Title: *HNRNPH1*-related syndromic intellectual disability: Seven additional cases suggestive of a distinct syndromic neurodevelopmental syndrome.

Short running title: *HNRNPH1*-related intellectual disability

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cge.13765

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Acknowledgments:

We gratefully acknowledge all the individuals and their families.

The work was funded by "Progetti di innovazione in ambito sanitario e socio sanitario Regione Lombardia, bando ex decreto n. 2713 del 28/02/2018" as part of the" GENE - Genomic analysis Evaluation NEtwork " project (MI)

This work was supported by the Centre for Biomedical Research on Rare Diseases (CIBERER) [ACCI14-759], the URDCat program (PERIS SLT002/16/00174), the Hesperia Foundation and the Secretariat for Universities and Research of the Ministry of Business and Knowledge of the Government of Catalonia [2017SGR1206] to AP

S.E. is a Wellcome Senior Investigator.

Conflict of Interest:

There are no conflicts of interest to disclose among the authors included in the preparation and publication of this manuscript.

Data Availability Statement:

There are no other data associated with this manuscript.

Abstract

Pathogenic variants in *HNRNPH1* were first reported in 2018. The reported individual, a 13 year old boy with a c.616C>T (p.R206W) variant in the *HNRNPH1* gene, was noted to have overlapping symptoms with those observed in *HNRNPH2*-related X-linked intellectual disability, Bain type (MRXSB), specifically intellectual disability and dysmorphic features. While *HNRNPH1* variants were initially proposed to represent an autosomal cause of MRXSB, we report an additional seven cases which identify phenotypic differences from MRXSB. Patients with *HNRNPH1* pathogenic variants diagnosed via WES were identified using clinical networks and GeneMatcher. Features unique to individuals with *HNRNPH1* variants include distinctive dysmorphic facial features; an increased incidence of congenital anomalies including cranial and brain abnormalities, genitourinary malformations, and palate abnormalities; increased incidence of ophthalmologic abnormalities; and a decreased incidence of epilepsy and cardiac defects compared to those with MRXSB. This suggests that pathogenic variants in *HNRNPH1* result in a related, but distinct syndromic cause of intellectual disability from MRXSB, which we refer to as *HNRNPH1*-related intellectual disability.

Key Words: Whole exome Sequencing, intellectual disability, microcephaly, congenital abnormalities, *HNRNPH1* gene

Introduction

The *HNRNPH1* [MIM 601035] and *HNRNPH2* [MIM 300986] genes produce the hnRNP H and hnRNP H' (also called hnRNP H2) proteins, respectively. These proteins belong to the heterogeneous nuclear ribonucleoprotein (hnRNP) family of RNA binding proteins which bind to pre-mRNA transcripts and assist in stabilizing, transporting, and targeting transcripts between the nucleus and cytoplasm for processing and alternative splicing prior to becoming mature mRNAs.[1] More than 20 hnRNPs have been identified, and hnRNP H and hnRNP H' share 96% homology. Both genes are ubiquitously expressed across multiple tissue types including the brain, eye, smooth muscle, small intestine, and stomach.[2] The functions of hnRNP H and H' on pre-mRNA processing include capping, splicing, polyadenylation, export, and translation and are mainly exerted within the nucleus.[3]

Given their central role in cellular function, it is not surprising that pathogenic variants affecting genes that code for hnRNPs are an emerging cause of disease. Altered expression of hnRNPs has been linked to tumorigenesis and germline variants in genes encoding hnRNPs have been implicated as a potential causes of adult-onset neurodegenerative conditions including frontotemporal dementia/amyotrophic lateral sclerosis, inclusion body myopathy with frontotemporal dementia (IBMPFM [MIM: 615424]), and Alzheimer disease.[1,4] Whole exome sequencing (WES) has identified multiple *HNRNP* genes as novel causes of early-onset syndromic intellectual disability.[5-7]

In 2016, pathogenic variants in *HNRNPH2* were reported as a novel cause for an X-linked intellectual disability syndrome in females.[6] This new syndrome, *HNRNPH2*-related X-linked intellectual disability, Bain type, (MRXSB [MIM: 300986]), was characterized by intellectual disability, dysmorphic features, feeding difficulties, seizures, and hypotonia. While

originally thought that pathogenic variants would be lethal in males, three groups have reported four males with pathogenic variants in *HNRNPH2* and symptoms consistent with MRXSB.[8-10] Among the eleven cases of MRXSB reported to date, seven have a common c.616C>T (p.R206W) variant, while another two have c.617G>A (p.R206Q) variants. The remaining two male cases carry c.626C>T (p.P209L) and c.340C>T (p.R114W) variants.

