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Bain type of X-linked Syndromic Mental Retardation in a Male with a Pathogenic Variant in *HNRNPH2*

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ABSTRACT

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are RNA binding proteins, which aid in maturation, stabilization and transport of mRNA. They have a significant role in cellular nucleic acid metabolism. The hnRNPs alter gene expression and are linked to various neurodegenerative disorders and cancers. Previously, six unrelated girls with developmental delay, intellectual disability and hypotonia were found to have *de novo* heterozygous pathogenic missense variants in *HNRNPH2*, located on the X chromosome. A gain-of-function effect was proposed for the variant and it was thought to be lethal in males as no surviving males were identified. We describe a family with two affected siblings, one male and one female, with a known pathogenic variant in *HNRNPH2*, possibly due to maternal germline mosaicism.

KEY WORDS

Heterogeneous nuclear ribonucleoproteins, developmental delay, intellectual disability, hypotonia, X-linked dominant, *HNRNPH2*

1. INTRODUCTION

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are RNA binding proteins, which play a significant role in nucleic acid metabolism by influencing alternative splicing, maturation, stabilization and transport of RNAs (Dreyfuss, Kim & Kataoka, 2002;

Dreyfuss, Matunis, Pinol-Roma & Burd, 1993; Geuens, Bouhy & Timmerman, 2016).

There are twenty types of hnRNPs named alphabetically from A1 to U (Geuens, Bouhy & Timmerman, 2016). Altered expression of hnRNPs has been associated with many types of cancer (Singh & Cooper, 2012). Misregulation of hnRNPs also causes aberrant splicing and has been implicated in the pathogenesis of several neurological disorders such as spinal muscular atrophy, amyotrophic lateral sclerosis and fragile X-associated tremor/ataxia syndrome (Feng & Xie, 2013).

X-linked syndromic mental retardation, Bain type (MRXSB, MIM # 300986) was first described among six unrelated females with intellectual disability, developmental delay and hypotonia with *de novo* missense variants in *HNRNPH2* (Bain et al., 2016). Here, we report two siblings, one male and one female, born to a consanguineous Indian couple, with features of MRXSB and a known pathogenic variant in *HNRNPH2*, identified by whole exome sequencing.

2. MATERIALS AND METHODS

2.1 Editorial policies and ethical considerations

The study was approved by the Institutional Ethics Committee. Written informed consents were obtained for the use of photographs and research findings.

2.2 Clinical report

Proband 1 (P1), the first-born boy of a third degree consanguineous couple was evaluated at 8-year-9 months, for global developmental delay, intellectual disability and seizures. He was born by normal vaginal delivery at term with a birth weight of 1.7 kg (-3 to -4 SD). He had delayed cry and was admitted to neonatal intensive unit. He

achieved head control at 10 months, rolling over at 12 months and sitting with support at 18 months. He reached out to objects at age 12 months and transferred objects at 18 months. He had generalized tonic-clonic seizures at 18 months of age following which he lost all his previously attained milestones. Myoclonic jerks were noted at 8 years of age. On examination, his weight was 19 kg (-3 SD), height was 115cm (-3 to -4 SD) and his head circumference was 48 cm (-2 SD). He had hypertelorism, medial flaring of eyebrows, bilateral ptosis, thick vermilion of upper and lower lips, short philtrum and high arched palate. He also had mild scoliosis, bilateral 5th finger clinodactyly. Skin hyperextensibility and significant joint hypermobility were present (Figure 1A-1D). He had abnormal flapping of hands and tremors, generalized hypotonia and preserved deep tendon reflexes. Karyotype, chromosomal microarray, tandem mass spectrometry, ophthalmic evaluation, electroencephalogram, magnetic resonance imaging (MRI) and computed tomography of brain revealed normal results.

Proband 2 (P2), the younger sibling of patient 1, a 5-year-9-months-old girl, also presented with global developmental delay, intellectual disability and neuroregression after 2 years of age. She was born at term with a birth weight of 2.75 kg (-1 to -2 SD). She achieved head control at 5 months, sat with support at 7 months and spoke monosyllables at 2 years. She did not have seizures. On examination her weight was 14.5 kg (-2 to -3 SD), height was 103 cm (-2 to -3 SD) and head circumference was 48 cm (-1 to -2 SD). No facial dysmorphism was noted in her (Figure 1E). Her fingers were long and slender. She had skin hyperextensibility and joint hypermobility, but of lesser severity when compared to her brother. She had generalized hypotonia and normal

deep tendon reflexes. She also had involuntary movements of her hands. Karyotype was normal. MRI of brain at three years of age, showed a hyperintense lesion in the inferior part of splenium of corpus callosum, suggestive of lipoma of corpus callosum. Parents were clinically normal.

