Early-onset Alzheimers and Cortical Vision Impairment in a Woman With Valosin-containing Protein Disease Associated With 2 *APOE ε4*/*APOE ε4* Genotype

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Abstract: Hereditary inclusion body myopathy is a heterogeneous group of disorders characterized by rimmed vacuoles and by the presence of filamentous cytoplasmic and intranuclear inclusions. Inclusion body myopathy with Paget disease of bone and frontotemporal dementia is a progressive autosomal dominant disorder associated with a mutation in valosin-containing protein (VCP) with typical onset of symptoms in the 30s. *APOE ε4* is a major risk factor for late-onset Alzheimer disease, a progressive neurodegenerative disorder that affects memory, thinking, behavior, and emotion as a result of the excessive buildup and decreased clearance of β-amyloid proteins resulting in the appearance of neuritic plaques and neurofibrillary tangles. In conclusion, we report a unique patient with an *APOE ε4*/*APOE ε4* genotype and atypical VCP disease associated with early Alzheimer disease and severe vision impairment. Future studies will elucidate the interaction of VCP mutations and *APOE ε4* alleles in understanding common mechanisms in AD and VCP disease.

Key Words: inclusion body myopathy, frontotemporal dementia and Paget disease of bone (IBMPFD), valosin-containing protein (VCP), Alzheimer disease (AD), *APOE ε4* allele, TAR DNA–binding protein-43 (TDP-43), ubiquitin (Ub), tau protein

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Hereditary inclusion body myopathy (IBM) is a heterogeneous group of disorders characterized by rimmed vacuoles and by the presence of filamentous cytoplasmic and intranuclear inclusions. IBM with Paget disease of bone (PDB) and frontotemporal dementia (FTD) is a progressive autosomal dominant disorder associated with a mutation in valosin-containing protein (VCP)[1] with typical onset of symptoms in the 30s.[2] Myopathy, the most prominent feature, is present in 90% of affected individuals, typically manifesting as weakness and atrophy of the shoulder and pelvic girdle muscles, marked scapular winging, and death in the age range of 40s to 60s from progressive muscle weakness and cardiac and respiratory failure.[2] Skeletal muscle in affected subjects contains rimmed vacuoles and ubiquitinated and TAR DNA–binding protein-43 (TDP-43)–positive inclusions.[3] PDB occurs in 50% of the affected individuals, in the age range of 30s or 40s and primarily involves the spine, pelvis, scapulae, and the skull. FTD is found in 33% of affected individuals, with a mean age of onset at 54 years. The early symptoms of FTD include mood or personality changes and language difficulties. Some individuals also develop amyotrophic lateral sclerosis–type features or Parkinsonism.[4]

VCP is a member of the AAA protein family of ATPases associated with a wide range of cellular activities involving cell division, fusing membranes within cells, apoptosis, and repairing damaged DNA.[5] There are currently >25 reported VCP disease mutations localized on chromosome 9p21.1-p12,[1,6] with >50% of the mutations affecting amino acid residue 155. VCP is a part of the ubiquitin-proteasome quality control system, the machinery that degrades proteins within cells.

APOE ε4 is a major risk factor for late-onset Alzheimer disease (AD), a progressive neurodegenerative disorder that affects memory, thinking, behavior, and emotion as a result of the excessive buildup and decreased clearance of β-amyloid proteins resulting in the appearance of neuritic plaques and neurofibrillary tangles. The association of 1 or 2 alleles of *APOE ε4* with late-onset AD is well documented. The risk of AD is highest with the *APOE ε4*/*APOE ε4* genotype, which occurs in approximately 1% of normal controls and in nearly 19% of the familial AD population. The probability that dementia is due to AD is approximately 90% in the presence of the *APOE ε4*/*APOE ε4* genotype, the risk of AD increasing by a factor of 2.84 for each additional *APOE ε4*.[7,8]

CASE REPORT

We have studied *APOE ε4* as a genetic modifier for the incidence of FTD in a cohort of 173 individuals including, 60 affected and 24 carriers. We identified 14 individuals with FTD associated with VCP disease (23%). We analyzed the incidence of dementia in this cohort with two, one, or no copies of *APOE ε4* and found an increased incidence of dementia with ≥1 copies of *APOE ε4*.[9] Among 4 affected individuals with 2 copies of *APOE ε4*, 3 had FTD and the patient we describe in this report is the only individual in our cohort with AD and an R155H mutation. Of the remaining individuals, 54 had 1 copy of *APOE ε4* (13 affected, 16 carriers, and 25 unaffected), we identified 5 with dementia (38.5% of affected individuals). Among 115 individuals with no *APOE ε4* allele (43 affected, 7 carriers, and 64 unaffected) there were only 3 individuals with dementia (6.9% of affected individuals). Among the unaffected individuals, 1 individual had 2 *APOE ε4* alleles and did not have dementia.

