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Lewy body pathology is a frequent co-pathology in familial Alzheimer's disease

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Abstract Our institution is currently engaged in ongoing genetic studies of familial Alzheimer's disease (AD), which include clinical ascertainment and brain autopsy of both affected and non-affected family members. Here we describe the analysis of 22 AD families, each with at least one family member with a postmortem diagnosis of dementia with Lewy bodies (DLB). For this study, 47 brains were examined according to NINCDS-Reagan Institute criteria for the diagnosis of AD. Lewy body pathology was evaluated with α-synuclein immunohistochemistry. Four families, with either one or two autopsies showing Lewy body pathology, demonstrated linkage to 12p. Five families had two or more autopsies with Lewy body pathology, but their linkage status was unknown. The remaining 13 families had one autopsy demonstrating Lewy bodies. These findings suggest that at least one pathological form of DLB may be familial. In some families, the pathological phenotype is identical in all examined affected family members; but in others, there may be several pathologies that coexist. Careful neuropathological examination of affected family members may prove critical for future genetic analysis of AD and DLB.

Keywords Alzheimer's disease · Dementia with Lewy bodies · Autopsy · Genetics

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Introduction

Understanding of the genetic basis of Alzheimer's disease (AD) is growing at a rapid pace. In recent years, three autosomal dominant genes that may be responsible for some forms of AD have been uncovered: (1) APP, (2) PS1, and (3) PS2 that code for amyloid precursor protein, presenilin 1 and presenilin 2, respectively [23]. The epsilon 4 allele of a fourth gene, apolipoprotein E (APOE), confers increased risk of late onset, sporadic and familial AD [27]. However, these four loci explain less than 50% of all AD cases [27]. Recently, several groups have reported late onset AD families with a fifth genetic locus on chromosome 12. However, different regions of maximum linkage have been reported [12, 21, 22, 31]. Further, fine mapping of chromosome 12 in late onset familial AD has uncovered potential genetic heterogeneity [27].

Dementia with Lewy bodies (DLB) represents the second most common form of dementia after AD alone, and consensus guidelines have been established for the neuropathological diagnosis of this disorder [17]. Estimates of the frequency of DLB range from 15% to 25% in autopsies of individuals with a clinical diagnosis of probable AD [6, 17]. Consensus guidelines define DLB clinically as progressive cognitive decline similar in magnitude to AD. There may also be visual hallucinations, behavioral abnormalities and/or clinical features of parkinsonism [6, 17]. Although familial cases of DLB are commonly recognized, little is known about the genetic epidemiology of this disorder [9].

Ongoing studies of familial dementia at our institution include clinical ascertainment, genetic analysis and postmortem examination of both affected and non-affected family members. The purpose of the present communication is to describe 22 families with DLB and their pathological findings at autopsy.

Materials and methods

The 22 families described here are part of an ongoing study examining individuals ascertained in 90 families and enrolled as possible participants in genetic research studies from 1985 through 1998. Each family had at least two clinically affected individuals, usually first degree relatives, and, by family history, affected individuals in multiple generations. Some follow-up data is available in 81 families. All affected individuals had possible or probable AD diagnosed according to National Institute of Neurological Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria by a neurologist or associated clinical personnel at the Joseph and Kathleen Price Bryan Alzheimer's Disease Research Center (ADRC) at Duke University Medical Center (DUMC) [18]. Research data was collected according to protocols reviewed and approved by the DUMC Institutional Review Board.

Table 1 Clinical data on the 22 families. Note that family 21 has been previously described and demonstrated to have a mutation in the APP [24] (*APP* amyloid-β precursor protein)

