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Casein Kinase 1 alpha associates with the tau-bearing lesions of inclusion body myositis

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Abstract

Inclusion body myositis and Alzheimer's disease are age-related disorders characterized in part by the appearance of intracellular lesions composed of filamentous aggregates of the microtubule-associated protein tau. Abnormal tau phosphorylation accompanies tau aggregation and may be an upstream pathological event in both diseases. Enzymes implicated in tau hyperphosphorylation in Alzheimer's disease include members of the casein kinase-1 family of phosphotransferases, a group of structurally related protein kinases that frequently function in tandem with the ubiquitin modification system. To determine whether casein kinase-1 isoforms associate with degenerating muscle fibers of inclusion body myositis, muscle biopsy sections isolated from sporadic disease cases were subjected to double-label fluorescence immunohistochemistry using selective anti-casein kinase 1 and anti-phospho-tau antibodies. Results showed that the alpha isoform of casein kinase 1, but not the delta or epsilon isoforms, stained degenerating muscle fibers in all eight inclusion body myositis cases examined. Staining was almost exclusively localized to phospho-tau bearing inclusions. These findings, which extend the molecular similarities between inclusion body myositis muscle and Alzheimer's disease brain, implicate casein kinase-1 alpha as one of the phosphotransferases potentially involved in tau hyperphosphorylation.

Keywords

Inclusion body myositis; Alzheimer's disease; tau protein; protein phosphorylation; protein kinases; casein kinase 1; immunohistochemistry

Sporadic inclusion body myositis (s-IBM) is a progressive muscle disease that leads to atrophy of specific muscle groups [12]. Characteristic histopathological findings include vacuolar degeneration of muscle fibers and mononuclear cell inflammation [3]. s-IBM muscle also shares pathobiochemical features with affected neurons in Alzheimer's disease (AD), including the accumulation of β-amyloid peptide [4,32] and the formation of intracellular filamentous inclusions composed of the microtubule associated protein tau [5]. In both s-IBM and AD, the tau proteins incorporated into filaments contain elevated stoichiometries of phosphorylation [23,24] and ubiquitination [6,20]. Depending on the sites occupied, tau hyperphosphorylation

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can lead to loss of microtubule binding activity and promotion of aggregation [33]. Thus, inappropriate phosphorylation of tau may contribute to lesion formation in both s-IBM and AD.

Tau phosphorylation reflects a balance among protein kinase and phosphoprotein phosphatase activities. Phosphotransferases implicated in tau hyperphosphorylation in AD include extracellular regulated kinase (ERK) [27] and Cdk5 [8,38]. Both enzymes associate with inclusions within degenerating muscle fibers in s-IBM [35,36,47,48], suggesting that common mechanisms may mediate tau hyperphosphorylation in both s-IBM and AD. Recently we showed that members of the casein kinase 1 (CK1) family of phosphotransferases fulfill criteria expected of protein kinases involved in AD pathogenesis [22]. The human CK1 family is composed of at least six gene products (Ckiα, γ1, γ2, γ3, δ and ε), each containing a conserved protein kinase domain joined to variable amino- and carboxyl-terminal tails [18]. CK1 isoforms are candidates for mediating protein hyperphosphorylation because their phosphotransferase domains selectively recognize acidic amino acid sequences including those containing phospho-amino acids. As a result, they can function processively or synergize with other protein kinases to support high-stoichiometry substrate phosphorylation [14]. CK1 activity associates with brain microtubules [42] and contributes to basal levels of tau phosphorylation in cultured cells [28]. Moreover, at least one isoform, Ckiδ, can phosphorylate tau and modulate its binding affinity for microtubules when highly overexpressed in cultured cells [28]. To identify which isoforms gain access to substrates under pathophysiologically relevant conditions, their colocalization with intact AD lesions has been investigated immunohistochemically in diseased tissue. In AD hippocampus, CK1 isoforms colocalize with ubiquinated cytoplasmic lesions including both granulovacuolar degeneration bodies and neurofibrillary tangles [15,22]. Isoforms Ckiδ and ε preferentially associate with the former whereas Ckiα predominates in the latter [22]. Consistent with these observations, Ckiα copurifies with paired helical filaments from AD brain, composing ~0.3% (w/w) of affinity purified preparations [25]. Because CK1 isoforms are expressed widely in tissues, including muscle [26], they may modulate tau phosphorylation and aggregation state in s-IBM as well as AD. If so, then CK1 isoforms may be expected to colocalize with tau inclusions in affected muscle fibers of s-IBM. Here we test this hypothesis by examining the distribution of Ckiα, δ and ε in degenerating muscle fibers of s-IBM.

