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Autosomal Dominant Inclusion Body Myopathy, Paget Disease of Bone, and Frontotemporal Dementia

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Abstract: Autosomal dominant proximal limb girdle or inclusion body myopathy, associated with Paget disease of bone and frontotemporal dementia (IBMPFD) is a recently described disorder that maps to chromosome 9p21.1-p12. We refined the critical locus and identified the gene as the Valosin Containing Protein (VCP) gene, a member of the AAA-ATPase superfamily using a candidate gene approach. Six missense mutations were found to co-segregate with affected individuals only, two of these representing mutation hot spots. We report the clinical and molecular findings in 99 individuals in 13 families. VCP is associated with a variety of cellular activities, including the control of cell cycle, membrane fusion, and the ubiquitin-proteasome degradation pathway. Previous studies have associated VCP mutants in cell lines with vacuole formation and aggregate formation. Identification of VCP as the gene causing IBMPFD has important implications for understanding the pathogenesis of neurodegenerative disorders.

Key Words: autosomal dominant inclusion body myopathy, frontotemporal dementia, Paget disease of bone

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Hereditary inclusion body myopathy associated with Paget disease of bone (PDB) and frontotemporal dementia (FTD)—IBMPFD—is a complex and ultimately lethal, autosomal dominant disorder (MIM 605382), which features adult-onset distal and proximal weakness clinically resembling limb girdle muscular dystrophy early-onset PDB in most cases, and premature frontotemporal dementia.[1,2]

The inclusion body myopathies are a clinically and molecularly diverse group of disorders that include both sporadic (s-IBM) and hereditary inclusion body myopathies (h-IBM 1, 2, and 3).[3] They are characterized by proximal or distal muscle weakness, absent or reduced deep-tendon reflexes, normal to mildly elevated creatine kinase (CK)s, and non-specific EMG myopathic changes; histologically, muscle fibers have rimmed vacuoles and cytoplasmic inclusions consisting of 15 to 18 nm filaments comprising aggregates including phosphorylated tau and β-amyloid, ubiquitin apolipoprotein E deposits similar to those seen in Alzheimer disease.[4] The genes have thus far been identified in the autosomal recessive IBM2 (GNE)[5] and autosomal dominant IBM3 (MYHC2A).[6]

Paget disease is a common bone disease seldom seen before the age of 50 years. It is characterized by foci of accelerated and disorganized bone remodeling featuring osteoclasts that are excessively large, multinucleated, and overactive. There is exaggerated bone turnover with accelerated bone resorption and new bone formation. The lesions are commonly found in the pelvis, spine, skull, femur, tibia, and elsewhere. Familial PDB is much more severe than sporadic cases. Cody et al.[7] assigned PDB2 to chromosome 18p21-22, the locus for expansile osteolysis where mutations in the gene TNFRSF11A were later found.[8] The gene for the 5q35 locus in French- Canadian families was identified as the ubiquitin binding protein, Sequestosome 1 (SQSTM1; p62), mutations of which have been associated with sporadically occurring PDB.[9,10]

Frontotemporal dementia causes a substantial proportion of cases of primary degenerative dementia occurring before age 65 years. FTD is diagnosed from impaired executive or other frontal lobe functions (changing behavior and conduct) early on, with relative sparing of memory and visuospatial abilities. Perhaps 38% to 45% of all FTD cases have a strong hereditary component, and 80% of these show autosomal dominant inheritance.[9] In disinhibition-dementia-parkinsonism-amyotrophy complex, mapping to chromosome 17q21-q22, mutations disrupt the *tau* gene.[11]

METHODS

Written consent from each subject was approved by the Springfield, IL Committee for Research Involving Human Subjects, and by Children's Hospital, Boston, MA. A diagnosis of myopathy was based on the presence of muscular weakness on physical examination, creatinine kinase measurements, and in several patients by EMG and/or muscle biopsy findings. PDB is often asymptomatic, but may manifest as spine or hip pain, reduced height, pathologic fractures, long bone or cranial bone deformity, or hearing loss due to eighth nerve compression by calvarial bony overgrowth. Onset of PDB is typically present for 10 to 15 years before diagnosis of PDB. Whenever possible, a clinical diagnosis of PDB is confirmed by skeletal

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radiologic surveys including views of the skull, spine, hips, long bones, hands, and feet. These typically show coarse trabeculation, cortical thickening, and spotty sclerosis. Radionuclide scans show focally increased bony uptake and are more sensitive indicators of PDB than plain survey films. Serum alkaline phosphatase (ALP) and urine pyridinoline (PYR) and deoxypyridinoline (DPD) measurements reflect increased bone turnover in PDB. The diagnosis of frontotemporal dementia is made by comprehensive neuropsychological assessments and imaging studies when available, together with typical clinical features of behavioral alteration (eg, personal/social unawareness, perseveration, abulia, disinhibition), early expressive or receptive language dysfunction, and relative preservation of memory, orientation, or praxis.

