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Identification of *FOXG1* mutations in infantile hypotonia and postnatal microcephaly

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Abstract

FOXG1, located at chromosome 14q12, is critical for brain development, and patients with *FOXG1* mutation exhibit developmental encephalopathy with high phenotypic variability, known as *FOXG1* syndrome. Here, we report 3 cases of *FOXG1* syndrome that presented with infantile hypotonia and microcephaly.

A total of 145 children with developmental delay and/or hypotonia were evaluated by whole-exome sequencing (WES) in the pediatric neurology clinic and medical genetics center at Asan Medical Center Children's Hospital, from 2017 to 2019. Each *FOXG1* mutation was confirmed by Sanger sequencing. The clinical findings of each patient with *FOXG1* mutation were reviewed.

WES identified de-novo, pathogenic, and heterozygous *FOXG1* mutations in 3 of 145 patients in our patient cohort with developmental delay and/or hypotonia. The characteristics of brain magnetic resonance imaging (MRI) were reported as callosal anomaly, decrease in frontal volume, fornix thickening, and hypoplastic olfactory bulbs. A phenotype-genotype correlation was demonstrated as a patient with a novel missense mutation, c.761A > C (p.Tyr254Ser), in the forkhead domain had better outcome and milder brain abnormalities than the other 2 patients with truncating mutation in the Groucho binding domain site, c.958delC (p.Arg320Alafs), or N-terminal domain, c.506dup (p.Lys170GlnfsThe). Importantly, all 3 patients had hypoplastic olfactory bulbs on their brain MRI, which is a distinct and previously unrecognized feature of *FOXG1* syndrome.

This is the first report of *FOXG1* syndrome in a Korean population; this condition accounts for 2% (3 of 145 patients) of our patient cohort with developmental delays and/or hypotonia. Our report contributes to understanding this extremely rare genetic condition in the clinical and genetic perspectives.

Abbreviations: EEG = electroencephalography, FOXG1 = Forkhead Box G1, MRI = magnetic resonance imaging, WES = whole-exome sequencing.

Keywords: *FOXG1*, hypoplastic olfactory bulbs, hypotonia, microcephaly, whole-exome sequencing

1. Introduction

FOXG1 is a transcription repression factor involved in the development of the telencephalon by differentiating cortical compartments.[1,2] After the first report in 2005 of a patient with a congenital variant of Rett syndrome due to a *FOXG1* mutation,[3] known as *FOXG1* syndrome, over 170 affected cases have been reported.[4,5] Compared to patients with classical Rett syndrome, patients with *FOXG1* syndrome are more severely affected in terms of receptive language and social interactions, and they are likely unable to walk and exhibit dyskinetic-hyperkinetic movement, seizures, irritability sleep disturbance. Interestingly, in contrast to Rett syndrome, *FOXG1* syndrome can affect both female and male and patients do not experience developmental regression.[6–8]

It is now considered that individuals harboring mutations in *FOXG1* belong to a distinct clinical entity, termed "*FOXG1* syndrome". It is a condition characterized by early onset movement disorders, absent language, autistic features, epilepsy, intellectual disability, and structural brain abnormalities.[8]

In the current report, we describe 3 Korean children with *FOXG1* syndrome, which was diagnosed by whole exome sequencing (WES). Our report further aids in understanding this extremely rare genetic condition.

2. Patients and methods

Totally, 145 children with developmental delay and/or hypotonia were evaluated by WES in the pediatric neurology clinic and

