecular genetic testing of a heterozygous pathogenic variant in TCF4 or a deletion of the chromosome region in which TCF4 is located (18q21.2) [1]. New variants were classified by using the American College of Medical Genetics and Genomics (ACMG) guideline [4]. In this study, we inspected patients who presented to our clinic with ID and PTHS phenotype, detected five patients with TCF4 mutation and presented genotype-phenotype correlations of the molecularly confirmed cases with PTHS.

MATERIAL AND METHOD

Patients who were followed up with severe ID and a variable association of features previously described as characteristic of the PTHS phenotype in the pediatric neurology clinic of Antalya Training and Research Hospital were screened for TCF4 mutations between 2017 and 2020. The genetic diagnosis was made by investigating the TCF4 gene using next-generation sequencing (NGS)-based tests. To confirm our results, we checked the reading frames by using an integrative genomics viewer (IGV) [5]. All obtained variants were classified by using the American College of Medical Genetics and Genomics (ACMG) guideline [4]. Electroencephalography (EEG), electroneuromyography (EnMG), echocardiography (ECHO), and magnetic resonance imaging (MRI) were performed for all patients with TCF4 mutations. Written informed consent was obtained from all parents of the children, which was approved by our Hospital Ethics Committee (Date: 04.03.2021 number: 1/43).

RESULTS

A total of 67 patients with ID were clinically evaluated and 13 patients were found to have clinical features compatible with PTHS. A genetic mutation associated with PTHS was detected in five patients. These patients were reported as case reports below.

Case 1

The first patient was a 2,5-year-old boy, who was born 3800 gr, by cesarean section, from healthy, nonconsanguineous parents. His prenatal period was uneventful. His head circumference was within normal intervals. During the first year of his life, severe psychomotor delay was observed. He was able to raise his head at 12 months. He was able to walk at age 2 years, but the walking was unsteady and ataxic. The speech was absent. He first presented to medical attention at the age 4 months, with hypotonia and lack of following with eyes. He had breath-holding spells at age 3 months and lasted until age of 7 months. He had a long-standing history of constipation after beginning to complementary foods.

In physical examination, he had a short neck, and facial dysmorphism includes coarse face, bitemporal narrowing, squared forehead, full cheeks, peculiar nose conformation, with a broad nasal bridge, down-turned nasal tip, and flaring nostrils, and M shaped upper lip. He had small hands and feet, pes planus, clinodactyly. He had a smiling appearance and lovable behavior. Stereotypic movements of the hands and the head were observed. Poor eye contact was noted. In the ocular examination, strabismus was detected, retinal and fundus examinations were normal. No abnormalities were detected in routine biochemical examinations, and metabolic studies such as ammonia, ceruloplasmin, homocysteine, folic

acid, vitamin B12, thyroid function tests, plasma amino acid analysis. Abdominal ultrasounds, echocardiography, cranial MRI, EEG, and EMG were normal as well. Conventional cytogenetic analysis and fluorescent in situ hybridization (FISH) analysis for AS were normal. By clinical exome sequencing (CES) analysis, we detected a novel heterozygous c.611-180dupT variant in the TCF4 gene which was classified as pathogenic according to ACMG variant interpretation guideline. The patient was diagnosed with Pitt Hopkins syndrome (OMIM: 610954) with this variant.

Case 2

The patient was a 3-year-old girl, who was born 2650 gr, by cesarean section, from healthy, nonconsanguineous parents. Her prenatal period was uneventful. She had microcephaly, hypotonia, and feeding difficulties and stayed in NICU for one week. She was able to control her head at age 1 year and sit without support at age 2.5 years. She cannot walk or talk.

At the age of 6 months, she was referred to pediatric neurology because of hypotonia and microcephaly. She had breath-holding spells at age of 4 months and lasted until age of 10 months. She suffered from constipation after age of 1 year.

In physical examination, she had a short neck and typical facial dysmorphism; includes coarse face, deep-set eyes, strabismus, thin eyebrows with flaring in their midline portion, a large nose with a high bridge and flared nostrils, M-shaped Cupid's bow, fleshy lips, and wide mouth with shallow and broad palate, dysplastic and thick ear helices, and full cheeks. She had a single palmar crease, small hands, and feet. Agitation, poor eye contact, stereotypic movements of the hands and the head were observed. In the ophthalmic examination, astigmatism was detected, retinal and fundus examinations were normal. Mild hypotonia persisted.