The first pathogenic variant in *HNRNPH1* was reported by Pilch et al. in 2018.[11] In the reported individual, a 13-year-old boy, a de novo c.616C>T (p.R206W) variant in *HNRNPH1* was identified by WES. His phenotype included dysmorphic facial features, microcephaly, hypermobile joints, hypotonia, non-verbal intellectual disability, and feeding difficulties.[11] Given the similar phenotypes, the conserved amino acid sequences at position 206 of hnRNP H and H', and the overall homology between these proteins, it was postulated that variants in *HNRNPH1* may represent an autosomal cause of MRXSB. However, some features reported by Pilch et al. were not observed in the MRXSB cohort, including arched eyebrows, blepharophimosis, congenital microcephaly, and hip dislocations.

Here, we present an additional seven cases of de novo pathogenic variants in *HNRNPH1* to further characterize and expand the phenotype initially described by Pilch et al.

Materials and methods:

Seven individuals with *HNRNPH1* pathogenic variants were identified using GeneMatcher and clinical networks.[12] One individual resides in the United States, the remaining in the European Union. DNA extracted form peripheral blood was analyzed by WES on all individuals using standard technologies. Details on case specific WES can be found in Supporting Materials. This series was reviewed by the VCU Health IRB and not found to meet the definitions of human subjects research, and thus did not require IRB review or approval. Written informed consent was obtained for individuals whose photographs are included within this report. We characterized their phenotype retrospectively and contrasted them with features reported previously by Pilch et al. as well as with individuals with MRXSB.

Results:

The seven individuals range in age from a fetus at 30w4d to a 23-year-old. The five surviving cases have intellectual disability, ranging from moderate to severe. Congenital anomalies were observed in this cohort, with 7/7 having abnormalities identified on brain MRI, 4/7 having palate abnormalities, and 3/7 having genitourinary malformations. Other notable features observed include ophthalmological abnormalities (6/7), short stature (5/7), microcephaly (5/7), and hypotonia (4/7). Developmental regression is not observed in this cohort. 4/6 surviving individuals are non-verbal or have very limited speech. A complete phenotypic review can be found in Table 1. Dysmorphic features were also observed within our cohort, including medial arched eyebrows (4/7), blepharophimosis (3/7), ptosis (3/7), and hypotelorism (3/7). (Figure 1, Table S1). These features are similar to what was reported by Pilch et al.[11] Additional clinical history can be found in Supporting Materials.

Overlapping features with MRXSB include intellectual disability, microcephaly, and gastrointestinal abnormalities. However, our cohort has other anomalies not consistently

reported among individuals with MRXSB. These include cerebellar anomalies, palate abnormalities, genitourinary abnormalities, micrognathia, and short stature. 3/7 individuals in this cohort were noted to have dystonia, versus 1/11 individuals with MRXSB. Individuals in this cohort were also more frequently observed to have ophthalmological abnormalities, most often strabismus. Epilepsy, reported in 5/11 individuals with MRXSB, was only observed in 2/7 individuals within our cohort. The constellation of dysmorphic facial features observed in this cohort also suggests a distinct phenotype when compared with MRXSB. Congenital microcephaly, blepharophimosis, ptosis, hypotelorism, posteriorly rotated hypoplastic ears, medial arched eyebrows, open bite, and syndactyly/clinodactyly are features observed in individuals with *HNRNPH1* pathogenic variants but not widely reported in MRXSB.

