

Depletion of wild-type huntingtin in mouse models of neurologic diseases

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Abstract

Huntington's disease (HD) is caused by a mutation in the gene encoding for huntingtin resulting in selective neuronal degeneration. Because HD is an autosomal dominant disorder, affected individuals have one copy of the mutant and one copy of the wild-type allele. Huntingtin has antiapoptotic properties and is critical for cell survival. However, the important role of wild-type huntingtin in both HD and other neurological diseases has not been fully recognized. We demonstrate disease-associated decreased levels of full-length huntingtin in brains of transgenic mouse models of HD,

ischemia, trauma, and in spinal cord after injury. In addition, overexpression of wild-type huntingtin confers *in vivo* protection of neurodegeneration after ischemia. We propose that in HD, in addition to a toxic gain-of-function of mutant huntingtin, a parallel depletion of wild-type huntingtin results in a detrimental loss-of-function, playing an important role in disease progression.

Keywords: BDNF, brain trauma, huntingtin, Huntington's disease, ischemia, spinal cord injury.

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Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disease caused by a mutation encoding an abnormal polyglutamine expansion in huntingtin (Huntington's Disease Collaborative Research Group, 1993). Huntingtin is widely expressed, most prominently in neurons in the striatum, cerebral cortex, and cerebellum (Li *et al.* 1993; Sharp *et al.* 1995; Ferrante *et al.* 1997). Previous work suggested a function of huntingtin in vesicle trafficking and transcription regulation (DiFiglia *et al.* 1995; Velier *et al.* 1998; Waelter *et al.* 2001; Zuccato *et al.* 2001). In addition, embryonic lethality in mice with targeted disruption of the *Hdh* gene demonstrates an essential function of huntingtin in development (Duyao *et al.* 1995; Nasir *et al.* 1995; Zeitlin *et al.* 1995). Inactivation of *Hdh* in the postnatal brain, results in progressive neurodegeneration, dem-

onstrating the importance of huntingtin in normal neuronal function and survival (Dragatsis *et al.* 2000). Moreover, overexpressed wild-type huntingtin confers resistance to

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Abbreviations used: ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotrophic factor; CBF, cerebral blood flow; HD, Huntington's disease; MCA, middle cerebral artery; PVDF, polyvinylidene difluoride; rCBF, regional cerebral blood flow; SCI, spinal cord injury; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBI, traumatic brain injury; TTC, 2,3,5-triphenyltetrazolium chloride.

apoptosis in cultured cells (Rigamonti *et al.* 2000). Providing additional support to a toxic gain of function, transgenic mouse models have demonstrated that mutant huntingtin itself is sufficient to cause a HD-like syndrome (Mangiarini *et al.* 1996; Hodgson *et al.* 1999; Reddy *et al.* 1999; Schilling *et al.* 1999). However, evidence demonstrating a mechanism for the involvement of wild-type huntingtin in neurodegeneration in HD as well as in other neurologic diseases, remains elusive. In this report, we demonstrate a progressive depletion of wild-type huntingtin associated with disease progression in a transgenic mouse model of HD. Furthermore, we provide evidence that depletion of wild-type huntingtin has significant detrimental functional consequences.

Materials and methods

Transgenic mice

R6/2 and mSOD1^{G93A} mice were obtained from Jackson Laboratory (Bar Harbor, MN, USA), and the colony was maintained in our animal facility. C57BL/6 mice were used for studies of trauma, ischemia, and spinal cord injury (SCI). YAC18 transgenic mice (line 212) and wild-type mice (FVB/NJ) were generated in Dr Hayden's laboratory. All efforts were made to minimize both suffering and number of animal used. All animal procedures were conducted in accordance with protocols approved by the Harvard Medical School.

