

# ALS phenotypes with mutations in *CHMP2B* (charged multivesicular body protein 2B)

**Abstract**—Mutation in the *CHMP2B* gene has been implicated in frontotemporal dementia. The authors screened *CHMP2B* in patients with ALS and several cohorts of control samples. They identified mutations (Q206H; I29V) in two patients with non-SOD1 ALS. Neuropathology of the Q206H case showed lower motor neuron predominant disease with ubiquitylated inclusions in motor neurons. Antibodies to p62 (sequestosome 1) showed novel oligodendroglial inclusions in the motor cortex.

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on behalf of the MRC Proteomics in ALS Study and the FReJA Consortium†

Approximately 10% of ALS cases are familial, linked at present to nine distinct loci. In 5 to 10% of motor neuron disease (MND) cases, a frontotemporal type dementia (FTD) is present.<sup>1</sup> Here we show that *CHMP2B* (charged multivesicular body protein 2B), a gene that was recently shown to be linked to FTD,<sup>2</sup> was altered in two unrelated patients with ALS-spectrum disorders. One mutation is predicted to alter a conserved functional domain. Pathology from this case strengthens the hypothesis of a common molecular pathology between ALS and FTD.<sup>3</sup> The second case showed a point substitution that has been previously described as a low-frequency variation in the *CHMP2B* gene.<sup>4</sup>

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**Case reports.** *Patient 1.* A 75-year-old man reported bulbar-onset weakness for 11 months, which progressed to involve his hands after 5 months. Examination findings at that stage were a wasted, weak, fasciculating tongue, flaccid dysarthria, and bilateral weakness and wasting of the intrinsic hand muscles. Tendon reflexes were depressed, and the plantar responses were flexor. There was no evidence of significant cognitive dysfunction on bedside testing. He had a right below-knee amputation for trauma 15 years previously. Neurophysiology showed widespread neurogenic changes in bulbar, upper limb, and lower limb territories, and a diagnosis of progressive muscular atrophy (PMA) (El Escorial category of suspected ALS) was made. His weakness rapidly progressed until his death from respiratory failure 15 months after symptom onset. There was no evidence of extramotor neurologic signs or symptoms or dementia throughout the illness. A cousin was also said to have died of ALS, but it was not possible to obtain DNA from other family members.

*Patient 2.* This 65-year-old man had behavioral and personality changes, including depression, excessive alcohol consumption, and inappropriate sexual behavior. In the next 4 years, this progressed to fulminant FTD. After 5 years, he developed motor disturbances including atrophy of the tongue and facial muscles. There was spastic dysarthria, pseudobulbar paresis causing dysphagia, and weight loss. Motor symptoms progressed to paresis of the right arm and hand and both legs. He retained normal sensation and autonomic function. There were brisk tendon reflexes and upgoing plantar responses. A diagnosis of ALS (El Escorial ALS + dementia) was established on clinical and neurophysiologic grounds. Six years after the onset of behavioral symptoms, he died suddenly, but no autopsy was performed. His father was reported to have frontal lobe dysfunction and motor disturbances. This familial history was not verified by case note review, and DNA could not be obtained from other family members because of lack of permission.

**Methods.** *Subjects.* The genetic studies, retention of tissues for diagnosis, and research histology were approved by local research ethics committees. Control DNA samples (n = 640) were obtained from Dr. Jørgen Nielsen (The Panum Institute, University of Copenhagen), the Centre d'Etude du Polymorphisme Humain (CEPH), and the European Collection of Cell Cultures (ECACC).

*PCR amplification of CHMP2b.* DNA was extracted from blood using Nucleon BACC2 DNA extraction kit (Amersham-Pharmacia). PCR was carried out using 10-ng genomic DNA, 35 cycles of 92°C for 30 seconds, 55°C for 45 seconds, and 72°C for 1 minute. PCR products were cleaned using Microclean (Microzone). Primers were designed to the acceptor splice site and start of exon 6 (forward GACGAAGAAGAAAGCCAGGA; reverse GAAATCTGCACTGTGCTTGG) and to flanking regions outside the start and finish of exon 1 (forward CCGCAGACGTGAGGAAAAG; reverse CTCAGGGACAGTAGGCAGA), exon 2 (forward GCGCCAGCCAATATAAGAT; reverse GCCATGTGCCTTCTTCCTAGT), exon 3 (forward CTTCATGATCGGGGACAAAG; reverse CAGGAGGTGCTTTTAAATCTGC), exon 4 (forward TTTGATGTGTTCCCTTTTGACTT; reverse TCATCATTTCTGCCTTCGTG), exon 5 (forward

