

# Haploinsufficiency of *NKX2-1* is likely to contribute to developmental delay involving 14q13 microdeletions

Osamu Machida<sup>1,2</sup>, Haruko Sakamoto<sup>3,4</sup>, Keiko Shimojima Yamamoto<sup>5,6</sup>, Yuiko Hasegawa<sup>7</sup>, Satoi Nii<sup>4</sup>, Hidenori Okada<sup>3</sup>, Kazuki Nishikawa<sup>3</sup>, Shin-Ichi Sumimoto<sup>4</sup>, Eriko Nishi<sup>7</sup>, Nobuhiko Okamoto<sup>7</sup>, Toshiyuki Yamamoto<sup>1,5,\*</sup>

<sup>1</sup>Division of Gene Medicine, Graduate School of Medicine, Tokyo Women's Medical University, Tokyo, Japan;

<sup>2</sup>Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan;

<sup>3</sup>Department of Pediatrics, Japanese Red Cross Osaka Hospital, Osaka, Japan;

<sup>4</sup>Otemae Rehabilitation Center for Children, Japanese Red Cross Osaka Hospital, Osaka, Japan;

<sup>5</sup>Department of Transfusion Medicine and Cell Processing, Tokyo Women's Medical University, Tokyo, Japan;

<sup>6</sup>Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan;

<sup>7</sup>Department of Medical Genetics, Osaka Women's and Children's Hospital, Osaka, Japan.

**SUMMARY** Nucleotide variations or deletions in the NK2 homeobox 1 gene (*NKX2-1*), located at 14q13.3, lead to symptoms associated with the brain, lungs, and thyroid, and the combination of these phenotypes is clinically recognized as the brain-lung-thyroid syndrome. Many types of nucleotide variants of *NKX2-1* have been identified, and phenotypic variability has been reported. Chromosomal deletions involving *NKX2-1* have also been reported; however, phenotypic differences between patients with nucleotide variants of *NKX2-1* and patients with chromosomal deletions involving *NKX2-1* have not been well established. Recently, we identified seven patients with 14q13 microdeletions involving the *NKX2-1*. Most patients exhibited developmental delay. This inquiry arises regarding the potential existence of haploinsufficiency effects beyond those attributed to *NKX2-1* within the 14q13 microdeletion. However, a literature review has shown that developmental delay is not rare in patients with nucleotide alterations in *NKX2-1*. Rather, motor function impairment may have affected the total developmental assessment, and the haploinsufficiency of genes contiguous to *NKX2-1* is unlikely to contribute to developmental delay.

**Keywords** Brain-lung-thyroid syndrome, chromosomal microarray testing, movement disorder, language delay

## 1. Introduction

The NK2 homeobox 1 gene (*NKX2-1*; MIM\* 600635), located on chromosome 14 long arm 13.3, encodes a protein initially identified as thyroid-specific transcription factor-1 (TTF-1) (*I*). This gene is highly expressed in the thyroid, lungs, and pituitary glands (<https://www.proteinatlas.org/>), and murine knockout studies have established its critical role during the embryogenesis of those organs (2). In 1998, Devriendt *et al.* first reported the case of an infant with neonatal thyroid dysfunction associated with an *NKX2-1* deletion (3). Subsequently, monoallelic pathogenic variants and heterozygous deletions of *NKX2-1* have been identified to be associated with a complex phenotype involving choreoathetosis, respiratory problems, and hypothyroidism (4), constituting the triad of brain-lung-thyroid syndrome (BLTS) (5). Patients with

BLTS typically experience respiratory failure during the neonatal period (6). In early infancy, a diagnostic evaluation conducted due to recurrent upper respiratory infections and stunted growth typically reveals the presence of subclinical hypothyroidism. Then, mildly delayed acquisition of motor milestones with involuntary movements including ataxia and chorea are gradually observed. Hence, these motor disorders are acknowledged as the primary manifestation of brain involvement in BLTS (7).

Recently, we identified seven new patients with 14q13 microdeletions including *NKX2-1*. Generally, patients with chromosomal microdeletions exhibit clinical phenotypes involving multiple contiguous genes owing to the haploinsufficiency effect of the deletion. Therefore, it is important to elucidate whether haploinsufficiency affects genes other than *NKX2-1* that are present within the 14q13 microdeletion region.

Herein, we discuss the phenotypic differences between microdeletions including *NKX2-1* and intragenic variants within *NKX2-1*.

