Short Report

Two percent of patients suspected of having Angelman syndrome have TCF4 mutations


The TCF4 gene encodes a basic helix–loop–helix (bHLH) transcription factor which belongs to the family of E-proteins. E-proteins form homo- and heterodimers with other members of the HLH family and bind to the common DNA sequence called E-box. Haploinsufficiency of the TCF4 gene has been found to be associated with the Pitt–Hopkins syndrome (PTHS). PTHS is characterized by severe mental retardation, a wide mouth plus other distinctive facial features (fleshy lips, beaked nose, broad nasal bridge) and breathing abnormalities. Because of some phenotypical overlap with Angelman syndrome (AS), it has been suggested that PTHS be considered in its differential diagnosis. To explore this possibility, we screened 86 patients who were suspected of having AS. All the patients were negative for UBE3A testing, and 53 were known to be negative for methylation analysis. We identified two TCF4 mutations in this cohort. The p.S384Tfsx7 mutation lacks the bHLH domain. The p.R582P mutation lies within the bHLH domain in which seven other missense mutations have been reported. Both mutations most likely affect the critical function of the bHLH domain of the TCF4 protein. In summary, we found two TCF4 mutations in 86 patients (2%) suspected to have AS. Screening for mutations in this gene should be considered in patients who present with findings of AS but who have been negative for methylation and UBE3A testing.

K TakanoA, M LyonsA, C MoyesB, J JonesA and CE SchwartzA,C

A Greenwood Genetic Center, Greenwood, SC, USA, B Whakatane Hospital, Whakatane, New Zealand, and C Department of Genetics and Biochemistry, Clemson University, Clemson, SC, USA

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Corresponding author: Charles E. Schwartz, Greenwood Genetic Center, 113 Gregor Mendel Circle, Greenwood, SC 29646, USA. Tel.: +1 864 941 8140; e-mail: ceschwartz@ggc.org

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Pitt and Hopkins described two unrelated patients with mental retardation, a wide mouth and intermittent overbreathing in 1978 (1). Pitt–Hopkins syndrome [PTHS; Online Mendelian Inheritance in Man (OMIM) 610954] is characterized by severe mental retardation, a wide mouth plus other distinctive facial features (fleshy lips, beaked nose, broad nasal bridge) and breathing abnormalities (2–7). In 2007, PTHS was found to be caused by haploinsufficiency of the transcription factor 4 (TCF4; OMIM 602272, also known as ITF2, SEF2 or E2–2) in 18q21 (2, 3). A total of 50 PTHS patients with haploinsufficiency of the TCF4 gene have been reported (2–7).

The TCF4 gene encodes a basic helix–loop–helix (bHLH) transcription factor which belongs to the family of E-proteins (8, 9). E-proteins form homo- and heterodimers with other members of the HLH family, specifically bind to a common DNA sequence called E-box (10, 11) and play important roles in the regulation of cell growth and differentiation, including the cortical development (9, 12–14). TCF4 has been shown to be involved in noradrenergic neuron development (15) and pontine nucleus differentiation (16), which may explain the neurological signs observed in patients with PTHS.

Because of some phenotypical overlap with Angelman syndrome (AS; OMIM 105830), it has been have suggested that PTHS be considered in its differential diagnosis (2–7). To elucidate the proportion of patients thought to have AS who have TCF4 mutations, we screened the TCF4 gene in 86 patients suspected of having AS.
Materials and methods

Patients

Eighty-six patients suspected of having AS were included in this study. All 86 patients utilized in this study had normal UBE3A sequencing. Fifty-three patients were negative for DNA methylation analysis of PWS/AS-IC as determined by the molecular diagnostic laboratory at the Greenwood Genetic Center. The remaining 33 patients were assumed to be negative for the DNA methylation test, as the DNA methylation test is commonly conducted first in patients suspected to have AS (17, 18).

