Brain & Development 41 (2019) 257–262

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Three Japanese patients with 3p13 microdeletions involving *FOXP1*

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Received 24 August 2018; received in revised form 29 October 2018; accepted 30 October 2018

Abstract

Background: FOXP1 is known as the gene responsible for neurodevelopmental delay associated with language impairment. Broad clinical findings also include feeding difficulty, muscular hypotonia, and distinctive features. These findings are common between patients with loss-of-function mutations in *FOXP1* and 3p13 microdeletion involving *FOXP1*. Thus, "*FOXP1*-related intellectual disability syndrome" is now recommended.

Methods: After obtaining informed consent, chromosomal microarray testing was performed for patients with unknown etiology.

Results: We identified three Japanese patients with 3p13 microdeletions involving *FOXP1*. One of the patients showed an additional 1q31.3q32.1 deletion as *de novo*, which was rather considered as a benign copy number variant.

Conclusion: This is the first report of patients with 3p13 microdeletions from Japan. All patients showed growth delay, moderate to severe developmental delay, hearing loss, and distinctive facial features including prominent forehead and mid facial hypoplasia. In addition, "square shaped face" commonly observed in all three patients may be a characteristic finding undescribed previously. From the obtained findings, "*FOXP1*-related intellectual disability syndrome" was considered to be clinically recognizable.

Keywords: Chromosomal microarray testing; Contiguous gene deletion; Facial dysmorphism

1. Introduction

The forkhead box P1 gene (*FOXP1*; MIM #605515) belongs to subfamily P of the forkhead box (FOX) transcription factor family. Forkhead box transcription factors play important roles in the regulation of tissue- and cell type-specific gene transcription during both development and adulthood [1]. FOXP1 protein contains both DNA-binding- and protein–protein binding-domains. *FOXP1* is located on 3p13. Because more than 10 patients with 3p13 microdeletions including *FOXP1* showed developmental delay, haploinsufficiency of *FOXP1* was suspected as a cause of developmental delay [2–4]. In 2010, de novo loss-of-function mutations of this gene were identified in several patients with intellectual disability [5,6]. Finally, *FOXP1* was confirmed as one of the genes responsible for neurodevelopmental delay associated with language impairment and with or without autistic features.

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https://doi.org/10.1016/j.braindev.2018.10.016
0387-7604/

Until now, many patients have been identified to show microdeletions involving *FOXP1* as well as *FOXP1* loss-of-function mutations [7,8]. Here, we report on the first Japanese cases of patients with severe intellectual disability due to 3p13 microdeletions.

2. Materials and methods

2.1. Subjects

Through our on-going study to identify genomic copy number aberrations in patients with developmental delay due to unknown etiology, three Japanese patients were identified to show microdeletion involving 3p13 region (Table 1). This study was approved by the ethics committee of Tokyo Women's Medical University. Written informed consent was obtained from all families of the patients in this study, for use of their blood samples, genotype data, and medical records, for research purposes. Blood samples were collected from patients and their parents if necessary.

2.2. Methods

Genomic DNA was extracted from the blood samples using the QIA quick DNA Extraction Kit (QIAGEN, Hamburg, Germany). Metaphase spreads were also prepared from blood samples and used for FISH analyses. Chromosomal microarray testing was performed using any of the Agilent Oligo Microarray Kits 60 K (Agilent Technologies, Santa Clara, CA), as described previously [9]. Genomic copy number aberrations were visualized using Agilent Genomic Workbench version 6.5 (Agilent Technologies). For patient 1, parental samples were also analyzed by microarray. To deny chromosomal translocation in this patient, metaphase spreads prepared from peripheral blood sample were used for fluorescence in-situ hybridization (FISH) analyses in accordance with the previous report [10]. All of the genomic regions are described according to build 19 in this study.

3. Results

3p13 deletions were identified in three patients (Fig. 1). Results were described as arr[hg19] 3p14.1p12.3(66,656,437–74,471,872)X1 (Patient 1), arr[hg19] 3p14.1p12.3(68,595,321–77,470,128)X1 (Patient 2), and arr[hg19] 3p14.1p12.3(68,722,841–76,918,117)X1 (Patient 3). As shown in Fig. 1, there are many segmental-duplications around the breakpoints. This may be a mechanism prone to lead the recurrent deletions in this region.

Patient 1 showed an additional deletion in chromosome 1 as described as arr[hg19] 1q31.3q32.1(196,573,625–203,198,025)X1 (Fig. 2). Both parents of patient 1 were similarly analyzed and there were no abnormalities.

Table 1
Summary of patients' clinical features.