Six variants were identified in this cohort including two missense variants, two frameshift variants, an in-frame deletion, and an entire gene duplication (Figure 2). One variant, c.616C>T (p.R206W) identified in Cases 1 and 7, was previously described by Pilch et al.[11] Another, c.617G>A (p.R206Q) was identified in Case 4 and is analogous to the *HNRNPH2* variant described by Bain et al.[6] The remaining four variants have not been described in either *HNRNPH1* or *HNRNPH2* to date. These include two small duplications, c.618dupG (p.Pro207fs) and c.1240_1243dup (p.Q415fs) and one in-frame deletion, c.1116_1175del (p.Glu373-Tyr392del). The final variant is a large duplication encompassing both the *HNRNPH1* and *RUFY1* [MIM 610327] genes.

Discussion:

The seven individuals reported in this series suggest a unique, but variable, phenotype due to pathogenic *HNRNPH1* variants. We hypothesize that much of the clinical variability observed among our cohort may be due to genotype/phenotype correlations. The hnRNP H and

H' proteins have three highly homologous RNA recognition motifs that allow them to specifically bind to G-rich RNA.[1] In addition, they also have two glycine-rich domains, designated GYR and GY. A non-classical nuclear localization sequence (NLS) has been identified within the GYR region between amino acids 194 and 220 with amino acids 205-213 being highly conserved and required for nuclear transport.[3] In vitro studies introducing point mutations into this NLS resulted in failure of these hnRNPs to shuttle from the cytoplasm to the nucleus, which is expected to compromise function.[3]

Individuals with missense variants affecting amino acids 206-208 appear to have a more severe clinical phenotype. This is likely because these variants are predicted to disrupt the NLS, thus impacting the overall function on the hnRNP H protein.[6,11] Individuals observed with the recurrent c.616C>T and c.617G>A variants (Cases 1, 7, and 4) presented immediately following birth or prenatally with issues including microcephaly, respiratory distress, hypotonia, and congenital anomalies. This early-onset severe presentation further highlights the importance of the NLS sequence in the hnRNP H protein and suggests that disruption of this sequence has severe clinical consequences.

The two frameshift variants reported in this cohort, c.618dupG (p.207fs) and c.1240_1243dup (c.Q415fs), are expected to result in either an abnormal, truncated protein or loss of protein from nonsense mediated mRNA decay. c.618dupG falls within the NLS and c.1240_1243dup in the GY domain. The discordant phenotypes of these two individuals with frameshift mutations may reflect the severity of the frameshift on the overall protein structure. As c.618dupG (p.P207fs) lies within the NLS, it is predicted to produce a protein with a malfunctioning NLS. This supports the above suggestion that disruption of the NLS results in severe clinical consequences, as Case 2, found to have c.618dupG (p.P207fs), presented with

hypotonia, respiratory distress, and congenital anomalies, similar to those with missense variants in the NLS. In contrast, Case 6, found to have c.1240_1243dup (p.Q415fs), has a less severe phenotype compared to cases 1, 2, 4, and 7. Case 6 did not come to medical attention in the neonatal period, is not noted to have microcephaly, and does not have palate abnormalities, short stature, or clinodactyly/syndactyly. Case 6 also has milder intellectual disability compared to other cases. Because c.1240_1243dup lies within the GY domain, it is predicted to impact the protein after the NLS, possibly resulting in an abnormal protein that may have a functional NLS region, which may ameliorate the severity of the observed phenotype.

Of the remaining *HNRNPH1* variants, one falls in the GY domain, c.1116_1175del (p.Glu373-Tyr392del) (Case 5), and is expected to remove 19 amino acids. The function of the GY domain is not as well understood as the GYR domain, however it is thought to be important for protein-protein interactions and splicing.[3] Thus, these pathogenic variants may have functional implications on protein/protein or protein/RNA interactions however it is difficult to say without additional functional studies. Case 5 has a less severe phenotype compared to those with pathogenic variants within the NLS, similar to Case 6 with a normal neonatal period, milder intellectual disability, and no microcephaly.

