Huntington’s disease is an autosomal dominantly inherited disease that usually starts in midlife and inevitably leads to death. In our effort to identify proteins involved in processes upstream or downstream of the disease-causing huntingtin, we studied the proteome of a well established mouse model by large gel two-dimensional electrophoresis. We could demonstrate for the first time at the protein level that α1-antitrypsin and αB-crystalline both decrease in expression over the course of disease. Importantly, the α1-antitrypsin decrease in the brain precedes that in liver and testes in mice. Reduced expression of the serine protease inhibitors α1-antitrypsin and contraspin was found in liver, heart, and testes close to terminal disease. Decreased expression of the chaperone αB-crystallin was found exclusively in the brain. In three brain regions obtained post-mortem from Huntington’s disease patients, α1-antitrypsin expression was also altered. Reduced expression of the major urinary proteins not found in the brain was seen in the liver of affected mice, demonstrating that the disease exerts its influence outside the brain of transgenic mice at the protein level. Maintaining α1-antitrypsin and αB-crystallin availability during the course of Huntington’s disease might prevent neuronal cell death and therefore could be useful in delaying the disease progression.

Huntington’s disease (HD)\(^1\) is an autosomal dominantly inherited progressive neurodegenerative disease that usually starts in midlife and inevitably leads to death (1). The disease mutation consists of an unstable expanded CAG trinucleotide repeat in the 5’ coding region of the HD gene that encodes a stretch of polyglutamines (2). Huntingtin has been implicated in vesicle trafficking (3), in the endosome/lysosome pathway (4), and in regulating the production of a cortically derived “brain-derived neurotrophic factor,” a prosurvival factor for striatal neurons that die in HD (5). The R6/2 mouse model expresses exon 1 of the human HD gene carrying highly expanded CAG repeats and develops a progressive neurological phenotype with many similarities to HD (6). This includes motor dysfunction (7), discrimination learning impairments (8), abnormal synaptic plasticity, and impaired spatial cognition (9). Learning impairments can be detected as early as at 3 weeks (8), brain weight reduction starts at 4 weeks (10), and motor deficits appear at 5 weeks of age (7). Investigating changes at the RNA level in R6/2 mice has yielded insights into the pathological process that causes HD. Early in the disease process, the mRNA levels of specific glutamate, dopamine (11), and cannabinoid (12) receptors, proteins in the dopamine signaling pathway (13), and met-enkephalin were decreased. These transcriptional changes were confirmed in brains of HD patients (14–16). Gene expression microarrays reiterated these results and showed that genes important in G-protein-coupled receptor signaling pathways, calcium homeostasis, and retinoid signaling, among others, were downregulated by 6 weeks in the striata of R6/2 mice (17).

When investigating the disease on the protein level it was found that human HD patients, as well as R6/2 mice, develop neuronal intranuclear inclusions and neuropil aggregates in the cerebral cortex and the striatum (3, 10, 18). Neuronal intranuclear inclusions are present in R6/2 mice at 4 weeks of age and do not increase greatly until close to terminal disease, although neuropil aggregates increase by as much as 14-fold during the disease course (19). Aggregates in neurodegenerative diseases may cause cellular toxicity via the recruitment of factors that are normally central to cell function, viability, and structure (20–22). In keeping with this, certain components of the proteasome (23), transcription factors (24, 25), chaperones (26, 27), and caspases (28) have so far been identified as aggregate components. Two members of the small heat shock protein (Hsp) family, the chaperones Hsp40 and Hsp70, have been shown to inhibit self-assembly of truncated polyglutamine proteins into amyloid-like fibrils in vitro (29). Another member of the small Hsp family, αB-crystallin (ABC), is upregulated during reactive gliosis (30, 31), a neuropathological feature of HD (32). It has been shown that overexpression of

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\(^1\) The abbreviation used are: HD, Huntington’s disease; AAT, α1-antitrypsin; ABC, αB-crystallin; CTS, contraspin; GFAP, glial fibrillary acidic protein; Hsp, heat shock protein; MUPs, major urinary proteins; SERPIN, serine protease inhibitor; 2D, two-dimensional; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight.
ABC can reduce the degree of aggregation caused by over-expression of glial fibrillary acidic protein (GFAP) in astrocytes (27) thereby implicating it in a mechanism for the maintenance of intermediate filament structure. ABC is also a component of plaques in Alzheimer’s disease (33).

