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Immunohistochemical Analysis of Activin Receptor-Like Kinase 1 (ACVRL1/ALK1) Expression in the Rat and Human Hippocampus: Decline in CA3 During Progression of Alzheimer's Disease

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Abstract

The pathophysiology of Alzheimer's disease (AD) includes signaling defects mediated by the transforming growth factor β —bone morphogenetic protein—growth and differentiation factor (TGF β -BMP-GDF) family of proteins. In animal models of AD, administration of BMP9/GDF2 improves memory and reduces amyloidosis. The best characterized type I receptor of BMP9 is ALK1. We characterized ALK1 expression in the hippocampus using immunohistochemistry. In the rat, ALK1 immunoreactivity was found in CA pyramidal neurons, most frequently and robustly in the CA2 and CA3 fields. In addition, there were sporadic ALK1-immunoreactive cells in the stratum oriens, mainly in CA1. The ALK1 expression pattern in human hippocampus was similar to that of rat. Pyramidal neurons within the CA2, CA3, and CA4 were strongly ALK1-immunoreactive in hippocampi of cognitively intact subjects with no neurofibrillary tangles. ALK1 signal was found in the axons of alveus and fimbria, and in the neuropil across CA fields. Relatively strongest ALK1 neuropil signal was observed in CA1 where pyramidal neurons were occasionally ALK1-immunoreactive. As in the rat, horizontally oriented neurons in the stratum oriens of CA1 were both ALK1- and GAD67-immunoreactive. Analysis of ALK1 immunoreactivity across stages of AD pathology revealed that disease progression was characterized by overall reduction of the ALK1 signal in CA3 in advanced, but not early, stages of AD. These data suggest that the CA3 pyramidal neurons may remain responsive to the ALK1 ligands, e.g., BMP9, during initial stages of AD and that ALK1 may constitute a therapeutic target in early and moderate AD.

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SUPPLEMENTARY MATERIAL

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Keywords

ACVRL1; ALK1; CA1; CA3; GAD67; hippocampus; immunohistochemistry

INTRODUCTION

The elucidation of the pathophysiologic mechanisms of Alzheimer's disease (AD) and the search for therapeutic targets for this illness has been approached by both unbiased experimental designs and hypothesis-driven studies. Recent investigations by Jaeger et al. [1] employing the former strategy of combining plasma proteomics [1], meta analysis of genome-wide association studies [2], and brain mRNA expression profiling [3] point to defects in the transforming growth factor β —bone morphogenetic protein—growth and differentiation factor (TGF β -BMP-GDF) signaling pathways in AD. These abnormalities include altered expression of TGF β -family proteins (e.g., GDF1, GDF3, myostatin), their receptors (e.g., ACVR1B, ACVR2B, ACVR1C), and co-receptors (e.g., cripto), as well as intracellular signal protein mediators, SMADs. In addition, there is evidence for increased BMP6 expression in the hippocampus of AD patients [4]. Our studies have utilized the hypothesis-based approach focusing on testing the idea that abnormalities in signaling by a member of the TGF β -BMP-GDF family protein, BMP9/GDF2, are a feature of AD pathology because of its actions on basal forebrain cholinergic neurons (BFCN). These neurons innervate the hippocampus and cerebral cortex and belong to critical neuronal systems that underlie learning, memory and attention [5, 6]. A decline in BFCN function and diminished cholinergic marker expression is apparent in aged humans and animals [7–9], in AD patients [10, 11] and in animal models of AD [10, 12–17]. Thus, it has been postulated that dysfunction and/or degeneration of BFCN contributes to the memory deficits seen in advanced age and in AD [8, 10, 11]. We [18, 19] and others [20] have obtained evidence that BMP9 is a key differentiating factor for BFCN during development and, when infused intracerebroventricularly in mice with experimental injury to these neurons, prevents BFCN loss [21]. Moreover, we subsequently showed that BMP9 infusion reverses the downregulation of BFCN markers in a transgenic mouse model of AD, ameliorates hippocampal amyloidosis, and increases the expression of several growth factors (NGF, NT3, and IGF1) [22]. These results were confirmed and extended by others who found that intranasal BMP9 administration in the same mouse model of AD improved learning and memory, reduced hippocampal amyloidosis and tau hyperphosphorylation, and suppressed brain inflammation [23]. BMP9 and a related protein, BMP10, signal by binding to- and activating a heterodimeric type II and type I receptor complex and their signaling specificity is conferred by ACVRL1/ALK1 type I receptor [24–28]. ALK1 is broadly expressed in the endothelium [29, 30] where its activity is central for normal vascular development and remodeling [31]. Mutations in the *ACVRL1* gene (reviewed in [32]), which encodes ALK1, cause hereditary hemorrhagic telangiectasia type II [OMIM #600376]—a disease characterized by arteriovenous malformations [31]. In addition to its expression in blood vessels, we found *Alk1* mRNA in fetal mouse BFCN [33] and König et al described ALK1 mRNA and protein in cultured rat neonatal hippocampal neurons and showed the upregulation of neuronal ALK1 expression upon excitotoxic and ischemic injury, possibly via a neuroprotective mechanism [34]. Thus, studies in animals indicate that hippocampal

