Huntington’s disease: Neural dysfunction linked to inositol polyphosphate multikinase

Ishrat Ahmed, Juan I. Sbodio, Maged M. Harraz, Richa Tyagi, Jonathan C. Grima, Lauren K. Albacary, Maimon E. Hubbi, Risheng Xu, Seyun Kim, Bindu D. Paul, and Solomon H. Snyder

The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205; McKusick–Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205; Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea; Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205; and Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205

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Huntington’s disease (HD) is a progressive neurodegenerative disease caused by a glutamine repeat expansion in mutant huntingtin (mHtt). Despite the known genetic cause of HD, the pathophysiology of this disease remains to be elucidated. Inositol polyphosphate multikinase (IPMK) is an enzyme that displays soluble inositol phosphate kinase activity, lipid kinase activity, and various noncatalytic interactions. We report a severe loss of IPMK in the striatum of HD patients and in several cellular and animal models of the disease. This depletion reflects mHtt-induced impairment of COUP-TF-interacting protein 2 (Ctip2), a striatal-enriched transcription factor for IPMK, as well as alterations in IPMK protein stability. IPMK overexpression reverses the metabolic activity deficit in a cell model of HD. IPMK depletion appears to mediate neural dysfunction, because intrastratal delivery of IPMK abates the progression of motor abnormalities and rescues striatal pathology in transgenic murine models of HD.

IPMK Protein Is Depleted in HD Striatum.

Huntington’s disease (HD) is an autosomal dominant disorder manifesting profound neurodegeneration and dementia with motor abnormalities deriving from the expansion of glutamine repeats in mutant huntingtin (mHtt) (1). This disease mainly affects the striatum, resulting in the dysfunction and death of striatal medium spiny neurons (2). Although the genetics of HD are well delineated, the specific mechanisms of disease mainly affects the striatum, resulting in the dysfunction and death of striatal medium spiny neurons (2). Striatal volume is most prominently affected in HD (2). The R6/2 transgenic murine model of HD involves about 150 glutamine repeats (21). We examined IPMK levels in the striatum of R6/2 mice, because this portion of the brain is most prominently affected in HD (2). Striatal IPMK protein levels are reduced in R6/2 striatum (Fig. 1C) and in

Results

IPMK Protein Is Depleted in HD Striatum. A useful model of HD is the immortalized striatal progenitor cell line with 111 glutamine repeats, STHdhQ111/Q111 (Q111), and the control cell line with seven glutamine repeats, STHdhQ7/Q7 (Q7) (20). IPMK protein is depleted by 75% in Q111 cells (Fig. 1A). This deficit appears to reflect, at least in part, defective IPMK transcription, because IPMK mRNA levels are reduced by 40% in Q111 cells (Fig. 1B). Furthermore, IPMK produces the soluble inositol phosphates IP₃ and IP₄, both of which are depleted in Q111 cells (Fig. S1A and B). Levels of inositol hexakisphosphate (IP₆), the catalytic product of IP₅ 2-kinase (IPPK), are unaltered, likely because of elevated IPPK protein expression in Q111 cells (Fig. S1C). The R6/2 transgenic murine model of HD involves about 150 glutamine repeats (21). We examined IPMK levels in the striatum of R6/2 mice, because this portion of the brain is most prominently affected in HD (2). Striatal IPMK protein levels are reduced in R6/2 striatum (Fig. 1C) and in

Significance

Huntington’s disease (HD) is a progressive neurodegenerative disorder affecting the striatum. The striatal-enriched transcription factor COUP-TF-interacting protein 2 (Ctip2) is depleted in HD and has been identified as a putative transcription factor for the enzyme inositol polyphosphate multikinase (IPMK). IPMK displays soluble inositol phosphate kinase activity, lipid kinase activity, and several noncatalytic activities including its role as a transcriptional coactivator. We describe severe depletion in IPMK protein in HD patients and several animal and cell models of the disease. IPMK overexpression rescues the metabolic impairments in a cell model of HD. Furthermore, delivery of IPMK in a transgenic HD model improves pathological changes and motor performance. The Ctip2–IPMK–Akt signaling pathway provides a previously unidentified therapeutic target for HD.