HD has been studied in mouse model systems (6) and human HD cases (18). Further downstream of the changes in RNA and protein levels described above, neurons dysfunction and/or die by an as yet unknown mechanism (34). Therefore proteins that act in pathways further upstream or downstream in the disease still need to be identified. We intended to find these disease-relevant proteins by studying the brain proteome of the well characterized mouse model system R6/2 (6). Large gel 2D electrophoresis was used to investigate the cytoplasmic, membrane, and DNase release fraction of murine brain tissue. Affected human brain regions were used to investigate the relevance of the results for human disease. Studies of mouse organs other than brain were conducted to obtain the tissue expression pattern of the proteins identified and to search for disease-related changes of protein expression outside of the brain.

It was demonstrated for the first time that the expression of α1-antitrypsin (AAT) and ABC was down-regulated at the protein level over the course of disease in R6/2 mice. The altered protein expression pattern of AAT was confirmed for human terminal disease bridging the mouse model with the human disease. Expression of ABC altered by disease could not be demonstrated in humans. The down-regulation of liver-specific proteins (major urinary proteins; MUPs) in mice suggests the involvement of tissues other than brain in HD.

**EXPERIMENTAL PROCEDURES**

*Animals and Tissues—Brains of 4-, 8-, and 12-week-old R6/2 transgenic mice, as well as CBAxC57Bl/6 control mouse brains, were used in our studies. These were supplied by in house breeding at our laboratory in the United Kingdom. R6/2 were maintained by back crossing to (C57Bl/6xCBA)F1 mice. Genotyping and CAG repeat sizing were as described previously (35). C57Bl/6 mice were acquired from Charles River, Sulzfeld, Germany. Human brain tissue samples were obtained from different sources. Post-mortem striata and parietal lobes of a male and a female HD patient, as well as age- and sex-matched controls, were provided by the Harvard Brain Tissue Resource Center, Belmont, MA.*

*Recovery of Brains, Brain Areas, and Other Tissues—Mouse tissue was prepared as described in detail elsewhere (36). Brain regions were prepared from three female C57Bl/6 mice. Human brain tissue was obtained in blocks frozen in liquid nitrogen in all cases (for procedure see Ref. 32).*

*Preparation of Protein Samples—The protein extraction procedure is described in detail elsewhere (36). The procedure ensures that the three fractions obtained cover the total spectrum of proteins present in a tissue sample. Any selective loss of protein species is avoided by omitting any purification steps. Protein concentrations in the extracts were close to in vivo conditions thereby precluding loss of protein classes through aggregation and precipitation. The components added to the tissues during the extraction procedure were always based on tissue weight thereby precluding differences in protein concentration because of tissue weight differences in R6/2 and control mice. An additional control for equal protein concentrations in both samples is the fact that most of the protein spots on the R6/2 and control 2D gels were expressed at the same intensity serving as an internal standard. The reproducibility of the extraction procedure was controlled strictly by comparing calculated experimental values to standard values (36).*

2D Large Gel Electrophoresis—Proteins were separated by high resolution, large gel 2D electrophoresis, a technique developed in our laboratory that was described in detail recently (37). The pl was determined as described in detail elsewhere (38). Briefly, the one-dimensional gel (40-cm separation distance; sample applied) was cut into pieces of 0.5-cm-length. The gel pieces were transferred individually into 1.5-ml test tubes (Eppendorf) containing 40 µl of bidistilled water that was degassed by boiling and vacuum exposure. During the process the tubes were kept in a nitrogen atmosphere. The tightly sealed tubes with the gel pieces were now sonicated in a water bath near 0°C for 15 min. The pH was measured for every other gel segment with a pH microelectrode. The complete gradient was determined after assembling the single measurements into a pH profile covering the separation distance. The pl of known protein spots was identified by comparing their separation distances to the pH profile generated. These proteins were used as reference points to determine the pl of unknown proteins in further 2D runs. The molecular mass was determined by their relative mobility in the second dimension as compared with marker proteins. Although run-to-run variations were small using this technique, pattern evaluations were performed only with R6/2 and control samples that were run in parallel throughout both electrophoresis procedures. These two samples were denoted a “sample pair.”