Muscle biopsy samples were obtained from the Neuromuscular Center at The Ohio State University College of Medicine. Muscle from four subjects undergoing biopsy for aching muscles were without pathological findings and these served as controls (mean age ± SD of 51 ± 13 yrs; Table 1). Muscle from eight subjects (mean age ± SD of 71 ± 9 yrs; Table 1) fulfilled diagnostic criteria for s-IBM [17]. The affected population was biased toward elderly males because s-IBM occurs predominantly in men aged over 50 years [12]. Transverse cryostat sections (10-μm thick) were cut from each biopsy, fixed in ice-cold acetone [46], and processed as described [35]. The hematoxylin and eosin staining pattern for each section was consistent with the diagnosis listed in Table 1, showing invading mononuclear inflammatory cells in s-IBM cases but not in control cases (data not shown).

To further characterize s-IBM lesions with respect to tau protein and CK1 isoforms, tissue sections were stained with primary antibodies PHF1 [16], 128a [15,22,25], Ckiε [22], and C19 [22], and subjected to single-label confocal fluorescence microscopy as described previously [22]. PHF1 is a mouse monoclonal antibody that binds tau phosphorylated at residues Ser396 and Ser404 [40]. It was chosen for analysis because tau phosphorylated at these residues colocalizes with a range of IBM pathological features, including cytoplasmic inclusions, atrophic fibers, and subsarcolemmal tau, but not with diffuse cytoplasmic tau [30,34]. In addition, Ser396/Ser404 are substrates CK1 [28] as well as for other protein kinases thought to contribute to tau hyperphosphorylation including GSK3 [2] and Cdk5 [41]. 128a (Icos

Corporation, Bothell, WA) and anti-Ckiε (BD Transduction Laboratories, CA) are mouse monoclonal antibodies raised against Ckiδ and ε, respectively. C19 (Santa Cruz Biotech, CA) is a goat polyclonal IgG recognizing the C-terminus of human Ckiα isoforms. These antibodies, which have been characterized for binding specificity by immunoblot analysis [22,25], have been used for double label staining of AD lesions [22]. All primary antibody labelings were conducted as described previously for 16 h at room temperature [22]. Immunostaining was visualized with Alexa 488 or Alexa 594 goat anti-mouse IgG and Alexa 594 donkey anti-goat IgG secondary antibodies (Molecular Probes, Inc., Eugene, Oregon). Images were collected on a Zeiss LSM 510 Meta Laser Scanning Confocal Microscope fitted with Argon and Helium/Neon I lasers.

Muscle fibers containing tau inclusions were found using PHF1 (Fig. 1A). The immunostaining pattern was specific for s-IBM tissue and never found in normal control samples. In AD brain sections, fluorescence microscopy is complicated by the presence of autofluorescent substances which can appear over large spectral ranges [43,44]. However, in muscle tissue, under single labeling conditions using Alexa 488-linked secondary, autofluorescence detected in the red channel was minor (Fig. 1B) and did not overlap with PHF1 staining appearing in the green channel (Fig. 1C). Among CK1 isoforms α, δ and ε, only Ckiα immunoreactivity was found in s-IBM tissue (shown for Ckiα only in Fig. 1 D-F). Like PHF1 immunoreactivity, detectable autofluorescence, this time in the green channel, was minimal (Fig. 1D). These data indicated that Ckiα was the major CK1 isoform associated with s-IBM lesions, and that autofluorescence background was sufficiently low to make double-label fluorescence methods feasible.