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confirmed the loss of Ctip2 in R6/2 striatum (Fig. S2). Accordingly, we explored its relevance to HD. We recently revealed Ctip2 as a putative transcription factor for cortex and hippocampus but not cerebellum (Fig. S1). Information on age, sex, and postmortem delay (PMD) of these striatal tissues is provided in Table S1.

Conversely, overexpression of Ctip2 reverses the loss of IPMK protein in Q111 cells, restoring them to normal levels in Q111 cells at the T308 and S473 sites (Fig. 3D). The lack of activity of IPMK-KASA indicates that the catalytic activity of IPMK is required for rescue. The inactivity of atIPK2β suggests that the PI3-kinase activity of IPMK is responsible for restoring the metabolic activity of Q111 cells.

### IPMK Expression Rescues mHtt-Induced Deficits in Mitochondrial Metabolic Activity.

We wondered whether the depletion of IPMK in HD is responsible for the molecular and behavioral abnormalities of HD. If so, restoring the depleted IPMK should be beneficial. We assessed mitochondrial metabolic activity of cells by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Fig. 3A). The metabolic activity of Q111 cells is only half that of Q7 cells (23). Overexpressing IPMK alleviates this abnormality (Fig. 3A). IPMK possesses both inositol phosphate kinase and PI3-kinase activities as well as displaying various noncatalytic actions (11, 14–17). To ascertain which of these activities mediates the beneficial effects of IPMK, we overexpressed IPMK K129A (IPMK-KASA), which is devoid of both inositol phosphate kinase and PI3-kinase activities. We also overexpressed the Arabidopsis thaliana ortholog, atIPK2β, which possesses inositol phosphate kinase but not PI3-kinase activity and has been shown to restore inositol phosphate production in IPMK−/− mouse embryonic fibroblasts (14). Neither IPMK-KASA nor atIPK2β rescue the depressed metabolic activity of Q111 cells (Fig. 3B). The lack of activity of IPMK-KASA indicates that the catalytic activity of IPMK is required for rescue. The inactivity of atIPK2β suggests that the PI3-kinase activity of IPMK is responsible for restoring the metabolic activity of Q111 cells.

### Adeno-Associated Virus Serotype 2–Mediated Delivery of IPMK Improves Psychomotor Performance in a Transgenic Mouse Model of HD.

Is the IPMK deficit in HD responsible for the pathological and motor abnormalities of HD? We investigated whether direct administration of IPMK-expressing adeno-associated virus serotype 2 (AAV2) in the striatum of R6/2 HD mice (Fig. 4A and B) influences the pathology and behavioral phenotype of these animals over time (Fig. S3A). Although no effects are observed on weight and survival (Fig. S3 B and C), viral overexpression of IPMK in the striatum of R6/2 mice reduces the number of mHtt aggregates and the size of these aggregates by ~75% and 30%, respectively, at 10 wk of age (Fig. 4 C–E). These pathological changes correspond with the delay in motor deficits observed in R6/2 animals. Repletion of IPMK restores central locomotor activity of the R6/2 mice to levels that are not significantly lower than those of wild-type mice at 6 wk (Fig. 4F). However, IPMK overexpression does not significantly improve rotarod performance (Fig. S4A), likely because of the earlier onset of this.
particular deficit. In a balance beam model, the time to cross is increased eightfold in R6/2 mice compared with wild-type animals (Fig. 4G). This time is reduced by half in IPMK-replenished mice. We also evaluated a composite phenotype (25) of HD abnormalities, which consist of hindlimb claspig, gait abnormalities, kyphosis, and ledge walking (Fig. 4F). The composite phenotype score is reduced almost by half with IPMK repletion. This reduction is consistent with improvement in gait, specifically stride length, in R6/2 mice receiving the IPMK-expressing virus (Fig. S4B). Furthermore, there is reduced forefootprint–hindfootprint overlap in the R6/2 animals, which appears to improve with IPMK delivery. We did not observe significant differences in balance beam and composite scores relative to wild-type mice before 10 wk of age.

