Parthanatos mediates AIMP2-activated age-dependent dopaminergic neuronal loss

Yunjong Lee1–4, Senthilkumar S Karuppagounder1,3,4, Joo-Ho Shin1,3–5, Yun-Il Lee1,3,12, Han Seok Ko1,3,6, Debbie Swing7, Haisong Jiang1,3,4, Sung-Ung Kang1,3,4, Byoung Dae Lee1,3,8, Ho Chul Kang1,3,9,10, Donghoon Kim1,3,6, Lino Tesserollo7, Valina L Dawson1,11,13 & Ted M Dawson1,3,4,9,11,13

The defining pathogenic feature of Parkinson’s disease is the age-dependent loss of dopaminergic neurons. Mutations and inactivation of parkin, an ubiquitin E3 ligase, induce Parkinson’s disease through accumulation of pathogenic substrates. We found that transgenic overexpression of a parkin substrate, aminoacyl-tRNA synthetase complex interacting multifunctional protein-2 (AIMP2), led to a selective, age-dependent, progressive loss of dopaminergic neurons via activation of poly(ADP-ribose) polymerase-1 (PARP1). AIMP2 accumulation in vitro and in vivo resulted in PARP1 overactivation and dopaminergic cell toxicity via direct association of these proteins in the nucleus, providing a path to PARP1 activation other than DNA damage. Inhibition of PARP1 through gene deletion or drug inhibition reversed behavioral deficits and protected against dopamine neuron death in AIMP2 transgenic mice. These data indicate that brain-permeable PARP inhibitors could effectively delay or prevent disease progression in Parkinson’s disease.

Mutations in the ubiquitin E3 ligase PARKIN (also known as PARK2) are an important cause of familial Parkinson’s disease1–2. In general, the majority of mutations in PARKIN result in a loss of E3 ligase activity3–5. In the more common sporadic form of Parkinson’s disease, there may be a loss of parkin function as a result of S-nitrosylation, oxidative and dopaminergic stress, and phosphorylation by the stress-activated kinase c-Abl6–13. Parkin is a multifunctional E3 ligase that ubiquitinates proteins using different ubiquitin lysine linkages. Monoubiquitination of parkin substrates is thought to lead to alterations in receptor trafficking and cell signaling5. Polyubiquitination via lysine 63 or 29 linkages may be involved in inclusion body formation and autophagy14,15. Parkin substrates that are polyubiquitinated via lysine 48 linkages are degraded by the ubiquitin proteosome system. The loss of parkin function in Parkinson’s disease would be expected to impair the ubiquitin proteasome system clearance of lysine 48 substrates and interfere with other parkin E3 ligase functions16. Given that parkin is inactivated in familial Parkinson’s disease with PARKIN mutations, sporadic Parkinson’s disease, Parkin−/− mice (also known as Park2−/−) and following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication in mice8, substrates elevated in all four conditions are candidates for parkin-mediated polyubiquitination via lysine 48 linkages and subsequent ubiquitin proteosome degradation. AIMP2, also known as JTV-1 or P38, is a parkin substrate that is present in Lewy body inclusions of Parkinson’s disease substantia nigra17,18. AIMP2 is a strong candidate as a pathogenic substrate in Parkinson’s disease that accumulates in Parkinson’s disease as a result of parkin inactivation, as AIMP2 levels are elevated in the ventral midbrain in Parkin−/− mice and post-mortem brain from patients with PARKIN mutations or sporadic Parkinson’s disease7,9,18. AIMP2 also accumulates in the MPTP model of Parkinson’s disease, consistent with the notion that parkin is inactivated following MPTP intoxication18. If accumulation of a parkin substrate is important in the pathogenesis of Parkinson’s disease, transgenic overexpression in an animal model should lead to an age-dependent progressive degeneration of dopamine neurons.

To explore a potential biologic mechanism for AIMP2 and to validate its role as a pathogenic substrate in Parkinson’s disease, we generated a tetracycline-regulated inducible transgenic mouse model19,20 of AIMP2 overexpression. AIMP2 overexpression at levels seen in Parkinson’s disease leads to an age-dependent degeneration of dopaminergic neurons that causes striatal dopaminergic deficits and impairment of motor coordination. AIMP2 toxicity is not mediated by its canonical function because gross protein translation is normal. Unexpectedly, AIMP2 directly activates PARP1, which is important for the DNA damage response through poly(ADP-ribose)ylation of...
PARP1 itself and chromatin-associated proteins. Excessive activation of PARP1 kills cells via the formation of poly(ADP-ribose) (PAR) polymer in a cell death mechanism known as parthanatos. During parthanatos, PAR polymer translocates from the nucleus to the mitochondria and binds apoptosis-inducing factor (AIF). PAR polymer binding to AIF facilitates the release of AIF from the mitochondria and translocation to the nucleus followed by large-scale DNA fragmentation and nuclear condensation leading to the execution phase of parthanatos. Knockout or inhibition of PARP1 completely prevents the degeneration of dopaminergic neurons resulting from AIMP2 overexpression. Thus, AIMP2-mediated dopaminergic cell death is mediated by parthanatos, suggesting that PARP1 inhibition may effectively delay the progression of Parkinson's disease.

RESULTS

Generation of inducible transgenic AIMP2 mice

To investigate whether AIMP2 causes neuronal degeneration in vivo, we generated a conditional transgenic mouse model in which the expression of a C-terminal FLAG-tagged human AIMP2 is under the control of a tetracycline-responsive regulator (Fig. 1a). We identified 29 founders expressing TetP-AIMP2 via PCR screening for the tetracycline promoter (Supplementary Fig. 1a, b). Five of the highest copy number male founder mice were crossed with CamkIIα-tTA transgenic mice, and mice expressing both CamKIIα-tTA and AIMP2 were identified by PCR (Supplementary Fig. 1c).

Expression of AIMP2 was detected in ventral midbrain and cortex and compared with that of littermate controls expressing either Camk2a-tTA or TetP-AIMP2 alone. The overexpression of AIMP2 was tetracycline responsive, as doxycycline administration attenuated the upregulation of AIMP2 (Supplementary Fig. 1d). Line 630 overexpressed AIMP2 ~14-fold in the cortex and ~4-fold in the ventral midbrain compared with controls (Fig. 1b, c). Line 634 overexpressed AIMP2 ~4-fold in the cortex and ~3-fold in the ventral midbrain (Fig. 1b, c). Line 323 overexpressed AIMP2 ~5-fold in the cortex and ~2-fold in the ventral midbrain (Fig. 1b, c). As line 630 expressed the highest levels of AIMP2, we selected it for detailed characterization. Western blot analysis indicates that AIMP2 is overexpressed 4–15-fold throughout the forebrain, olfactory bulb, ventral midbrain and cerebellum with no overexpression in the pons and medulla (Fig. 1d, e). Immunohistochemistry with an antibody to AIMP2 revealed that the majority of AIMP2 overexpression occurred in neurons (Fig. 1f). To determine whether AIMP2 overexpression in the substantia nigra is localized to dopaminergic neurons, we performed colocalization studies with AIMP2 and tyrosine hydroxylase (Fig. 1g). AIMP2 was overexpressed in the majority of tyrosine hydroxylase-containing neurons in the substantia nigra (Fig. 1g).