**2. Patients and Methods**

This study was performed in accordance with the Declaration of Helsinki, and requisite permission was obtained from the ethics committee of Tokyo Women's Medical University. Peripheral blood samples were drawn from patients after obtaining written informed consent from their parents. Genomic DNA was extracted from the blood samples using a QIAamp DNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Chromosomal microarray analysis (CMA) was performed using an Agilent Microarray 60 K kit (Agilent Technologies, Santa Clara, CA, USA) as previously described (8). Aberrations in the genomic copy number were visualized using the Agilent Genomic Workbench version 7 (Agilent Technologies). In this study, all genomic coordinates are referred to as GRCh37/hg19.

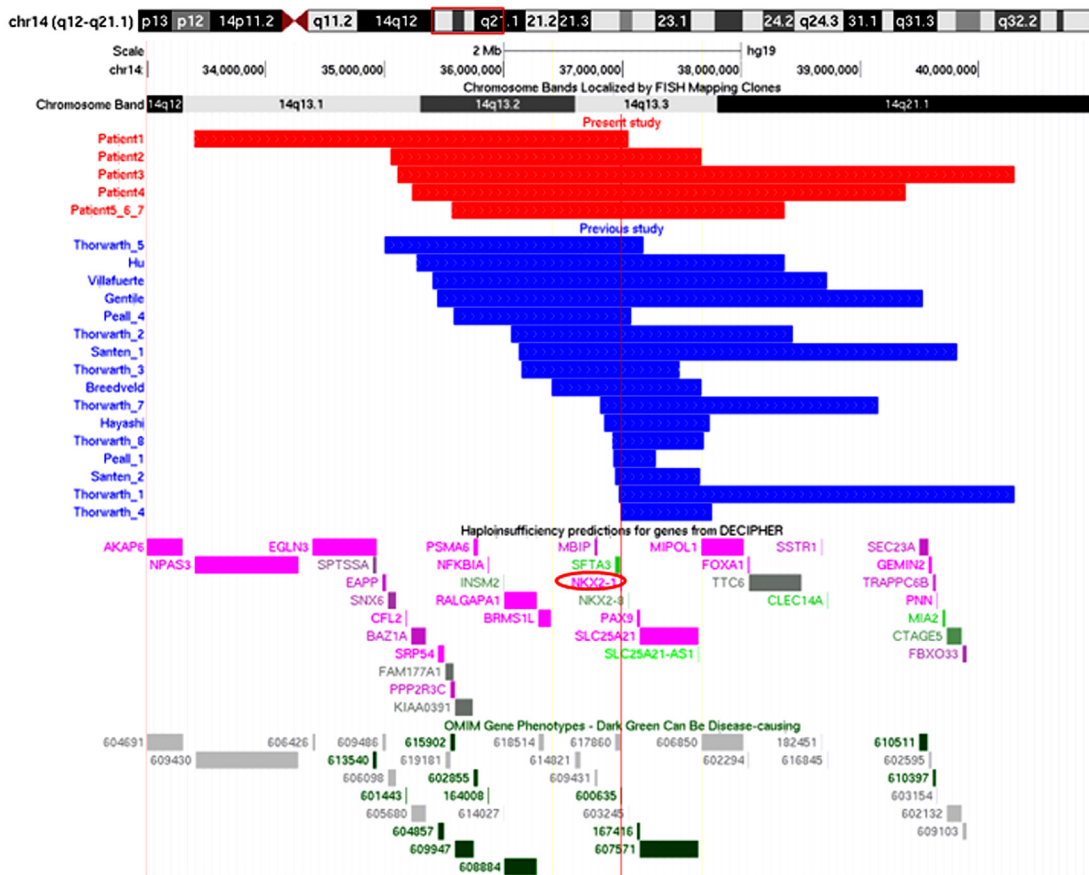
The clinical information of patients with 14q13

microdeletions was obtained from their attending physicians. Genotype-phenotype correlation was analyzed for patients with 14q13 microdeletions, in which previously reported patients were also included. The patients who showed microdeletions within chr14:33,000,000–41,000,000 were included in this analysis.

**3. Results**

**3.1. Microdeletions**

Five different microdeletions involving *NKX2-1* were detected in the seven patients (Figure 1). One of the microdeletions was detected in a sibling (an older sister and a male-female twin; patients 5–7). The genotypes and phenotypes of the patients are summarized in Table 1. In the literature, we identified sixteen previously reported patients, whose detailed information was available and who had microdeletions within chr14:33,000,000–41,000,000. These patients were included in Figure 1 and Table 1 for comparison with the patients in this study (9-15).



