ABSTRACT: A family with hypokalemic periodic paralysis (HypoPP) and motor neuron degeneration is reported. In conjunction with HypoPP, the index patient developed progressive muscle atrophy. The calcium channel gene *CACNA1S* showed a mutation encoding p.R528H, which has been related previously to HypoPP. We propose that *CACNA1S* mutations may comprise a previously unrecognized genetic risk factor in a greater spectrum of motor unit disorders including amyotrophic lateral sclerosis.

Muscle Nerve **37:** 120–124, 2008

PROGRESSIVE MUSCLE ATROPHY WITH HYPOKALEMIC PERIODIC PARALYSIS AND CALCIUM CHANNEL MUTATION

THOMAS MEYER, MD,[1] KARIN JURKAT-ROTT, PhD,[2] ANGELA HUEBNER, MD,[3] FRANK LEHMANN-HORN, MD,[2] PETER LINKE, MD,[1] FRANK VAN LANDEGHEM, MD,[4] JÖRN S. DULLINGER, MD,[1] and SIMONE SPULER, MD[1]

[1] Department of Neurology, Charité University Hospital, Berlin, Germany
[2] Institute of Applied Physiology, Ulm University, Germany
[3] Children's Hospital, Technical University Dresden, Germany
[4] Department of Neuropathology, Charité University Hospital, Berlin, Germany

Accepted 16 April 2007

Hypokalemic periodic paralysis (HypoPP) is a rare autosomal-dominant channelopathy characterized by attacks of disabling muscle weakness lasting from several hours to days.[7] The majority of cases of HypoPP are caused by two mutations in the calcium channel *CACNA1S* gene, R528H and R1239H.[2,4,6] Among these patients, 80% develop late-onset vacuolar myopathy resulting in a fixed weakness.[7] Biemond and Daniels[1] were the first to note that permanent muscle wasting can occur and resembles spinal muscle atrophy distinct from HypoPP-related myopathy. As early as 1934, they noted that "the relation of spinal muscle atrophy and paroxysmal paralysis is a problem which we feel can only find a solution in the genetics."[1] One author has described rare familial cases with classic histories of HypoPP that showed additional clinical and morphological evidence of progressive muscle atrophy (PMA), which is a progressive lower motor neuron disease.[3] Most cases of PMA are linked both clinically and pathologically to amyotrophic lateral sclerosis (ALS).[5,9] Herein we report a patient with familial HypoPP and PMA that had a fatal course. The disorder was associated with the R528H mutation in the calcium channel protein Cav1.1. This finding extends the phenotype related to calcium channel mutations and suggests that genetic variants of *CACNA1S* may act as a susceptibility factor to motor neuron degeneration.

Abbreviations: ALS, amyotrophic lateral sclerosis; ATPase, adenosine triphosphatase; CSF, cerebrospinal fluid; EMG, electromyography; HypoPP, hypokalemic periodic paralysis; MRC, Medical Research Council; MRI, magnetic resonance imaging; MUAP, motor unit action potential; NADH, nicotinamide adenine dinucleotide (reduced form); PMA, progressive muscle atrophy; SOD1, superoxide dismutase

Key words: amyotrophic lateral sclerosis; *CACNA1S;* calcium channel; hypokalemic periodic paralysis; progressive muscle atrophy

Correspondence to: T. Meyer; e-mail: thomas.meyer@charite.de

Published online 22 June 2007 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.20825

MATERIALS AND METHODS

All specimens and clinical data were collected from consenting subjects and handled with approval of the Review Board of Charité Hospital.

Case Reports. ***Patient 1.*** The index patient (II:4, Fig. 1), a 67-year-old man, was born to unrelated parents. At age 17 he developed period paralysis which was responsive to potassium administration. Permanent residual weakness began at the age of 50 with proximal weakness of the lower limbs. The working diagnosis was myopathy due to HypoPP. However, the weakness continued to worsen and affected the right foot and left lower leg. Nine years after the onset of fixed weakness, the disorder progressed to the point where he required a walking aid. On neurological investigation in another hospital, proximal weakness was rated 3 and power

