REPORT

De Novo Truncating Mutations in the Last and Penultimate Exons of *PPM1D* Cause an Intellectual Disability Syndrome

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Intellectual disability (ID) is a highly heterogeneous disorder involving at least 600 genes, yet a genetic diagnosis remains elusive in ~35%–40% of individuals with moderate to severe ID. Recent meta-analyses statistically analyzing de novo mutations in >7,000 individuals with neurodevelopmental disorders highlighted mutations in *PPM1D* as a possible cause of ID. PPM1D is a type 2C phosphatase that functions as a negative regulator of cellular stress-response pathways by mediating a feedback loop of p38-p53 signaling, thereby contributing to growth inhibition and suppression of stress-induced apoptosis. We identified 14 individuals with mild to severe ID and/or developmental delay and de novo truncating *PPM1D* mutations. Additionally, deep phenotyping revealed overlapping behavioral problems (ASD, ADHD, and anxiety disorders), hypotonia, broad-based gait, facial dysmorphisms, and periods of fever and vomiting. *PPM1D* is expressed during fetal brain development and in the adult brain. All mutations were located in the last or penultimate exon, suggesting escape from nonsense-mediated mRNA decay. Both *PPM1D* expression analysis and cDNA sequencing in EBV LCLs of individuals support the presence of a stable truncated transcript, consistent with this hypothesis. Exposure of cells derived from individuals with *PPM1D* truncating mutations to ionizing radiation resulted in normal p53 activation, suggesting that p53 signaling is unaffected. However, a cell-growth disadvantage was observed, suggesting a possible effect on the stress-response pathway. Thus, we show that de novo truncating *PPM1D* mutations in the last and penultimate exons cause syndromic ID, which provides additional insight into the role of cell-cycle checkpoint genes in neurodevelopmental disorders.

Next-generation sequencing (NGS) techniques have accelerated the discovery of genes associated with intellectual disability (ID).[1–7] Mutations in more than 600 autosomal and X-linked genes have been implicated,[8] but many more are likely to be elucidated. Recently, two separate meta-analyses used the de novo mutations identified in >7,000 individuals affected by a neurodevelopmental disorder to identify mutations that might also cause ID.[9,10] In both meta-analyses, *PPM1D* (protein phosphatase, Mg^{2+}/Mn^{2+}-dependent 1D [MIM: 605100]), encoding a negative regulator of cellular stress-response pathways, had significantly more damaging de novo mutations than expected given the cohort size. However, the clinical characteristics of these individuals were not provided in detail, and insights on the pathophysiological mechanism remained unidentified. Including seven individuals identified in the meta-analyses, we collected a total of 14 unrelated individuals with mild to severe ID and/or developmental delay (DD) through international collaboration with colleagues and data-sharing resources such as

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GeneMatcher.[9–12] One individual was previously described as part of the Simons Simplex Collection cohort.[13] Herein, we report on an ID syndrome caused by de novo germline mutations in the last and penultimate exons of *PPM1D*, as well as the implication of such mutations in the role of *PPM1D* in stress responses.

We collected clinical data by inviting individuals back to the clinic for re-evaluation and deep phenotyping. Detailed clinical information of the 14 individuals (2–21 years old) is described in the Supplemental Note and summarized in Table 1. All but one individual had mild to severe ID (93%), and 11 individuals (79%) had behavioral problems, such as anxiety disorders, attention deficit hyperactivity disorder (ADHD), obsessive behavior, sensory integration problems, and autism spectrum disorder (ASD). The individual with a normal IQ of 96 did, however, need extra tutoring at school and showed an anxiety disorder and attention problems. Seven individuals were hypersensitive for sounds. Hypotonia was a common feature in the individuals for whom this information was available (10/14 [71%]), and several individuals had a broad-based gait (5/10 [50%]). Brain MRI was performed for nine individuals (64%) without any substantial findings, except for moderate cortical and cerebellar atrophy and abnormal vascular structures in individual 13, who was also diagnosed with Potocki-Shaffer syndrome. Eight individuals (62%) had short stature, but weight and head circumference were variable. Feeding difficulties were a common feature (10/14 [71%]), and remarkably, eight individuals (62%) had periods of illness with fever and/or vomiting. In addition, nine individuals had a high pain threshold (90%). One individual had problems emerging from anesthesia. Vision problems, such as myopia, hypermetropia, and strabismus, were seen in nine individuals (64%). There was no apparent shared or consistent facial gestalt despite the presence of overlapping facial features, including a broad forehead, low-set posteriorly rotated ears, upturned nose, and broad mouth with thin upper lip (Figure 1A). Ten individuals (91%) had small hands often with brachydactyly, seven individuals had small feet, and six individuals had hypoplastic toenails. To further delineate the clinical spectrum associated with de novo mutations in *PPM1D*, we established a website to collect detailed clinical information of additional individuals to be identified over the coming year (see Web Resources).