Selective dopaminergic degeneration in AIMP2 mice

All lines of AIMP2 transgenic mice are viable and develop normally. AIMP2 transgenic mice and littermate control mice appeared to have comparable body weight for up to 3 months of age. Starting at 5 months of age, the AIMP2 mice gained less body weight than littermate controls and we observed a significant reduction in body weight at 14 and 20 months of age (P = 0.0000152 and P = 0.0092, respectively; Supplementary Fig. 2). To determine the effect of AIMP2 expression on dopamine neuronal viability, we monitored tyrosine hydroxylase immunoreactivity and Nissl staining in the substantia nigra zona compacta (SNpc) using unbiased stereologic methods. At 2–3 months of age, there was a trend toward a loss of tyrosine hydroxylase–positive and Nissl-stained neurons. At 8 months of age, there was a 37% loss of tyrosine hydroxylase–positive and Nissl-stained neurons in the SNpc that progressed to 53% at 20 months (Fig. 2a, b). In addition, approximately 28% of dopamine neurons in the ventral tegmental area (VTA) had degenerated in AIMP2 transgenic mice by 20 months of age (Supplementary Fig. 3a), consistent with the loss of VTA dopamine neurons in Parkinson’s disease. Thus, VTA dopamine neurons are less sensitive to AIMP2 toxicity than those in the SNpc, similar to the differential vulnerability of the SNpc and VTA dopamine neurons in Parkinson's disease. To further confirm that dopamine neuronal loss in the SNpc depends on AIMP2 accumulation, we characterized another transgenic line expressing AIMP2 at lower levels (line 634). At 10 months of age, there was a significant loss of dopamine neurons in this second line of AIMP2 transgenic mice (21%, P = 0.016; Supplementary Fig. 3b, c).

In the highest AIMP2-expressing line, no substantial loss of neurons was identified in other brain regions. In the cortex, which overexpressed AIMP2, ~14-fold there was a small reduction (10%) of neurons at 2 months of age, but there was no substantial difference in neuron number at 20 months of age between AIMP2 transgenic mice and age-matched littermate controls, suggesting that the degeneration of dopamine neurons induced by AIMP2 is selective (Fig. 2c, d).

To evaluate whether there are other neuropathologic abnormalities, we examined the brains of AIMP2 mice and littermate controls for astrocytosis. In 20-month-old AIMP2 transgenic mice, there was a substantial increase in glial fibrillary acidic protein (GFAP) immunoreactivity in the substantia nigra, consistent with the loss of dopaminergic neurons (Fig. 2e). There was no substantial difference in GFAP immunoreactivity in the cortex between the AIMP2 transgenic mice and age-matched littermate controls (Fig. 2f). Western blot analyses of the levels of GFAP confirmed that there was a significant, greater than threefold and twofold, upregulation of GFAP in the substantia nigra of 20-month-old (P = 0.00172) and 3-month-old (P = 0.0217) AIMP2 transgenic mice, respectively, but no change in the cortex (Fig. 2f, g).

Behavioral and dopaminergic deficits in AIMP2 mice

We examined the motor function of AIMP2 transgenic mice by rotarod and open field testing. AIMP2 transgenic mice performed normally in the open field test compared with age-matched littermate controls at 8 and 20 months of age (Fig. 3a, b). In an accelerating rotarod procedure, AIMP2 transgenic mice showed a progressive loss of their ability to remain on the rod compared with age-matched littermate controls (Fig. 3b). In a dopamine-sensitive pole test, AIMP2 transgenic mice exhibited deficits in climbing down the pole as assessed at 20 months of age (time to ground in pole test, mean ± s.e.m.: control, 43.8 ± 11.2 s; transgenic, 97.9 ± 10.2 s; P < 0.05 determined by Mann-Whitney test, n = 13 control and 8 transgenic mice). High-performance liquid chromatography (HPLC) analysis of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), was performed to monitor dopaminergic integrity. At 3 months of age, AIMP2 transgenic mice showed a 20% reduction in dopamine, DOPAC and HVA compared with age-matched littermate controls. The loss of dopamine was progressive, with a 60% reduction of dopamine content at 20 months of age in the AIMP2 transgenic mice. DOPAC and HVA levels remained reduced at 20 months of age (Fig. 3c).

Assessment of the (DOPAC + HVA)/dopamine ratio suggests that there is a trend toward increased dopamine metabolism in the AIMP2 transgenic mice (data not shown). Dopamine terminal density in the striatum was assessed in the AIMP2 transgenics compared with age-matched littermate controls. There was a 40% reduction of dopamine terminal density in 20-month-old AIMP2 transgenic mice compared with age-matched littermate controls (Fig. 3d). The mean ± s.e.m. dopamine terminal density at 20 months of age was 2.8 ± 0.4 pmol/mg for AIMP2 transgenic mice and 4.6 ± 0.5 pmol/mg for littermate controls. A comparison of the density of dopamine terminals in the AIMP2 transgenic mice with age-matched littermate controls was performed using a Mann-Whitney test (P = 0.0217) (Fig. 3e).
mice (Fig. 3d–f). Taken together, these data indicate that expression of AIMP2 leads to an age-dependent loss of dopaminergic neurons that is accompanied by motor deficits.

AIMP2 accumulation leads to PARP1 activation

To explore the potential mechanism by which AIMP2 overexpression leads to neurodegeneration, we developed a conditional AIMP2 PC12 cell model by expressing AIMP2 under the control of the tetracycline-responsive promoter (Supplementary Fig. 4a). The canonical function of AIMP2 is in aminoacyl-tRNA synthesis, which is essential for peptide extension during protein synthesis27. To determine whether AIMP2 overexpression affects protein synthesis, we monitored 35S-methionine incorporation into proteins in the stable PC12-conditional AIMP2 cell model in the presence or absence of doxycycline. No substantial difference in protein synthesis was observed between doxycycline-stimulated or unstimulated conditions (Supplementary...
AIMP2 accumulation leads to a progressive and selective degeneration of dopaminergic neurons in the substantia nigra of transgenic mice. (a) Representative tyrosine hydroxylase immunohistochemistry of the substantia nigra of AIMP2 transgenic mice and age-matched littermate controls. Scale bar, 500 µm. (b) Stereological assessment of tyrosine hydroxylase- and Nissl-positive neurons in the substantia nigra (n = 9 mice per group at 2–3 months; 8 months: control, n = 5; transgenic, n = 7; n = 5 per group at 20 months). (c) Representative images of Nissl-stained cortex. Scale bar, 50 µm. (d) Stereological assessment of Nissl-positive cortical neurons (n = 3 per group). (e) Representative GFAP immunohistochemistry of the substantia nigra and cortex of the AIMP2 transgenic and age-matched littermate control mice. Scale bars, 100 µm. (f) Representative western blots of GFAP, AIMP2 and β-actin in the cortex and the ventral midbrain of 3-month-old and 20-month-old AIMP2 transgenic and littermate control mice. (g) Quantification of GFAP protein levels normalized to β-actin (n = 3 mice per group of 3 month olds n = 4 mice per group of 20 month olds). Quantified data (b, d, g) are expressed as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001. Kruskal-Wallis ANOVA test (tyrosine hydroxylase counts of Fig. 2b), ANOVA test followed by Student-Newman-Keuls post hoc analysis (Nissl counts of Fig. 2b, d), unpaired two-tailed Student t test (g). Full-length blots are presented in Supplementary Figure 14.

Fig. 4b). To determine whether AIMP2 overexpression might regulate stress-induced protein synthesis, we evaluated the effects of H2O2, thapsigargin and MPP+ in the presence or absence of doxycycline.