Protein Staining—Protein spots were revealed by an acidic silver staining procedure described in detail elsewhere (37). With this staining technique spots containing 2 ng of protein can be detected. Coomassie Brilliant Blue G 250 staining was used when protein spots had to be analyzed by mass spectrometry (39).

Spot Detection—2D gels were evaluated visually by a trained observer comparing the two gels of a sample pair on a light box (BIOTEC-FISCHER, Reiskirchen, Germany). Changes of spots were considered with respect to presence/absence, quantitative variation, or altered mobility. Mobility variants are spots that “move” in the gel to another position indicating a shift of isoelectric point and/or molecular weight. Changes in spot intensities were recorded only if quite obvious after visual inspection (40). Simultaneous intensity changes of spot groups and the expression profile over time were important criteria for evaluating the significance of the changes observed.

In-gel Digestion—Proteins were extracted from gel pieces as described elsewhere recently (41). Two different procedures were used for MALDI target preparation. The first method was the conventional dried droplet technique including sample concentration with Poros 10 R1 beads (PerSeptive Biosystems, Cambridge, United Kingdom) (42). After evaporation of the solvent, samples containing Poros beads (with peptides) were suspended in 1 µl of matrix solution (α-cyano-4-hydroxycynamic acid) in acetonitrile/0.1% trifluoroacetic acid (1:1, v/v) and transferred onto a conventional MALDI target. Another sample preparation method utilized AnchorChip™ technology. Anchor-Chips™ are equipped with hydrophilic patches (“anchors”) in a hydrophobic surrounding causing the relatively hydrophilic analyte to concentrate on the anchors. One-µl droplets of the combined super-
located too close to another spot of higher intensity (see Fig. 1A). The protein spot is hard to identify accurately, because it was present at low amounts in the membrane fractions and undetectable in the fraction of proteins released by DNase in control mice, arguing for a mainly cytoplasmic localization of the protein (data not shown). ABC was found in the absence of AAT expression below the detection limit were detected in all three fractions. A consistently lower expression of ABC and AAT reduction of AAT and ABC availability during the course of disease—The brain tissue of 12-week-old transgenic R6/2 mice represented a stage close to terminal disease. Two earlier stages of the disease were now investigated. The results are shown in Fig. 2A and Table I for AAT and Fig. 2B and Table I for ABC. When compared with control, AAT was expressed heterogeneously at 4 weeks of age (Table I). In one R6/2 mouse AAT expression was at a level similar to 12-week-old control mice whereas other mice showed no expression in the brain at all. However, the AAT expression at 4 weeks of age in controls is also heterogeneous, mirroring the levels of expression in R6/2 mice. This suggests that AAT was expressed initially at about control levels in R6/2 mice. At 8 weeks controls showed high levels of expression whereas the

ALTERATIONS IN MOUSE AND HUMAN PROTEOME BY HD

RESULTS

Close to Terminal Disease R6/2 Brains Show Exhaustion or Reduced Expression of α1-Antitrypsin and β-Crystallin, Respectively—The brain proteome of HD exon 1 transgenic mice (R6/2) was investigated and compared with age-, sex-, and genetic background-matched controls using large gel 2D electrophoresis. Twelve-week-old R6/2 mice showed significant differences at the expression level of two proteins in the brain. In the pH range of 4 to 6 the R6/2 2D gels lacked a three-spot group in eight HD/control sample pairs (see Fig. 1A and Table I). In the pH region of 6 to 9.5 a quantitative difference in one spot was observed in all eight sample pairs tested (see Fig. 1B and Table I). All three protein spots detected in the low pH region were investigated by mass spectrometry and identified as a serine protease inhibitor (SERPIN), AAT (Swiss-Prot Q00898). One additional spot located in the neighborhood of the three-spot group was also absent in R6/2 mice close to terminal disease. Its expression pattern over time did not correlate fully with the three-spot pattern at the time points studied though the overall trend was the same. The protein spot is hard to identify accurately, because it was located too close to another spot of higher intensity (see Fig. 1A and Fig. 2A). The spot located in the high pH region was determined to be ABC (Swiss-Prot P23927). The observed pl of AAT of 5.3 coincided well with the calculated pl of 5.4. The observed molecular mass of 61 kDa differed by 15 kDa from the one calculated (46 kDa). The molecular mass of 23 kDa determined for ABC and the calculated value of 20.5 kDa differed by 2.5 kDa. The pl of 7.5 was determined by the mobility of ABC in the gel relative to marker proteins. It differs by 0.6 pH units from the calculated value. Both aforementioned alterations could indicate that some kind of protein modification might have occurred. Apart from the differential expression of these two proteins the 2D gels showed highly reproducible spot patterns as can be seen in Fig. 1.