Western blot

According to the protocol provided by Santa Cruz Biotechnology (Santa Cruz, CA, USA), tissue was homogenized at 4°C in RIPA buffer [1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (SDS)] with protease inhibitor cocktail set III (Calbiochem, San Diego, CA, USA), sodium orthovanadate (1 mM), phenylmethylsulfonyl fluoride (0.1 mg/mL), and aprotinin (150 mM; Sigma, St Louis, MO, USA). The lysate was centrifuged twice at 10 000 *g* for 10 min and mixed with an equal volume of 2 × electrophoresis loading buffer (1 mL glycerol, 0.5 mL β-mercaptoethanol, 3 mL 10% SDS, 1.25 mL 1.0 M Tris-HCl, pH 6.7, 0.1 mg bromophenol). Protein concentrations were determined by Bradford assay. Proteins were separated in SDS-PAGE (polyacrylamide gel electrophoresis) gels (4% stacking gel in 0.125 M Tris, pH 6.8, and 7.5% separating gel in 0.375 M Tris, pH 8.8) and transferred electrophoretically to polyvinylidene difluoride (PVDF) membranes. A mouse monoclonal antibody recognizing the N-terminal huntingtin from 181 to 810 (mAb2166, Chemicon, Temecula, CA, USA) was used for immunoblotting in 1 : 5000 dilution. The PVDF membrane was striped and reblotted with a mouse anti-tubulin (mouse monoclonal antibody, 1 : 5000; Sigma). The signal of huntingtin was normalized as the ratio to tubulin. The final result was expressed comparing each huntingtin/tubulin signal in the diseases group with the signal in the control group (/Control).

Injury models

Traumatic brain injury (TBI) and SCI were induced as previously described (Fink *et al.* 1999; Li *et al.* 2000). For the SCI experiments, mice were anesthetized with an intraperitoneal injection of

2,2,2-tribromoethanol (0.02 mL of a 2.5% solution/g body weight). After the depth of anesthesia was adequate, the mouse was placed prone in a modified spinal stereomicroscope. The skin was shaved and decontaminated. A 15–20 mm midline incision was made, and the T7–T12 levels were exposed by laterally separating the dorsal para-spinous muscles. Trauma was performed using a modification of the weight-drop method. Briefly, T8, T9, and T10 laminectomies were made with a high-speed microdrill (Harvard apparatus, Inc. Holliston, MA, USA) and a microrongeur (Fine Science Tools, Inc. Foster City, CA, USA). Following the laminectomy, a window over the dura of at least 1.6 mm in diameter was made to accommodate a stainless steel impact rod with a diameter of 1.4 mm and weight of 1.8 g. Part of the dura mater was carefully removed through the window for better penetration. Two horizontal bars of the stereotactic frame were used for vertebral column stabilization by clamping the T9–T10 transverse processes bilaterally. A vertical bar of the stereotactic frame held the cylinder supporting the weight rod, which was raised 10 mm above the dura and dropped onto the spinal cord at the T9 level. Following trauma, mice were kept in a 37°C incubator until fully alert. The bladder was manually expressed until return of reflexive bladder control. The sham group of mice was operated in the same manner as the traumatized group, with the exception of dropping the rod on the spinal cord. Mice were killed 24 h after surgery to extract spinal cord tissue for analysis.

For TBI, C57BL/6 mice were initially anesthetized with halothane in 70% N₂O and 30% O₂, and positioned fixed in a stereotactic frame. Before trauma, an atraumatic craniectomy was performed by removing the right parietal bone posterior to the bregma, lateral to the sagittal, and anterior to the lambdoid suture. Laterally, the craniectomy was extended to the insertion of the temporal muscle. A piston that is 3 mm in diameter, and has an excursion of 3 mm was then placed over craniectomy window. A 20-g weight was dropped inside a cylinder from a height of 150 mm on to the piston (final speed $V = 1.70$ m/s). Rectal temperature was maintained between 36.6 and 37.2°C using a feedback-regulated heating pad (Harvard Apparatus). The sham group of mice was operated in the same manner as the traumatized group, with the exception of weight drop injury. Mice were killed 24 h after surgery to extract brain tissue for analysis.