No substantial difference in protein synthesis was observed under these conditions. H2O2 (200 µM) suppressed protein synthesis, as previously reported28, and served as a positive control (Supplementary

Figure 2 AIMP2 accumulation leads to a progressive and selective degeneration of dopaminergic neurons in the substantia nigra of transgenic mice. (a) Assessment of spontaneous exploration in an open field chamber for AIMP2 transgenic and littermate control mice (8 months: control, n = 7 mice; transgenic, n = 8; 20 months: control, n = 6; transgenic, n = 8). (b) Assessment of retention time in an accelerated rotarod test (2 months: control, n = 11; transgenic, n = 5; n = 8 per group at 8 months; 20 months: control, n = 13; transgenic, n = 8). (c) HPLC assessment of the striatal content of dopamine and its metabolites, DOPAC and HVA (3 months: control, n = 5; transgenic, n = 6; 20 months: control, n = 10; transgenic, n = 6). (d) Tyrosine hydroxylase immunohistochemistry of the striatum. Darkfield photomicrographs (bottom rows) of dorsal and ventral striatum. (e) Quantification of optical densities of (d) (control, n = 3; transgenic, n = 4). Optical density of the striatum was subtracted by background before normalization. Densitometric analysis was processed using ImageJ software. (f) High magnification of tyrosine hydroxylase–positive dopaminergic nerve fibers in the striatum. Quantified data (a–c, e) were expressed as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, unpaired two-tailed Student’s t test (a, e), or Kruskal-Wallis ANOVA test (b, c).
 AIMP2 overexpression led to PARP1 activation and cell death. (a) Viability of SH-SY5Y cells transfected with AIMP2 and mock DNA (DPQ; 10 µM; ZVAD; 50 µM; n = 4 per group). (b) Western blot analysis of PARP1 self-PARsylation and PAR conjugation to proteins in SH-SY5Y cells (empty triangle, FLAG-AIMP2; filled triangle, endogenous AIMP2). (c) Immunoprecipitation of FLAG-AIMP2 and endogenous PARP1 in SH-SY5Y cells. (d) PARP1 pulldown of recombinant AIMP2 (rAIMP2) and immunopurified PARP1 (IP PARP1). (e) FLAG pulldown of recombinant PARP1 (rPARP1) and recombinant AIMP2-FLAG (rAIMP2-FLAG). (f) Subcellular localization of AIMP2 and PARP1 in SH-SY5Y cells. (g) Confocal microscopic images in SH-SY5Y cells transfected with FLAG-AIMP2. (h) Co-immunoprecipitation of AIMP2 using PARP1 antibody in the ventral midbrain of mice. (i) Co-immunoprecipitation of PARP1 using FLAG antibody in the ventral midbrain of mice. (j) In vitro PARP1 activation assay (n = 5 per group). (k) Subcellular localization of AIMP2 and PARP1 in the ventral midbrain of mice. (l)Western blots of AIMP2 and PAR in the ventral midbrain of 12-month-old mice. Quantified data (a-j) are expressed as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 ANOVA test followed by Student-Newman-Keuls post hoc analysis (a) or Mann-Whitney U test (j). Similar results were reproduced in multiple experiments (c-f,i,k). β-actin serves as a loading control (b,c,e,h,i,l). Full-length blots are presented in Supplementary Figure 14.

Fig. 4b. Given that AIMP2 overexpression did not have any observable effects on overall protein synthesis, we monitored cell death in a transient conditional AIMP2 overexpression model in SH-SY5Y cells. In this cellular model, AIMP2 overexpression resulted in approximately 20% cell death in a caspase-independent manner, as the broad spectrum caspase inhibitor ZVAD did not prevent cell death. However, AIMP2 toxicity was PARP dependent, as the PARP inhibitor 3,4-Dihydro-5-4-(1-piperidinyl)butoxy-1(2H)-isoquinoline (DPQ) completely prevented cell death (Fig. 4a).

AIMP2 overexpression activated PARP and led to a 3.5-fold increase in PAR levels (relative levels, mean ± s.e.m.: mock, 1.4 ± 0.2; AIMP2, 3.9 ± 0.3; P < 0.01, determined by unpaired Student’s t test) and a greater than fivefold increase in PARylated PARP1 (relative levels, mean ± s.e.m.: mock, 1.2 ± 0.4; AIMP2, 6.1 ± 1.0; P < 0.05 determined by unpaired Student’s t test) (Fig. 4b). To determine whether AIMP2 interacts with PARP1 directly, we carried out co-immunoprecipitation experiments and found that immunoprecipitation of FLAG-AIMP2 in SH-SY5Y cells pulled down endogenous PARP1 (Fig. 4c). In an immunopurified PARP1 pulldown assay, recombinant AIMP2 bound to immunopurified PARP1 (Fig. 4d). Recombinant AIMP2-FLAG also pulled down recombinant PARP1 in an immunoprecipitation with an antibody to FLAG (Fig. 4e). Taken together, these data indicate that the AIMP2 and PARP1 proteins interact.

Deletion analysis revealed that PARP1 interacted with amino acids 1–83 of AIMP2 (Supplementary Fig. 5a) and AIMP2 interacted with the automodification domain of PARP1 (Supplementary Fig. 5b). Although AIMP2 was primarily enriched in the post-nuclear fraction, there was also a smaller pool present in the nuclear fraction that was available to interact with PARP1, as determined by subcellular fractionation and monitored by AIMP2, the nuclear marker PARP1 and the mitochondrial marker manganese superoxide dismutase (MnSOD) immunoreactivity (Fig. 4f). Confocal image analysis revealed that AIMP2 was primarily localized to the cytosol, but a small percentage was found in the nucleus (Fig. 4g). In the ventral midbrain of AIMP2 transgenic mice, immunopurificated FLAG-AIMP2 pulled down PARP1 and immunopurificated of PARP1 pulled down FLAG-AIMP2 (Fig. 4h, l). The interaction of AIMP2 and PARP1 in the ventral midbrain in vivo was also confirmed in transgenic line 322, which overexpressed AIMP2 at levels similar to the adult conditional Parkin+/− mouse brains (Supplementary Figs. 6a and 7a,b). To ascertain whether AIMP2 binding to PARP1 directly activates PARP1, we performed a PARP ribosylation activity assay in the presence of recombinant GST-AIMP2, using GST as a control. GST-AIMP2 significantly increased the ribosylation activity of PARP1 (P = 0.008, 0.032, 0.008 for 0.125, 0.25 and 1.0 units of PARP1, respectively; Fig. 4j).

We then monitored the subcellular localization of AIMP2 in the ventral midbrain of AIMP2 transgenic mice versus age-matched littermate controls. AIMP2 was found in both the nuclear and post-nuclear fractions, as monitored by PARP1 and MnSOD immunoreactivity, respectively (Fig. 4k). Accompanying the nuclear localization of
AIMP2, there was an approximately 2.5-fold increase in the level of PAR (relative levels, mean ± s.e.m.: control, 1.1 ± 0.1; transgenic, 2.4 ± 0.3; P < 0.05 determined by unpaired Student’s t test, n = 6 per group) in the ventral midbrain of AIMP2 transgenic mice as compared with age-matched littermate controls (Fig. 4I). The association of overexpressed AIMP2 and nuclear PARP1 appeared to be region specific, as we failed to detect nuclear translocation of AIMP2 or interaction of AIMP2 and PARP1 in cortical tissues of AIMP2 transgenic mice (Supplementary Fig. 6b,c). Consistent with these results, no significant change in PAR levels was observed in the cortex of AIMP2 transgenic mice versus age-matched control mice (P = 0.0577; Supplementary Fig. 8a,b).

To further explore the AIMP2–PARP1 pathway, we treated SH-SY5Y cells with H2O2 or N-methyl-N′-nitro-N′-nitosoguanidine (MNNG), a DNA-damaging reagent and strong PARP1 activator. Following treatment with H2O2 or MNNG, endogenous AIMP2 translocated into the nucleus (Supplementary Fig. 9a), leading to a strong association with PARP1 and subsequent PARP1 production (Supplementary Fig. 9b). Taken together, these data indicate that AIMP2 interacts with PARP1 in the nucleus, leading to PARP1 activation and PAR formation in dopaminergic neurons of the substantia nigra.

