Journal of the Neurological Sciences 315 (2012) 77–81

Contents lists available at SciVerse ScienceDirect

Journal of the Neurological Sciences

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

Hypoperfusion in caudate nuclei in patients with brain–lung–thyroid syndrome

Mitsugu Uematsu [a,*], Kazuhiro Haginoya [b], Atsuo Kikuchi [a], Tojo Nakayama [a], Yousuke Kakisaka [a], Yurika Numata [a], Tomoko Kobayashi [a], Naomi Hino-Fukuyo [a], Ikuma Fujiwara [a], Shigeo Kure [a]

[a] Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan
[b] Department of Pediatric Neurology, Takuto Rehabilitation Center for Children, Sendai, Japan

ARTICLE INFO

Article history:
Received 20 August 2011
Received in revised form 11 November 2011
Accepted 15 November 2011
Available online 12 December 2011

Keywords:
Brain–lung–thyroid syndrome
NKX2-1
Array CGH
ECD-SPECT
eZIS

ABSTRACT

Mutations in *NKX2-1* cause neurological, pulmonary, and thyroid hormone impairment. Recently, the disease was named brain–lung–thyroid syndrome. Here, we report three patients with brain–lung–thyroid syndrome. All patients were unable to walk until 24 months of age, and still have a staggering gait, without mental retardation. They have also had choreoathetosis since early infancy. Genetic analysis of *NKX2-1* revealed a novel missense mutation (p.Val205Phe) in two patients who were cousins and their maternal families, and a novel 2.6-Mb deletion including *NKX2-1* on chromosome 14 in the other patient. Congenital hypothyroidism was not detected on neonatal screening in the patient with the missense mutation, and frequent respiratory infections were observed in the patient with the deletion in *NKX2-1*. Oral levodopa did not improve the gait disturbance or involuntary movement. The results of ^{99m}Tc-ECD single-photon emission computed tomography (ECD-SPECT) analyzed using the easy Z-score imaging system showed decreased cerebral blood flow in the bilateral basal ganglia, especially in the caudate nuclei, in all three patients, but no brain magnetic resonance imaging (MRI) abnormalities. These brain nuclear image findings indicate that *NKX2-1* haploinsufficiency causes dysfunction of the basal ganglia, especially the caudate nuclei, resulting in choreoathetosis and gait disturbance in this disease.

1. Introduction

NK2 homeobox 1 (*NKX2-1* or *TITF-1*; MIM #600635), which maps on chromosome 14q13, is a member of the *NK-2* gene family of highly conserved homeodomain-containing transcription factors [1,2]. The gene is expressed in the thyroid, bronchial epithelium, and specific areas of the forebrain during development in the mouse [3–5]. Mice homozygous for the disrupted gene are born dead and lack a thyroid gland, lung parenchyma, and pituitary gland, while heterozygous mice develop normally [4]. An abnormality of the gene in humans was first reported in patients with congenital hypothyroidism [6]. Subsequently, heterozygous point mutations in *NKX2-1* were identified in affected members of a family with benign hereditary chorea [7]. Recently, *NKX2-1* was reported as the gene responsible for brain–lung–thyroid syndrome (MIM #610978), which involves symptoms of neurological impairment, pulmonary disorders, and hypothyroidism [8–13]. Respiratory distress during the neonatal period, recurrent respiratory tract infection, and hypothyroidism are common clinical findings. The neurological impairment is characterized by gait disturbance with delayed first walking and choreoathetosis, in the absence of mental retardation or brain magnetic resonance imaging (MRI) abnormalities [13]. However, some affected individuals have had low-average intelligence, learning problems, psychosis and seizures [14–16].