The final variant reported is an entire gene duplication of *HNRNPH1* and *RUFY1* (Case 3). This de novo 100.1kb duplication was consistently identified by WES and whole genome sequencing (WGS) independently, with coordinates identified by three different CNV identification tools, ExomeDepth v1.1.10, XHMM and Conifer v0.2.2 (Figure S1). To confirm this duplication, a qPCR assay was run on fibroblasts from the proband and parents to confirm the *de novo* duplication (Figure S1). The *RUFY1* gene encodes the RUN and FYVE domain containing 1 protein, and is thought to be involved in early endosomal trafficking.[13] To date, no

human disease has been associated with the *RUFY1* gene. The impact of this *HNRNPH1* gene duplication on protein function is unclear, however given the overlap of features observed in other individuals with missense, frameshift, and small deletions within *HNRNPH1*, it is likely that it would result in abnormal hnRNP H function. It is also worth considering that a small rearrangement within the duplicated region can not be ruled out, as it would go undetected by short read WES or WGS. Further functional studies are required to better understand the implications of this duplication on protein function. The clinical features of Case 3 are not as severe as those with pathogenic variants impacting the NLS, however they are more severe than those with pathogenic variants in the GY glycine rich domain. Specifically, Case 3 was found to have white matter and cerebellar abnormalities,dysmorphic features, short stature, genitourinary abnormalities, and microcephaly along with dysmorphic traits present in other reported cases. However, he has moderate intellectual disability, is verbal, and is ambulatory.

While limited due to a small number of individuals, these findings provide additional evidence that pathogenic variants in *HNRNPH1* cause a related, but unique syndrome from MRXSB. Given the observations presented in this report, genotype/phenotype correlation does appear to exist, since individuals with variants impacting the NLS have a more severe phenotype. Given the absence of predicted loss of function variants in healthy controls, haploinsufficiency is a likely pathogenic mechanism for variants in *HNRNPH1*. Future endeavors focusing on functional studies will provide insights into the pathological mechanisms of *HNRNPH1* variants.

Our findings suggest that pathogenic variants in *HNRNPH1* represent a related, but distinct, syndrome from MRXSB with unique dysmorphic features, increased incidence of congenital anomalies, and an increased incidence of ophthalmological abnormalities. Importantly, identification of additional individuals with pathogenic *HNRNPH1* variants will

continue to shape the observed phenotype and provide further insights into the potential genotype/phenotype correlation. While this represents a rare form of syndromic intellectual disability, given the severity observed in individuals with variants impacting the NLS, consideration of rapid-WES in critically ill newborns with microcephaly, congenital anomalies, and respiratory distress may identify pathogenic variants in *HNRNPH1*. Common features reported among the majority of individuals with *HNRNPH1* variants include short stature, microcephaly, intellectual disability, congenital anomalies, and dysmorphic features, specifically blepharophimosis, ptosis, hypotelorism, medial arched eyebrows, and micrognathia. We therefore propose that individuals with *HNRNPH1* pathogenic variants be described as having *HNRNPH1*-related syndromic intellectual disability.

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Table 1. Clinical characteristics of Individuals with *HNRNPH1* variants (NM 005520.2) in comparison with individuals with HNRNPH2-related X-linked intellectual disability, Bain type (MRXSB). Abbreviations are as follows: (+), present; (-), not present; (+/-), mild; (FTT), Failure to Thrive; (GERD), gastroesophageal reflux disease; (d), deceased.