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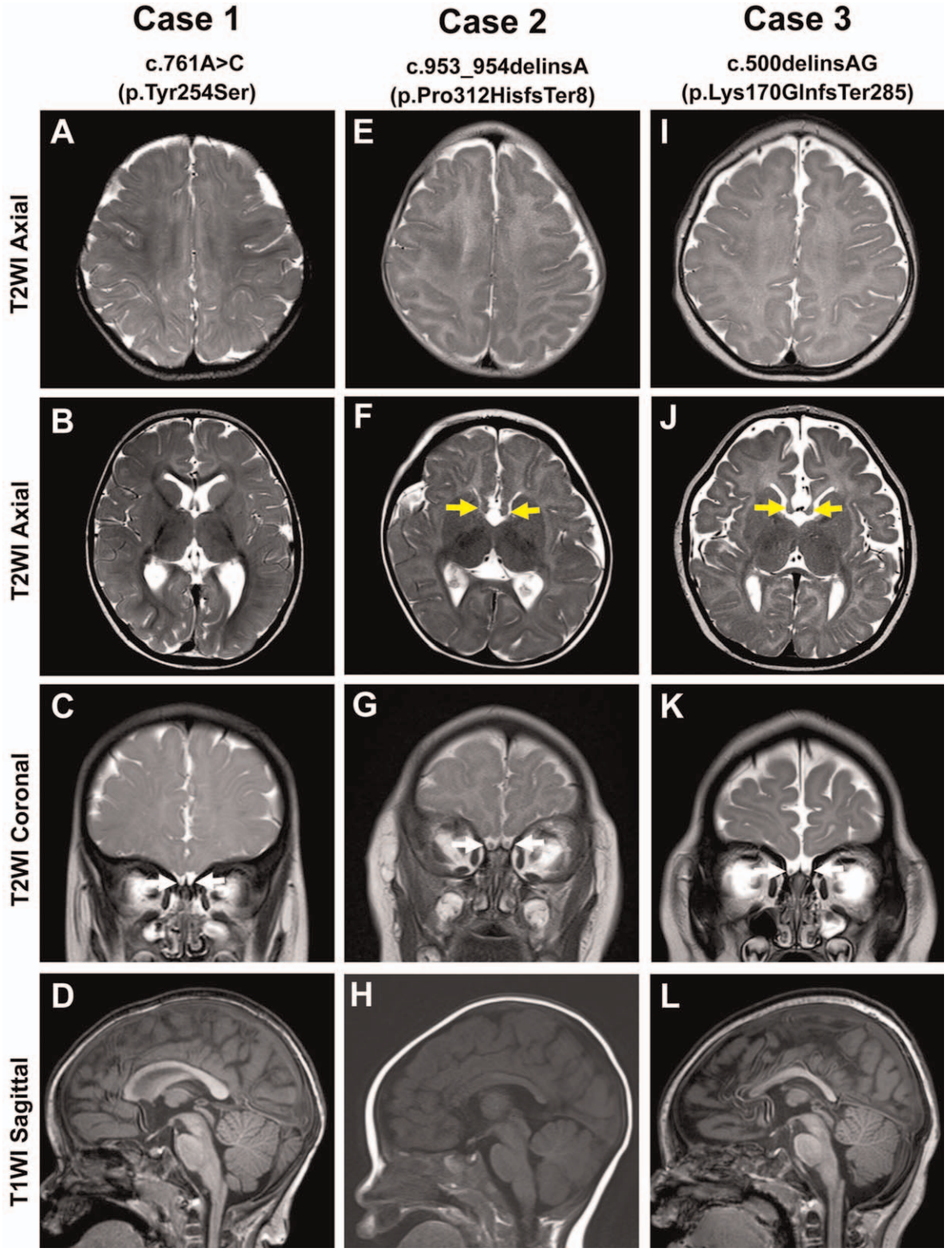