The pathological mechanism of *NKX2-1* haploinsufficiency has been clarified for the hypothyroidism [17] and pulmonary impairment [18,19], but it is still unclear for the neurological symptoms. Most of the neurological deficits, i.e., the gait disturbance and involuntary movements sometimes accompanied with dystonia, dysarthria, action tremor and saccadic abnormalities [20], reflect dysfunction of the control of movement. Therefore, the basal ganglia were considered to be the most important causal lesion [8,14]. The *NKX2-1* null mouse showed severe morphological changes in the basal ganglia, including absence of the globus pallidus and enlargement of the striatum [4]. *NKX2-1* gene expression has been identified as the origin of the pallidum in the mammalian and avian embryonic archistriatum. These studies indicated that *NKX2*-1 is essential for development of the striatum, especially the pallidum rather than the caudate nuclei [5,21,22].

Brain MRI of patients with brain–lung–thyroid syndrome showed no notable abnormalities, except one case report of reduced size and intensity in the pallidum [8]. Previous brain nuclear imaging studies described various findings regarding the basal ganglia, including reduced blood flow in the striatum and thalamus [23], and hypometabolism in the basal ganglia, more prominent in the caudate nuclei [15].

* Corresponding author at: Department of Pediatrics, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan. Tel: +81 22 717 7287; fax: +81 22 717 7290.
E-mail address: uematsu@bk9.so-net.ne.jp (M. Uematsu).

0022-510X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2011.11.025

Here, we report three patients with brain–lung–thyroid syndrome in whom the diagnosis was confirmed by genetic examinations. We performed brain nuclear image analysis to investigate the causal lesion for the neurological symptoms.

2. Method

2.1. Clinical findings

We studied three patients (5, 6, and 7 years old; one male and two females) with gait disturbance who visited Tohoku University Hospital between 2008 and 2009 (Table 1).

Patient 1 was the second female child of healthy non-consanguineous parents (Fig. 1). She was born at term without neonatal respiratory problems. Congenital hypothyroidism was noted on neonatal screening and she has been given thyroxin replacement therapy since then. After the age of 1.6 years, she developed recurrent respiratory infections and was admitted to hospital five times in one year. She had normal mental development, but delayed gross motor development. She could sit alone at the age of 12 months and first walked at 38 months. A staggering gait persists. Her trunk and extremes were mildly hypotonic and continuous choreoathetosis was observed during wakefulness and exacerbated by stress.

Patients 2 and 3 were cousins via their maternal families (Fig. 1). Patient 2 was the third female and Patient 3 was an only male child. Both sets of non-consanguineous parents were healthy fathers and affected mothers with mild involuntary movement and a history of delayed first walking. Both patients were born at term without any perinatal complications. Congenital hypothyroidism was diagnosed in the neonatal period by screening in Patient 2, but at the age of 5 years in Patient 3, despite a neonatal screening test. Unlike Patient 1, they had no severe respiratory infections during infancy. Similar to Patient 1, first walking was observed at 30 months in Patient 2 and at 24 months in Patient 3. They also have persistent gait disturbance and choreoathetosis without mental retardation. The neurological examinations in all three patients did not detect any abnormalities, such as muscle weakness, abnormal deep tendon reflexes, or cerebellar manifestations.

Brain MRI in all three patients showed normal brain size, form, and intensity, including the basal ganglia. Oral levodopa (20 mg/kg/day) was given to all three patients, but no obvious improvement in the neurological symptoms was observed.

2.2. Brain nuclear image analysis

All three patients underwent single photon emission computed tomography (SPECT) to evaluate brain function at Tohoku University Hospital using technetium-99 m ethyl cysteinate dimer (ECD, approximately 12 MBq/kg of body weight) as the radiotracer. Twenty minutes after the injection, SPECT images were acquired using a PRISM IRIX (Shimadzu, Kyoto, Japan), with a low-energy, high-resolution, fan-beam collimator. In total, 120 projection datum points in a 128×128 matrix were obtained in 20 min. Using an ODYSSEY computer (Shimadzu), tomograms two pixels thick (5.8 mm) were reconstructed after a high-frequency cutoff with a Butterworth filter.

