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SHORT REPORT

Evidence for *HNRNPH1* being another gene for Bain type syndromic mental retardation

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The *HNRNPH2*-associated disease (mental retardation, X-linked, syndromic, Bain type [MRXSB, MIM #300986]) is caused by *de novo* mutations in the X-linked *HNRNPH2* gene. MRXSB has been described in six female patients with dysmorphy, developmental delay, intellectual disability, autism, hypotonia and seizures. The reported *HNRNPH2* mutations were clustered in the small domain encoding nuclear localization signal; in particular, the p.Arg206Trp was found in four independent *de novo* events. *HNRNPH1* is a conserved autosomal paralogue of *HNRNPH2* with a similar function in regulation of pre-mRNAs splicing but so far it has not been associated with human disease. We describe a boy with a disease similar to MRXSB in whom a novel *de novo* mutation c.616C>T (p.Arg206Trp) in *HNRNPH1* was found (ie, the exact paralogue of the recurrent *HNRNPH2* mutation). We propose that defective function of HNRNPH2 and HNRNPH1 nuclear localization signal has similar clinical consequences. An important difference between the two diseases is that the *HNRNPH1*-associated syndrome may occur in boys (as in the case of our proband) which is well explained by the autosomal (chr5q35.3) rather than X-linked localization of the *HNRNPH2* gene.

KEYWORDS

de novo mutation, *HNRNPH1*, *HNRNPH2*, nuclear localization signal, pre-mRNAs splicing, syndromic mental retardation, whole exome sequencing

1 | INTRODUCTION

Recently, pathogenic *de novo* mutations in the X-linked *HNRNPH2* gene were reported in patients with a neurodevelopmental disorder with features including dysmorphy, developmental delay, intellectual disability, autism, hypotonia and seizures. All six reported patients were females suggesting that the *HNRNPH2*-associated disease is lethal in hemizygous males.[1] The *HNRNPH2*-related disease has been registered in OMIM (http://omim.org) as mental retardation, X-linked, syndromic, Bain type (MRXSB, MIM #300986). The reported *HNRNPH2* mutations were clustered in the small glycine-rich domain encoding nuclear localization signal. In particular, the p.Arg206Trp was found in four independent *de novo* events among these patients.[1] Here, we report a boy with a disease similar to MRXSB in whom we found a novel *de novo* mutation c.616C>T (p.Arg206Trp) in the *HNRNPH1* gene which is a highly conserved autosomal paralogue of *HNRNPH2*.

2 | CASE REPORT

Legal parental consent was obtained for the study.

A 13-year-old boy was born at term by vaginal delivery with Apgar score 5/9/10 to nonconsanguineous parents, from first pregnancy complicated by gestational diabetes, with body weight: 2650 g (3pc), length: 52 cm (50-75pc). OFC was 31 cm (<3pc). After the birth blepharophimosis, high-arched palate, retrognathia, penile hypospadias and talipes valgus have been noted. Ultrasound of the head, abdomen and echocardiography were normal. Since birth muscle tone

was low, physiotherapy was started. In infancy a significant failure to thrive and gastroesophageal reflux were noted. Despite the physiotherapy, there was no relevant progress in development. He is unable to sit, even unsupported and never reached standing position. At the age of 13 years his weight was 16 kg (≪3pc), length: 140 cm (<3pc) and OFC: 48 cm (<3pc). Many dysmorphic features were noted (Figure 1A-C): microcephaly, long face, high forehead, arched eyebrows, blepharophimosis, down slanting palpebral fissures, divergent squint, high nasal root, thin nasal ridge, long hanging columella, small ale nasi, malar hypoplasia, short and smooth philtrum, open bite with dental crowding, high-arched palate, retrognathia, low-set dysplastic ears with small ear lobe, severe rotational scoliosis, chest deformity, hypermobile joints, hip and elbow dislocations, knee contractures, pes planus, arachnodactyly, clinodactyly of V fingers, clubbed fingers and toes. Boy is severely intellectually disabled. He does not speak, but his hearing is intact. Due to a significant intellectual disability and lack of cooperation, formal IQ testing could not be performed.

Magnetic resonance imaging (MRI) of the head showed partial defect of anterior falx with asymmetry of frontal lobes, moderately wide supratentorial ventricular system with features of colpocephaly, atypically shaped body, isthmus and splenium of corpus callosum, hypoplasia of cerebellar vermis (Figure S1A-C). Additionally, MRI revealed craniofacial abnormality – developmental defect of the skull base in the form of oblique anterior cranial fossa with vertically aligned clivus of sphenoid bone, plagiocephaly of right occipital bone, narrowing of foramen magnum and first cervical vertebra anomaly with narrowing of perimedullary fluid space. Formation of the brain structures was in accordance with the shape of the skull.