Discussion

In the present study we report a dramatic depletion of IPMK in the striatum of humans with HD as well as in Q111 HD cells and in the R6/2 and zQ175 murine models of HD. The depletion of IPMK occurs at both the transcriptional and protein stability levels and corresponds with decreased Akt signaling (Fig. 4F). IPMK depletion appears to mediate, at least in part, the pathology and motor deficits in HD, because viral expression of IPMK in the striatum of R6/2 mice, the brain region primarily affected in clinical HD, delays locomotor deficits of the animals and reduces the number and size of aggregates. Although grade III and IV HD are advanced and are characterized by severe neuronal loss in the striatum, numerous proteins are down-regulated at these stages because of mHtt-mediated transcriptional dysregulation rather than merely reflecting pathogenic tissue loss (5, 6, 8, 26–28). Additional studies during earlier stages of HD may discriminate differential sensitivities of specific neuronal populations in HD.

The striatal-enriched transcription factor Ctip2 appears to determine IPMK transcription. Its overexpression reverses the IPMK depletion in Q111 cells. Ctip2 protein itself is also selectively expressed in striatal medium spiny neurons (29), the cell type uniquely lost in HD (2). The depletion of Ctip2 in the striatum, cortex, and hippocampus of R6/2 mice is consistent with the altered expression pattern of IPMK in these mice, which reflects the HD pathology in the cortex and additional brain tissues (30). Interestingly, Ctip2 overexpression in Q7 cells does not change IPMK protein levels, suggesting a potential negative feedback effect of IPMK or other targets on Ctip2 transcriptional activity.

Altered IPMK protein stability and lysosomal degradation also contribute to the loss of IPMK protein in the cellular model of HD. Although both macroautophagy and the ubiquitin proteasome system are impaired in HD, chaperone-mediated autophagy is constitutively up-regulated in early stages of the disease, thereby increasing the turnover of both wild-type Htt and mHtt fragments (31). The selective interaction of IPMK with the N-terminal fragment of Htt might explain the loss of IPMK through lysosomal degradation in Q111 cells but not Q7 cells.

We provide several lines of evidence that the depletion of IPMK is pathogenic in HD. Overexpressing IPMK restores to normal the depressed mitochondrial metabolic activity of Q111 cells, an action that appears to be determined by the PI3-kinase activity of IPMK. IPMK and the p110 PI3-kinase act co-ordinately in generating PIP3 (14), a classic stimulant of Akt activity. IPMK and the p110 PI3-kinase act co-ordinately in generating PIP3 (14), a classic stimulant of Akt activity. IPMK occurs at both the transcriptional and protein stability levels and corresponds with decreased Akt signaling (Fig. 4).

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protein arfaptin2, which rescues mHtt-induced proteosomal impairment (34).

mHtt is phosphorylated by Akt at the S421 site, which restores fast axonal transport by altering the mHtt interaction with dynactin (35). Conversely, excitotoxic stimulation of NMDA receptors (NMDARs) reduces mHtt S421 phosphorylation (36). Because the R6/2 animals used in this study express the N-terminal fragment of mHtt rather than full-length mHtt, the observed effects of IPMK likely do not require direct phosphorylation of mHtt by Akt. Thus, through the multiple downstream effects of Akt, IPMK deficit may account for the notably pleiotropic manifestations of HD. IPMK also may have additional functions, because its lipid kinase activity is required for the selective export of mRNA (37).

Several signaling pathways implicated in HD converge on Akt. The BDNF pathway is altered in HD because of decreased transcription and release of BDNF at the corticostriatal synapses (4, 38) as well as impaired TrkB receptor signaling (39). Similarly, synaptic (but not extrasynaptic) NMDARs enhance Akt phosphorylation and neuroprotection (40).

Intrastrial delivery of IPMK in R6/2 mice improved motor deficits in the R6/2 model of HD. IPMK overexpression had the greatest effects on balance beam performance and gait. The motor deficits assessed using these tests appear during later symptomatic stages (after age 8.5 wk) in R6/2 animals (41). Mild to no effects were observed in open field and rotarod testing, respectively, likely because the corresponding motor deficits occur at age 5 wk. Thus, earlier delivery of IPMK may have greater effects on the behavioral phenotype of these R6/2 animals. In addition to motor deficits, early clinical manifestations of HD include cognitive symptoms (42). IPMK regulates the induction of immediate early genes required for learning, memory, and behavior, because IPMK-deleted mice display aberrant spatial memory (17).

