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RESEARCH ARTICLE

PSMD12 haploinsufficiency in a neurodevelopmental disorder with autistic features

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Abstract

Protein homeostasis is tightly regulated by the ubiquitin proteasome pathway. Disruption of this pathway gives rise to a host of neurological disorders. Through whole exome sequencing (WES) in families with neurodevelopmental disorders, we identified mutations in *PSMD12*, a core component of the proteasome, underlying a neurodevelopmental disorder with intellectual disability (ID) and features of autism spectrum disorder (ASD). We performed WES on six affected siblings from a multiplex family with ID and autistic features, the affected father, and two unaffected mothers, and a trio from a simplex family with one affected child with ID and periventricular nodular heterotopia. We identified an inherited heterozygous nonsense mutation in *PSMD12* (NM_002816: c.367C>T: p.R123X) in the multiplex family and a *de novo* nonsense mutation in the same gene (NM_002816: c.601C>T: p.R201X) in the simplex family. *PSMD12* encodes a non-ATPase regulatory subunit of the 26S proteasome. We confirm the association of *PSMD12* with ID, present the first cases of inherited *PSMD12* mutation, and demonstrate the heterogeneity of phenotypes associated with *PSMD12* mutations.

KEYWORDS

autism spectrum disorder, intellectual disability, neurogenetics, proteasome

1 | INTRODUCTION

The ubiquitin proteasome pathway (UPP) functions in protein turnover and ubiquitin-mediated signaling. Ubiquitination is a posttranslational modification that involves conjugating a ubiquitin moiety to target proteins through sequential steps mediated by three classes of enzymes: ubiquitin-activating enzymes, ubiquitin-conjugating enzymes, and ubiquitin ligases. In addition to its traditional role of targeting proteins for degradation, ubiquitination functions as a regulatory mechanism involved in various signaling processes in the cell. Studies have shown that protein ubiquitination plays a key part in brain development, through the regulation of neuronal formation, migration, and maturation (Kawabe & Brose, 2011). Furthermore, ubiquitin-like proteins, acetyl groups, and phosphate groups can be added to ubiquitin chains on substrate proteins leading to a complex post-translational ubiquitin code that directs the function and lifespan of the substrate (Swatek & Komander, 2016). Following ubiquitination, substrate proteins are then targeted for degradation by the proteasome. The 26S proteasome consists of a 20S catalytic subunit responsible for substrate degradation, and a 19S regulatory subunit that facilitates interaction with the substrate and removal of the ubiquitin chain (da Fonseca, He, & Morris, 2012). The regulatory subunit itself is composed of a base containing six ATPase subunits and two non-ATPase subunits, and a lid of 10 non-ATPase subunits. The proteasome functions in all eukaryotic cells to degrade substrate proteins through a highly regulated ATP and ubiquitin-dependent process (Bhattacharyya, Yu, Mim, & Matouschek, 2014).

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that affects ~1% of the population. It is characterized by deficits in communication, diminished social skills, and stereotyped behaviors. The most common co-morbid condition with ASD is intellectual disability (ID), occurring in over 70% of individuals with ASD (Betancur, 2011). ASD is highly heritable (Colvert et al., 2015; Sandin et al., 2014) and it is extremely heterogeneous, both phenotypically and genetically (Betancur, 2011; Mitchell, 2011). It is estimated that hundreds of genes contribute to ASD risk, and genes identified to date encode molecules involved in a myriad of molecular pathways. Mutations in genes that encode for members of the UPP have been shown to result in a host of neurodevelopmental disorders with ASD and ID (Abrahams et al., 2013; Kanehisa & Goto, 2000). Examples include disruptions in ubiquitin-conjugating enzymes (*UBE2A* mutations in syndromic X-linked ID [Nascimento, Otto, de Brouwer, & Vianna-Morgante, 2006], MIM 300860), ubiquitin ligases (*UBE3A* mutations in Angelman syndrome [Kishino, Lalande, & Wagstaff, 1997], MIM 105830; *UBE3B* mutations in Kaufman oculocerebrofacial syndrome (Basel-Vanagaite et al., 2012), MIM 244450; *HUWE1* mutations in X-linked Turner type syndromic ID (Froyen et al., 2008), MIM 300706), and deubiquitinating enzymes (*OTUD6B* mutations in syndromic neurodevelopmental disability [Santiago-Sim et al., 2017], MIM 617452).

Herein, we report nine individuals affected with a neurodevelopmental disorder involving ASD, ID, and periventricular nodular heterotopia (PVNH), because of mutations in *PSMD12* (*RPN5*, MIM 604450), encoding the 26S proteasome non-ATPase regulatory subunit 12, a non-ATPase subunit of the lid component of the proteasome. *De novo* mutations in *PSMD12* were recently reported in individuals with ID and one individual with ID and ASD from the Simons Simplex Collection (Küry et al., 2017). Here we identify, using whole exome sequencing (WES), the first familial cases of inherited heterozygous mutations in *PSMD12*, and find that *PSMD12* mutations are associated with a range of neuropsychiatric features including ASD and ID, and demonstrate and extend the heterogeneity of phenotypes arising from *PSMD12* mutations.

2 | MATERIALS AND METHODS

2.1 | Subjects

Subjects were identified and evaluated in a clinical setting as having an unexplained neurodevelopmental disorder and referred for participation in a genetic research study. Peripheral blood samples were collected from the affected individuals and family members after obtaining written informed consent. Affected individuals of family AU28900 were evaluated by a team of clinicians (genetics, psychology, developmental pediatrics, and neurology) and assessed using the Goodenough–Harris Draw-A-Person Test (Goodenough, 1926; Goodenough & Harris, 1950; Harris, 1963). The affected individual of family PH23300 was evaluated by a clinical geneticist.