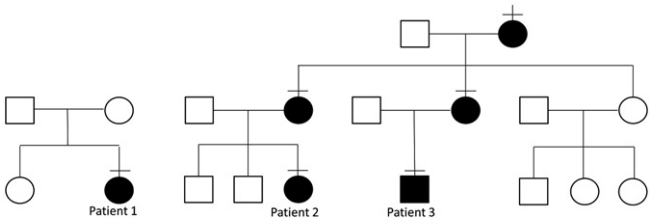

Fig. 1. Family pedigrees of three patients. Affected members are indicated by black squares and circles; unaffected members, white squares and circles. Patient 2 and 3 are cousins on mother's side.

The easy Z-Score Imaging System (eZIS; Fuji Film RI Pharma), used for the statistical analysis of SPECT images, standardizes brain images using Statistical Parametric Mapping (SPM99) [24]. Each SPECT image of the subjects after anatomical standardization followed by isotropic 12-mm smoothing was compared with the mean and SD of SPECT images of the age-matched healthy controls already incorporated in the eZIS program as a normal database using voxel-by-voxel Z-score analysis after voxel normalization to global mean values: Z score = (control mean − individual value)/control SD. These Z-score maps were overlain on tomographic sections and projection with an averaged Z-score of 14-mm thickness to surface rendering of the anatomically standardized MRI template.

Positron emission tomography (PET) was performed in Patients 2 and 3, 1 h after administering [^{18}F]-fluorodeoxyglucose (^{18}FDG) (approximately 3 MBq/kg of body weight) using a Biograph Duo, ECAT EXACT HR^{+} (Siemens, Hoffman Estates, IL) or SET-2400 W (Shimadzu) after fasting for at least 4 h. Emission scans were performed for 10 min for the entire brain. Attenuation was corrected. Fourteen 6-mm-thick slices parallel to the orbitomeatal line, encompassing virtually the entire brain, were analyzed visually by two investigators independently. When the interpretation was inconsistent, a third investigator was called to make a decision.

2.3. Gene analysis

Gene analyses were performed with the informed consent of the patients' parents. Genomic DNA was extracted from peripheral blood lymphocytes using a Sepa Gene kit (Sanko Junyaku, Tokyo, Japan). All coding exons and flanking introns in *NKX2-1* were amplified by PCR. All primers were based on the NCBI reference sequence (accession number NG_013365; the primer sequences are available upon request). The PCR products were separated on 3% agarose gels and purified with a QIAquick Gel Extraction kit (QIAGEN, Chatsworth, CA, USA). The PCR products were sequenced directly using a Big Dye Primer Cycle Sequencing kit and ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

Subsequent array-based comparative genomic hybridization (CGH) analysis was performed using an Agilent 244 K oligonucleotide array (Agilent, Santa Clara, CA; www.agilent.com) with a resolution of approximately 15 kb following the protocols provided by Agilent. The array was analyzed with the Agilent scanner and the Feature Extraction software (v. 9.1.3).

Table 1
Clinical characteristics in three patients.

	Patient 1	Patient 2	Patient 3
Age/sex	7 years/female	5 years/female	6 years/male
Recurrence of respiratory infection	Yes	No	No
Neonatal respiratory problems	No	No	No
Hypothyroidism	Yes (neonatal screening)	Yes (neonatal screening)	Yes (diagnosed at 5 years)
Initiation of walking	3 years and 2 months	2 years and 6 months	2 years
Mental retardation	No	No	No
Choreoathetosis	Yes	Yes	Yes
Response to L-dopa	No	No	No
Brain MRI	Normal	Normal	Normal
NKX2-1 analysis	del 14q12–13	p.V205P	p.V205P

3. Results

From the raw nuclear image ECD-SPECT findings in all three patients (Fig. 2, lower figures) and FDG-PET in Patients 2 and 3