Mutation screening

Genomic DNA was isolated from whole blood according to standard methods. The 18 coding exons of TCF4 (exons 2–19) were amplified using intronic primer pairs flanking each exon. Polymerase chain reaction (PCR) products were prescreened by denaturing high-performance liquid chromatography (dHPLC) (WAVE System, Transgenomic, Inc., Omaha, NE) and patients with abnormal profiles were sequenced using an ABI3730 x/ DNA analyzer (Applied Biosystems, Inc., Foster City, CA). DNA mutation numbering was based on cDNA sequence NM_001083962. The polymorphism study for the c.1745G>C mutation was conducted using dHPLC. Primer sequences PCR conditions and dHPLC protocols are available upon request.

Protein secondary structure analysis

The secondary structure of the bHLH domain of the p.R582P mutation was analyzed using the software program Protean (DNASTAR, Inc).

Results

Screening of 86 patients thought to have AS identified two novel TCF4 mutations. One is a frameshift mutation (c.1151delG; p.S384Tfsx7) and the other one is a missense mutation (c.1745G>C;p.R582P) in the bHLH domain.

Patient 1 is a 7-year-old son of healthy, unrelated Hispanic parents. He has a healthy brother and three half siblings. A cousin of his mother has mental retardation and seizures. Although the pregnancy was complicated by preterm labor requiring monitoring and partial bed rest, his birth at term was an uneventful cesarean section. His birth weight was 3600 g. Developmental delay was noticed around 1 year of age. He had difficulties with eating solid foods in early infancy.

At the age of 18 months, he was referred to the Greenwood Genetic Center because of developmental delay. On the physical examination, his height was 75.2 cm (<3rd percentile), his weight was 9.5 kg (<3rd percentile), and his head circumference was 45.5 cm (3rd to 5th percentile). He had an open-mouth expression with a tented upper lip and a full lower lip with a thick vermilion border, small epicanthal folds and long and curly eyelashes (Fig. 1a). He had a depressed nasal bridge with anteverted nares. Dermatoglyphics showed bilateral bridge transverse palmer creases. There were a few small café-au-lait spots on his left rib cage and flank, a capillary hemangioma and a small nevus on his right buttock. He had persistent drooling. His muscle tone and reflexes were normal. He had difficulties with constipation. He had staring spells which had existed since 6–8 months of age. They lasted for 5–10 s and he seemed unconscious during spells. Brain magnetic resonance imaging (MRI) and electroencephalogram (EEG) were normal.

At a follow-up evaluation at the age of 4 years 8 months (Fig. 1b), he was in a special needs class in pre-K. He could sit at the age of 12 months and walk at the age of around 3 years. Although he started to speak ‘Mama’ and ‘Dada’ at the age of 3 years, he has not obtained additional words. He had some problems of fighting with his siblings and biting his teachers. He showed some stereotypic movements, such as spinning. He had no obvious seizures and staring spells. On physical examination, his height was 96 cm (<3rd percentile), his weight was 11.3 kg (<3rd percentile), and his head circumference was 48.9 cm (<10th percentile). He had fifth finger clinodactyly bilaterally and slightly prominent fingertip pads. Feet turned out with pes planus evident and there was mild 2–3 toe syndactyly bilaterally. He exhibited a wide-based unsteady gait. His fingers were somewhat long and thin. He showed mild to moderate hypotonia.

Presently, at 7 years old, he attends elementary school in a special education classroom and receives physical and occupational therapies. His expressive language has not progressed since he was 3 years of age. His disposition is usually happy. He shows unusual hand posturing, finger crossing and washing hand movement. On the physical examination, his height was 115 cm (<3rd percentile), his weight was 16 kg (<3rd percentile), and his head circumference was 50 cm (5th to 10th percentile). In addition to his characteristic nose, mouth and lips, he has very
prominent, wide-spaced central incisors (Fig. 1c). The palate appears thick and high. Chest shows a supernumerary nipple on the left. He does not have myopia. He has slender hands (3rd to 25th percentile). He has extra distal flexion creases on 3rd and 4th fingers bilaterally. He has prominent finger and toe pads. He has mild hypotonia and normal deep tendon reflexes. He has a wide-based gait. He did not have any problems with breathing. He had neither staring spells nor obvious seizures.

