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Capzb2 PROTEIN EXPRESSION IN THE BRAINS OF PATIENTS DIAGNOSED WITH ALZHEIMER'S DISEASE AND HUNTINGTON'S DISEASE

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Abstract

The silencing of actin capping protein β2, Capzb2, by RNAi in developing cultured neurons results in short, dystrophic neurites reminiscent of cytoskeletal changes seen in diverse neurodegenerative diseases, including Alzheimer's disease (AD) and Huntington's disease (HD). Actin and tubulin are two major cytoskeletal proteins indispensable for normal neurite development and regenerative responses to injury and neurodegenerative stimuli. We have previously shown that Capzb2 binds tubulin and, in the presence of microtubule- associated protein tau, affects microtubule polymerization necessary for neurite outgrowth and normal growth cone morphology. Accordingly, Capzb2 silencing in hippocampal neurons results in short neurites with abnormal growth cones. Decreased neurite length is found in both AD and HD. In the first step towards uncovering the possible role of Capzb2 in these diseases, we studied Capzb2 protein expression in the postmortem brains of AD and HD patients. To determine whether disease-specific changes in Capzb2 protein accompany the progression of neurodegeneration, we performed Western Blot analysis of prefrontal cortices (PFC) and hippocampi (HPC) in AD patients and of PFC and heads of caudate nuclei (HCN) in HD patients. Our results show disease- and area-specific dynamics in the levels of Capzb2 protein expression in the progressive stages of AD and HD.

Keywords

Actin capping protein; Alzheimer's; Huntington's

1. Introduction

Abnormalities in the cytoskeleton are found in many neurodegenerative diseases including AD. How these abnormalities affect neurodegeneration remains unclear. In *Drosophila*,

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neurodegeneration stems directly from mutations in alpha and beta subunits of the actin capping protein (CP), demonstrating that a mutation in a gene encoding an actin cytoskeleton regulator can lead to the demise of neurons [1]. Recently, we showed that RNAi-mediated silencing of the actin capping protein β2 subunit (Capzb2) in cultured hippocampal neurons resulted in short, dystrophic neurites [2] reminiscent of cytoskeletal changes associated with neurodegeneration in AD and HD. Interestingly, apolipoprotein E4 isoform (apoE4), the only confirmed genetic risk factor for late onset AD [3], inhibits neurite outgrowth in cultured neuronal cells [4], while the simplification of dendritic branching patterns in the brains of AD patients correlates with the presence of apoE4 allele [5]. Furthermore, amyloid precursor protein (APP), similar to Capzb2, is concentrated in lamellipodia [6], consistent with the idea that APP may play a role in growth cone motility and neurite outgrowth. The targets of the aggregations initiated by the interaction with mutant huntingtin are considered responsible for the neuronal morbidity in HD; these targets include cytoskeletal proteins [7]. Accordingly, dystrophic neurites containing mutant huntingtin are found in deep cortical layers of HD patients [8]. Moreover, decrease in neurite density and abnormal distribution of cytoskeletal markers by immunohistochemistry have been found in cortices of pre-symptomatic HD patients [9]. These neuropathological findings are thought to underlie cognitive and behavioral disturbances that often precede motor deficits [10,11].

CP is an F-actin binding protein that functions as an α/β heterodimer. By binding the barbed end of F-actin, the CP heterodimer blocks the access of actin monomers to the fast growing end. Both *Drosophila* and mammalian CP subunits have been shown to play a critical role in the organization and dynamics of lamellipodia and filopodia in non-neuronal cells by regulating the actin cytoskeleton [12,13]. In mammals, the β-subunit is encoded by one gene that gives rise to three isoforms [14]; one of these isoforms, Capzb2, is predominantly expressed in the brain [14]. While we have shown that Capzb2 function is indispensable for the normal morphology of growth cones and neurite length [2], it remains unclear what Capzb2 expression may mean for neurons during neurodegenerative disease or following injury. The established first critical step in response to axotomy is the initiation of microtubule polymerization and F-actin cytoskeleton rearrangement leading to the formation of a motile growth cone [15]. We have demonstrated that Capzb2 in the growth cones not only caps the F-actin barbed end, but also binds βIII-tubulin directly to affect the rate and extent of microtubule polymerization [2]. Interestingly, the interaction between actin capping protein and β-tubulin has been uncovered in a mass spectrometry screen for the alterations in protein-target binding in vivo in response to spatial learning [16].

