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BRIEF COMMUNICATION

Phenotypic expansion in *DDX3X* – a common cause of intellectual disability in females

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Abstract

De *novo* variants in *DDX3X* account for 1–3% of unexplained intellectual disability (ID) cases and are amongst the most common causes of ID especially in females. Forty-seven patients (44 females, 3 males) have been described. We identified 31 additional individuals carrying 29 unique *DDX3X* variants, including 30 postnatal individuals with complex clinical presentations of developmental delay or ID, and one fetus with abnormal ultrasound findings. Rare or novel phenotypes observed include respiratory problems, congenital heart disease, skeletal muscle mitochondrial DNA depletion, and late-onset neurologic decline. Our findings expand the spectrum of DNA variants and phenotypes associated with *DDX3X* disorders.

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*Members are listed in Table S3.

Introduction

Intellectual disability (ID) affects 1–3% of the population and is more prevalent in males versus females.[1] Although over 100 genes on the X chromosome were found to be associated with ID in males,[2,3] relatively less is known about X-linked ID genes in females.[4] Whole-exome sequencing (WES) is finding *de novo* variants in X-linked ID genes in females of all ages.[5–8] However, limited information is available regarding such cases. Some of the genes causing ID in females are known to cause disease in males, including *PHF6*,[9] *NEXMIF*,[8] and *USP9X*, with the latter causing congenital malformations not observed in affected males.[10] Further evidence for gender-specific variant pathogenicity comes from *DDX3X* located on Xp11.4, with pathogenic *de novo* variants causing syndromic ID in 39 females; in the same study, three males inherited *DDX3X* variants from apparently unaffected mothers.[11] Differences in predicted variant severity or X-chromosome inactivation studies from blood DNA did not explain the gender-specific disease expression. Five additional females with *DDX3X* variants have been described in the literature.[12–14] These reports led us to hypothesize that females with *de novo* variation in *DDX3X* may show additional clinical phenotypes. We report 31 individuals with *DDX3X*-related disorders, and provide comprehensive clinical presentations for 13, including expanding the age range of molecular diagnosis with the oldest reported individual and a fetus. These data expand the number of *DDX3X* pathogenic variants and their associated phenotypic spectrum.

Methods

Variants in *DDX3X* were identified by WES, performed according to previously described methods,[5,6,13] either on a clinical basis at Baylor Genetics (Females 1–24, Males 1–2, Fetus 1) or on a research basis by the Baylor Hopkins Center for Mendelian Genomics (BHCMG, Females 25–27) or through the Centre de Génétique Humaine, Université de Franche-Comté (Female 28). Deidentified reporting of aggregated demographic and molecular data for all clinically referred cases was approved by the Institutional Review Board at Baylor College of Medicine (BCM). Additional, informed consent for publication of clinical details was obtained for a subset of clinically referred cases and all research-based cases according to IRB-approved protocols: at Baylor College of Medicine (Female 8, 14, 17, 23, 24, 26), through the Undiagnosed Diseases Network (UDN) protocol (Female 13), and through the BHCMG (Females 7, 19, 25, 27); and at Centre de Génétique Humaine, Université de Franche-Comté (Female 28). Females 7 and 19 were previously reported in a study of research-based reanalysis of clinical WES data.[13] *DDX3X* variants were annotated using transcript NM_001193416. Variant pathogenicity was determined based on the ACMG guidelines[15] and the internal guidelines developed at Baylor Genetics (https://www.baylorgenetics.com/variant-classification/). For the interpretation of *de novo* variants, the PS2 evidence is used if rare and/or private variants in the proband were detected in both parents by WES (Trio WES) or Sanger sequencing (proband only WES). Otherwise the PM6 evidence is used. 0.1–1 μg total RNA from patient fibroblast cells was extracted for library preparation with TruSeq Stranded mRNA kit

Table 1. Subjects with causal variants in *DDX3X*.