2.2 | WES and data analysis

DNA samples were sequenced at the Broad Institute (Cambridge, MA). Exome enrichment was performed on 1 μg of whole blood genomic DNA using SureSelect v4 (Agilent Technologies, Santa Clara, CA), according to the manufacturer's protocol. The kit targets 98.2% of consensus coding sequences of the human exome. The captured, purified, and amplified library targeting the exome from each individual was sequenced on the Illumina HiSeq. Paired-end sequences were obtained at a read length of 72 bp.

Sequence analysis was performed according to a customized bioinformatic pipeline using open-source tools for aligning reads, variant calling and annotation, and filtering out benign variation. Briefly, sequence reads were aligned to the reference human genome build GRCh37/hg19 using the Burrows–Wheeler Aligner (Li & Durbin, 2009). Alignments underwent base quality score recalibration, PCR duplicate removal, and realignment around indels. Variants were called using the Genome Analysis Toolkit (McKenna et al., 2010) and annotated using ANNOVAR (Wang, Li, & Hakonarson, 2010). Variants were loaded into a MySQL database for further annotation, including predicted consequences (noncoding, coding synonymous, coding nonsynonymous, frameshift, or splice site), allele frequency in public databases (1000 Genomes Project, dbSNP135, NHLBI-GO Exome Sequencing Project, and Exome Aggregation Consortium [ExAC]), and mode of inheritance. The functional effect of the mutation on the protein was assessed using PolyPhen-2 (Adzhubei et al., 2010) and SIFT (Kumar, Henikoff, & Ng, 2009). Sequence data were visualized using the UCSC Genome Browser.

2.3 | Sanger sequencing validation

For variant validation and confirming segregation within the family, variants were sequenced by capillary electrophoresis according to standard molecular biology practices at SeqWright, Inc. (Houston, TX).

2.4 | PSMD12 expression analysis in cell lines

Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines from two family members (affected father AU28905 and unaffected mother AU28904) were generated at the Partners HealthCare Center for Personalized Genetic Medicine BioSample Services Facility. Cells were cultured in RPMI-1640 with 2 mM L-glutamine, 10% fetal bovine serum, and 1% v/v Penicillin–Streptomycin solution. For immunoblotting, cells were lysed in 50 mM Tris pH 7.5, 150 mM NaCl, 0.5% NP40, 2 mM OPT, with cOmplete ULTRA EDTA-free protease inhibitor cocktail, and PhosSTOP phosphatase inhibitor cocktail tablets (Roche, Germany). Protein content was quantified, and for each sample 10 μg of total protein was loaded. Samples were boiled for 10 min, separated on an 8% SDS-PAGE gel, and transferred to a polyvinylidene difluoride (PVDF) membrane. Following antibody incubation, signal was detected with an enhanced chemiluminescense-based approach (SuperSignal West Pico chemiluminescent substrate, Thermo Fisher Scientific). Mouse monoclonal antibodies against PSMD12 (1:500, sc-398279, Santa Cruz Biotechnology, Dallas, TX) and β-actin (1:10,000, ab6276, Abcam, Cambridge, MA), and peroxidase AffiniPure donkey anti-mouse secondary antibody were used (1:5,000, 715-035-150, Jackson ImmunoResearch, West Grove, PA).

2.5 | Electronic resources

1000 Genomes Project: http://www.1000genomes.org/
dbSNP: http://www.ncbi.nlm.nih.gov/projects/SNP/
ExAC Browser: exac.broadinstitute.org
HGVS: http://www.hgvs.org/mutnomen/recs-DNA.html
KEGG: http://www.genome.jp/kegg/
NHLBI-GO Exome Sequencing Project: http://evs.gs.washington.edu/EVS/
OMIM: http://www.omim.org/.
SWISS-MODEL: https://swissmodel.expasy.org/.
UCSC Genome Browser: http://genome.ucsc.edu

3 | RESULTS

In this study, we report on the identification of two nonsense mutations in *PSMD12* in two families with ID, ASD, and PVNH.

3.1 | CLINICAL PRESENTATION

We ascertained two nonconsanguineous families, one of Middle Eastern descent with nine affected individuals (including the father; AU28900), and a second from the United States with one affected individual (PH23300; Figure 1(a)). Affected individuals in family AU28900 presented with variable ID and autistic features (Figure 1(a)). Family PH23300 consisted of a trio in which the proband presented with PVNH and mild developmental delay.

3.1.1 | Family AU28900

We identified a nonconsanguineous family of eight children in three branches that exhibit ASD and ID. The children share the same father (AU28905), who is also affected and is from the United Arab Emirates. The mother of the first family branch is deceased and was of Indian ancestry; she and the father had two affected daughters. The mother of the second family branch (AU28911) is from the United Arab Emirates, and she and the father had three affected children, one son and two daughters. The mother of the third family branch (AU28904) is Omani, and she and the father had three affected children, one daughter and two sons.

Upon evaluation, the father (AU28905) of all of the children showed significant cognitive limitation. He attended school until fourth grade. He was immature for his age of about 42 years but was social with good eye contact and engagement. His speech was unclear and he was confused when asked about his age and the age of his children, though was able to name his occupation (janitor) and write his name in Arabic. Paying close attention to detail, he drew a simple picture of a man.

The first wife of AU28905 is deceased and her medical information was not available but she was reportedly unaffected. She and AU28905 had two affected children. Their first daughter, AU28906, has ID and learning delays. Evaluation of AU28906 at 21 years 8 months of age revealed a head circumference of 54 cm (39th percentile) and no obvious dysmorphic features. She was able to write her name and draw a very detailed cartoon character but did not know her age and displayed delays in math skills. The second daughter of AU28905 and his first wife, AU28907, has learning delay and possibly mild ID. Evaluation of AU28907 at 19 years 7 months of age revealed a head circumference of 52 cm (2nd percentile) and high arched feet. She was reported to have been born with unilateral hand polydactyly that was corrected shortly at birth. She had delayed math abilities, drew an immature cartoon character, was socially shy, and had a somewhat obsessive interest in cartoons, which together give the impression that she might have ASD, though this was not formally assessed.