Figure 1. MRI findings of patients with *FOXG1*-related syndromes. Case 1 MRI obtained at 13 months of age. The lateral ventricles are mildly enlarged on the T2W axial images of the brain (A, B), and the bilateral olfactory bulbs appear small (white arrows) on the T2W coronal image (C). Sagittal T1-weighted MRI of the brain shows mild thinning of the corpus callosum (D). Case 2 MRI obtained at 6 months of age. The fronto-temporal lobes and the bilateral basal ganglia appear slightly small (E), the fornices appear enlarged and separated (yellow arrows, F), and the olfactory bulbs appear slightly small (white arrows, G). Sagittal T1W image displays the dysgenetic corpus callosum with absent rostrum and thinning of the posterior body and splenium (H). Case 3 MRI obtained at 7 months of age. The fronto-temporal lobes and the bilateral basal ganglia appear slightly small with suspicious slightly simplified gyral pattern (I, J), and the fornices appear enlarged and separated (yellow arrows, J). The ventricles are not dilated, but there is prominence in the extra-axial CSF (J), and the olfactory bulbs appear hypoplastic (white arrows, K). Sagittal T1W image displays the dysgenetic corpus callosum with absent rostrum and overall thinning especially of the posterior body and splenium (L).

medical genetics center at Asan Medical Center Children's Hospital, Seoul, Korea, from 2017 to 2019. Genomic DNA was isolated from either whole blood or saliva. All exons of all human genes (approximately 22,000) were captured using a SureSelect kit (Version C2; Agilent Technologies, Inc., Santa Clara, CA, USA) and sequenced using a NovaSeq platform (Illumina, San Diego, CA, USA). Raw genome sequences were aligned to the reference sequence (NCBI genome assembly GRCh37; accessed in February 2009). Each of the *FOXG1* mutations was confirmed by Sanger sequencing. WES was performed as previously described,[9] and each *FOXG1* mutation was confirmed by Sanger sequencing. The clinical findings of each patient were reviewed. The parents of all patients provided written informed consent for the study, approved by the Medical Sciences Ethics Committee (IRB number 2017-0988).

3. Results

We found that *FOXG1* mutation is accounted for 2% (3 of 145 patients) in our pediatric patients with developmental delay and/or hypotonia. Each patient's characteristics and clinical outcomes were described as below.

3.1. Case 1

This female infant was the second child of non-consanguineous Korean parents. Her birth was uneventful after 40 weeks of gestation. At 11 months of age, her head circumference (HC) was 42 cm (<the 3rd percentile), and she could sit only with hand support, but could not crawl. The brain magnetic resonance imaging (MRI) at 1 year of age showed slightly delayed myelination and mildly enlarged lateral ventricles, hypoplastic olfactory bulbs, and mild thinning of the rostrum of the corpus callosum (Fig. 1A–D, Supplemental Digital Content Table S1, http://links.lww.com/MD/G501). WES at 29 months of age revealed a novel missense, likely pathogenic[9] mutation in the forkhead domain of the *FOXG1* gene, c.761A > C (p.Tyr254Ser) (Fig. 2A). Her parents did not carry the mutation.

At her latest visit at the age of 4.6 years, microcephaly (HC: 46.5 cm, <the 3rd percentile) was persistent. She was able to walk alone and communicate verbally. Denver developmental scale screening test showed profound global delay (gross motor development quotient (DQ) = 67.9, fine motor DQ = 58.4, language DQ = 64.2, personal DQ = 52.8, social DQ = 62.2). Her electroencephalography (EEG) reported focal epileptiform discharges, but she had no clinical seizure.

3.2. Case 2

This female infant was the first child of healthy non-consanguineous Korean parents. At 7 months of age, motor developmental delay and microcephaly (HC: 40 cm, <the 3rd percentile) was noted. She was also noted for sleep problems and irritable hypersensitivity to external stimuli, and her EEG was normal. Her brain MRI at 6 months of age revealed slightly decreased volume in the frontal lobes accompanied by delayed myelination, hypoplastic olfactory bulbs, and dysgenetic corpus callosum (Fig. 1E–H, Supplemental Digital Content Table S1, http://links.lww.com/MD/G501).

WES revealed a pathogenic,[9] frameshift *FOXG1* mutation, c.958delC (p.Arg320Alafs), near the Groucho binding domain, which her parents did not carry (Fig. 2B). At latest follow-up at age of 1.6 years, microcephaly was persistent, and she could not sit without support and speak any meaningful word.