Therefore, double-label confocal immunohistochemistry was performed to determine whether Ckiα colocalized with tau-bearing lesions in s-IBM sections. On average, 6.4 ± 1.1 (SEM) PHF1-positive vacuolated fibers were quantified per each of the eight s-IBM cases, with multiple PHF-1 positive cytoplasmic inclusions, including those with “squiggly” morphology [3], being observed per fiber (Figs. 2 and 3). Similarly, C19-positive fibers were found in every s-IBM case examined (Table 1), with 3.5 ± 0.7 (SEM) C19-positive fibers found per case (Fig. 3). In these fibers, C19-positive immunoreactivity overlapped extensively with PHF1 immunoreactivity within cytoplasmic inclusions and also rimmed vacuoles (Fig. 2). When quantified using the Wilson score statistical method [37], the proportion of all PHF1-positive muscle fibers (n = 51) containing C19 immunoreactivity was 51 ± 13% (95% C.I.) (Fig. 3). Conversely, 93 ± 16% (95% C.I.) of C19-positive muscle fibers were also PHF1-positive fibers (Fig. 3).

These findings extend the observation that CK1 isoforms differentially associate with tau pathology. In normal tissues, Ckiα activity is widely distributed within cells [9,18] where it binds diverse proteins including nuclear protein regulator of chromosome condensation 1 (RCC1), high mobility group proteins 1 and 2, synaptotagmin IX, centaurin-α1, and members of various transcription factor families [9,10,21,39]. Some of these proteins have important functions in muscle. For example, centaurin-α1 activates ERK kinases implicated in the pathological phosphorylation of tau in IBM [47], whereas deficiency in at least one member of the synaptotagmin family (synaptotagmin VII) results in an inflammatory myopathy resembling IBM [7]. In many cases, CK1-mediated phosphorylation precedes ubiquitination and subsequent intracellular trafficking or proteasome-mediated turnover of substrates. For example, Ckiα mediates phosphorylation-dependent turnover of transcription factor Cubitus Interruptus [21]. Other mammalian CK1 homologs modulate turnover of substrates in involved in circadian rhythm [11] and the Wnt [31] and Hedgehog [21] signaling pathways. In lower eukaryotes, CK1 isoforms play a similar role in the regulation of plasma membrane-bound substrates including mating type receptors Ste2p and Ste3p [13,19] and also components of the permeases and sensors involved in the detection and transport of extracellular nutrients

[1,29,45]. These observations suggest that CK1 isoforms function in part to mediate ubiquitination of diverse proteins in different biological contexts. Immunohistochemical studies indicate that Ckiα is positioned to contribute to tau hyperphosphorylation and ubiquitination in both AD [22] and s-IBM (herein). In contrast, Ckiδ is more closely associated with ubiquitinated inclusions associated with granulovacuolar degeneration in hippocampal neurons [15].

In summary, these data extend the pathological similarity between the tau-bearing lesions of AD and IBM to include CK1 colocalization. The results implicate CK1 isoform Ckiα in the upstream pathological events that lead to accumulation of tau phospho-epitopes in both diseases.