AU28905 and his second wife, AU28911, had three affected children. Their first daughter, AU28910, has ID and possible learning delay. Evaluation of AU28910 at 15 years of age revealed a head circumference of 52 cm (2nd percentile), a few beats of endgaze nystagmus, and a somewhat beaked nose. She did not know her age, had delayed math skills, wrote her name repetitively, and drew an immature drawing of a girl. The second daughter of AU28905 and AU28911, AU28909, also showed ID and possible learning delay. At 13 years of age, her head circumference was 52.5 (19th percentile) and she had a somewhat beaked nose similar to her sister. AU28909 was shy but friendly and made an immature drawing. The third child of AU28905 and AU28911 is a son, AU28908, with more significant ID as well as speech delay. Evaluation of AU28908 at 11 years of age revealed a head circumference of 54 cm (76th percentile). He was socially engaged but had difficulty following directions. His speech

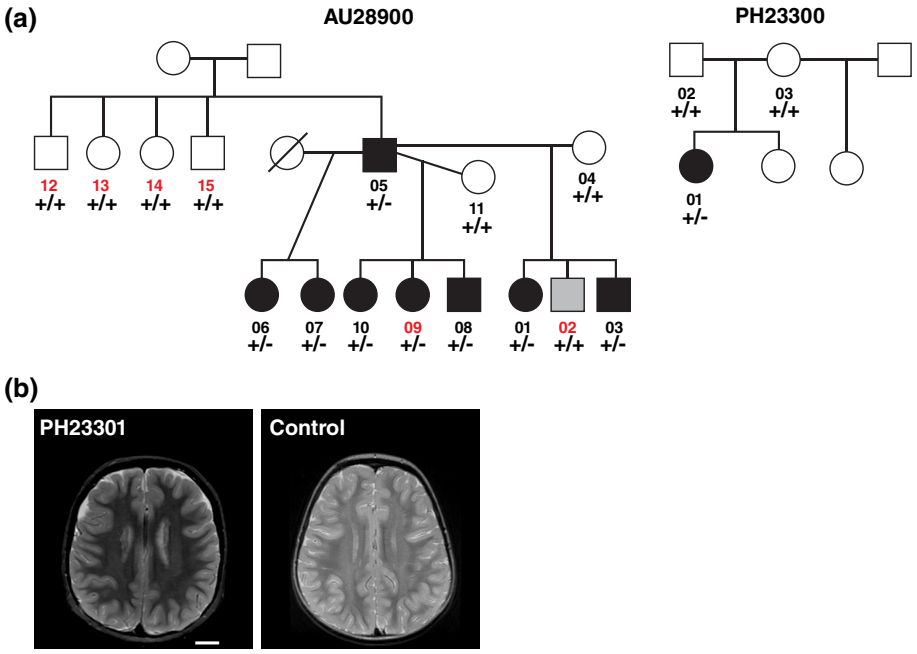


FIGURE 1 Pedigrees of families with ID, ASD, and PVNH. (a) Family AU28900 (left) with nine individuals affected with ID and ASD, and family PH23300 (right) with one individual affected with ID. Filled symbols indicate affected individuals, and empty symbols indicate unaffected individuals. WES was performed on all numbered individuals except for AU28902 and AU28909. Genotypes for *PSMD12* are indicated (+, reference allele; –, mutant allele). AU28902 presents with mild ID, and is wild type for *PSMD12*. Additional family members recruited for Sanger validation are indicated in red (02, 09, 12, 13, 14, 15). (b) Axial brain MRI of affected individual from family PH23300 (left) and normal control (right; 5 years of age). Abnormal brain structural findings included multiple small PVNH in the lateral walls of the frontal horns and the anterior bodies of lateral ventricles, mild enlargement of cerebral ventricles, large anterior commissure, and slightly diminished white matter volume. Scale bar is 2 cm [Color figure can be viewed at wileyonlinelibrary.com]

was unclear and he did not know his age or grade in school. He wrote his name with poor handwriting, drew an immature figure of a man and had delayed math skills. The mother of these three children, AU28911, is reportedly unaffected and had children with another partner: a healthy son and daughter, and a daughter that died with congenital heart disease.

AU28905 and his third wife, AU28904, had three affected children. Their only daughter and eldest child, AU28901, has delayed speech and likely ID. Examination at 11 years 1 month of age revealed a head circumference of 53 cm (60th percentile) and no obvious dysmorphic features. Although not formally assessed, she was noted to have very prominent autistic features, including frequent echolalia, reduced eye contact, inconsistent response to her name, and repetitive hand movements. She was also reported to have limited interest in interactions with peers in school and a history of writing numbers repetitively. The second child of AU28905 and AU28904 is a son, AU28902, who is the least affected child of this father, likely having cognitive or at least learning delay, as well as hyperactivity. At 8 years 1 month of age, AU28902 had a head circumference of 51.8 cm (35th percentile) and no obvious dysmorphic or autistic features. He spoke in 3–4 word sentences that were mostly comprehensible and followed directions easily. He could do basic math operations but performed well below his grade level. The third child of AU28905 and AU28904 is another son, AU28903, who was diagnosed with autism and speech delay at 3 years of age. Evaluation of AU28903 at age 6 years 4 months of age revealed a head circumference of 52.5 cm (72nd percentile) and frontal bossing. He knew about 50 words and was more socially engaged than previously noted but his level of interaction was limited. He also displayed repetitive behaviors and poor eye contact. It was reported that he does not accept variety in his diet. The medical information for the mother of these three children, AU28904, is not available but she is reportedly unaffected.