Whole-exome sequencing was performed in all individuals as previously described,[9,10] and all were identified to have a deleterious *PPM1D* mutation. This study was approved by the institutional review board Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen NL36191.091.11 and received UK research ethics committee (REC) approval (10/H0305/83 granted by the Cambridge South REC and GEN/284/12 granted by the Republic of Ireland REC). Written informed consent was obtained from all individuals. Subsequent confirmation by Sanger sequencing and investigation of parental DNA samples of 13 individuals indicated that *PPM1D* mutations had occurred de novo. Interestingly, all 14 ID-associated *PPM1D* mutations are located in the last and penultimate exons (Figure 1B) and are predicted to result in a premature stop codon in exon 6 or in the last 55 nucleotides of exon 5 (Figure 1C). The truncated mRNA is therefore presumed to escape nonsense-mediated decay (NMD) and result in a truncated PPM1D still containing its functional protein phosphatase Mg^{2+}/Mn^{2+}-dependent (PPM)-type phosphatase domain but lacking its nuclear localization signal (NLS). To analyze *PPM1D* mRNA expression on cDNA derived from lymphoblastoid cell lines (LCLs) of individuals with a mutation in *PPM1D*, we obtained LCLs from human blood by immortalization via Epstein-Barr virus (EBV) transformation according to standard procedures. *PPM1D* mRNA expression analysis using two different sets of primer pairs showed no significant difference in mRNA levels between control lines and cDNA derived from LCLs of individuals 2 and 3, indeed confirming that the truncated mRNA was not subjected to NMD (Figure 2B). Subsequent Sanger sequencing, performed in four individuals (1–3 and 7) with a primer set targeting the mutated area (PPM1D_1 and PPM1D_3), confirmed the presence of truncated *PPM1D* transcripts. We showed that the de novo mutations in *PPM1D* lead to stable transcription of truncated mRNA with normal expression levels. Because the truncated protein lacks its NLS, it might no longer reach the nucleus to exert its function. Immunohistochemical staining of PPM1D showed cytosolic and nuclear staining in LCLs in a control line (Figure S1). Similar staining in EBV-transformed LCLs from an individual with a heterozygous de novo mutation in *PPM1D* showed a similar localization (Figure S1). However, the latter can be explained by the presence of the wild-type allele, which still results in a fully functional PPM1D. Notably, given the specific need of PPM1D in cellular stress, it is possible that localization of the mutant PPM1D is mostly affected during this state. Further quantification of PPM1D in the different cell compartments and under different physiological scenarios could help to improve our understanding of the biological mechanism underlying *PPM1D* pathology.

Importantly, de novo mutations in *PPM1D* have not been observed in over 2,000 control trios.[1,15–18] Interrogation of large databases, such as the Exome Aggregation Consortium (ExAC) Browser, shows that *PPM1D* is under constraint for missense mutations (*Z* score 3.13). Interestingly, however, *PPM1D* seems to be tolerant of loss-of-function mutations (with pLI = 0.00).[19] Together, these scores could indicate that the pathophysiological mechanism underlying ID-associated *PPM1D* mutations is more complex and that a mechanism involving a C-terminally truncated protein is more disruptive than complete loss of it, similar to what has been identified for *DVL1* and *DVL3* frameshift mutations causing Robinow syndrome.[20–22] This highlights the importance of not disregarding these genes without consideration, given that

