ANNALS of Clinical and Translational Neurology
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AMERICAN NEUROLOGICAL ASSOCIATION

RESEARCH ARTICLE

Horizontal gaze palsy with progressive scoliosis: Further expanding the *ROBO3* spectrum

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Received: 9 February 2024; Revised: 3 June 2024; Accepted: 3 June 2024

Annals of Clinical and Translational Neurology **2024; 11(8): 2088–2099**

doi: 10.1002/acn3.52129

Abstract

Objective: Horizontal gaze palsy with progressive scoliosis (HGPPS) is a rare, autosomal recessive disorder resulting from axonal midline crossing defect due to variants in *ROBO3*. **Methods**: We retrospectively evaluated demographics, clinical phenotype, course of spinal deformities, and neuroimaging findings of six Turkish patients with HGPPS. We performed targeted gene testing by next-generation sequencing. **Results**: The median age at symptom onset and diagnosis was 1.5 years (0.5–4), and 11 years (2–16), respectively. Oculomotor signs were the most common presenting symptom ($n = 4$), followed by scoliosis ($n = 2$). The course of scoliosis was progressive and accompanied by kyphosis, showed intrafamilial variability, and was corrected surgically in three of the patients. Intellectual disability ($n = 4$), hypergonadotropic hypogonadism ($n = 2$), hearing loss ($n = 2$), and tranisent movement disorders ($n = 1$) were additional features. Targeted gene sequencing revealed five distinct homozygous variants. Of the four novel variants, two of them were located in the acceptor site of the non-coding region of the gene, remaining two were missense and frameshift variants, located in immunoglobulin-like domain-2, and cytoplasmic signaling motif 2, respectively. Structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) showed the absence of decussation of superior cerebellar peduncle and dorsal transverse pontine fibers. **Interpretation**: Spectrum of HGPPS is further expanded with novel variants in the *ROBO3* with clinical and radiological fingerprints. Spinal deformities require close orthopedic screening and individualized approach. Intellectual disability and hearing loss emerge as additional features. Hypogonadism and transient subtle movement disorders require further attention and confirmation from other series.

Introduction

Horizontal gaze palsy with progressive scoliosis (HGPPS; MIM# 607313) is a rare autosomal recessive disorder, typically characterized by congenital or early-onset absence of conjugate horizontal eye movements and progressive scoliosis.[1–4] HGPPS is among the human disorders of axon guidance, which also include congenital mirror movements and congenital fibrosis of the extraocular muscles, type III.[2] The disorder results from axonal midline crossing defects of specific populations of neurons in the hindbrain and possibly spinal cord. Historically, it was described in 1970s, and variants in *ROBO3* resulting in loss of function were identified in 2004.[1,5] Animal models and in vitro neuronal culture techniques help our understanding of this complex interplay within the developing nervous system.[2,6–8]

ROBO3 is a member of roundabout transmembrane receptor family (ROBO), which plays a key role in axon guidance and brain connectivity.[6,7] It is located at

chromosome 11q23-q25 and contains 28 exons that encode a protein of 1386 amino acids, which has five immunoglobulin (Ig)-like loops, three fibronectin (Fn) type III repeats, a transmembrane segment, and three conserved signaling motifs (CC0, CC2, and CC3).[2,6–9]

Homozygous or compound heterozygous variants in *ROBO3* are responsible for typical clinical and neuroradiological findings.[9–13] Robo3 is an axon guidance receptor predominantly expressed by commissural neurons in the developing embryonic hindbrain and spinal cord. Failure of the corticospinal and dorsal column tracts to decussate at the hindbrain is a pathognomonic finding, which is demonstrated by structural magnetic resonance imaging (MRI) studies.[14–16] Radiological signatures of HGPSS include (a) hypoplasia of the brainstem with a so called "butterfly" medulla oblongata, (b) midsagittal cleft extending ventrally from the fourth ventricle, called "split pons," as well as, (c) absence of the facial colliculi.[15,16] There are a few diffusion tensor imaging (DTI) studies reported in patients with HGPPS, which show absence of major crossing fiber tracts within the pons and midbrain with preserved interhemispheric connections in the corpus callosum.[17–20] Our aim is to present clinical phenotype, structural MRI, and DTI findings, along with the course of our patients with HGPPS due to variants in *ROBO3*.

Subjects and Methods

Subjects

This is a retrospective study at Hacettepe University Faculty of Medicine, Division of Pediatric Neurology. Six patients with HGPPS were enrolled. The study was approved by the Hacettepe University Non-interventional Clinical Studies Institutional Review Board (Number: 2021/07-45).

Characteristics and molecular findings of the patients are presented in the Results section, and summarized in Table 1.

Neuroimaging

Routine structural brain MRI and DTI were obtained for all patients. Patient 4 also had a hypothalamo-pituitary imaging. The DTI protocol included a matrix size of 256 × 256, FOV (field of view) of 250 × 250 mm, 24 slices with a thickness of 5 mm and no gap, TR (repetition time) of 4.2 s, TE (echo time) of 110 ms, b-value = 1000 mm^2/s, and 12 diffusion directions. The cranial volume covered the motor cortex, while the caudal volume covered the medulla oblongata. All the neuroradiological results of the patients have been combined and stated in an additional section.

Molecular investigations

Genomic DNA was extracted from a peripheral blood sample of each participant using an automated DNA isolation kit (QIAamp DNA Blood Midi Kit, Qiagen, Hilden, Germany) after obtaining the written informed consent form from the patient's legal guardians. Targeted *ROBO3* testing by next-generation sequencing (NGS) was performed. To amplify all 28 exons and splice sites of the *ROBO3*, a set of NGS compatible primers was designed according to the MANE (Matched Annotation of NCBI and Ensemble) selected transcript (NM_022370.4). NGS analyses were carried out by the MiSeq system (Illumina, San Diego, CA, USA). QIAGEN Clinical Insight Interpret was used for the interpretation of the data according to the recommendations of current guidelines.[21]

The guidelines for variant assessment include 16 pathogenic and 12 benign criteria, with evidence levels distributed into supporting, moderate, strong, and very strong.[22,23] The criteria used in this study are summarized as the following:

1 PM1 was used if the location of the variant is in a mutational hotspot or a critical protein domain (Uniprot-https://www.uniprot.org/)
2 PM2 was used if the allele frequency of the variant in healthy population data is not found or extremely low (GnomAD-https://gnomad.broadinstitute.org/).
3 PVS1 was used according to variant type and effect (for truncating variants; nonsense and frameshift).
4 PP3 was used as the pathogenicity predictions of computational in silico tools (MetaRNN score (http://www.liulab.science/metarnn.html) for non-synonymous variant prediction, and *SpliceAI* (https://spliceailookup.broadinstitute.org/) for possible splice region variants).
5 PP5 was used for the database information that a reliable source has reported the variant as pathogenic (Clinvar-https://www.ncbi.nlm.nih.gov/clinvar/; HGMD-https://www.hgmd.cf.ac.uk/ac/index.php; LOVD-https://www.lovd.nl/).
6 PP1 was used for the segregation analysis (detection of the variant in homozygous state in multiple affected (PP1-strong) or heterozygous wild type in the unaffected members in a family (PP1-supporting)). PP1 is included in the classification of all of the variants since the segregation analyses were performed for each family using Sanger sequencing.
7 PP4 criteria was used for all the variants, as the patients had the clinical pre-diagnosis for a specific disease with a single genetic etiology.

Table 1. Characteristics of patients with *ROBO3* variants

	Patient 1 (F)	Patient 2 (F)	Patient 3 (F)	Patient 4 (M)	Patient 5 (M)	Patient 6 (M)
Consanguinity/FH	+/+	+/+	+/−	+/−	+/+	+/−
Developmental milestones	Mild GDD	Mild GDD	Moderate GDD	Normal	Mild GDD	Mild GDD
Presenting symptom/Age at onset	Nystagmus/1 year	Scoliosis/2 year	Horizontal gaze palsy/4 year	Scoliosis/2 year	Horizontal gaze palsy/6 month	Nystagmus/6 month
Age at diagnosis	12 year	4 year	16 year	15 year	10 year	2 year
Age at scoliosis	7 year	2 year	11 year	2 year	10 year	2 year
Age at scoliosis surgery	12.5 year	10 year	14.3 year	NP	NP	NP
Age at onset of ophthalmological findings	1 year	NA	4 year	NA	6 month	6 month
Cognitive status	Mild ID	Mild ID	Mild ID	Mild ID	Normal (low average)	Normal (low average)
Follow-up duration	8 year	8 year	7 year	5 year	5 year	3.5 year
Functional status at last visit	Ambulatory	Ambulatory	Ambulatory	Ambulatory	Ambulatory	Ambulatory
Additional features			Hearing loss, hypogonadism	Hypogonadism, osteoporosis, bicuspid aortic valve		Hearing loss, cleft lip and palate
Variant, (cDNA and protein)	c.906-14G>A (IVS5- 14G>A)	c.906- 14G>A (IVS5-14G>A)	c.1366G>T, (p.Gly456*)	c.290G>C (p.Trp97Ser)	c.1034-12T>A (IVS6- 12T>A)	c.3663delT (p.Ser1222AlafsTer17)
Variant type and possible effect	Intronic, possible splicing effect	Intronic, possible splicing effect	Nonsense Truncating (PVS1)	Missense variant in mutational Hotspot-PM1	Intronic, possible splicing effect	Frameshift truncating (PVS1)
Population Data (GnomAD frequency; PM2)	NF	NF	NF	NF	NF	0.000000686/no homozygous
In-silico tool prediction (PP3)	Splice AI: 0.16 acceptor loss PD	Splice AI: 0.16 acceptor loss PD	PD	MetaRNN = 0.975 PD	Splice AI: 0.99 acceptor gain PD	PD
Database information (PP5)	NR	NR	Clinvar (RCV000002260)	NR	NR	NR
Segregation analysis (PP1)	Heterozygous in parents and healthy siblings, homozygous in the affected sibling	Heterozygous in parents and healthy siblings, homozygous in the affected sibling	Heterozygous in parents and healthy siblings	Heterozygous in parents and healthy siblings	Heterozygous in parents and healthy siblings	Heterozygous in parents and healthy siblings
ACMG Criteria and Classification	PM2, PPS, PP3, PP4; LP	PM2, PP1-S, PP3, PP4; LP	PVS1, PM2, PP1, PP4, PP5; P	PM1, PM2, PP1, PP3, PP4: LP	PM2, PP1, PP3-S, PP4; LP	PVS1, PM2, PP1, PP4; P

F, Female; FH, Family History; GDD, Global Developmental Delay; ID, Intellectual Disability; LP, Likely Pathogenic; M, Male; NA, Not Available; NF, Not found in healthy population; NR, Not Reported; P, Pathogenic; PD, Possibly Damaging; PP3-S, PP3-Strong; PP4-S, PP4-Strong.

Results

Patients 1 and 2

A 12-year-old girl was presented with abnormal head movements and thoracolumbar scoliosis. Prenatal and natal histories were uneventful. She was able to sit unsupported at 6 months, walked independently at 24 months, had single words at 12 months. Her parents recognized abnormal eye movements and head titubation at age 1 year, and scoliosis at age 7 years. Parents were first cousins. A younger sister was also affected (Patient 2). Physical examination revealed nystagmus, horizontal gaze palsy, head titubation, thoracolumbar (T7-L2) scoliosis, and kyphosis (T2-T12)

(Cobb angle: 100° and 68°, respectively). Wechsler intelligence scale for children-revised (WISC-R) test revealed mild intellectual disability. The evoked potential study showed abnormal ipsilateral cortical-sensory response. Scoliosis surgery 2 months after presentation was followed by thoracic-lumbar-sacral orthosis. Postoperative lateral X-rays revealed thoracolumbar (T7-L2) scoliosis and kyphosis (T2-T12) (Cobb angle: 33° and 45°, respectively). At the most recent visit 8 years after surgery, scoliosis remained stable.