3.1.2 | Family PH23300

We identified a nonconsanguineous family from the United States with one daughter affected with PVNH and mild developmental delay. The parents are reported to be healthy, as is an older maternal half-sister and a younger full sister. The affected daughter, PH23301, was noted to have intrauterine growth restriction and was the product of a premature vaginal delivery around 35–36 weeks gestation. Her birth weight was 1.87 kg (11th percentile for 35 weeks gestational age). Whereas motor development was normal, she was reported to have mild cognitive and language delay. At 7 years 11 months of age her head circumference was 51.5 cm (48th percentile), height was 122 cm (21st percentile), and weight was 38.5 kg (98th percentile). Upon physical examination, she was noted to have mild ptosis, a very small mouth and normal muscle tone. There was no history of seizures. Clinical testing performed included a karyotype, array CGH, and *FLNA* sequencing, all of which were normal. Brain magnetic resonance imaging (MRI) at 7 years 6 months of age revealed multiple small PVNH in the lateral walls of the frontal horns and the anterior bodies of lateral ventricles (Figure 1(b)). The cerebral ventricles were mildly enlarged, the anterior commissure was large, and there was slightly diminished white matter volume (Figure 1(b)).

TABLE 1 Summary of the whole exome sequencing performed on samples from family AU28900

Sample	Reads (million)	% Reads aligned	Bases (billion)	% >Q20 bases	Mean target depth	% target coverage at 2×	% target coverage at 10×	% target coverage at 20×	% target coverage at 30×	Total variants	SNVs	Indels	Known SNPs	Novel SNPs	% SNPs known	Known indels	Novel indels	% Indels known	Non-synonymous variants
AU28901	73.2	97.78	7.3	90.42	78.01	99.52	95.95	88.43	79.44	27,522	26,219	1,303	24,794	1,425	94.57	555	748	42.59	10,065
AU28903	71.9	97.72	7.2	89.94	73.28	99.63	95.98	87.91	78.14	27,395	26,053	1,342	24,662	1,391	94.66	546	796	40.69	10,057
AU28904	54.9	97.87	5.5	90.26	61.53	99.38	94.27	83.9	72.04	27,356	26,053	1,303	24,664	1,389	94.67	553	750	42.44	10,043
AU28905	94.3	97.68	9.4	90.17	99.45	99.71	97.36	92.1	85.41	26,897	25,564	1,333	24,212	1,352	94.71	542	791	40.66	9,839
AU28906	53.6	98.16	5.4	90.18	57.87	99.39	94.19	83.13	70.55	27,086	25,762	1,324	24,380	1,382	94.64	545	779	41.16	9,840
AU28907	38.6	98.08	3.9	90.45	45.05	99.08	91.01	75.65	59.6	26,751	25,488	1,263	24,063	1,425	94.41	540	723	42.76	9,846
AU28908	59.1	98.09	5.9	90.25	63.42	99.49	94.67	84.63	73.07	27,295	25,988	1,307	24,695	1,393	94.64	544	763	41.62	10,006
AU28910	143.6	99.66	14.4	95.85	148.37	99.74	98.81	96.5	93.17	27,750	26,356	1,394	24,913	1,443	94.52	549	845	39.38	10,186
AU28911	119.4	99.54	11.9	95.75	124.24	99.73	98.47	95.35	90.92	27,696	26,294	1,402	24,843	1,451	94.48	557	845	39.73	10,113

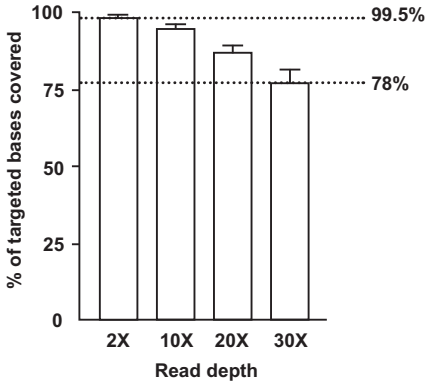
3.2 | IDENTIFICATION OF THE DISEASE-CAUSING MUTATIONS

We performed WES in family AU28900 on six of the eight affected siblings, the affected father, and two unaffected mothers. The WES yielded a mean target coverage of 96% at ≥10× and a mean read depth of 83×. We identified an average of 27,305 variants, ~95% of which were single nucleotide variants and 5% small insertions and deletions (indels; Table 1). We filtered for nonsynonymous variants that are rare with a minor allele frequency of less than 1% in several public databases of genomic variants (1000 Genomes Project, dbSNP135, NHLBI-GO Exome Sequencing Project, and ExAC) and an internal dataset of 831 whole exomes from the Middle Eastern population. Based on the pedigree structure and the segregation of the phenotype, we assumed a dominant mode of inheritance and focused on the remaining 513 heterozygous variants, of which 16 segregated with the phenotype and were potentially pathogenic (nonsynonymous, frameshift, at conserved splice site; Figure 2). Out of the 16 variants, the majority were missense mutations and only two had a predicted severe effect on protein, a nonsense mutation in *PSMD12* (NM_002816: c.367C>T: p.R123X) and a loss of stop codon mutation in *CLEC1A* (NM_016511: c.A843G: p.X281W; Table 2). To systematically rule out nonpathogenic variants and narrow in on a disease-causing mutation, we recruited extended family members and performed comprehensive Sanger sequencing of the 16 variants in all family members, including the two affected children that were not included in the original WES, AU28902 and AU28909 (Figure 1(a) and Table 2). Following this validation, we identified only one variant that segregated and validated: an inherited heterozygous nonsense mutation in *PSMD12*, (NM_002816: c.367C>T: p.R123X; Figure 3a,b). To confirm the functional impact of the nonsense p.R123X mutation, we performed western blotting on lymphoblastoid cell lines from the affected father (AU28905) and unaffected mother (AU28904). We found that the mutation in the affected individual results in a reduction of PSMD12 protein expression compared to the unaffected control (Figure 3(c)).

We then examined WES data from 433 other families with neurodevelopmental disorders in our study for potential mutations in *PSMD12* and identified one additional allele in a simplex trio family, PH23300, with one child affected with mild developmental delay (Figure 1(a)). WES on the affected child and both parents identified a rare *de novo* nonsense mutation in *PSMD12* (NM_002816: c.601C>T: p.R201X) in the affected child (PH23301; Figure 3(b)). Brain MRI on PH23301 revealed multiple small PVNH in the lateral walls of the frontal horns and the anterior bodies of lateral ventricles. In addition, the cerebral ventricles were mildly enlarged, the anterior commissure was large, and there was slightly diminished white matter volume (Figure 1(b)).