Subject	Genotype[1]	Inheritance	Nucleotide change	Amino acid change	Mutant/total reads by WES	WES type	Variant interpretation	SIFT	Polyphen2	Mutation taster	Scaled CADD score	Ref
Female 1	Het	*De novo*	c.14_17delCAGT	p.A5 fs	93/203	Trio	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	34	
Female 2	Het	*De novo*	c.949T>C	p.C317R	130/265	Proband	Likely pathogenic, PS2, PM2, PP3	deleterious	probably damaging	disease causing	27.9	
Female 3	Het	*De novo*	c.126_129delTTTA	p.H42 fs	38/88	Proband	Pathogenic, PVS1, PM2, PM6	NA	NA	NA	33	
Female 4	Mosaic 21%	*De novo*	c.573_575del	p.I191del	69/327	Proband	Likely pathogenic, PS2, PM2	NA	NA	NA	22.3	
Female 5	Het	*De novo*	c.1244T>A	p.I415N	205/435	Proband	Likely pathogenic, PS6, PM2, PP3	deleterious	probably damaging	disease causing	31	
Female 6	Het	*De novo*	c.971C>G	p.P324R	146/292	Proband	Likely pathogenic, PS2, PM2, PP3	deleterious	probably damaging	disease causing	28.3	
Female 7	Het	*De novo*	c.1703C>T	p.P568L	239/448	Proband	Pathogenic, PS2, PS4, PM1, PM2, PP3, PP5	deleterious	probably damaging	disease causing	34	[13]
Female 8	Het	*De novo*[2]	c.336dupC	p.R113 fs	107/233	Proband	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	33	
Female 9	Het	*De novo*	c.873_874insTATA	p.R292 fs	97/257	Proband	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	35	
Female 10	Het	*De novo*	c.874C>T	p.R292*	119/229	Proband	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	39	
Female 11	Het	*De novo*	c.887G>C	p.R296P	183/407	Proband	Likely pathogenic, PS2, PM2, PP3	deleterious	probably damaging	disease causing	34	
Female 12	Het	*De novo*	c.1180_1185dupCGTGAT	p.R394_D395dup	70/182	Proband	Likely pathogenic, PS2, PM2	NA	NA	NA	19.5	
Female 13	Het	*De novo*	c.1600C>T	p.R534C	30/88	Proband	Pathogenic, PS2, PS4, PM1, PM2, PP3	deleterious	probably damaging	disease causing	31	
Female 14	Het	*De novo*	c.1600C>T	p.R534C	50/114	Proband	Pathogenic, PS2, PS4, PM1, PM2, PP3	deleterious	probably damaging	disease causing	31	
Female 15	Mosaic 14%	*De novo*	c.1805G>A	p.R602Q	19/137	Trio	Likely pathogenic, PS2, PM2, PP3	deleterious	probably damaging	NA	27.9	
Female 16	Het	*De novo*	c.1804C>T	p.R602*	67/141	Proband	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	51	
Female 17	Het	*De novo*	c.453_454del	p.S152 fs	173/372	Proband	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	34	
Female 18	Het	*De novo*	c.173C>A	p.S58*	53/100	Proband	Pathogenic, PVS1, PM2, PM6	NA	NA	NA	36	
Female 19	Het	*De novo*	c.192dupA	p.D65 fs	98/210	Proband	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	34	[13]

(*Continued*)

Table 1. Continued.

Subject	Genotype[1]	Inheritance	Nucleotide change	Amino acid change	Mutant/total reads by WES	WES type	Variant interpretation	SIFT	Polyphen2	Mutation taster	Scaled CADD score	Ref
Female 20	Het	*De novo*	c.1595C>T	p.T532M	79/171	Proband	Likely pathogenic, PS6, PM2, PP3	deleterious	probably damaging	disease causing	33	
Female 21	Het	*De novo*	c.1033G>C	p.V345L	79/155	Proband	Likely pathogenic, PS2, PM2, PP3	deleterious	possibly damaging	disease causing	26.6	
Female 22	Het	No parental samples	c.1386C>G	p.Y462*	159/342	Proband	Likely pathogenic, PVS1, PM2	NA	NA	NA	37	
Female 23	Het	*De novo*	c.284+1G>A	p.?	95/191	Proband	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	26.9	
Female 24	Het	*De novo*	c.865-1G>A	p.?	58/163	Proband	Pathogenic, PVS1, PS2, PM2	NA	NA	NA	24.6	
Female 25	Het	*De novo*	c.1021T>C	p.C341R	65/126	Trio	Likely pathogenic, PS2, PM2, PP3	deleterious	damaging	disease causing	26.4	
Female 26	Het	*De novo*	c.1244T>A	p.I415N	46/87	Proband	Likely pathogenic, PS6, PM2, PP3	deleterious	damaging	disease causing	31	
Female 27	Het	*De novo*	c.1206_1208delCTT	p.F402del	108/235	Trio	Likely pathogenic, PS2, PM2, PP3	NA (Provean: deleterious)	NA	disease causing	18.88	
Female 28	Het	*De novo*	c.1438A>G	p.R480G	1821/3806	Trio	Likely pathogenic, PS2, PM2, PP3	deleterious	probably damaging	disease causing	25.6	
Male 1	Hemi	From asymptomatic mother	c.1052G>A	p.R351Q	50/50	Proband	Likely pathogenic, PS6, PM2, PP5	deleterious	benign	NA	25.1	[11]
Male 2	Hemi	*De novo*	c.443+3A>T	p.?	87/88	Proband	Likely pathogenic, PS2, PM2	NA	NA	NA	13.91	
Fetus 1	Het	*De novo*	c.1304T>C	p.L435P	260/513	Trio	Variant of unknown significance, PS2, PM2, phenotypic match uncertain	deleterious	probably damaging	NA	28.4	