Family	Sex	Age of onset (years)	Age of death (years)	Lewy bodies	Lewy body location	Linkage	Braak stage
1	M	52	57	Yes	Limbic	Uncertain	IV
	F	63	78	Yes	Neocortex	Uncertain	III
2	F	63	70	Yes	Neocortex, limbic	Uncertain	IV
3	M	70	77	Yes	Unknown	Uncertain	Unknown
	F	74	86	No		Uncertain	III
	F	68	83	Yes	Neocortex	Uncertain	VI
4	M	64	79	Yes	Limbic	Uncertain	III
	M	63	76	No		Uncertain	V
5	F	75	86	Yes	Neocortex	12p	IV
	M	56	74	Yes	Neocortex	12p	II
6	M	61	69	Yes	Brain stem	12p	I
	F	75	82	No		12p	IV
	M	68	71	No		12p	III
	F	82	90	Yes	Brain stem	12p	II
7	M	?	80	Yes	Neocortex	Uncertain	III
8	M	69	77	Yes	Neocortex	Uncertain	III
	M	70	75	Yes	Neocortex	Uncertain	II
9	F	?	71	Yes	Neocortex	Uncertain	III
10	F	59	71	Yes	Brain stem	Uncertain	IV
	F	64	74	Yes	Neocortex	Uncertain	II
11	M	74	85	Yes	Limbic	Uncertain	III
	F	73	90	No		Uncertain	Unknown
	F	69	88	No		Uncertain	V
	F	65	80	No		Uncertain	Unknown
	M	76	82	No		Uncertain	V
12	F	68	82	No		Uncertain	IV
	F	70	87	Yes	Limbic	Uncertain	IV
13	M	73	84	Yes	Neocortex, limbic	Uncertain	V
	F	74	105	No		Uncertain	V
	F	82	100	No		Uncertain	IV
	F	72	77	No		Uncertain	III
14	M	66	71	Yes	Neocortex	Uncertain	IV
15	M	60	73	Yes	Neocortex	Uncertain	II
16	F	70	91	Yes	Neocortex	12p	V
	F	73	84	No		12p	IV
17	F	65	84	No		Uncertain	IV
	F	70	77	Yes	Unknown	Uncertain	Unknown
18	M	73	76	Yes	Neocortex	Uncertain	III
19	M	70	76	Yes	Limbic	12p	IV
	M	75	78	No		12p	V
20	M	63	72	Yes	Neocortex	Uncertain	III
21	F	49	59	Yes	Limbic	APP	VI
	F	50	65	Yes	Neocortex	APP	VI
	F	41	61	Yes	Neocortex	APP	VI
	F	60	75	Yes	Neocortex	Uncertain	V
	F	47	64	No		APP	VI
22	F	Unknown	58	Yes	Limbic	Uncertain	Unknown

One hundred and twenty-seven brain autopsies have been performed on affected members of 70 of the 90 families. Of these, 47 were performed on affected members of the 22 families which form the basis of this report. Forty-two of the 47 brain autopsies reported here were performed at our institution (DUMC). At least 1 member of each of these 22 families exhibited DLB according to consensus guidelines at postmortem examination [17]. Four of the families whose neuropathology is reported here were part of a multi-center genomic screen of 54 dementia families [26, 27].

Brains were examined and sectioned according to standardized protocols used by the Neuropathology Core of the Joseph and Kathleen Price Bryan Alzheimer's Disease Research Center [7]. Each of the cases described here met the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria for the neuropathological diagnosis of AD. None of the individual affected family members were diagnosed antemorterm with DLB.

Sections of each brain were submitted according to CERAD guidelines, including sections of the frontal, parietal and temporal lobes, hippocampal formation and substantia nigra [19]. Neuritic plaques were scored as recommended by CERAD guidelines using a silver stain. Neurofibrillary change was staged according to Braak based on sections of the amygdala and entorhinal cortex, hippocampal formation and inferior temporal lobe and the primary visual cortex [1]. NIA Reagan Institute criteria for the diagnosis of Alzheimer's Disease were applied [8].

