

The Gln-Ala repeat transcriptional activator CA150 interacts with huntingtin: Neuropathologic and genetic evidence for a role in Huntington's disease pathogenesis

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Huntington's disease (HD) is a neurodegenerative disease caused by polyglutamine expansion in the protein huntingtin (htt). Pathogenesis in HD appears to involve the formation of ubiquitinated neuronal intranuclear inclusions containing N-terminal mutated htt, abnormal protein interactions, and the aggregate sequestration of a variety of proteins (noticeably, transcription factors). To identify novel htt-interacting proteins in a simple model system, we used a yeast two-hybrid screen with a *Caenorhabditis elegans* activation domain library. We found a predicted WW domain protein (ZK1127.9) that interacts with N-terminal fragments of htt in two-hybrid tests. A human homologue of ZK1127.9 is CA150, a transcriptional coactivator with a N-terminal insertion that contains an imperfect (Gln-Ala)₃₈ tract encoded by a polymorphic repeat DNA. CA150 interacted *in vitro* with full-length htt from lymphoblastoid cells. The expression of CA150, measured immunohistochemically, was markedly increased in human HD brain tissue compared with normal age-matched human brain tissue, and CA150 showed aggregate formation with partial colocalization to ubiquitin-positive aggregates. In 432 HD patients, the CA150 repeat length explains a small, but statistically significant, amount of the variability in the onset age. Our data suggest that abnormal expression of CA150, mediated by interaction with polyglutamine-expanded htt, may alter transcription and have a role in HD pathogenesis.

Huntington's disease (HD) is a dominant neurodegenerative disorder characterized by motor abnormalities, cognitive impairment, and psychiatric disturbances (1) and is caused by a polyglutamine (polyQ) expansion tract in huntingtin (htt), a ubiquitously expressed protein of unknown function (2, 3). HD results in selective neuronal loss, especially in the striatum and cerebral cortex (4). The polyQ size in HD patients is inversely correlated with the age of onset and severity of symptoms (1).

The pathogenesis in HD and other inherited polyQ diseases remains unclear (5). In transgenic mice expressing the htt exon 1 product (6, 7), the appearance of ubiquitinated neuronal intranuclear inclusions containing truncated polyQ-expanded htt before the onset of neurological symptoms has suggested that neuronal intranuclear inclusions may be toxic to neurons (8). However, a cellular model for HD has suggested that the translocation of soluble polyQ-expanded htt cleavage products in the nucleus may be required for neuronal death (9). Transgenic mice that express full-length htt (10, 11) and immunohistochemical analysis of human HD brain tissue (12) have suggested that polyQ aggregates may not be essential for initiation of neuronal death. Although the role of aggregation in HD is not clear (13), the appearance of misfolded truncated htt species may constitute a primary mechanism of HD pathogenesis (13). The importance

of misfolded polyQ-expanded proteins has been illustrated by the ability of molecular chaperones to reverse polyQ neuronal toxicity in transgenic *Drosophila* models (14–17) and muscular toxicity in a transgenic *Caenorhabditis elegans* model (18).

The misfolding of htt may also alter the interaction with proteins essential for neuronal survival (19–24). Several reports shown the abnormal interaction of mutated htt with transcription factors, including the TATA-binding protein (TBP) (25), N-Cor (26), mSin3a (26, 27), and the cAMP-responsive element-binding protein-binding protein (CBP) and p53 (27). These proteins have abnormal locations in cells carrying polyQ-expanded htt, often as components of aggregates with TB, mSin3a, CBP, and p53 (25–27). The repression of p53-regulated promoters by polyQ-expanded htt exon 1 product has pointed to aberrant transcription in HD (27), which may lead to a greater understanding of selective neuronal degeneration in HD and other polyQ-related diseases (26–28).

We hypothesized that the simple model system *C. elegans* might provide useful clues to unravel the complexity of HD pathogenesis. Although *C. elegans* appears to be no homolog for the human htt gene (29), we explored the possibility that *C. elegans* might have other proteins that could interact htt. We also speculated that simplified *C. elegans* protein families and knowledge of a nearly complete set of predicted proteins would provide an enhanced framework for protein-interaction screens. Thus, we identified a *C. elegans* protein (ZK1127.9) that interacts with N-terminal htt in two-hybrid tests. The human homologue of ZK1127.9 is the transcriptional coactivator CA150 (30, 31). Herein, we report biochemical, neuropathologic, and genetic evidence for a role of CA150 in HD pathogenesis.