neurons express the specific BMP9 receptor, ALK1, and that BMP9 administration ameliorates AD-like pathology in the hippocampus of mouse models of AD. Here we report that ALK1 protein signal pattern in normal human and rat hippocampus is similar and that expression in human CA3 neurons is reduced in advanced, but not early, stages of AD.

MATERIALS AND METHODS

Rat brain

Young adult 2.5-month-old Wistar rats (strain 003, Charles River Laboratories International, MA) were anesthetized with 10 mg/kg xylazine and 80 mg/kg ketamine hydrochloride given intraperitoneally and subsequently perfused transcardially with 0.1M phosphate buffer (PB) followed by 4% paraformaldehyde with 0.01% glutaraldehyde in 0.1M PB (EM fixative), pH 7.4. All animal procedures were approved by the Institutional Animal Care and Use Committee of Boston University.

Human postmortem hippocampi

Human formalin-fixed paraffin-embedded (FFPE) tissue blocks of hippocampi of twenty-two subjects were acquired through the Framingham Heart Study Brain Donation Program, Framingham, MA, the Netherlands Brain Bank, Amsterdam, Netherlands, and Boston Medical Center as described in Table 1. Boston University Medical Center's Institutional Review Board approved this study and the authors state adherence to these standards. The analyzed subjects were stratified into three groups based on Clinical Dementia Rating (CDR) score [35–37] and Braak and Braak (BB) stage [38]. The CDR was assigned based on antemortem assessment months prior to death and a postmortem *retrospective* CDR based on a family interview with one or more family members [39]. Group 1 consisted of true control individuals defined as cognitively healthy subjects without any presence of neurofibrillary tangles (NFTs) in the CA fields (CDR0, BB0; n = 7, age mean 66.0 ± 5.4 (SD) years, 2 female (F)/5 male (M)); Group 2 included subjects either cognitively intact or with minimal cognitive dysfunction (CDR0-0.5) in the limbic BB stages (CDR0-0.5, BBI-III; n = 8, age mean 91.6 ± 6.2 years, 6F/2M); and Group 3 consisted of subjects with definite AD by NINCDS-ADRDA criteria and in the isocortical BB stages (CDR1-3, BBIV-VI; n = 7, age mean 86.7 ± 6.2 years, 2F/5 M) (Table 1). Group 1 subjects (true controls without any evidence of NFTs in the hippocampus) are inherently younger than Group 2 and 3 subjects, as NFTs increase with age even in cognitively intact subjects. No Lewy body pathology was reported in any of the subjects. All subjects were de-identified and authors were blinded to subjects' CDR score and BB stage during data acquisition. Quantitative analysis of ALK1 immunoreactivity within the CA3 subregion, distinctly identifiable at the level of the lateral geniculate nucleus, was conducted on the twenty-two subjects (Table 1).