He has a heterozygous c.1151delG (p.S384Ter) mutation (Fig. 2a). The mutation is de novo, as it was not present in either parent.

Patient 2 is a 17-year-old male with severe mental retardation. His unrelated parents and two brothers are well. A cousin and a maternal uncle are reported to have schizophrenia. The pregnancy was uneventful. He was born by normal delivery at 37 weeks with a birth weight of 2900 g. His development was slow from the very beginning. He had difficulty feeding. At the age of 10 months, he was referred to the pediatrician because he could not sit and did not babble. He walked at the age of 4 years.

He has suffered from the spells with cyanosis and dystonic movements during wakefulness as he was 7. He hyperventilates prior to the onset

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**Fig. 1.** Facial features of patient 1 at the ages of 18 months (a), 4 years and 8 months (b), and 7 years (c). Note the open-mouthed appearance with a tented upper lip, a full lower lip, depressed nasal bridge with anteverted nares, and small epicanthal folds. Wide-spaced central incisors become prominent with advancing age.

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**Fig. 2.** Identification of TCF4 mutations in patients. Sequence chromatograms of patient (top panel) and wild-type (bottom panel) genomic DNA, with corresponding amino acids. (a) Patient 1 has a heterozygous c.1151delG mutation, which leads to a frameshift mutation p.S384Ter. (b) Patient 2 has a heterozygous c.1745G>C mutation changing amino acid arginine 582 to a proline.
of the event, followed by apnea. Then he has writhing movements and becomes significantly cyanotic with consciousness. This event lasts for a minute or less. At the age of 12 years, the video EEG monitoring showed that these episodes were not of epileptic origin. His background EEG had extremely low amplitude 3–4 Hz activity seen in a diffuse distribution.

His first seizure occurred at the age of 10 years. He experienced several episodes of generalized tonic–clonic seizure.

On physical examination at the age of 12 years, his height was 144.5 cm (<25th percentile), his weight was 30.2 kg (3rd percentile), and his head circumference was 50.8 cm (<3rd percentile). He had a large mouth with prominent lips and drooling, slight beaked and broad nasal bridge, convergent strabismus, familial blond woolly hair, mild supraorbital ridging, thin eyebrows, cryptorchidism and planovalgus deformities of his feet. He had long fingers and short 4th toes bilaterally. He was hypotonic with poor coordination. He walked with an ataxic jerky gait. His meaningful use of words was limited to ‘Mum’ and ‘Car’.

At the age of 16, he continues to suffer from epilepsy and cyanotic spells. Both events are relatively well controlled on his antiepileptic medication, Lamotrigine and Clobazam.

He has a heterozygous c.1745G>C (p.R582P) mutation (Fig. 2b). DNA samples of his parents were not available. This alteration was not found in 277 control individuals.

Discussion

PTHS is an autosomal dominant syndrome characterized by severe mental retardation, distinct facial features and hyperventilation (Table 1) (2–7). Initially, PTHS was thought to be a rare syndrome as only eight patients had been reported in the literature until recently (2, 3). However, once it was determined that haploinsufficiency of the TCF4 gene causes PTHS in 2007 (2, 3), a total of 50 patients have been published with TCF4 mutations (2–7). With a better understanding of the patients with PTHS, it has been suggested that PTHS be an important consideration in the differential diagnoses of other mental retardation syndromes, e.g. AS, Rett syndrome (RTT; OMIM 312750) and Mowat–Wilson syndrome (MWS; OMIM 235730) (2–7). AS is characterized by severe mental retardation with severe impairment of speech, balance disorder, characteristic behaviors, epilepsy and dysmorphic facial features (17, 18). Because some of the clinical features of PTHS overlap those of AS (Table 1), we screened 86 patients who were suspected to have AS but had normal UBE3A sequencing and found two TCF4 mutations.