Patient 2 was the younger sister of patient 1, a 4-year-old girl, who had scoliosis and titubation noticed after the age of 2 years. She was born at term with a birthweight of 3000 g. She was able to sit unsupported at 12 months, walked independently at 24 months, had first words at 2 years, and was able to talk with two-word sentences at 3.5 years. She had bilateral horizontal gaze palsy, nystagmus, unsteady gait, and in-toeing. Initial X-rays showed thoracolumbar (T5-L2) scoliosis (Cobb angle: 42°) (Fig. 1). Kyphosis at the level of T2-T12 was within normal limits (30°). The Stanford–Binet test showed mild intellectual disability. She underwent serial Mehta castings during follow-up. Lateral X-rays obtained at the age of 10 years revealed thoracolumbar scoliosis and kyphosis (Cobb angle: 83° and 50°, respectively), and thereafter she was operated. At the last visit, 2 years after surgery, thoracolumbar scoliosis and kyphosis (Cobb angle: 17° and 42°, respectively) were stable. WISC-R test revealed moderate intellectual disability. Age at onset of scoliosis showed an intrafamilial variability.

A novel c.906-14G>A(IVS5-14G>A) variant in *ROBO3* was identified in both siblings (Fig. 2).The variant was not found in the healthy population (PM2) and segregated in homozygous state in affected members (PP1-Strong) in the family. We used PP3 for this variant, as SpliceAI score was not enough to include PVS1.[22]

Patient 3

A 15-year-old girl presented with abnormal eye movements and thoracolumbar scoliosis. She was born at 34th gestational weeks with a birthweight of 1500 g via normal spontaneous vaginal delivery. Parents were first cousins. Early developmental milestones were delayed with independent walking at 3 years, and first words at 5 years.

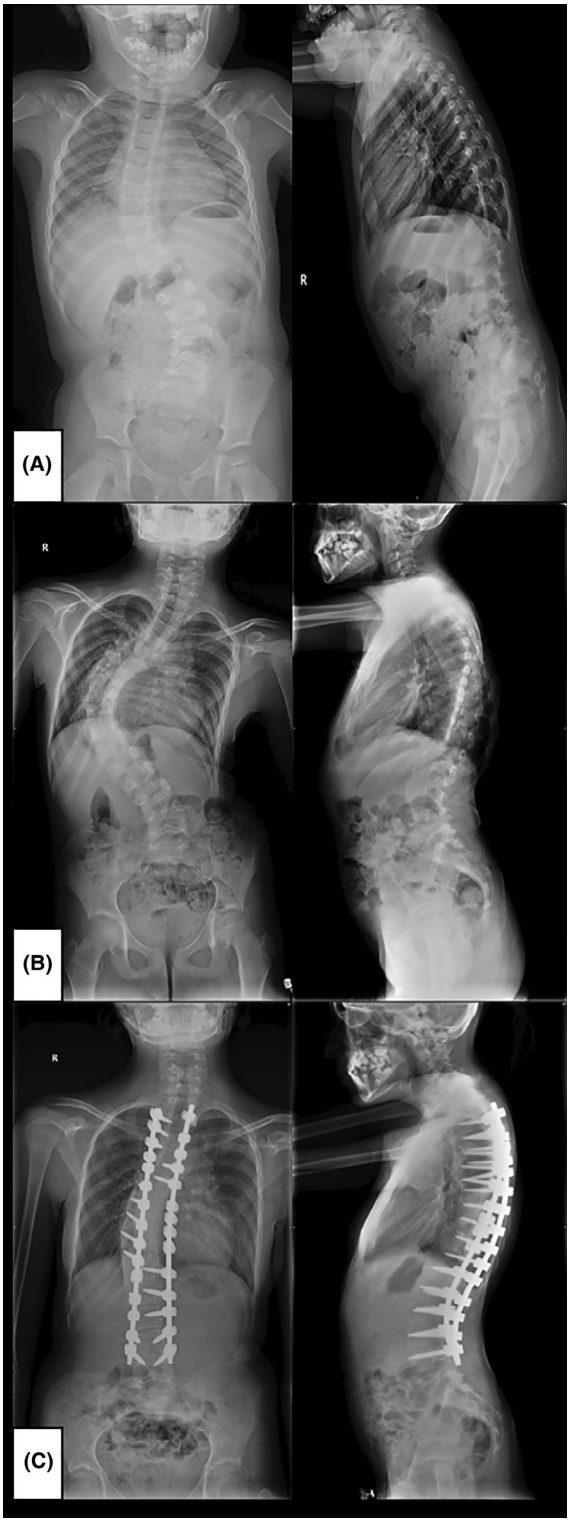


Figure 1. Four-year-old girl, she presented with thoracolumbar 42° scoliosis. (A) Sagittal alignment was uneventful. With the diagnosis of neuromuscular early-onset scoliosis, treatment was started with Mehta body cast under general anesthesia. The cast was changed three times in 3 months intervals. Afterwards, she was followed up with a brace. There was a poor compliance. At the age of 10 years, when the deformity was found to have reached significant degrees (T5-L2: 83°) (B), she had a spinal surgery with posterior instrumentation and fusion (C).