Materials and Methods

Yeast Two-Hybrid Screens. We subcloned DNA fragments encoding normal 18-Gln or mutated 128-Gln N-terminal htt species, the first 50 aa of normal 23-Gln ataxin3, and lamin C into the pGBT9 bait vector (CLONTECH). DNA fragments encoding htt species were derived from cDNAs encoding htt amino acids

Abbreviations: CBP, cAMP-responsive element-binding protein-binding protein; HD, Huntington's disease; htt, huntingtin; polyQ, polyglutamine; TBP, TATA-binding protein; GST, glutathione S-transferase.

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1–546 (Centre for Molecular Medicine and Therapeutics, Vancouver, BC, Canada). CG1945 yeast cells were transformed with pGBT9 encoding amino acids 1–546 of normal htt. A two-hybrid screening was performed as described (32) by mating transformed CG1945 yeast cells with Y187 yeast cells transformed with a random-primed *C. elegans* cDNA activation domain library subcloned into the pACT vector (R. Barstead, Oklahoma Medical Research Foundation, Oklahoma City, OK). Diploid clones were grown on minimal medium lacking Leu, Trp, and His and tested for β -galactosidase activity by filter assays. Independent interactor clones were identified from the *Bam*HI and *Eco*RI digestion profiles and tested for htt-dependent activation of *His3* and *LacZ* reporters in plate assays. Prey cDNAs in true independent interactor clones were sequenced as described (33). Sequence analysis was performed with BLAST (GenBank) and PFAM (<http://www.sanger.ac.uk>) searches. Relevant cDNAs were also tested in two-hybrid liquid-phase β -galactosidase assays as described (19) and results were analyzed with ANOVA (MacIntosh, Statview, Berkeley, CA).

In Vitro Binding Experiments. We subcloned DNA fragments encoding full-length CA150 (from C. Sune and M. A. Garcia-Blanco, Duke University Medical Center, Durham, NC) into the pGEX vector (Amersham Pharmacia) to generate glutathione *S*-transferase (GST) fusion proteins. GST-CA150 fusion proteins were produced in *Escherichia coli*. Purified GST-CA150 fusion protein (30 μ g) was incubated with protein extracts (60 μ g) from lymphoblastoid cell lines of HD family subjects (NIGMS Cell Repository, Coriell Institute for Medical Research, Camden, NY) in 0.2 ml of HNTG buffer (20 mM Hepes-KOH, pH 7.5/100 mM NaCl/0.1% Triton X-100/10% glycerol) (21) for 2 h at 4°C. Glutathione-agarose beads (150 μ l) were added, and the incubation was continued for 90 min. Beads were then collected by centrifugation and washed three times with 1 ml of HNTG buffer to remove unbound proteins. Bound proteins were eluted from the beads, separated by SDS/PAGE, and analyzed by Western blotting with a monoclonal antibody specific to htt (HU-4C8; diluted 1:2,500; Euromedex, Mundolsheim, France). The CAG repeat size in the htt gene from individuals tested was determined as described (34, 35).

Brain Tissue Specimens. Postmortem striatal and cortical tissue specimens from 10 adult-onset HD patients (2 grade 1 patients, 2 grade 2, 2 grade 3, and 4 grade 4; mean age, 62.4 years; range, 52–72 years) and 4 age-matched patients without any known neurologic disease (mean age, 66.8 years; range 45–76 years) were dissected fresh and placed in ice-cold (4°C) 2% paraformaldehyde/lysine/0.2% periodate for 24–36 h. Brain tissue specimens were received from the Bedford Veterans Affairs Medical Center Brain Tissue Archive and Emory University. The postmortem intervals did not exceed 14 h (mean time, 11.6 h; range, 6–14 h). CAG repeat length analysis was performed on 8 of the 10 HD specimens (mean number of CAG repeats, 44.8). Each HD patient had been clinically diagnosed based on a family history of HD and symptoms of HD. The diagnosis of HD was confirmed by neuropathologic examination and graded by severity. Tissue blocks were rinsed in 0.1 M sodium phosphate, pH 7.4 (SPB) and placed in ice-cold cryoprotectant in increasing concentrations of 10% and 20% glycerol/2% DMSO over a 36-h period. Frozen 50- μ m serial sections of the striatal and cortical tissue blocks were cut in the coronal plane and stored in SPB/0.08% sodium azide at 4°C for immunocytochemistry and immunofluorescence with ubiquitin and CA150 antibodies.