For the ischemia experiments, male YAC18 mice and wild-type mice, weighing 18–22 g were anesthetized initially with 1.5% isoflurane and thereafter maintained in 1.0% isoflurane in 70% N₂O and 30% O₂ using a fluotec 3 vaporizer (Colonial Medical, Amherst, NH, USA). Rectal temperature was maintained between 36.6 and 37.2°C using a feedback-regulated heating pad. The arterial blood pressure was recorded in a non-invasive manner using a tail pulse sensor (Harvard Apparatus) before and during surgery. Regional cerebral blood flow (rCBF) was monitored by using laser Doppler flowmeter (PeriFlux System, Perimed Inc., Stockholm, Sweden) before and during middle cerebral artery (MCA) occlusion, as well as after reperfusion. To induce focal cerebral ischemia with reversible occlusion of the MCA, an 8.0 nylon monofilament suture coated with a silicone/hardener mixture (Heraeus Kulzer) was inserted into the right external carotid artery and threaded via the common carotid artery into the internal carotid artery. The suture was advanced 9–10 mm from the insertion site through the internal carotid artery, occluding the MCA. A two-hour MCA occlusion was

followed meanwhile the wound was closed and anesthesia was discontinued. Two hours later, the filament was taken out and mice were reperused for 24 h. Laser Doppler flow measurement of CBF indicated that the MCA occlusion was successful in both groups because the CBF dropped to 20% of base line. The volume of the ischemic lesion, 24 h after MCA occlusion was calculated. The mice were given an overdose of pentobarbital, and the brains were rapid removed and sliced into six (1-mm thick) coronal sections using a mouse brain matrix (RBM-2000C; Activational System, Warren, MI, USA). The brain sections were then stained in 2% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma) at room temperature in the dark for 30 min, then placed in 10% neutral buffered formalin overnight. Brain slices were directly scanned on an image scanner. The lesion was measured on the posterior surface of each section (NIH Image 1.61, US National Institutes of Health, Bethesda, MD, USA). A direct measurement of infarct area in the cortex and striatum was carried out, which was then corrected to eliminate the effect of edema using the following formulas: $(\text{contralateral area} - \text{ipsilateral non-ischemic area}) / \text{contralateral area} \times 100\%$. z-VAD-fmk (100 ng in 2 μL) was injected intracerebroventricularly 30 min before ischemia and immediately before reperfusion. The sham group of mice was operated in the same manner as the mice undergoing ischemia, with the exception of the arterial occlusion.

Brain-derived neurotrophic factor determination

Brain-derived neurotrophic factor (BDNF) quantification was performed following the manufacturer's protocol for the BDNF Emax-Immunoassay System (Promega Co., Madison, WI, USA). The samples were not acid treated to allow for measurement of the amount of free mature BDNF.

Data analysis

Data are presented as means \pm SEM. Statistical comparisons were made by Student's *t*-test.

Results

Progressive huntingtin depletion in brains of R6/2 mice

To evaluate whether wild type huntingtin might play a role in HD, we evaluated the levels of huntingtin protein at different disease stages in R6/2 mice. R6/2 mice, a transgenic model of HD, express exon-1 of human huntingtin with an expanded poly-glutamine repeat that develops a progressive phenotype with some features of HD (Mangiarini *et al.* 1996). In brains of wild-type mice there is an increase in full-length huntingtin protein in 5-, 7- and 12-week-old mice when compared to 1-week-old mice (Fig. 1a). Similar to wild-type mice, at 5 weeks of age, R6/2 mice demonstrate increased full-length huntingtin levels compared to 1-week-old mice; however, by 7 and 12 weeks there is progressive reduction of full-length huntingtin in R6/2 mice (Fig. 1b). Disease progression in R6/2 mice, as evaluated by weight loss and Rotarod performance, closely parallels depletion of full-length wild-type htt (Figs 1b,c). As demonstrated in humans with HD (Li *et al.* 1993),