Routine biochemical examinations and metabolic studies such as ammonia, ceruloplasmin, homocysteine, folic acid, vitamin B12, thyroid function tests, plasma amino acid analysis were within normal limits. Conventional cytogenetic analysis, FISH analysis for AS, and Prader-Willi (PWS)/AS-specific methylation analyses were normal. By CES analysis, we detected a heterozygous c.1113delC variant in the TCF4 gene which was classified as pathogenic according to the ACMG variant interpretation guideline. The patient was diagnosed with Pitt Hopkins syndrome (OMIM: 610954) with this variant.

Case 3

The third patient was a 7-year-old boy, who was born 820 gr in the 24th gestational week from consanguineous parents. He had intracranial hemorrhage at birth and stayed in NICU for 4 months. He received mechanical ventilator support. He had apnea and breath-holding spells during his NICU stay. He had hypotonia and microcephaly during the first year of his life. He had a psychomotor developmental delay. He was unable to raise his head until age of 12 months. He was able to walk at age 6 years, but walking was unsteady and ataxic. Speech development was absent. He had a constipation complaint at age 1 year. At the age 4 years, several episodes of hyperventilation were observed. He was checked and treated for retinopathy of prematurity (ROP).

Physical examination showed short neck and typical facial dysmorphism; heavy supraorbital regions, a broad and beaked nose with a high bridge and flaring nostrils, a wide mouth, a bow-shaped upper lip, broad palate, widely spaced teeth, dysplastic and thick ear helices, and protruding lower face, and micropenis. He had clubbing fingers, small hands, and feet. In the ophthalmic examination, astigmatism was detected, retinal and fundus examinations were normal. He suffered from chronic constipation. He showed autistic features such as low frustration tolerance, sleep disturbances, stereotypical hand and head movements, and poor eye contact. His full-scale intelligence quotient (IQ, Stanford Binet Intelligence Scales, 5th Edition) was 57.

Routine biochemical examinations and metabolic studies such as ammonia, plasma, and urine amino acid analysis, long-chained fatty acid analysis, urine organic acid analysis, and tandem mass spectrometry were within normal limits. Abdominal ultrasounds, echocardiography, MR spectroscopy, EEG, and EMG were normal. His cranial MRI showed diffuse cerebellar atrophy. Conventional

cytogenetic analysis and FISH analysis for AS were normal. By whole-exome sequencing (WES) analysis, we detected a novel heterozygous c.611-180dupT variant in the TCF4 gene which was classified as pathogenic according to ACMG variant interpretation guideline. The patient was diagnosed with Pitt Hopkins syndrome (OMIM: 610954) with this variant.

Case 4

Patient four; was an 8-year-old girl, who was born 2750 gr, by cesarean section, from healthy, consanguineous parents. Her prenatal period was uneventful. During the first year of her life, severe psychomotor delay, and microcephaly were observed. She was able to raise her head at 3 months and sit unsupported at age 3 years. She was able to walk at age 4.5 years yet still she was unable to walk independently.

Speech development was absent. Urinary control had not developed. She had a long-standing history of constipation. She had never experienced apnea, breath-holding spells, or episodes of hyperventilation. She first presented to medical attention at the age 9 months, with hypotonia and developmental delay.

In physical examination, she had a short neck and typical facial dysmorphism; includes coarse face, bitemporal narrowing, thin eyebrows with flaring in their midline portion, peculiar nose conformation, with a broad nasal bridge, down-turned nasal tip, large nostrils, wide mouth, bow-shaped upper lip, broad palate, widely spaced teeth, dysplastic and thick ear helices, well-developed chin and protruding lower face. She had a smiling appearance. Anxiety behaviors and stereotypic movements of the hands (wringing and swaying) and the head were observed. Poor eye contact was noted. Her full-scale intelligence quotient (IQ, Stanford Binet Intelligence Scales, 5th Edition) was 62.

No abnormalities were detected in routine biochemical examinations and metabolic studies such as ammonia, plasma, and urine amino acid analysis, long-chained fatty acid analysis, urine organic acid analysis, and tandem mass spectrometry. She had congenital hypothyroidism. Ophthalmic examination was normal. Abdominal ultrasound, EEG, and EMG were normal as well. She had minimal ASD and VSD in echocardiography. Her cranial MRI showed corpus callosum agenesis.