Variant interpretation column contains the clinical significance of the variant and the type of evidences supporting the interpretation based on the ACMG guidelines[15] and the internal guidelines developed at Baylor Genetics (https://www.baylorgenetics.com/variant-classification/).

NA: not applicable.

WES type: Trio: trio WES; Proband: proband only WES.

None of the variants above has been seen in ExAC (http://exac.broadinstitute.org/) or gnomAD.[22]

[1]Het: heterozygous. Hemi: hemizygous.

[2]Heterozygous in the similarly affected monozygotic twin sibling, negative in two other siblings.

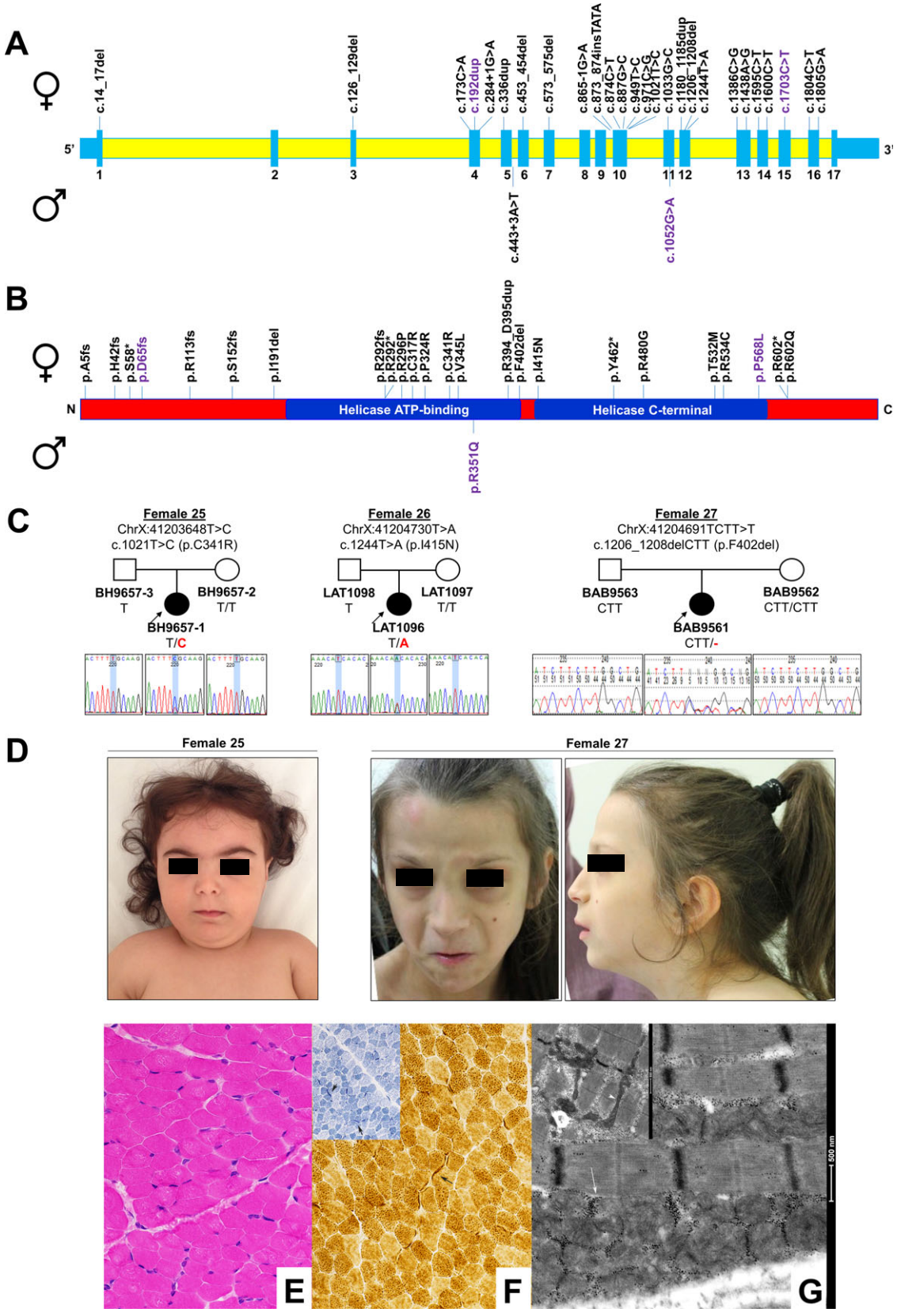

Figure 1. Location of *DDX3X* variants identified in this study, Female individuals (25, 26, 27) ascertained through the BHCMG, and muscle biopsy results in Female 17 showing abnormal mitochondrial morphology. (A) Schematic view of the *DDX3X* exon–intron structure based on NM_001193416. Blue boxes represent exons and yellow fields represent introns. Exon number is listed below each exon. cDNA change is listed for each variant. (B) Schematic view of the DDX3X protein structure based on Snijders Blok et al.[11] Amino acid change is listed for each variant. (C) Pedigree and Sanger tracings demonstrate de *novo* inheritance in three unrelated female probands. (D) Female 25 demonstrated synophrys, a broad nasal root with upturned nostrils, a long philtrum, and thin upper lip. Female 27 demonstrated cupped ears, a long philtrum, and a thin upper lip. (E–G) Muscle biopsy results in Female 17 (E) Skeletal muscle cross-section showing mild variation in fiber size (H&E; magnification ×400). (F) Skeletal muscle cross-section showing few fibers with mild subsarcolemmal increase