REPORT

Variants in HNRNPH2 on the X Chromosome Are Associated with a Neurodevelopmental Disorder in Females

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Via whole-exome sequencing, we identified six females from independent families with a common neurodevelopmental phenotype including developmental delay, intellectual disability, autism, hypotonia, and seizures, all with de novo predicted deleterious variants in the nuclear localization signal of Heterogeneous Nuclear Ribonucleoprotein H2, encoded by *HNRNPH2*, a gene located on the X chromosome. Many of the females also have seizures, psychiatric co-morbidities, and orthopedic, gastrointestinal, and growth problems as well as common dysmorphic facial features. HNRNPs are a large group of ubiquitous proteins that associate with pre-mRNAs in eukaryotic cells to produce a multitude of alternatively spliced mRNA products during development and play an important role in controlling gene expression. The failure to identify affected males, the severity of the neurodevelopmental phenotype in females, and the essential role of this gene suggests that male conceptuses with these variants may not be viable.

A number of X-linked disorders have been extensively associated to intellectual disability as well as neurodevelopmental disabilities including autism.[1–6] Here we describe six unrelated females who have de novo missense variants in two specific amino acids predicted to be deleterious in the protein product of Heterogeneous Nuclear Ribonucleoprotein H2 (*HNRNPH2* [MIM: 300610]). *HNRNPH2* (GenBank: NM_019597.4) is located at Xq22.1 and encodes a member of a family of ubiquitous heterogeneous nuclear ribonucleoproteins (HNRNP). The HNRNPs are a large group of RNA binding proteins that have distinct nucleic acid binding properties that act as a shuttle between the nucleus and the cytoplasm and act on pre-mRNA to positively or negatively affect spliceosome assembly at nearby splice sites, thereby controlling pre-mRNA splicing.[7,8] These proteins are designated HNRNPA1 through HNRNPU, and each contains one or more RNA binding domains, such as RNA recognition motifs (RRM) or ribonucleoprotein (RNP) consensus sequences (Figure 1).[9,10] Alternative RNA splicing allows for the production of multiple mRNAs from a single gene, allowing for control of gene expression by cell type and developmental stage. Aberrant splicing causes many neurological diseases, including various hematolymphoid neoplasias, autosomal-dominant retinitis pigmentosa, microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1 [MIM: 210710]), amyotrophic lateral sclerosis (ALS [MIM: 105400]), spinal muscular atrophy (SMA [MIM: 600354]), and X-linked syndromic mental retardation (XLMR [MIM: 309530]).[11–13] We report an association of de novo predicted pathogenic missense variants in *HNRNPH2* with a range of neurodevelopmental features including developmental delay (DD), intellectual disability (ID), autism, hypotonia, and seizures.

Informed consent was obtained from all participants included in the study and the study was approved by the Institutional Review Board of Columbia University. Magnetic resonance imaging (MRI) and photographs of affected individuals were obtained with consent from each individual's guardians. For the five females tested at GeneDx, a commercial clinical genetic testing laboratory, genomic DNA was extracted from whole blood from the affected children and their parents. Exome sequencing was performed on exon targets captured using the Agilent SureSelect Human All Exon V4 (50 Mb) kit or the Clinical Research Exome kit (Agilent Technologies) as previously described.[14] After automated filtering of variants with a MAF of >10%, manual curation was performed to filter less common variants with MAF of 1%–10%, single variants in genes inherited from unaffected parents, evaluate predicted effects of rare variants and known function of the genes and associated human conditions, and examine overlapping phenotypes of individuals with de novo variants in the same gene as previously described.[14] All de novo variants

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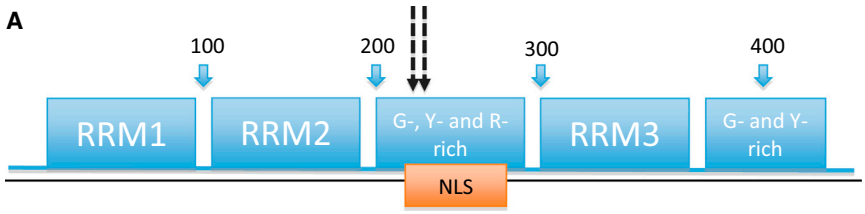