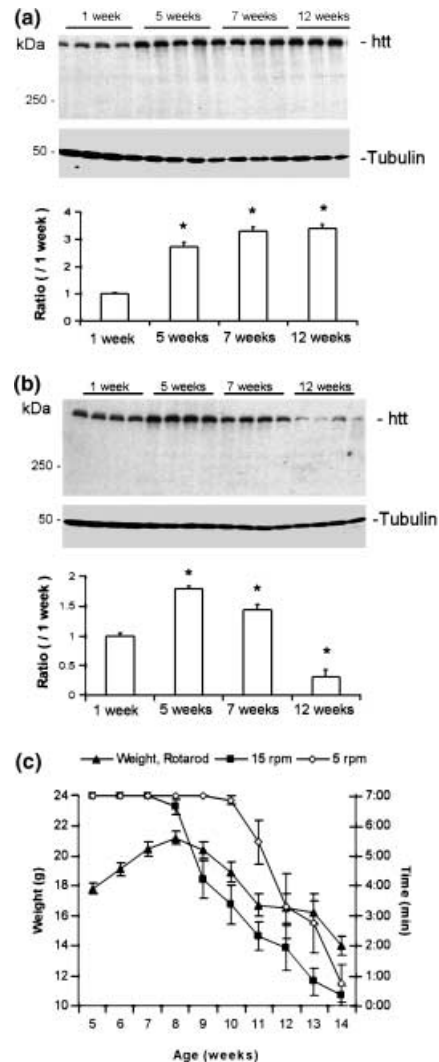


Fig. 1 Reduction of wild-type huntingtin is concomitant with disease progression. (a) The amount of wild-type huntingtin in hemispheric brains of 1-, 5-, 7-, and 12-week-old wild-type mice was evaluated by western blot with antihuntingtin antibody (mAb2166). Protein lysates (50 μg) prepared from half brains of individual mice were loaded in each lane. Densitometric analysis of full-length huntingtin was normalized to tubulin levels. The final result was expressed comparing each huntingtin/tubulin signal with it in the 1-week group (/1 week; $*p < 0.05$ vs. 1 week, $n = 3-4$, repeated three times in different blots). (b) As in (a) but for R6/2 mice. (c) Rotarod performance and weight of R6/2 mice (▲, weight, Rotarod; ■, 15 rpm; ◇, 5 rpm). Age-matched wild-type littermates gained weight and remained on the Rotarod for 7 min ($n = 11$). In R6/2 mice, weight loss and deterioration of Rotarod performance begins by 7–8 weeks of age. Data are mean \pm SEM. Error bars indicate SEM.

huntingtin/GAPDH mRNA ratios did not differ in R6/2 and wild-type mice, confirming that reduction of full-length huntingtin results not from reduced production (data not shown).

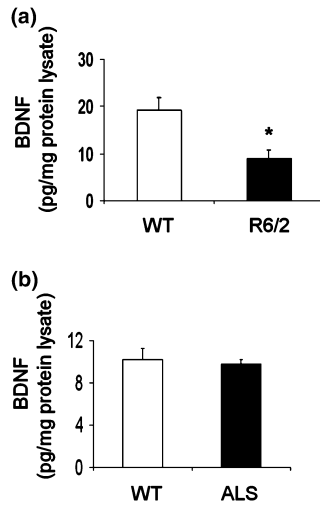


Fig. 2 (a) Reduction of BDNF in hemispheric brains of 12-week-old R6/2 mice evaluated by enzyme-linked immunosorbent assay (ELISA; $n = 5$ mice in each group, $*p < 0.01$). Error bars indicate SEM. (b) BDNF levels in spinal cords of end-stage ALS (■) and age-matched wild-type (□) mice ($n = 5$ in each group). Error bars indicate SEM.