in oxidative activity [cytochrome oxidase (long arrow) and NADH tetrazolium reductase (inset – arrow heads; magnifications ×400)]. (G) Electron microscopic images showing mild subsarcolemmal mitochondrial proliferation (long arrow) with inset in the upper corner showing pleomorphic abnormally elongated and irregularly shaped mitochondria (arrow heads). Variant color in (A) and (B): black, first reported in this study; purple, previously reported. The c.1304T>C (p.L435P) variant from Fetus 1 was not listed in (A) and (B).

Table 2. Comparison of clinical presentations in this study and in the published cohort.

Clinical features	Number of subjects in this study	Percentage in this study	Percentage in the published cohort
DD and/or ID	28/28	100%	100%
Hypotonia	19/28	68%	76%
Dysmorphic features	19/28	68%	NA
Structural brain abnormalities	18/20	90%	81%
Movement disorders	17/28	61%	45%
Visual impairments	9/28	32%	34%
Microcephaly	7/28	25%	32%
Autism spectrum disorders and other behavior problems*	6/28	21%	53%
Respiratory problems	5/28	18%	NA
Congenital heart disease	5/7	71%	NA
Skin abnormalities*	5/28	18%	37%

NA, not specified or reported in the published study.[10]

*In comparison to published data, autism spectrum disorder and other behavior problems and skin abnormalities are underrepresented in our cohort: 6/28 versus 20/38 ($P = 5.2 \times 10^{-3}$) and 5/28 versus 14/38 ($P = 4.6 \times 10^{-2}$), respectively. One-tailed Z score test for two population proportions is used.

and was sequenced by Illumina NextSeq 550. Genes with expression at the top/bottom 5% were used for pathway enrichment analysis by Ingenuity Pathway Analysis (IPA, QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis).