Table 1. Main Clinical Features of Affected Individuals

	Individual														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**Total**
General Information															
Age	14 y	5 y	5 y	2 y	5 y	10 y	9 y, 9 m	21 y	16 y	6 y	18 y	15 y	7 y	7 y	2–21 y
Gender	F	M	F	M	M	M	M	M	F	F	M	F	F	F	7 M, 6 F
Mutation	c.1221T>A (p.Cys407*)	c.1216del (p.Thr406 Profs*3)	c.1260+ 1dup (p.Ser421 Thrfs*12)	c.1210C>T (p.Gln404*)	c.1269_ 1270dup (p.Glu424 Glyfs*8)	c.1339G>T (p.Glu447*)	c.1188_ 1191del (p.Asp397 Alafs*11)	c.1250dup (p.Pro418 Thrfs*16)	c.1270dup (p.Glu424 Glyfs*10)	c.1270dup (p.Glu424 Glyfs*10)	c.1281G>A (p.Trp427*)	c.1281G>A (p.Trp427*)	c.1654C>T (p.Arg552*)	c.1404_ 1411del (p.Lys469 Argfs*4)	NA
Inheritance	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	NK[a]	de novo	NA
Growth															
Birthweight (g)	2,780	4,000	2,655	3,742	3,527	3,180	2,200	2,211	3,061	2,730	3,200	3,429	NK	3,140	NA
Height (SD)	−2.7	−1.5	−2.8	0	NK	−3	<−3	<−2.5	−2.3	−2.5	−2.7	+0.41	−2.6	−2	NA
Weight (SD)	+2.3	+0.5	−1.9	0	NK	+2	+0.5	<−2.5	−2.2	−2	−1.8	>+2.5	−4.9	0	NA
Head circumference (SD)	+0.7	−0.5	−1.5	−0.5	−0.5	−1	−1.8	<−2.5	−3.1	−2.5	−2.5	+3.49	NK	−1.5	NA
Neurological															
ID (severity)	+ (mild to moderate)	+ (mild to moderate)	+ (mild)	+	+	−[b]	+ (mild to moderate)	+ (mild to moderate)	+ (severe)	+ (moderate)	+ (moderate)	+ (severe)	+ (severe)	+ (mild)	13/14 (93%)
Hypotonia	+	+	+	+	+	−	−	+	+	+	+	−	+	−	10/14 (71%)
Broad-based gait	+	NK	+	+	+	−	−	−	+	NK	NK	−	NK	−	5/10 (50%)
Sensitivity to sounds	NK	NK	+	+	NK	NK	+	+	+	+	NK	NK	NK	+	7/7 (100%)
Behavioral features	ADHD, ODD, anxiety disorder	sensory integration problems	short attention, panic attacks	sensory integration problems, hyperarousal, short attention	−	anxiety disorder, attention difficulties, biting	sensory integration problems, anxiety, short attention	anxiety	ASD	−	−	ASD, attention problems, oppositional, aggression	ASD	ASD	11/14 (79%)
Facial															
Broad forehead	+	+	−	−	NK	−	−	+	+	+	+	NK	+	+	8/12 (67%)
Low-set, posteriorly rotated ears	+	+	+	+ (right)	NK	NK	−	−	+	+	+	NK	NK	+ (only posteriorly rotated)	8/10 (80%)
Upturned nose	+	−	+	−	NK	−	−	−	−	+	+	NK	+	−	5/12 (42%)
Thin upper lip	−	+	+	+	NK	+	−	+	+	+	+	NK	+	+	10/12 83%
Broad mouth	+	+	+	−	NK	+	−	−	+	−	+	NK	−	−	6/12 50%

(*Continued on next page*)