On X-ray of the pelvis developmental abnormalities such as right hip dislocation, coxa valga and narrowing of iliac bones were present (Figure S2A). On the elbow X-rays there were multiple abnormalities including wide humeral, radial and ulnar metaphyses, delayed development of the dysplastic ossification centers with improper formation of the articular surfaces and subluxation of the radial bones. Also, narrowing of the long bone shafts with osteopenia was present (Figure S2B-D).

Dysmorphic features such as long face, high forehead, blepharophimosis, high nasal root, thin nasal ridge, malar hypoplasia, high-arched palate, low-set dysplastic ears, hypospadias, arachnodactyly and clinodactyly prompted the initial clinical suspicion of Schilbach-Rott/Blepharofacioskeletal syndrome (OMIM #164220).[2–6]

3 | GENETIC STUDIES

Karyotype analysis and aCGH (Agilent SurePrint G3 CGH ISCA v2, 8x60K) were normal. Whole exome sequencing (WES) was performed in proband's DNA extracted from peripheral blood (trio analysis was not performed). Enrichment was done using SeqCap EZ MedExome (Roche) according to the manufacturer's instructions. MedExome library was paired-end sequenced (2 × 100 bp) on Illumina HiSeq1500 platform. Bioinformatics analysis of WES data was performed using previously reported pipeline (Hg19 genomic build was used for alignments).[7] We prioritized four heterozygous variants in *WHSC1*, *GCK*, *SULF2* and *HNRNPH1*. All these variants were potentially pathogenic according to bioinformatics analyses and were absent from available databases including GnomAD (http://gnomad.broadinstitute.org) and an in house database of >1000 Polish exomes. The presence of *HNRNPH1* chr5:179045245-G>A, c.616C>T (p.Arg206Trp) mutation in proband was confirmed by amplicon sequencing on HiSeq 1500 (Illumina) as well as by Sanger sequencing; family study showed that it was a *de novo* event (Figure 2). The p.Arg206Trp mutation was predicted as damaging by *in-silico* programs (https://varsome.com/): DANN (score: 0.9933), FATHMM-MKL (result: damaging, coding score: 0.9102), MutationTaster (result: disease causing, converted rank score: 0.8103), SIFT (result: damaging, converted rank score: 0.4813) and PROVEAN (result: damaging, converted rank score: 0.8981). The variants in *WSHC1* and *SULF2* were inherited from the father and *GCK* was inherited form the mother thus excluding their pathogenicity.

4 | DISCUSSION

The HNRNPH1 and HNRNPH2 proteins have similar function in regulation of pre-mRNAs splicing; in particular, they are both involved in

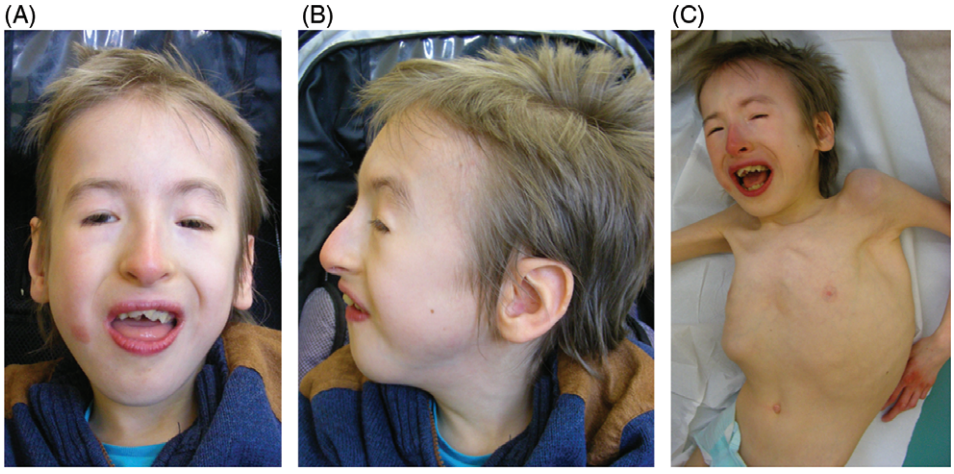

FIGURE 1 (A-C) Patient photograph (13 years): long face, high forehead, blepharophimosis, high nasal root and thin nasal ridge, low hanging columella, short and smooth philtrum, open bite with dental crowding, retrognathia (not significant), low-set ears [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 2 Amino acid sequence (A) and exon/intron organization (B) of the *HNRNPH1* and *HNRNPH2* genes. (C) IGV view of verification of the HNRNPH1 c.616C>T (p. Arg206Trp) variant in proband and his parents obtained by sequencing (NGS) of the amplicon indicated in (B). (D) Variant Reporter 1.1 view of verification of the HNRNPH1 c.616C>T (p.Arg206Trp) variant in proband and his parents using Sanger sequencing [Colour figure can be viewed at wileyonlinelibrary.com]