B

	190	200	210
HNRNPH	EVRTHYDPPRKLMAMQ	R	PGP
HNRNPH'	EVRTHYDPPRKLMAMQ	R	PGP
HNRNPF	EVRSYS DPPLKFMSVQ	R	PGP

C

	190	200	210
Homo sapiens	EVRTHYDPPRKLMAMQ	R	PGP
Pan troglodytes (chimpanzee)	EVRTHYDPPRKLMAMQ	R	PGP
Oryctolagus cuniculus (rabbit)	EVRTHYDPPRKLMAMQ	R	PGP
Canis lupus familiaris (dog)	EVRTHYDPPRKLMAMQ	R	PGP
Felis catus (cat)	EVRTHYDPPRKLMAMQ	R	PGP
Mus musculus (mouse)	EVRTHYDPPRKLMAMQ	R	PGP
Cavia porcellus (guinea pig)	EVRTHYDPPRKLMAMQ	R	PGP
Rattus norvegicus (rat)	EVRTHYDPPRKLMAMQ	R	PGP
Danio rerio (zebrafish)	EVRTHYEPQRKVMGMQ	R	PS

Figure 1. HNRNPH2 Domains and Conservation among Various Family Members and across Species

(A) HNRNPH2 domains. The numbers above (100, 200, 300, and 400) represent the amino acid sequence. Dotted arrows indicate the location of de novo variants at amino acid positions 206 and 209. Abbreviations are as follows: NLS, nuclear localization sequence; RRM, RNA recognition motifs; G, glycine-rich domain; Y, tyrosine-rich domain; R, arginine-rich domain.

(B) Highly conserved sequences among members of the HNRNPH family, including HNRNPs H1 (H), H2 (H'), and F proteins. Highlighted boxes indicate amino acid residues 206 and 209 reported.

(C) Cross species conservation of amino acids 206 and 209. Highlighted boxes indicate amino acid residues 206 and 209 reported.

were confirmed by Sanger sequencing. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page.

In the GeneDx cohort, five female individuals were identified out of 2,030 females and 2,486 males with DD and/or ID analyzed by WES with de novo predicted pathogenic missense variants in *HNRNPH2* (Table 1). WES on these five samples produced an average of ~10 GB of sequence per sample. Mean coverage of captured regions was ~138× per sample, with >98% covered with at least 10× coverage, an average of 91% of base call quality of Q30 or greater, and an overall average mean quality score of >Q36. Filtering of common SNPs (>10% frequency present in 1000 Genomes database) resulted in ~5,050 variants per proband sample. Among these five case subjects, four had de novo missense variants at amino acid 206: three with c.616C>T (p. Arg206Trp) (GenBank: NM_019597.4) and one with c.617G>A (p.Arg206Gln) (Figure 1). Individual 4 harbored a de novo missense variant located three amino acids away (c.626C>T [p.Pro209Leu]). According to a model that predicts gene-specific background mutation rate based on sequence context,[15] the expected number of de novo missense variants in *HNRNPH2* among 2,030 unrelated females is about 0.051. Using a previously established mutation rate and Poisson test,[15] the observed five de novo missense variants in these individuals is highly significant (p value = 2.3×10^{-9}). Putative de novo variants were filtered for variant call quality and then for presence in unaffected internal control WES samples. In two case subjects, *HNRNPH2* was the only de novo change whereas in the other three case subjects, there were a small number of other variants. These other de novo variants were manually evaluated at the gene level in the context of the individuals' phenotypes and at the variant level for cross-species conservation and