Additional clinical features of HD include peripheral organ dysfunction such as weight loss, skeletal muscle wasting, and metabolic and endocrine alterations (43). IPMK influences metabolism through its inhibition of AMP-activated kinase (AMPK) activation (44). AMPK promotes catabolic pathways while inhibiting various anabolic pathways such as cholesterol and triglyceride synthesis (45). Interestingly, a pathologic increase in AMPK phosphorylation has been demonstrated in HD patients and in the R6/2 model (46). IPMK delivery in R6/2 animals did not improve body weight and survival, probably because IPMK was expressed only in the striatum. Conceivably, widespread expression of IPMK in HD models may improve peripheral organ dysfunction.

The small G protein Ras homolog enriched in striatum (Rhes) sumoylates mHtt, resulting in cytotoxicity (47) (Fig. 4J), which is further enhanced through the interaction of Rhes and mHtt with acyl-CoA binding domain containing 3 (ACBD3) (48). An in vivo screen for SUMO1 substrates demonstrated that Ctip2 is sumoylated (49). Perhaps Rhes also modulates the Ctip2–IPMK–Akt signaling pathway. Interestingly, Rhes has been shown to recruit the regulatory subunit of PI3K to the cell membrane, subsequently enhancing Akt phosphorylation in healthy cells (Fig. 4J) (50). We also reported a marked depletion of cystathionine γ-lyase (CSE) in HD (51). CSE is a rate-limiting enzyme in the biosynthesis of cysteine and generation of the gasotransmitter hydrogen sulfide (51, 52). Treating R6/2 mice with cysteine or cysteine precursors markedly improves the motor performance of R6/2 mice, similar to the improvement that we have observed with viral delivery of IPMK.

Fig. 3. The lipid kinase activity of IPMK rescues the mitochondrial metabolic activity deficit in Q111 striatal cells and restores Akt signaling. (A) IPMK overexpression rescues the mitochondrial metabolic activity deficit in Q111 cells as measured by the MTT assay. (B) Overexpression of the kinase-dead mutant of IPMK (IPMK-KASA) or atIPK2 δ does not rescue the metabolic activity deficit measured by the MTT assay. In A and B, bars represent means ± SEM (n = 4). **P < 0.01, ****P < 0.0001 relative to the Q7 empty vector control unless otherwise indicated. (C) Akt phosphorylation at the T308 site [p-Akt (T308)] is decreased in Q111 cells. (D) Akt phosphorylation at the S473 site [p-Akt (S473)] also is reduced in Q111 cells. In C and D, bars represent means ± SEM normalized to total Akt (n = 3). **P < 0.01 relative to the Q7 control. (E) IPMK overexpression similarly rescues the loss of p-Akt (T308) in Q111 cells but not in Q7 cells. (F) IPMK overexpression rescues the loss of p-Akt (S473) in Q111 cells. In E and F, bars represent means ± SEM normalized to total Akt (n = 4). *P < 0.05 relative to the Q711 empty vector control sample.
aggregates per 40 field of view measuring 0.1 mm². Bars represent the mean number of aggregates ± SEM (n = 3 animals). **P < 0.01 relative to either wild-type or R6/2 mice injected with control virus. (F) General phenotype based on clasping, kyphosis, gait, and ledge walking is presented as a composite score and is improved by IPMK overexpression. Bars represent the mean of the total time required to cross the beam ± SEM (n = 9–10 animals per group). *P < 0.05 relative to either wild-type or R6/2 mice injected with control virus. (G) IPMK restores motor coordination and balance assessed by balance beam performance. Bars represent the mean of the total time required to cross the beam ± SEM (n = 7–10 animals per group). **P < 0.01, ****P < 0.0001 relative to either wild-type or R6/2 mice injected with control virus. (H) General phenotype based on clasping, kyphosis, gait, and ledge walking is presented as a composite score and is improved by IPMK overexpression. Bars represent the mean of the total time required to cross the beam ± SEM (n = 9–10 animals per group). *P < 0.05 relative to R6/2 mice receiving control virus. (I) In normal striatal cells, Ctip2 up-regulates IPMK expression. IPMK displays several functions, including the lipid kinase activity, which enhances Akt signaling, as well as a soluble inositol phosphate kinase activity and various noncatalytic activities. In HD, Ctip2 transcriptional activity and expression is inhibited by mHtt, resulting in decreased IPMK transcription. Decreased IPMK protein stability, likely caused by the selective interaction with mHtt, further reduces IPMK protein levels, resulting in the loss of Akt phosphorylation. (J) Our current model indicates that Ctip2 up-regulates IPMK, which in turn enhances Akt phosphorylation. Rhes also has been shown to increase Akt phosphorylation in healthy cells. Additional mechanisms in HD cells (indicated in red), include Akt-mediated phosphorylates and inhibition of mHtt. Furthermore, Ctip2 and IPMK are both impaired by mHtt. The roles of Rhes in modulating Ctip2 function in healthy and HD cells and the effect of Rhes on Akt in the presence of mHtt remain to be elucidated.