4 | DISCUSSION

We identified an inherited and a *de novo* nonsense mutation in *PSMD12* in two families with ASD, ID, and PVNH. *PSMD12* encodes a component of the regulatory subunit of the proteasome (Figure 3(a)),



	AU28900
Total variants	27,305
Nonsynonymous	9,872
...and rare	581
...and heterozygous	513
...and potentially pathogenic	16
...and validated	7
...and segregates	1

FIGURE 2 Whole exome sequencing and variant identification. WES in AU28900 was performed to a mean target coverage of 96% at ≥10× (left). Variant filtration and prioritization of WES data from AU28900. On average, we identified a total of 27,305 variants per exome, of which only one was nonsynonymous, rare, potentially pathogenic, segregated with phenotype, and was inherited (right)

a large multi-subunit enzymatic complex responsible for ATP-dependent, ubiquitin-mediated degradation of proteins. PSMD12 is a highly conserved, tightly regulated, non-ATPase component of the regulatory lid of the proteasome. The lid is responsible for proper substrate recognition, deubiquitination, and unfolding for subsequent translocation into the proteolytic cavity of the proteasome (Bedford, Paine, Sheppard, Mayer, & Roelofs, 2010). PSMD12 is required for proper proteasome assembly and localization (Yen, Espiritu, & Chang, 2003). It is highly expressed in the brain based on data from the Allen Mouse Brain Atlas of the Allen Institute for Brain Science. The gene is extremely intolerant to heterozygous loss of function mutations, with a pLI score of 1 according to ExAC (the ExAC database, containing WES data from 60,706 individuals); the closer pLI is to one, the higher the likelihood that a gene is intolerant to loss-of-function (pLI ≥ 0.9 indicates extreme intolerance to loss of function; Lek et al., 2016). Although the p.R123X mutation segregated with phenotype in family AU28900, there was one family member, AU28902, who was homozygous for the reference allele for every variant tested but nonetheless showed a mild learning disability without deficits in social behavior. The learning disability could potentially reflect being raised in a household where all other siblings have significant cognitive limitations.

Mutations in *PSMD12* had not been associated with any human disorders, until a recent report of *de novo* mutations in a cohort with ID (Küry et al., 2017). Table 3 summarizes the features of individuals with *PSMD12* mutations reported to date. The two *PSMD12* mutations we identify (p.R123X and p.R201X) were also reported by Küry et al. (2017) as occurring *de novo* in one individual with ID and another with ID and ASD. Our study reports on nine individuals with ID because of *PSMD12* mutations, confirming the association of *PSMD12* mutations with ID. We describe the first case of a *PSMD12* mutation being inherited in a dominant fashion, indicating that the condition does not exclusively occur *de novo*. By a near doubling of the total number of reported cases (from 10 to 19), we further refine the

TABLE 2 Candidate heterozygous variants identified from whole exome sequencing in family AU28900

Symbol	Name	Transcript	Mutation	Type	Effect	Segregation
PSMD12	Proteasome 26S subunit, non-ATPase, 12	NM_002816	c.C367T	Stopgain	p.R123X	Yes
CLEC1A	C-type lectin domain family 1, member A	NM_016511	c.A843G	Stoploss	p.X281W	No
ABI	Abelson interactor 1	NM_001178124	c.C853T	Missense	p.P285S	No
ACACA	Acetyl-CoA carboxylase alpha	NM_198834	c.C907T	Missense	p.R303C	No
ARHGAP23	Rho GTPase activating protein 23	NM_001199417	c.G552C	Missense	p.E184D	No
DYSF	Dysferlin, limb girdle muscular dystrophy 2B	NM_001130982	c.C2201T	Missense	p.T734M	No
GNB3	Guanine nucleotide binding protein (G protein), beta polypeptide 3	NM_002075	c.G82A	Missense	p.V28I	No
HTT	Huntingtin	NM_002111	c.G1241A	Missense	p.R414Q	No
IDUA	Alpha-L-iduronidase	NM_000203	c.T965A	Missense	p.V322E	No
IKZF3	IKAROS family zinc finger 3 (Aiolos)	NM_183230	c.G712A	Missense	p.E238K	No
RNF4	Ring finger protein 4	NM_002938	c.G352A	Missense	p.D118N	No
SMCR7	Smith–Magenis syndrome chromosome region, candidate 7	NM_148886	c.G788A	Missense	p.R263H	No
SREBF1	Sterol regulatory element binding transcription factor 1	NM_001005291	c.C3227T	Missense	p.A1076V	No
TBX2	T-box 2	NM_005994	c.C905A	Missense	p.P302Q	No
TEKT4	Tektin 4	NMJ44705	c.G734C	Missense	p.R245P	No
FNBP4	Formin binding protein 4	NM_015308	c.825_836del	Nonframeshift deletion	p.275_279del	No