Table 1. Predicted Pathogenicity and Allele Frequencies of *HNRNPH2* Variants

Nucleic Acid Variant	cDNA Change	Amino Acid Change	Position	SIFT	PolyPhen (HDIV Score)	Mutation Taster	CADD Phred	ExAC Allele Frequency
CGG>TGG	c.616C>T	p.Arg206Trp	chrX: 100,667,592	damaging (.97)	tolerated (0.025)	disease causing	20.6	0
CGG>CAG	c.617G>A	p.Arg206Gln	chrX: 100,667,593	damaging (.99)	tolerated (0.025)	disease causing	22.1	0
CCC>CTC	c.626C>T	p.Pro209Leu	chrX: 100,667,602	damaging (1)	damaging (0.938)	disease causing	22.3	0

Table 2. Clinical Characteristics of Individuals with De Novo *HNRNPH2* Variants

Individual	Variant	Age (Years)	Microcephaly? (Congenital or acquired?)	DD/ID	Regressions	ASD	ADHD	Other Psychiatric Co-morbidities	Seizures	Tone Abnormalities (Hypo or Hyper)
1	c.616C>T (p.Arg206Trp)	34	Y (A)	Y	Y	Y	Y	anxiety, OCD, aggressive behavior, self-injurious	Y	hypo, hyper
2	c.616C>T (p.Arg206Trp)	8	N	Y	N	Y	N	repetitive stereotypic behavior	Y	hypo
3	c.617G>A (p.Arg206Gln)	4	N	Y	Y	N	N	N	N	hypo
4	c.626C>T (p.Pro209Leu)	6	Y (A)	Y	Y	N	N	anxiety	Y	hypo
5	c.616C>T (p.Arg206Trp)	21	N	Y	N	Y	Y	N	N	hypo
6	c.616C>T (p.Arg206Trp)	2	N	Y	N	N	N	N	N	hypo

Abbreviations are as follows: GERD, gastroesophageal reflux disease; FTT, failure to thrive; MVP, mitral valve prolapse; ?, unknown; A, acquired; NA, not applicable.

in silico predicted pathogenicity. These other variants were ruled out as likely contributing to the individuals' phenotypes. A table of the filtering and remaining variants is provided in Table S1. One additional individual (individual 6) was identified with the same c.616C>T (p.Arg206Trp) variant by WES at another laboratory, and sequencing methods for that individual have been previously described.[19] It is notable that five of the six missense variants alter the same amino acid. The missense variants are not present in ExAC, and there are no truncating variants in *HNRNPH2* in the NHLBI Exome Variant Server or ExAC (accessed December 2015) or in a local database of more than 23,000 exomes.

These six individuals range in age from 2 years to 34 years and all have DD or ID (Table 2). Three individuals have histories of developmental regression. Three individuals were diagnosed with autism spectrum disorders and three have behavioral disturbances, ranging from aggressive or self-injurious behavior to psychiatric diagnoses such as anxiety, attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), or stereotyped behaviors. Neurological exams were notable for tone abnormalities, including all six with hypotonia and one also with hypertonia (spasticity), as well as ataxia and gait disturbance. Three of the individuals were diagnosed with seizures and two with acquired microcephaly. Two had cerebellar anomalies on brain imaging whereas the MRIs of two others were unremarkable (Figure 2). All individuals have dysmorphic facial features, and Figure 3 illustrates three individuals (#1, #2, and #5) who share many similar facial features including almond-shaped eyes, short palpebral fissures, a short philtrum, full lower lip, long columella, hypoplastic alae nali, and micrognathia. Orthopedic problems were common and included scoliosis, lordosis, pectus carinatum, pes planus, and joint laxity and likely are at least partially secondary to the neurological problems. Feeding difficulties, gastresophageal reflux disease, and constipation were noted in several individuals, as was short stature.

HNRNPH2 is located on chromosome Xq22.1, contains 449 amino acids, and is a member of the HNRNP family. The protein product has several different domains, including three quasi-RRMs that bind RNA and two glycine-rich domains (Figure 1A).[16] Of note, there are highly conserved sequences between HNRNPH, H2 (also referred to as H'), and F (Figure 1B) and therefore are often referred to as HNRNP H/F. HNRNP H and F proteins are highly conserved in the RRM and have an extensive glycine-rich region near the carboxyl terminus, which further allows members of the H/F family to homo- or heterodimerize.[9] Furthermore, these glycine-rich domains in this gene are critical for nuclear localization of the protein.[16] A nuclear localization sequence (NLS) located within a glycine-rich domain between amino acids 194 and 220 has been identified to interact with the import receptor transportin 1 (Trn1). This NLS encompasses our two missense variants, a region highly conserved across species (Figure 1C).[16] Proteins in the HNRNP family localize to