Genomic analysis

Genomic DNA was isolated by standard phenol-chloroform method from EDTA blood samples. Whole exome sequencing (WES) was performed using Illumina's Nextera Rapid Capture Exome Kit and NextSeq500 Sequencer with NextSeq™500 High Output Kit (Illumina, Inc., San Diego, CA, USA) with a coverage of >95% of all amplicons at minimum of 20X depth for proband 1 and 2 as previously described (Shukla et al., 2017). Additionally, OMIM phenotypes, HPO terms and in-house allele frequency from exome data of 583 individuals of Indian origin were integrated into the WES data. Filtering strategy used to identify the candidate variant is provided in supplementary table 1. Validation and segregation analysis of the variant was done by Sanger sequencing.

3. RESULTS

WES of the siblings identified a known missense pathogenic variant, c.616C>T [p.(Arg206Trp), rs886039763] in *HNRNPH2* (NM_001032393.2). Patient 1 was hemizygous and patient 2 was heterozygous for the variant. Parents were found to have wild type allele (Figure 1F). This variant was not found in population databases such as gnomAD, 1000 Genome Project and our in-house WES data of 583 individuals of Indian origin. The variant is conserved across species with GERP score of 3.06. *In-silico*

prediction tools such as MutationTaster, SIFT and CADD Phred predicted the variant to be damaging to the protein function.

4. DISCUSSION

We identified a known missense pathogenic variant, c.616C>T [p.(Arg206Trp)] in *HNRNPH2* in hemizygous state in P1 and heterozygous state in P2. Bain et al., previously described this *de novo* missense heterozygous variant in four unrelated females with developmental delay and intellectual disability. Two other *de novo* missense heterozygous variants, c.617 G>A [p. (Arg206Gln)] and c.626C>T [p. (Pro209Leu)] in *HNRNPH2* were identified in two other girls with a similar phenotype (Bain et al., 2016).

All the females described by Bain et al., had developmental delay, intellectual disability and hypotonia. These features were consistently seen in the siblings we describe. Neuroregression was not a constant feature and was observed only in three of the six females described by Bain et al. The two subjects in our study had regression of milestones. P1 was more severely affected than P2. P1 had seizures, which were not seen in P2. Seizures were observed in three of the six females previously described. Two individuals described by Bain et al had acquired microcephaly. The head circumference of both probands in our study was within normal range. Speech was either absent or severely delayed in five of the six girls. The siblings we describe had absent speech. All the previously described individuals had variable facial dysmorphic features like hypertelorism, thick vermilion of lower lips, concave eyebrows and almond shaped eyes. Though P1 had facial dysmorphism, P2 did not show any dysmorphic features. Three reported individuals had features of autistic spectrum disorder and two of the

older individuals had behavioral issues like attention deficit hyperactivity disorder and obsessive-compulsive disorders. Though both P1 and P2 had repetitive hand flapping and involuntary movements, formal assessment for autism was not carried out. Skin hyperextensibility and joint hypermobility were prominent findings in both probands. These findings were observed only in one individual in the previously described cohort. A detailed comparison of clinical features is given in Table 1.

HNRNPH2 is located on the Xq22.1 and the HNRNPH2 protein has five domains including three homologous quasi-RNA recognition motif (RRMs) and two glycine-rich domains (GRDs). RRMs are involved in binding to G-rich RNA and GRDs are essential for protein-protein interactions and nuclear localization of the protein. GRDs contain highly conserved nuclear localization sequence between amino acid residues 194 to 220, which interacts with transportin 1, a nuclear transport receptor. HNRNPH2 protein is ubiquitously expressed and localizes predominantly in the nucleus as well as cytoplasm. It is mainly involved in alternating splicing either by enhancing or silencing splicing of pre-RNA and nucleo-cytoplasmic shuttling mRNA (Geuens, Bouhy & Timmerman, 2016; Grammatikakis et al., 2016; Van Dusen, Yee, McNally & McNally, 2010). The variant, p.(Arg206Trp) is located in the highly conserved nuclear localization sequence of GRD of HNRNPH2 protein.