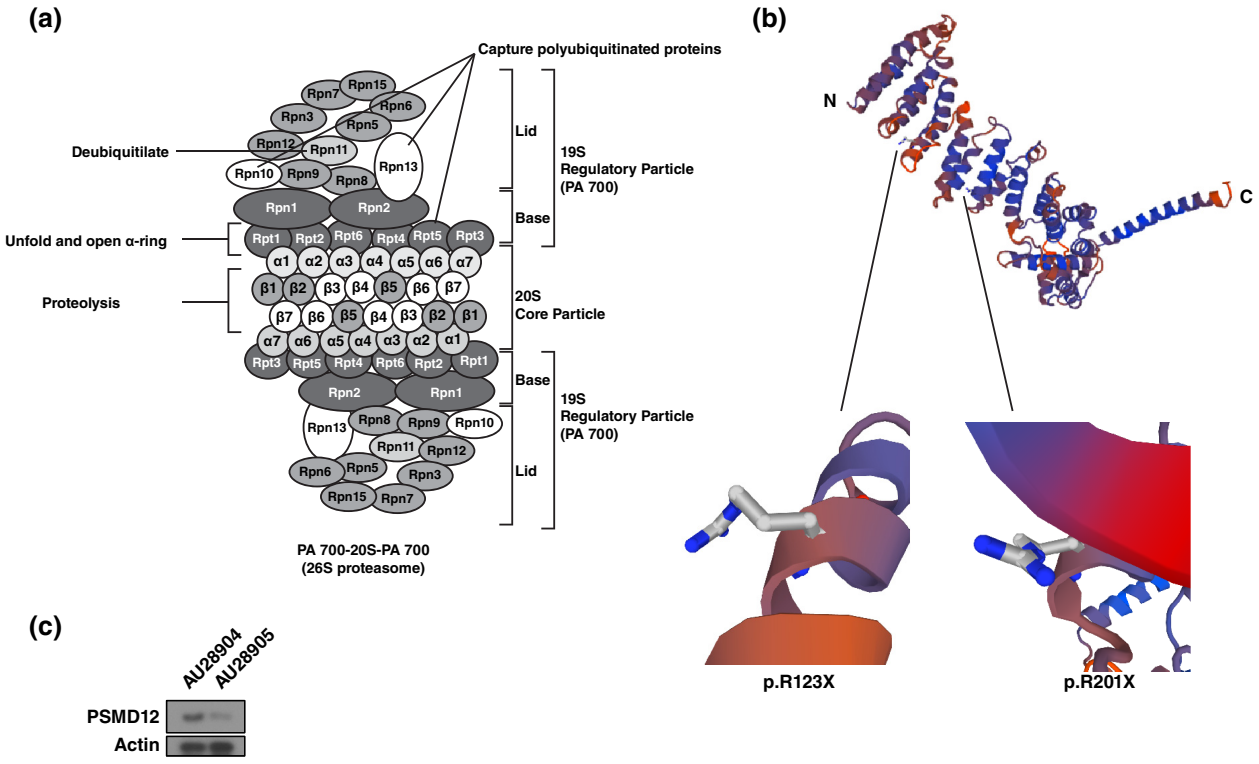


FIGURE 3 Modeling of the *PSMD12* mutations and expression analysis. (a) Schematic of the 26S proteasome showing the location of PSMD12 (Rpn5) in the regulatory lid (highlighted in red). Known functions for some subunits are indicated. Schematic was obtained and modified with permission from Kyoto Encyclopedia of Genes and Genomes (KEGG; [Ogata et al., 1999]). (b) Mapping of the p.R123X and p.R201X mutations onto the human PSMD12 crystal structure using the SWISS-MODEL workspace (Arnold, Bordoli, Kopp, & Schwede, 2006; Biasini et al., 2014; Bordoli et al., 2009; template ID: 5l4k.1.H). The lines point to zoomed in views of the two arginine (R) residues. (c) Western blot analysis on lymphoblastoid cell lines from two family members, the affected father AU28905 and unaffected mother AU28904. There was a decrease in PSMD12 protein in the affected individual heterozygous for the p.R123X mutation compared to the unaffected individual. PSMD12 size is 53 kDa, actin was used as a loading control [Color figure can be viewed at wileyonlinelibrary.com]

phenotype associated with *PSMD12* mutations and describe additional associated features, including ASD and possibly PVNH, rather than only ID.

In our study, the p.R201X mutation was associated with PVNH but brain imaging was not available for the individual with p.R201X reported by Küry et al. to confirm this association. The *de novo* p.R123X case they reported had normal brain MRI findings, but our family with the same mutation did not have brain imaging to assess for PVNH or other malformations. Of the two cases Küry et al. reported to have abnormal brain imaging findings, one had a *de novo PSMD12* splice variant of unknown functional impact, and the other had a *de novo* 4.06 Mb deletion including numerous genes in addition to *PSMD12*. The brain MRI of the individual with the splice variant showed abnormality in the white matter of the centrum semiovale bilaterally, related punctate areas of gliosis versus demyelination, and a lesion (most likely a cyst) in the pineal gland region. In the individual with the large deletion, the brain MRI abnormalities included moderate reduction in cerebral white matter volume (particularly posteriorly), thinning of the posterior corpus callosum, periventricular hypomyelination, and dysmorphic subcallosal frontal lobes, with interdigitation of gyri. These findings suggest the need for future brain imaging studies in individuals with *PSMD12* mutations to confirm their possible association with structural brain abnormalities. Another notable finding is the surprising heterogeneity of the clinical features of the individuals reported both in our series and that of Küry et al. (2017).

Mutations in genes encoding members of the UPP are associated with ASD and ID. Importantly, modulators of the proteasome system are already being explored as cancer therapies (Micel, Tentler, Smith, & Eckhardt, 2013; Tsukamoto & Yokosawa, 2009), making the UPP a pharmacologically accessible target for ASD therapies that explore strategies using these modulators. Only ~56% of ASD-associated UPP genes have phenotyped mouse models, and only eight phenotypic descriptions report abnormalities of brain development, morphology, or altered behavior (Eppig et al., 2015). Thus future work in animal models will be crucial to understand the underlying molecular mechanisms in this class of ASD-associated genes. Interestingly, recent work suggests that *PSMD12* is a transcriptional target of CHD8 in human neural stem cells (Cotney et al., 2015). *CHD8*, chromodomain helicase DNA-binding protein 8, is one of the most penetrant ASD genes identified through several large-scale WES studies (Iossifov et al., 2012; Neale et al., 2012; O'Roak, Vives, Fu, et al., 2012; O'Roak, Vives, Girirajan, et al., 2012; Sanders et al., 2012). It encodes an ATP-dependent chromatin remodeler of the SNF2 family. *PSMD12* was also reported to be upregulated in rat fetal brain in response to maternal immune activation (Lombardo et al., 2018). The mechanism of how *PSMD12* haploinsufficiency gives rise to the neurodevelopmental phenotype