Other Neurological Findings	MRI Findings	Ophthalmologic Findings	Dysmorphic Features	Skeletal Anomalies	GI Symptoms and Growth Parameters	Other Clinical Findings
abnormal gait, ankle clonus	normal	Y, exotropia	hypotelorism, wide mouth, full lips, high narrow nasal bridge, curly hair	lordosis, short stature, bilateral femoral osteotomies, arachnodactyly	FTT, short stature	N
ataxia, muscle weakness	aplasia /hypoplasia cerebellar vermis	?	epicanthal folds, midface hypoplasia, almond shaped eyes	N	constipation	mild hearing loss, anemia, epistaxis
N	NA	N	small palpebral fissures	pes planus	GERD as an infant	happy demeanor, sensitive to noise, hand flapping
incoordination	normal	N	hypertelorism, fetal finger pads	short stature, talus valgus	FTT, feeding issues	cardiac (atrial septal defect and MVP), sensory disorder
N	normal	N	highly arched palate, mild micrognathia, elongated fingers	pectus carinatum, pes planus, scoliosis, stretchable skin, joint laxity	GERD as a child; underweight	cardiac (MVP)
torticollis, dystonic posturing left hand, dyspraxia	possible distorted cerebellar vermis	N	symmetrically concave eyebrow, pseudo-fissure in upper lip	N	feeding difficulties, drooling	N

the nucleus, bind to heterogeneous nuclear RNA, and shuttle between the nucleus and cytoplasm for pre-mRNA processing and transport. This protein is ubiquitous and highly co-localized in the nuclear compartments in the brain, gastrointestinal tract, lung, skin, spleen, and testes, which may be the foundation for the multisystem abnormalities noted in this study. Most studies for *HNRNPH2* have been in vitro functional assays, and there have been no knockout mouse models to further study gene function.

Five do novo variants identified in our series are located at amino acid 206, and the last variant involves nearby amino acid 209. Both of these amino acids are located within the NLS, suggesting that this is a critical region for proper gene function. Furthermore, given that all six individuals are female, it is possible that these variants are lethal in males and that only heterozygous females survive, similar to our recently reported findings with *DDX3X* (MIM: 300160).[17]

HNRNPs mediate pre-mRNA alternative splicing, and aberrations in splicing are increasingly recognized as a cause of developmental disorders.[18] HNRNPs A1B2, H, and F have each been identified in neurons and are important in cellular proliferation, differentiation, and apoptosis. Developmentally, HNRNP H/F splicing factors

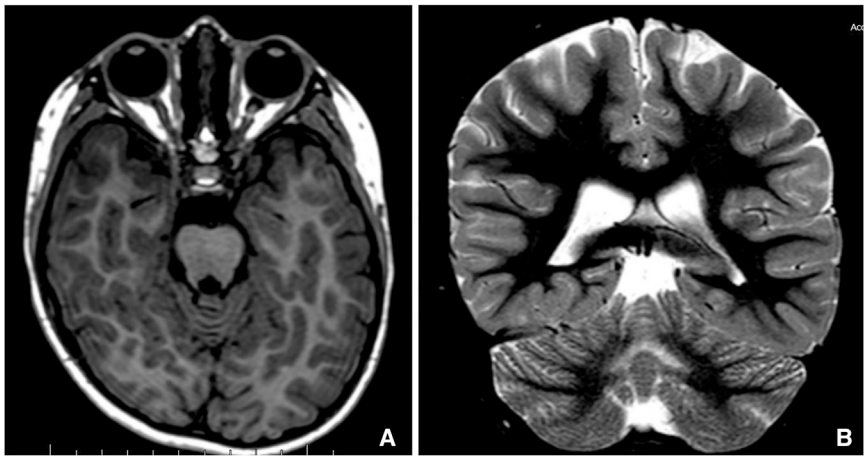

Figure 2. Magnetic Resonance Images of Affected Individuals
Axial T1-weighted (A) and coronal (B) STIR images of individual 2 demonstrating hypoplasia of cerebellar vermis at age 7 years old. Images obtained on a 1.5 Tesla MRI.