Lewy body pathology was examined and quantified according to consensus guidelines [17]. In addition, all submitted sections were immunostained with α-synuclein to highlight Lewy body formation and to provide a semiquantitative analysis of the frequency and distribution of Lewy Body pathology. Immunostaining for α-synuclein was performed as follows: paraffin-embedded sections, 8 μm thick, were deparaffinized in xylene and hydrated through graded ethanols. Endogenous peroxidase activity was blocked with 2% hydrogen peroxide in methanol for 10 min followed by washing in deionized H_2O. The sections were treated with 95% formic acid (Sigma, St. Louis, Mo.) for 1 min and washed in deionized H_2O. Background staining was blocked by incubating with 5% w/v nonfat dry milk in 0.05 M TRIS buffer. A PBS buffer wash was used to remove excess milk. Sections were incubated with the monoclonal primary antibody Synuclein-1 (1:100, Transduction Laboratories, Lexington, Ky.) for 1 h at 37°C followed by incubations with a prediluted biotinylated secondary antibody and a prediluted streptavidin-peroxidase antibody from Biomeda (Foster City, Calif.), each for 20 min at 37°C. The sections were developed using diaminobenzidine (Sigma) and counterstained using Biomeda's aqueous hematoxylin. The sections were treated in ammonical water for 10 s and washed in tap water for 5 min before dehydrating through graded ethanols and clearing in xylene. The sections were mounted with Permount (Fisher Scientific, Fair Lawn, N.J.) and cover slipped. Positive and negative controls were performed, the latter by leaving out the primary antibody, for quality assurance.

Results

Of the 47 autopsies reported here, 19 were male and 28 were female. Age of onset varied from 52 to 76 years for male affected family members compared to 41 to 82 years for female affected family members (Table 1). Autopsy was offered to all affected individuals in each of the 22 families, and there was an overall autopsy rate amongst affected individuals of 64%.

Four families were part of a prior genetic study and demonstrated linkage to 12p (Table 1, Fig. 1) [26]. Of these four families, two had two autopsies with Lewy body pathology and the remaining two families had one autopsy each demonstrating Lewy body pathology.

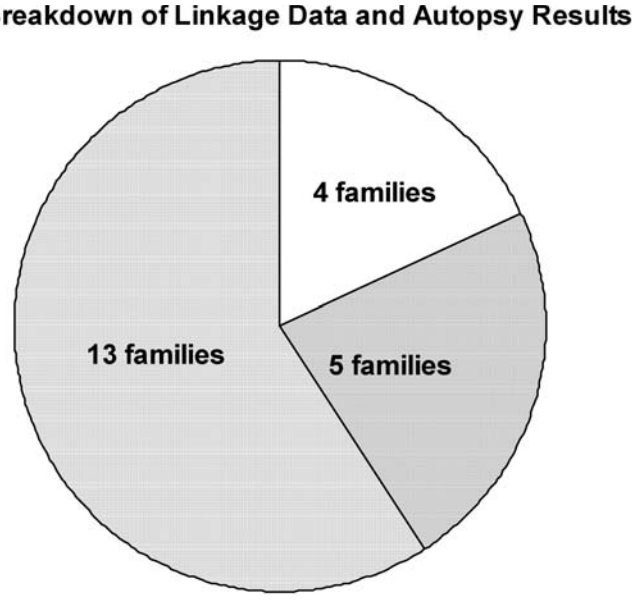

Fig. 1 Breakdown of the 22 families in terms of linkage status. Four of the 22 families demonstrated linkage to chromosome 12p. Five of the 22 families had two or more autopsies, but their linkage status was unknown. The remaining 13 families had only one autopsy and none of these have been screened for linkage

Five of the remaining 18 families each had two or more autopsies, with limbic or neocortical Lewy body pathology present in each autopsy. However, linkage status on these five is unknown. One family had four autopsies with Lewy body pathology, and the remaining four families had two autopsies each (Table 1, Fig. 1).

The remaining 13 families had only one autopsy which showed either limbic or neocortical Lewy bodies. In general, these were smaller families; and none of these families were included in the previously reported genomic screen. None of these families has been yet screened for linkage (Table 1, Fig. 1).

The location of Lewy body pathology was variable, ranging from the brain stem to the neocortex. Alzheimer's neuropathological changes (NIA-Reagan criteria) were also quite variable across all 22 families, ranging from stage II to stage V neurofibrillary change (Table 1). In all examined cases, there were frequent neuritic plaques adequate to meet CERAD criteria for the diagnosis of AD. Pathological changes ranged from AD alone with no Lewy bodies detectable by α-synuclein immunohistochemistry to neocortical category DLB. In five subjects, the location of the Lewy body pathology and/or the Braak stage was not determined as the autopsies were performed at an outside institution.