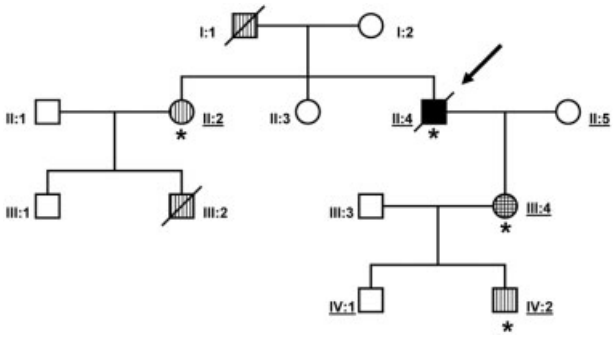

FIGURE 1. Pedigree of the family. The index patient is indicated by an arrow. Black symbol: patient with hypokalemic periodic paralysis (HypoPP) in conjunction with progressive muscle atrophy (PMA); symbols with vertical lines: individuals with HypoPP; symbols with crossed lines: individual with electrophysiological and morphological features of motor neuron degeneration; white symbols: healthy family members. Individuals tested for the p.R528H mutation of the *CACNA1S* gene are underlined; carriers of the p.R528H mutation are shown by an asterisk.

in the distal legs was rated 4 using the Medical Research Council (MRC) scale. At age 64 he showed progressive impairment of upper-limb motor function and respiratory muscle weakness. At 67 death resulted from progressive hypoventilation and respiratory failure. Consent to an autopsy was given.

On neurological examination at age 65 the patient had severe muscle atrophy of the shoulder- and hip-girdle muscles and wasting of the distal legs and arms. Right and left first dorsal interosseus, abductor digiti minimi, abductor pollicis brevis, flexor carpi ulnaris, biceps brachii, triceps brachii, deltoid, tibialis anterior, gastrocnemius, quadriceps femoris, and gluteus maximus were especially affected. Fasciculations were observed in the four limbs and torso. Motor examination revealed weakness of all limbs, especially the right and left abductor digiti minimi (MRC 0), and first dorsal interosseus (1), extensor digitorum communis (2), flexor carpi ulnaris (2), biceps brachii (3), triceps brachii (3), deltoid muscles (3), flexor hallucis brevis (1), tibialis anterior (2), gastrocnemius (3), quadriceps femoris (3), and hip flexors (2). There was marked weakness of the cervical paravertebral, abdominal, and respiratory muscles. There was no bulbar syndrome or upper motor neuron involvement. Tendon reflexes were absent in all four limbs, and Babinski and Hoffmann signs were not present. Vibration sense was mildly reduced in the ankles. Sphincter functions remained normal. Cognitive, cerebellar, and autonomic functions were preserved and remained so throughout the disease.

Cerebral, brainstem, and spinal cord magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) examination were normal. Electromyographic (EMG) examination performed in proximal and distal muscles in the upper and lower limbs showed widespread denervation in the right and left first dorsal interosseus, abductor digiti minimi, abductor pollicis brevis, extensor digitorum communis, flexor carpi ulnaris, biceps brachii, triceps brachii, deltoid, flexor hallucis brevis, tibialis anterior, vastus lateralis, vastus medialis, gastrocnemius, and cervical and thoracic paraspinal muscles. EMG examination of bulbar muscles was normal. There was no myotonic activity. The amplitudes of the compound motor action potentials of the median and peroneal nerves were markedly decreased. A biopsy taken from the right biceps muscle revealed single or multiple, centrally placed vacuoles (maximum 40 μm), as is typical for HypoPP-related vacuolar myopathy. Furthermore, severe neurogenic atrophy was found. The fibers were rounded and the caliber spectrum showed a marked shift to the left, with a range from 4 to 130 μm. Nicotinamide adenine dinucleotide (NADH) activity was increased in atrophic groups (10 fibers, $<$20 μm). Adenosine triphosphatase (ATPase) reactivity demonstrated fiber atrophy of type 1 and 2 fibers. The electrophysiological and morphological studies did not support the diagnosis of HypoPP-related myopathy as the primary cause for fixed weakness and atrophy. A diagnosis of periodic paralysis and vacuolar myopathy in conjunction with motor neuron degeneration was made. The motor neuron disease fulfilled the clinical criteria of PMA. Postmortem studies demonstrated prominent loss of lower motor neurons and Betz cells of the motor cortex, degeneration of corticospinal tracts, and atrophy of anterior roots. Bunina bodies and ubiquinated inclusions were found. The morphological features were typical for ALS (Fig. 2).[5]

Patient 2. The daughter of Patient 1 (III:4, Fig. 1), a 43-year-old woman, developed periodic paralysis at the age of 14, with about one attack per month, which was quickly responsive to potassium administration. At the age of 40 she developed fixed weakness of the lower extremities. She presented with increasing difficulty in climbing stairs and in sitting upright from a supine position. Over the ensuing 3 years weakness of the proximal legs continued to progress but remained comparatively mild.