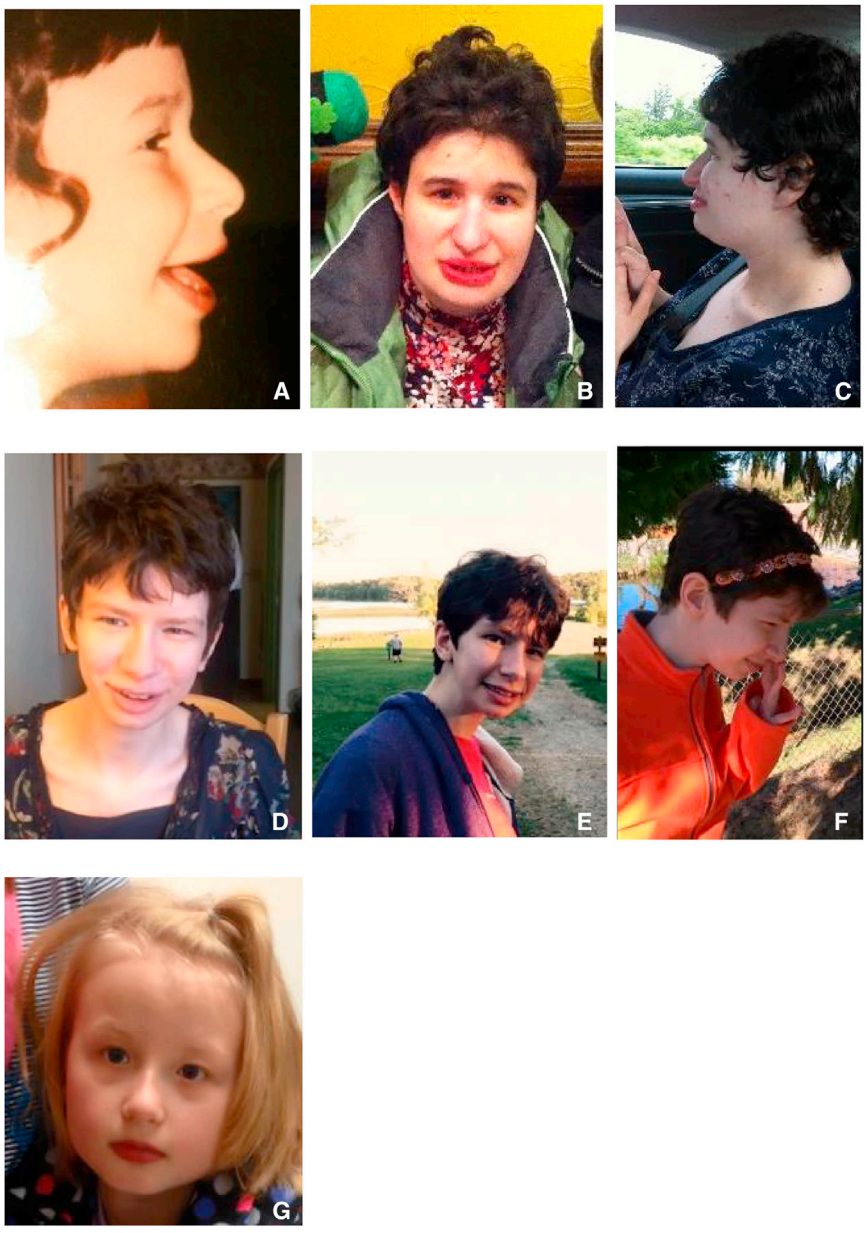

Figure 3. Dysmorphic Features in Affected Individuals
Photographs of individual 1 (A–C) and individual 5 (D–F) with dysmorphic features including almond shaped eyes, short palpebral fissures, short philtrum, full lower lip, long columella, and hypoplastic alae nasi. Individual 1 is shown at 5 years of age (A) and 34 years of age (B, C). Individual 5 is shown at 21 years of age. Individual 2 (G) is shown at 8 years of age.