We performed tau immunohistochemistry (IHC) on all obtained hippocampi to corroborate reported BB stages in the accompanying neuropathological reports. No neuritic plaques were present in cognitively intact subjects of Group 1 (CDR0, BB0; Table 1) while amyloid- β pathology was reported in nearly all Group 3 subjects - patients diagnosed with AD clinically and neuropathologically (CDR1-3, BBIV-VI; Table 1). Group 2 included subjects with only hippocampal/limbic NFTs (BBI-III) and heterogeneous in terms of both amyloid-

β pathology and cognitive status (CDR0-0.5; Table 1). Neuropathology reports for three subjects (one cognitively intact individual, CDR0, and two with minimal cognitive deficits, CDR 0.5; Group 2, Table 1) reported absence of any amyloid- β pathology. It is possible that some or all of these three individuals (ages 89-97) could nowadays meet criteria for Primary Age-Related Tauopathy (PART) [40] and not develop AD.

Immunohistochemistry

FFPE blocks were sectioned at 5 μ m thickness, dried at room temperature for 24 h, and heated at 80°C for 24 h before IHC processing. Deparaffinization, antigen retrieval, and subsequent staining was performed with Ventana Benchmark Ultra automated IHC instrument using Ventana Medical System reagents including ultraView Universal DAB (Cat#760-500), Hematoxylin II (Cat#790-2208), and Bluing Reagent (Cat#760-2037) (Ventana Medical Systems, Inc., Roche Diagnostics Ltd., Tucson, AZ) at the Boston Medical Center Pathology Department.

Examined protein expression of ALK1 was analyzed using the following paradigm: three independent IHC experiments of each subject were processed collectively. Therefore, experiments performed yielded three independently stained step-wise sections separated by at least 10 μ m per subject for analysis. Automated IHC with the Ventana Benchmark Ultra allowed for maximally replicative conditions in IHC experiments, eliminating variability in reagent composition, quantity, incubation time, and human error, minimizing variability between experiments. Internal control sections from established subjects were stained collectively with any newly added subjects to ensure reproducibility of staining for the protein of interest. Quantitative analysis of ALK1 was generated from the imaged triplicate sections. Data from triplicate sections was averaged to obtain representative values for each subject.

Western blot

C57BL6 wild type mouse hippocampal protein lysates, 40 μ g per sample, were subjected to PAGE electrophoresis using 4-12% Bis-Tris Midi Gel (Invitrogen) and transferred to a blotting membrane with the iBlot system (Invitrogen). The membrane was blocked with 5% milk in TBS/1.5% Tween (TBS-T), washed with TBS-T, and probed overnight with rabbit anti-ALK1 antibody (1:1000, Atlas Antibodies, Stockholm, Sweden #HPA007041). Following incubation with the primary antibody, the blot was incubated in anti-Rabbit-HRP (1:4000, Bio-Rad). Reactive bands were detected with SuperSignal West Femto chemiluminescent substrate (Pierce, Rockford, IL). Chemiluminescence was captured with a Kodak ImageStation 440CF.

Antibodies

Primary antibodies included rabbit polyclonal anti-ALK1 (1:25, HPA007041, Atlas Antibodies). This anti-ALK1 antibody immunohistochemical specificity in FFPE tissues was characterized by immunoabsorption assay with the ALK1 immunogen (APrEST71390, Atlas Antibodies) incubated at 5X molar excess of the ALK1 antibody at room temperature for 1 h before applying primary and immunogen or primary alone to control tissue in the identical IHC protocol used for ALK1 analysis (Supplementary Fig. 1A-D). Specificity of