AIMP2 neurodegeneration occurs through parathanatos

Given that CamKIIα-tTA-driven expression of AIMP2 resulted in a broad expression of AIMP2 in the mouse forebrain and that the degeneration of dopamine neurons in the midbrain takes 8–20 months to occur, we sought to develop a conditional model in which AIMP2 overexpression is largely confined to the ventral midbrain and the loss of dopamine neurons occurs on a shorter timescale. We constructed an adeno-associated virus type 1 (AAV1) vector in which the chicken β-actin promoter drives tTA, and IRES2 zsGreen is used for monitoring expression (AAV1-tTA-IRES-zsGreen; Supplementary Fig. 10a). AAV1-tTA-IRES-zsGreen was stereotaxically injected into the ventral midbrain of TetP-AIMP2 transgenic mice or non-transgenic control mice (Fig. 5a). The AAV1-tTA-IRES-zsGreen robustly transduced the ventral midbrain of non-transgenic mice, with a majority of dopaminergic neurons being transduced as monitored by colocalization of tyrosine hydroxylase immunostaining with zsGreen (Supplementary Fig. 10b). We monitored AIMP2 levels 17 d after injection by immunoblot, and AIMP2 levels were increased by more than twofold (Fig. 5b,c). To determine whether these mice exhibit comparable levels of AIMP2 expression as Parkin−/− mice, we assessed the level of AIMP2 in adult conditional Parkin−/− mice. AIMP2 was overexpressed approximately twofold in adult conditional Parkin−/− mice (Supplementary Fig. 7b,a). At 25 d post injection, there was a robust loss of tyrosine hydroxylase immunoreactivity in the substantia nigra zona compacta (Fig. 5d). Unbiased stereologic analysis of tyrosine hydroxylase–immunoreactive and Nissl-stained neurons revealed a greater than 95% loss of dopamine neurons (Fig. 5e). Consistent with the lack of cortical neurotoxicity in the AIMP2 transgenic mice, virally induced AIMP2 overexpression in the cortex of TetP-AIMP2 mice (at similar levels of overexpression of AIMP2 in the ventral midbrain) failed to activate PARP1 (Supplementary Fig. 11a–c) and there was no obvious degeneration of cortical neurons (Supplementary Fig. 11d,e).
To determine whether parthanatos is involved in AIMP2-induced dopamine cell loss, we monitored PARP1 activation 17 d after the AAV1-tTA–IRES–zsGreen injection. PAR levels were increased two-fold (Fig. 5b,c). We then crossed TetP–AIMP2 transgenic mice with jpark1/c− mice to create TetP–AIMP2; Park1/c− mice (Supplementary Fig. 1e). These TetP–AIMP2; Park1/c− mice were stereotaxically injected with AAV1–IRES–zsGreen, and dopamine cell loss was monitored and compared with that of TetP–AIMP2 mice with and without doxycycline treatment. AIMP2 induction and PARP1 activation were examined via western blot analysis using antibodies to AIMP2 and PAR (Fig. 5f). AIMP2 induction was efficiently suppressed by doxycycline treatment in TetP–AIMP2 mice, and the elevation of PARsylated proteins in the injected side of TetP–AIMP2 was reduced to basal levels (Fig. 5f,g). Knockout of PARP1 completely rescued the degeneration of dopamine neurons, similar to the absence of loss of dopamine neurons in the presence of doxycycline, which prevents the expression of AIMP2 (Fig. 5b,i). PARP1 activation was monitored by PAR immunoreactivity in AAV1–IRES–zsGreen–injected TetP–AIMP2 transgenic mice and TetP–AIMP2; Park1/c− mice. There was an approximately twofold increase in PAR in the TetP–AIMP2 transgenic mice, and we found no increase in the TetP–AIMP2; Park1/c− mice (Fig. 5f,g). As the injection was unilateral, we used amphetamine-induced rotation as a functional behavioral readout of dopaminergic degeneration. AIMP2 overexpression led to an eightfold increase in rotational behavior, consistent with a loss of dopaminergic neurons. Knockout of PARP1 prevented amphetamine-induced rotation, indicating that the dopamine neurons that had been spared were functional (Fig. 5j).

We administered the PARP inhibitor AG014699 (ref. 29) to Tet-AIMP2 mice 3 d before injection of AAV1–IRES–zsGreen and then continuously until the mice were analyzed (Fig. 5a). AG014699 prevented the degeneration of dopamine neurons in a dose-dependent manner, as monitored by unbiased stereologic counting of tyrosine hydroxylase– and Nissl-positive neurons (Fig. 6a). AG014699 treatment reduced PARP activation, as monitored by PAR immunoreactivity (Fig. 6b,c). To evaluate the potential pathophysiological relevance of PARP1 activation and PAR accumulation in human disease, we monitored PAR by western blot in human postmortem substantia nigra brain tissue from Parkinson’s disease patients and age-matched controls (Supplementary Table 1). It is well known that substantial numbers of dopamine neurons are present in patients with Parkinson’s disease26,30, which we confirmed via immunoblot with antibody to tyrosine hydroxylase (Fig. 6d.e). As previously reported, there was a 2–3-fold increase in AIMP2 levels in the substantia nigra of Parkinson’s disease patients as compared with controls, similar to the level of overexpression of AIMP2 that we observed in our models. Accompanying the increase in AIMP2, we observed a ~10-fold increase in PAR levels (Fig. 6d,e). It has been reported that there is no increase in the level of AIMP2 in areas of the Parkinson’s disease brain that are relatively non-affected, such as the cortex7,9. We found no increase in PAR in the cortex of Parkinson’s disease patients (Supplementary Fig. 12), suggesting that the increase in the substantia nigra is a result of the elevation of AIMP2 levels. Taken together, these data suggest that elevation of AIMP2 leads to pathological activation of PARP1 and an accumulation of PAR that results in a loss of dopaminergic neurons (Supplementary Fig. 13). This signaling may participate in the disease process of sporadic Parkinson’s disease.

DISCUSSION

We found that transgenic expression of the parkin substrate AIMP2 led to age-dependent selective degeneration of dopaminergic neurons in the substantia nigra. Mutations in PARKIN are a common cause of autosomal recessive Parkinson’s disease1,2. Postmortem studies from sporadic Parkinson’s disease have found protein modifications of parkin that lead to its inactivation and the accumulation of the K48 ubiquitinated proteins6–13,18,31,32, such as PARIS, FBP-1 and AIMP2. Thus, parkin inactivation could be a common pathogenic feature in both familial and sporadic Parkinson’s disease. Expression of either PARIS or AIMP2 leads to neuronal degeneration18,32. We found that expression of AIMP2 at levels similar to those in observed in Parkinson’s disease7,9,18 led to an age-dependent selective neurodegeneration of dopaminergic neurons, which models the human disease. The progressive degeneration of dopaminergic neurons is a characteristic and unique pathological feature of Parkinson’s disease33. However,
an extensive investigation of the molecular mechanisms underlying neurodegeneration in Parkinson's disease has been hindered by the limited availability of animal models that include age-dependent neurodegeneration. Our conditional AIMP2 transgenic mouse models, in which AIMP2 expression is sustained at levels in dopaminergic neurons similar to those found in sporadic Parkinson's disease, reveal that the canonical nigrostriatal degeneration of human Parkinson's disease can be successfully recapitulated in rodents. The characteristic age-dependent loss of SNpc dopaminergic neurons reached up to 60% at 20 months of age. VTA dopaminergic neurons also degenerated, but to a lesser extent than the SNpc dopaminergic neurons, mirroring human Parkinson's disease. Moreover, striatal dopamine content and the behavior of the transgenic mice corresponded with the deficits in nigral dopamine-producing neurons. The neuronal degeneration in AIMP2 transgenic mice was confined to dopamine neurons in the substantia nigra and VTA when compared with other brain regions such as cortex, which did not develop signs of age-dependent degeneration despite much higher levels of AIMP2. These results suggest that overexpression of AIMP2 leads to selective degeneration of dopamine neurons.