have been proposed as "master orchestrators" of differentiation, integral to the developmental programming in nervous system differentiation.[19,20] There have not been any studies specifically demonstrating *HNRNPH2* expression throughout development. However, other HNRNPs have been more studied. For example, HNRNP A2 and HNRNP E1 co-localize in oligodendrocytes and neuronal dendrites. Overexpression of or microinjection of exogenous *HNRNPE1* in rat neural cells inhibited translation of *cis*-acting A2 response element (A2RE) RNAs, which forms a complex with HNRNPA1 called a RNA granule.[20] These granules are then transported along microtubules to distal dendrites to regulate myelin basic protein production.[20]

As in the adult neurodegeneration literature, it is possible that mutant HNRNPH2 leads to trafficking problems that keep HNRNPH2 in the cytoplasm.[21–23] Recently, several RNA binding proteins including FUS, ataxin 2, HNRNPA1, and HNRNPB2 have been implicated in the frontotemporal dementia/amyotrophic lateral sclerosis (FTD/ALS) phenotype pathology. The proposed pathogenesis includes deposition of poorly soluble assemblies on the mutant RNA-binding protein in the nucleus and cytoplasm of neurons in the brain and spinal cord of ALS/FTD-affected individuals with mis-localization of the protein due to incorrect nuclear localization signaling.[22,23] Specifically, sequestration of HNRNPH by potentially toxic GGGGCC intronic repeat expansions in ALS/FTD disrupts appropriate RNA processing and contributes to neurodegeneration.[24] Similarly, a family was identified with a missense variant in HNRNPA2B1 with dominantly inherited degeneration called multisystem proteinopathy, also known as inclusion body myopathy with frontotemporal dementia (IBMPFD [MIM: 615424]) which affects muscle, bone, brain, and motor neurons.[21] Wild-type HNRNP A1 and A2 have an intrinsic tendency to assemble into self-seeding fibrils as it contains a distinctive prion-like domain (PrLD) enriched in uncharged polar amino acids and glycine, but mutant proteins greatly accelerate this process.[21] Pathogenic variants in PrLDs of HNRNPs A2B1 and A1 have been described in families with this inherited neurodegeneration.[21] The reported disease variants promote excess incorporation of HNRNPA2 and HNRNPA1 into stress granules and drive the formation of cytoplasmic inclusions in *Drosophila* and mouse models that may initiate the degenerative disease. In another related study, HNRNPA2B1 has been previously implicated in fragile-X-associated tremor ataxia syndrome (FXTAS [MIM: 300623]), and the protein sequesters with RNA foci in postmortem human tissue and then is recapitulated in *Drosophila* models.[25–27] Three individuals in our series have developmental regression suggesting an underlying neurodegenerative process, and the lack of consistent

neurodegenerative phenotype across individuals could be due to the random X inactivation pattern.

Here we describe six females with de novo missense variants in *HNRNPH2*, a gene on the X chromosome that is critical for pre-mRNA processing and trafficking between the nucleus and cytoplasm, allowing for heterogeneity of protein products in different tissues and at different developmental checkpoints. All of the individuals share a common neurological phenotype of developmental delay, some with regression, autism, and tone abnormalities. Many have additional neurological problems such as seizures or psychiatric co-morbidities such as ADHD, anxiety, and OCD. Most of the individuals also have systemic abnormalities, including orthopedic, gastrointestinal, and growth problems. Five of the individuals share the same altered amino acid, 206, and the sixth individual carries a variant at the nearby amino acid position 209, both of which are located within a glycine-rich domain containing a very particular nuclear localization sequence. Recent studies show that similar HNRNP proteins that are dysfunctional can accumulate in the cytoplasm, leading to neurodegenerative conditions in adults. We propose a similar gain-of-function variant in these six individuals that produces a distinct neurodevelopmental phenotype. Critical checkpoints in the development of the brain as well as other organ systems are managed by the HNRNP family members, and aberrant gene function can lead to aberrant development of such systems. In the case of this gene located on the X chromosome, variants in males may be embryonic lethal, and the corresponding phenotype in females leads to one with a range of neurodevelopmental disability, including developmental delay, intellectual disability, autism, psychiatric disease, tone abnormalities, seizures, and other systemic abnormalities. More functional assays including animal models of this critical mRNA regulator is imperative to better understand the implications of alterations in this developmental pathway and potential neurodegenerative implications.