Experimental Procedures

Cell Cultures and Reagents. The immortalized striatal cell lines STMitR Q7/Q7 (Q7) and STMitR Q111/Q111 (Q111) express endogenous wild-type Htt and mHtt with seven or 111 glutamine repeats, respectively. These cell lines were provided by M. MacDonald of the Department of Neurology, Massachusetts General Hospital, Boston. The Q7 and Q111 cells were maintained at 33 °C in DMEM supplemented with 10% (vol/vol) FBS, 2 mM l-glutamine, 400 μg/mL Geneticin, and antibiotics (penicillin and streptomycin). Experiments were performed in the absence of Geneticin.

Animals. Animals were housed and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (53), and animal experiments were approved by the Johns Hopkins University Animal Care and Use Committee (JHU ACUC). Animals were kept on a 12-h light/dark cycle and were provided food and water ad libitum.

Postmortem Brain Tissues. Striatal tissues from control and HD patients were obtained from J. Troncoso and O. Pletnikov (Brain Resource Center, Johns Hopkins University).

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Stereotoxic Surgery. AAV2 containing either a GFP-only control vector or IPMK was generated by Vector BioLabs at a titer of 3.1 × 10^12 genome copies (GC)/mL. Three-week-old male mice were anesthetized using 300 μL Avertin (20 mg/mL solution). Virus was injected at the following coordinates: anterior (A) −0.8, lateral (L) 2, ventral (V) −3.3; A −0.8, L 2, V −3.3; A −0.8, L 2, V −3.1; and A −0.8, L 2, V −2.9 for a total of 4 μL virus in each striatum.

Statistical Analysis. Statistical analysis was performed using Excel software (Analysis ToolPak). Student’s t test and single-factor ANOVA were performed. All error bars represent ± SEM. Significance was determined as P < 0.05.

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SI Experimental Procedures

Cell Cultures and Reagents. HEK293 cells were transfected with PolyFect (Qiagen). The plasmids encoding wild-type IPMK, kinase dead IPMK (KASA), and the Arabidopsis thaliana ortholog atIPK2β were described previously (14, 16, 18). The Ctip2 plasmid was purchased from OriGene. The N-terminal fragments of wild-type Htt (N171–180Q) and mHtt (N171–82Q) were described previously (47). Ctip2 siRNA and scrambled siRNA (Santa Cruz) were used at a concentration of either 50 pmol or 100 pmol. For translational blocking, cells were treated with 30 μg/mL cycloheximide (Sigma). Q7 and Q111 cells were treated with MG132 and bafilomycin, inhibitors of proteasomal degradation and lysosomal degradation, respectively, for 13 h at the indicated concentrations.

Animals. The R6/2 mice [B6CBA-Tg(HDexon1)62Gpb/1J], a transgenic model of HD which expresses exon 1 of human Htt, were purchased from Jackson Laboratory. This line carries ∼150 polyglutamine repeats. The breeding strategy involves ovarian transplant hemizygous females and B6CBAF/1J males. Only F1 R6/2 and noncarrier sibling males were used for the experiments. zQ175 mice, obtained from the Cure Huntington’s Disease Initiative Foundation, are a knockin model of HD and contain one chimeric human/mouse exon 1 allele with ∼190 polyglutamine repeats (22).