Since no surviving male was identified previously, it was postulated that variants in *HNRNPH2* were incompatible with survival in males and only females survived, suggesting an X-linked dominant inheritance with male lethality. Recently, an earlier reported variant, c.617G>A (p.Arg206Gln), has been described in a 3-years-old male

with clinical findings similar to those of females with MRXSB (Harmsen, Buchert, Mayatepek, Haack & Distelmaier, 2019). Ascertainment of another male with this condition, though with more severe clinical manifestations indicates that pathogenic variants in *HNRNPH2* are compatible with postnatal survival. Germline mosaicism in mother has been previously described in X-linked dominant conditions like periventricular nodular heterotopias due to variants in *FLNA* gene (Lapointe, Spriggs & Mhanni, 2014). In the present study, both the parents had wild type alleles. The most likely explanation for recurrence in two siblings could be of maternal germline mosaicism. A *de novo* variant, c.616C>T in *HNRNPH1*, an autosomal paralogue of *HNRNPH2*, was reported in a boy with similar phenotypic features of MRXSB (Pilch et al., 2018).

5. CONCLUSIONS

The study expands the phenotype of MRXSB and reports a surviving male with a variant in *HNRNPH2*. The recurrence in the two siblings could be due to gonadal mosaicism of the variant in the mother. This aspect is important while offering counseling to families with apparent *de novo* variants in *HNRNPH2*. Also, additional report of subjects with this condition and functional validation of variants would aid in further elucidating the role of *HNRNPH2* as a cause of syndromic X-linked intellectual disability.

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CONFLICTS OF INTEREST

Authors declare no conflict of interest.

Data Availability Statement

The data providing the evidence of the study is available from the corresponding author upon reasonable request.

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TABLE

Table 1: Clinical features of proband 1 and proband 2 in comparison to the previously reported patients.

FIGURE LEGEND

Figure 1: Photographs of affected individuals. Patient 1 shows hypertelorism, medial flaring of eyebrows, ptosis, short philtrum, thick vermilion of upper and lower lips (A), skin hyperextensibility (B and C), severe joint hypermobility (D). Patient 2 with no facial dysmorphism (E). Pedigree of the family and electropherograms show c.616C>T in *HNRNPH2* in patient 1 and patient 2 in hemizygous and heterozygous state respectively. Parents had wild type allele (F).