Functional role of huntingtin in production of BDNF

A novel function of wild-type huntingtin is upregulation of BDNF expression (Zuccato *et al.* 2001). We therefore evaluated whether decreases in BDNF brain concentrations correlate with the decrease in full-length huntingtin levels in symptomatic R6/2 mice. BDNF plays an important role in development and protection in neurodegeneration (Shen *et al.* 1997). In parallel with reduction of full-length huntingtin protein, there is a significant reduction of BDNF concentration in brains of 12 week-old R6/2 mice when compared to age-matched wild-type littermates (Fig. 2a). This evidence supports a loss-of-huntingtin function in R6/2 mice. Adding relevance to this finding, Zuccato *et al.* (2001) have demonstrated reduced brain BDNF levels in humans with HD. Considering the transcriptional regulatory effect of mutant huntingtin, decreased BDNF concentration in R6/2 mice could also be a result from gain of function. To evaluate whether reduced BDNF levels is a result of the advanced stage of the disease, we compared BDNF levels in spinal cord of end-stage amyotrophic lateral sclerosis (ALS) mice (mSOD1^{G93A}) with age-match wild-type littermates. In spinal cord of end-stage ALS mice, there is significant neuron loss and marked neuropathology, there is normal levels of BDNF (Fig. 2b). This provides support to the notion that reduced BDNF levels are not the result of the mice being at the end stage of the disease.

Depletion of huntingtin in neurologic disease models featuring caspase activation

Previous work showed inhibition of cell death conferred by overexpression of wild-type huntingtin in striatal cell lines

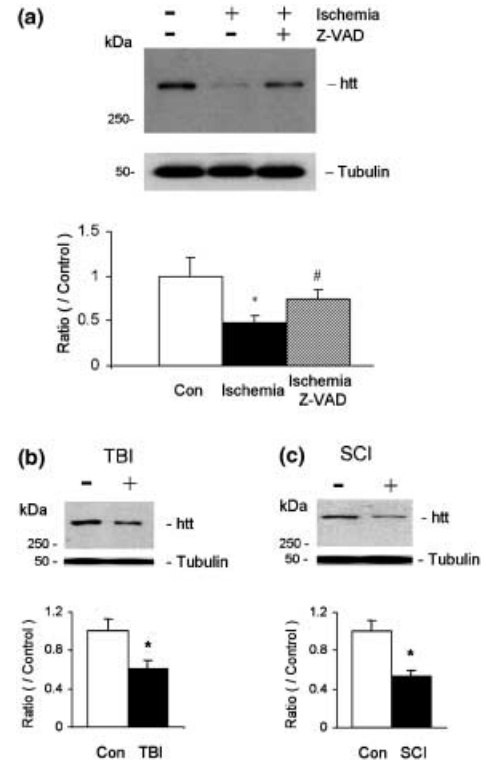


Fig. 3 Depletion of huntingtin in experimental mouse models of ischemia, traumatic brain injury (TBI), and spinal cord injury (SCI). (a–c) The blots for determining huntingtin reduction in injured hemispheric brains (ischemia and TBI) and spinal cords (SCI) were probed with an antihuntingtin antibody (mAb2166). Reduction in full-length huntingtin was detected 24 h following the induction of ischemia (a), TBI (b), and SCI (c). Densitometric analysis of full-length huntingtin was normalized to tubulin levels. Value is expressed as a ratio of the densitometric huntingtin/tubulin value of the disease group to the value of the control group (/Control). Injection of z-VAD-fmk (100 ng in 2 μ L) intracerebroventricularly 30 min before ischemia and immediately after reperfusion attenuated the depletion of huntingtin (a; $n = 7$ control, $n = 12$ ischemia, $n = 9$ ischemia pre-treated with zVAD-fmk, $*p = 0.011$ vs. control group; $\#p = 0.027$ vs. ischemia group). TBI (b; $n = 6$ in each group, $*p = 0.028$ vs. control group), SCI (c; $n = 3$ of control, $n = 4$ of SCI, $*p = 0.01$ vs. control group).