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We report a unique subject with an R155H mutation in addition to severe manifestations of VCP disease, including IBM and severe early-onset dementia associated with the presence of the neuropathologic changes of AD. On reviewing her disease progression at the age of 45 years, we found that she developed generalized motor weakness and muscle pain in her legs causing her to walk slowly. She also had trouble using her hands for basic tasks. She had a high normal creatinine kinase levels [219 U/L normal (20 to 222 U/L)]. Electromyography at 46 years revealed myopathic and complex repetitive discharges of myotonic type in her right deltoid muscle, vastus medialis, biceps, iliopsoas, and upper thoracic paraspinous muscles with short duration and polyphasic recruitment units. Muscle histology from the right deltoid at the age of 48 years showed degenerating and focally regenerating myofibers and centrally located nuclei, which are normally seen in the periphery of the muscle fibers. Repeat muscle biopsy from the left biceps 8 months later showed several atrophic angular fibers (10 to 30 μ), which stained excessively with NADH, with evidence of denervation and reinnervation. Gomori Trichrome staining revealed red-rimmed vacuoles and some ragged-red fibers in these samples. The patient was diagnosed with moderate neurogenic atrophy with inclusion body disease. She showed no evidence of PDB.

At 45 years, she complained of visual problems such as seeing "stars" and "large floaters," a decrease in peripheral vision worse on left, diplopia, night blindness, and a distorted perception. Visually evoked responses were absent and she was diagnosed with legal blindness 2 years later. She experienced lack of energy, increased frustration, and depression at 45 years. Neurological evaluation revealed a dull mental status, delay in understanding commands, slow wide-based gait, and a borderline Romberg test. Neuropsychological evaluation revealed cognition and verbal dysfluency, difficulty in performing complex calculations, bilateral finger agnosia, and an IQ of 60. Within the next 8 months, she went