	MRXSB (n=11)	**Pilch et al. 2018**	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**	**HNRNPH1 (n=8)**
Variant	c.616C>T (p>R206W) n=7, c.617G>A (p.R206Q) n = 2, c.626C>T (p.P209L) n=1, c.340C>T (p.R114W) n=1	c.616C>T (p.R206W)	c.616C>T (p.R206W)	c.618dupG (p.Pro207fs)	178977572-179050134 duplication	c.617G>A (p.R206Q)	c.1116_1175del (p.Glu373-Tyr392del)	c.1240_1243dup (p.Q415fs)	c.616C>T (p.R206W)	
Sex	7F, 4M	M	M	M	M	M	F	F	M	2F, 6M
Age		13y	14 y	11y	14y	30w3d (d)	5 years	23 years	10.5 m (d)	30w3d – 14y
Intellectual disability	+ (11/11)	+	+	+	+		+	+	+ global developmental delay	7/8
Verbal skills	Variable: nonverbal to short sentences	Non-verbal	Non-verbal	Non-verbal	Minimal		Minimal			3/8 nonverbal; 2/8 minimal verbal skills
Abnormalities of the cerebellar vermis	+ (2/11)	+	-	+	+		+	-	+	5/8
Other Brain Abnormalities	+ (1/11) Lipoma in the corpus callosum region; (1/11) white matter	Anomaly of clivus and atlantooccipital joint	Tethered Cord, Anomaly of clivus and atlantooccipital joint		T2 periventricular white matter hyperintensities with cysts	Foramen magnum stenosis		White Matter Abnormalities	Dysmorphic midbrain, delayed myelination	6/8

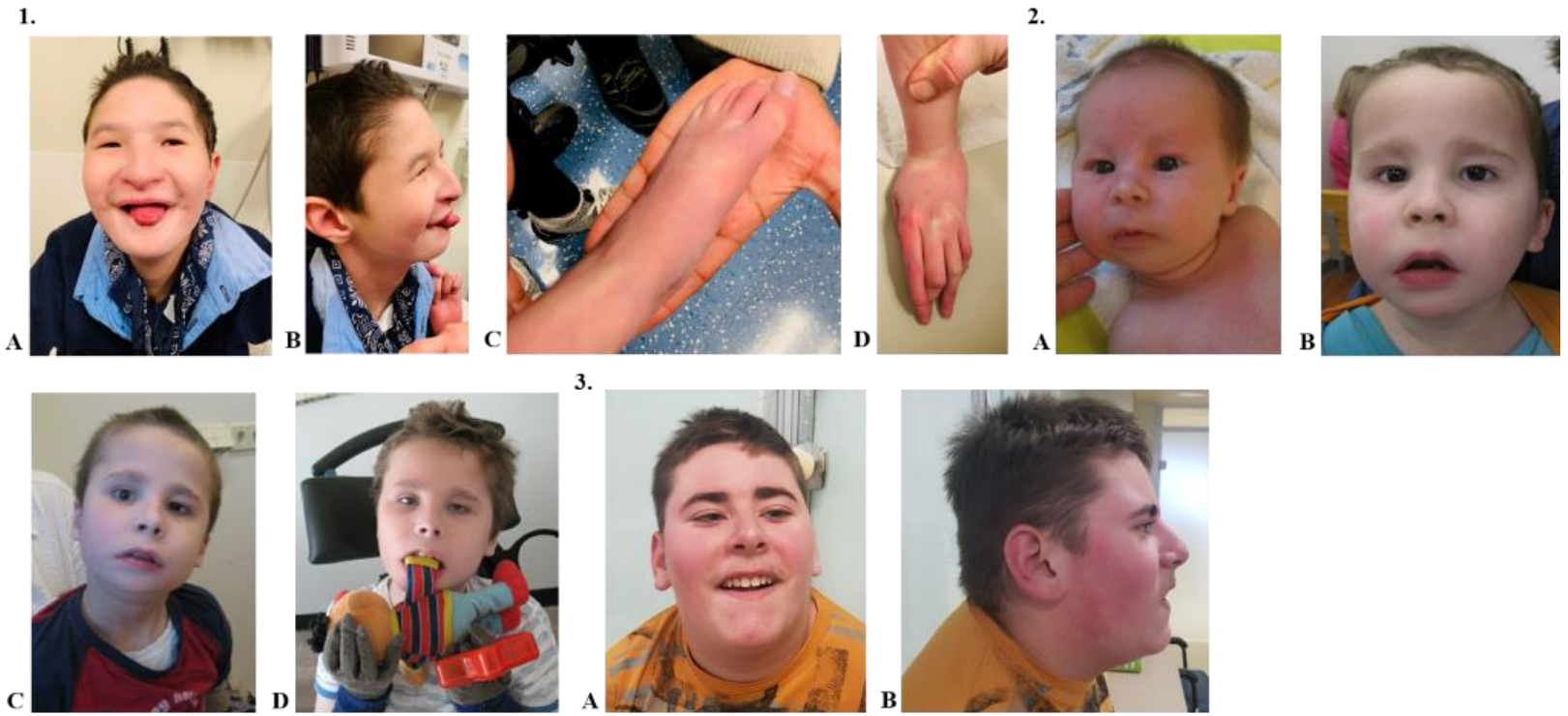
	abnormalities									
Joint laxity	+ (3/11)	+	+	+	-		-	+		4/8
Short stature	+ (4/11)	+	+	+	+	+ (IUGR)		-	+	6/8
Skeletal Issues	+ (4/11) Scoliosis; (1/11) Pectus carinatum; (2/11) Pes Planus	Pectus Carniatum, Scoliosis/ Lordosis, contractures	Scoliosis/ lordosis			Bilateral clubfoot, camptodactyly	Clenched fists, elbows and wrists flexed, toe walking	Prognathism	Bilateral Hip Dysplasia	6/8
Gastrointestinal abnormalities	+ (8/11) FTT, GERD, constipation, feeding difficulties	GERD	FTT, G-tube dependent, Hiatal hernia, constipation	Feeding difficulties, G-tube, GERD, constipation	-		-	-	Feeding difficulties, G-tube	4/8
Tone Abnormalities	+ (11/11) Hypotonia; (1/11) Hypertonia	+ Hypotonia	+ Hypotonia	+ Hypotonia	+ Hypertonia			+ Hypotonia	+ Hypotonia	6/8
Genitourinary anomalies	-	Hypospadias	Horseshoe kidney, hypospadias	-	Posterior urethral valves, Cryptorchidism			-	Cryptoorchidism	4/8
Ophthalmologic findings	+ (1/11) exotropia, cortical visual impairment	Strabismus	Strabismus, Myopic astigmatism	Motility issue, retinal dystrophy/ RP?	Strabismus, nystagmus Optic disk pallor		Left Squint	Hypermetropia, strabismus	Retinopathy of prematurity	7/8
Movement disorder	+ (1/11) gait disturbance; (1/11) ataxia; (1/11) athetoid movements; (1/11) involuntary	Non-ambulatory	Non-ambulatory	Non-ambulatory	Ataxia, tremor, wide-based gait, Dystonia		Dystonia		Dystonia	3/8 dystonia 1/8 ataxia