neuronal cell differentiation by regulating alternative splicing.[8] A study in rat cortical neurons showed that although HNRNPH2 had a slightly lower effect than HNRNPH1, both paralogs acted similarly in controlling splicing of *Trf2* through promoting the longer splice variant which inhibited neurogenesis.[9] The HNRNPH1 and HNRNPH2 proteins are highly conserved at the protein level with only 15 of 449 amino acid differing (Figure 2A).

Van Dusen et al. performed a detailed functional study on the role of various HNRNPH2 domains/amino acids for proper nucleocytoplasmic localization of the protein.[10] In particular, these authors expressed the aa 205-213 HNRNPH2 fragment containing the glycine-tyrosine-arginine-rich domain (GYR domain) and showed that mutating Arg206 into Ala disrupted its nuclear localization. Because amino acid sequence of this fragment is identical in HNRNPH2 and HNRNPH1, we propose that the p.Arg206Trp mutation found by us most likely leads to improper nuclear localization of the HNRNPH1 protein as well.[10] Importantly, the *HNRNPH1* p.Arg206Trp mutation found by us (Figure 2) is the exact paralogue of the p.Arg206Trp *HNRNPH2* mutation found in three patients with MRXSB.[1] However, in another MRXSB patient, the *HNRNPH2* in the Arg206 position was mutated into Gln.[1]

The phenotype of our proband is similar to the phenotype of patients with the *HNRNPH2* mutations, however, the clinical picture is not homogeneous (Table 1). In all patients, low muscle tone and developmental delay were reported. Additionally, various gastrointestinal signs were found in childhood: gastroesophageal reflux, drooling, feeding issues or constipation. In two patients with different mutations in *HNRNPH2* gene (patients 1, 4) microcephaly was acquired, whereas in our proband small head circumference was congenital being present since the neonatal period. In all the three patients short stature has been noted. Most patients including ours, had different skeletal anomalies, mainly deformations of the chest, spine or limbs. Hip and elbow dislocations were not reported earlier, however, joint laxity was seen in one of them. An abnormal behavior or seizures were not constant features, but in 3 out of 7 patients, autism spectrum disorder, anxiety or seizures were reported.

While all reported MRXSB patients had somewhat different dysmorphic features, the highest similarity was observed among those with the *HNRNPH2* p.Arg206Trp mutation (patients 1, 5).[1] Interestingly, these patients were also most similar to our proband having short palpebral fissures, short philtrum, long columella, hypoplastic alae nasi. Other features such as highly arched palate, scoliosis,

TABLE 1 Clinical characteristics of patients reported by Bain et al and our patient