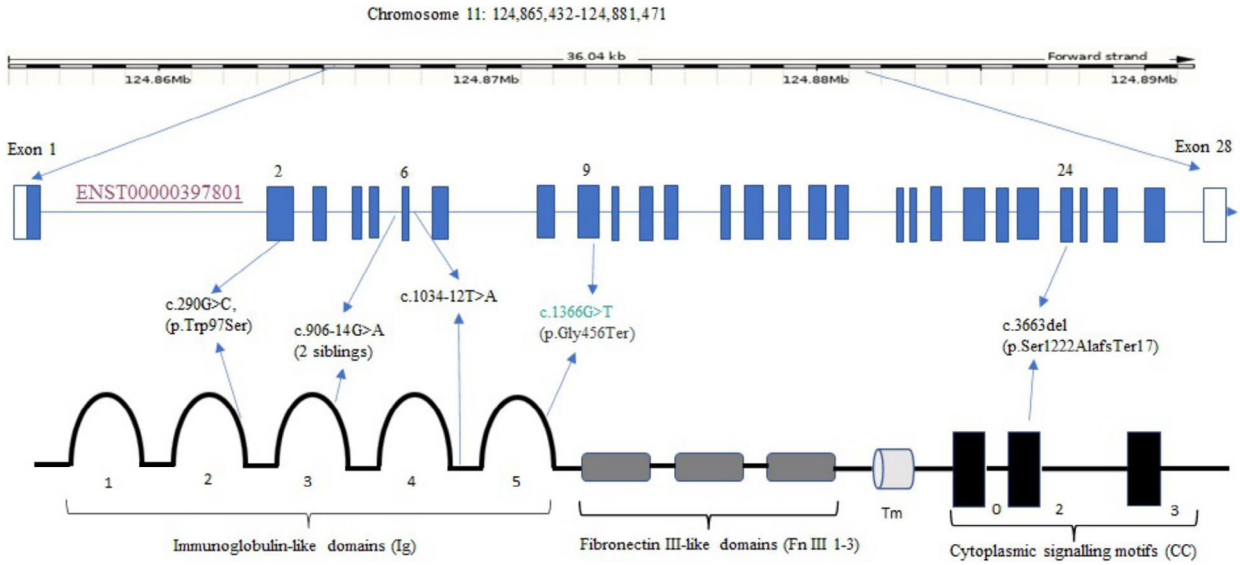


Figure 2. Visualization of the genomic placement and general structure of the *ROBO3* gene. The localization of the variants detected in this study has been demonstrated on the MANE-selected transcript (ENST00000397801) and on the protein domain level. The variants written in black are novel. Tm; Transmembrane segment. "The figure is created using the ensemble (https://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000154134;r=11:124865432-124881471;t=ENST00000397801) database and adapted from the literature."[9]

Her parents recognized abnormal eye movements and scoliosis at the ages of 4 and 11 years, respectively. Hearing loss was noticed at 6 years, and hearing aid was provided at 9 years. She had nystagmus and horizontal gaze palsy, short stature (weight: 34 kg, height: 141 cm, both <5th percentile), and thoracolumbar (T6-L2) scoliosis and kyphosis (T2-T12) (Cobb angle: 55° and 63°, respectively). WISC-R test revealed mild intellectual disability. She underwent scoliosis surgery 5 months after admission, and postoperative lateral X-rays revealed 21° scoliosis and 31° kyphosis. The evoked potential study showed abnormal ipsilateral cortico-sensory response. At the age of 16.5 years, she was diagnosed with hypergonadotropic hypogonadism during evaluation for primary amenorrhea. She is still on medications for regulation of menstrual cycle at the age of 23 years, and her spine X-ray showed 19° scoliosis and 43° kyphosis.

A nonsense c.1366G>T, (p.Gly456*) variant in *ROBO3* was identified (Fig. 2). This nonsense variant which causes a truncated protein (PVS1) was not found in the healthy population (PM2). The variant was reported as disease causing[1], and submitted to ClinVar database (PP5). Parents and healthy siblings were heterozygous for this variant (PP1).

Patient 4

A 15-year-old male presented with early-onset scoliosis and limitation of eye movements. Parents were first cousins. He was able to walk independently at 18 months. There was no parental concern for developmental delay. He developed scoliosis around the age of 2 years, and spinal bracing was introduced since the age of 5 years. Initial severity of scoliosis and age at onset of gaze palsy were unknown.

Physical examination revealed short stature (weight: 25.8 kg, height: 128 cm, both <5th percentile), pectus carinatum, and severe spinal deformity. X-rays demonstrated thoracolumbar (T6-L2) scoliosis and kyphosis (T2-T12) (Cobb angle: 118 and 96°, respectively) (Fig. 3). He had nystagmus, horizontal gaze palsy, titubation, kinetic tremor, and in-toeing. WISC-R test revealed mild intellectual disability. The echocardiogram showed mild aortic regurgitation and cardiac MRI revealed axis rotation. Pediatric endocrinology evaluation revealed right undescended testicle, delayed puberty (hypogonadism), and osteoporosis leading to initiation of testosterone and pamidronate treatments, consecutively. MRI of the hypothalamo-pituitary region was normal (not shown). Unfortunately, there has been a gap in his follow-up, and last examination was performed at the age of 20 years. The most recent spine X-ray revealed a neglected spinal deformity with 145-degree scoliosis and 127-degree kyphosis.

A novel missense c.290G>C (Trp97Ser) variant classified as likely pathogenic in exon 2 of the *ROBO3* was identified (Fig. 2). The variant was located on a mutational hotspot and functional domain of Robo3 (Ig-like

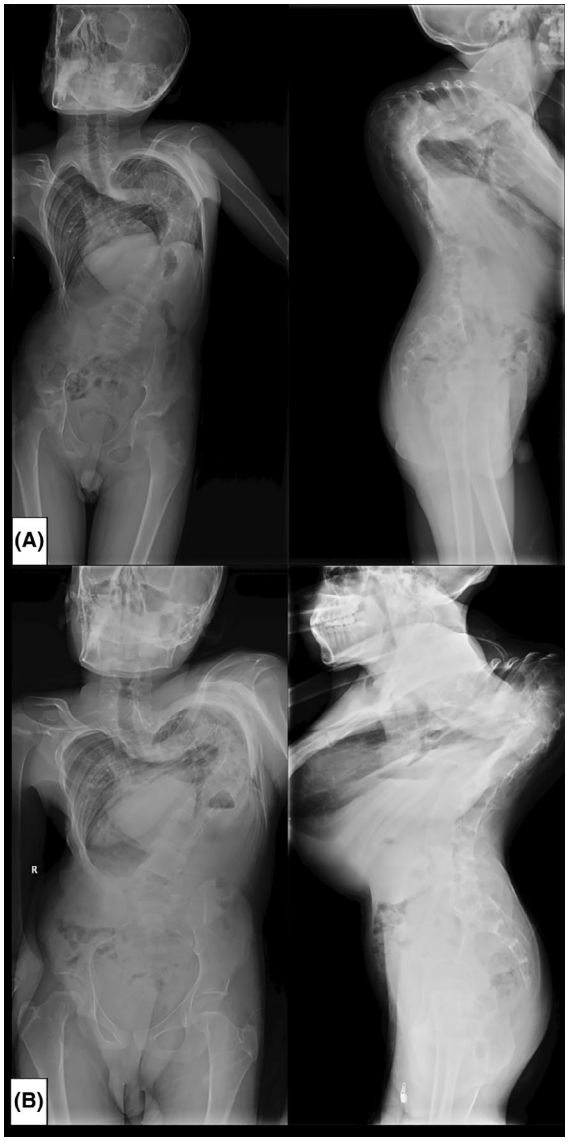


Figure 3. Fifteen-year-old male, although his spinal deformity was first noticed at the age of 2 years and brace treatment was recommended at the age of 5 years, the brace was not used effectively. Severe rotational scoliosis and kyphosis were detected when he presented to our clinic at the age of 15 years for the first time (A). The patient was recommended halo-gravity traction followed by posterior instrumentation and fusion. The treatment was refused by the family. Follow-up visit 5 years later, revealed further progression of the spinal deformity (B) leading to severe cardiopulmonary compromise.