(Rigamonti *et al.* 2000). Because huntingtin has a neuroprotective effect, we wished to evaluate whether huntingtin depletion is a finding specific to HD, or whether it is also detected in other neurologic diseases featuring cell death. We therefore evaluated whether huntingtin is depleted in experimental models of stroke, and in TBI and SCI. We demonstrate a significant depletion of huntingtin following cerebral ischemia, TBI, and SCI (Figs 3a–c). Because huntingtin is a caspase substrate, and caspases are activated in ischemic injury, we then evaluated whether caspase activation might play a role in huntingtin depletion *in vivo*. zVAD-fmk (a broad caspase inhibitor) was injected into the lateral ventricle 30 min prior to ischemic injury and immediately

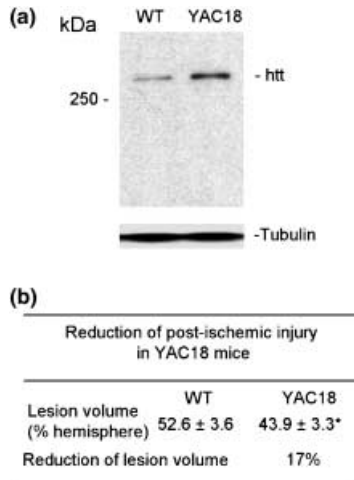


Fig. 4 Overexpression of wild-type huntingtin decreases neurodegeneration in YAC18 mice. (a) Western blot demonstrates that the amount of full-length huntingtin is approximately two to three times greater in YAC18 mice than in wild-type mice. The blot was probed with antihuntingtin mAb2166, an antibody recognizing both human and mouse huntingtin. (b) Reduction in post-ischemic brain injury in YAC18 transgenic mice. The ischemic paradigm was 2 h of middle cerebral artery occlusion followed by 24 h of reperfusion ($n = 8$ in wild-type, $n = 9$ in YAC18). Data are mean \pm SEM; * $p < 0.05$, compared with wild-type.

before reperfusion. Treatment with zVAD-fmk significantly inhibited caspase-mediated depletion of full-length huntingtin (Fig. 3a). The above-described data provide evidence for a functional role for caspases in huntingtin depletion.

Wild-type huntingtin confers neuroprotection *in vivo*

As demonstrated above, endogenous huntingtin is reduced following ischemic injury (Fig. 3a). To evaluate whether htt reduction has a detrimental role in ischemia, we performed MCA occlusion in transgenic mice expressing wild-type human huntingtin (YAC18) at two to three times the levels of endogenous huntingtin (Fig. 4a). As demonstrated above, full-length huntingtin levels are decreased following ischemic injury (Fig. 3a). Following ischemic injury, YAC18 mice had a 17% reduction of ischemic tissue injury when compared to their wild-type littermates (Fig. 4b). These results demonstrate that wild-type huntingtin plays a protective role in reducing brain damage *in vivo*, providing further support that huntingtin depletion is associated with increased vulnerability to cell death.

Discussion

We have provided evidence that compounded upon the recognized toxic gain of function of mutant huntingtin, depletion of wild-type huntingtin likely results in a detrimental

additional loss of protective function. In contrast to wild-type mice, after the age of 7 weeks, brains of R6/2 mice demonstrate progressive depletion of wild-type huntingtin (Fig. 1). Reduction of wild-type huntingtin was also noted in mouse models of ischemia, TBI, and SCI featuring caspase activation (Fig. 3). In the stroke model, a pan-caspase inhibitor, z-VAD significantly inhibited ischemia-induced reduction of huntingtin, suggesting that huntingtin depletion is a caspase-mediated process. Because huntingtin is also calpain substrate, and zVAD-fmk at high concentrations may also inhibit calpain activity, our results are also consistent with a potential role for calpain in huntingtin depletion (Kim *et al.* 2001; Gafni and Ellerby 2002). A detrimental role of huntingtin depletion is demonstrated at two levels in this paper. First, the loss of a known physiological function of wild-type huntingtin. As huntingtin has been demonstrated to be an important regulator of BDNF expression, the association of huntingtin depletion with reduced BDNF concentrations, provides evidence for a possible functional consequence of loss of function. Although reduced levels of BDNF in brains of R6/2 mice could be related to a gain of function of mutant huntingtin. Second, as we demonstrate, increasing levels of wild-type huntingtin resulted in a resistance to apoptotic neurodegeneration *in vivo*, providing further support to its neuroprotective role in a pathological condition (Fig. 4).