To determine the presence of VCP in normal, IBM, and IBMPFD muscle, postmortem sections were subjected to immunohistochemistry with anti-VCP polyclonal antibody. Immunohistochemistry was performed as described previously.[2] The immune reactivity was detected by light microscopy using horseradish peroxidase.

IBMPFD linkage to chromosome 9p21.1-p12 was known in four families,[2] and the locus was further refined in nine new kindred by analysis of the disease haplotype. Sequencing of candidate genes included VCP, the gene encoding the Valosin Containing Protein (MIM #601023).[12,13] Restriction site mapping from PCR amplified exons was used to confirm that the mutation co-segregated with disease in the families containing mutation 695 C > A (family 6), which destroys an *Mfe*I site, 283 C > G (family 9), which destroys an *RSA*I restriction site, and 464 C > T (family 11), which creates a *BSSK*I restriction site. Co-segregation for mutations 463 C > T, 464 G > A, and 572 G > C was confirmed using dHPLC in a blinded study of 79 individuals from seven families.

SUMMARY OF CLINICAL RESULTS

Myopathy

The myopathy is characterized by variability and mild asymmetric muscle weakness with initial involvement typically of the limb girdle muscle groups.[1,2] Individuals have an abnormal gait with lumbar lordosis from the proximal weakness and several demonstrated mild weakness of the hands. Tendon reflexes are absent or severely reduced. Within 99 (46 M, 53 F) members in 13 families studied, 82 (84%) of the patients had myopathy at a mean age of presentation of 42 years (range 3–61 years). EMG shows primarily myopathic changes, with neurogenic changes noted in some individuals. CK levels are normal to mildly elevated (mean 195 U/L, range 40–1145 U/L; normal range 20–222 U/L). Muscle biopsies in 33% of individuals showed non-specific myopathy with atrophy, rimmed vacuoles, and inclusion bodies. Immunocytochemistry showed normal staining for dystrophin, merosin, and α-sarcoglycan. Ultrastructural studies revealed paired helical filaments 15 to 20 nm long in the nucleus and cytoplasm.

Paget Disease of Bone

Paget disease of bone was present in 50 of 99 (51%) individuals. Unlike sporadically occurring PDB, onset was early at a mean age of 42 years with involvement of hip and scapulae, which later became widespread.[1,2] Alkaline phosphatase is elevated in all individuals with PDB (mean 359 U/L, range 58–1724 U/L; normal range 30–130 U/L). Urine deoxypyridinoline and pyridinoline was elevated in affected and asymptomatic carriers. Many individuals were not diagnosed with PDB before our study. PDB is successfully managed with bisphosphonates. Electron microscopy revealed similar paired helical filaments in Pagetic bone.[14]

Frontotemporal Dementia

Frontotemporal dementia was seen in 30 cases (31%) at a mean age of 55 years. Some individuals even within the same family have been erroneously diagnosed with Alzheimer disease. An individual from family 1 illustrates the progression of his FTD.[2] At age 44 years he was diagnosed with semantic dementia after a 6-year history of language difficulties. He remained oriented and had a good memory being able to continue his job delivering soda. When examined he had minimal proximal weakness and no evidence of PDB. He had rapid intellectual decline and died at age 50 years. His brain MRI showed diffuse cerebral atrophy and brain histology was considered to show nonspecific changes.

Molecular Studies

The locus was initially mapped to 9p21-p12 in family 1 and the critical region refined in three additional families.[2] Haplotype analysis of additional IBMPFD families identified two ancestral, disease-associated haplotypes, distinguishing families 1, 3, 7, and 16 (Group A) of English/American origin from families 2 and 5 (Group B). We excluded *GNE* (UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase), which causes IBM2 or Nonaka myopathy and other candidate genes.[12] Subsequently, we identified six missense mutations within VCP only in affected individuals (Fig. 1). Families 1, 3, 4, 7, 10, 15, and 16 share a 464 G > A (R155H) change in exon 5. In families 4, 10, and 15 with unique haplotypes, the mutations probably arose independently from Group A. Families 2 and 5 have an alteration at the first base of the same codon 463 C > T (R155C). Families 6, 9, 11, and 13 did not share haplotypes and their *VCP* mutations were also unique. Family 6 has a transition mutation 695 C > A in exon 6. Family 9 has a base change in exon 3 at 283 C > G (R95G), whereas family 13 has a G > C change at base 572 (R191Q) in exon 5. Family 11 also has an alteration at base 464 involving a G > C (R155P) change.