TABLE 3 Clinical features of individuals with *PSMD12* mutations

	AU28901	AU28903	AU2890S	AU28906	AU28907	AU28908	AU28909	AU28910	PH23301	Küry et al.
PSMD12 mutation (NM_002816)	c.367C>T p.R123X	c.367C>T p.R123X	c.367C>T p.R123X	c.367C>T p.R123X	c.367C>T p.R123X	c.367C>T p.R123X	c.367C>T p.R123X	c.367C>T p.R123X	c.601C>T p.R201X	4 SNV and 6 CNV
Sex	Female	Male	Male	Female	Female	Male	Female	Female	Female	6 male and 4 female
Age at assessment	11 y 1 m	6 y 4 m	42 y	21 y	19 y	11 y	13 y	15 y	7 y 11 m	21 m to 14 y 19 m
Head circumference (SD)	53 cm (0.31)	52.5 cm (0.62)	ND	54 cm (−0.29)	52 cm (−2.17)	54 cm (0.56)	52.5 cm (−0.86)	52 cm (−1.97)	51.5 cm (−0.05)	(−2 to 2.32)
Intellectual disability	Yes	ND	Yes	Yes	Mild	Yes	Yes	Yes	Mild	10/10
Motor delay	No	No	No	No	No	No	No	No	No	7/10
Speech or language delay	Yes	Yes	Unclear speech	Not observed	ND	Yes, unclear speech	ND	ND	Mild language delay	Speech delay 9/10
Abnormal behavior	Echolalia, poor eye contact, repetitive hand movements and writing	Autism, repetitive behaviors, and poor eye contact	None observed	None observed	Socially shy and obsessive interests	None observed	Socially shy	Repetitive writing	ND	7/10 (2 ND)
Seizures	No	No	No	No	No	No	No	No	No	3/10
Hypotonia	ND	ND	ND	ND	ND	ND	ND	ND	No	6/10 (1 ND)
Brain MRI abnormalities	ND	ND	ND	ND	ND	ND	ND	ND	PVNH, mild ventriculomegaly, and decreased white matter	2/10 (3 ND)
Other abnormalities	ND	Frontal bossing	ND	ND	Unilateral hand polydactyly and high arched feet	ND	Beaked nose	Nystagmus and beaked nose	IUGR and premature delivery, mild ptosis, and small mouth	Cardiac: 5/10 Renal: 6/10 (1 ND) Skeletal: 5/10 Genital: 6/10 Opthalmologic: 8/10 (1 ND) Craniofacial: 9/10 (1 ND) Other: 8/10 (1 ND)

Only one variant segregated with disease in family AU28900, mapping to *PSMD12*. Mutation positions are based on the reference human genome build GRCh37/hg19. Variants were reported according to HGVS nomenclature version 2.0. For nucleotide numbering we used 1 as the initiation codon and +1 as the A of the ATG translation initiation codon in the reference sequence. Abbreviations: CNV = copy number variant; IUGR = intrauterine growth restriction; m = months; ND = not determined; PVNH = periventricular nodular heterotopia; SD = standard deviation; SNV = single nucleotide variant; y = years.

remains under investigation, and future functional studies will be required to address this.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTION

L.A-G., C.A.W., and M.H.C. conceived and designed the experiments. R.K., C.K., and M.H.C. performed the experiments. R.K., R.S.H., and M.H.C. analyzed the data. G.H.M. and R.H.N. performed clinical phenotyping. J.N.P., B.B., M.A.-S., and C.E. organized clinical information and subject samples. C.R.S. coordinated and S.B.G. oversaw whole exome sequencing at the Broad Institute. L.A-G. (AU28900) and J.W.E. (PH23300) referred and performed clinical phenotyping of subjects and characterized the clinical phenotype, and A.J.B. reviewed the clinical phenotype (PH23300). M.H.C. wrote the manuscript and all authors participated in reviewing and editing of the manuscript. C.A.W. and M.H.C. oversaw the project.

ETHICS COMPLIANCE

The Institutional Review Boards of the United Arab Emirates University and Boston Children's Hospital approved this study.

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REFERENCES

Abrahams, B. S., Arking, D. E., Campbell, D. B., Mefford, H. C., Morrow, E. M., Weiss, L. A., … Packer, A. (2013). SFARI gene 2.0: A community-driven knowledgebase for the autism spectrum disorders (ASDs). *Molecular Autism*, *4*(1), 36.

Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., … Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. *Nature Methods*, *7*(4), 248–249.

Arnold, K., Bordoli, L., Kopp, J., & Schwede, T. (2006). The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. *Bioinformatics*, *22*(2), 195–201.

Basel-Vanagaite, L., Dallapiccola, B., Ramirez-Solis, R., Segref, A., Thiele, H., Edwards, A., et al. (2012). Deficiency for the ubiquitin ligase UBE3B in a blepharophimosis-ptosis-intellectual-disability syndrome. *American Journal of Human Genetics*, *91*(6), 998–1010.

Bedford, L., Paine, S., Sheppard, P. W., Mayer, R. J., & Roelofs, J. (2010). Assembly, structure, and function of the 26S proteasome. *Trends in Cell Biology*, *20*(7), 391–401.

Betancur, C. (2011). Etiological heterogeneity in autism spectrum disorders: More than 100 genetic and genomic disorders and still counting. *Brain Research*, *1380*, 42–77.

Bhattacharyya, S., Yu, H., Mim, C., & Matouschek, A. (2014). Regulated protein turnover: Snapshots of the proteasome in action. *Nature Reviews. Molecular Cell Biology*, *15*(2), 122–133.

Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., et al. (2014). SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Research*, *42*(Web Server issue), W252–W258.

Bordoli, L., Kiefer, F., Arnold, K., Benkert, P., Battey, J., & Schwede, T. (2009). Protein structure homology modeling using SWISS-MODEL workspace. *Nature Protocols*, *4*(1), 1–13.