C2-type 1), and the variant frequency in the healthy population was not reported (PM2). We used PP3-S criteria, as the MetaRNN score of the variant is >0.939 (0.975).[23] PP1 criteria is used as the variant was segregated as expected.

Patient 5

A currently 15-year-old boy was referred at the age of 6 months because of mild developmental delay and restricted eye movements. Prenatal and natal histories were uneventful. Parents were first cousins. On admission, he had bilateral horizontal gaze palsy without nystagmus and upward gaze palsy in the right eye. Head control and independent walking were achieved at the age of 12 months and 2 years, respectively. He had his first words at 10 months of age. He presented at the age of 10 years with abnormal rotatory repetitive head movements and right-sided tilt (Video S1, supplementary material) in addition to horizontal gaze palsy. There was resolution of these stereotypical movements upon distraction (Video S2, supplementary material). These movements were noticed to disappear during sleep. He also had intentional movements on the left side, resulting in involuntary movements on the right side affecting mainly fingers, mimicking mirror movements (Video S3, supplementary material). Spine X-ray at the age of 10 years was normal. Follow-up lateral X-rays demonstrated thoracolumbar (T5-L1) scoliosis and mild kyphosis (T2-T12) (Cobb angle: 27° and 47.5°, respectively).

His most recent examination, at the age of 15 years, revealed postural and kinetic tremor with resolution of rotatory repetitive head movements and mirror movements. He was followed by spinal bracing for the last 12 months, and the latest spine X-ray revealed stable scoliosis and normal kyphosis (Cobb angle: 17.5 and 43°, respectively). The WISC-R test revealed low average scores. The evoked potential study demonstrated abnormal ipsilateral cortico-sensory responses. There was a history of another affected individual within the family, who was unfortunately not able to attend our clinic.

A novel intronic variant, c.1034-12T>A in *ROBO3* was identified, which is assessed to be disease-causing by disturbing the splicing (Fig. 2). This variant was evaluated as likely pathogenic depending on PP1 and PP4 criteria, PM2 criteria (the allele frequency was not reported in the healthy population), and PP3-S criteria (SpliceAI score was >0.5).[22]

Patient 6

A 9-month-old boy was referred for developmental delay and nystagmus, which was recognized at the age of 6 months. Parents were first cousins. At birth, he was noticed to have cleft palate and hearing loss. Auditory brainstem response (ABR) test was abnormal. He underwent cleft palate surgery and an ear tube was inserted at 9 months of age. He achieved head control at 4 months, sat unsupported at 8 months, and started to babble at

8 months. He had global developmental delay, nystagmus and absence of the horizontal eye movements. He was able to walk with support at the age of 2 years, and had speech delay. Lateral X-rays revealed mild thoracolumbar (T10-L4) scoliosis (Cobb angle: 23°). A follow-up ABR test after tube insertion was not available because of poor compliance. At the age of 5.5 years, he was able to walk without support, unable to climb stairs, talk with two-word sentences, and had no toilet training. Stanford–Binet intelligence scale subtests reveal low average scores, though assessment was suboptimal due to hearing problems.

The most recent spine X-ray at the age of 5.5 years showed stable thoracolumbar (T10-L1) scoliosis (Cobb angle: 25°), and sagittal plane alignment was normal (T2-12: 36.3 degree). Thoracic-lumbar-sacral orthosis was recommended.

A novel frameshift homozygous variant c.3663delT (p.Ser1222AlafsTer17), which causes premature termination of the protein synthesis by creating a stop codon after 17 amino acids was identified (PVS1) (Fig. 2). In addition to PVS1 and PP4 criteria; allele frequency of the variant in healthy population is too low (0.000000686) according to PM2 criteria, and familial segregation helps to classify the variant as pathogenic according to PP1 criteria.

Summary of neuroimaging findings

Structural MRI findings

Hypoplasia of the pons and medulla oblongata, with a prominent ventral midline cleft at the level of the pons, as well as both ventral and dorsal midline clefts at the level of the medulla were detected in all six patients (Fig. 4). A butterfly configuration of the medulla oblongata and tent-like configuration of the pons were observed in all patients. The dorsal nodular surface contour representing the facial colliculus at the level of the pons was absent in all patients. Three patients had a thick corpus callosum (Patients 1, 2, and 4). Superior cerebellar peduncles (SCPs) could not be detected in the structural