Conventional cytogenetic analysis and FISH analysis for AS were normal. By WES analysis, a known pathogenic, heterozygous c.1459C>T variant in the TCF4 gene was detected, and the patient was diagnosed with Pitt Hopkins syndrome (OMIM: 610954) with this variant.

Case 5

The last patient was a 6-year-old girl, who was born 2900 gr, by cesarean section, from healthy, nonconsanguineous parents. Her prenatal period was uneventful. Her head circumference was less than 3 percentiles. During the first year of her life, severe psychomotor delay was observed. She was unable to raise her head until age of 12 months. She was able to walk at age 4 years, but she has been still walking unsteady and ataxic. Speech development was absent. She first presented to medical attention at age 3 months, with hypotonia and microcephaly. Apnea and breath-holding spells were observed but she had no seizures. She had a long-standing history of constipation.

In physical examination, she had a short neck and typical facial dysmorphism includes coarse face, full cheeks, flaring eyebrows, hypertelorism, broad nasal bridge, down-turned nasal tip and flaring nostrils, wide mouth, bow-shaped upper lip, broad palate, widely spaced teeth, dysplastic and thick ear helices. She had clubbing fingers, small hands, and feet.

Anxiety behaviors, stereotypic movements of the hands (hand clapping and flapping), and the head were observed. Ophthalmic examination was normal. Poor eye contact was noted. Her full-scale intelligence quotient (IQ, Stanford Binet Intelligence Scales, 5th Edition) was 53.

Routine biochemical examinations and metabolic studies such as ammonia, plasma, and urine amino acid analysis, long-chained fatty acid analysis, urine organic acid analysis, and tandem mass spectrometry were within normal limits. Abdominal ultrasounds, echocardiography, MR spectroscopy, and EMG were normal. Her cranial

MRI showed mild cerebellar atrophy and corpus callosum agenesis. EEG studies revealed focal temporal epileptic activity however she had no seizure. The patient was diagnosed with Pitt Hopkins syndrome at a different genetic testing center. Conventional cytogenetic analysis and FISH analysis for AS were normal. They detected a known pathogenic c.2039G>A variant in the TCF4 gene by using NGS panel for Mendelian diseases.

Figure-1: Facial features of the patients. Images are numbered in order as mentioned in the article. Coarse face, bitemporal narrowing, squared forehead, full cheek, broad nasal bridge, down-turned nasal tip and flaring nostrils, m shaped upper lip are seen in all patients. Patient 3 also has dysplastic and thick ear helices and protruding lower face.

DISCUSSION

PTHS is a rare, nonprogressive encephalopathy, characterized by distinct facial features, severe developmental delay with moderate-to-severe intellectual disability and behavioral disturbances, loss of speech, tendency to epilepsy, episodic hyperventilation, and/or breath-holding while awake, and a variety of additional clinical findings. PTHS is caused by haploinsufficiency of the TCF4 gene at 18q21.2 due to deletions, stop, splice-site, and de novo missense mutations [6]. TCF4 gene contributes to human development by regulating the expression of several genes. Therefore, apart from TCF4 gene mutations, changes in the function and expression of the genes controlled by TCF4 may also affect the phenotype of PTHS patients [7].

Characteristic facial features of PTHS contain bitemporal narrowing, full cheeks with a prominent lower face, deep-set eyes, large nasal bridge, large mouth with M-shaped upper lip, protruding and thick lower lip vermillion, cup-shaped ears, and widely spaced teeth. Sweet personality, subtle brain abnormalities, seizures, postnatal microcephaly, ataxic gait/motor incoordination, stereotypic movements, strabismus, myopia, and astigmatism were the most frequent additional clinical manifestations of PTHS. Breathing abnormalities are a prominent part of PTHS, could be seen as breath-holding spells or hyperventilation episodes.