Immunocytochemistry. We used a rabbit polyclonal antibody specific to CA150 (30). Immunohistochemical localization of antibodies to CA150 (diluted 1:600) was performed with a

conjugated second antibody as described (36). All dilutions of primary antiserum contained 0.08% Triton X-100/2% normal goat serum. Tissue sections were preincubated in absolute methanol/0.3% hydrogen peroxide for 30 min, washed for three 10-min periods in PBS (pH 7.4), incubated in 10% normal goat serum (GIBCO) for 1 h, incubated free-floating in primary antiserum at room temperature for 12–18 h, washed as above, placed in periodate-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (both, diluted 1:300 in PBS, Roche Molecular Biochemicals), washed as above, and incubated with 3,3'-diaminobenzidine-HCl (1 mg/ml) in 1M Tris-HCl (pH 7.0) containing 0.005% hydrogen peroxide. To complete double immunocytochemical, selected striatal tissue sections that had been immunoreacted with CA150 antiserum were not preincubated in absolute methanol/0.3% hydrogen peroxide. To test for specificity for the antisera used in this study, we performed preabsorption with excess target proteins (e.g., homologous CA150 fusion proteins) and omission of the primary antibody to determine the amount of background generated from the detection assay. The CA150 antibody was tested by preadsorption of dilute primary antisera with an excess of appropriate fusion protein (12 μ g/ml) for 6 h at room temperature.

Fluorescent Immunocytochemistry. Immunofluorescence was performed as described (36) by incubating striatal tissue sections in the CA150 polyclonal antiserum (diluted 1:600) and in a anti-ubiquitin mouse mAb (Zymed; diluted 1:250) in 1M Tris-HCl (pH 7) buffer containing 0.3% Triton X-100 for 24–72 h at 4°C. Sections were then rinsed in PBS (three 10-min washes), incubated in the dark with a goat anti-rabbit-FITC conjugate for 2 h at 20°C (Roche Molecular Biochemicals; diluted 1:15), rinsed three times in PBS, and incubated with a goat anti-mouse-tetramethylrhodamine B isothiocyanate conjugate (Roche Molecular Biochemicals; diluted 1:10) for 2 h at 20°C. Deletion of the CA150 antiserum resulted in no green fluorescence, whereas deletion of the ubiquitin antiserum resulted in no red fluorescence. Sections were wet-mounted and cover-slipped in 50% glycerol. Identical microscopic fields were immediately photographed with a Nikon fluorescent microscope, to show the locations of CA150 and ubiquitin in the same striatal section.

Polymorphism Analysis. A pilot study of the polymorphism triplet repeat in the CA150 gene was performed in 20 CEPH (Centre d'Étude du Polymorphisme Humain) pedigree parents (American families 102, 104, 1331, 1332, 1340, 1347, 1362, 1413, 1416, and 1423) as described (33) by using the forward primer CA1 (5'-AACTGACACCTATGCTTG-3') and the reverse primer CA2 (5'-GTTGAAGTGGATACTGCA-3') flanking the (CAGGCC)₃₈ imperfect repeat that encodes the Gln- and Ala-rich sequence. CA1 and CA2 were used as indicated below. Large-scale polymorphism analysis was performed in 432 HD subjects recruited from the Baltimore Huntington's Disease Center, at Johns Hopkins Hospital, which covers the State of Maryland and portions of the mid-Atlantic region of the United States. The age of onset was the age at which the characteristic extrapyramidal movement disorder was first diagnosed. It was ascertained by interview of the patient and family members, by review of medical records, and, in some instances, by clinical observation of an individual previously examined and found asymptomatic. After obtaining informed consent, blood was drawn from HD subjects and DNA was extracted. The repeat region of the CA150 gene was amplified as follows. The reaction mixture contained 500 nM forward primer CA1 (fluorescently tagged) and 500 nM reverse primer CA2, approximately 500 ng of DNA, buffer G (Epicenter Technologies, Madison, WI), and 2.5 units of *Taq* polymerase (Life Technologies). After a 5-min denaturation at 95°C, PCR was performed for 33 cycles (95°C for