Our cell and mouse models provide a unique platform for understanding the cell death signaling events that result in AIMP2-mediated neurodegeneration. In the AIMP2-inducible PC12 cells, toxicity was independent of protein translation efficiency, implicating non-canonical functions of AIMP2 in the execution of the cell death. Pharmacological screening following transient expression of AIMP2 in SH-SY5Y cells identified that AIMP2 toxicity was caspase independent and was mediated by PARP1. Activation of PARP1 typically involves DNA strand nicks and breaks, but AIMP2 seemed to activate PARP1 in the nucleus through a direct protein-protein association, providing a previously unknown mode of PARP1 activation. The dose-dependent protection of dopaminergic neurons against AIMP2 toxicity in mice treated with a PARP1 inhibitor and the complete protection of dopaminergic neurons and prevention of amphetamine-induced stereotopic rotations in Parp1−/− mice suggest that the suppression of PARP1's enzymatic activity was sufficient to rescue the amphetamine-induced behavior. Given that the glial expression of AIMP2 accelerated the phenotype.

AIMP2 upregulation is not likely to be the sole mechanism by which inactivation of parkin contributes to the loss of dopamine neurons. How and whether AIMP2 contributes to other putative mechanisms of loss of dopamine neurons resulting from parkin inactivation is not known. These additional mechanisms will need to be evaluated in future studies to determine their relative contributions to dopamine neuron degeneration as a result of parkin inactivation. In particular, it will be important to determine whether AIMP2 and PARIS or other mechanisms of parkin-induced dopamine neurodegeneration intersect in a common pathway or whether they are separate pathways. Given that these other mechanisms may involve perturbations in mitochondrial control, either through PARIS impairment of mitochondrial biogenesis or mitochondrial degradation via mitophagy, it is conceivable that AIMP2 acts downstream of these mitochondrial perturbations. Indeed, under physiological conditions, AIMP2 is trapped in the aminoacyl-tRNA synthetase complex as a structural cofactor in the cytosol and functions to maintain the stability of this complex. Thus, AIMP2 without any stress is only found in the cytosol as a complex with aminoacyl-tRNA synthetase. Dopamine neurons in Parkinson's disease may be particularly vulnerable to oxidative stress, which could account for the AIMP2 elevation and PARP1 activation observed in the substantia nigra of patients with Parkinson's disease, as well as the selective vulnerability of dopamine neurons. Consistent with this notion is the selective degeneration of dopamine neurons in the AIMP2 transgenic mice, as AIMP2 only interacts with and activates PARP1 in dopamine neurons.

In postmortem brain tissue from control and Parkinson's disease patients, there are increased levels of PAR-conjugated proteins in total substantia nigra lysates implicating PARP1 activation in Parkinson's disease pathogenesis. In previous reports, AIMP2 accumulation was noted in familial and sporadic forms of Parkinson's disease in which mutations of PARKIN or loss of function of this E3 ligase have been observed. The presence of AIMP2 accumulation and elevated PARP1 activation in Parkinson's disease patient samples supports the pathological relevance of AIMP2-PARP1 signaling in Parkinson's disease. Consistent with the notion that PARP1 may be involved in Parkinson's disease is the observation that knockout or inhibition of PARP1 is protective against the MPTP model of Parkinson's disease, supporting a role for parthanatos in Parkinson's disease.

In conditional overexpression of AIMP2 at levels equivalent to those produced by CamKII-tTA/TetP–AIMP2 expression was restricted to dopamine neurons similar to those found in sporadic Parkinson's disease, reveal that the canonical nigrostriatal degeneration of human Parkinson's disease can be successfully recapitulated in rodents. The characteristic age-dependent loss of SNpc dopaminergic neurons reached up to 60% at 20 months of age. VTA dopaminergic neurons also degenerated, but to a lesser extent than the SNpc dopaminergic neurons, mirroring human Parkinson's disease. Moreover, striatal dopamine content and the behavior of the transgenic mice corresponded with the deficits in nigral dopamine-producing neurons. The neuronal degeneration in AIMP2 transgenic mice was confined to dopamine neurons in the substantia nigra and VTA when compared with other brain regions such as cortex, which did not develop signs of age-dependent degeneration despite much higher levels of AIMP2. These results suggest that overexpression of AIMP2 leads to selective degeneration of dopamine neurons.

Our cell and mouse models provide a unique platform for understanding the cell death signaling events that result in AIMP2-mediated neurodegeneration. In the AIMP2-inducible PC12 cells, toxicity was independent of protein translation efficiency, implicating non-canonical functions of AIMP2 in the execution of the cell death. Pharmacological screening following transient expression of AIMP2 in SH-SY5Y cells identified that AIMP2 toxicity was caspase independent and was mediated by PARP1. Activation of PARP1 typically involves DNA strand nicks and breaks, but AIMP2 seemed to activate PARP1 in the nucleus through a direct protein-protein association, providing a previously unknown mode of PARP1 activation. The dose-dependent protection of dopaminergic neurons against AIMP2 toxicity in mice treated with a PARP1 inhibitor and the complete protection of dopaminergic neurons and prevention of amphetamine-induced stereotopic rotations in Parp1−/− mice suggest that the suppression of PARP1's enzymatic activity was sufficient to halt the dopaminergic neuronal loss resulting from AIMP2 overexpression. Given that the amphetamine-induced behavior was rescued in Parp1−/− mice even with AIMP2 induction, AIMP2 accumulation itself does not seem to impair nigrostriatal dopamine release, but rather affects the viability of dopaminergic neurons via PARP1 activation and cell death.

Notably, we found that AAV1-tTA mediated overexpression of AIMP2 at levels equivalent to those produced by CamKII-tTA-mediated overexpression in the ventral midbrain of the transgenic mice, and markedly accelerated the pathogenesis of AIMP2-mediated neurodegeneration. The mechanisms underlying the acceleration of the pathogenesis with viral delivery of tTA are not clear. One possibility is the acuteness of the virally induced model, which bypasses compensatory mechanisms, which in many cases are strongly induced during embryonic development. Consistent with this notion, virally induced adult onset deletion of Parkin results in age-dependent degeneration of dopamine neurons, which is absent in germ-line Parkin knockouts. Moreover, viral induction of other Parkinson's disease genes, such as LRRK2 (ref. 37) or α-synuclein, leads to degeneration of dopamine neurons, whereas expression of LRRK2 or α-synuclein via conventional approaches does not cause degeneration of dopamine neurons. Conditional overexpression of α-synuclein via a CamKII-tTA/TetP–α-synuclein expression cassette takes months to cause degeneration of dopamine neurons, whereas viral expression of α-synuclein occurs in a much shorter time frame, similar to our observations. Given that adeno-associated virus serotype 1 transduces both neurons and glia cells, whereas CamKII-tTA/TetP–AIMP2 expression was restricted to neurons, it is possible that the glial expression of AIMP2 accelerates the phenotype.