In addition to the cytoplasmic brain fraction that was used in the above experiments, proteins solubilized by urea and detergent treatment, yielding an extract enriched in membrane proteins, as well as DNA-binding proteins released by DNase, were screened to expand the range of protein classes studied. In both fractions no additional proteins were consistently different between R6/2 and control mice (data not shown). AAT was present at low amounts in the membrane fraction and undetectable in the fraction of proteins released by DNase in control mice, arguing for a mainly cytoplasmic localization of the protein (data not shown). ABC was found in all three fractions. A consistently lower expression of ABC and AAT expression below the detection limit were detected in R6/2 samples in all three fractions (data not shown).

Reduction of AAT and ABC availability during the course of disease—The brain tissue of 12-week-old transgenic R6/2 mice represented a stage close to terminal disease. Two earlier stages of the disease were now investigated. The results are shown in Fig. 2A and Table I for AAT and Fig. 2B and Table I for ABC. When compared with control, AAT was expressed heterogeneously at 4 weeks of age (Table I). In one R6/2 mouse AAT expression was at a level similar to 12-week-old control mice whereas other mice showed no expression in the brain at all. However, the AAT expression at 4 weeks of age in controls is also heterogeneous, mirroring the levels of expression in R6/2 mice. This suggests that AAT was expressed initially at about control levels in R6/2 mice. At 8 weeks controls showed high levels of expression whereas the
protein was present at reduced amounts in R6/2 mice (see Fig. 2A and Table I). The decrease of the three spots was linked, hinting at an involvement of all three variants in the same or related processes (see Fig. 1A and Fig. 2A). The amount of ABC expressed increased steadily over time in controls but remained at about the level of 4 weeks of age in R6/2 mice (see Fig. 2B and Table I) indicating utilization and/or reduced production of the protein during disease.

**Gender Specificity of Differentially Expressed Proteins**—The results reported so far were obtained in male mice. 2D gel spot patterns of CBAxC57Bl/6 and C57Bl/6 mice matched well, and the positions and intensities of spots belonging to the proteins of interest were the same in C57Bl/6 and CBAxC57Bl/6 control mice. When investigating brain areas of 20.5-week-old female C57Bl/6 mice, AAT was not detected on the whole organ level. However, certain brain areas expressed medium to low levels of AAT (Table II). One of the most prominent expression sites was the trigeminal nerve, but other areas like the pituitary gland and the motor cortex, the hippocampus, the olfactory bulb, and the cerebellum tested positive for the SERPIN, as well (Table II). The lack of detection on the whole organ level might be because of the fact that large brain areas such as cortex, midbrain, and cerebellum did not express AAT at detectable levels (Table II) thereby diluting the protein below the detection limit of the silver stain.

ABC is expressed uniformly in males and females (Table II) and at a roughly homogenous level in different brain regions with the exception of the trigeminal nerve (very high) and the pituitary gland (low) (Table II).

**Tissue Distribution of AAT and ABC**—In the R6/2 mice, protein aggregates in the form of nuclear inclusions are found in peripheral tissues, in addition to the central nervous system (45). Therefore, we investigated the organ specificity of the proteins identified. AAT was also expressed in heart, liver, and testes of C57Bl/6 male mice (Table II). An additional SERPIN, contraspin (CTS) (Swiss-Prot Q62257; pl about 5.0, observed molecular mass 75 kDa), was identified in the liver, and ex-

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**Table I**

**AAT and ABC expression over time**

Declaration of symbols: spot is present (+++; spot intensity of control mice at 12 weeks of age); spot is present but the intensity is reduced (++); spot is present, but the intensity is severely reduced (+); spot is not detectable (−).