Results

Among 4839 (2152 females, 2687 males) patients referred to the Baylor Genetics laboratory for clinical WES with developmental delay (DD) and/or ID, 26 postnatal individuals (24 females, 2 males) were found to carry pathogenic or likely pathogenic variants in *DDX3X*, and 1 female fetus was found to carry a *de novo* variant of unknown significance in *DDX3X*. Through collaboration with the BHCMG and Centre de Génétique Humaine, Université de Franche-Comté, an additional four unrelated female cases (Females 25–28) were identified. The ages at molecular diagnosis of the postnatal individuals ranged from 1 to 47 years (Table S1). Twenty-nine unique variants were identified (26 novel and 3 reported previously), including 13 missense, 6 frameshift, 3 splice site, 4 nonsense, and 3 in-frame deletion/duplication changes (Table 1 and Fig. 1A and B). In 29 individuals with available parents (27 female, 1 male, 1 fetus), the *DDX3X* variants were confirmed as *de novo*, supporting the variant pathogenicity. Two *de novo* variants, c.573_575del (p.I191del) and c.1805G>A (p.R602Q) are mosaic in the proband, with allele fractions of 21% and 14%, respectively (Table 1). The most frequent clinical presentations in the 28 females include DD and/or ID (28/28), hypotonia (19/28), dysmorphic features (19/28), structural brain abnormalities (18/20 who had brain MRI, Fig. S1), movement disorders (17/28), visual impairments (9/28), and microcephaly (7/28) (Table 2). The most commonly observed dysmorphic facial features include a high-arched palate (5/19), thin upper lip (5/19), large ears (5/19), and long/smooth/large philtrum (4/19). Clinical presentations that are not present in published studies include respiratory problems (5/28): obstructive sleep apnea, tachypnea, and chronic respiratory failure, as well as congenital heart disease (5/7 who had

echocardiogram): atrial/ventricular septal defect, double orifice mitral valve with small patent ductus arteriosus, mild concentric left ventricular hypertrophy and bicuspid aortic valve. In comparison to published data, autism spectrum disorder and other behavior problems and skin abnormalities are underrepresented in our cohort (Table 2). For 13 subjects, we obtained additional informed consent and provide detailed clinical descriptions (Table S1), as well as clinical images for two subjects (Fig. 1D).

In two subjects undergoing muscle biopsy, skeletal muscle mitochondrial DNA content was reduced. The first subject (Female 17) is a 6-year-old nondysmorphic girl with a history of neonatal hypotonia, esophageal reflux, and global developmental delay. A quadriceps muscle biopsy demonstrated mild fiber type variation and abnormal pleomorphic mitochondria on electron microscopy (Fig. 1E–G). After correction for the reduced citrate synthase activity, respiratory chain enzyme activity analysis demonstrated reductions of multiple complexes, with relative sparing of complex II activity. Sequencing of mitochondrial DNA from the muscle sample did not detect any known or likely pathogenic variants. Mitochondrial DNA content in muscle was 39% of age-matched control muscle. Clinical WES demonstrated a *de novo* heterozygous c.453_454del (p.S152 fs) pathogenic variant in *DDX3X*, with no other variants in known disease-associated genes that explain the patient's clinical presentations. The second subject is a 47-year-old woman (Female 13) with history of global developmental delay, intellectual disability, short stature, dysmorphic features, microcephaly, and unilateral renal agenesis. She learned to sit at two years of age and walk at eight, and she only learned to say simple words. In her early 40 sec, she regressed, becoming nonverbal and unable to ambulate or to use her arms. She was found to carry a *de novo* heterozygous c.1600C>T (p.R534C) pathogenic variant in *DDX3X* (Table 1). The same variant was also observed in Female 14 in our cohort, and a variant involving the same codon (p.R534H) has been reported in a patient with ID.[11] A quadriceps muscle biopsy demonstrated severe mitochondrial and lipid depletion, and reduction in mitochondrial size similar to Female 17. Mitochondrial DNA content in muscle was 26% of the mean value for age- and tissue-matched controls. A reduction in all mitochondrial respiratory chain complex activities was observed. However, the reduction do not meet diagnostic criteria after correction for the low citrate synthase activity.