AIMP2 upregulation is not likely to be the sole mechanism by which inactivation of parkin contributes to the loss of dopamine neurons. How and whether AIMP2 contributes to other putative mechanisms of loss of dopamine neurons resulting from parkin inactivation is not known. These additional mechanisms will need to be evaluated in future studies to determine their relative contributions to dopamine neuron degeneration as a result of parkin inactivation. In particular, it will be important to determine whether AIMP2 and PARIS or other mechanisms of parkin-induced dopamine neurodegeneration intersect in a common pathway or whether they are separate pathways. Given that these other mechanisms may involve perturbations in mitochondrial control, either through PARIS impairment of mitochondrial biogenesis or mitochondrial degradation via mitophagy, it is conceivable that AIMP2 acts downstream of these mitochondrial perturbations. Indeed, under physiological conditions, AIMP2 is trapped in the aminoacyl-tRNA synthetase complex as a structural cofactor in the cytosol and functions to maintain the stability of this complex. Thus, AIMP2 without any stress is only found in the cytosol as a complex with aminoacyl-tRNA synthetase. Dopamine neurons in Parkinson's disease may be particularly vulnerable to oxidative stress, which could account for the AIMP2 elevation and PARP1 activation observed in the substantia nigra of patients with Parkinson's disease, as well as the selective vulnerability of dopamine neurons. Consistent with this notion is the selective degeneration of dopamine neurons in the AIMP2 transgenic mice, as AIMP2 only interacts with and activates PARP1 in dopamine neurons.

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METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.
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AUTHOR CONTRIBUTIONS
Y.L. designed and performed the in vitro and in vivo experiments. S.K.K. performed the HPLC analysis and behavior tests. J.H.S. and Y.-L.L. performed stereotaxic intranigral virus injection and stereological counting of tyrosine hydroxylase-positive neurons. Y.-L.L. performed subcellular fractionation and confocal microscopy. H.S.K., B.D.L., H.C.K., S.-U.K. and D.K. provided materials and helped with the analysis of the results. H.J. performed immunofluorescence and cell counting for the intracortical virus injection samples. D.S. and L.T. performed pronuclear injections of the TetR-AIMF2 construct and provided founder mice. V.L.D. and T.M.D. formulated the hypothesis, initiated and organized the study and wrote the manuscript. Y.L., V.L.D. and T.M.D. contributed to the final manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Plasmid constructions. The Tet off transgenic construct (C-terminal FLAG human AIMP2) was cloned into the XhoI site of pPrTetEp vector (9 kb), pCMV-Tag2A-AIMP2 (ref. 18), EGFP-Parp1 (provided by G.G. Poirier, Laval University), and MYC-AIMP2 (ref. 18) and its deletion mutants have been described. Deletion mutants of EGFP-Parp1 (amino acids 1–372 and 1–555) were cloned into the restriction sites (XhoI and XmaI) of EGFP-N1 vector. The GST-AIMP2-FLAG plasmid was generated by using the XhoI restriction site of pGEX-6p1 vector. CRA-pWPRE-BGH (5,374 bp) was used to clone the rAAV1-1TA-IRE5-2zeGreen viral construct into BamHI/EcoRV sites. The integrity of the cloned constructs was verified by sequencing.

Antibodies. The following antibodies were used. For primary antibodies, we used rabbit antibody to AIMP2 (cat# 10424-1-1AP, 1:1,000 western blot; 1:20,000 for immunoblot analysis), mouse antibody to AIMP2 (cat# 10424-1-1AP, 1:1,000 western blot; 1:20,000 for immunoblot analysis), rabbit antibody to MnSOD to tyrosine hydroxylase (NB300-109, 1:2,000, Novus Biologicals), mouse antibody to poly(ADP-ribose) polymerase (cat# 556494, mouse, Roche Diagnostics), mouse antibody to FLAG (M2, 1:5,000, Sigma-Aldrich), rabbit antibody to eGFP (cat# ab290, 1:1,000, Abcam), mouse antibody to MYC (cat# 11 667 149 001, 1:1,000, Roche Diagnostics), monoclonal antibody to GFAP (GA5, 1:5,000, Cell Signaling Technology), rabbit antibody to MEF2C (cat# 5030, 1:1,000, Cell Signaling). For secondary antibodies, we used horseradish peroxidase (HRP)-conjugated mouse antibody to β-actin (AC15, 1:10,000, Sigma-Aldrich), HRP-conjugated mouse antibody to FLAG (cat# A8592, 1:5,000, Sigma-Aldrich), HRP-conjugated sheep antibody to mouse IgG (cat# RPN4301, 1:5,000, GE Healthcare), HRP-conjugated donkey antibody to rabbit IgG (cat# RPN4101, 1:5,000, GE Healthcare), biotin-conjugated goat antibody to mouse IgG (cat# BA-2000, 1:1,000, Vector Laboratories), biotin-conjugated goat antibody to rabbit IgG (cat# BA-1000, 1:1,000, Vector Laboratories), Alexa Fluor 488–conjugated donkey antibody to mouse or rabbit IgG (H+L) (cat# A21202, A21206, 1:1,000, Invitrogen), Alexa Fluor 568–conjugated donkey antibody to mouse or rabbit IgG (cat# A10037, A10042, 1:1,000, Invitrogen).

Conditional AIMP2 transgenic mouse generation. The linearized transgenic constructs (NotI digestion, 7 kb) were microinjected into the embryos of the B6C3F2 strain and the one- or two-cell embryos were transferred into B6D2F1 pseudopregnant female mice (Transgenic Animal Core of National Cancer Institute). Using genomic DNA prepared from tail snap (Proteinase K, Roche Diagnostics; direct PCR (tail) Lysis, Viagen), pups were genotyped by PCR (GoTag Green Master Mix, Promega) using T7P-AIMP2 primers (forward: CCG GTC GAC TAG GGG TGT TGT AC; reverse: TCT AAG TGA TCC CCG GGT ACC GAC; PCR product = 173 bp) to select positive founders. Positive founders were further subjected to semi-quantitative PCR and normalized by GAPDH PCR (forward: AAA CCC ATC ACC ATC TTC CAG; reverse: AGG GGC CAT CCA CAG TCT TCT; PCR product = 300 bp) to screen for high copy-number founders. The top three high copy founders were mated with C57/BL6 mice for two–three generations to establish the transgenic lines. The following primer sets were used for genotyping of CamKIIe–tTA (forward: TGA AAG TGG GTCC CGC GTA C; reverse: TAC TCG TCA ATT CCA AGG GC; PCR product = 391 bp) or Parp1 (primer 1: AGG TAT GAT GAC AGG AGG AGC; primer 2: CCA CAG CCG CTC AGA GAA GGC; primer 3: CAT GTT CAG TGA GGG AAG GCC; PCR products: null = 350 bp, wild type = 112 bp). AIMP2 induction in conditional transgenic mice was suppressed by feeding the mice with doxycycline–containing food (doxycycline Diet-Sterile, 200 mg per kg doxycycline, Bio-Serv). All procedures involving mice were approved by and conformed to the guidelines of the Institutional Animal Care Committee of the Johns Hopkins University. Mice were kept in a 12-h dark, 12-h light cycle.