In order to understand fully the consequences of depletion of wild-type huntingtin, it is critical to understand its normal function. In addition to BDNF transcriptional upregulation, other proposed functions of huntingtin include regulation of vesicle trafficking (Waelter *et al.* 2001) and modulation of NMDA receptor-mediated excitotoxicity (Sun *et al.* 2001). Depletion of wild-type huntingtin, either in HD or in other neurologic diseases, may result either in cell dysfunction or increased vulnerability to excitotoxic damage.

Understanding that loss of huntingtin function plays a role in neurodegeneration provides another avenue of potential therapeutic intervention. Strategies aimed at inhibiting depletion of wild-type huntingtin (caspase inhibitors or gene therapy) or restoring huntingtin function (BDNF) merit evaluation as therapy aimed at reducing cell dysfunction and ameliorating the progression of HD and other neurological diseases featuring caspase activation.

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References

- DiFiglia M., Sapp E., Chase K., Schwarz C., Meloni A., Young C., Martin E., Vonsattel J. P., Carraway R., Reeves S. A., Boyce F. M. and Aronin N. (1995) Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron* **14**, 1075–1081.
- Dragatsis I., Levine M. S. and Zeitlin S. (2000) Inactivation of *Hdh* in the brain and testis results in progressive neurodegeneration and sterility in mice. *Nat. Genet.* **26**, 300–306.
- Duyao M. P., Auerbach A. B., Ryan A., Persichetti F., Barnes G. T., McNeil S. M., Ge P., Vonsattel J. P., Gusella J. F., Joyner A. L. and MacDonald M. (1995) Inactivation of the mouse Huntington's disease gene homolog *Hdh*. *Science* **269**, 407–410.
- Ferrante R. J., Gutekunst C. A., Persichetti F., McNeil S. M., Kowall N. W., Gusella J. F., MacDonald M. E., Beal M. F. and Hersch S. M. (1997) Heterogeneous topographic and cellular distribution of huntingtin expression in the normal human neostriatum. *J. Neurosci.* **17**, 3052–3063.
- Fink K. B., Andrews L. J., Butler W. E., Ona V. O., Li M., Bogdanov M., Endres M., Khan S. Q., Namura S., Stieg P. E., Beal M. F., Moskowitz M. A., Yuan J. and Friedlander R. M. (1999) Reduction of post-traumatic brain injury and free radical production by inhibition of the caspase-1 cascade. *Neuroscience* **94**, 1213–1218.
- Gafni J. and Ellerby L. M. (2002) Calpain activation in Huntington's disease. *J. Neurosci.* **22**, 4842–4849.
- Hodgson J. G., Agopyan N., Gutekunst C. A., Leavitt B. R., LePiane F., Singaraja R., Smith D. J., Bissada N., McCutcheon K., Nasir J., Jamot L., Li X. J., Stevens M. E., Rosemond E., Roder J. C., Phillips A. G., Rubin E. M., Hersch S. M. and Hayden M. R. (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron* **23**, 181–192.
- Kim Y. J., Yi Y., Sapp E., Wang Y., Cui B., Kegel K. B., Qin Z. H., Aronin N. and DiFiglia M. (2001) Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis. *Proc. Natl Acad. Sci. USA* **98**, 12784–12789.
- Li S. H., Schilling G., Young W. S., Li X. J., Margolis R. L., Stine O. C., Wagster M. V., Abbott M. H., Franz M. L., Ranen N. G., Hedreen J., Folstein S. E. and Ross C. A. (1993) Huntington's disease gene (IT15) is widely expressed in human and rat tissues. *Neuron* **11**, 985–993.