Accession Numbers

The ClinVar accession numbers for the reported sequences in this paper are SCV000267837, SCV000267838, and SCV000267839.

Supplemental Data

Supplemental Data include case reports and one table and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.06.028.

Conflicts of Interest

M.T.C., A.T., K.R., and K.G.M. are employees of GeneDx. W.K.C. is a former employee of GeneDx.

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Web Resources

ClinVar submission page (GeneDx), http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/
ExAC Browser, http://exac.broadinstitute.org/
GenBank, http://www.ncbi.nlm.nih.gov/genbank/
OMIM, http://www.omim.org/

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Supplemental Data

Variants in HNRNPH2 on the X Chromosome Are Associated with a Neurodevelopmental Disorder in Females

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Supplemental Information: Case Reports

Individual 1 is a 34-year-old female. Her prenatal history was unremarkable but she had a pneumothorax at birth requiring resuscitation. Her head circumference was 32 cm (10^{th} percentile) at birth but by 3 months of age the head circumference fell to the 2^{nd} percentile and remained less than 2^{nd} percentile, measuring 48 cm at 7 years. As an adult with short stature (154 cm, 5-10^{th} percentile) and weight at 56 kg (25-50^{th} percentile). Her development was delayed, as she did not walk until 5 years of age, and she began to speak at 7 years of age. She has autism and speaks in single words and short sentences. She is not toilet trained. She is intellectually disabled, and her intellectual quotient (IQ) is untestable. Her neurological examination is notable for truncal hypotonia, appendicular hypertonia and an abnormal gait. She had rare seizures, both generalized and absence, and co-morbid psychiatric diagnoses including attention deficit hyperactivity disorder (ADHD), and obsessive-compulsive disorder. She has stereotyped behaviors, as well as self-injurious behavior including hand biting. Dysmorphic features include hypotelorism, a wide mouth, full lips, high narrow nasal bridge and curly hair in this photograph taken at 5 years (Figure 2A) and 34 years of age (Figure 2 B,C). She has had orthopedic issues including lordosis, arachnodactyly and bilateral femoral osteotomies. A brain MRI was normal.

Individual 2 is an 8-year-old female with an unremarkable prenatal and perinatal course. Her birth parameters were within normal limits, including a birth weight of 2.6 kg and length 45 cm, both at the 10^{th} percentiles. At 2.5 and 6 years of age, her weight was at the 10^{th} and 48^{th} percentiles respectively. Her head circumference at birth was not available, but at 6.5 years of age was 51 cm at the 50^{th} percentile. Her development was delayed, as she was able to sit

independently at 18 months of age. Currently, she does not walk or talk. She is autistic, intellectually disabled (IQ not testable but estimated as severe) and has epilepsy, which is well controlled on oxcarbazepine. Her neurological examination is significant for hypotonia, weakness and ataxia. Dysmorphic features include bilateral epicanthal folds, midface hypoplasia and almond shaped eyes in a photograph taken at 8 years of age (Figure 2 G). She also has mild hearing loss. Brain MRI showed cerebellar vermis hypoplasia. (Figure 3).

Individual 3 is a 4-year-old female with normal birth and subsequent growth parameters. Her birth weight was 2.7 kg (10-25th percentile) and birth length was 46 cm (10th percentile). She has developmental delay and a history of developmental regression. Abnormal behaviors include hand flapping but Ωno formal diagnosis of ASD. Her neurologic exam is notable for hypotonia and dysmorphic features include small palpebral fissures.