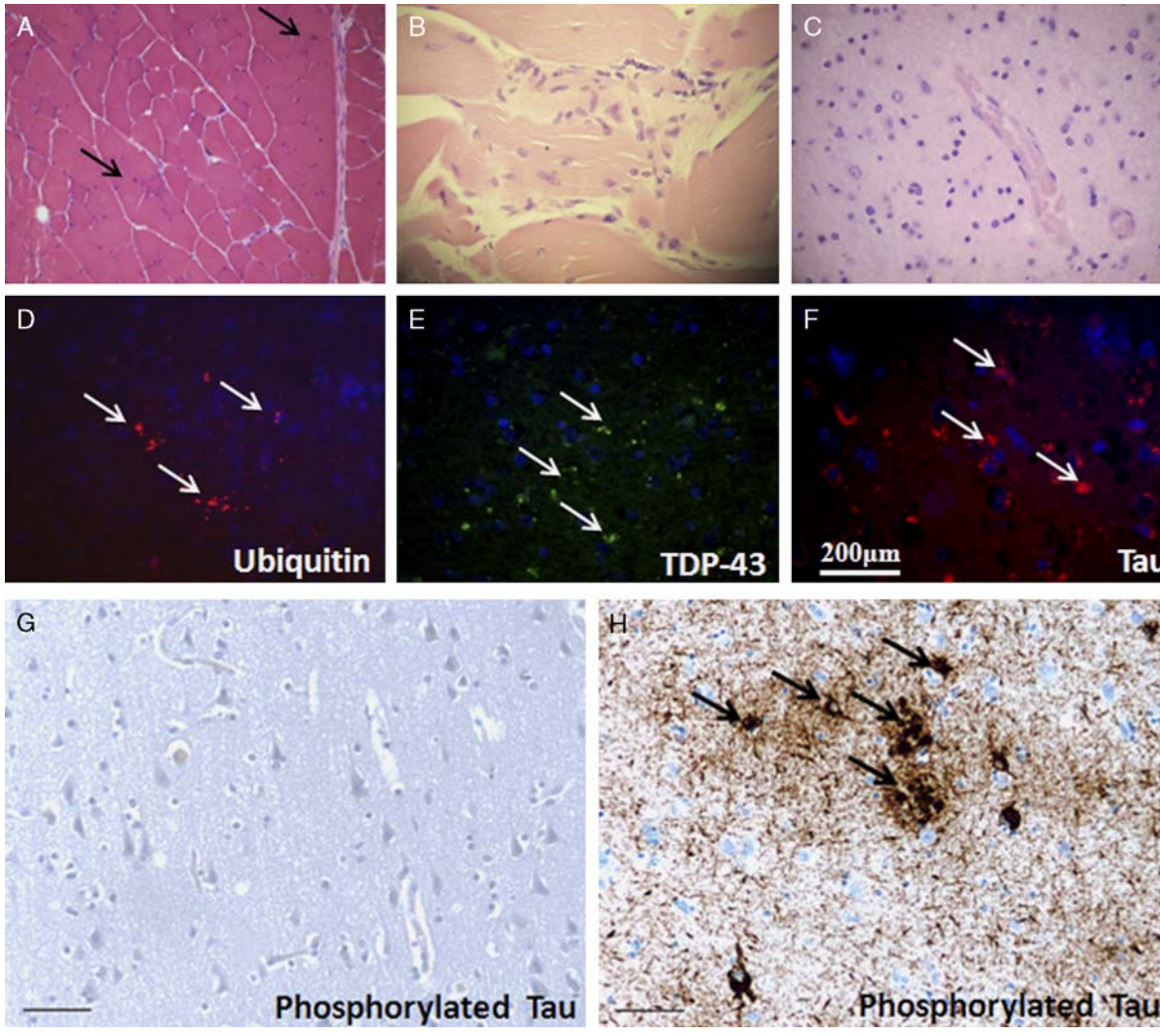

FIGURE 1. Histologic analysis of the patient's left deltoid and frontal cortex. A, Hematoxylin and eosin (H&E) image of left deltoid showed myopathic features including variation in fiber size and centrally located nuclei. B, H&E image of a different region of deltoid showed moderate neurogenic atrophy with reinnervation (magnification: ×40). Mononuclear cells are observed invading a necrotic fiber. C, Histologic evaluation of the frontal cortex shows evidence of pathology along with expressions of (D) ubiquitin, (E) cytoplasmic TDP-43 inclusions, and (F) tau protein (as shown by white arrows) (magnification: ×40). G, An AT8 immunostaining pattern from inferior temporal cortex from a 45-year-old subject with no history of neurological disease. No p-tau immunoreactivity was seen. H, Immunohistochemical analysis of the inferior temporal neocortex stained for phosphorylated tau (which recognizes phospho tau at pSer202 and pThr205) and counterstained with hematoxylin shows neurofibrillary plaques and tangles (as shown by black arrows) (Scale bar = 50 μm; magnification: ×60 before enlargement). full color online

into a state of confusion and had frequent hallucinations. Magnetic resonance imaging of the brain at 46 years revealed moderate neuron loss and gliosis in her occipital lobe. A second magnetic resonance imaging 2 years later depicted extensive generalized cortical atrophy with the evidence of an arachnoid cyst in the left parietal lobe without any pressure effect. Brain perfusion scan revealed decreased perfusion in the bilateral posteroparietal lobes and the left posterooccipital area, which was considered significant in the etiology of her extinguished visual field. Brain angiogram was normal. EEG for drowsy and awake states was normal. Test for Huntington disease, by sequencing the entire gene to include presenilin1 (*PSEN1*) and presenilin 2 (*PSEN2*) genes was negative. She did not have diabetes, hypertension, infarction, cancer, or stroke. Subsequently, she remained noncommunicative, unable to walk, and had generalized myoclonus. She had increased spasticity bilaterally, distal atrophy, a high-frequency low-amplitude tremor of the right arm at rest, severe dystonia, and chorea.

There was a history of limb-girdle myopathy, presenile dementia, and Paget disease on her father's side of the family spanning 4 generations. Her father also became blind and died from respiratory failure related to his myopathy at the age of 59 years. Red-rimmed vacuoles were detected in the muscles of affected individual consistent with IBM.