Colvert, E., Tick, B., McEwen, F., Stewart, C., Curran, S. R., Woodhouse, E., … Bolton, P. (2015). Heritability of autism Spectrum disorder in a UK population-based twin sample. *JAMA Psychiatry*, *72*(5), 415–423.

Cotney, J., Muhle, R. A., Sanders, S. J., Liu, L., Willsey, A. J., Niu, W., … Noonan, J. P. (2015). The autism-associated chromatin modifier CHD8 regulates other autism risk genes during human neurodevelopment. *Nature Communications*, *6*, 6404.

da Fonseca, P. C., He, J., & Morris, E. P. (2012). Molecular model of the human 26S proteasome. *Molecular Cell*, *46*(1), 54–66.

Eppig, J. T., Blake, J. A., Bult, C. J., Kadin, J. A., Richardson, J. E., & Mouse Genome Database, G. (2015). The mouse genome database (MGD): Facilitating mouse as a model for human biology and disease. *Nucleic Acids Research*, *43*(Database issue), D726–D736.

Froyen, G., Corbett, M., Vandewalle, J., Jarvela, I., Lawrence, O., Meldrum, C., et al. (2008). Submicroscopic duplications of the hydroxysteroid dehydrogenase HSD17B10 and the E3 ubiquitin ligase HUWE1 are associated with mental retardation. *American Journal of Human Genetics*, *82*(2), 432–443.

Goodenough, F. L. (1926). A new approach to the measurement of intelligence of young children. *Journal of Genetic Psychology*, *33*, 185–211.

Goodenough, F. L., & Harris, D. B. (1950). Studies in the psychology of children's drawings: II 1928-1949. *Psychological Bulletin*, *47*(5), 369–433.

Harris, D.B. (1963). *Children's drawings as measures of intellectual maturity. A revision and extension of the Goodenough Draw-A-Man Test*. New York, NY: Harcourt, Brace, and World.

Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., … Wigler, M. (2012). De novo gene disruptions in children on the autistic spectrum. *Neuron*, *74*(2), 285–299.

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, *28*(1), 27–30.

Kawabe, H., & Brose, N. (2011). The role of ubiquitylation in nerve cell development. *Nature Reviews. Neuroscience*, *12*(5), 251–268.

Kishino, T., Lalande, M., & Wagstaff, J. (1997). UBE3A/E6-AP mutations cause Angelman syndrome. *Nature Genetics*, *15*(1), 70–73.

Kumar, P., Henikoff, S., & Ng, P. C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature Protocols*, *4*(7), 1073–1081.

Küry, S., Besnard, T., Ebstein, F., Khan, T. N., Gambin, T., Douglas, J., et al. (2017). De novo disruption of the proteasome regulatory subunit PSMD12 causes a syndromic neurodevelopmental disorder. *American Journal of Human Genetics*, *100*(4), 689.

Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., et al. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, *536*(7616), 285–291.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*, *25*(14), 1754–1760.

Lombardo, M. V., Moon, H. M., Su, J., Palmer, T. D., Courchesne, E., & Pramparo, T. (2018). Maternal immune activation dysregulation of the fetal brain transcriptome and relevance to the pathophysiology of autism spectrum disorder. *Molecular Psychiatry*, *23*(4), 1001–1013.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., … DePristo, M. A. (2010). The genome analysis toolkit: A

MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297–1303.

Micel, L. N., Tentler, J. J., Smith, P. G., & Eckhardt, G. S. (2013). Role of ubiquitin ligases and the proteasome in oncogenesis: Novel targets for anticancer therapies. *Journal of Clinical Oncology*, *31*(9), 1231–1238.

Mitchell, K. J. (2011). The genetics of neurodevelopmental disease. *Current Opinion in Neurobiology*, *21*(1), 197–203.

Nascimento, R. M., Otto, P. A., de Brouwer, A. P., & Vianna-Morgante, A. M. (2006). UBE2A, which encodes a ubiquitin-conjugating enzyme, is mutated in a novel X-linked mental retardation syndrome. *American Journal of Human Genetics*, *79*(3), 549–555.

Neale, B. M., Kou, Y., Liu, L., Ma'ayan, A., Samocha, K. E., Sabo, A., et al. (2012). Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature*, *485*(7397), 242–245.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., & Kanehisa, M. (1999). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, *27*(1), 29–34.

O'Roak, B. J., Vives, L., Fu, W., Egertson, J. D., Stanaway, I. B., Phelps, I. G., … Shendure, J. (2012). Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science*, *338*(6114), 1619–1622.

O'Roak, B. J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B. P., et al. (2012). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature*, *485*(7397), 246–250.

Sanders, S. J., Murtha, M. T., Gupta, A. R., Murdoch, J. D., Raubeson, M. J., Willsey, A. J., … State, M. W. (2012). De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature*, *485*(7397), 237–241.

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C. M., & Reichenberg, A. (2014). The familial risk of autism. *JAMA*, *311*(17), 1770–1777.

Santiago-Sim, T., Burrage, L. C., Ebstein, F., Tokita, M. J., Miller, M., Bi, W., … Zara, F. (2017). Biallelic variants in OTUD6B cause an intellectual disability syndrome associated with seizures and dysmorphic features. *American Journal of Human Genetics*, *100*(4), 676–688.

Swatek, K. N., & Komander, D. (2016). Ubiquitin modifications. *Cell Research*, *26*(4), 399–422.

Tsukamoto, S., & Yokosawa, H. (2009). Targeting the proteasome pathway. *Expert Opinion on Therapeutic Targets*, *13*(5), 605–621.

Wang, K., Li, M., & Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*, *38*(16), e164.

Yen, H. C., Espiritu, C., & Chang, E. C. (2003). Rpn5 is a conserved proteasome subunit and required for proper proteasome localization and assembly. *The Journal of Biological Chemistry*, *278*(33), 30669–30676.

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