| Animals | R6/2 | Controls |
|---------|------|----------|
|         | AAT  | ABC      | AAT  | ABC      |
| 4 Weeks |      |          |      |          |
| 1       | +++  | +        | −    | +        |
| 2       | +++  | +        | +++  | +        |
| 3       | +++  | +        | +++  | +        |
| 4       | ++   | +        | +    | +        |
| 5       | +    | +        | +    | +        |
| 6       | +    | +        | −    | +        |
| 7       | +    | +        | −    | +        |
| 8       | +    | +        | +    | +        |
| 9       | +    | +        | +    | +        |
| 10      | +    | +        | ++   | +        |
| 11      | +    | +        | +    | +        |
| 12      | +    | +        | +    | +        |
| 8 Weeks |      |          |      |          |
| 1       | ++   | +        | +++  | +        |
| 2       | +    | +        | +++  | +        |
| 3       | +    | +        | +++  | +        |
| 4       | ++   | +        | +++  | +        |
| 5       | +    | +        | +    | +        |
| 6       | −    | +        | +    | +        |
| 7       | −    | +        | +    | +        |
| 8       | −    | +        | +    | +        |
| 12 Weeks|      |          |      |          |
| 1       | −    | +        | +++  | +        |
| 2       | −    | +        | +++  | +        |
| 3       | −    | +        | +++  | +        |
| 4       | −    | +        | +++  | +        |
| 5       | −    | +        | +++  | +        |
| 6       | −    | +        | +++  | +        |
| 7       | −    | +        | +++  | +        |
| 8       | −    | +        | +++  | +        |

* The sample pairs (R6/2 and control animals) are grouped according to AAT expression level starting with the highest expression in R6/2.
expression was confirmed in heart and testes (Fig. 3A). As already found with AAT the molecular mass determined by mobility in the 2D gel deviates by about 28 kDa from the one calculated (46.7 kDa). ABC was detected in the brain and heart only (Table II and data not shown). We studied the expression of the three proteins in the liver, testes, and heart of R6/2 mice. AAT and CTS expression was reduced strongly in the liver (see Fig. 3A and Table III), testes (see Fig. 3A and Table III), and heart (Fig. 3A and data not shown) of transgenic mice at 12 weeks of age. The number of AAT spots varied from a three-spot group and an affiliated spot in the brain to at least five spots in the liver, testes, and heart (Fig. 3A and data not shown). Of the at least five spots affected in the liver of R6/2, three were below detection limit. In the liver four spots that belong to CTS were identified by mass spectrometry in the 2D gel sections from heart and testes show spot patterns similar to liver. A slight decrease of expression was only found in one of four R6/2 gels (data not shown). One striking difference in protein expression was detected in the liver that was not present in the brain, testes, or heart; a pattern consisting of at least nine spots, which was identified to belong to the MUPs of the mouse (Swiss-Prot P11588), was strongly reduced in expression in transgenic mice at 12 weeks of age (Fig. 3A). A pl in the range of 4.9 to 5.3 and a molecular mass of 22 kDa were determined according to protein moilities in the gel. This correlates with data published for the pl of 4.6 to 5.3 (46) and a molecular mass of about 21 kDa of the unprocessed precursor (data supplied with Swiss-Prot P11588 data sheet entry). The degree of reduction on the single-spot level was heterogeneous although the strong reduction in almost all spots showed clearly that the decrease itself was homogenous. MUPs were also strongly decreased in the urine of R6/2 mice at 12 weeks of age (data not shown). When looking at the time course of AAT (Table III) and MUPs (Table III) expression, it became obvious that AAT in liver and testes and MUPs in the liver started to decrease after about 8 weeks whereas the decrease of AAT in the brain had already begun after 4 weeks of age (see Fig. 1A, Table I, and Table III). This suggests different kinetics of AAT consumption in the three tissues starting in the brain, the tissue primarily affected by the disease, followed by liver and testes.