**Figure 1. Genome map around 14q13 depicted by identified deletions.** The map was captured from the UCSC genome browser (<https://genome.ucsc.edu/>). Regions of the identified deletions are depicted by custom tracks with red and blue bars (red for the deletions identified in this study, and blue for previously reported deletions). For haploinsufficiency prediction track, genes with magenta shades indicate a higher expectation of being haploinsufficient and genes with green shades indicate a lower expectation of being haploinsufficient. For OMIM gene phenotypes track, dark green and light gray indicate whether the genes are associated with OMIM phenotype or not, respectively. *NKX2-1* is highlighted by a red line and its location is shown by a red dotted line. All data are converted to GRCh37/hg19.



### 3.2. Patient reports

Clinical features of the patients are summarized in Table 1.

Patient 1, a 9-year-5-month-old boy, was born at 38 weeks of gestation without asphyxia. His birth weight was 3008 g. The right undescended testis was observed. He showed generalized hypotonia and psychomotor developmental delay since infancy, walking alone at three years of age. At present, his height is 118.6 cm (-2.5 SD), weight is 21 kg (-2.3 SD), and occipitofrontal circumference (OFC) is 51.5 cm (-0.7 SD), indicating short stature. He has developed established patterns for toilet habits and wearing clothing. Although he could walk by himself, he fell easily because of his ataxic gait. Thus, he required a handrail when going up and down the stairs. His intellectual quotient (IQ) was 33, indicating a moderate intellectual disability.

Patient 2 is a 5-year-6-month-old boy, who was born at 38 weeks of gestation without asphyxia. His birth weight was 2,928 g. Due to transient neonatal hypercapnia, he required transient ventilatory management. Pulmonary artery bifurcation stenosis was also observed. He showed distinctive facial features, including frontal bossing, epicanthus, long philtrum, large ear cups, and a congenital ear fistula. Although the patient showed elevated levels of thyroid-stimulating hormone, no medication was prescribed. His development was mildly delayed, with laughter noted at three months, head control at five months, turning over at six months, crawling and sitting at 12 months, and standing with support at 18 months. He uttered a two-word sentence after two years. At present, his height is 98.8 cm (-2.4 SD), weight is 21 kg (-2.3 SD), and OFC is 51.5 cm (-0.7 SD), indicating short stature. He has established his activities in daily life. His running and jumping movements were ataxic; however, dysarthria has been noted. The patient's IQ was 75.

Patient 3, a 3-year-1-month-old girl, was born at 40 weeks of gestation without asphyxia. The patient's birth weight was 3,182 g. After birth, she showed temporary respiratory distress but no feeding problems. Due to hypothyroidism, levothyroxine treatment was initiated on day eight. There was a history of viral infection at 2 months. She showed a mild motor developmental delay, with head control at 3 months, turning over at 7 months, sitting at 12 months, crawling and standing with support at 13 months, and walking alone at 15 months of age. However, her language development was not delayed with three-word sentences spoken at three years. Her developmental quotient was 60, indicating a mild delay. She shows gait instability and athetosis, although she can climb stairs using a handrail. At present, her height is 89.4 cm (-0.7 SD), weight is 13.5 kg (+0.2 SD), and OFC is 47.8 cm (-0.4 SD).

Patient 4 is a 17-year-old boy, who was born at 37 weeks of gestation without asphyxia. His birth weight

was 3,278 g. The patient experienced transient neonatal hyperpnea associated with pulmonary hypertension. The patient had a history of recurrent pneumonia accompanied by bronchomalacia and gastroesophageal reflux. His motor development was severely delayed, with head control at 2 years, sitting at 6 years, and standing with support at 10 years. However, his total IQ score was 46, indicating a moderate intellectual disability. At present, his height is 146.2 cm (-4.2 SD), weight is 36.6 kg (-2.5 SD), and OFC is 52.5 cm, (+0.3 SD), indicating short stature. His daily life skills have not yet been fully established. The patient still shows an ataxic gait and falls easily.

Patients 5, 6, and 7 are siblings. Interview revealed that their mother had shown developmental delay during childhood. The mother exhibits a short stature. Due to hypothyroidism, she has continued to take levothyroxine. The father of patient 5 and the father of patients 6 and 7 are different. Patient 5 is a 9-year-old girl born as a result of her mother's second pregnancy. The patient was born at 41 weeks of gestation with a birth weight of 3,748 g. She showed developmental delay with head control at 10 months, walking with support at 16 months, and use of simple words at 12 months. The patient experienced recurrent pneumonia during early childhood. Levothyroxine was prescribed for hypothyroidism. She also had short stature. Involuntary movement and dysarthria were also observed. When necessary, patients used a wheelchair. She also had anodontia in some teeth.