3.3. Case 3

This male infant was the second child of non-consanguineous Korean parents. His pre- and perinatal periods were uneventful. At the age of 6 months, he showed microcephaly (HC: 40 cm, <the 3rd percentile) and a slightly dysmorphic face with a thin vermilion border of the upper lip and a rather prominent ear. He also had strabismus, intermittent orolingual dyskinesia, and recurrent reflux and vomiting. He could sit with a tripod for a few seconds but was not able to maintain his head. His brain MRI showed hypoplasia of the corpus callosum as well as decreased frontal volume and simplified gyral formation with fornix thickening, and hypoplastic olfactory bulbs (Fig. 1I–L, Supplemental Digital Content Table S1, http://links.lww.com/MD/G501). At 11 months of age, tonic seizure was noted, and his

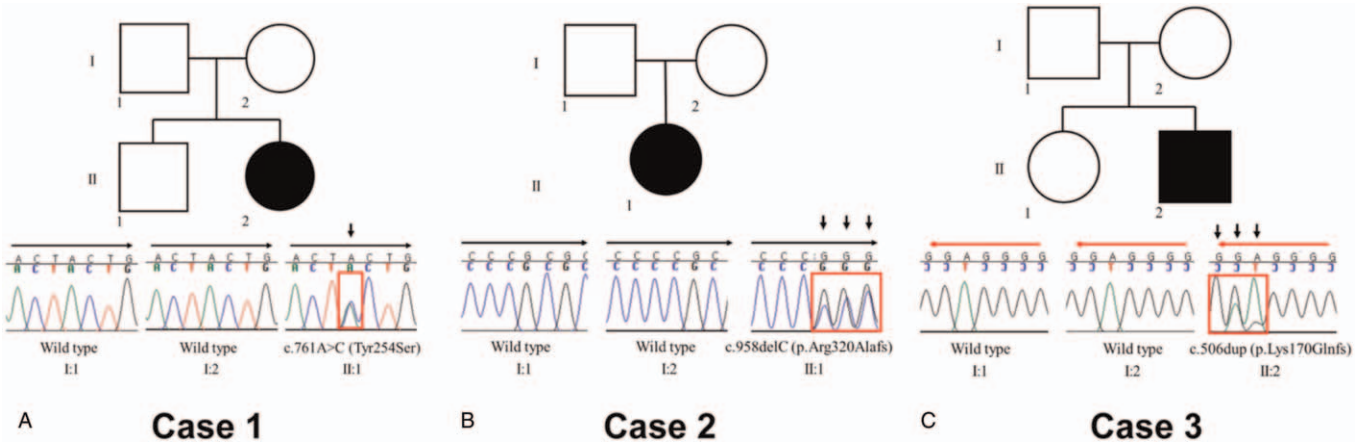

Figure 2. Sequence tracing of *FOXG1* mutations and pedigree of each patient. These figures reveal sequence tracing of the *FOXG1* mutations in each of the 3 patients. Case 1 patient with a novel missense mutation in the forkhead domain, c.761A > C (p.Tyr254Ser) (A), Case 2 patient with a truncating mutation in the GBD site, c.958delC (p.Arg320Alafs) (B) and Case 3 patient had truncating mutation at or N-terminal domain, c.506dup (p.Lys170Glnfs) (C). Mutated bases are indicated by black arrows above the line. GBD = Groucho binding domain.