Table 1. ***Continued***

	Individual														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**Total**
Gastrointestinal															
Feeding difficulty	+ (neonatal)	+ (neonatal)	+ (neonatal)	+	+	−	+	+	−	−	+	−	+	+ (neonatal)	10/14 (71%)
GER and/or vomiting	+	+	+	+	NK	+	+	+ (infancy)	+	+	−	−	+	−	10/13 (77%)
Constipation	−	+	−	+	+	−	+	+ (infancy)	+	−	NK	−	+	+	8/13 (55%)
Skeletal															
Small hands	NK	+	+	−	NK	+	+	+	+	+	+	NK	+	+	10/11 (91%)
Small feet	NK	NK	+	−	NK	NK	+	+	+	NK	+	NK	+	+	7/8 (88%)
Hyperlordosis	+	−	−	−	+	NK	+	+	+	NK	+	NK	NK	+	7/10 (70%)
Other															
Periodic illness[c]	+	+	−	−	+	+	+	+	+	+	−	−	NK	−	8/13 (62%)
High pain threshold	NK	+	+	+	NK	+	+	+	+	+	+	NK	NK	−	9/10 (90%)
Congenital abnormalities	bicuspid aortic valve	retractile testes, small genital	small VSD and small ODA	−	bilateral cryptor-chidism	−	−	−	−	laryngo-malacia	−	−	bilateral parietal foramina, exostoses, diaphragmatic hernia, volvulus intestine[d]	−	6/14 (43%)
Vision problems	myopia, nystagmus, amblyopia	hyper-metropia, cilinder, strabismus	hyper-metropia	myopia, strabismus, astigmatism, CVI	−	−	hyper-metropia, strabismus, astigmatism, nystagmus	myopia, strabismus	−	hyper-metropia, strabismus	hyper-metropia, strabismus	−	strabismus, nystagmus, iridocyclitis, retinal detachment	−	9/14 (64%)
Hypoplastic nails	+ (toenails)	+ (toenails)	+ (toenails)	−	+ (toenails)	−	−	+ (fifth toenails)	−	−	+	NK	NK	−	6/12 (50%)
Recurrent infections	−	−	NK	−	NK	NK	+	NK	+	NK	+	+	+	−	5/9 (56%)

Abbreviations are as follows: +, present; −, absent; y, years; m, months; M, male; F, female; NK, not known; ID, intellectual disability; ADHD, attention deficit hyperactivity disorder; ODD, oppositional defiant disorder; ASD, autism spectrum disorder; GER, gastro esophageal reflux; VSD, ventricle septum defect; ODA, open ductus arteriosus; and CVI, cerebral visual impairment.
[a]Parental DNA not available.
[b]Individual did have learning difficulties.
[c]Including cyclic vomiting.
[d]Individual 13 also had a confirmed diagnosis of Potocki-Shaffer syndrome.