this antibody was further shown in western blot analysis of mouse hippocampus, revealing a single band at 70 kDa, corresponding to ALK1 signal (Supplementary Fig. 1E). Mouse anti-GAD67 monoclonal antibody (1:125, GR419, Sigma, Dallas, TX), mouse anti-human amyloid- β (A β) [6F/3D] monoclonal antibody (1:25, Dako, Glostrup, Denmark) rabbit anti-human tau [A0024] polyclonal antibody (1:3200, Dako), and mouse anti-human phospho-PHF-tau [AT8] monoclonal antibody (1:2000, Pierce) were also used for confirmation of neuropathological report data and qualitative analyses.

Quantitative image analysis

Slides were imaged using an Olympus BX60 light microscope, QImaging Retiga 2000R camera, and QCapture Suite and Suite-PLUS software. All images used in quantitation were analyzed with ImageJ, version 1.48, National Institutes of Health, Bethesda, MD [41, 42]. ImageJ Stitching plugin was used to compose high resolution, low magnification images [43].

Three 40 $\times$ field images, encompassing the anatomically distinct CA3 field (Supplementary Fig. 2), were used in quantitative ALK1 signal analysis of de-identified hippocampal sections to examine the CA3 subregion (n=22, see Table 1). RGB images were converted to 8-bit grayscale with intensity values ranging from 0 to 255, and the threshold value for ALK1 IHC signal in CA3, including ALK1-immunoreactivity in the pyramidal neurons and in the neuropil, was determined empirically for each case, within the range of threshold values from 100 to 175 on the 255 scale as described [44, 45]. The above-threshold ALK1 signal was subsequently calculated as a percent area without size exclusion using ImageJ v1.48 software (Supplementary Fig. 2). Mean data values from the 40 $\times$ field images in triplicate experiments comprised representative values for each human subject.

Semi-quantitative analyses

In CA1 and CA4 analyses, de-identified slides were viewed at 20 $\times$ magnification for semi-quantitative analysis of CA1 horizontally oriented neurons at the alveus border, consistent with the location of GABAergic neurons (see Fig. 3), and of CA1 neuropil signal in triplicate IHC experiments. Similarly, ALK1-immunoreactivity in CA4 neuropil and pyramidal neurons was scored semi-quantitatively in each triplicate IHC experiment. All CA1 and CA4 analyses used a scale of 0-3; 0 indicating no immunoreactivity, and 3 representing the strongest immunoreactivity. Semi-quantitative scoring was evaluated by 2 independent, blinded observers. All data values were averaged to find the mean score of ALK1 signal in each CA subregion for each subject.

Statistical analyses

Quantitative log-transformed data among subject groups were analyzed by one-way ANOVA and post-hoc Fisher's Least Significant Difference comparison test in CA3 field analysis of 22 subjects. This examined the percent area of ALK1-immunoreactivity in CA3 compared across subject groups, testing the null hypothesis that no significant difference should be observed across groups ($\alpha = 0.05$). Similar statistical analysis was conducted on semi-quantitative data. Statistical analyses were performed with GraphPad Prism, version 5.0b,

GraphPad Software Inc., San Diego, CA, and with JMP®, Version 11.2.1. SAS Institute Inc., Cary, NC, 1989-2013.

RESULTS

ALK1 protein expression in the hippocampus

Initial characterization of ALK1 immunoreactivity in the rat brain (Fig. 1) revealed prominent staining in cells of the hippocampal pyramidal layer of the CA2 and CA3 fields, while CA1 cells were infrequently stained. Moreover, multiple strongly stained cells were present in the subgranular zone and granule cell layer of the dentate gyrus. In addition, there were sporadic ALK1-immunoreactive (ALK1-ir) cells in the stratum oriens, mostly in CA1.