Quantitative Real-Time PCR. RNA was isolated from Q7 and Q111 cells using the RNeasy mini kit (Qiagen). Quantitative real-time PCR was performed using the TaqMan RNA-to-Ct 1-Step kit and the StepOnePlus Real-Time PCR system (Life Technologies). TaqMan probes for mouse IPMK and mouse Ctip2 were used. Data were normalized to β-actin.

Western Blotting. Q7 and Q111 cells were lysed in a buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 10% (vol/vol) glycerol, and protease and phosphatase inhibitors (standard lysis buffer). Animals were killed with CO2 in accordance with the JHU ACUC protocol. The striata of wild-type and R6/2 mice were extracted at wk 9. The striata of wild-type and zQ175 mice were extracted at 12 mo. These tissues were homogenized in standard lysis buffer using a hand-held homogenizer. Postmortem human tissue was homogenized in RIPA buffer. Cell lysates and brain homogenates were centrifuged at 16,000 × g for 30 min, and the supernatant was collected for Western blotting. The antibodies used were against mouse IPMK (Covance), which was described previously (14), human IPMK (Genetex), total Akt, phospho-Akt (T308), and phospho-Akt (S473) (Cell Signaling Technology), and IPPK, GAPDH, and β-actin (Santa Cruz). The secondary anti-rabbit and anti-mouse antibodies were purchased from GE Healthcare. Densitometric analysis of Western blot bands was performed using ImageJ and normalized to β-actin, GAPDH, or total Akt.

Coimmunoprecipitation. Cells were lysed in standard lysis buffer 24 h after transfection. Lysates were further homogenized using a 25-gauge needle and then were centrifuged. Samples were pre-clearned using rabbit IgG-agarose (Sigma) for 1 h. Following protein measurement, supernatant containing 500 μg of protein was incubated overnight with EZView Red c-myc-agarose (Sigma). The beads were washed five times with wash buffer [50 mM Tris-HCl (pH 7.5), 300 mM NaCl, 1% Triton X-100, and 10% (vol/vol) glycerol]. Protein was eluted by boiling in 2x LDS sample buffer (Invitrogen) with 5% (vol/vol) 2-mercaptoethanol. n = 3 for coimmunoprecipitations.

MTT Assay. The MTT assay, which measures mitochondrial succinate dehydrogenase activity, was used to assess mitochondrial metabolic activity. Q7 and Q111 cells were transfected (in triplicate) 1 d after plating. After 48 h, cells were treated with 250 μg/mL MTT and incubated at 33°C for 1.5 h. After removal of the medium, 2 mL DMSO was added to each well to dissolve the formazan crystals. The absorbances at 570 nm and 630 nm were determined using a SpectraMax M5 plate reader (Molecular Devices) and Softmax Pro software. n = 6 for MTT experiments.

Inositol Polyphosphate Measurement. Q7 and Q111 cells were plated in six-well dishes. After reaching 60% confluency, the cells were labeled with 200 μCi of [3H]inositol (PerkinElmer) for 2 d. Soluble inositol phosphates were extracted using an acid (0.6 M perchloric acid and 2 mM EDTA), neutralized with 1 M K2CO3, and then centrifuged. EDTA (1 mM) was added to the supernatant. Following extraction, the inositol phosphates were resolved by anion-exchange HPLC as previously described (15). The soluble inositol phosphates were detected using a scintillation counter. IP5, IP6, and IP7 levels were normalized to myo-inositol levels in each sample. n = 6 for all inositol phosphate levels.