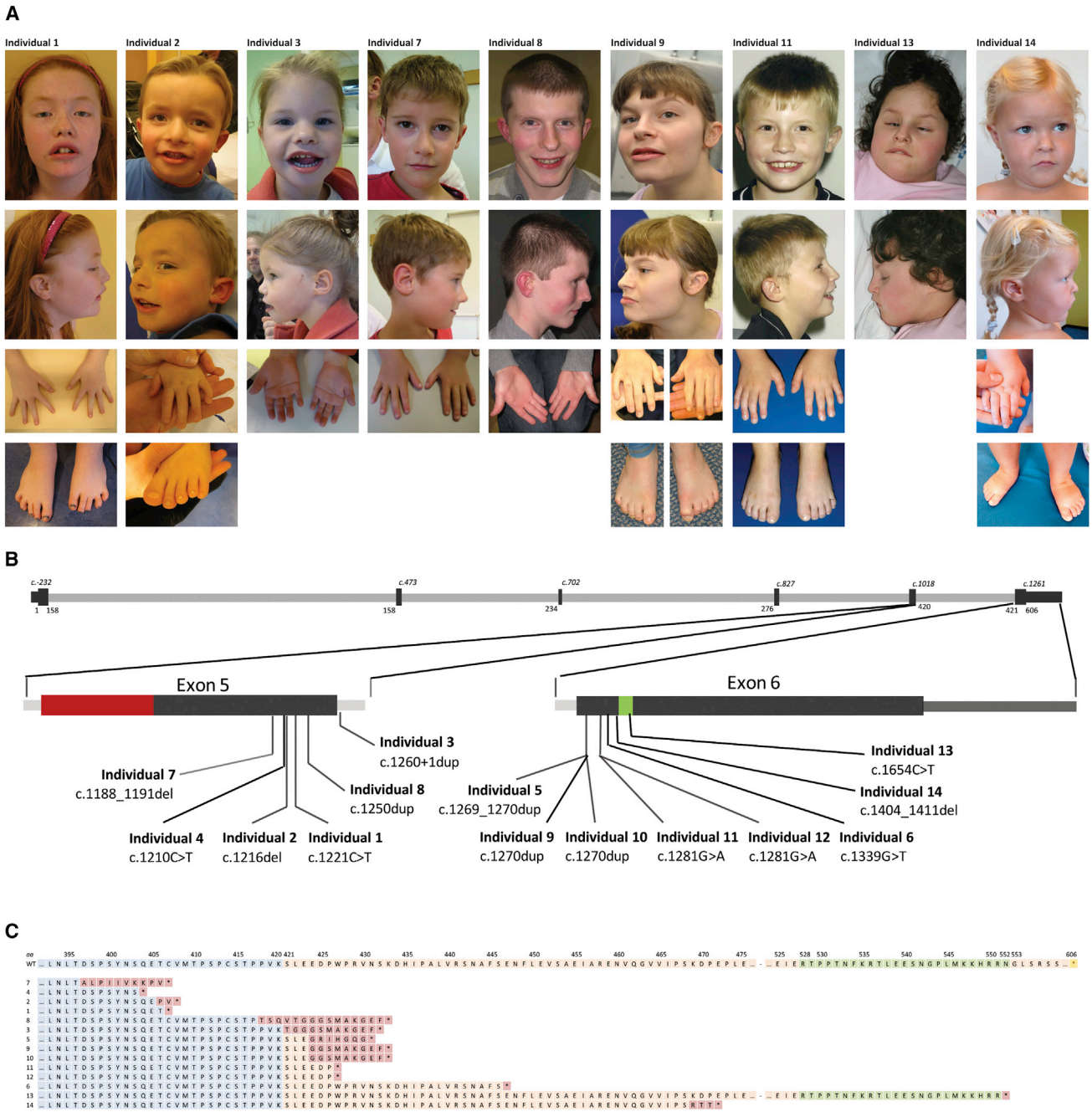

Figure 1. Photographs of Nine Individuals with a Truncating Mutation in *PPM1D*, De Novo Mutations in *PPM1D*, and Predicted Consequences at the Protein Level

(A) Shared facial features including a broad forehead, upturned nose, broad mouth with thin upper lip, and low-set posteriorly rotated ears. Extremities show small hands and feet with brachydactyly and hypoplastic toenails. Individual 13 also had a confirmed diagnosis of Potocki-Shaffer syndrome. Parents provided informed consent for the publication of these photographs.

(B) Schematic representation of the coding sequence of *PPM1D* (GenBank: NM_003620.3), including zoomed-in exons 5 and 6. All de novo *PPM1D* mutations identified are depicted according to their location in the coding sequence. Protein domain structures encoded by exons 5 and 6 are highlighted in color: red for the PPM-type phosphatase domain (in exon 5) and green for the nuclear localization signal (in exon 6).

(C) Predicted protein sequences in individuals 1–14. The last part of the translated sequence of exon 5 is in blue, and the amino acids encoded by the first part of exon 6 are in orange. For individuals 1–14, the predicted mutant amino acids are depicted in red. Abbreviations are as follows: WT, wild-type; and aa, amino acid.

the disease-causing mutations could have other pathophysiological mechanisms not directly inferable from such metrics in the ExAC Browser.

PPM1D has previously been shown to be expressed in both mouse and human brain,[23,24] but a second hint toward a role for *PPM1D* in the occurrence of ID would