	Patient 1 5Y	Patient 2 8Y	Patient 3 5Mo	Incidence*
Gender	Female	Male	Male	
Birth weight (g)	2333	2650	2290	
Growth delay	+	+	+	41%
Feeding problem	–	+	+	69%
Neurodevelopment				
Motor development	Severe delay	Moderate delay	Moderate delay	96%
Speech delelopment	Severe delay	Severe delay	NA	100%
Behavior abnormality	–	Aggressive/self-injuries	NA	69%
Abnormal tonicity	–	Hypotonic	Hypotonic	74%
Visceral findings				
MRI abnormality	–	–	Enlarged lateral ventricles	57%
Congenital heart defect	–	–	+	54%
Hearing loss	+	+	+	
Congenital dislocation of hip	–	+	–	
Distinctive features				
Square shaped face	+	+	+	
Prominent forehead	+	+	+	87%
Hypertelorism	+	+	+	50%
Downslanting palpebral fissures	–	+	+	55%
Low-set ears	–	+	+	
Flat nasal bridge	–	+	+	72%
Short upturned nose	–	+	+	69%
Short/flat philtrum	–	+	+	
High arched palate	–	+	+	
Bilateral undescended testes	–	–	+	

Y, years; Mo, months; NA, not applicable.
* Incidence is referred to the data reported by Meerschaut et al. 2017.

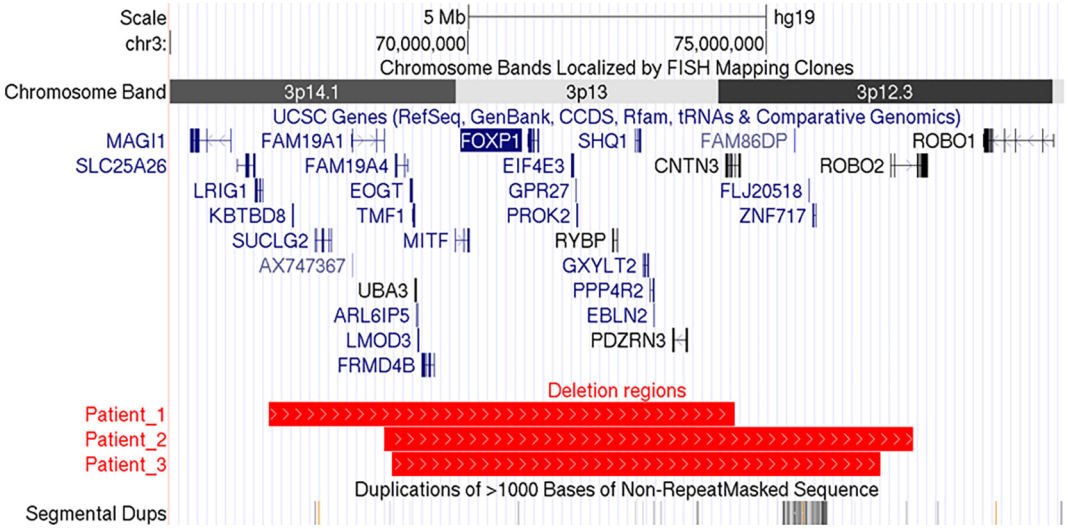

Fig. 1. The identified 3p13 deletions in this study. The three deletion regions are depicted on the genome map captured from UCSC genome browser (https://genome.ucsc.edu/). As shown, *FOXP1* is commonly deleted. The identified deletions are shown by red rectangles by use of the custom track. In the bottom, segmental duplications are shown. Some of them are located near the breakpoints identified in patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