**Altered AAT Expression Was Demonstrated in Humans with Terminal Disease**—The question of whether altered protein expression of the same or related proteins found in R6/2 mice is also present in human brain regions affected by HD was
investigated next. A six-spot group was detected on the low pH side of gels from human HD brain regions that corresponded to only three spots in control gels hinting at spot duplication by an increase in molecular weight or alteration of protein structure (Fig. 4). This altered expression was seen in the striatum and parietal lobe of a male and a female individual affected with HD. Two of four female anterior cingulate cortex samples tested displayed the same pattern (Fig. 4). The four strongest staining spots of the six-spot group were identified by mass spectrometry to be the SERPIN AAT (SwissProt P01009), the same protein that was found to be differentially expressed in the murine model system. The pI of this group is about 4.7, and the molecular mass is about 40 kDa. The altered spots in human HD cases differed in pI (0.6) and molecular mass (20 kDa) from those found in mice. These differences probably indicate processing of the protein. In one case the spot duplication was also seen in an anterior cingulate cortex control brain (data not shown). According to the neuropathology report supplied by the Harvard Brain Tissue Resource Center this brain was affected by a fresh hemorrhage in the cerebellar and subarachnoid space and a moderately acute encephalopathy of hypoxic-ischemic-type. There was also an increase in the expression of GFAP (data not shown), hinting at gliosis. In two of three of the remaining anterior cingulate control regions no abnormalities were diagnosed. The third control brain showed a poorly differentiated tumor consistent with metastatic melanoma but did not show the spot duplication. An expression pattern for ABC altered by disease could not be found consistently in the human brain regions studied (data not shown). The ABC isoform found in mice does exist in human tissue. Its presence was confirmed recently in human myocardial tissue (47). Therefore the inability to identify ABC in the brain regions studied could be because of an expression level that cannot be detected by silver staining.

DISCUSSION

We could demonstrate for the first time on the protein level that AAT and ABC decrease in expression over time in the brain of HD exon 1 transgenic mice until below detection limit or present in reduced amount, respectively, close to terminal disease. AAT and CTS expression were found to be strongly reduced in heart, liver, and testes. AAT is expressed at detectable amounts only in male tissue and certain brain regions of female mice like the trigeminal nerve and the pituitary gland. The onset of AAT decrease in the brain preceded that in liver and testes. A novel six-spot pattern was found in human Huntington’s disease cases, which was identified as AAT, thereby bridging the mouse model with the human disease. ABC expression, found in both genders, was increased in brains of control but not R6/2 mice past 4 weeks of age. The down-regulation of the liver-specific MUPs demonstrates that at least the HD transgene in mice exerts its influence past the brain.

AAT is a member of the proteins participating in the acute phase response, which is produced mainly in the liver (48). It is secreted in an effort to prevent ongoing tissue damage (48). The primary function of AAT is the inhibition of neutrophil elastase (49). Astrocytes are thought to be a source of AAT in brain tissue (50). AAT (50, 51) and ABC (33) have been implicated recently in the pathology of Alzheimer’s disease, another devastating neurodegenerative disorder. The protection of the rat pancreatic islet tumor subclone cell line I5 from cell death in the presence of Aβ1–42, Aβ1–40, and Aβ25–35 by...
AAT required that the SERPIN retained its inhibitory capacity (51). AAT was also implicated in decreasing cell death by preventing the degradation of the extracellular matrix in vascular smooth muscle cells thereby precluding the activation of caspases (52). The SERPIN CTS might act synergistically with AAT. In the present study we could show that the expression of AAT was already down-regulated at 8 weeks in the brain (Fig. 2). Lower expression of AAT (see Fig. 3A and Table III) and CTS (Fig. 3A) was only found close to terminal disease (12 weeks) in the liver, heart, and testes of R6/2 mice. Based on the data on the disease progression (7, 8, 19) the AAT decrease in the brain seems to be a later event coinciding with an increase in severity of the disease whereas the decrease of AAT and CTS in the liver, heart, and testes manifested itself only close to its terminal stage. It has already been demonstrated that high levels of AAT inhibit the activation of caspases 1 and 3 in a kidney ischemia/reperfusion experiment (53) and caspases 3 and 7 in a hepatitis model experimentally induced with tumor necrosis factor and d-(+)-galactosamine (54). Caspases 1 and 3 were also up-regulated in brains of R6/2 transgenic mice at 7 weeks of age (55) at about the time when AAT availability decreases (see Table I and Fig. 2A). The observed loss of brain weight in R6/2 mice (10) coincided with altered AAT expression observed in our study (see Table I and Fig. 2). AAT does not act directly upstream of procaspase 3 or 7, because it did not have any caspase-inhibitory capacity for recombinant caspases and in tumor necrosis factor cytoxicity assays (56). However, the mechanism underlying cell death in HD brains is unknown currently, and there is no evidence for apoptosis occurring in the R6/2 mouse brains (34). The AAT expression in the anterior cingulate cortex was investigated further, because a brief examination of the human cingulate cortex at the Harvard Brain Tissue Resource Center showed that it was unaffected morphologically by HD even in Grade 3 and 4 cases. Pathological alterations in this region might therefore be similar to the early changes occurring in severely affected brain regions such as the striatum. Consistent with this hypothesis this brain region displayed changes in AAT spot pattern in only two of a total of four cases studied (Fig. 4 and data not shown).