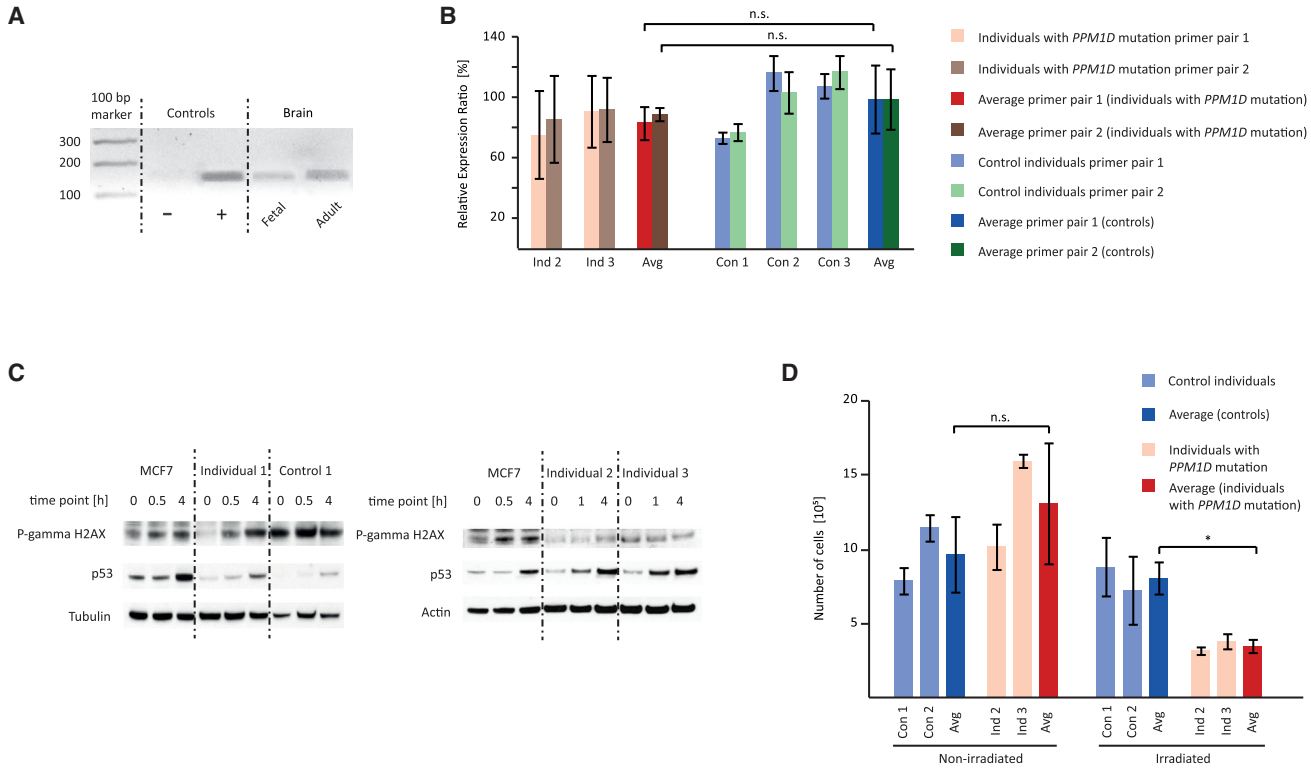

Figure 2. Functional Effects of *PPM1D* Mutations at the RNA and Protein Levels and Downstream Effects on p53 Activation and Cell Growth

(A) Semiquantitative PCR using primers PPM1D_3 (forward: 5′-AACCTGACTGACAGCCCTTC-3′; reverse: 5′-ACCAGGGCAGGTA TATGGTC-3′) on tissue-specific cDNA libraries shows *PPM1D* expression in fetal and adult brain. EBV-LCL cDNA is the positive control (+), and ddH_2O is the negative control (−).

(B) Expression levels of *PPM1D* were quantified by qPCR using cDNA obtained from EBV-LCLs derived from individuals with a mutation in *PPM1D* (individuals 2 and 3). Experiments were performed in triplicate with two sets of primer pairs: PPM1D_1 (forward: 5′-TGC TTGTGAATCGAGCATTG-3′; reverse: 5′-CCCTGATTGTCCACTTCTGG-3′) and PPM1D_2 (forward: 5′-AAGTCGAAGTAGTGGTGCT CAG-3′; reverse: 5′-TCTTCTGGCCCCTAAGTCTG-3′). Analysis was performed with SDS software according to standard procedures, and *GUSB* expression was used as a calibrator (forward: 5′-AGAGTGGTGCTGAGGATTGG-3′; reverse: 5′-CCCTCATGCTCTAGC GTGTC-3′).[14] There was no significant change in *PPM1D* mRNA expression between affected individuals and control lines. Abbreviations are as follows: n.s., not significant; Ind, individual; Con, control individual; and Avg, average. Results are presented as the average ± SD.