Immunohistochemistry (tyrosine hydroxylase, AIMP2 and GFAP) and stereological assessment of the number of tyrosine hydroxylase– and Nissl-positive cells. Mice were intracardially perfused with ice-cold phosphate-buffered saline (PBS) and 4% paraformaldehyde/PBS (wt/vol, pH 7.4) after deep anesthesia by intraperitoneal injection of Nembutal sodium solution (50 μl of twofold dilution in PBS of pentobarbitall sodium 50 mg ml−1, 1 ml/kg, 1 ml/kg). Brains were removed and postfixed 16 h in the same fixative. After cryoprotection in 30% sucrose/PBS (wt/vol, pH 7.4), brains were frozen on dry ice, and serial coronal sections (40-μm sections) were cut with a microtome, and the striatum and ventral midbrain regions were saved. Every four sections were collected for subsequent procedures. Free-floating sections in one group were blocked with 4% goat serum (vol/vol, Sigma-Aldrich)/PBS plus 0.2% Triton X-100 (vol/vol) and incubated with antibodies to tyrosine hydroxylase, AIMP2 or GFAP, followed by incubation with biotin-conjugated antibody to rabbit (tyrosine hydroxylase and AIMP2) or mouse (GFAP), ABC reagents (Vector Laboratories) and VectorGold 1:30 3.3′-diaminobenzidine (DAB) tablets (Sigma–Aldrich). Sections were counterstained with Nissl (0.09% thionin, wt/vol) after tyrosine hydroxylase staining as described previously25. Sections were dehydrated in 100% ethanol and cleared in Xylene (Fisher Scientific) followed by mounting with DPX (Sigma–Aldrich) before imaging using a microscope. Cell counting was performed with the aid of the optical fractionator software, which is an unbiased method for the counting of tyrosine hydroxylase–positive or Nissl–positive cells in the substantia nigra region of the left hemisphere of AIMP2 transgenic mice (experimenters were blinded for genotypes of mice during stereological counting) or in the injected and non-injected sides of mice used in the virus-injection procedure. This unbiased stereological counting was done with a computer-assisted image analysis system consisting of an Axioptophotomicroscope (Carl Zeiss Vision) equipped with a computer-controlled motorized stage (Ludl Electronics), a Hitachi VC20 video camera and Stereo Investigator software (MicroBrightField). The total numbers of tyrosine hydroxylase– and Nissl-stained neurons were calculated as previously described25.

Immunofluorescence. 4% paraformaldehyde/PBS (pH 7.4)-fixed coronal brain sections or SH-SY5Y cells were blocked with 4% donkey serum (Sigma–Aldrich)/PBS plus 0.2% Triton X-100 and incubated with antibodies to tyrosine hydroxylase, AIMP2, PARP1 or MEF2C. After brief washes with PBS containing 0.2% Triton X-100, floating brain sections or cells were incubated with corresponding secondary antibodies conjugated with fluorescent dyes (Alexa Fluor 488–conjugated donkey antibody to mouse IgG for tyrosine hydroxylase and PARP1, Alexa Fluor 568–conjugated donkey antibody to rabbit IgG for AIMP2). For immunostaining of endogenous and transgenic AIMP2 in mouse brain sections, TSA Fluorescence Systems Tyramide signal amplification (Streptavidin–HRP, Blocking reagent, amplification diluent and Cyanine 3 dye), PerkinElmer Life Sciences) was used to amplify the signal following the manufacturer's instructions. Images were obtained using fluorescence microscope (Zeiss Axiosvert 200M) or confocal microscope (Zeiss Confocal LASM 710).

Co-immunoprecipitation and immunoblot analysis. Human brain tissue or mouse brains or cells were homogenized and prepared in lysis buffer (for tissue, 10 mM Tris–HCl (pH 7.3), 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40 (vol/vol), 1% Triton X-100, 0.5% sodium deoxycholic acid (wt/vol); for cells, PBS, 1% Nonidet P-40 supplemented with Phosphatase Inhibitor Cocktail I and II (P2850, I, P5726, II, Sigma–Aldrich) and Complete Protease Inhibitor Mixture). Protein levels were quantified using a BCA protein assay kit (Pierce). Co-immunoprecipitation was performed with antibodies and Protein G Sepharose and Glutathione Sepharose 4B (GE Healthcare Sciences). The immunocomplexes were then washed with immunoprecipitation buffer four times and samples were prepared by adding 2× sample loading buffer (Bio–Rad). Brain tissue lysates (40 μg) or immunoprecipitated samples were electrophoresed on SDS–PAGE gels and transferred to nitrocellulose membranes. Membranes were blocked with 5% skim milk (wt/vol) in TBS-T and incubated with primary antibodies. After HRP-conjugated secondary antibody incubation, the immunoblot signal was detected using chemiluminescent substrates (Thermo Scientific).

Subcellular fractionation. Ventral midbrain tissues from mice or ice-cold PBS-washed SH-SY5Y cells were homogenized in a hypotonic buffer (10 mM HEPES (pH 7.5), 10 mM KCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM DTT and 0.1% NP-40) supplemented with a complete protease inhibitor cocktail and passed through 26G syringe needle three times. After incubation on ice for 20 min with 10 s of vortexing twice, the lysates were centrifuged at 720g for 5 min. The supernatant was used as a postnuclear fraction and the pellet was washed with the same buffer twice and used as a nuclear fraction for subsequent western blot analysis (loading for western blot: 2% of postnuclear fraction and 8% of nuclear fraction).
Measurement of neurotransmitters in the striatum. Biogenic amine concentrations were measured by HPLC with electrochemical detection (HPLC-ECD). Briefly, mice were decapitated and the striatum was quickly removed. Striatal tissue was weighed and sonicated in 0.2 ml of ice-cold 0.1 M perchloric acid containing 0.01% EDTA (wt/vol) and 60 mM 3,4-dihydroxybenzylamine (DHBA) as an internal standard. After centrifugation (15,000g, 30 min, 4 °C), the supernatant was passed through a 0.2-μm filter. 20 μl of the supernatant were analyzed in the HPLC column (4.6 × 150 mm C-18 reverse phase column, MC Medical) with detection by a dual channel Coulchem III electrochemical detector (Model 5300, ESA). The protein concentrations of tissue homogenates were measured using the BCA protein assay kit (Pierce). Data were normalized to protein concentrations (ng neurotransmitters per mg tissue).

Stereotoxic intranigral virus injection. For stereotoxic injection of AA V1-IRESz-zsGreen—overexpressing tTA and zsGreen, 8-week-old mice of indicated genotypes were anesthetized with pentobarbital (60 mg per kg). An injection cannula (26.5 g) was applied stereotaxically into the SNpc (anteroposterior, 3.2 mm from bregma; mediolateral, 1.3 mm; dorsoventral, 4.3 mm) or the cortex (anteroposterior, 3.2 mm; mediolateral, 2 mm; dorsoventral, 0.5 mm) unilaterally (applied into the right hemisphere). The infusion was performed at a rate of 0.2 μl per min, and 2 or 1 μl of a high-titer AA V1-TTA-IRESz-zsGreen (3.5 × 1013 AAV vector genomes per ml in PBS) was injected into each mouse. After the final injection, the injection cannula was maintained in the substantia nigra for an additional 5 min for a complete absorption of the virus and then slowly removed from the mouse brain. The head skin was closed by suturing, and wound healing and recovery were monitored following surgery. For western blot analysis, brains were removed 17 d (nigral injection) or 25 d (cortical injection) after viral infection and protein samples were prepared as noted above. For stereological analysis, brains were perfused and fixed intracardially with ice-cold PBS followed by 4% paraformaldehyde 25 d after intranigral viral injection. The brain was removed and processed for immunohistochemistry or immunofluorescence. Amphetamine-induced stereotypic rotation was performed 3 weeks after the unilateral intranigral virus injection. PARP1 inhibition was accomplished by AG014699 (PF-01367338, Selleck Chemicals) treatment (no randomization was used in the drug treatment protocol) that was initiated 3 d before intranigral AA V1-TTA injection and continued until perfusion.

Behavioral tests. All behavior tests were performed in the Behavioral Core Facility at the Johns Hopkins University School of Medicine. Because behavioral data for open field and rotarod tests were obtained automatically using instruments detailed below, no particular blinding strategy was employed.