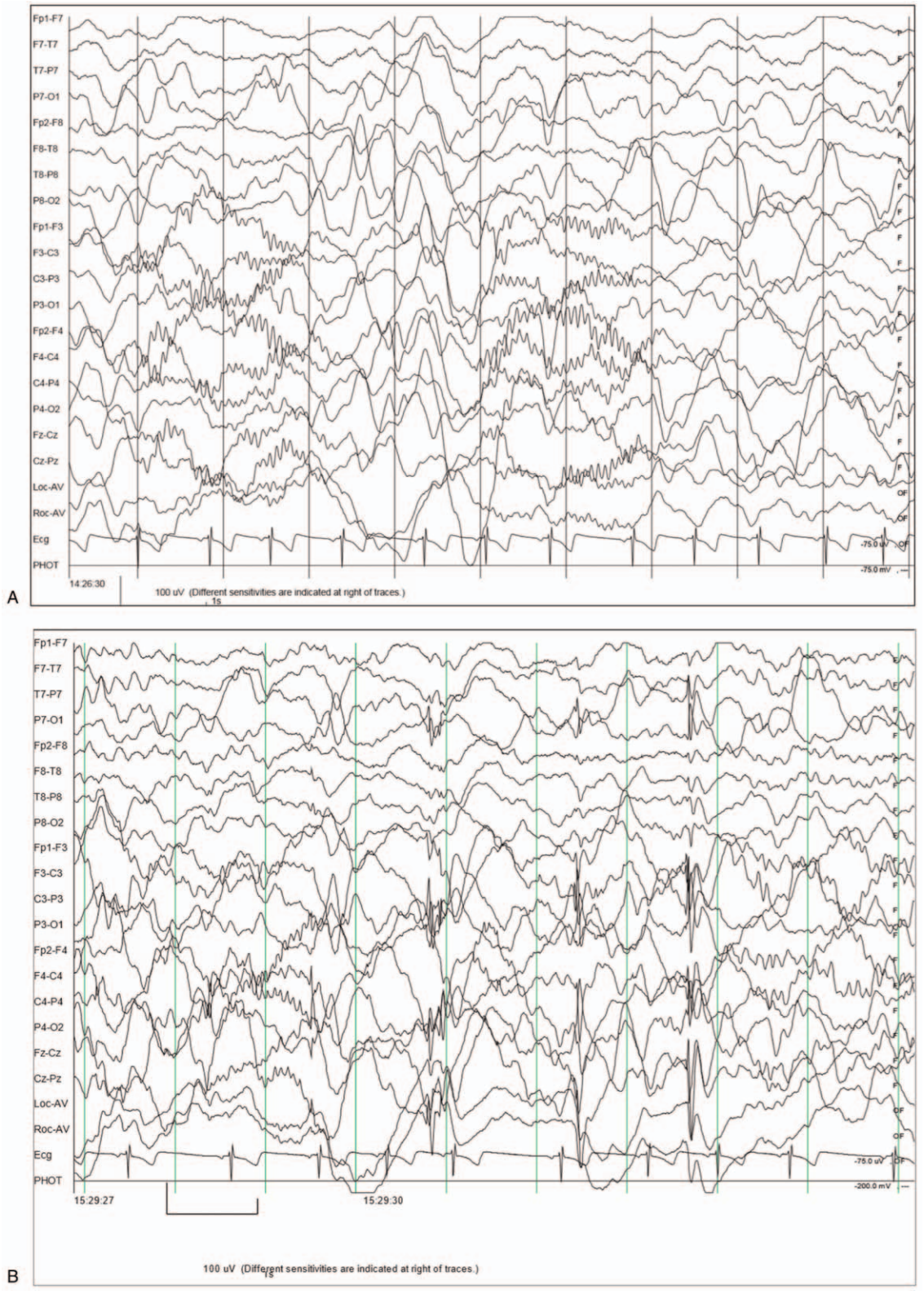

Figure 3. Serial EEG findings of the case 3 patient. The background activities consisted of high amplitude delta activities intermixed with sleep spindle activities at 13 months of age (A). The background activities consisted of high amplitude slow activities, concomitantly with high amplitude spikes or polyspikes and slow discharges over the left parieto-occipital areas or less from the left fronto-central areas at 51 months of age (B).

initial EEG showed high amplitude posterior slow activities with normal sleep spindle activities (Fig. 3A). At 4.3 years of age, the multifocal epileptiform discharges over the high amplitude delta activities became more evident (Fig. 3B). At the age of 3 years, WES revealed a pathogenic,[10] frameshift *FOXG1* mutation, c.506dup (p.Lys170Glnfs), which his parents did not carry (Fig. 2C). At the

latest follow-up at the age of 4 years, microcephaly (HC: 44.2 cm, <the 3rd percentile) was persistent; he could not fully control his head and barely said any meaningful word.