The p.S384Tfsx7 mutation results in the loss of the bHLH domain. mRNA with this frame shift mutation is predicted to either be degraded by nonsense-mediated mRNA decay (19) or produce a truncated protein lacking the bHLH domain. Either of these events would lead to a loss of function of TCF4 and haploinsufficiency.

The p.R582P mutation lies within the bHLH domain in which seven other recurrent or individual missense mutations have been reported (Fig. 3) (2–4, 6). The bHLH domain consists of a DNA-binding basic region followed by two α-helices separated by a loop region (Fig. 3) (10, 11). The HLH domain forms homo- and heterodimers with other HLH proteins and the basic region is required for dimarized bHLH proteins binding a specific DNA motif (10, 11). Amino acid residue R582 lies within the helix 1 region of the bHLH domain and is highly conserved from Drosophila to human among E-proteins (Fig. 3). The substitution of this arginine 582 with proline is predicted to disrupt the α-helix using the Garnier–Robson algorithm (data not shown) (20). Proline substitution in helix 1 abolished oligimerization in MyoD, another bHLH protein (10). Consequently, the substitution of this arginine 582 with proline very likely affects the critical function of the bHLH domain of the TCF4 protein and leads to haploinsufficiency for TCF4.

Our patients have characteristic neurological and facial findings, which are quite similar to the clinical features of PTHS (Table 1). However, patient 1 has neither hyperventilation nor epilepsy. It has been reported that 30–80% of PTHS patients have hyperventilation and the age of its onset varies from the first month to 17 years (4–6). Epilepsy was observed 30–55% of patients and the age of its onset varies from birth to 9 years (4–6). Postnatal microcephaly has been observed in 60–90% of PTHS patients. Patient 1 is not microcephalic (4–6). Some clinical features of PTHS vary among patients, which complicates the diagnosis of PTHS.

Both patients have severe mental retardation with limited speech, a wide-based unsteady gait, wide mouth, hypotonia and drooling, which overlap the clinical findings of AS (Table 1) (17). Their methylation analysis of PWS/AS-IC and sequencing analysis of UBE3A were normal. About 10–15% of patients with clinical findings of AS have normal genetic testing for AS (17). Mutations in TCF4 may therefore account for a significant proportion of these patients.
Males affected with X-linked α-thalassaemia mental retardation (ATR-X; OMIM 301040) have severe mental retardation, dysmorphic features, genital abnormalities and mild α-thalassaemia (21). Some of these findings also overlap those of PTHS (Table 1). Patient 1 was suspected having ATR-X because of his open-mouth expression, a tented upper lip, a full lower lip, a depressed nasal bridge with anteverted nares and wide-spaced incisors (21). His ATRX testing was negative.

Our study would appear to indicate that at least 2% of patients with an AS-like phenotype may have mutations in TCF4. As we did not screen for cryptic large deletions or exonic deletions and as direct sequencing is incapable of detecting large deletions, the prevalence of TCF4 mutations may
TCF mutations in suspected Angelman pts

Fig. 3. Alignment of E-proteins orthologs. An arrow shows the location of R582P mutation. Shadowed amino acids are conserved in orthologs. Filled circle and triangles indicate the positions of nonsense and missense mutations in the literature, respectively.

be understated. It is important to consider PTHS in the differential diagnosis of AS, especially those whose usual AS testing has been negative. Additionally, for males thought to have ATR-X but are negative for gene testing, TCF4 testing should be considered.

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Web resources

The URL for data presented herein is as follows: Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/OMIM/

Accession numbers: The GenBank Accession No. for the TCF4 cDNA sequence used for mutation numbering was NM_001083966.

Conflict of interest

There are no conflicts of interest or financial disclosures to report with regard to this article.

References