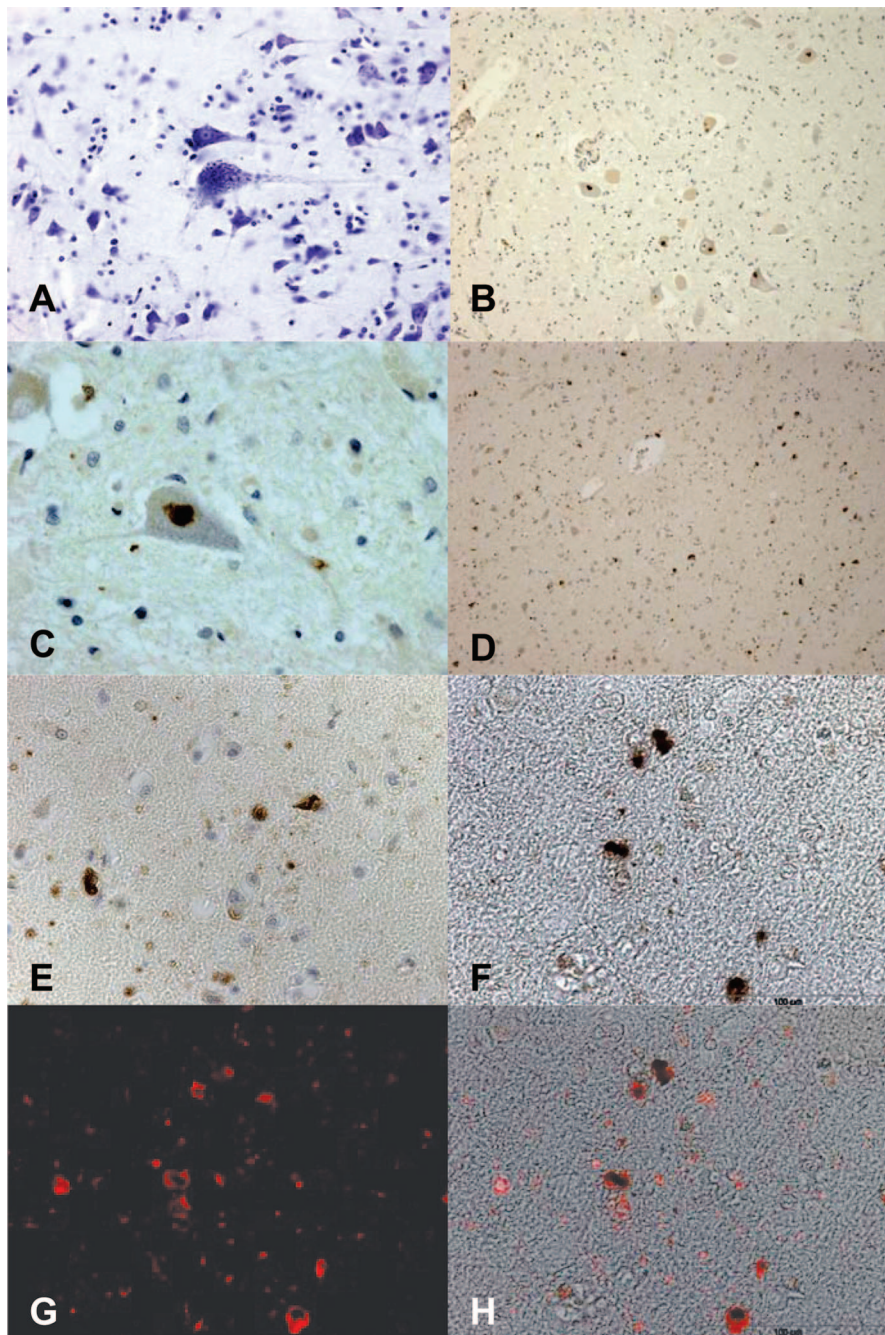


Figure. Photomicrographs of motor system regions from Patient 1. (A) Motor cortex showing intact Betz cells. (B) Low-power view of spinal anterior horn showing numerous motor neuron compact inclusions typical of ALS. These lesions were immunoreactive both to ubiquitin and p62/sequestosome 1 but negative for tau, neurofilament, and  $\alpha$ -synuclein. (C) Compact inclusion in a spinal cord motor neuron. (D) Motor cortex showing numerous cell profiles immunoreactive for p62/sequestosome 1. (E) At high power, these cortical inclusion bodies show coiled body morphology. They were not demonstrated by conventional ubiquitin immunocytochemistry or to antibodies to tau, neurofilament, and  $\alpha$ -synuclein. Double-labeling immunocytochemistry was performed using either SMI32 (neurons), glial fibrillary acidic protein (astrocytes), CD68 (microglia), or carbonic anhydrase II (oligodendroglia) and p62/sequestosome 1 to identify the cell type involved by coiled body inclusion formation. (F) Coiled bodies immunostained for p62/sequestosome 1. (G) As in F under fluorescence illumination showing cell profiles stained by carbonic anhydrase II. (H) Merged images of parts F and G showing colocalization of coiled bodies to oligodendroglia. (Cresyl fast violet [A], immunoperoxidase [3,3'-diaminobenzidine chromogen] for p62/sequestosome 1 [B, C, D, E, F, H]; immunofluorescence [Texas Red for carbonic anhydrase II] [G, H]; magnification:  $\times 2$  [B,D];  $\times 40$  [A, C, E, F, G, H]).

TTCAGTGGTTTGCCTTCTGT; reverse CGTGCATTAGGAAA-CATTTGG), and exon 6 (forward GGAGGTGCATGGTTTT-TATTTTC; reverse TTGGCAGCTGTAACCACCTA).

Sequencing reactions for *CHMP2B* were carried out using dynamic ET terminator chemistry (Amersham-Pharmacia) on a MegaBACE 3000 instrument (Amersham-Pharmacia).

**Neuropathology.** The brain and spinal cord from Patient 1 were donated for research. The tissues were dissected so that one cerebral hemisphere, the midbrain, left hemibrainstem and left cerebellar hemisphere were sliced for rapid freezing. Selected spinal cord segments were also frozen. The remaining tissues were fixed in formalin for processing to paraffin wax. These fixed tissues were used in routine staining and immunocytochemistry from all levels of the CNS. Standard immunocytochemical methods, including antigen retrieval where appropriate, were used to demonstrate localization of ubiquitin, p62/sequestosome 1,<sup>5</sup> CD68,<sup>6</sup>  $\alpha$ -synuclein, and AT8 (table E-1 on the *Neurology* Web site at [www.neurology.org](http://www.neurology.org)).