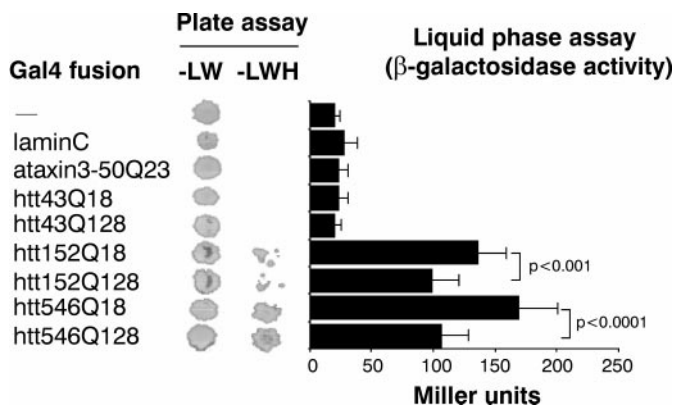


Fig. 1. Interaction of *C. elegans* ZK1127.9(187–758) with human htt in yeast two-hybrid tests. The first 43 (htt43), 152 (htt152), or 546 (htt546) amino acids of normal (18 Gln, Q18) or mutated (128 Gln, Q128) htt were tested for interaction with ZK1127.9(187–758). In plate assays with selective medium lacking Leu (L), Trp (W), and His (H), only normal and mutated htt152 and htt546 bound to ZK1127.9(187–758). In liquid-phase assays, mutated htt152 and htt546 bound weakly to ZK1127.9(187–758) compared with normal htt152 and htt546, respectively (ANOVA). No interaction was detected with the Gal4-binding domain alone (–), a random bait protein (lamin C), the first 50 aa of normal ataxin3 with 23 consecutive glutamines (ataxin3–50Q23), and N-terminal htt lacking the Pro-rich region (htt43Q18 and htt43Q128). Results for β -galactosidase activity are the mean \pm SD ($n = 15$).

45 sec, 51°C for 45 sec, and 72°C for 1 min), with a 7-min final extension at 72°C. The CAG repeat length in the htt gene was determined by PCR amplification across the CAG repeat or across the combined length of the CAG and CCG repeats, with a correction for the CCG repeat as described (34, 35). Allele sizes were determined by using the application Genotyper (MacApp). Regression tests of htt and CA150 allele lengths against HD onset age were performed by using the SPSS 8.0 statistical package (SPSS, Chicago).

Results

***C. elegans* ZK1127.9 Interacts with Htt in Two-Hybrid Tests and Is Homologous to Human CA150.** Screening more than 12×10^7 cDNAs with a normal N-terminal htt sequence by using a yeast mating-based procedure (mating efficiency, 33.6%) yielded 16 interactor clones. Fifteen clones, represented by multiple cDNAs, activated *HIS* and *LacZ* reporters in the absence of bait. One clone, represented by a single cDNA, was a true interactor and encoded amino acids 187–758 of the *C. elegans* ZK1127.9 (chromosome II). Two-hybrid analysis showed that binding of htt to the ZK1127.9(187–758) peptide was diminished in presence of an expanded polyQ region and depended on the Pro-rich region (Fig. 1). The Pro-rich region in htt may be required for binding to WW domain proteins, these interactions being modulated by the length of the adjacent polyQ region (22, 37). Consistently, PFAM analysis of ZK1127.9 revealed three WW domains that could mediate the binding to htt (Fig. 2). BLAST searches (GenBank version 114) revealed one human homolog for ZK1127.9, the coactivator CA150 (30, 31). Compared with ZK1127.9, CA150 shows a well-conserved protein structure, suggesting interaction with htt. Human CA150 also shows an N-terminal insertion between the first two WW domains that contains an imperfect (Gln-Ala)₃₈ repeat (Fig. 2), raising the possibility that the binding of CA150 and htt may differ from the binding of ZK1127.9 and htt.

CA150 Interacts *in Vitro* with Full-Length htt from Lymphoblastoid Cells. GST fusion proteins purified from *E. coli* were used to determine whether full-length CA150 binds to full-length htt.