Discussion

Normal RNA metabolism requires the function of RNA helicases (RH), and yet, the exact function of most human RH remains unknown. There are six superfamilies of RH known with more than 50 human members in superfamily two that are characterized by a DExH and DExD signature in their Walker B motifs, thus termed DHX and DDX proteins. Genetic studies have begun to address the role of altered RH function in human disease (e.g., *DHX37* and *DHX30*).[16,17] *DDX3X* encodes a DEAD-box RNA helicase important in transcription, splicing, RNA transport, and translation.[18,19] In a diagnostic laboratory referral cohort of 4839 subjects with DD and/or ID, we have identified 26 postnatal individuals (24 females, 2 males) with syndromic ID or DD carrying pathogenic or likely pathogenic variants in *DDX3X*, and one fetus with abnormal ultrasound findings carrying a *de novo* variant of unknown significance in *DDX3X*; an additional four females were identified through research WES at BHCMG. The overall frequency of pathogenic or likely pathogenic *DDX3X* variations in our diagnostic laboratory referral cohort is 0.54% of the total (26/4839) and 1.12% of females (24/2152), similar to a previous report (0.6% and 1.5% respectively),[10] confirming mutations in *DDX3X* are one of the most common genetic causes of unexplained ID in females. In our diagnostic laboratory, *DDX3X* ranks third among approximately 450 genes for the occurrence of *de novo* variants, with *ARID1B* first (43 individuals) and *ANKRD11* sec (29 individuals).

In addition to confirming and extending published mutational data, our phenotypic analyses expand the phenotypic spectrum associated with *DDX3X* variants in females. For instance, we found respiratory problems and congenital heart disease in 5/28 and 5/7 of our subjects, phenotypes not previously described in the original description of *DDX3X* related disorders,[11] although observed in a subsequent report of two females.[12] We found no evidence for genotype–phenotype correlations between the mutations we identified and age at onset or phenotypic severity. Previously reported individuals ranged in ages from 1 to 33 years. We report the phenotype of a 47-year-old woman (Female 13) who had manifestations consistent with *DDX3X* disorder and a clinical picture of previously unreported late-onset neurologic decline. The decline is unrelated to intercurrent illness, and her motor function is at least in part responsive to physical therapy. Of note, other X-linked DD/ID loci, exemplified by female *FMR1* premutation [MIM: 300623] and *MECP2* duplication carriers,[20] are notable for late adult onset neurological or neurocognitive phenotypes.

Two variants reported in this study, c.1600C>T (p.R534C) and c.1703C>T (p.P568L), and three previously reported, c.641T>C (p.I214T), c.931C>T (p.R311*), and c.1084C>T (p.R362C),[11] have also been observed to occur somatically in association with medulloblastoma,

malignant melanoma, and esophageal squamous cell carcinoma (http://cancer.sanger.ac.uk/cosmic). Malignancy has not been reported in the 31 patients included in this study. However, pathway analysis for the highest 5% and lowest 5% genes expressed in RNAseq data from dermal fibroblasts obtained in one subject (Female 13) showed enrichment in cell cycle control of chromosomal replication and double-strand break repair pathways (Table S2). Future studies will elucidate whether individuals carrying *DDX3X* variants are at risk for the development of malignancies.[21] In summary, we identified 31 unrelated patients with causal variants in *DDX3X* and expanded the genotypic and phenotypic spectrum of *DDX3X*-related disorders. The collective data suggest that *DDX3X* defects are a frequent cause of syndromic ID in females, and the causal variants are likely to be loss-of-function (ExAC database showed pLI = 1.00 for *DDX3X*).[22]

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Author Contributions

Conception and design of the study: X. W., B. L., J. R. L., B. H. G., P. M. Acquisition and analysis of data: X. W., J. E. P., J. A. R., B. L., J. R. L., B. H. G., P. M. C. A. B., F. S., L. I., J. M. H., S. E. H., T. M. M., A. S., J. Z., J. B., M. S. L., W. H., F. V., M. A. W., W. B., R. X., P. F. L., Y. S., A. G., E. Y. G., Y. J., S. A. D., A. W. H., M. M. K., D. P., J. P., D. M. M., N. H., J. W. B., L. V. M., R. A. G., M. K. E., Z. C. A., T. H., A. M. A., S. C., C. M. E., F. X., Y. Y. Drafting a significant portion of the manuscript or figures: X. W., J. E. P., J. A. R., J. R. L., B. H. G., P. M.

Conflicts of Interest

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing done at Baylor Genetics. J. R. L. has stock ownership in 23 and Me, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen, Inc and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting.

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Supporting Information

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

Figure S1. Brain MRI images of subjects with *DDX3X* variants.
Table S1. Clinical features and *DDX3X* variants in subjects enrolled in this study. Detailed clinical features are only reported for the subjects in whom we were able to obtain additional informed consent.
Table S2. RNAseq pathway analysis for Female 13.
Table S3. Members of the Undiagnosed Diseases Network.