The ALK1 signal distribution pattern in human CA and dentate gyrus neurons was similar to that of rat. Human hippocampal projection fibers of alveus and fimbria harbored ALK1-immunoreactive axons (Fig. 2A). As in rat, multiple neurons within the CA2, CA3 (Fig. 2B), and CA4 pyramidal layer were ALK1-ir. Dentate granule layer neurons were lightly positive for ALK1, though, as in the rat, a few neurons in subgranule zone yielded strong ALK1 signal. Human CA1 neuropil yielded strong ALK1 signal, unlike rat CA1 (see Fig. 1). Consistent with the rat, only rare human CA1 pyramidal somata were highlighted by ALK1 antibody giving rise to a punctate signal (Fig. 2C). Finally in both human and rat, horizontally oriented neurons at the stratum oriens of CA1 were strongly ALK1-immunoreactive (Fig. 3). The location and morphology of these cells is consistent with the GABAergic phenotype. Therefore, we analyzed adjacent hippocampal sections using antibodies against ALK1 and glutamic acid decarboxylase 1 (GAD67), the enzyme that synthesizes GABA. Indeed, these cells were immunoreactive to both antibodies indicating that the horizontal GABAergic neurons in the stratum oriens of the human hippocampus express ALK1 (Fig. 3). A blinded, semi-quantitative analysis of the intensity of ALK1 signal of the horizontally oriented neurons at the stratum oriens revealed no significant differences between our subject groups. Similarly, in a preliminary analysis the intensity of ALK1 signal in the CA1 neuropil also did not differ among subject groups (data not shown).

ALK1 protein expression in CA3 decreases during progression of AD

We analyzed the ALK1 immunoreactivity patterns in the human hippocampus quantitatively in 22 subjects (Table 1), across stages of AD-associated pathological changes with advancing disease based on dementia ratings and AD-associated pathological changes (Table 1). The CA3 subregion was chosen for quantitative analysis due to the dense cholinergic innervation to CA3 from the basal forebrain neurons, which respond positively to treatment with neurotrophic factor and ALK1 ligand BMP9 in mice with experimental fimbria fornix injury [21]. In quantitative analysis we found that AD progression was associated with overall reduction of the ALK1 signal in CA3 (one-way ANOVA, $F_{2/19}=3.47$, $p=0.05$). AD patients (Group 3; CDR1-3; BBIV-VI) showed over 30% decrease in ALK1 signal in CA3 as compared to controls ($p=0.05$; Fisher's LSD test) and subjects with early AD-associated pathological changes (CDR0-0.5; BBI-III) ($p=0.02$; Fisher's LSD test) (Fig. 4). The ALK1 immunoreactivity in CA3 in the latter subjects did not differ from that of controls. Semi-

quantitative analysis of the ALK1 signal in CA4 neurons and neuropil found no significant change among subject groups (data not shown).

DISCUSSION

In this study we discovered the presence of ALK1 in populations of rat and human hippocampal neurons, consistent with the report by König et al [34] who found ALK1 mRNA and protein in cultured hippocampal rat neurons. We extend their findings by demonstrating similarities of ALK1 protein expression in adult rat and human hippocampus.

Using postmortem human brain samples from control subjects and from individuals with various stages of AD we documented a decrease in ALK1 protein signal in CA3 in patients with AD-associated dementia and histologically advanced disease (CDR1-3; BBIV-VI). CA3 participates in short-term spatial memory, associative memory recall, and pattern completion processes [46–48]. CA3 receives septal GABA-ergic afferents [49] synapsing on GABAergic interneurons in the hippocampal subfields (CA1, CA3, and DG) [50]. This process of disinhibition from the septohippocampal GABAergic afferents modulates memory processing by promoting CA pyramidal neurons to fire. Further, Schaffer collaterals from CA3 pyramidal cells project onto CA1 pyramidal neurons which stimulate horizontal interneurons near the stratum oriens-alveus border. These horizontal interneurons, shown here to be ALK1- and GAD67-immunoreactive, provide feedback inhibition to the CA1 pyramidal cells [51]. The ALK1-ir GABA-ergic interneurons would be expected to respond to the ALK1 agonists, BMP9 or BMP10, and may benefit from the documented BMP signaling support for GABAergic neuron differentiation and survival [52, 53]. Consistently, studies of postmortem human brain have revealed a dramatic downregulation of GAD67 expression concomitant with abnormalities in the expression of TGF β /BMP signaling molecules in this neuronal population in patients with schizophrenia and bipolar disorder [54], suggesting that defects in the TGF β /BMP signaling pathways may underlie the underappreciated neurodegeneration in these diseases.