Stereotoxic Surgery. IPMK is expressed downstream of an eSYN promoter and an EGFP cassette followed by a T2A linker. EGFP is cleaved from IPMK once the proteins are expressed. For the stereotoxic injections, the zero coordinates were set at the bregma. Two holes were drilled, and the virus was delivered using a Hamilton syringe with a 33-gauge blunt-tip needle bilaterally. The needle was lowered at a rate of 1 mm/min into the striatum (A = −0.8, L 2, V −3.7). pulld back to A = −0.8, L 2, V −3.5, and left in place for 2 min. One microliter of virus was injected at this site and three additional sites along the ventral axis for a total of 4 μL virus in each striatum. After the last injection and hold, the needle was raised 0.2 mm and left in place for 5 min. The needle then was withdrawn at a rate of 0.5 mm/min.

Immunohistochemistry and Immunofluorescence. Male mice that had received AAV2 injections were anesthetized with 100 mg/kg pentobarbital and were perfused transcardially with 4% (wt/vol) paraformaldehyde (PFA) at 5 wk and 10 wk of age. Brains were postfixed for 24 h in 4% PFA, cryo-protected in 30% (wt/vol) sucrose. Free-floating sections 40-μm thick were harvested with a microtome. EM48 (Millipore) immunohistochemistry was detected using ABC Vectastain (Vector Labs). The number and size of aggregates were quantified using ImageJ. For immunofluorescence, the sections were mounted with ProLong Gold antifade reagent with DAPI (Life Technologies) and were visualized using a Zeiss LSM 510 confocal microscope.

Behavioral Assays. Locomotor activity was assessed during the wake cycle using open field activity chambers with infrared photo beams (San Diego Instruments). Data were collected over 1 h following a 30-min acclimatization period. Horizontal and vertical activities were recorded automatically as beam breaks. For open field, n = 9 wild type with control virus, n = 10 wild type with IPMK virus, n = 11 R6/2 with control virus, and n = 12 R6/2 with IPMK virus. These tests were performed in the dark.

Motor coordination and balance were assessed using the rotarod and the balance beam tasks. For the rotarod test, mice were trained for 10 min at a constant speed. An accelerating protocol was then used and repeated three times at each time point. For the rotarod test, n = 7 wild type with control virus, n = 10 wild type with IPMK virus, n = 11 R6/2 with control virus, and n = 9 R6/2 with IPMK virus.
virus. For the balance beam task, the time to traverse a 60-cm-long wooden rod (50 cm above ground) away from a light source was recorded. Three measurements were taken for each animal \((n = 7)\) wild type with control virus, \(n = 10\) wild type with IPMK virus, \(n = 9\) R6/2 with control virus, and \(n = 9\) R6/2 with IPMK virus). The balance beam tests were performed in the dark.

Mice were evaluated for hindlimb clasping, ledge walking, kyphosis, and gait to obtain a composite phenotype score (25). A score of three is given for the worst performance in each category (for a maximum total score of 12). The wild-type mice received a perfect score of 0 \((n = 9)\) wild type with control virus, \(n = 9\) wild type with IPMK virus, \(n = 10\) R6/2 with control virus, and \(n = 9\) R6/2 with IPMK virus).

Gait analysis was performed using the footprint test. Hindpaws and forepaws were painted with blue and red, respectively. Animals walked on paper lining the floor of an elongated box starting from a light source at one end to a dark box at the other end. Three stride-length measurements were taken for each animal \((n = 6)\) wild type with control virus, \(n = 9\) wild type with IPMK virus, \(n = 10\) R6/2 with control virus, and \(n = 7\) R6/2 with IPMK virus).

