LETTER TO THE EDITOR

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Novel variants and phenotypes of *ROBO3* gene associated with horizontal gaze palsy with progressive scoliosis

To the editor:

Horizontal gaze palsy with progressive scoliosis (HGPPS) is a rare autosomal recessive neurologic disorder characterized by eye movement abnormalities apparent from birth and childhood-onset progressive scoliosis. Based on the clinical features and molecular diagnosis, HGPPS could be classified into two subtypes. HGPPS1 (OMIM1#607313) is caused by mutations of the *ROBO3* gene.[1] With exceptions of inability to move eyes horizontally and scoliosis, patients may have symptoms such as nystagmus, strabismus, facial palsy, sensory hearing loss, nodding of the head, and motor delay.[2] HGPPS2 (OMIM1#617542) is caused by mutations of the *DCC* gene and distinguished from HGPPS1 by impaired intellectual development, global developmental delay, agenesis of the corpus callosum, and absence of the anterior and hippocampal commissures.[3,4] Here, we report new phenotypes of abnormal horizontal tongue movements and brachycephaly and reveal deviation from normal developmental trajectories in gross motor rather than fine motor development by the developmental assessment scale in our HGPPS patient. To the best of our knowledge, it is the first report of developmental assessment in HGPPS patients.

A 32-month-old boy was referred to our clinic because of his inability to move his eyes horizontally from the age of 6 months. Family history revealed that he came from a non-consanguineous family, with no similar profile in his family members. He was delivered by Cesarean section at full term, weighing 4100 g. In addition, he was found to have paroxysmal head jerks (Video S1), presenting at the age of seven months. The head jerk had a relationship with his emotions, aggravated when excited, and stopped under emotional control. He fell on his knees because of his head jerking after he could walk. He gained the ability to control his head at 6 months, to sit unsupported at 10 months, to stand with support at 17 months of age, and to walk independently but unsteadily until the age of 22 months. Moreover, he was found to have difficulty in tongue movement and swallowing. He was diagnosed with gross motor developmental delays through evaluations using the Gesell Development Schedules (GDS) and Peabody Developmental Gross and Fine Motor Scales-2 (PDMS-2), with decreased Gross Motor Quotient scores of 87 and 68 at the ages of 7.63 and 25.37 months, respectively (Table S1).

Craniofacial anomalies consist of large low-set ears and brachycephaly (Figure 1A). The clinical head circumference is 49 cm in the 50th percentile. The fundus and anterior segment of his eyes were normal. Ocular alignment was good at the primary gaze position. His horizontal eye movements were damaged, including versions and convergence, with his vertical movements preserved (Figure 1B). Nystagmus occurred when he looked up and down and was disassociated and more pronounced in the right eye. Asynchronous blinking could be observed (Video S2). In addition, he had a problem with horizontal tongue movement in that he could not touch the corners of his mouth using his tongue with the lingual frenulum intact (Figure 1C and Video S3). Spinal radiographic images showed mild scoliosis that deviated to his right side with a Cobb angle of 11.6° at the age of 32 months (Figure 1D–F). Brain magnetic resonance imaging revealed hypoplastic pons with a prominent midline cleft, a "butterfly-like" medulla, and an enlarged fourth ventricle (Figure 1G–I and Figure S1). Diffusion tensor imaging showed no decussation of corticospinal tracts in the ventral and dorsal segments of the lower medulla (Figure 1J).

Next-generation sequencing and Sanger sequencing identified a compound heterozygous variation of c.841delG (p.Val281*) and c.2779+1G>A in the *ROBO3* gene (NM_022370.4) in the affected boy, in which the variant

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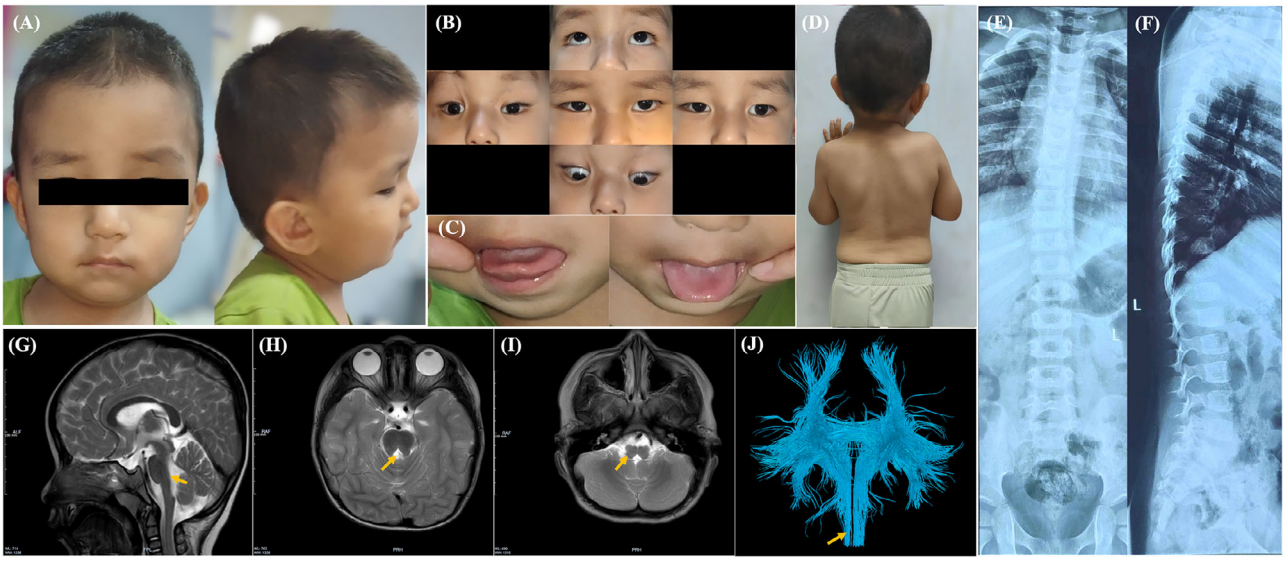