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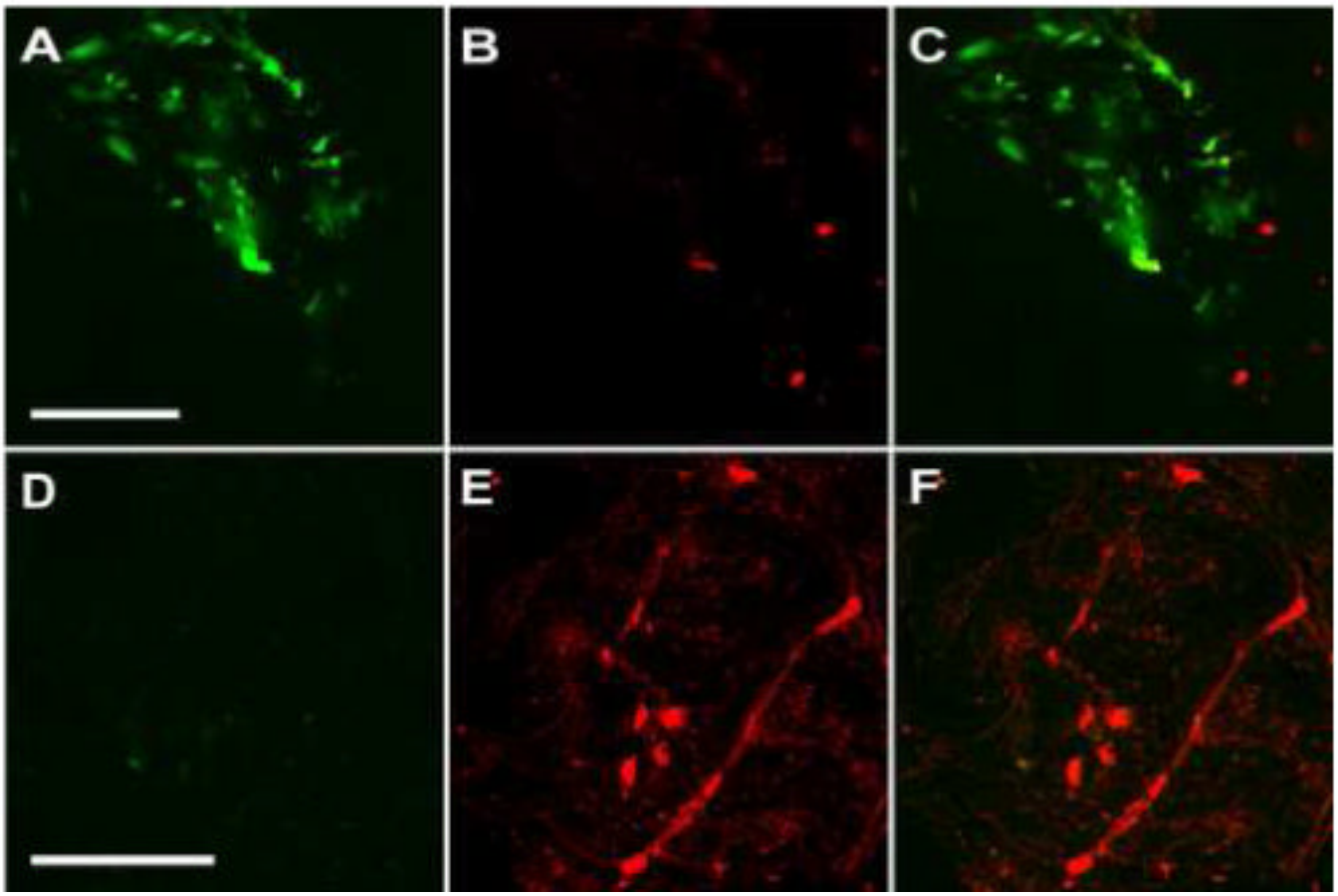

Fig. 1.

Histochemical analysis of tissue sections (10-μm thick) prepared from s-IBM vacuolated muscle fibers. Single label immunofluorescence confocal microscopy performed with monoclonal anti-phospho tau antibody PHF1 (~4 μg/ml) and Alexa 488-linked secondary showing phospho-tau inclusions in the (A) green channel, with minimal autofluorescence background in (B) the red channel. (D-E), Single label immunofluorescence confocal microscopy performed with polyclonal anti-Ckiα antibody C19 (3 μg/μl) and Alexa 594-linked secondary showing Ckiα-bearing inclusions in the (E) red channel, with minimal autofluorescence background in (D) the green channel. (C, F), merged images, where yellow color corresponds to colocalization. Scale bars for each row represent 10 μm.