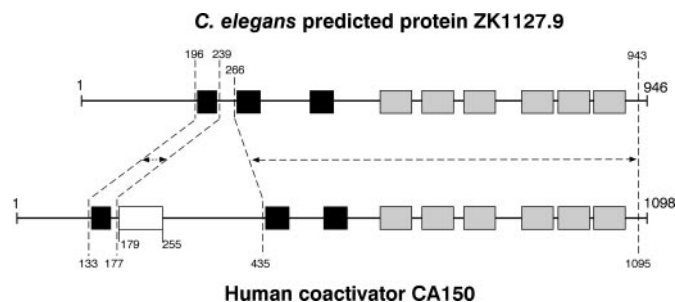


Fig. 2. Structures of *C. elegans* ZK1127.9 and human CA150 proteins (a compilation of BLAST and PFAM analyses). Regions of homology (tblastn score, 405) are shown (double arrows) and indicated by amino acid numbers. The two proteins contain WW (solid squares) and FF (shaded squares) domains. CA150 contains a 255-amino acid N-terminal insertion, with an imperfect repeat (open square) QAQV(QA)₂₅QVQAQVQAQV(QA)₂ (Q, Gln; A, Ala; V, Val).

Protein extracts of lymphoblastoid cells from three members of an HD-affected Venezuelan family were incubated with the GST-CA150 fusion protein. Glutathione-agarose beads were then added, and similar amounts of bound protein were resolved by SDS/PAGE. Western blots were performed with mAb HU-4C8 specific for amino acids 181–810 of htt (Fig. 3). Full-length htt from unaffected individual GM04858 with two normal polyQ alleles, HD patient GM05542 homozygous for mutation, and HD patient GM04477 heterozygous for mutation produced a similar binding signal (a single band corresponding to the two allelic forms of htt), suggesting that full-length CA150 binds to full-length htt.

CA150 Aggregates in Human HD Brain Tissue. In comparison to normal age-matched tissue specimens from the neostriatum (head of the caudate nucleus) and superior frontal cortex, CA150 immunoreactivity was markedly increased in all HD samples. CA150 was faintly detected in the nuclei of cells in nonneurologic control specimens (Fig. 4). The level of CA150 was increased in many neurons within the caudate nucleus, putamen, and superior frontal cortex in low-grade HD (grade 1), and significantly more CA150 was observed in moderate- and severe-grade HD specimens (Fig. 4). The number of immuno-

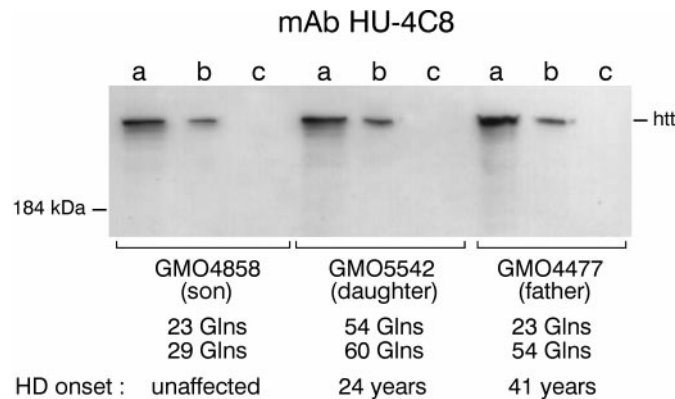


Fig. 3. Interaction of full-length htt with GST-CA150 fusion protein *in vitro*. Equivalent amounts of protein extracts from lymphoblastoid cells from three members of an HD-affected family were incubated with purified GST (lanes c) or GST-full length CA150 (lanes b). The input of protein extracts (lanes a) was also analyzed. Western blotting was performed with anti-htt mAb HU-4C8. Experiments were in triplicate. The intensity of HU-4C8 signal in lanes with GST-CA150 was similar in the three individuals tested, suggesting that CA150 binds equally to both normal and mutated full-length htt.