The diagnosis of PTHS is based on the typical clinical presentation confirmed by molecular genetic methods. PTHS is clinically overlapping with other several neurodevelopmental disorders such as AS, Rett Syndrome, and MWS. Differential diagnosis is mainly based on clinical findings. Although AS has a characteristic EEG pattern, MRI and EEG studies do not suggest specific diagnostic leads for the mentioned syndromes [8]. AS mostly presents with unmotivated laughing episodes, seizures, and microcephaly. In addition, AS patients lack the typical facial features observed in PTHS. In MWS, Hirschsprung disease is seen in almost half of the cases and cardiac and urogenital (hypospadias) malformations are common which could be useful for differential diagnosis. Rett syndrome is a progressive encephalopathy, with a normal early development is followed by arrest and regression of motor and cognitive skills. Intractable epilepsy presents in many patients with Rett syndrome. Importantly, patients lack the facial characteristics associated with PTHS [6,9].

Genotype-phenotype correlation analyses led researchers to define a clinical score system for targeted genetic testing for PTHS. Whalen et al. and Marangi et al. defined different scoring systems. Both studies attributed most of the points to characteristic facial configurations. Absent speech, ataxic gait, hyperventilation, and strabismus were the other common signs in both scoring systems. The main aim is to detect patients who need TCF4 screening as a first choice [9,10].

With NGS technologies, the rates of diagnosis, detection of new genes, and mutations have increased. This increase allows for the development of follow-up and treatment protocols depending on the clinical heterogeneity resulting from different mutations. Therefore, comparing the clinical findings of patients with different mutations in the same gene can provide important information. Here, in this study, we shared the clinical findings of 5 Pitt-Hopkins patients with 4 different TCF4 mutations by comparing them with the findings reported in the literature.

Genetic analyses were performed for all of our patients, and we detected a novel variant in patients 1 and 3 that was not reported in the literature previously. In order to confirm our results, we checked the reading frames by using integrative genomics viewer (IGV) [5]. All obtained variants were classified by using the American College of Medical Genetics and Genomics guideline [4]. After that, we reviewed the literature for each variant that was found in our patients and compared our clinical findings with the reported patients' clinical findings. Patients 2,4 and 5 had mutations that were previously reported.

Case 1 and 3 were diagnosed in our center and had the same variant in the TCF4 gene that had been not reported in the literature before. They shared several clinical features including hypotonia, developmental delay, absent speech, autistic features, stereotypic movements, poor eye contact, ocular findings, and dysmorphic features as shown in Table 1. Unlike Case 1, Case 3 had prematurity with intracranial hemorrhage and ROP, intellectual disability, and cerebellar atrophy. Both TCF4 gene mutation, and prematurity and intracranial hemorrhages at birth can cause intellectual disability and atrophy. As far as we know, a male patient with a preterm labor complication was reported in the literature, however, the patient's mutation was affecting another domain of the protein [11]. Therefore, the relationship between TCF4 gene mutation and preterm birth remains unclear. (Table 1)

Case 2 had TCF4 c.1113delC variant and classic features of Pitt-Hopkins syndrome. In the literature, only a VarSome user from Turkey reported this variant as disease-causing, but clinical findings of the patient were unavailable [12]. So, we could not compare the patients' clinical findings.

Case 4 had c.1459C>T variant in the TCF4 gene and Case 5 had c.2039G>A variant in the TCF4 gene, both had similar findings with the previously reported patients, and additionally, patient 4 had congenital hypothyroidism and urinary incontinence. The comparisons of clinical findings with the literature are shown in Table 2 and Table 3, respectively [13–16].

Limitations of the study

Our study has several limitations mainly related to the study population size The number of patients meeting the inclusion criteria was small because it was a single center study. However, this present study may contribute to national data and/or the systematic reviews and meta-analyzes [17] which will be done together with other studies originating from our country. In our opinion, It would be more effective to identify new variants and define the phenotype-genotype characteristics of these variants in a multi-center study.

CONCLUSION

Pitt-Hopkins syndrome should be considered in children with specific craniofacial dysmorphism, severe developmental delay, intellectual disability, breathing abnormalities, and disturbances of intestinal motility. The diagnosis of PTHS is a clinical one; clinical evaluation and genetic confirmation must be performed together. It is well known that a certain degree of genetic heterogeneity exists for well-defined clinical conditions, including PTHS. Therefore, more studies about genotype-phenotype correlations and novel variant reports are needed to make a precise diagnosis.