Although the expression pattern of AAT differed in human disease and the mouse model this does not necessarily mean that there is a different mechanism at work. It is important to note that we detected the mouse and human AAT at two different locations in the protein pattern. The spots in the murine and the human gels were separated in pI by 0.6 pH units, and the molecular mass was about 20 kDa lower in HD post-mortem brains. The 61-kDa form of AAT that ceased to be detectable in the murine system was most likely the unprocessed 46-kDa protein with 413 amino acids. The 15-kDa difference in molecular mass of AAT between the calculated and observed value was most likely because of its globular conformation, which caused decreased SDS binding and therefore lower mobility in SDS-PAGE (57). Considering the sequence identity of 44% of the SERPINs CTS and AAT (58), the molecular mass shift in SDS gels of about 30 kDa of CTS might also be because of the reduced binding of SDS. Contrasting humans possessing one AAT gene it has been shown that mice belonging to the species Mus musculus have at least five (59). In C57Bl/6 mice two orthodox, with human-like specificity at the reactive center, as well as three unorthodox genes are located on chromosome 12. The reactive center of a SERPIN is the region where the serine protease cuts between a variable amino acid at position P1 and the conserved serine at position P1’ before it can form an inseparable inhibitory complex with the SERPIN (59, 60). The amino acid at P1 varies between methionine (humanlike, orthodox), tyrosine, or leucine (both not human-like and therefore unorthodox) (59). Because of rapidly diverging reactive center sequences in unorthodox genes there may be differences in protease specificity between the two groups (61). In this study, though, there is a simultaneous reduction in expression of two different SERPINs, AAT and CTS, in R6/2 tissue (liver, heart, and testes). This indicates that a common inhibitory activity of both SERPINs or some other mechanism involving SERPINs might be at work in the pathological process of HD that is not dependent on a single specificity in the reactive center. Nevertheless it has already been shown that both inhibitors possess a common inhibitory potential against one protease but not others (62).

The six spots of AAT detected in human HD possess an observed molecular mass of about 40 kDa. It has been reported in the literature that AAT was cleaved in the presence of heparin and glucose. The protein fragment emerging after cleavage had a molecular mass of about 45 kDa (63), which is within range of the 40-kDa protein identified in human HD cases indicating a similar processing of AAT in HD. The double spot pattern was also seen in one of the anterior cingulate cortex control cases with a fresh hemorrhage in the cerebellar and subarachnoid space and a moderately acute encephalopathy of hypoxic-ischemic-type with increased GFAP expression (data not shown). It seems therefore conceivable that the emergence of this AAT pattern in humans belonged to a more general mechanism, maybe an acute phase-like response of the brain, to deal with excessive stress and tissue damage.