Patients 6 and 7 are 3-year-old male and female twins, respectively. They were born at 37 weeks of gestation with birth weights of 2,550 g and 2,631 g, respectively. Both patients needed respiratory management at the NICU due to neonatal respiratory failure. Nitric oxide inhalation was administered to patient 6 (first twin) for persistent pulmonary hypertension. Surfactant administration was required for patient 7 (second twin) because of associated pulmonary hemorrhage. After extubation, both patients experienced difficulty in weaning from oxygen and continued home oxygen therapy. Both patients showed developmental delay, turning over at 18 months of age without sitting. They also exhibited hypotonia and short stature. Due to hypothyroidism, levothyroxine was prescribed to both patients.

### 4. Discussion

The classical triad of BLTS is not always present, and only 50% of patients with *NKX2-1* involvement develop the complete triad (16). The severity of the phenotypes also varies, even within the same family (17). As shown in Table 1, not all patients identified in this study fulfilled the triad.

Movement disorders, including choreoathetosis, are thought to be the main neurological symptoms



associated with *NKX2-1*-related abnormalities. However, most patients in this study showed mild-to-severe developmental delay (Table 1). Thus, we hypothesized that developmental delay may be a symptom specific to microdeletions and may be attributed to haploinsufficiency affecting another gene within the deletion.

Santen *et al.* reported seven patients with 14q deletions (9). Two of them showed microdeletions including *NKX2-1*, and one of the two patients showed mild developmental delay (Table 1). Hamvas *et al.* reported five patients with *NKX2-1* deletions and 16 patients with *NKX2-1* variants (18). All patients with *NKX2-1* deletions exhibited developmental delay in association with language delay or behavior problems. On the other hand, only three of 16 patients with *NKX2-1* variants showed language delay. The "Patient 2", reported by Shetty *et al.* (19), demonstrated 14q13-q21.1 microdeletion; however, details are unavailable. The patient was diagnosed as having autism; whereas, such clinical features are considered as a nonmotor neurological manifestations of *NKX2-1* abnormalities. Thorwarth *et al.* (12) reported 32 patients with *NKX2-1* involvement. Eleven patients had microdeletions including *NKX2-1*. Twelve patients showed low-normal levels of IQ (12); however, whether they had microdeletions or nucleotide variants in the gene remains unknown. Peall *et al.* (11) reported ten patients with *NKX2-1*-related abnormalities. Two of ten patients exhibited microdeletions involving *NKX2-1*, and exhibited developmental delay. In contrast, developmental delay was also observed in four of the eight patients with *NKX2-1* intragenic nucleotide variants (50%). Parnes *et al.* reported five patients with *NKX2-1* variants. Four of five patients show speech delay together with motor delay (20). From these findings, we concluded that the comorbidity rate of developmental delay does not change significantly depending on whether patients had microdeletions involving *NKX2-1* or nucleotide variants of *NKX2-1*.

As shown in Figure 1, in the neighboring region of *NKX2-1*, there are some genes with a higher expectation of being haploinsufficient. Although some of them are related to OMIM phenotypes, most of them are related to unknown inheritance pattern. Previously, haploinsufficiency of the Ral GTPase activating protein alpha subunit 1 gene (*RALGAP1*) was considered to be related to developmental delay and epilepsy (21), and *RALGAP1* was included in the commonly deleted region in this study (Figure 1). However, *RALGAP1* was later identified as a gene related to developmental epileptic encephalopathy associated with an autosomal recessive trait (22), and carriers of loss-of-function variants of *RALGAP1* showed no symptoms. Hence, haploinsufficiency of *RALGAP1* was not related to clinical symptoms, such as the developmental delay observed in patients with 14q13 microdeletions.

Because *RALGAP1* is the only gene highly expressed in the brain in the contiguous region of *NKX2-1*, the haploinsufficiency of other genes contiguous to *NKX2-1* is unlikely to contribute to developmental delay.

In conclusion, alteration of *NKX2-1* itself would contribute to developmental delay in patients.

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**Conflict of Interest:** The authors have no conflicts of interest to disclose.

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- \*Address correspondence to:*  
Toshiyuki Yamamoto, Division of Gene Medicine, Graduate School of Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ward, Tokyo 162-8666, Japan.  
E-mail: yamamoto.toshiyuki@twmu.ac.jp
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