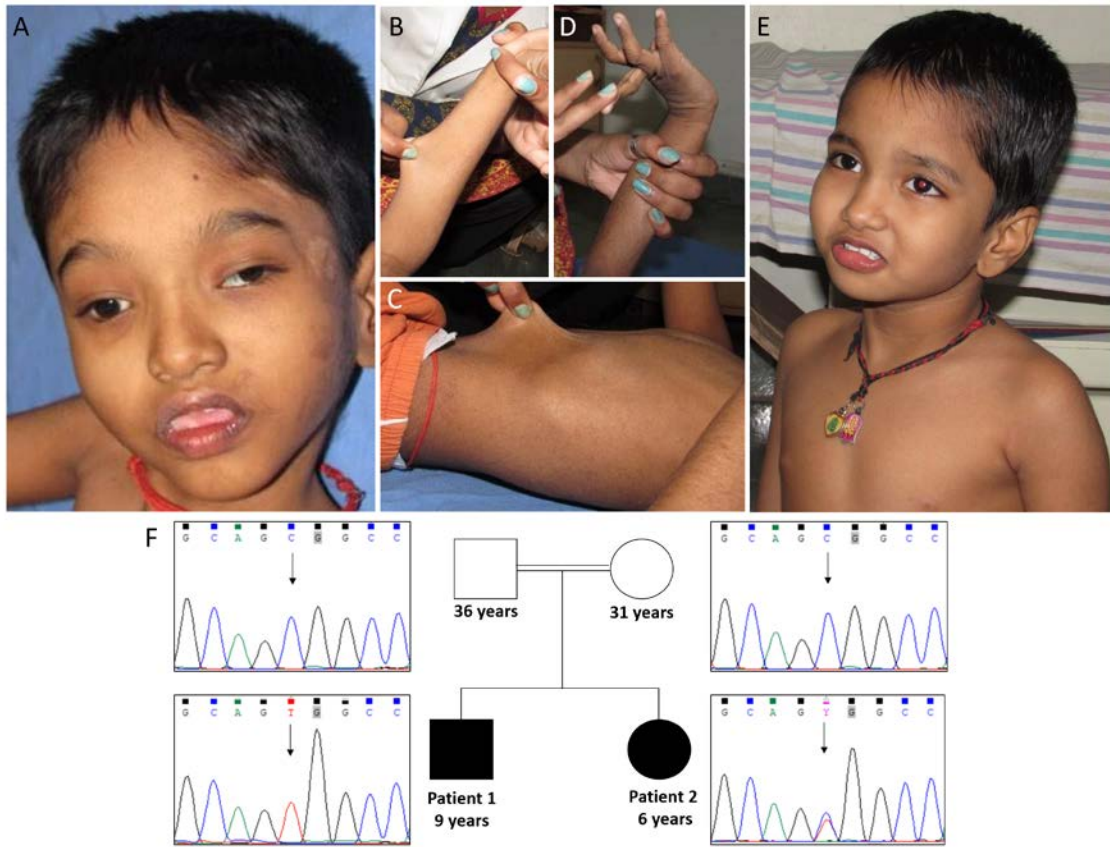
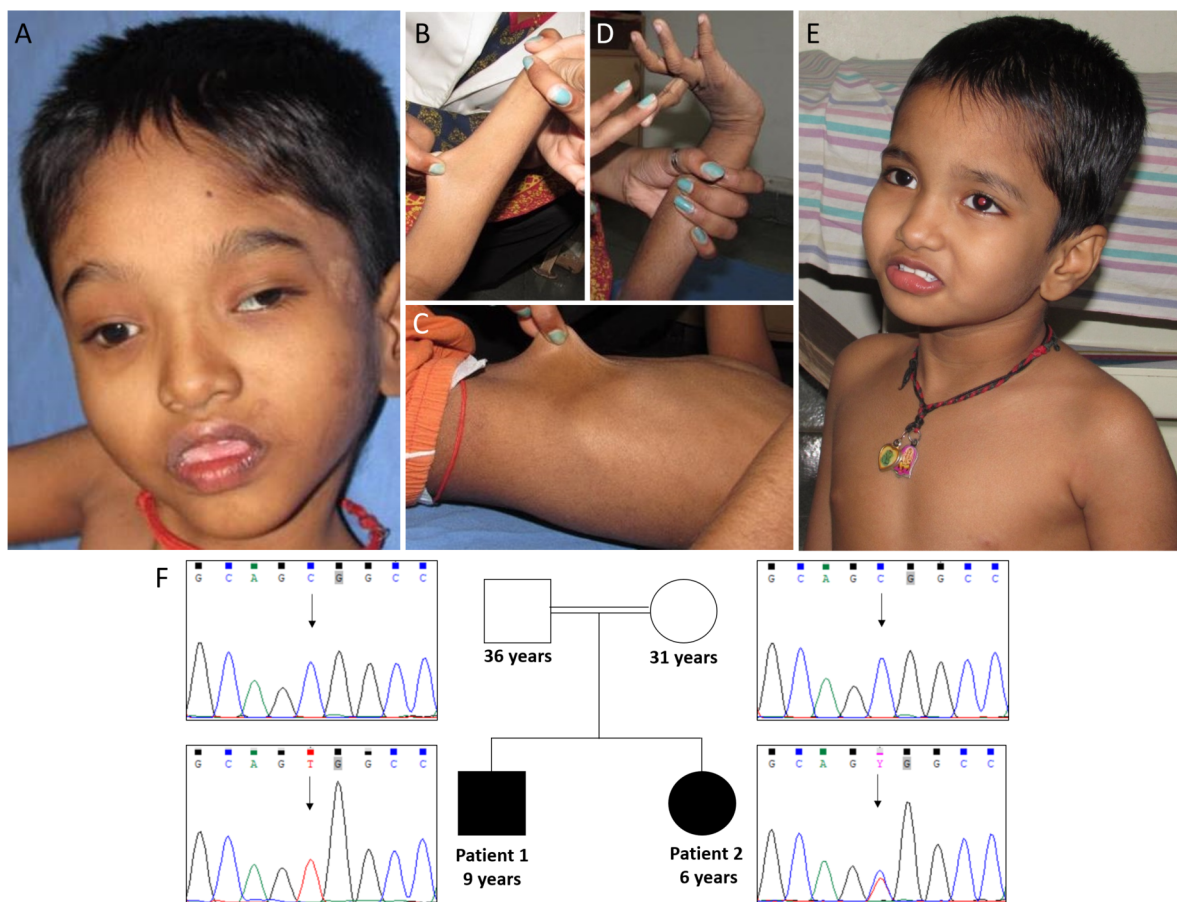