	movements; (1/(11) dystonic posturing									
Epilepsy	+ (5/11)	-	-	-	+		+		-	2/8

Figure Legends:

Figure 1: Dysmorphic Features in Affected Individuals. Photographs of Case 1 at 14 years of age (1A-1D) with dysmorphic features including long face, hypotelorism, belpharophimosis, ptosis, downslanting palpebral fissures, medial arched eyebrows, long prominent nose with hypoplastic alae nasi and low hanging columella, small mouth, open bite, micrognathia, posteriorly rotated hypoplastic ears, arachnodactyly, clinodactyly, campodactyly, and clubbed fingers. Photographs of Case 2 at 1 week, 3 years, 8 years, and 11 years of age respectively (2A-D) with dysmorphic features including belpharophimosis, ptosis, medial arched eyebrows, mild downslanting palpebral fissures, hypoplastic alae nasi, micrognathia, posteriorly rotated hypoplastic ears, and open bite. Case 2 was also noted to have clinodactyly (not shown). Photographs at Case 3 at 14 years of age (3A-B) with dysmorphic features including blepharophimosis, medial arched eyebrows, hypoplastic alae nasi, micrognathia, and posteriorly rotated ears.

Figure 2: Characteristics of *HNRNPH1* pathogenic variants. A. Location of *HNRNPH1* pathogenic variants in the HNRNP H protein. B. Predicted pathogenicity and mutational consequence of *HNRNPH1* pathogenic variants.