Open field test. General locomotor and exploratory activities were assessed in open field square-shaped chambers equipped with an automated beam tracking system. Unless otherwise noted, the tests were performed in the afternoon. Briefly, a mouse was placed in the center of the open field arena and allowed to explore the area for a total of 30 min. The activities of a mouse were recorded over 30 min, which consisted of six 5-min sessions, by photobeam activity system software installed in a computer connected to the open field equipment. Before and after each testing, the surface of the arena was cleaned with 70% ethanol. The total number of beam breaks during the 30-min period was used to determine gross locomotor activity of a mouse. These mice were also used for rotarod testing.

Rotarod test. Motor coordination of mice was measured as the retention time on an accelerating rotarod of the rotamex V instrument equipped with photobeams and a sensor to automatically detect mice that fell from the rotarod. Prior to the actual rotarod test, mice were trained on the rotarod at 4.0 r.p.m. for 5 min and allowed to rest for at least 30 min. After training and resting, four mice were placed on separate rods and the durations on the accelerating rods were recorded automatically by the software installed on a computer connected to the instrument. The setting of the rotamex was as follows and remained constant throughout all trials. Start speed, 4.0 r.p.m.; maximum speed, 40 r.p.m.; acceleration interval, 30 s; acceleration step, 4 r.p.m. The tests were repeated three times and the average retention time and end speed were recorded for each mouse. The retention time was used to determine the motor coordination of the mouse.

Amphetamine induced stereotypic rotation. 25 d after mice received the AA V1-tTA (2 μl) intranigral injection into the right hemisphere, we intraperitoneally administered 5 mg per kg amphetamine (Sigma-Aldrich). Mice were placed into a white paper cylinder of 20-cm diameter and monitored for 30 min. The behavior of mice was filmed at three 1-min intervals between 20 and 30 min following amphetamine administration. Full body ipsilateral rotations (clockwise) during 1-min sessions were counted for each mouse from the video recordings.

In vitro binding assay of recombinant AIMP2. The GST fusion protein, GSTAIMP2, was prepared following a standard protocol and GST was cleaved by prescision protease (GE Healthcare). For in vitro binding assays, recombinant AIMP2 was incubated with immunoprecipitated human PARP1 from SH-SY5Y using a PARP1-specific antibody and protein G beads. After washing three times with a binding buffer (1% Triton X-100 in PBS), the bound proteins were separated by SDS-PAGE and immunoblotted with the indicated antibodies. Antibody to FLAG pulldown was performed by incubating recombinant AIMP2-FLAG and recombinant PARP1 with protein G sepharose beads overnight at 4 °C using a similar procedure as described for the anti-PARP1 pulldown.

Cell culture and transfection. SH-SY5Y cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (vol/vol) and 100 U per ml of penicillin/streptomycin antibiotics and were grown at 37 °C, 7% CO2, and 90% humidity. AIMP2-inducible tet-off PC12 cell lines were maintained in DMEM supplemented with 10% horse serum, 5% tet-approved fetal bovine serum (Clontech) and the following antibiotics: 100 U per ml penicillin/streptomycin (Invitrogen), 100 μg per ml G418 (Invitrogen), 200 μg per ml hygromycin (Invitrogen) and 200 ng per ml doxycycline (Invitrogen) for suppression of AIMP2 induction. For differentiation, PC12 cells were plated onto collagen-coated plates and provided with 100 ng ml−1 nerve growth factor (NGF, Roche) in low serum media (1% horse serum (vol/vol) in DMEM). NGF was replenished every other day during differentiation. All transient transfections of SH-SY5Y cells were performed using Fugene transfection reagent (Roche).

Cell viability analysis. AIMP2-inducible PC12 cells or SH-SY5Y cells were plated in a 6-well plate (collagen coating for PC12 cells) at a seeding density of 0.5 × 104 cells per well. 7 d following differentiation and AIMP2 induction (AIMP2-inducible PC12 cells) or 2 d after transient transfection (SH-SY5Y cells) with the treatment of indicated chemicals (Z-VDAD-FMK (N-CBZ-Val-Ala-Asp(O-Me) fluoromethylene ketone, Sigma-Aldrich; DPPQ, sc-207255, Santa Cruz Biotechnology), trypan blue exclusion was performed to assess cell toxicity by counting the number of dead (blue) and live cells. After the indicated days of differentiation and induction, cultures were briefly washed with PBS, followed by trypsinization. Resuspended cells were mixed with equal volume of 0.4% trypan blue (wt/vol). Live and dead cells were counted under light microscope using a hemacytometer.

35S-pulse methionine incorporation assay. Differentiated AIMP2-inducible PC12 cells grown in regular media were pulse incubated in media containing 35S-labeled methionine together with appropriate chemicals such as 1-methyl-4-phenylpyridinium (MPP+), H2O2 and thapsigargin (all purchased from Sigma-Aldrich). After 10-min incubation, total lysates were prepared and separated in SDS-PAGE followed by transferring to nitrocellulose membrane and taking signals in X-ray film to visualize newly synthesized proteins with 35S-methionine incorporation.

In vitro PARP1 activity assay. In vitro PARP1 activity assay was performed following the manufacturer’s instructions (Trevigen, Universal PARP Colorimetric Assay Kit). As a modification, activated DNA was left out of the reactions to prevent PARP1 activation saturation. Briefly, GST and GST-tagged recombinant AIMP2 were added to different units of recombinant PARP1 in histone-coated wells. After reaction for 60 min at (15–25 °C), the levels of PARsylation of the PARP1 substrate histone were measured using a colorimetric assay (absorbance at 450 nm) and normalized as fold change compared to the level at 0.125 units of PARP1 with GST.

Statistics. Power analysis was performed by using G’Power 3.1 software to determine approximate sample sizes for behavior tests or tyrosine hydroxylase stereological analysis of Camk2a-tTA; TetP-AIMP2 mice and AAV-tTA virus injection mice. On the basis of mean difference from our preliminary experiments with
AIMP2 transgenic mice, a total sample size of six mice was calculated for behavior, tyrosine hydroxylase stereology in Camk2a-tTA; TetP-AIMP2 mice (effect size \( f = 3.33 \) for 20% mean difference, 0.2 s.d. value in each group; \( \alpha = 0.05 \)). A total sample size of four mice was calculated for tyrosine hydroxylase stereology with virally induced AIMP2 transgenic mice (effect size \( f = 22.5 \) for 90% mean difference, 0.2 s.d. in each group; \( \alpha = 0.05 \)). Normality of the data was tested with the Shapiro-Wilk test. The equality of variance was determined with Levene statistics. All quantitative data are expressed as the mean ± s.e.m. Statistical significance was determined either by an unpaired two-tailed Student’s \( t \) test and nonparametric Mann-Whitney \( U \) test for comparison of two groups (control and test) or a one-way ANOVA test and Student-Newman-Keuls post hoc analysis and nonparametric Kruskal-Wallis ANOVA test for comparison among multiple groups of more than three as indicated in each figure legend. \( P \) values lower than 0.05 were considered to indicate significant difference among groups.

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Corrigendum: Parthanatos mediates AIMP2-activated age-dependent dopaminergic neuronal loss

Yunjong Lee, Senthilkumar S Karuppagounder, Joo-Ho Shin, Yun-II Lee, Han Seok Ko, Debbie Swing, Haisong Jiang, Sung-Ung Kang, Byoung Dae Lee, Ho Chul Kang, Donghoon Kim, Lino Tessarollo, Valina L Dawson & Ted M Dawson

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In the version of this article initially published, the image in Figure 2c described as being from 20-month-old transgenic mice was actually from 2-month-old control mice. New representative images showing a wider field of view have been provided for all of Figure 2c, and Figure 2d has been replaced by a new quantification performed independently of the original one. This quantification (n = 3) yielded a P value for the comparison between the 2-month-old control and transgenic of <0.001, as compared to <0.01 in the original. The errors have been corrected in the HTML and PDF versions of the article.

Initial version

Corrected version