- Li M., Ona V. O., Chen M., Kaul M., Tenneti L., Zhang X., Stieg P. E., Lipton S. A. and Friedlander R. M. (2000) Functional role and therapeutic implications of neuronal caspase-1 and -3 in a mouse model of traumatic spinal cord injury. *Neuroscience* **99**, 333–342.
- Mangiarini L., Sathasivam K., Seller M., Cozens B., Harper A., Hetherington C., Lawton M., Trotter Y., Leach H., Davies S. W. and Bates G. P. (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **87**, 493–506.
- Nasir J., Floresco S. B., O'Kusky J. R., Diewert V. M., Richman J. M., Zeisler J., Borowski A., Marth J. D., Phillips A. G. and Hayden M. R. (1995) Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell* **81**, 811–823.
- Reddy P. H., Charles V., Williams M., Miller G., Whetsell W. O. Jr and Tagle D. A. (1999) Transgenic mice expressing mutated full-length HD cDNA: a paradigm for locomotor changes and selective neuronal loss in Huntington's disease. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **354**, 1035–1045.
- Rigamonti D., Bauer J. H., De-Fraja C., Conti L., Sipione S., Sciorati C., Clementi E., Hackam A., Hayden M. R., Li Y., Cooper J. K., Ross C. A., Govoni S., Vincenz C. and Cattaneo E. (2000) Wild-type huntingtin protects from apoptosis upstream of caspase-3. *J. Neurosci.* **20**, 3705–3713.
- Schilling G., Becher M. W., Sharp A. H., Jinnah H. A., Duan K., Kotzuk J. A., Slunt H. H., Ratovitski T., Cooper J. K., Jenkins N. A., Copeland N. G., Price D. L., Ross C. A. and Borchelt D. R. (1999) Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum. Mol. Genet.* **8**, 397–407.
- Sharp A. H., Loev S. J., Schilling G., Li S. H., Li X. J., Bao J., Wagster M. V., Kotzuk J. A., Steiner J. P., Lo A., Hedreen J., Sisodia S., Snyder S. H., Dawson T. M., Ryugo D. K. and Ross C. A. (1995) Widespread expression of Huntington's disease gene (IT15) protein product. *Neuron* **14**, 1065–1074.
- Shen L., Figurov A. and Lu B. (1997) Recent progress in studies of neurotrophic factors and their clinical implications. *J. Mol. Med.* **75**, 637–644.
- Sun Y., Savanenin A., Reddy P. H. and Liu Y. F. (2001) Polyglutamine-expanded huntingtin promotes sensitization of *N*-methyl-D-aspartate receptors via post-synaptic density 95. *J. Biol. Chem.* **276**, 24713–24718.
- Huntington's Disease Collaborative Research Group. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* **72**, 971–983.
- Velier J., Kim M., Schwarz C., Kim T. W., Sapp E., Chase K., Aronin N. and DiFiglia M. (1998) Wild-type and mutant huntingtins function in vesicle trafficking in the secretory and endocytic pathways. *Exp. Neurol.* **152**, 34–40.
- Waelter S., Scherzinger E., Hasenbank R., Nordhoff E., Lurz R., Goehler H., Gauss C., Sathasivam K., Bates G. P., Leach H. and Wanker E. E. (2001) The huntingtin interacting protein HIP1 is a clathrin and α -adaptin-binding protein involved in receptor-mediated endocytosis. *Hum. Mol. Genet.* **10**, 1807–1817.
- Zeitlin S., Liu J. P., Chapman D. L., Papaioannou V. E. and Efstratiadis A. (1995) Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nat. Genet.* **11**, 155–163.
- Zuccato C., Ciammola A., Rigamonti D., Leavitt B. R., Goffredo D., Conti L., MacDonald M. E., Friedlander R. M., Silani V., Hayden M. R., Timmusk T., Sipione S. and Cattaneo E. (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* **293**, 493–498.