(C) Radiation-induced activation of p53 was investigated in fibroblasts derived from individual 1, EBV-LCLs derived from individuals 2 and 3, healthy control cells, and the cancer cell line with active PPM1D (MCF7 as the positive control). Cells were exposed to gamma irradiation (5 Gy) from an X-ray source. Whole-cell lysates were generated from cells 30–60 min and 4 hr after irradiation and subjected to protein electrophoresis. Immunoblotting of electrophoresed lysates was performed with antibodies specific to p53 (9282S), phospho-Histone γH2AX (ser139) (9718S), and actin (I-19), or β-tubulin (D-10). Anti-rabbit, anti-mouse, and anti-rabbit secondary antibodies were incubated with the blot (1:5,000) for 1 hr at room temperature, and then exposure using enhanced chemiluminescent detection followed. Western blot analysis showed no difference between case and control cell lines but did show increased p53 activation in the *PPM1D* mutant breast cancer cell line MCF7.

(D) Growth behavior of EBV-LCLs derived from individuals with a mutation in *PPM1D* (individuals 2 and 3) was compared with that of age- and sex-matched control EBV-LCLs. Cells were irradiated (UV light: 60 J/m^2) and cultured in a concentration of 3×10^5 cells/mL. Cell numbers were counted in triplicate after 48 hr, and the experiment was repeated three times. 48 hr after irradiation, the number of EBV-LCLs derived from individuals with a mutation in *PPM1D* ($n = 2$) was significantly lower than that of control cells ($n = 2$), whereas the growth of untreated cells was unaffected. Abbreviations are as follows: *, $p < 0.05$; n.s., no significance; Ind, individual; Con, control individual; and Avg, average. Results are presented as the average ± SD.

be its expression in the developing brain. We therefore investigated *PPM1D* expression in cDNA libraries obtained from fetal and adult brain by using semiquantitative PCR. Human cDNA libraries from different human tissues were purchased from Stratagene. Expression of *PPM1D* in embryonic and adult brain and EBV-LCLs (positive control) was investigated with *PPM1D*-transcript-specific semiquantitative PCR using the primer set PPM1D_3. We detected *PPM1D* expression in both fetal and adult brain (Figure 2A). In addition, our analysis showed wider fetal (developmental), whereby *PPM1D* expression was detected in fetal liver and skeletal muscle, but not in their adult counterparts (data not shown). The expression of *PPM1D* in the fetal brain suggests a role during fetal brain development and thus potentially in developing normal cognition. Although we were not able to further narrow down

the expression to detailed brain regions, the highest *Ppm1d* expression in mice has been reported in the cerebellum,[23] which is the center for coordination. Several of our affected individuals had a broad-based gait, a possible sign of cerebellar disturbance, which might therefore be associated with the mutation in *PPM1D*. However, the individuals did not display other symptoms of coordination defects, and in the individuals who had received brain MRI, no structural abnormalities of the cerebellum could be identified.

PPM1D has also been reported to be an important regulator of global heterochromatin silencing and thus critical in maintaining genome integrity.[25] The latter was examined in germ cells of *Ppm1d*-deficient mice, which showed enlarged heterochromatin centers with enriched immunofluorescent staining for H3K9me3 and HP1γ, both markers for transcriptional repression.[25] When PPM1D dysfunction indeed alters gene expression, this might have an effect on fetal (brain) development. Moreover, *Ppm1d*-deficient mice show an increase in anxiety and depression-like behavior,[26] suggesting a potential protective function of PPM1D in mood stabilization.[26] Interestingly, four of the individuals with a *PPM1D* mutation showed anxiety.