4. Discussion

In the current report, we describe 3 patients with infantile hypotonia and postnatal microcephaly, who were shown to have a de novo *FOXG1* mutation by WES. This is the first report of *FOXG1* syndrome in a Korean population, and 1 novel missense mutation was found.

FOXG1, as a transcriptional repressor, is essential for forebrain development, including the cerebral cortex, hippocampus, and basal ganglia, which are derived from the telencephalon.[1] The previous studies reported the major clinical features of *FOXG1* syndrome as postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum anomaly.[4,6,7,11] Patients with *FOXG1* syndrome can also be affected by variable types of movement disorders and epilepsy.[12,13] In addition, abnormal sleep patterns, irritability, and gastrointestinal symptoms have been observed in some patients.[14] Since clinical features vary among patients, characteristics determined from brain MRI findings provide important clues for diagnosis, such as filiform thinning of the corpus callosum rostrum, gyral malformation, and thickened fornix.[15]

Our 3 study patients with *FOXG1* syndrome also manifested with axial hypotonia during infancy and postnatally developed microcephaly with normal somatic growth; however, at presentation, they did not exhibit any symptoms suggestive of typical Rett syndrome (Table 1). Case 2 patient had nonspecific symptoms such as irritability, abnormal sleep patterns, and hypersensitivity to external stimuli, but these symptoms were relieved by clonazepam. Case 3 patient had epilepsy, which required multiple anti-epileptic drugs, and his orolingual-dyskinesia was sustained until his latest follow-up. All 3 patients were affected in the corpus callosum and showed myelination delay with microcephalic features in varying degrees on their brain MRI (Supplemental Digital Content Table S1, http://links.lww.com/MD/G501).

The phenotypical variability of *FOXG1* syndrome has been reported as being correlated with the type of mutation.[7,14,16] Truncating mutations in the N-terminal domain and the forkhead domain have been associated with severe phenotypes, whereas truncating mutations affecting the C-terminal domain or missense variants in the forkhead domain have been associated with favorable developmental milestones and brain abnormalities.[6,7,14,15] In case 1 of our study, the patient with a novel missense mutation in the forkhead domain, c.761A>C (p.Tyr254Ser), had a better outcome and milder brain abnormalities than the other 2 patients with a truncating mutation in the

Table 1

Summary of clinical features and neurodevelopmental profiles.

	Case 1	Case 2	Case 3
Age at first visit	11 m	7 m	6 m
Birth history			
Gestational age	40 wks	39 wks	40+4 wks
Birth weight	3.1 kg	3.2 kg	3.5 kg
Mode of delivery	Cesarean section	Spontaneous	Spontaneous
Anthropometric data			
Height	80.4 cm (85 p)	69.7 cm (85 p)	68.4 cm (50 p)
Body weight	8.7 kg (50 p)	6.8 kg (10 p)	8.8 kg (75 p)
Head circumference	42 cm (<3 p)	40 cm (<3 p)	40 cm (< 3p)
FOXG1 mutation			
Nucleotide change	c.761A>C	c.958delC	c.506dup
Amino acid change	p.Tyr254Ser	p.Arg320Alafs	p.Lys170Glnfs
Inheritance	de novo	de novo	de novo
Type of mutation	Missense	Frameshift	Frameshift
ACMG	Likely pathogenic	Pathogenic	Pathogenic
Last follow-up	60 m	18 m	56 m
Motor development			
Sitting	13 m (unassisted)	18 m (assisted)	14 m (assisted)
Walking alone	24 m	No	No
Functional hand use	43 m	No	14 m
Speech development			
Can speak words	35 m	No	No
Expressive speech	43 m	No	No
Behavior			
Social interactions	13 m	Poor	8 m (social smile)
Eye contact	11 m	11 m	9 m
Abnormal sleep pattern	No	Yes	Yes
Neurological feature			
Epilepsy	No	No	Focal epilepsy
Stereotypic/dyskinetic movement	No	No	Orolingual dyskinesia
Spasticity	No	Yes, lower leg	No
Strabismus	No	No	Yes

Table 2

Clinical summary of previously reported adolescent and adult patients with *FOXG1* mutations.