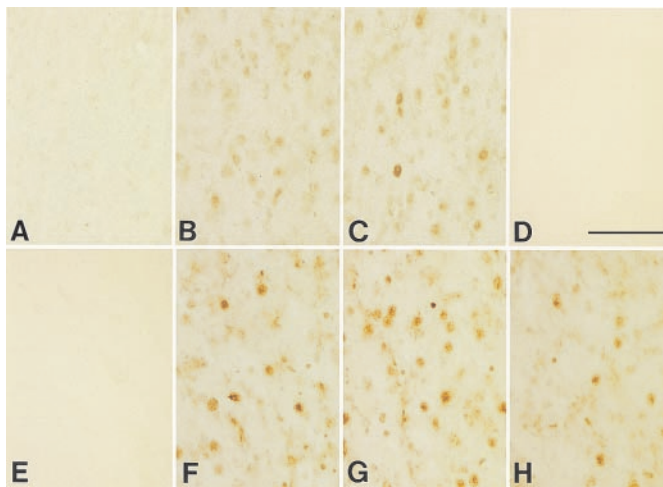


Fig. 4. CA150 immunohistochemistry in the superior frontal neocortex (A–D) and caudate nucleus (E–H) of HD and normal age-matched nonneurologic control. There is little CA150 immunostaining in the neocortex of a control patient (A), but it is markedly increased in grade 1 HD (B) and more intense in grade 3 HD (C). Immunostaining is primarily in the nucleus with less activity in the nucleolus, with a dark annulus surrounding the nucleolus. No CA150 immunoreactivity was observed after preadsorption (D). As in the neocortex, little or no CA150 immunostaining was observed in the normal caudate nucleus (E). Increasing expression of CA150 was seen in grade 1 HD (F) and grade 3 HD (G), respectively. Intense immunostaining was observed in the dorsal caudate nucleus and in remaining neostriatal neurons (H) (compare to F and G). (Bar = 100 μm .)

stained cells decreased with disease severity. The nuclei were intensively immunoreactive for CA150, with little or no immunostaining in the nucleolus. A dark annulus of CA150 immunoreactivity surrounded the nucleolus (Fig. 4). CA150 immunoreactivity

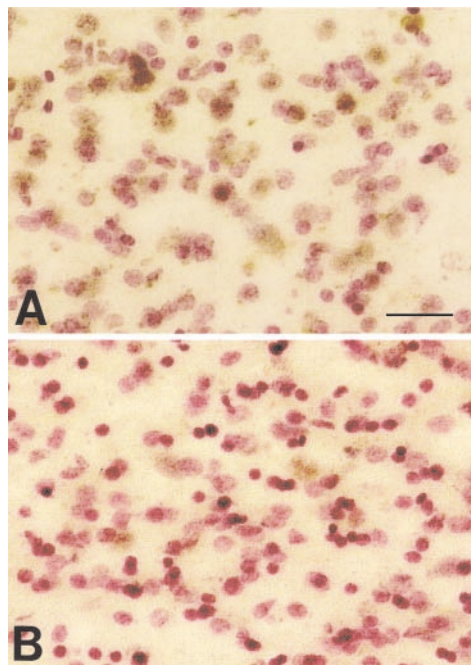


Fig. 5. Combined CA150 immunoreactivity (brown) and hematoxylin staining (red) in section of caudate nucleus of grade 1 HD (A) and grade 3 HD (B). CA150 immunostaining is primarily localized to the nucleus of neurons. Intense staining of puncta resembling nuclear aggregates is present in neurons. (Bar = 50 μm .)

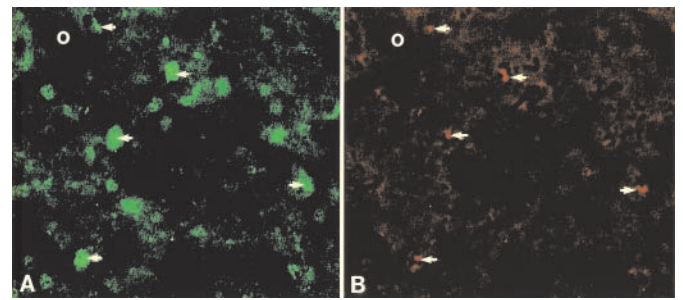


Fig. 6. Double immunofluorescence for CA150 (FITC) (A) and ubiquitin (tetramethylrhodamine B isothiocyanate) (B) immunoreactivities in the same tissue section of caudate nucleus of a patient with grade 3 HD. Circles represent a fiduciary mark. Almost all ubiquitin-positive aggregates (B) (arrows) colocalized with intense staining in puncta in CA150-positive neurons (A) (arrows).

was present, in large part, within neurons and nuclei (Fig. 5). However, not all striatal and cortical neurons expressed activity, and there was heterogeneous expression in the immunopositive neurons. This variable cell labeling did not show dorso-ventral differences within the striatum. To further characterize any correlation between CA150 immunoreactivity and the disposition of aggregates, the same tissue section was double labeled for CA150 (FITC) and ubiquitin (tetramethylrhodamine B isothiocyanate). CA150 and ubiquitin were colocalized within the HD neostriatum and cortex. Almost all ubiquitin-positive aggregates colocalized with CA150-positive neurons or neuropil activity (Fig. 6). In contrast, much of the CA150 immunofluorescence was not colocalized with ubiquitin immunofluorescence. CA150 immunoreactivity was significantly greater than that of ubiquitin in the striatum (Fig. 6).