In contrast to a dramatic neuronal loss and neurofibrillary tangle accumulation seen in CA1 in AD [38, 55–60], the CA3 neurons are relatively spared (e.g., [55, 60–62]) and there are few examples of AD-selective abnormalities in this neuronal population. These include, increased GABA β receptor 1 [63] and AMPA receptor [64] immunoreactivities, overall increase in tau mRNA expression in the pyramidal neurons [65], reduced mRNA levels of several subunits of protein phosphatase 2A that dephosphorylates the phosphorylated species of tau [66], highly abnormal morphology of the postsynaptic components of the stratum lucidum synapses between mossy fibers of granule cells and dendrites of CA3 pyramidal neurons called the thorny excrescences [67] as well as abnormal phosphorylation of tau protein in those structures [68].

Recently, we analyzed most of the subjects utilized in the current study to determine the hippocampal expression of methionine sulfoxide reductase B3 (MSRB3)—a protein encoded by a polymorphic gene with an AD-risk allele [69, 70]. Similar to MSRB3 [71], ALK1 is expressed not only in hippocampal neurons, but also in choroid plexus and ependymal lining of the human brain (data not shown). We found reduced MSRB3

expression in the CA3 stratum lucidum in AD as compared to controls [71], consistent with abnormalities in the mossy fiber synapses. Our current data provide additional evidence for AD-related defects in CA3. It is worth noting that our control subjects, though elderly, were on average 25 years younger than the individuals with early dementia. Yet, ALK1 expression in the latter group was similar to that of controls, indicating that ALK1 levels are stable during aging, and that the changes in the ALK1 signal that we observe in advanced AD are therefore related to the pathophysiology of AD and not to age.

Overall this study suggests that the CA3 pyramidal neurons may remain responsive to the ALK1 ligands, BMP9 and BMP10 [26, 28], during initial stages of AD. Given that BMP9 administration ameliorates hippocampal AD-like pathology in mouse models of this illness [22, 23], the data suggest that ALK1 may constitute a viable therapeutic target in early and moderate AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

ALK1 protein expression in the rat hippocampus. ALK1 immunohistochemistry in a 2.5-month-old rat. Prominent staining in the pyramidal neurons in CA2 and CA3, subgranular zone of the dentate gyrus (arrows) and scattered cells in the CA1 stratum oriens (arrowheads). Scale bar = 500 μ m.

Fig. 2.

ALK1 protein expression in the healthy human hippocampus. A) Robust ALK1 signal is found in the CA pyramidal layer and in the axons of alveus and fimbria. B) CA3 pyramidal cells show stronger cytoplasmic ALK1 signal in comparison to the neuronal somata of CA1 (C). Overall, the quality of ALK1 cytoplasmic signal in pyramidal neurons is granular (C, asterisk). In comparison, ALK1 neuropil signal in CA1 appears more prominent than in CA3. Scale bar A = 1500 μ m.

Fig. 3. GAD67-immunoreactive interneurons in human CA1 are ALK1-immunoreactive. In adjacent sections of healthy (CDR0, BB0) CA1 region, GAD67-immunoreactive horizontally oriented interneurons (A, arrows) are also ALK1-ir (B, arrows). Among pyramidal neurons (GAD67 non-ir), few are weakly ALK1-ir (solid arrowheads A, B). ALK1-immunoreactivity is found in fibers within alveus (B, striped arrowheads) which are not GAD67-ir (A, striped arrowheads). * Indicates landmark vessels; # indicates alveus adjacent to the lateral ventricle. Scale bars = 50 μ m.