In this study, we investigate the expression of Capzb2 protein in the postmortem brains of patients at progressive stages of two neurodegenerative diseases accompanied by cognitive decline, AD and HD. We conducted our analyses in the regions affected early and specifically in each disease (hippocampi in AD and heads of caudate nuclei in HD) as well as in the region affected as both diseases progress (prefrontal cortex). We provide evidence that Capzb2 protein expression exhibits region-specific changes in the HD brains and increases in the AD brains in spite of the known progressive neuronal loss.

2. Experimental Procedures

We examined Capzb2 protein expression levels in the postmortem brain tissue from patients diagnosed with Alzheimer's disease (11) and Huntington's disease (9) from Massachusetts Alzheimer Disease Research Center (MADRC) at Massachusetts General Hospital. Tissue from 10 control (neuropathology- absent) brains was obtained from either MADRC or Boston Medical Center (BM) (Table 1).

Total protein extracts were prepared from the prefrontal cortex (Brodman area 9), hippocampus, and head of the caudate nucleus in RIPA buffer. The final protein concentration of each sample was determined by BCA assay. Protein samples were subjected to SDS-PAGE and immunoblotting with anti-Capzb2 1:1000 (DSHB) and anti-GAPDH 1:10000 (Ambion) primary antibodies. A Chemiluminescent Detection System (Pierce) was used to visualize protein expression on an electronic capture Imager (Kodak). Densitometry was performed using ImageJ version 1.37v software (National Institutes of Health) and measurements were expressed as a relative value: disease samples were compared to the average value of control cases considered 100% (Relative Protein Expression, %, Figures 1–4).

3. Results

In AD HPC (Figure 1), about a half of the examined cases (6 out of 11) have increased Capzb2 protein expression in comparison to controls. Moreover, the increase in Capzb2 protein expression (on average 150% of the control level) in these cases was more pronounced than the decrease (on average 25% less than the control level) noted in the remaining 5 examined cases. The highest individual increase was observed in case 972 at BB stage III–IV (close to 230%). The highest individual decrease was recorded in case 1325 at BB stage V–VI (−50%). A comparable trend was seen in AD PFC (Figure 2). In comparison to controls 8 out of 11 AD PFCs showed an increase in Capzb2 protein expression. The decrease in protein expression, noted in three cases, was relatively small (−10% to −20%). The highest individual increase was observed in one of the advanced AD cases, BB stage V–VI (case 1325, ~ 220%). The highest individual decrease was recorded in a less advanced AD case, BB stage III–IV (case 972), −20%. Thus, the case with the highest increase in Capzb2 protein expression in HPC had the highest decrease of Capzb2 expression in PFC. Conversely, the case with the highest decrease in Capzb2 protein expression in HPC (1325, BB stage V–VI, −50%) had the highest recorded increase in PFC expression (~ 220%).

In the heads of caudate nuclei (HCN) of HD patients there was a uniform decrease in the Capzb2 expression (Figure 3). Regardless of the stage of the disease, eight out of nine examined cases showed uniform decrease (on average −65%) in Capzb2 expression level in comparison to controls. In sharp contrast, in the majority of the cases (7 out of 9), the PFC showed increase in Capzb2 expression ranging from 110% to over 200% of the control level (Figure 4). Thus, Capzb2 expression in HD brains shows starkly divergent, region-specific trends.