Discussion

DLB is the second most common form of dementia [17]. Ongoing genetic studies would suggest that at least one form of DLB is familial [9]. DLB patients, in general, do

not demonstrate neocortical tangles and have a different histological and biochemical profile compared to typical AD cases, leading DLB to be considered at least a subtype of, if not a distinct entity from, AD [2, 3, 17]. In addition to histological and biochemical differences, some authors have noted differences between the two diseases in terms of disease length, sex ratios, APOE genotype, and Braak stages [24]. The importance of differentiating DLB from other neuropathological diagnoses is threefold: (1) correct diagnosis improves the ability to recognize a characteristic and rapidly progressive clinical syndrome, thus aiding both clinicians and families, (2) DLB cases mandate increased caution with neuroleptic medications, and (3) DLB patients may be significantly more sensitive to cholinesterase inhibitors [17, 29].

Our study found four families, each with at least one autopsy demonstrating LB and with linkage to 12p [26]. Several studies support the idea that there are at least two dementia loci located on chromosome 12. Three studies support linkage to chromosome 12p in at least a subset of dementia families [12, 22, 31]. These studies centered on late-onset AD families, and at least one of these studies found that evidence for linkage was stronger in families with affected individuals with the neuropathological diagnosis of DLB [21, 22, 27]. Neuropathological confirmation of the clinical diagnosis has not yet been reported in many of the other studies, thus it is not possible to determine how many cases of DLB actually existed in each group.

The previously reported fine mapping of chromosome 12 in late-onset AD analyzed 8 families with DLB. These families had at least one autopsied family member and were separate from the 46 families in which AD only at autopsy was found. This work suggested linkage for DLB to chromosome 12p [26, 27]. Of the various studies of chromosome 12-linked AD, each study has found different regions of maximum linkage. It is conceivable that two or more different loci may exist, one for AD and another for late-onset AD/DLB [12, 21, 22, 26, 31].

Without a particular candidate gene, the molecular basis of DLB is a matter of conjecture. Lewy bodies have been demonstrated to consist of α-synuclein, the function of which is unclear, although it is suggested that it may play a role in neurotransmission [13, 14, 30]. Overexpression of mutant forms of α-synuclein is harmful to neuronal cells [11, 20, 25, 28, 32]. Transgenic mice that express human α-synuclein accumulate α-synuclein neuronal inclusions and reproduce a Parkinson's disease phenotype [10, 15]. Recently, it has been demonstrated that α-synuclein can protect cells from oxidative stress via the C-Jun N terminal kinase pathway [4]. However, hypothalamic cells with overexpression of α-synuclein have mitochondrial alterations and increased levels of free radicals, possibly resulting in excessive oxidative stress to the cell [5]. Thus, oxidative changes may be one way in which α-synuclein promotes neuronal damage. In addition, transgenic mice with neuronal expression of human α-synuclein and β-amyloid show severe deficits in learning and memory, motor deficits, and age-dependent degeneration of cholinergic neurons and presynaptic terminals compared to mice that only expressed neuronal human α-synuclein, suggesting a mouse model of DLB [16].

The evidence from the mouse models described above may mean that several adverse genetic changes are necessary to predispose individuals to DLB. DLB also lacks a clear Mendelian inheritance pattern that lends itself to linkage studies. Combined with the lack of sensitive antemortem diagnostic criteria, these conditions indicate association studies of individual candidate genes in postmortem tissue may be a complementary approach to determining the etiology of DLB and AD.

Additional conclusions can be drawn from the data presented here. In other large families with several members affected with dementia, more than one neuropathological process, including AD, may be present. At autopsy, neuropathological analysis would help differentiate these individuals and avoid confounding genetic studies by combining different disease groups. Consistent autopsy confirmation and neuropathological examination of affected family members with the clinical diagnosis of AD or DLB thus remains critical to further genetic studies of these disorders.

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