**Results.** Mutation analysis of *CHMP2B* identified previously undescribed heterozygous mutations. A single nucleotide change, A161G, was identified in Patient 2 predicting an isoleucine to valine substitution (I29V) (figure E-1A). A different single nucleotide change, A694C, in exon 6 (RefSeq NM\_014043) was identified in Patient 1 predicting a glutamine to histidine substitution (Q206H) (figure E-1B). Q206H and I29V were not identified in 640 control samples (120 CEPH individuals, 100 Danish individuals, 420 UK white individuals), in the public SNP databases, or any of the other 170 ALS samples screened as part of this study, nor in 400 FTD samples previously published.<sup>2</sup>

Neuropathology in Patient 1 showed no upper motor neuron pathology in the motor cortex (figure, A, or in mul-

multiple levels of the corticospinal tracts based on conventional stains and immunocytochemistry for CD68. Lower motor neurons (LMNs) in the ventral horn of the spinal cord and hypoglossal nuclei were depleted. Surviving LMNs showed classic ubiquitylated inclusion bodies, negative for tau and  $\alpha$ -synuclein, characteristic of ALS/MND, but there were no Bunina bodies. The case was assigned a pathologic diagnosis of PMA. After the discovery of Q206H genetic change in *CHMP2B*, the histology was reviewed with additional stains including p62/sequestosome1, a marker of ubiquitylated inclusions in a variety of neurodegenerative disorders.<sup>5</sup> Antibody to p62/sequestosome1 labeled LMN inclusions intensely (figure, B and C) and revealed a previously unrecognized pathology in the motor cortex comprising both neuritic profiles and coiled body type inclusions (figure, D and E). These coiled bodies were localized to oligodendroglia by double-labeling immunocytochemistry to carbonic anhydrase II (figure, F through H) but were negative for ubiquitin, glial fibrillary acidic protein, tau, and  $\alpha$ -synuclein. They appear to represent a novel pathology, indicate that there was motor cortex involvement at a pathologic but not clinical level, and provide a potential candidate pathology that may characterize *CHMP2B*-related familial FTD. These lesions were also present at much lower densities in the premotor cortex (Brodmann area 6) and in a prefrontal block (Brodmann area 9) and were not identified in the neocortex of a series of 25 sporadic ALS cases.