PPM1D (also known as *Wip1*) is, like other genes encoding PPM and PP2C phosphatases, a regulator of stress response.[27] In particular, PPM1D regulates the DNA damage response (DDR) pathway by inhibiting p53 and other tumor suppressors (p38, ATM, Chk1, and Chk2) through dephosphorylation of these proteins.[27] Previously substantial amounts of work have gone into the role of *PPM1D* in tumorigenesis, given that acquired *PPM1D* mutations have been identified in individuals with breast, ovarian, colon, and lung cancer and are postulated to exert their effect through gain of function.[27–31] However, these mosaic mutations in lymphocytes were shown to occur only in individuals who had undergone chemotherapy and were shown to be absent in DNA isolated from the germline prior to chemotherapy.[27–31] The gain-of-function effect was shown by overexpression of cancer-associated *PPM1D* mutations in tumor cells, which suppressed ionizing radiation (IR)-induced molecular responses.[28] In normal conditions, exposure to IR causes upregulation of p53 levels and phosphorylation of the histone H2AX (γH2AX), events that are normally prevented by PPM1D activity. In tumor cells with overexpression of cancer-associated *PPM1D* mutations, p53 and γH2AX upregulation after IR exposure is impaired, suggesting that in tumor cells, truncating *PPM1D* mutations are hyperactive.[28] We tested the upregulation of p53 and γH2AX in MCF7 cells, a breast cancer cell line serving as a positive control, which showed the expected upregulation of p53 and γH2AX, suggesting that the IR exposure was successful (Figure 2C). Compared with healthy control cells, fibroblasts from individual 1 and EBV-LCLs derived from individuals 2 and 3 showed normal p53 and γH2AX responses (Figure 2C). In conclusion, these data indicate that ID-associated *PPM1D* mutations do not cause p53 depletion, which suggests a pathophysiological mechanism different from the acquired *PPM1D* cancer-associated mutations.

As a regulator of the DDR, PPM1D plays an important role in cell-cycle control by positively upregulating G1-to-S phase progression.[32] We hypothesized that the ID-associated mutations in *PPM1D* would lose this positive upregulation and thereby cause cells to stall in the G1-to-S phase, leading to reduced cell proliferation. We therefore next tested whether EBV-LCLs derived from individuals 2 and 3 showed growth abnormalities in comparison with age- and sex-matched control EBV-LCLs. For this, cells were exposed to IR and analyzed for growth characteristics (Figure 2D). Indeed, cells derived from individuals showed 50% less growth than control lines, whereas the growth of untreated cells was unaffected (Figure 2D), showing that heterozygous PPM1D truncation leads to growth disadvantage after radiation. Hence, if the effect of the truncating mutations in our cases is a gain of function, this does not seem to affect the role of PPM1D on p53. However, a cell-growth disadvantage was observed after IR, suggesting that another function related to PPM1D cell-cycle checkpoints might be compromised.

Several genes are known to have somatic mutations that lead to cancer but germline mutations that cause an ID phenotype. Examples include genes encoding components of the RAS-MAPK pathway, *SETBP1* (SET binding protein 1 [MIM: 611060]), and *CTNNB1* (catenin beta 1 [MIM: 116806]).[33–36] Also, some of these germline mutations give rise to a higher cancer risk, whereas others do not.[33–36] Germline *PPM1D* mutations in individuals with cancer have to our knowledge not yet been reported. Although it is known that germline mutations in some genes, for instance *NF1* (neurofibromin 1 [MIM: 613113]), cause ID and a higher risk of (benign) tumors,[37] none of the individuals studied here (2–21 years old) have developed cancer. Thus, we cannot exclude nor confirm the possibility that the *PPM1D* mutations in the individuals with ID predispose to cancer.

In conclusion, de novo truncating germline mutations in the last and penultimate exons of *PPM1D* lead to an ID syndrome with behavioral problems, hypotonia, broad-based gait, periods of fever and vomiting, high pain threshold, short stature, small hands and feet, and overlapping facial dysmorphisms. Exposure of affected cells to IR resulted in normal p53 activation, suggesting that p53 signaling is not affected by the truncated protein. Nonetheless, a cell-growth disadvantage after IR was observed. The significant enrichment of de novo mutations in individuals with ID, the expression in the developing and mature brain, and this clinical ID syndrome underscore the role of *PPM1D* in neurodevelopment.

Supplemental Data

Supplemental Data include a Supplemental Note and one figure and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2017.02.005.

Conflicts of Interest

K.G.M. is an employee of GeneDx, Inc.

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Web Resources

Allen Brain Atlas, http://human.brain-map.org
Allen Mouse Brain Atlas, http://mouse.brain-map.org
DECIPHER, https://decipher.sanger.ac.uk/
ExAC Browser, http://exac.broadinstitute.org/
GeneMatcher, https://genematcher.org/
OMIM, http://www.omim.org/
Our *PPM1D* website, http://www.ppm1dgene.com
RefSeq, https://www.ncbi.nlm.nih.gov/refseq/

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