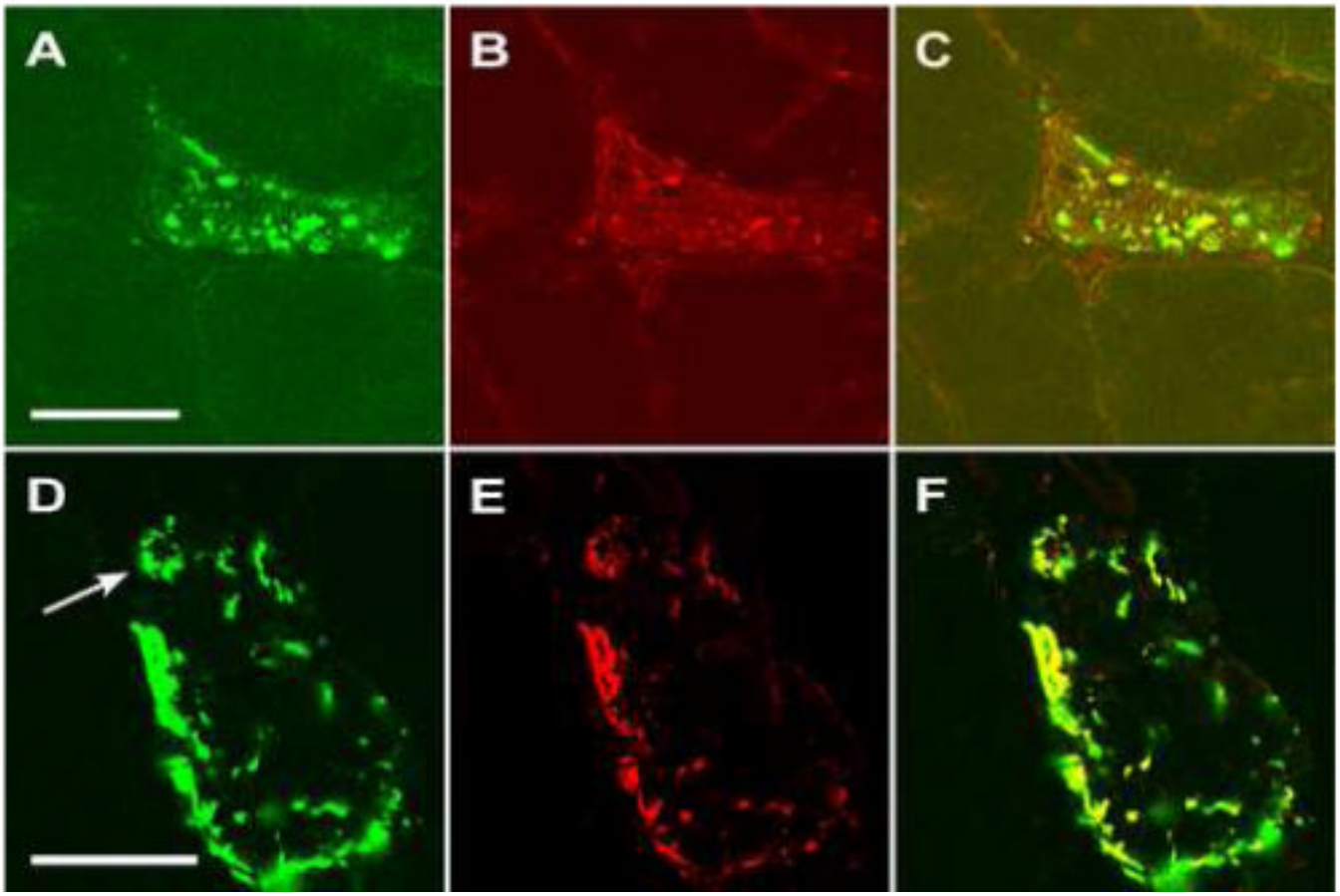

Fig. 2.

Double-label confocal images from two s-IBM vacuolated muscle fibers stained with (A, D) PHF1/Alexa 488-linked secondary antibodies to visualize phospho-tau, and (B, E) C19/Alexa 594-linked secondary antibodies to visualize Ckiα. (D, F) Merged images, where yellow color corresponds to colocalization. Scale bars for each row represent 10 μm. Ckiα and phospho-tau colocalize in both cytoplasmic inclusions and rimmed vacuoles (*arrow*).