The CA150 Gene May Influence the Age of HD Onset. Because the HD onset age might be influenced by polymorphic repeat loci other than that of *htt* (38, 39), we explored whether the length of the imperfect (Gln-Ala)₃₈ repeat in CA150 influences the HD onset age. In 20 CEPH pedigree parents, the heterozygosity of the corresponding DNA repeat was 30%, with four alleles of 295, 298, 304, and 310 bp, including one allele (295 bp) with a 3-bp variation. In 432 American HD patients (Johns Hopkins University, Baltimore), heterozygosity was 15%, with six alleles (287, 292, 298, 304, 307, and 313 bp) including one major allele (304 bp). Only a few genotypes were frequent (genotype [304, 292] in 21 patients; genotype [313, 304] in 18; genotype [304, 304] in 373; all other genotypes in ≤ 10). Regression analyses of *htt* and CA150 allele length against HD onset age indicated that, when the longest of the two CA150 alleles was added as a second variable, a statistically significant ($P < 0.05$) small amount of the variability in onset age was explained (Table 1). We also performed regression analyses by using, instead of the longest CA150 allele, the shorter allele or the combined length of the

Table 1. Regression analysis of *htt* and CA150 alleles against HD the onset of age

Regression	Variables	R^2	ΔR^2	P
Model 1	<i>htt</i>	0.468	–	<0.0001
Model 1	<i>htt</i> + CA150	0.474	0.006	0.032
Model 2	<i>htt</i>	0.628	–	<0.0001
Model 2	<i>htt</i> + CA150	0.632	0.004	0.043

Model 1 uses larger *htt* and CA150 repeat allele sizes as independent variables and age of onset as a dependent variable. Model 2 uses $\log(\text{age of onset})$. When CA150 is added as a second independent variable, increase in R^2 (ΔR^2) was detected in models 1 and 2. –, Does not apply.

two alleles. However, neither improved the regression of the CAG repeat length in the *htt* gene with onset age, by the linear or logarithmic model. The significance derived from a slightly earlier than expected age of onset in individuals whose longest allele is 313 bp compared with individuals whose longest allele is 304 bp. The 19 individuals carrying the 313-bp allele did not have a particular age of disease onset (19–51 years) or a specific CAG repeat size (39–64 copies).

Discussion

In this report, we use *C. elegans* to explore HD mechanisms and present biochemical, neuropathologic, and genetic evidence for a role of human CA150, a Gln-Ala repeat transcriptional coactivator (30, 31), in HD pathogenesis. We identified a *C. elegans* protein (ZK1127.9) that binds to *htt* at its N-terminal end. CA150, a human homolog of ZK1127.9, was then found to be associated with HD pathogenesis, suggesting that *C. elegans* is of use in defining disease mechanisms, an aspect also illustrated by transgenic *C. elegans* (40). Compared with ZK1127.9, CA150 contains an imperfect (Gln-Ala)₃₈ repeat and, hence, may be prone to aggregation on interaction with the polyQ-expanded *htt* protein. CA150 indeed appears to interact preferentially with mutated *htt*, as inferred from CA150 immunohistochemistry in HD brain tissue. *In vitro*, CA150 bound equally to normal and mutated full-length *htt*, consistent with the proposal that in HD, abnormal protein interactions involve N-terminal cleavage products of mutated *htt* (41).

Misfolding of N-terminal cleavage products of polyQ-expanded *htt* may be a primary event of HD pathogenesis (13) and provides a basis for inappropriate interaction with proteins essential for neuronal survival (19–24), as illustrated by transcription factors with different localization or aggregate sequestration in HD brain, including TBP (25), N-Cor (26), mSin3a (26, 27), and CBP and p53 (27). Human TBP-associated factor TAF_{II}-130 is a coactivator involved in cAMP-responsive element-binding protein-dependent transcriptional activation, which interacts preferentially with the expanded polyQ in the dentatorubral-pallidolusian atrophy (DRPLA) gene product (42). PQBP-1 is a transcriptional modulator that binds to the polyQ tract in the brain-specific transcription factor Brn-2 and interacts preferentially with polyQ-expanded *htt* in two-hybrid tests (43). Both TAF_{II}-130 and PQBP-1 might participate to HD. TBP (25), CBP (27), and Brn-2 (43) contain a normal polyQ likely to mediate the interaction with polyQ-expanded *htt* and the accumulation of the protein. CA150 contains a long Gln-Ala repeat, and, as shown herein, proteins rich in both Gln and Ala may be involved in HD pathogenesis.