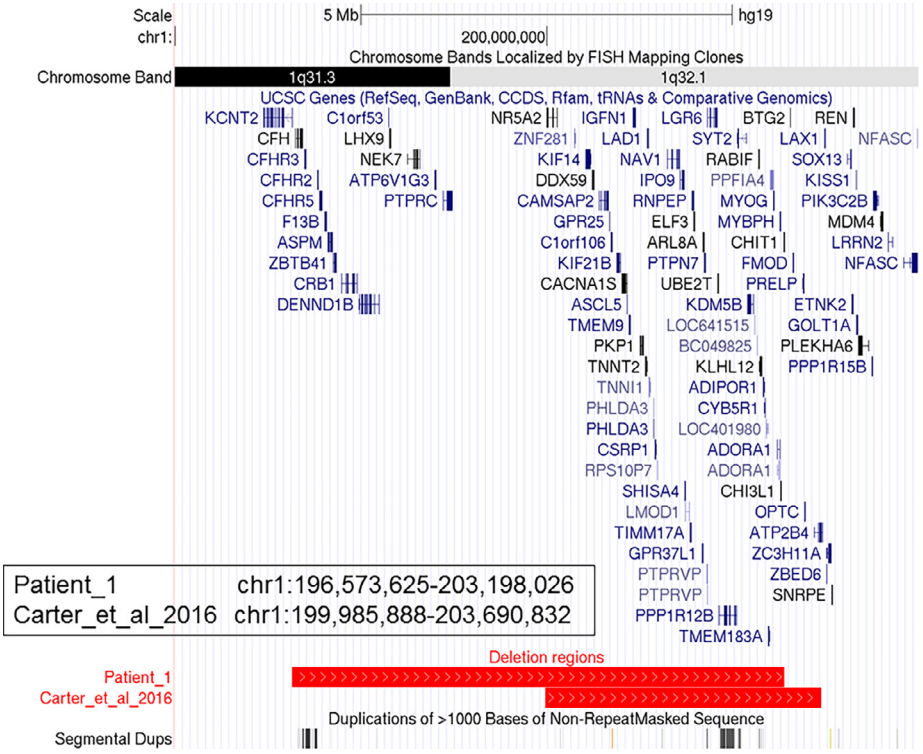

Fig. 2. Genome map around 1q31q32 region. The deletion identified in patient 1 is compared to that in a patient reported by Carter et al. (2016).

Subsequent FISH analyses for patient 1 showed no translocation between chromosome 1 and 3 (data not shown). Thus, both deletions in 1q31.3q32.1 and 3p14.1p12.3 observed in patient 1 were confirmed as *de novo* origin. For patient 2, deletions were re-confirmed by FISH (not shown). Parents of patients 2 and 3 declined their own genotyping.

4. Patient reports

4.1. Patient 1

A 5-year-old girl was born at 37 weeks of gestation, with a birth weight of 2,333 g (10th∼50th centile). At birth, there were distinctive features, including square

shaped face, prominent forehead and hypertelorism. In early infancy, bilateral sensorineural hearing impairment and retinitis pigmentosa was noted. Her development was delayed with walking at 4 years. She is still aphasic. Although she can use spoon, she requires supports for all activities of the dairy life. Her developmental quotient was evaluated by Enjoji with the score of 15. At present, her height is 95 cm (<3rd centile), weight is 13.1 kg (<3rd centile), occipitofrontal circumference (OFC) is 50.5 cm (mean), indicating growth impairment. Brain magnetic resonance imaging (MRI) showed no abnormality. Behavior abnormality is not observed.

4.2. Patient 2

An 8-year-old boy is a first child of healthy and non-consanguineous Japanese parents. He was born at 38 weeks of gestation with weight 2,650 g (10th∼50th centile). Soon after birth, generalized hypotonia, bilateral hearing loss, and congenital dislocation of hip joint were noted. Nasogastric tube feeding was necessitated for several months owing to failure to thrive since early infancy. Although special educational care with hearing aid has been provided, there is no meaningful word. Motor development was also delayed with walking at 2 years.

At present, his height was 110 cm (<3rd centile), weight was 16 kg (<3rd centile) and OFC was 48.8 cm (<3rd centile), indicating growth impairment and microcephaly. There are dysmorphic features including square shaped face, hypertelorism, down slanting palpebral fissures, low set ears, a flat nasal bridge, a short upturned nose, a short philtrum and a high arched palate (Fig. 3A). He does not understand words that express abstract concepts such as colors. Due to severe developmental delay, he requires supports for daily life with usage of diapers. Aggressive and hyperkinetic behaviors and self-injurious activities are observed. Brain MRI showed no significant findings. Routine cytogenetic investigations revealed normal results.

4.3. Patient 3

A 5-month-old male infant is a first child of healthy and non-consanguineous Japanese parents. He was born at 38 weeks of gestation with a birth weight of 2,290 g (3rd∼10th centile), length of 44 cm (3rd∼10th centile), and OFC of 33 cm (mean). Congenital heart defects with atrial septal defect, patent ductus arteriosus, and pulmonary hypertension were detected. His feeding activity was poor. He was hypotonic and his development was delayed. Bilateral undescended testes and right hearing loss was noted. He was suspected to have CHARGE syndrome. At 5 months, he was referred to our hospital for medical investigation.

He showed dysmorphic features including square shaped face, hypertelorism, down slanting palpebral fissures, low set ears, a flat nasal bridge, a short upturned nose, a flat philtrum, a high arched palate and thin lips (Fig. 3B). His height was 54.9 cm (<3rd centile), weight was 3.9 kg (<3rd centile), and OFC was 40.4 cm (3rd∼10th centile), indicating growth impairment. Brain MRI showed enlarged lateral ventricles (not shown).