ABC was reported to have chaperone function (64) and can be induced by heat and other stresses conveying thermotolerance to cells like other molecules belonging to the group of small Hsp (65). It is expressed in many different tissues at mRNA level (66). In the normal brain ABC is expressed by cells in the deeper layers of the white matter (31). Tumor necrosis factor-α-stimulated human astrocytes also express the protein (67). Up-regulation has also been demonstrated in pathological conditions such as brain tumors (68). ABC has been implicated as a first line of defense against damaged or disorganized intermediate filaments, which lead to inclusion body formation, because of its debundling properties (27). In
our study the appearance of intranuclear inclusions and neuropil aggregates in R6/2 mice at 3 to 4 weeks coincides with decreased ABC expression after 4 weeks (see Table I and Fig. 2B). Interestingly this decrease is seen in the brain but not in the heart of R6/2 mice (data not shown). This could be because of a different disease progression of HD in different tissues or the higher expression level of ABC in the heart. Therefore it might be possible that this protein is responsible for keeping proteins such as the truncated N terminus of huntingtin from precipitating into intranuclear neuronal inclusions and neuropil aggregates in the brain (10, 18, 19) prior to 4 weeks of age. The lack of sufficient ABC that was found at about 8 weeks of age might therefore contribute to the increase in the formation of aggregates. This lack could be because of lower expression with increasing severity of disease and/or rapid utilization by aggregates. ABC is not expressed ubiquitously. It was detected in the brain (see Fig. 1, Fig. 2, Table I, and Table II) and heart (Table II and data not shown) but not in the spleen (Table II), liver, and testes (see Tables II and III). The difference in the isoelectric point of 0.6 pH units could be because of post-translational modifications and/or processing of the proteins by enzymes. Other soluble Hsps such as Hsp70 and Hsp40 were already shown to possess an inhibitory capacity for huntingtin fibril formation (29). A recent study of sHsp implicates Hsp40, Hsp70, and N-ethylmaleimide-sensitive factor in caspase 3 and 9 inhibition although only Hsp40 inhibited aggregate formation significantly (69).

The expression of a protein group, the MUPs with no known human homologue, was strongly reduced during the disease in liver (see Table III and Fig. 3) and urine (data not shown). These proteins are known to be expressed specifically in the liver (70). Their expression is considerably higher in male than in female mice (71). MUPs are ~20-kDa proteins that have a β-barrel structure enclosing a hydrophobic cavity (72), which serves as a binding site for 3,4-dehydro-exo-breviscrom and 2-s-butyl-4,5-dihydrothiazole (73). Binding to MUPs allows these volatile ligands to be released over prolonged periods from dried urine (73). The release of these ligands communicates aggressiveness to other males (74) and stimulates estrus in females (75). Therefore male transgenic mice close to terminal disease would no longer attract the attention of female mice by scent marks, decreasing reproductive success (6). Individual recognition of mice seems also to be dependent on MUPs, so that a decrease in the level of this protein could jeopardize the social behavior of mice (76). It is already established that the weight of testes in transgenic mice starts to decrease at about 8 weeks (45) coinciding with the decrease in MUPs availability. MUPs were also shown to be downregulated in an experimental model for stroke (77). This raises the possibility that these proteins are sensitive to pathological changes. MUPs are readily accessible for analysis by means of urine collection. Therefore these proteins could qualify as a good marker in R6/2 mice for screening and evaluation of drugs for the treatment of HD. The results obtained with this study also suggest that maintaining AAT and ABC availability during the course of HD might prevent neuronal cell death and therefore could be useful in delaying the disease progression.

The four proteins identified in this study, AAT and CTS, both SERPINs, ABC, a chaperone and member of the small Hsp family, and MUPs, which are regulated differentially between R6/2 and healthy controls, do not belong to the group of very low abundance proteins associated with HD. Very low abundance proteins identified previously include interaction partners of huntingtin (24, 78), neurotransmitter receptors, or their ligands (11, 12). Nonetheless SERPINs and chaperones have already been implicated in neurodegenerative diseases (26, 33, 50, 51). We have shown that by utilizing a proteomics approach it has been possible to identify disease-related proteins on a broad basis. To screen low and very low abundance proteins, subfractionation of tissues and cells followed by protein extraction that prevents dilution would be required.

Acknowledgments—We thank the Harvard Brain Tissue Resource Center, supported in part by Public Health Service Grant MH/NS 31862, for providing the anterior cingulate cortex tissue and ongoing support. We are indebted to Dr. Schweger for dissecting the brain regions used for the protein expression study. We thank Anj Mahal for the genotyping of mice and appreciate the excellent technical support by Marion Hermann and Yvonne Kläre.

† This work was supported in part by grants from the Wellcome Trust, Human Frontiers Science Programme, and Huntington’s Disease Society of America (to G. P. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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