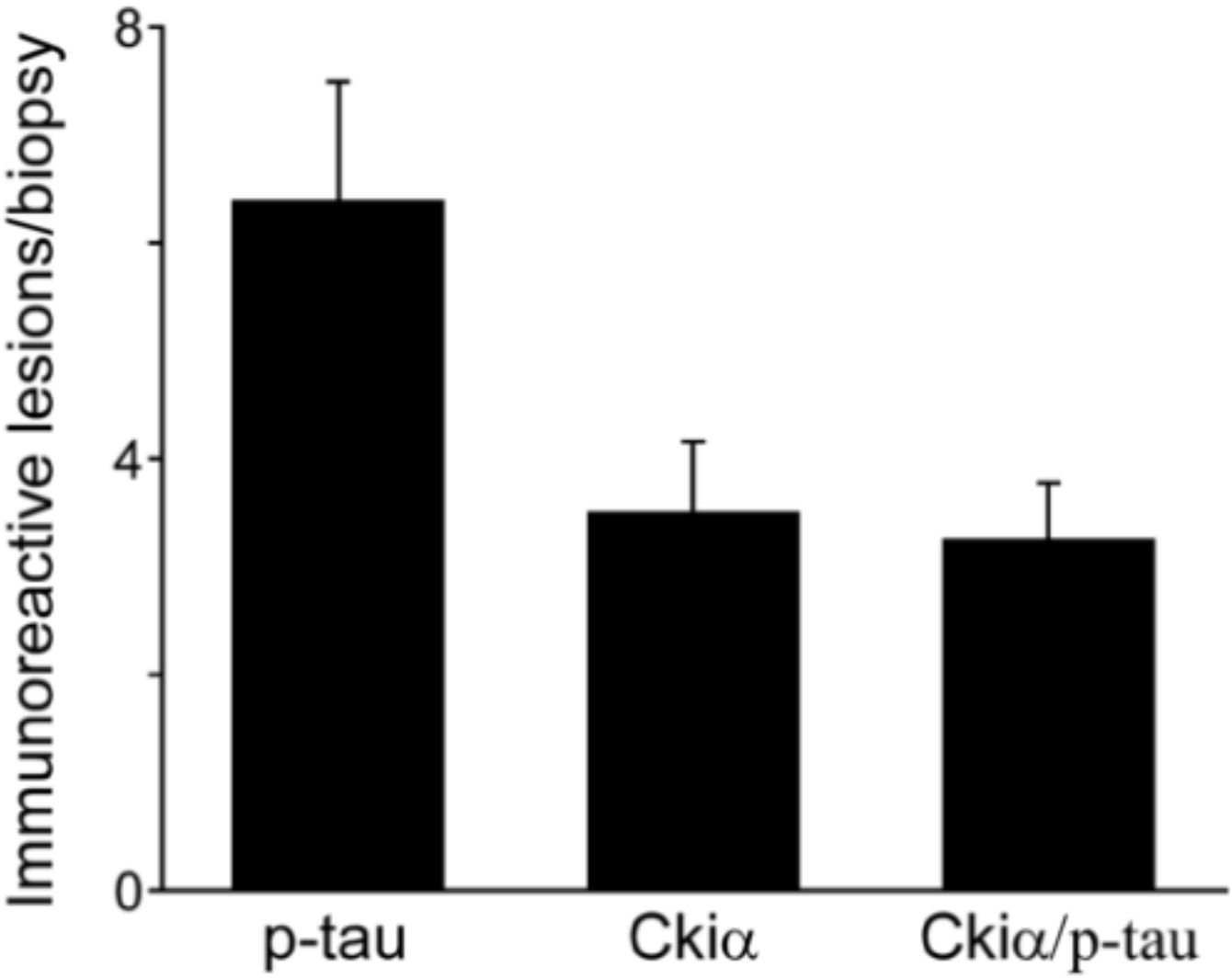

Fig. 3.

Colocalization of phospho-tau and Ckiα in s-IBM lesions is extensive. Double-label immunofluorescence images (similar to those shown in Fig. 2) were collected from all s-IBM cases summarized in Table 1 (n = 8 cases). Numbers of muscle fibers positive for PHF1 (p-tau), for C19 (Ckiα), or for both C19 and PHF1 (Ckiα/p-tau) were then quantified ± SEM. Approximately 50% of PHF1-positive lesions colocalized with Ckiα immunoreactivity, whereas nearly all Ckiα immunoreactivity was associated with PHF-1 positive lesions.

Table 1

Case demographics and results

Case (#)	Age (y)	Gender	Diagnosis	Vacuolated fibers positive for Ckiα
1	38	F	Control	-
2	40	F	Control	-
3	61	F	Control	-
4	63	M	Control	-
5	66	M	s-IBM	+
6	82	M	s-IBM	+
7	79	M	s-IBM	+
8	65	F	s-IBM	+
9	75	F	s-IBM	+
10	56	M	s-IBM	+
11	77	M	s-IBM	+
12	70	M	s-IBM	+