5. Discussion

In this study, we identified 3p13 microdeletions harboring *FOXP1* in three Japanese patients. This is the first report of the cases from Japan. All patients showed

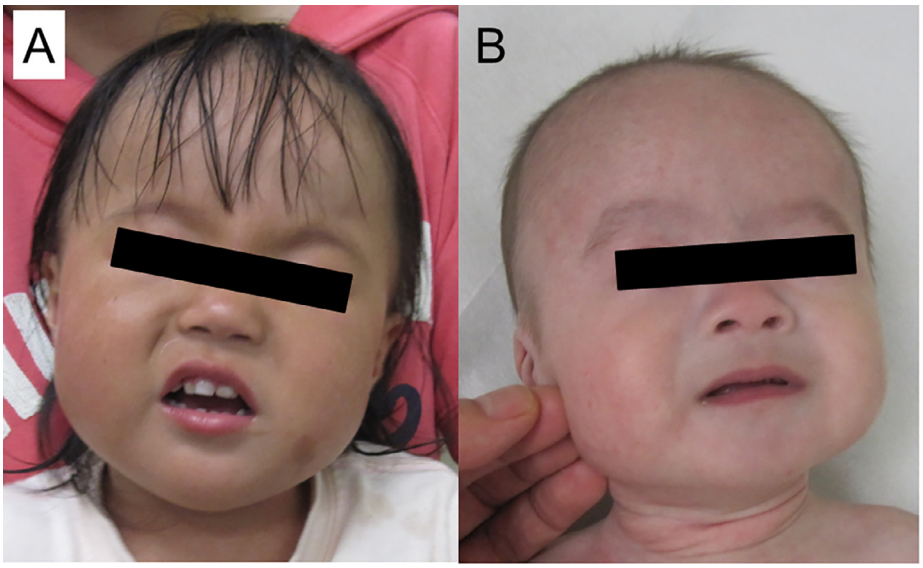

Fig. 3. Distinctive features of the patients. Both patients show square shaped face, arched eyebrows, and mid-facial hypoplasia. A; patient 2, B; patient 3. We obtained informed consent from patient's parents for the usage of this photo with masked eyes.

severe to moderate developmental delay. In addition, growth impairments were also observed in all patients. Muscular hypotonia and feeding difficulty were observed in two patients. These findings are consistent with that described in the previously reported patients from abroad associated with 3p13 microdeletions harboring *FOXP1* [11].

Most of the previous patients also showed distinctive features including relative macrocephaly and a broad and high forehead [11]. Similarly, present patients exhibited distinctive findings such as prominent forehead and down-slanting palpebral fissures. In addition to that, square shaped face was observed in all three patients in this study. Previously, the term "square shaped face" has never been used in the patients' reports of 3p13 microdeletions. However, broad forehead is commonly observed in patients with 3p13 microdeletions and "square shaped face" is recognized in some of the provided images of the reported patients [3,4,11]. Thus, this may be an undescribed characteristic for patients with 3p13 microdeletions.

Meerschaut et al. compared clinical features of the patients with contiguous gene deletions in 3p13 harboring *FOXP1* with that observed in patients with monogenic *FOXP1* defects [12]. As a result, most of the clinical features except for hearing impairment are common among them. The only difference is whether the microphthalmia-associated transcription factor gene (*MITF*), which is neighboring to *FOXP1* and responsible for Waardenburg type IIA syndrome, is deleted together or not. Patients with 3p13 microdeletion including *MITF* invariably show deafness. This is consistent that all of the three patients reported here showed hearing problem. Because there is no other phenotypic difference between 3p13 microdeletions and *FOXP1* mutations, other neighboring genes would not be related to the phenotypic difference. Now, the name of clinical entity "*FOXP1*-related intellectual disability syndrome" (MIM #613670) is proposed by Meerschaut *et al.* [12]. Le Fevre *et al.* suggested clinical variability of this syndrome because patients with this condition show variable degrees of intellectual disability and specific language impairment, with or without autistic features and other minor anomalies including distinctive features are recognizable [13].

Patient 1 showed another deletion at 1q32.1 region. Previously, a patient with similar deletion was reported to show developmental delay and dysmorphism [14]; however, such clinical features were mild and nonspecific. Thus, it is difficult to distinguish which phenotypic features are derived from 1q32.1 deletion per se in this patient.

In conclusion, we presented the first Japanese cases of patients with *FOXP1*-related intellectual disability syndrome. Previously undescribed square shaped facial features were documented. This report will make it easier to get clinical diagnosis of this syndrome.

Acknowledgement

We would like to thank the patients' families for their cooperation. This research was supported by the Practical Research Project for Rare/Intractable Diseases from the Japan Agency for Medical Research and Development (AMED) (TY), and the Japan Society for the Promotion of Science (JSPS) KAKENHI grant number 15K09631 (TY).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2018.10.016.

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