Table 1: Clinical features of proband 1 and proband 2 in comparison to the previously reported patients.

	Current study		JM Bain et al., 2016						Harmse n et al., 2019
	1	2	1	2	3	4	5	6	1
Proband	1	2	1	2	3	4	5	6	1
Gender	Male	Female	Female	Female	Female	Female	Female	Female	Male
Age (years)	9	6	34	8	4	6	21	2	3
Variant	c.616C>T	c.616C>T	c.616C>T	c.616C>T	c.617G>A	c.626C>T	c.616C>T	c.616C>T	c.617G>A
Birth weight	1.7 kg (-3 to -4 SD)	2.75 kg (-1 to -2 SD)	Not available	2.6 kg (-1 to -2 SD)	2.7 kg (-1 to -2 SD)	2.9 kg (-1 SD)	3.9 kg (+1 to +2 SD)	Not available	3.92 kg (+1 to +2 SD)
Weight	19 kg (-3 SD)	14.5 kg (-2 to -3 SD)	56 kg (0 to -1 SD)	20 kg at 6 years (normal)	Not available	11.8 kg (-4 to -5 SD)	42.5 kg at 19 years (-2 to -3 SD)	Not available	Not available
Length	115cm (-3 to -4 SD)	103 cm (-2 to -3 SD)	154 cm (-1 to -2 SD)	Not available	Not available	104 cm (-2 to -3 SD)	159.8 cm at 19 years (-1 SD)	Not available	Not available
Head circumference	48 cm (-2 SD)	48 cm (-1 to -2 SD)	48 cm at 7 years (-1 to -2 SD)	51 cm at 6.5 years (normal)	Not available	46.5 cm (-2 to -3 SD)	55 cm at 19 years (normal)	46 cm (-1 to 2 SD)	45.6 cm (-2 to 3 SD)
Developmental delay	+	+	+	+	+	+	+	+	+
Intellectual disability	+	+	+	+	+	+	+	+	+
Regression	+	+	+	-	+	+	-	-	+
Autism spectrum disorder	-	-	+	+	-	-	+	-	-
Seizures	+	-	+	+	-	+	-	-	-

Speech	Absent	Absent	Single words and short sentences achieved at 7 years	Absent	Not available	Few words	Speaking in sentences achieved at 8 years	Absent (babbling)	Absent
Tone	Hypotonia	Hypotonia	Truncal hypotonia and limb hypertonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Axial hypotonia
Skin hyperextensibility	+	+	-	-	-	-	+	-	-
Joint hypermobility	+	+	-	-	-	-	+	-	-
Facial features	Hypertelorism, medial flared eyebrow, short philtrum, thick vermilion of upper and lower lips, high arched palate, ptosis	Hypertelorism, short philtrum	Hypotelorism, high narrow nasal bridge, wide mouth, full lips	Bilateral epicanthal folds, midface hypoplasia, almond shaped eyes	Short palpebral fissures	Hypertelorism	Mild micrognathia, high arched palate	Concave eyebrows, fissure in the upper lip	Mild facial dysmorphism with almond-shaped eyes
Skeletal abnormalities	Scoliosis mild, bilateral 5 th digit clinodactyly,	Long slender fingers	Lordosis, bilateral femoral osteotomies,	-	Pes planus	Talus valgus	Thoracolumbar scoliosis, pectus carinatum, pes	-	Scoliosis