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Table 1: Comparison of clinical findings of TCF4 novel c.611-80dup variant

		Physical exam	Developmental exam	Radiologic/Ophthalmologic exam	Other
Present study	Case 1	•Coarse face •Bitemporal narrowing •Squared forehead •Full cheeks •Peculiar nose conformation, broad nasal bridge, down-turned nasal tip and flaring nostrils •M shaped upper lip •Short neck •Small hands and feet •Pes planus •Clinodactyly	•Psychomotor delay •Hypotonia •Absent speech •Autistic features •Stereotypic movements •Poor eye contact	•Strabismus	-
	Case 3	•Microcephaly •Heavy supraorbital regions •Dysplastic and thick ear helices •Protruding lower face •Broad and beaked nose with high bridge and flaring nostrils •Wide mouth, bow-shaped upper lip •Broad palate •Widely spaced teeth •Short neck •Clubbing fingers •Small hands and feet •Micropenis	•Psychomotor delay •Hypotonia •Absent speech •Intellectual disability •Autistic features •Stereotypic movements •Poor eye contact	•Astigmatism •Diffuse cerebellar atrophy	•Prematurity •Intracranial hemorrhage at birth •ROP •Apnea •Constipation •Hyperventilation episodes
Literature review	N/A				

Table 2: Comparison of clinical findings of TCF4 c.1459C>T variant

		Physical exam	Developmental exam	Radiologic/Ophthalmologic exam	Other
Present study	Case 4	•Microcephaly •Coarse face •Bitemporal narrowing •Squared forehead •Full cheeks •Peculiar nose conformation, with a broad nasal bridge, down-turned nasal tip and flaring nostrils, •M shaped upper lip •Short neck •Small hands and feet •Pes planus •Clinodactyly	•Severe psychomotor delay •Ataxia •Hypotonia •Absent speech •Happy appearance •Stereotypic movements •Poor eye contact •Anxiety •Constipation	•Minimal ASD and VSD •Strabismus •Corpus callosum agenesis	•Congenital •Hypothyroidism •Urinary incontinence
Literature review	Zweier et al.	•Microcephaly •Short hands and feet •Hyperconvex nails •Bilateral supernumerary digital flexion crease on 3 and 4th fingers •Single palmar crease	•Severe psychomotor delay •Ataxia •Hypotonia •Absent speech •Episodes of hyperventilation-apnea •Unmotivated laughter episodes •Very anxious and auto aggressive behavior •Constipation	•Strabismus	•MRI N/A
	Hamdan et al. N/A				

Table 3: Comparison of clinical findings of TCF4 c.2039G>A variant

		Physical exam	Developmental exam	Radiologic/Ophthalmologic exam	Other
Present study	Case 5	•Coarse face •Bitemporal narrowing •Squared forehead •Full cheeks •Peculiar nose conformation, with a broad nasal bridge, down-turned nasal tip and flaring nostrils •M shaped upper lip •Short neck •Small hands and feet •Pes planus	•Microcephaly •Severe psychomotor delay •Intellectual disability •Hypotonia •Absent speech •Apnea and breath-holding spells •Constipation •Anxiety stereotypic movements •Poor eye contact	•Mild cerebellar atrophy and corpus callosum agenesis •Focal temporal epileptic activity without seizure	-
Literature review	Mary et al.	•Mildly fascial gestalt	•Microcephaly •Intellectual disability •Delayed walking •Absent speech •Hyperventilation/apnea •Happy appearance •Sleep disturbances	•Duane anomaly	•Headaches
	Zweier et al (a) P13	•Typical fascial gestalt •Single palmar crease	•Intellectual disability •Hypotonia •Happy appearance •Stereotypic movements		•No microcephaly •No ventilation anomalies •Inability to use hands •Fetal pads of toes •Cold hands and feet
	Zweier et al (a) P16 (total patient number: 6)	•Typical fascial gestalt (all patients) •Single palmar crease (in 4 patients)	•Severe intellectual disability (all patients) •Seizures (in 2 patients) •Hypotonia (all patients) •Ventilation anomalies (in 5 patients) •Happy appearance (all patients)	•Arachnoidal cyst	•No microcephaly (but other 4 patients had) •No constipation (but in 3 patients) •No scoliosis (but in 2 patients) •Recurrent ear infections •One patient had accessory nipple, and 2 patients had strabismus

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