Individual 4 is a 6-year-old female. At birth, growth parameters were within normal limits including both weight of 2.9 kg and length of 48 cm, both at the 25th percentile, and head circumference measured 34.5 cm at the 75th percentile. Her subsequent growth was at <3% for all growth parameters, with weight 11.8 kg, height 104 cm and head circumference 46.5 cm at 6 years of age, thereby with acquired microcephaly. She is developmentally delayed, with a history of developmental regression and an estimated mild degree of intellectual disability. She was using approximately 7 words at 3 years of age, but then stopped consistently using words after a seizure in the setting of a fever at 3.5 years of age. She now has only a few word approximations. Her walking was once steady and unassisted, but she now is less consistent preferring to hold a hand for support while walking. She had previously been finger feeding

independently at 2.5 years old, but now requires full assistance with feeding. She has an anxiety disorder, sensory issues, and has had one convulsion seizure in the setting of fever at 3.5 years of age and one breakthrough seizure at 4.5 years of age, currently on levetiracetam monotherapy. Her neurological exam is remarkable for hypotonia and poor coordination. Dysmorphic features include hypertelorism and fetal finger pads. She has had chronic difficulties with feeding, failure to thrive, and wears orthotics for talus valgus. She also has cardiac abnormalities including an atrial septal defect and mitral valve prolapse. Brain MRI was normal.

Individual 5 is a 21-year-old female. Prenatal care was complicated by advanced maternal age, preeclampsia and gestational diabetes but birth history and perinatal course were unremarkable. Her birth weight was 3.9 kg and length was 53 cm, both above the 90^{th} percentile, and birth head circumference was not available. At 19 years of age, her weight is 42.5 kg (< 5^{th} percentile), height is 159.8 cm (2^{nd} percentile) and head circumference is 55 cm (50^{th} percentile). She was globally developmentally delayed, with walking achieved at 3 years of age and speaking in sentences achieved at 8 years of age. She is intellectually disabled with a full scale IQ of 60; she is able to read and do some addition and subtraction, but functions at a level of about 6 years of age. Her neurological exam is remarkable for hypotonia. She has dysmorphic features including highly arched palate, mild micrognathia and elongated fingers observed at 21 years of age (Figure 2 D-F). She has been diagnosed with both autism and ADHD. She has severe skeletal abnormalities including thoracolumbar scoliosis, joint laxity, pectus carinatum, pes planus and stretchable skin. She also has mitral valve prolapse and mitral regurgitation, but her aorta is within normal limits.

Individual 6 is a 2-year-old female. Her head circumference measured 37 cm at 2 months of age (5^{th} - 10^{th} percentile) and then grew to 46 cm (10^{th} and 25^{th} percentiles) at 2 years of age. She has been developmentally delayed, sitting at 14 months of age and still unable to walk or say any discernible words, although she can babble. Her neurological exam is remarkable for hypotonia, as well as an early right hand preference and abnormal posturing of the left hand suggestive of dystonia. She has dyspraxia, causing difficulty with language and feeding, including handling secretions and solid foods. She has dysmorphic features including concave eyebrows and a fissure in the upper lip. A MRI performed at 4 months of age for hypotonia was suggestive of a distorted cerebellar vermis.

Supplemental Table 1.

Individual #	HNRNPH2 variant	Number of additional *de novo* variants	Non-HNRNPH2 *de novos*
1	c.C616T p.Arg206Trp	2	*HAUS6*:NM_001270890:c.A1943G:p.Q648R; *CHD6*:NM_032221:c.G4229A:p.R1410H
2	c.C616T p.Arg206Trp	2	*B3GNTL1*:NM_001009905:c.A430G:p.R144G
3	c.G617A p.Arg206Gln	3	*COL14A1*:NM_021110:c.A1706G:p.Y569C; *MYO7A*:NM_000260:c.A1373G:p.N458S; *TMEM10*:NM_198563:c.C740T:p.A247V
4	c.C626T p.Pro209Leu	0	none
5	c.C616T p.Arg206Trp	0	none
6	c.C616T p.Arg206Trp	0	none