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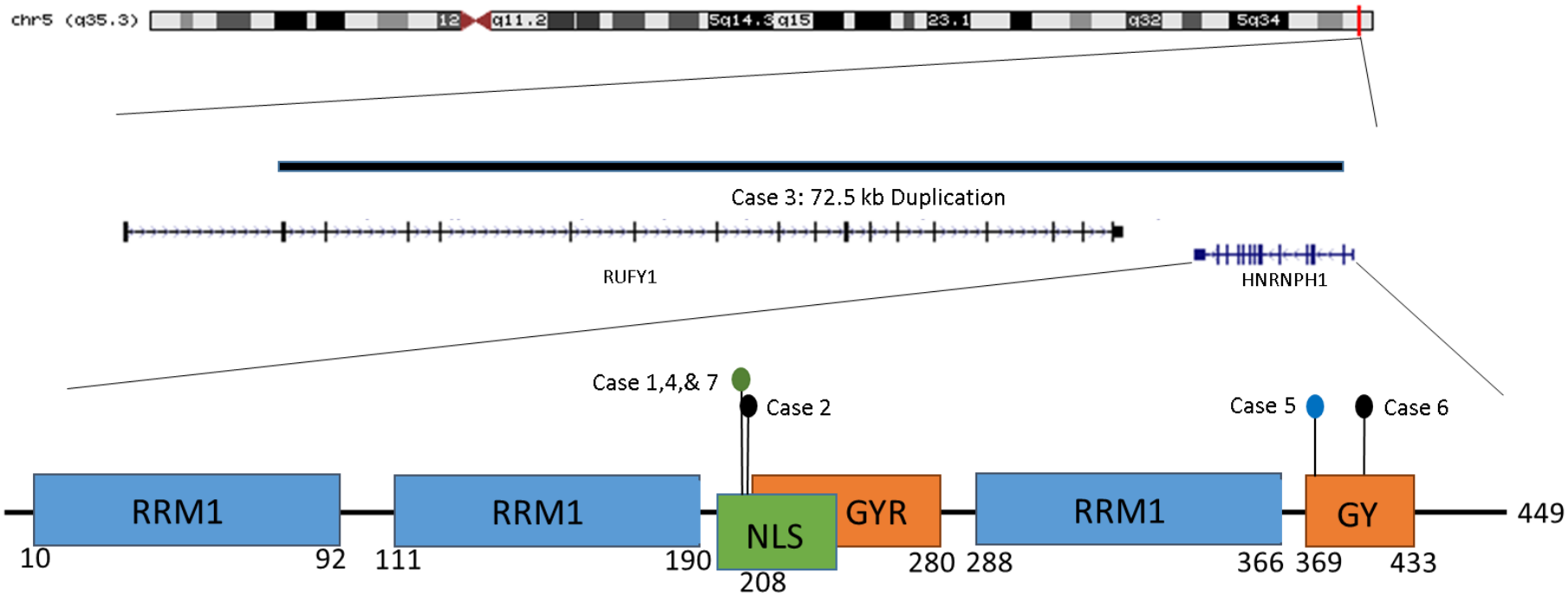
A.



B.

	Variant (NC_000005.9, NM_001257293.1, hg19)	Mutational Consequence	CADD	SIFT	Mutation Assessor	Provean	PolyPhen-2
Cases 1 and 7	c.616C>T p.Arg206Trp chr5:g.179045245G>A	Missense	Deleterious (25.2)	Damaging (0.024)	2.02 (medium)	Deleterious (-4.63)	Benign (0.028)

Case 2	c.618dupG p.Pro207Alafs*5 chr5:g.179045243dupC	Frameshift	NA	NA	NA	NA	NA
Case 3	NA chr5:178977572-179050134dup	Partial Gene Duplication	NA	NA	NA	NA	NA
Case 4	c.617G>A p.Arg206Gln chr5:g.179045244C>T	Missense	Deleterious (24.0)	Damaging (0.025)	1.42 (low)	Neutral (-1.95)	Benign (0.051)
Case 5	c.1116_1175del152 p.Glu373_Tyr392del chr5:g.179043902_179044053del	In-Frame Deletion	NA	NA	NA	NA	NA
Case 6	c.1240_1243dup p.Gln415Profs*30 chr5:g.179043193_179043196dup	Frameshift	NA	NA	NA	NA	NA

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