4. Discussion

In the caudate nuclei of HD patients overall, the Capzb2 protein expression is reduced in comparison to controls (Figures 3). However, in AD (Figures 1 and 2) as well as in PFC of HD patients (Figure 4), several individuals show increased levels of Capzb2 expression even in the advanced stages of each disease.

While the accumulation of hyperphosphorylated tau was demonstrated in the dystrophic dendrites of the affected (tangled) neurons in AD, the remaining non-tangled neurons exhibited proliferation of perisomatic dendrites as well as sprouting of distal dystrophic neurites [17]. The presence of growth cone-like structures on distal ends of processes has been interpreted as regenerative response occurring simultaneously with degenerative changes [17]. We found that the Capzb2 protein expression was increased in both the hippocampus and the prefrontal cortex of several AD patients. Previously we identified Capzb2 as a link between microfilament and microtubule assembly in the growth cone [2]. Together, these data raise the possibility that Capzb2 may be involved in morphological changes associated with regenerative response in neurons.

We found that Capzb2 protein levels exhibit diverging trends in the head of caudate nucleus (HCN) and the prefrontal cortex (PFC) during the progression of HD (Figures 3 and 4). A profound decrease in Capzb2 expression is noted at the earliest HD grade grossly diagnosable by the atrophy of the medial paraventricular portion of the caudate nucleus (Vonsattel grade 2/4) [18]. At this stage there is a severe neuronal loss (>50%)[18], which could explain the approximately 50% reduction in Capzb2 expression in HCN (Figure 3). However, PFCs of the same patients exhibit increased Capzb2 expression (Figure 4). PFC pyramidal neurons of layers III and V in HD patients have been found to augment their dendritic tree [19]. The observed increase in Capzb2 expression in the PFC of HD patients may reflect cytoskeletal reorganization during regenerative responses to the ongoing degeneration of cortical neurites [8, 9].

Thus, two clinically, morphologically, and biologically different neurodegenerative diseases, AD and HD, have in common decreased neurite length and the occurrence of growth cone formation followed by sprouting. These morphological features were shown to be affected by the changing levels of Capzb2 [2]. The here reported Capzb2 expression trends during the development of AD and HD warrant further evaluation of the potential role of Capzb2 as the regulator of neurite length and growth cone morphology in neurodegeneration.

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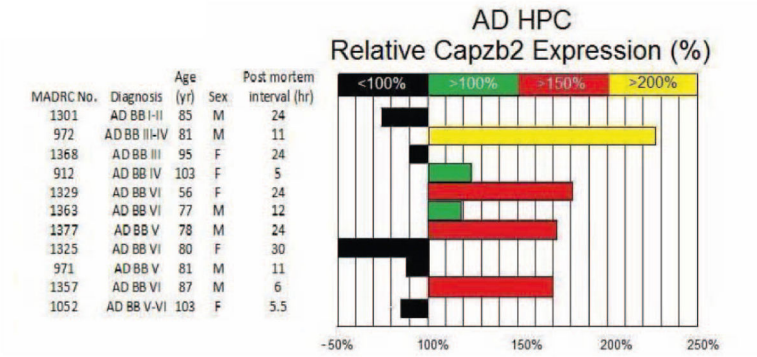
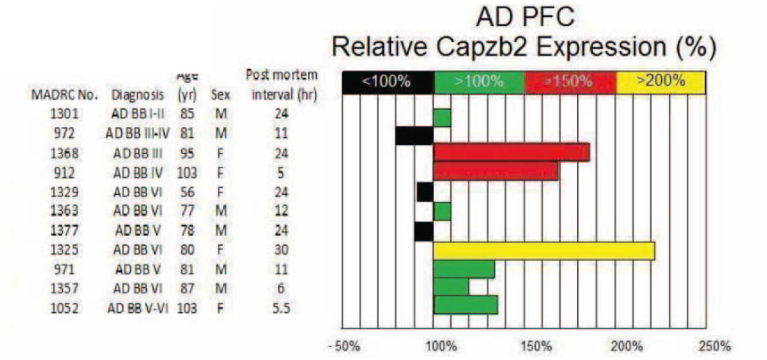


Figure 1.