Mice were tested at 6, 8, and 10 wk of age. All behavior assays were performed blind to virus treatment. For body weight, \(n = 10\) wild type with control virus, \(n = 9\) wild type with IPMK virus, \(n = 17\) R6/2 with control virus, and \(n = 13\) R6/2 with IPMK virus. For survival, \(n = 10\) wild type with control virus, \(n = 11\) wild type with IPMK virus, \(n = 11\) R6/2 with control virus, and \(n = 11\) R6/2 with IPMK virus.
Fig. S1. Catalytic activities of IPMK. (A) Both IPMK and the p110 PI3-kinase (PI3K) phosphorylate PtdIns(4,5)P$_2$ (PIP$_2$) to produce PtdIns(3,4,5)P$_3$ (PIP$_3$). PtdIns(4,5)P$_2$ also can be cleaved by phospholipase C (PLC), thereby generating Ins(1,4,5)P$_3$ (IP$_3$). IPMK additionally phosphorylates IP$_3$ to produce inositol-1,4,5,6-tetrakisphosphate [Ins(1,4,5,6)P$_4$] and inositol-1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P$_4$], both known as IP$_4$. Ins(1,3,4,5)P$_4$ also results from the catalytic activity of Ins(1,4,5)P$_3$-kinase (IP$_3$K). IPMK phosphorylates IP$_4$ to produce inositol-1,3,4,5,6-pentakisphosphate (IP$_5$), [Ins(1,3,4,5,6)P$_5$], which is subsequently phosphorylated by inositol 1,3,4,5,6-pentakisphosphate 2-kinase (IPPK or IPK1) to produce Ins(1,2,3,4,5,6)P$_6$. The higher inositol phosphates, containing pyrophosphate moieties, are not shown. Circles indicate phosphate groups. (B) IP$_4$, IP$_5$, and IP$_6$ levels are depleted in Q111 cells, but IP$_3$ levels remain normal. Bars represent mean disintegrations/min (D.P.M.) ± SEM normalized to myo-inositol levels ($n = 6$). *P < 0.05, **P < 0.01 relative to Q7 controls. (C) IPPK levels are elevated in Q111 cells. Bars represent means ± SEM normalized to β-actin ($n = 3$). *P < 0.05 relative to Q7 control. (D) IPMK protein levels are depleted in the cortex and hippocampus of R6/2 mice but not in the cerebellum. Bars represent means ± SEM normalized to β-actin ($n = 4$). *P < 0.05 relative to wild-type control.
Fig. S2. Ctip2 protein is depleted in HD. (A) Ctip2 protein levels are reduced in the R6/2 striatum relative to wild-type mice. Bars represent means ± SEM normalized to β-actin (n = 3). *P < 0.05 relative to wild type. (B) Ctip2 protein levels are also reduced in the cortex and hippocampus of R6/2 mice. Ctip2 is not expressed in the cerebellum. Bars represent means ± SEM normalized to β-actin (n = 3). *P < 0.05, **P < 0.01 relative to wild-type control. (C) Treatment of Q7 and Q111 cells with the proteasomal inhibitor MG132 did not alter IPMK protein expression. Bars represent means ± SEM normalized to β-actin (n = 3). *P < 0.05, **P < 0.01 relative to Q7 control.
Fig. S3. Survival and weight of wild-type and R6/2 mice injected with either control or IPMK-expressing virus. (A) Intrastriatal injections were performed at age 3 wk followed by behavior testing at 6, 8, and 10 wk of age. Pathology was assessed at 10 wk. (B) R6/2 mice injected with either control or IPMK virus show a similar and gradual decrease in weight over time. IPMK overexpression did not affect wild-type mice (n = 10 for wild type with control virus, n = 9 for wild type with IPMK virus, n = 17 for R6/2 with control virus, and n = 13 for R6/2 with IPMK virus). (C) The Kaplan–Meier curve indicates that striatal overexpression of IPMK did not alter survival in wild-type or R6/2 mice (n = 10 for wild type with control virus, n = 11 for wild type with IPMK virus, n = 11 for R6/2 with control virus, n = 11 for R6/2 with IPMK virus).
**Fig. S4.** Effect of virus-mediated expression of IPMK on additional motor performance. (A) IPMK overexpression does not affect rotarod performance at 6, 8, and 10 wk of age. Bars represent the mean time to fall in seconds ± SEM (n = 7–11 animals per group). *P < 0.05, **P < 0.01, ***P < 0.001 relative to wild-type mice injected with either control or IPMK virus. (B) IPMK overexpression improves R6/2 animal gait, specifically stride length at 10 wk of age. (Upper) Forepaw and hindpaw prints are shown in red and blue, respectively. (Lower) Bars represent mean stride length in centimeters ± SEM (n = 6–10 animals per group). *P < 0.05, **P < 0.01, ****P < 0.0001 relative to wild-type mice or R6/2 mice injected with control virus as indicated.
Table S1. Patient demographics for postmortem control and HD striatal tissues

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