DISCUSSION

Valosin Containing Protein, also called CDC48 or p97 (a member of the AAA-ATPase super family *–ATPase Associated with a variety of cellular Activities*), characterized by the presences of two conserved energy-generating ATPase domains is ubiquitous, constituting 1% of the total protein content in yeast. Structurally, VCP is divided into several domains: a cofactor and poly ubiquitin binding N domain (1-187), N-D1 linker, D1 weak ATPase (209-460), flexible D1-D2 linker, D2, the major ATPase (481-761), and C (762-806) domains. VCP is presumed to act as a chaperone in the ubiquitin-proteasome–mediated degradation pathway in which

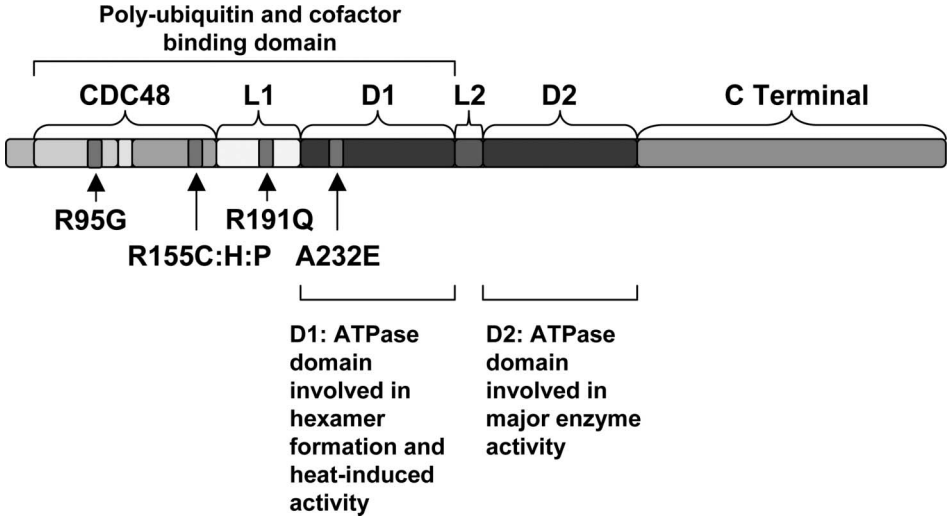

FIGURE 1. Valosin Containing Gene (VCP). Schematic of domain structure in VCP: CDC48 domain composed of; double ψ barrel (amino acids 25–106, orange) and the four-stranded β barrel (amino acids 112–186, cyan), connected by a short linker region (amino acids 107–111, green). The CDC48 domain connects the D1 AAA-ATPase domain (amino acids 208–459, blue) by a linker region (amino acids 187–208, yellow), linker region (L2, dark gray), second AAA ATPase domain (amino acids 481–761, D2, dark blue) and C-domain (amino acids 762–806, Gray) are indicated. The R155 residue, mutated in IBMPFD, is colored red.

the targeted substrate is first conjugated with ubiquitin and then guided to the proteasome for final degradation. With the help of cofactors (Ufd1, Np14, and p47) VCP has been associated with ubiquitin-proteasome–mediated distinct and crucial cell protein pathways: namely homotypic membrane fusion, nuclear envelope reconstruction, postmitotic Golgi reassembly, ERAD (Endoplasmic Reticulum Associated Degradation), DNA damage repair function, and suppressor of apoptosis.[15–19] VCP also binds to expanded poly-glutamine (poly-Q) protein aggregates, this binding domain mapping to the N-domain and N-D1 linker domain that contains two of the mutations we identified. A *Drosophila* VCP (ter94) loss-of-function mutant identified as a dominant suppressor of expanded poly-glutamine (poly-Q) induced neuronal degeneration.[20] VCP has been identified as co-localizing with ubiquitin-containing nuclear inclusions in the cerebral cortex from a number of neuronal degenerative disorders involving protein quality control and the ubiquitin protein degradation pathways, such as Huntington, Alzheimer, Creutzfeldt–Jakob, and Parkinson disease (in particular the Lewy bodies) as well as motor neuron disease[21] with dementia. A K524A VCP mutant substituted in the Walker A motif of the second ATP binding domain induced vacuoles in cells transfected with the mutant.[22]

In 13 IBMPFD families, only four amino acid residues (three in the N-terminal domain and one in the D1 domain) are mutated. Interestingly 10 of the 13 IBMPFD families have an amino acid change at codon 155 in *VCP*, suggesting either a mutation hot-spot in the N-domain of VCP or that VCP has such tight operational constraints that other types of mutation elsewhere are lethal. Indeed, homozygous loss-of-function mutants in *Drosophila* were embryonic lethal[23] as was a knockout mouse model for VCP (Personal communication, Deinhardt et al.).

We propose that mutations in *VCP* compromises ubiquitin-binding and targets similar cellular pathways or proteins because of the similarity of the pathology seen in cell models [22,23] and in the muscle, bone, and brain in IBMPFD. Because this is a progressive disorder it is possible that the mutations we have identified are relatively subtle, and that the accumulative effects of aging, oxidative stress, and ER stress result in the IBMPFD phenotype. Further work is essential in identifying the mechanism by which VCP mutations result in such a diverse phenotype. Understanding these critical steps will hopefully lead to molecular targets in alleviating the progression of this and other neurodegenerative disorders.

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