**Discussion.** We report two patients with ALS-spectrum disorders and mutations in *CHMP2B*. Both have a possible family history. Both cases were negative for other known ALS mutations. Both were screened for SOD1 and angiogenin, Patient 1 was screened for VAPB, and Patient 2 for SOD2, SOD3, VEGF-A1, and dynactin. They were phenotypically dissimilar: Patient 1 showed PMA during life (El Escorial category suspected ALS) confirmed by conventional neuropathologic methods. Patient 2 showed features of ALS-dementia presenting with frontal lobe features before developing ALS (El Escorial category ALS + syndrome). Q206H is highly conserved from humans to *Drosophila*, whereas I29V is positioned within two conserved regions, the snf-7 and coiled coil domains (figure E-2). In silico prediction of structure with the I29V mutation suggests that stability would be significantly reduced in the mutant protein. The Q206H change likely represents a pathogenic mutation because it was not found in this study in 450 controls or in 400 samples from FTD patients,<sup>2</sup> or in 141 patients with frontotemporal lobar degeneration (FTLD).<sup>4</sup> The I29V change has been suggested to be a nonpathogenic variation on the basis that it was present in one of 141 probands with FTLD, and at a frequency of 0.5% in 200 control chromosomes.<sup>4</sup> Whether I29V represents a benign variation or a pathogenic mutation of variable penetrance remains to be elucidated. In this context, it is of interest that at least 16 of the 128 disease-associated mutations in the SOD1 gene in patients with ALS are associated with reduced disease penetrance.<sup>7</sup> The finding that

a gene implicated in FTD is also present in patients with ALS supports the hypothesis that pathogenic mechanisms in ALS underlie a diverse phenotypic spectrum that spans from pure LMN disease (PMA; Patient 1) through to FTD with no clinical motor system neurology.<sup>3</sup>

Mutation within the 3' acceptor splice site of exon 6 and a point mutation in exon 5 of *CHMP2B* were previously identified in FTD.<sup>2</sup> Those patients were not recognized to have features of ALS. *CHMP2B* is a component of the endosomal sorting complex (ESCRT) involved in sorting cargoes to form multivesicular bodies. Dysfunction of the ESCRT impairs the ability to internalize membrane bound cargoes and leads to dysmorphic endosomes.<sup>8</sup> Overexpression of mutant *CHMP2B* causes the formation of aberrant endosomes in cell culture.<sup>2</sup> The changes in *CHMP2B* reported here support endosomal dysfunction as a potential mechanism of motor system degeneration. This mechanism is also postulated in *Alsin*-related ALS<sup>9</sup> and in vesicle-associated membrane protein (VAMP)/synaptobrevin-associated membrane protein B gene (*VAPB*)-related ALS.<sup>10</sup>

The upper motor neuron pathology of Patient 1 is unusual and apparently novel. Inclusions in oligodendroglia are a feature of the cortical pathology of ALS-dementia and FTD where they are immunoreactive either for ubiquitin or tau epitopes. The combination of p62 immunoreactivity, in the absence of tau,  $\alpha$ -synuclein, and ubiquitin, is unusual and suggests the possible utility of p62 as a potential molecular pathologic signature of *CHMP2B* gene-related neurodegeneration.

## Appendix

Frontotemporal Dementia Research in Jutland Association (FReJA) Consortium: Peter Johannsen, MD, Anders Gade, PhD, Jørgen Nielsen, MD, and Tove Thusgaard, RN, Memory Disorders Research Unit, Copenhagen University Hospital, Copenhagen, Denmark; Susanne Gydesen, MD, Department of Psychiatry, Central Hospital, Holbaek, Denmark; Ida Holm, MD, Department of Pathology Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; Elisabet Englund, MD, Department of Pathology, Lund University Hospital, Lund, Sweden; Jerry Brown, MD, Department of Neurology, Addenbrooke's Hospital, Cambridge, UK; Martin Rossor, MD, Dementia Research Group, Institute of Neurology, UK; John Collinge, MD, MRC Prion Unit, and Elizabeth Fisher, PhD, Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, UK. MRC Proteomics in ALS Study: Maria Spillantini, PhD, University of Cambridge, Cambridge, UK; Robert Layfield, University of Nottingham, Nottingham, UK; Paul G. Ince, MD, and Pamela J. Shaw, MD, University of Sheffield, Sheffield, UK.

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