We have shown herein that, in HD brain, all ubiquitin-containing aggregates also contain CA150, but all CA150 does not colocalize with ubiquitin. CA150 is higher in HD brain than in normal brain tissue. In HD brain tissue, CA150 immunoreactivity increases as the disease grade increases. Its level may correspond to increased nuclear accumulation of the protein, perhaps as a response to aggregated *htt* protein or secondary processes associated with the severity of cell death and reactive astroglia (4, 44). We observed the accumulation of far more CA150, particularly within the neostriatum, than of ubiquitin, suggesting that expression of CA150 and ubiquitin may be independent of each another. From neurohistopathological examination of human HD brain tissue and various model systems, protein accumulation in HD appears to be a progressive process with a threshold for polyQ length. This process leads to various aggregate types detected by light microscopy, including ubiquitin-positive neuronal intranuclear inclusions and neuropil aggregates (12, 44, 45). Electron microscopy also reveals what has been referred to as microaggregate formation, not visible by light microscopy (44). Much of the increased CA150 immunoreactivity detected in HD tissue may be in microaggregates. Large

aggregates are present in relatively spared neurons within the neocortex and neostriatum in HD (12). Microaggregates and unaggregated *htt* have been hypothesized to occur in the more vulnerable spiny neurons and may result in their untimely demise (12, 44). CA150 may colocalize with these components, which could explain its increased expression. Hence, CA150 may act in concert with or independently of aggregate formation and ubiquitin immunoreactivity.

CA150 is involved in Tat-dependent HIV-1 transcriptional activation (30, 31). The transcriptional targets of CA150 are not well understood. In contrast, CBP is a well-characterized coactivator that interacts with multiple signaling factors (46) and is involved in aberrant transcription in HD (27), spinal bulbar muscular atrophy (47), and DRPLA pathogenesis (42). Because CA150 and CBP are required for HIV-1 Tat-dependent transcriptional activation (30, 48) and carry a Gln-rich region, it would be intriguing to test for a functional alteration of CA150 in HD that may involve genes regulated by CBP.

Seventy percent of the variability in HD onset age is attributed to the size of the CAG repeat in the *htt* gene (39). Two studies have suggested that a rare untranslated repeat allele of the kainate receptor gene *GluR6* is associated with a younger onset age of HD (38, 39). We have observed that a rare repeat allele (313 bp) corresponding to the (Gln-Ala)₃₈ region of CA150 may account for a small proportion of the variability in HD onset age. Among the 432 HD subjects, a slightly earlier than expected age of onset was detected in individuals whose longest allele is 313 bp than in individuals whose longest allele is 304 bp. The 313-bp allele may have three more amino acids (that may be Gln or Ala) than the 304-bp allele. Short expansions of polyalanines are suspected to be more cytotoxic than polyQ expansions, as suggested by the length of mutated polyalanines (an additional one to seven alanines) in the oculopharyngeal muscular dystrophy gene product (49). Thus one to three additional alanines in the Gln-Ala repeat of CA150 may significantly modify the biochemical properties of the protein. The influence of the 313-bp allele on HD onset age would be consistent with a more severe aggregation of CA150 mediated by stronger binding to a mutated *htt*. Binding of CA150 to *htt* may also be influenced by polymorphisms in the Pro-rich region of *htt*. Although the effect of CA150 on age of HD onset is significant, it is small and will need to be confirmed in other cohorts of HD patients. The small magnitude of the CA150 effect may be attributed partly to the low frequency (4.4%) of the 313 allele. It is also possible that genetic modifiers will consist of multiple small effects such as the ones of *GluR6* (38, 39). Consequently, small effects should not be ignored.

Collectively, these findings suggest that the coactivator CA150 may be involved in HD pathogenesis. Its accumulation within cortical and striatal neurons of HD brain tissue suggests that it could interfere with the transcription of genes essential to neuronal survival. The influence of CA150 on the variability of the HD age of onset suggests that transcription factors and the genes they regulate may be potential modifier genes in HD pathogenesis.

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