The patient passed away at 62 years of age. An autopsy was performed, and brain and muscle samples were obtained for detailed pathologic studies. Muscle histology showed myopathic features including variation in fiber size and centrally located nuclei, in addition to moderate neurogenic atrophy with reinnervation (Figs. 1A, B). Analysis of her brain revealed marked generalized atrophy and severely atrophic hippocampus. On further histologic analysis, high density of neurofibrillary tangles (Braak stage VI) and senile plaques in the limbic and neocortical regions (CERAD C) were seen; neuron loss and gliosis were most prominent in the areas with high levels of senile plaques and neurofibrillary tangles; TDP-43-positive (Abcam) neurodegenerative disease inclusions, expression of tau (Thermo Scientific), and ubiquitin (Abcam) were noted in the neocortex and limbic areas (Figs. 1C–H); and the lymphocytes, macrophages, and microglia in the inflammatory infiltrate were highlighted using immunostaining for leukocyte common antigen and CD68. The microscopic data from the patient's brain autopsy are shown in Table 1. The concluded diagnosis from the autopsy report was AD along with perivascular chronic inflammation.

TABLE 1. Microscopic Data From the Patient's Brain Autopsy

	Area of the Brain												
	Hippocampus			Neocortical Regions					Subcortical Regions			Brainstem/Spinal Cord		
	Amygdala	CA1/ Subiculum	Entorhinal Cortex	Middle Frontal Gyrus	Angular Gyrus	Sup/ Midtemporal Gyrus	Cingulate Gyrus	Neocortical Regions	Caudate/ Putamen	Thalamus/ Subthalamus	Midbrain/ Substantia Nigra	Pons/ Locus Ceruleus	Medulla
Tau	3 +	3 +	3 +	2 +	2 +	2 +	2 +	3 +	1 +	1 +	1 +	1 +	1 +
Plaques	2 +	1 +	2 +	3 +	2 +	3 +	2 +	3 +	1 +	0	0	0	0
Ubiquitin	2 +	2 +	2 +	1 +	1 +	1 +	1 +	1 +	1 +	Rare	1 +	0	Rare
Intranuclear TDP-43 inclusions	0	0	Rare	Rare	Rare	1 +	Rare	0	0	0	0	0	0
Gliosis	3 +	3 +	3 +	2 +	2 +	2 +	2 +	2 +	0	2 +	3 +	1 +	0
Neuron loss	3 +	3 +	3 +	2 +	2 +	1 +	2 +	2 +	0	2 +	0	1 +	0
Amyloid angiopathy	0	0	0	2 +	2 +	2 +	1 +	1 +	0	0	0	0	0
Dystrophic neurites	0	1 +	1 +	1 +	1 +	1 +	1 +	1 +	0	0	0	0	0

0 = none; 1 + = mild; 2 + = moderate; 3 + = severe.
TDP-43 indicates TAR DNA–binding protein-43.

DISCUSSION

VCP-associated IBM with PDB and FTD is a heterogeneous disease linked with amyotrophic lateral sclerosis and Parkinson disease. Mehta et al[9] discovered a significant association between the *APOE ε4* genotype and FTD but not IBM or PDB in patients with VCP disease. Our patient with VCP disease is the only 1 in the study database known to have 2 *APOE ε4* alleles and AD.

Previous studies have explored pathologic similarities between the sporadic IBM and AD. For instance, proteins like tau, APOE, amyloid β, and ubiquitin accumulate in the Alzheimer patients' brains and also accumulate in the sporadic IBM patients' muscle fibers. A VCP mutation has previously been identified in an individual with late-onset AD;[10] however, the *APOE* allele status in the individual was not reported. VCP/p97 has been recently identified as a putative substrate of active Caspase-6 (Casp6) in primary human neurons.[11] As Casp6 is activated in mild cognitive impairment and in AD patients' brains, the targeting of p97 by Casp6 may represent an important step that leads to the ubiquitin-proteasome system (UPS) impairment in AD.[11] Griciuc et al[12] showed colocalization of VCP with misfolded rhodopsin in retinal cells, and decreased activity of VCP function resulted in the suppression of retinal

degeneration and blindness in Drosophila (Rh^{P37H}). The etiology of the patient's vision loss may be complex, associated with degeneration of the left occipital lobe and her VCP mutation on rhodopsin function.

In conclusion, we report a unique patient with an *APOE ε4/APOE ε4* genotype and atypical VCP disease associated with early AD and severe vision impairment. Future studies will elucidate the interaction of VCP mutations and *APOE ε4* alleles in understanding common mechanisms in AD and VCP disease.

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