	proximally placed thumb		arachnodactyly				planus, joint hypermobility, elongated fingers		
Brain MRI	Normal	Lipoma in Corpus callosum region	Normal	Cerebellar vermis hypoplasia	Not available	Normal	Normal	Distorted cerebellar vermis	Normal
Other findings	Stereotypic hand flapping, tremors while trying to hold objects	Involuntary movements	Abnormal gait, ankle clonus, attention deficit hyperactivity disorder, obsessive-compulsive disorder	Ataxia, mild hearing loss, anemia, epistaxis, constipation	Gastroesophageal reflux disease, hand flapping	Incoordination, Failure to thrive, walks with support, feeding difficulty, anxiety, sensory issues, atrial septal defect and mitral valve prolapse, fetal finger pads Incoordination	Attention deficit hyperactivity disorder, mitral valve prolapse, mitral regurgitation, gastroesophageal reflux disease	Dystonia of left hand, Dyspraxia, feeding difficulty, drooling	Lack of independent movement, spasticity of the extremities, feeding difficulties



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Variant	c.616 C>T	c.616C>T	c.616C>T	c.616C>T	c.617G>A	c.626C>T	c.616C>T	c.616C>T	c. 617G>A
Birth weight	1.7 kg (-3 to -4 SD)	2.75 kg (-1 to -2 SD)	Not available	2.6 kg (-1 to -2 SD)	2.7 kg (-1 to -2 SD)	2.9 kg (-1 SD)	3.9 kg (+1 to +2 SD)	Not available	3.92 kg (+1 to +2 SD)
Weight	19 kg (-3 SD)	14.5 kg (-2 to -3 SD)	56 kg (0 to -1 SD)	20 kg at 6 years (normal)	Not available	11.8 kg (-4 to -5 SD)	42.5 kg at 19 years (-2 to -3 SD)	Not available	Not available
Length	115cm (-3 to -4 SD)	103 cm (-2 to -3 SD)	154 cm (-1 to -2 SD)	Not available	Not available	104 cm (-2 to -3 SD)	159.8 cm at 19 years (-1 SD)	Not available	Not available
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Developmental delay	+	+	+	+	+	+	+	+	+
Intellectual disability	+	+	+	+	+	+	+	+	+
Regression	+	+	+	-	+	+	-	-	+
Autism spectrum disorder	-	-	+	+	-	-	+	-	-
Seizures	+	-	+	+	-	+	-	-	-
Speech	Absent	Absent	Single words and short	Absent	Not available	Few words	Speaking in sentences	Absent (babbling)	Absent

			sentences achieved at 7 years				achieved at 8 years		
Tone	Hypotonia	Hypotonia	Truncal hypotonia and limbs hypertonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Axial hypotonia
Skin hyperextensibility	+	+	-	-	-	-	+	-	-
Joint hypermobility	+	+	-	-	-	-	+	-	-
Facial features	Hypertelorism, medial flared eyebrow, short philtrum, thick vermilion of upper and lower lips, high arched palate, ptosis	Hypertelorism, short philtrum	Hypotelorism, high narrow nasal bridge, wide mouth, full lips	Bilateral epicanthal folds, midface hypoplasia, almond shaped eyes	Short palpebral fissures	Hypertelorism	Mild micrognathia, high arched palate	Concave eyebrows, fissure in the upper lip	Mild facial dysmorphism with almond-shaped eyes
Skeletal abnormalities	Scoliosis mild, bilateral 5 th digit clinodactyly, proximally placed thumb	Long slender fingers	Lordosis, bilateral femoral osteotomies, arachnodactyly	-	Pes planus	Talus valgus	Thoracolumbar scoliosis, pectus carinatum, pes planus, joint hypermobility, elongated fingers	-	Scoliosis
Brain MRI	Normal	Lipoma in Corpus callosum region	Normal	Cerebellar vermis hypoplasia	Not available	Normal	Normal	Distorted cerebellar vermis	Normal

Other findings	Stereotypic hand flapping, tremors while trying to hold objects	Involuntary movements	Abnormal gait, ankle clonus, attention deficit hyperactivity disorder, obsessive-compulsive disorder	Ataxia, mild hearing loss, anemia, epistaxis, constipation	Gastroesophageal reflux disease, hand flapping	Incoordination, Failure to thrive, walks with support, feeding difficulty, anxiety, sensory issues, atrial septal defect and mitral valve prolapse, fetal finger pads Incoordination	Attention deficit hyperactivity disorder, mitral valve prolapse, mitral regurgitation, gastroesophageal reflux disease	Dystonia of left hand, Dyspraxia, feeding difficulty, drooling	Lack of independent movement, spasticity of the extremities, feeding difficulties
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