Relative protein expression in the hippocampi (HPC) of the individual patients in the progressive stages of AD (Braak and Braak Stages, BB, I–VI) in comparison to normal controls.



MADRC No.	Diagnosis	Age (yr)	Sex	Post mortem interval (hr)
1301	AD BB I-II	85	M	24
972	AD BB III-IV	81	M	11
1368	AD BB III	95	F	24
912	AD BB IV	103	F	5
1329	AD BB VI	56	F	24
1363	AD BB VI	77	M	12
1377	AD BB V	78	M	24
1325	AD BB VI	80	F	30
971	AD BB V	81	M	11
1357	AD BB VI	87	M	6
1052	AD BB V-VI	103	F	5.5

Figure 2.

Relative protein expression in the prefrontal cortices (PFC) of the individual patients in the progressive stages of AD (Braak and Braak Stages, BB, I–VI) in comparison to normal controls.

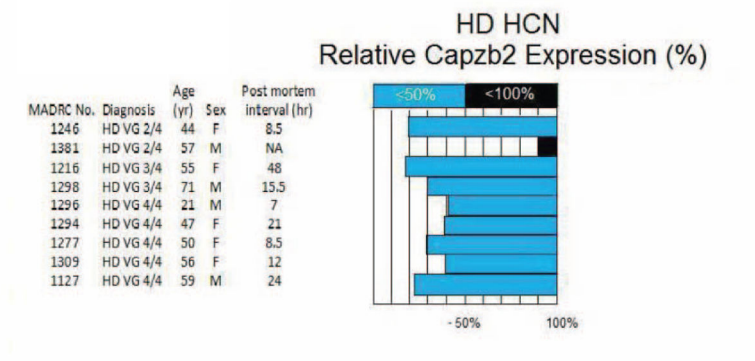


Figure 3.

Relative protein expression in the heads of caudate nuclei (HCN) of the individual patients in the progressive stages of HD (Vonsattel Stages, VS, 2–4) in comparison to normal controls.

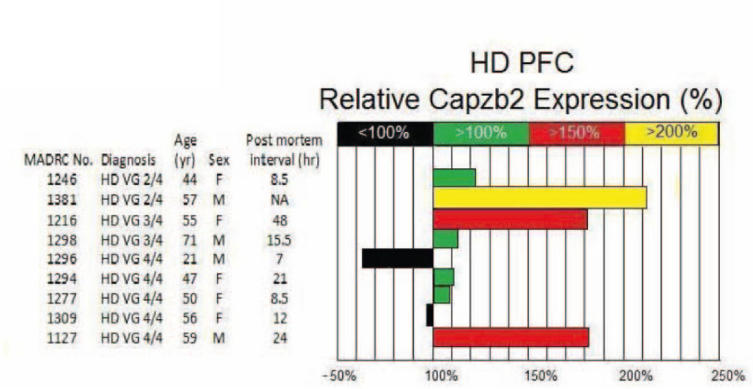


Figure 4.

Relative protein expression in the prefrontal cortices (PFC) of the individual patients in the progressive stages of HD (Vonsattel Stages, VS, 2/4, 3/4, and 4/4) in comparison to normal controls.

Table 1

Control brains used to obtain the average (normal, 100%) Capzb2 expression in each of the examined regions (HPC, PFC, HCN).

Case no.	Age [yr]	Sex	Post mortem interval [hr]
BM 1	58	M	19
BM 6	65	M	4
BM 7	81	M	37
BM 9	72	F	23
BM 11	73	F	13
BM 12	33	M	45
BM 13	68	F	25
MADRC 1314	56	M	36
MADRC 1339	79	F	48
MADRC 1388	85	F	27