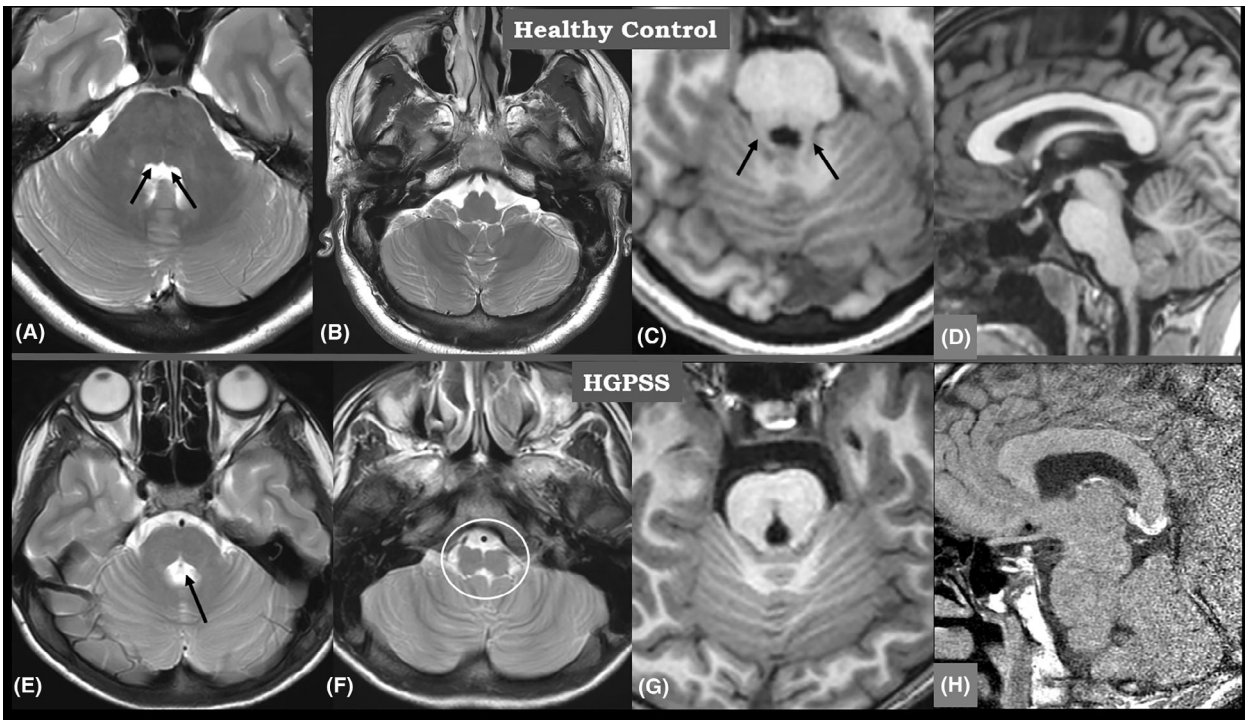
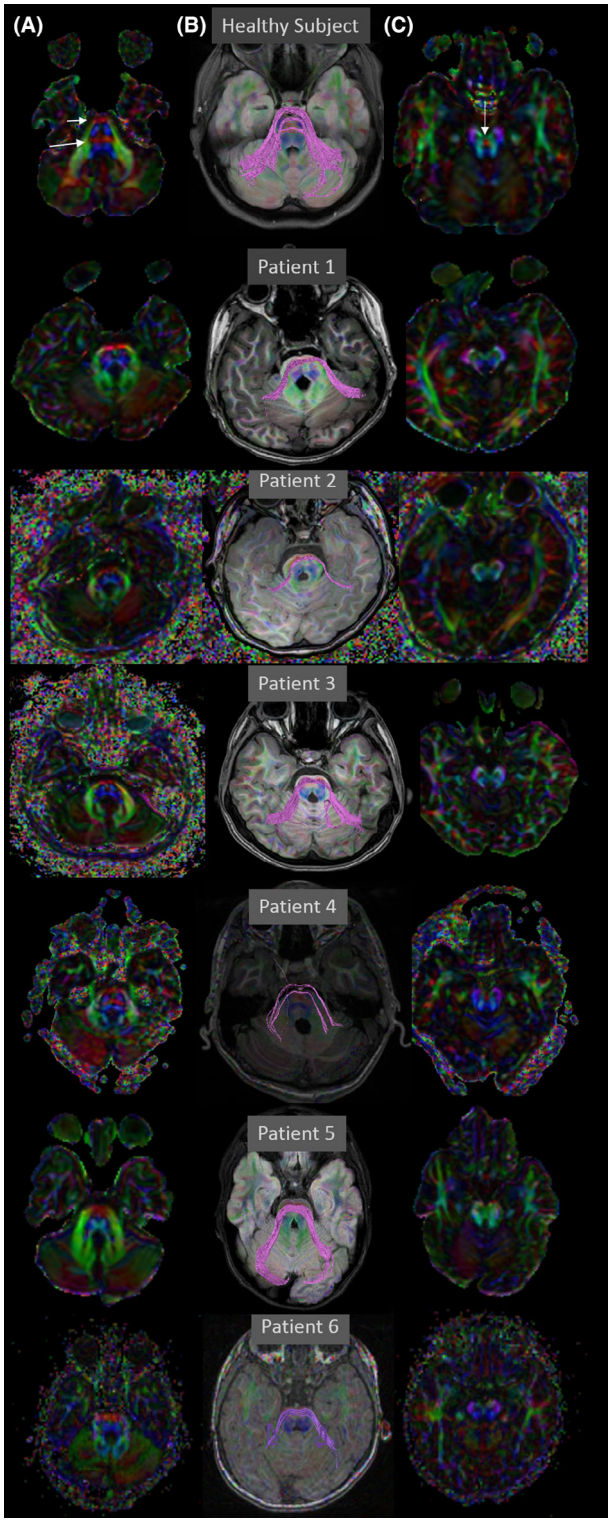


Figure 4. Structural magnetic resonance imaging findings at the brainstem level in a healthy individual (A–D) and the patient with horizontal gaze palsy with progressive scoliosis (HGPPS) (E–H). Axial T2-weighted images showing normal pons (A), facial colliculi (A, arrows), medulla oblongata (B), superior cerebellar peduncles (C), and corpus callosum (D). Hypoplasia of the pons (C) and medulla (D) are seen in patient with HGPPS (Patient 1). The pons shows diminished anteroposterior dimensions, particularly dorsally, with a dorsal midline cleft and a tent-like appearance (E, arrow). Also, note the absence of the contour of the facial colliculi and compare it with a healthy individual. The medulla oblongata exhibits a prominent midline cleft both ventrally and dorsally, as well as a butterfly configuration (F, circle). The superior cerebellar peduncles cannot be distinguished separately in the patient with HGPPS. Sagittal T1-weighted image demonstrates thick corpus callosum (Patient 4).

MRI of all other patients except one (due to being very thin or absent). Patient 4 had normal SCPs. MRI of the hypothalamo-pituitary region was normal in Patient 4 (not shown).