	Age	Sex	Mutation type	Microcephaly	Sitting	Walking	Functional hand use	Speech	Social interaction	Seizure	Movement	Others
Arini et al[16]	22y	M	c.765G>A p.Trp255X	+	–	–	+	–	–	+	+	Arrhythmic breathing
Mencarelli et al[4]	13y 2 m	F	c.681C>G; p.Asn227Lys	+	N/A	–	N/A	–	N/A	+	+	Scoliosis
Phillipe et al[17]	22y	F	c.924A>G p.Trp308X	+	N/A	–	+	–	–	+	+	–
Seltzer et al[12]	16y 3 m	F	c.460dupG p.Glu154Glyfs*301	+	N/A	–	–	–	–	+	+	–
	13y 9 m	M	c.577G>A p.Ala193Thr	+	N/A	–	+	–	+	+	+	–
	25y 10m	F	c.460dupG p.Glu154Glyfs*301	+	N/A	–	–	–	+/–	+	+	–
	22y	M	c.460dupG p.Glu154Glyfs*301	+	N/A	–	+	–	+/–	+	+	–
Cellini et al[10]	17y	F	c.298delC; p.Gln100Serfs*92	+	N/A	–	N/A	–	–	+	+	Facial dysmorphism
Wong et al[3]	17.3y	N/A	c. 250delC p.Gln86Argfs*106	+	+	–	N/A	N/A	–	+	+	–

N/A = not available.

groucho binding domain site, c.958delC (p.Arg320Alafs), or N-terminal domain, c.506dup (p.Lys170Glnfs).

Interestingly, we found that all 3 patients were affected by hypoplastic olfactory bulbs in varying degrees with rather preserved olfactory sulci. The absence of recognizable olfactory epithelium, bulbs or vomeronasal organs was also observed in *Foxg1* knock-out mice at older embryo stages.[16] Therefore, hypoplastic olfactory bulbs may be another new phenotype suggestive of *FOXG1* syndrome. However, the development of any clinical signs or symptoms related to olfactory bulb hypoplasia, such as hyposmia, anosmia, and parosmia, cannot be assessed due to due to their language and cognitive deficits.

As the follow-up periods for our patients were short, we reviewed the clinical outcomes of nine previously reported adolescent or adult patients aged over 12 years.[4,8,11–13,17,18] Unfortunately, all these patients showed persistent microcephaly, epilepsy, and movement disorders, and most of them could not achieve unassisted walking (9/9, 100%), functional hand use (4/6, 66.6%), expressive speech (8/8, 100%), or social interaction (3/8, 37.5%) (Table 2).

This is the first report of *FOXG1* syndrome in a Korean population, which accounts for 2% (3 of 145 patients) in our patient cohort with developmental delay and/or hypotonia. Early-onset axial hypotonia with microcephaly and brain MRI findings related to telencephalon development (such as callosal anomaly, decreased frontal volume, fornix thickening, and hypoplastic olfactory bulbs) were distinctive features of *FOXG1* syndrome, and phenotypic severity was associated with the type of *FOXG1* mutation. Our report contributes to further understanding this extremely rare genetic condition in the clinical and genetic perspectives.

Author contributions

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Resources: Taeho Kim, Ah Young Jung.
Supervision: Tae-Sung Ko.
Writing – original draft: Han Na Jang.
Writing – review & editing: Han Na Jang, Beom Hee Lee, Mi-Sun Yum.

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