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 forhead 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.\[^2\]\[^1\]\ 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 patients and 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
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.
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 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 (<3 p) | 68.4 cm (50 p) | 68.4 cm (50 p) |
| Body weight | 8.7 kg (50 p) | 8.8 kg (75 p) | 8.8 kg (75 p) |
| Head circumference | 42 cm (<3 p) | 40 cm (<3 p) | 40 cm (<3 p) |
| 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 |
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. 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 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 and adult patients with FOXG1 mutations. Our report contributes to further understanding this extremely rare genetic condition in the clinical and genetic perspectives.

**Author contributions**

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**References**

[1] Eagleson KL, McFadyen-Ketchum LS, Ahrens ET, et al. Disruption of Foxg1 expression by knock-in of cre recombinase: effects on the development of the mouse telencephalon. Neuroscience 2007;148:385–99.

[2] Hou P-S, hAlin DO, Vogel T, Hanashima C. Transcription and beyond: delineating FOXG1 function in cortical development and disorders. Front Cell Neurosci 2020;14:35.

[3] Shochet SA, Kunde S-A, Viertel P, et al. Haploinsufficiency of novel FOXG1B variants in a patient with severe mental retardation, brain malformations and microcephaly. Hum Genet 2005;117:536–44.

[4] Mencarelli MA, Spanhol-Rosseto A, Artuso R, et al. Novel FOXG1 mutations associated with the congenital variant of Rett syndrome. J Med Genet 2010;47:49–53.

[5] Wong L-C, Singh S, Wang H-P, et al. FOXG1-related syndrome: from clinical to molecular genetics and pathogenic mechanisms. Int J Mol Sci 2019;20:4176.

[6] Kortum F, Das S, Flindt M, et al. The core FOXG1 syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. J Med Genet 2011;48:396–406.

[7] Mitter D, Pringsheim M, Kaulisch M, et al. FOXG1 syndrome: genotype-phenotype association in 83 patients with FOXG1 variants. Genet Med 2018;20:98–108.

[8] Wong L-C, Wu Y-T, Hsu C-J, et al. Cognition and evolution of movement disorders of FOXG1-related syndrome. Front Neurol 2019;10:641–1641.

[9] See-Gi, Park J-y, Kim S, et al. High diagnostic yield and clinical utility of WES for patients with undiagnosed genetic disorder by automating variant interpretation. Clin Genet 2020;98:562–70.

[10] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–23.
[11] Cellini E, Vignoli A, Pisano T, et al. The hyperkinetic movement disorder of FOXG1-related epileptic–dyskinetic encephalopathy. Dev Med Child Neurol 2016;58:93–7.

[12] Papandreou A, Schneider RB, Augustine EF, et al. Delineation of the movement disorders associated with FOXG1 mutations. Neurology 2016;86:1794–800.

[13] Seltzer LE, Ma M, Ahmed S, et al. Epilepsy and outcome in FOXG1-related disorders. Epilepsia 2014;55:1292–300.

[14] Vegas N, Cavallin M, Maillard C, et al. Delineating FOXG1 syndrome: from congenital microcephaly to hyperkinetic encephalopathy. Neurol Genet 2018;4:e281.

[15] Pringsheim M, Mitter D, Schröder S, et al. Structural brain anomalies in patients with FOXG1 syndrome and in Foxg1+/- mice. Ann Clin Transl Neurol 2019;6:655–68.

[16] Duggan CD, DeMaria S, Baudhuin A, Stafford D, Ngai J. Foxg1 is required for development of the vertebrate olfactory system. J Neurosci 2008;28:5229–39.

[17] Ariani F, Hayek G, Rondinella D, et al. FOXG1 is responsible for the congenital variant of Rett syndrome. Am J Hum Genet 2008;83:89–93.

[18] Philippe C, Amsallem D, Francannet C, et al. Phenotypic variability in Rett syndrome associated with FOXG1 mutations in females. J Med Genet 2010;47:59–63.