FIGURE 1 Clinical manifestations of our patient. (A) The front and side craniofacial photos. (B) Images of ocular motility—limited conjugate horizontal movements but preserved vertical movements. (C) Restricted horizontal tongue movements. (D–F) Spine photo and radiographs including anteroposterior and lateral views showed mild scoliosis deviating to his right side with a Cobb angle of 11.6° at the age of 32 months. (G-J) Brain T2-weighted magnetic resonance image and diffusion tensor image. A sagittal scan revealed a hypoplastic medulla and pons and an enlarged fourth ventricle (G). An axial image showed a deep posterior mid-sagittal cleft of the pons (H). Flattening and butterfly-like morphology in the medulla oblongata (I). Diffusion tensor imaging showed no decussation of corticospinal tracts at the level of the lower medulla (J).

of c.841delG (p.Val281*) came from his mother, and c.2779+1G>A came from his father. Both the small deletion of c.841delG (p.Val281*) and the splicing variant c.2779+1G>A were predicted to result in a frameshift of the coding region and to produce a premature termination codon signal in advance, leading to mRNA degradation due to nonsense-mediated mRNA decay. Both two variants were classified as likely pathogenic following the guidelines of the American College of Medical Genetics and Genomics.[5]

As a member of the roundabout gene family, the *ROBO3* gene encodes transmembrane receptors and interacts with the Slit ligand to form Slit-Robo signaling, playing a crucial role in mediating midline crossing of neurons during embryogenesis of the spinal cord.[6,7] Mutations in *ROBO3* may lead to a stall of the axon crossing to the contralateral side, resulting in a loss of horizontal conjugate movement. There are abundant nerve fiber crossings in the brain, such as the corticospinal and somatosensory axon tracts to cross the midline in the medulla, and pathogenic *ROBO3* variations will affect their formation, leading to dysgenesis of the brainstem, showing a flattened pontine tegmentum, markedly reduced facial colliculi, and reduced volume of pons and medulla.[8] Currently, the mechanism of scoliosis onset in HGPPS patients remains unknown. This may be due to chronic muscle tone abnormalities caused by brainstem malformation involving posterior column structures and associative descending fibers from the brain to the spinal cord, including the reticulospinal and corticospinal tracts.[9,10]

With exceptions of loss of conjugate horizontal movement and scoliosis, our patient presents a series of atypical phenotypes, such as vertical nystagmus, poor convergence, asynchronous blink, paroxysmal head jerk or nodding, inflexible tongue movements, difficulty swallowing, gait instability, and motor development delay, among which the phenotypes of abnormal horizontal tongue movement and brachycephaly have not been reported in HGPPS patients previously. Because of logistical reasons, we were unable to perform skull radiographs to further confirm the suspected craniosynostosis, which may be considered as an additional unreported deformity. Besides, it is reported that about one-third of HGPPS patients had motor developmental delay according to literature review,[2] which only described motor development abnormalities. There are lack of detailed data on motor development assessment revealing any deviation from normal developmental trajectories. In our report, GDS and PDMS-2 were used to assess and record motor developmental trajectories, which suggested that the HGPPS patient had disorders in gross motor development along his developmental process while fine motor development was intact. To the best of our knowledge, it is the first report of developmental assessment in HGPPS patients. We take the view that the motor developmental delay in our patient is related to the *ROBO3* mutation and may be an earlier sign of sensorimotor integration

imbalance before the onset of scoliosis. Further evaluation of the natural neurodevelopmental trajectories of HGPPS patients with pathogenic variants and their benefits from physical rehabilitation is needed in the future for precise genetic counseling and clinical management.

In conclusion, we found novel phenotypes and pathogenic variants in the *ROBO3* gene, which expanded the clinical and mutational spectrum of HGPPS syndrome. Mutations of *ROBO3* may result in extensive neurologic problems in the human body, including motor and sensory systems. It is a difficult task for long-term follow-up and giving appropriate treatment to HGPPS patients.

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CONSENT FOR PUBLICATION

Written informed consent was obtained from the parents of the patient for the publication of any potentially identifiable images or data included in this article.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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