Diffusion tensor imaging findings

Fractional anisotropy (FA) maps showed the absence of the central red dot in the central midbrain representing the decussation of the SCPs in all patients except one (Patient 4). Furthermore, FA maps revealed the absence of dorsal transverse pontine fibers (dTPF), while ventral transverse pontine fibers (vTPF) were preserved in all patients except one (Patient 4). Patient 4 had normal vTPF and very thinned dTPF (Fig. 5). In all patients except one, pyramidal decussation could not be evaluated optimally because DTI imaging was terminated just caudal to the medulla oblongata. Decussation pyramidalis was absent in this single patient (Patient 5).

Discussion

Absence of lateral gaze accompanying scoliosis was first reported by Dretakis and Kondoyannis in 1974[5]. In 2004, variants in *ROBO3*, located on chromosome 11q23-25 were identified as the underlying cause of HGPPS.[1] To date, based on a systematic analysis and review articles, almost 100 patients with more than 50 variants in *ROBO3* have been reported.[9–11] In addition to typical symptoms, patients also presented with strabismus, bilateral early-onset sensorineural hearing loss, ipsilateral stroke, and intermittent nodding of the head.[9–11] Motor developmental delay and intellectual disability are also reported; however, detailed information on developmental milestones, formal neurocognitive tests and cognitive outcomes are not available for majority of the reported patients.[9–11]

The reported *ROBO3* variants are missense, nonsense, frameshift, and splice site variants, which affect multiple subdomains of the Robo3 axon guidance receptor, supporting a complete loss of function.[9–11] These variants are highly diverse, identified in all exons and exon-intron boundaries, and mostly located on the extracellular protein. It is unclear if *ROBO3* variants alter ligand recognition, protein folding, or targeting, and whether resultant changes in protein function might have a differential effect on developing nerve fiber tract decussation and/or

Figure 5. Diffusion tensor imaging findings of all patients with HGPPS in our cohort and a comparison with a healthy individual. The upper row demonstrates the fractional anisotropy (FA) map, showing ventral (A, short arrow) and dorsal (A, long arrow) transverse pontine fibers (vTPF and dTPF) coded in red, fiber tractography showing vTPF and dTPF, and an FA map depicting a red dot at the center of the midbrain, representing the decussation of the superior cerebellar peduncle (SCP) (C, arrow) in a healthy individual. In all patients with HGPPS except one, the dTPFs and SCP decussations are absent while vTPFs are preserved with normal thickness. In patient 4, thinning of dTPF and the presence of SCP decussation can be seen.

clinical expression. Missense variants are reported to be the most common, and there is not a clear genotype–phenotype correlation.[9–11]

In this study, we report five distinct homozygous *ROBO3* variants in six patients from five unrelated families. Two of these variants are truncating (a nonsense variant p.Gly456* in Patient 3 located in the Ig-like domain 5, and a frameshift variant p.Ser1222AlafsTer17 in Patient 6 located in cytoplasmic signaling motif 2) possibly leading to a loss of function. The presence of the p.Gly456* variant only in patients with Turkish origin questions whether it might be a founder variation of Turkish ethnicity.[1,11,24] Two novel variants located in the non-coding region of the gene (the c.906-14G>A variant in two siblings (Patient 1 and 2) and c.1034-12T>A variant in Patient 5) are predicted to be deleterious by probably resulting in a new premature acceptor site. The last variant, missense Trp97Ser located in exon 2, a functional domain of the protein (Ig-like C2-type 1), which is identified in Patient 4, is also novel. Unfortunately, as we were not able to complement this result with functional studies, we could not delineate the possible effect of this variant on phenotype. However, we believe that the patient's delayed diagnosis was due to presentional delay.

Horizontal gaze palsy stems from uncrossed and/or absent oculomotor and abducens internuclear tracts. In our study, majority of our patients (four out of six) presented with oculomotor signs (nystagmus, horizontal gaze palsy) in infancy. Xiu et al. reviewed 76 HGPPS patients, and they also showed the tendency toward recognition of oculomotor signs before scoliosis.[10] Nystagmus, esotropia, exotropia, hypertropia, and strabismus may accompany the horizontal gaze palsy, without major impairment in vertical gaze and visual acuity.[9–11,25]

Scoliosis was noticed between the ages of 2–10 years in our cohort, and three out of four of the patients with progressive scoliosis were treated surgically. Scoliosis is often recognized in early childhood, however it has been reported as early as infancy and even in the neonatal period.[10,11,24] It is typically progressive, though a group of Tunisian patients had showed a stable course.[9–11,26–28] A small proportion of individuals with HGPPS have also been reported to have kyphosis in addition to scoliosis.[9] We should emphasize that, all of our patients also had severe kyphosis. Spinal deformities should be closely monitored not only in the coronal plane, but also in the sagittal plane. This highlights the importance of precise orthopedic assessment and follow-up in HGPPS. Treatment decisions should be individualized.

Contrary to the previous reports, we reported higher rates of intellectual disability in this study. All patients, other than two having a low-average IQ, had intellectual disability. Information on motor and mental developmental milestones and cognitive evaluations are not available for all reported patients.[9–11] Therefore, it is not possible to draw conclusions based on the available data. Of note, patients may seem neurologically unimpaired at first visit, but may require repeated evaluations and formal testing during follow-up visits. Although fragmented, developmental trajectories and formal test results were available to conclude on the intellectual disability as part of the clinical expression in our patients with *ROBO3* variants.

Hearing loss is defined previously in a Turkish patient with a homozygous missense *ROBO3* variant, who required hearing aids.[24] Preclinic and clinic studies revealed that *ROBO3* variants also cause impairment in auditory pathways and alterations in brain stem auditory evoked potentials.[29] Sensorineural hearing loss is documented in two of our patients. Based on our findings and previous reports, sensorineural hearing loss can be a component of the clinical phenotype.