Fig. 4.

ALK1 immunoreactivity in CA3 declines in advanced AD. A) Robust ALK1-immunoreactivity in CA3 pyramidal neurons (arrows) and surrounding neuropil of a control subject (CDR0 BB0). B) Weak ALK1 signal in CA3 pyramidal neurons (arrows), and almost absent ALK1-immunoreactivity in surrounding neuropil of an AD subject (CDR1 BBIV). C) ALK1-immunoreactivity in the pyramidal neurons and in the neuropil, was determined empirically and the percent area of above-threshold ALK1 signal was determined using ImageJ software (see Methods). The original data $\pm$ SEM are plotted on the graph. The data were log-transformed and analyzed by one-way ANOVA. There was a statistically significant decrease in ALK1 signal in advanced AD patients (CDR1-3; BBIV-VI) compared to control subjects (CDR0; BB0) (* $p=0.05$, Fisher's LSD) and to subjects with early AD-associated pathological changes (CDR0-0.5; BBI-III) (** $p=0.02$, Fisher's LSD). Scale bars = 20 μ m. Blue squares represent the examples shown in A and B.

Table 1

Analyzed subjects organized according to Braak and Braak (BB) stage, hippocampal plaque score (CERAD), cognitive status Clinical Dementia Rating (CDR) score, age, gender, and postmortem interval (PMI).

Subjects in Groups 1-3	BB stage	Hippocampal plaques (CERAD)	Cognitive status/CDR score	Age (y)	Gender	PMI (h)
Group 1 (CDR 0, BB 0)						
NBB 2012 052	0	0	No evidence of dementia	64	F	5.7
NBB 2010 115	0	0	No evidence of dementia	70	M	3.6
NBB 2011 091	0	1+ (D)	No evidence of dementia	76	M	6.8
BM 23	0	0	No evidence of dementia	66	F	24
BM 29	0	0	No evidence of dementia	61	M	24
BVAX 85	0	0	No evidence of dementia	61	M	7.5
BM 33	0	0	No evidence of dementia	64	M	27
Group 2 (CDR 0-0.5, BB I-III)						
BVAX 255	I	0	0	92	F	16
NBB 2012 059	II	1+ (D)	0	98	F	4.6
NBB 2013 011	III	2+ (D)	0	92	F	4.4
NBB 2012 001	II	3+ (D)	0.5	79	M	5.7
BVAX 219	II	0	0.5	97	F	6
BVAX 226	II	0	0.5	97	F	unknown
BVAX 147	III	1+ (D), 1+ (N)	0.5	89	M	unknown
NBB 2013 010	III	0	0.5	89	F	6.6
Group 3 (CDR 1-3, BB IV-VI)						
NBB 2013 009	IV	1+ (D), 3+ (N)	1	92	F	7.4
NBB 2008 075	IV	3+ (D), 1+ (N)	Overt dementia; CDR not available	88	M	5.0
BVAX 100	IV	1+ (D), 2+ (N)	1	89	M	3.0
BVAX 205	IV	2+ (N)	1	96	F	3.5
BVAX 250	VI	0	3	82	M	6.5
BVAX 368	VI	2+ (D), 2+ (N)	3	80	M	6.5
BVAX 216	VI	2+ (D), 3+ (N)	3	80	M	Unknown

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0 = none; 1+ = sparse; 2+ = moderate; 3+ = frequent. BB, Braak & Braak; CERAD, The Consortium to Establish a Registry for Alzheimer's Disease; D, diffuse plaques; N, neuritic plaques; NBB, Netherlands Brain Bank; BM, Boston Medical Center; BVAX, Brain Bank of the Boston University Alzheimer