Clinical features	1	2	3	4	5	6	Our patient
Age (y)/sex	34/F	8/F	4/F	6/F	21/F	2/F	13/M
Gene/mutation	*HNRNPH2* c.616C>T (p.Arg206Trp)	*HNRNPH2* c.616C>T (p.Arg206Trp)	*HNRNPH2* c.617G>A (p.Arg206Gln)	*HNRNPH2* c.626C>T (p.Pro209Leu)	*HNRNPH2* c.616C>T (p.Arg206Trp)	*HNRNPH2* c.616C>T (p.Arg206Trp)	*HNRNPH1* c.616C>T (p.Arg206Trp)
DD/ID	Y	Y	Y	Y	Y	Y	Y (severe)
Developmental regression	Y	N	Y	Y	N	N	N
ASD	Y	Y	N	N	Y	N	N
ADHD	Y	N	N	N	Y	N	N
Other psychiatric comorbidities	Anxiety, OCD, aggressive behavior, self-injurious	N	N	Anxiety	N	N	Anxiety
Seizures	Y	Y	N	Y	N	N	N
Muscle tone	Decrease, increase	Decrease	Decrease	Decrease	Decrease	Decrease	Decrease
Other neurological findings	Abnormal gait, ankle clonus	Ataxia, muscle weakness	N	Incoordination	N	Torticollis, dystonic posturing left hand, dyspraxia	Muscle weakness, incoordination, dyspraxia
MRI findings	N	Aplasia/hypoplasia cerebellar vermis	NA	N	N	Possible distorted cerebellar vermis	Skull base/craniofacial abnormality, falx hypoplasia, atypical shape of corpus callosum, enlargement of supratentorial ventricles with colpocephaly, vermis hypoplasia
Ophthalmologic findings	Exotropia	?	N	N	N	N	Exotropia
Growth parameters	FTT, short stature, microcephaly (a)	N	N	FTT, short stature, microcephaly (a)	N	N	FTT, short stature, microcephaly (congenital)
Dysmorphic features	Hypotelorism, short palpebral fissures, high narrow nasal bridge, short philtrum, long columella, hypoplastic alae nasi, wide mouth, full lips, curly hair	Epicanthal folds, midface hypoplasia, almond-shaped eyes, short palpebral fissures	Short palpebral fissures	Hypertelorism, fetal finger pads	Short palpebral fissures, short philtrum, long columella, hypolastic alae nasi, highly arched palate, mild micrognathia, elongated fingers	Symmetrically concave eyebrow, pseudo-fissure in upper lip	Arched eyebrows, blepharophimosis, high narrow nasal bridge, short philtrum, long columella, hypoplastic alae nasi, highly arched palate, retrognathia
Skeletal anomalies	Lordosis, bilateral femoral osteotomies, arachnodactyly	N	Pes planus	Talus valgus	Pectus carinatum, pes planus, scoliosis, stretchable skin, joint laxity	N	Scoliosis, chest deformity, joint laxity, hip and elbow dislocations, clinodactyly, pes planus
GI symptoms	N	Constipation	GERD as an infant	Feeding issues	GERD as a child, underweight	Feeding difficulties, drooling	GERD as a child, drooling
Other findings	N	Mild hearing loss, anemia, epistaxis	Happy demeanor, sensitive to noise, hand flapping	Cardiac (atrial septal defect and MVP), sensory disorder	Cardiac (MVP)	N	Hypospadias, bone remodeling

Abbreviations: a, acquired; ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; DD/ID, developmental delay/intellectual disability; F, female; FTT, failure to thrive; GERD, gastroesophageal reflux disease; GI, gastrointestinal; M, male; MVP, mitral valve prolapse; NA, not applicable; OCD, obsessive-compulsive disorder; ?, unknown.

arachnodactyly, joint laxity were reported in single MRXSB patients. In patients 2 and 6 similar as in our proband cerebellar vermis anomaly was found in MRI. However, in our patient MRI revealed additionally corpus callosum, falx cerebri and skull base abnormalities. We noted that among six previously reported patients, patient 1 was most similar to our proband. However, for proper delineation of clinically significant features it will be necessary to assess larger number of patients with *HNRNPH1/2* mutations.

We propose that the *de novo HNRNPH1* mutation causes the disease in our proband and that defective function of *HNRNPH1* nuclear localization signal may have similar consequences as mutations affecting the paralogous domain of *HNRNPH2*. An important difference between the two diseases is that the *HNRNPH1*-associated syndrome may occur in boys (as in the case of our proband) which is well explained by autosomal location as opposed to the *HNRNPH2* gene on the X chromosome.

No anomalies in the base of the skull, nor dislocations or narrow iliac wings were reported in any of the analyzed patients with the mutations in *HNRNPH2* gene. However, some brain and skeletal developmental anomalies and various dysmorphic features identified in our patient are also reported in patients with autosomal recessive microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1, OMIM #210710), caused by homozygous or compound heterozygous mutations in the *RNU4ATAC* gene (http://omim.org). MOPD1 belongs to the group of diseases affecting pre-mRNAs splicing, too. Thus, presence of similar clinical symptoms may suggest a shared pathomechanism of both disorders.

Clinical signs present in our patient and patients with the *HNRNPH2* disease are reminiscent of Schilbach-Rott/Blepharofacioskeletal syndrome, although lack of hypotelorism and cleft palate would represent a difference.[5] Because the causative gene of Schilbach-Rott/Blepharofacioskeletal syndrome is not known, it should be interesting to search for mutations in *HNRNPH1/2* or other genes affecting pre-mRNAs splicing also in this disease.

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Ethics approval

Ethics approval was granted by the Institutional Review Board of Warsaw Medical University.

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 section at the end of the article.

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