Although head nodding and jerks (usually accompanying nystagmus and gaze palsy) are reported in patients with *ROBO3* variants[9–11], mirror movements and transient nature of these subtle movement disorders that we observed in our Patient 5, are not previously documented in HGPPS. Congenital mirror movements can be syndromic, non-syndromic, and/or isolated. Another midline axon guidance defect, developmental split brain syndrome (DSBS, MIM# 617542) is caused by biallelic DDC netrin 1 receptor (*DCC*) variants leading to loss of function.[30,31] It is associated with agenesis of corpus callosum and widespread failure of commissural tracts throughout the rest of the CNS, with or without mirror movements.[30,31] Several features of HGPPS and DSBS, horizontal gaze palsy and scoliosis, overlap. Mirror movements are associated with the reduced midline crossing of descending corticospinal tract projections at the pyramidal decussation.[30–32] Like, HGPPS, not all patients with DSBS present with mirror movements which may be due to a complete, rather than a partial, failure of the descending corticospinal tracts to cross the midline. The exact nature of these transient subtle movement disorders in our Patient 5, is unfortunately not supported by standard scales and transcranial magnetic stimulation, which is reported to have a role to characterize characteristics of movement disorders with expanding genetic heterogeneity.[32] It is challenging to speculate on these subtle movement disorders, since at the last follow-up visit, our patient outgrew these findings. Confirmation of these findings from other series will be of interest, since Robo3 is a functional, intracellular binding partner of Dcc, expressed by commissural axons in the brainstem and spinal cord.[2,30–32] Moreover, as a targeted NGS-based molecular analysis was performed, we may be missing

other relevant variants and/or modifiers that may interfere with the clinical expression of the disorder.

We report hypogonadism during the adolescence period, in patients with HGPSS (Patients 3 and 4). We reviewed the structural cranial MRI studies in all of our patients, paying attention to hypothalamo-pituitary region, and all are normal. Having said that, an imaging dedicated to hypothalomo-pituitary region was available only for one of the two patients with hypogonadism, Patient 4, which was normal. Overall, hypogonadism can be overlooked in patients with *ROBO3* variants, and this observation again requires attention, and has an impact on management.

From a developmental perspective, gonadotrophin-releasing hormone (GnRH) neurons migrate from the nasal area to hypothalamus where they modulate gonadotrophin release from the pituitary gland. Defective migration of GnRH-1 neurons to the brain, lack of GnRH secretion, or signaling cause hypogonadotrophic hypogonadism, characterized by delayed or absence of puberty. Slits are secreted proteins that bind to Robo receptors. Slit-Robo signaling in the developing nervous system is complex.[8] Genetically altered mouse models showed that migration of GnRH neurons is directly modulated by Slit2 and Robo3, and postulated to modulate GnRH-1 cell motility and basal forebrain access during migration.[33] However, a recent study do not confirm these results, and showed that Slit2 loss of function affects GnRH-1 cell positioning in the brain in a Robo3 independent fashion.[34] Therefore, it is challenging to tie hypogonadism with complex GnRH-1 neuronal migration mechanisms. Long term follow-up and natural history of patients with *ROBO3* variants from other series will better help us to understand this relationship.

Brain MRI is a valuable tool in the diagnosis and evaluation of HGPPS, as it provides detailed information on structural abnormalities in HGPPS. The classic findings include split brainstem with tent-like pons and butterfly medulla oblongata.[15–17] Subsequently, the findings of thinning of the SCPs and absence of facial colliculus have also been reported. Every structural abnormality listed above was consistently present in our cohort. We could not demonstrate the SCPs in any of the patients, except one, probably due to its absence or being too thin. DTI is a specialized MRI technique that is used to evaluate the microstructural integrity and connectivity of white human disorders of axon guidance tracts in the brain. It can be particularly useful in understanding the underlying microstructural and connectivity abnormalities in the HGPPS. Thus, uncrossed pyramidal tracts, absence of decussation of the SCPs at the level of midbrain (absence of red dot sign on FA maps) have been previously reported.[17] In all our patients, except one, pyramidal decussation could not be evaluated because of technical issues. Absence of the decussation pyramidalis was detected in this single patient. The absence of decussation of the SCPs was observed in all of our patients, except one. Crossing fibers, also known as transverse pontine fibers, at the pons level were not present in our patients, except for one. Pontine crossing fibers typically consist of two bundles referred to as ventral and dorsal pontine fibers in healthy individuals. These two distinct crossing fibers are readily visible as two red bands on FA maps. Dorsal transverse pontine fibers were absent in all of our patients, except one, but ventral transverse pontine fibers were present. The absence of pontine crossing fibers has been previously reported in patients with HGPPS.[9–11,17–19] To the best of our knowledge, this study is the first to analyze these two distinct crossing fibers independently. Consequently, DTI has demonstrated that microstructural connectivity abnormalities occur in the dorsal rather than the ventral part of the brainstem, beyond structural MRI changes. Our imaging findings support the hypothesis of a midline crossing defect of the hindbrain in HGPPS.

Neurophysiological studies also present uncrossed corticospinal and dorsal column-medial lemniscal tracts as motor and somatosensory evoked potentials (MEP and SSEP) are obtained ipsilaterally.[1] In our study, MEP and SSEP in three patients (Patients 1, 3 and 5) also revealed abnormal ipsilateral motor and sensory response. Haller et al., reported a 14-year-old HGPPS patient together with SSEP, MEP, functional MRI, and DTI, and suggested that any of the above studies can serve as an ancillary test.[18]

Study limitations

Although our study had a relatively small sample size, it represents the largest HGPPS patient series from the Turkish population with almost a median follow-up of 8 years. Retrospective nature of the study is a limitation; however, we tried to delineate the developmental trajectories and course of the presenting symptoms. One of the major limitations of this study was inadequate data regarding precise ophthalmologic examination. Because of the molecular confirmation of the diagnosis, electrophysiological evaluations are not available as a complementary tool for all patients.

Conclusions

Horizontal gaze palsy and progressive scoliosis (HGPPS) is a rare autosomal recessive disorder characterized by congenital and early-onset gaze palsy with progressive scoliosis. Recognition of the clinical phenotype combined with neuroimaging signatures on structural and

functional MRI can accelerate the diagnostic pathway through targeted *ROBO3* testing. This study broadens the spectrum of *ROBO3* variants.

Acknowledgments

We are grateful to our patients and their families who supported this work.

Funding Information

No funding was received for conducting this study.

Conflict of Interest

The authors declare no potential conflict of interest with any commercial entities relating to this study.

Author Contributions

Conception and design of the study (CG and GH). Acquisition and analysis of data (CG, GH, BÇ, MY, ÇMT, RG, SÇ, and ÖÖ). Drafting a significant portion of the manuscript or figures (CG, RG, and GH).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Video S1.
Video S2.
Video S3.
Captions.