On neurological examination at the age of 43, the patient revealed wasting and weakness of the lower limbs, with pelvic girdle muscles being the most affected. Motor examination revealed weakness with a symmetrical and proximal pattern. Hip flex-

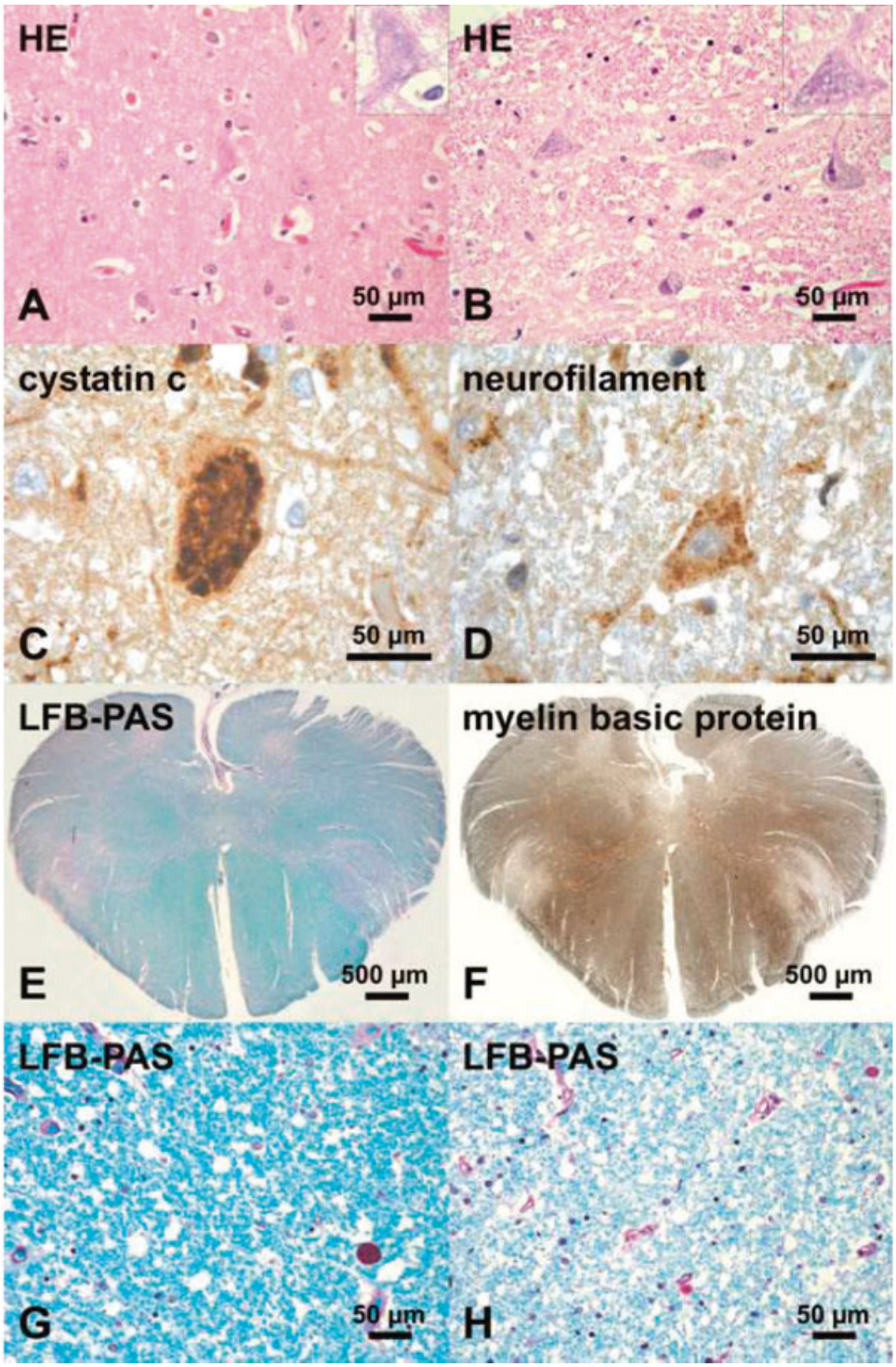

FIGURE 2. Postmortem studies of the brain and spinal cord. **(A)** Apparent reduction or shrinkage of Betz cells is demonstrated in contrast to the clinical presentation of pure lower motor neuron involvement. **(B)** Lower motor neuron loss is more prominent and is found within medullary and spinal regions. **(C,D)** Immunohistochemistry using cystatin C and neurofilament antibodies labeled several Betz cells and anterior horn cells for Bunina bodies that are neuronal inclusions typical for ALS. **(E–H)** A section from a cervical segment (C4) of the spinal cord shows degeneration of the corticospinal projection pathway with myelin pallor.

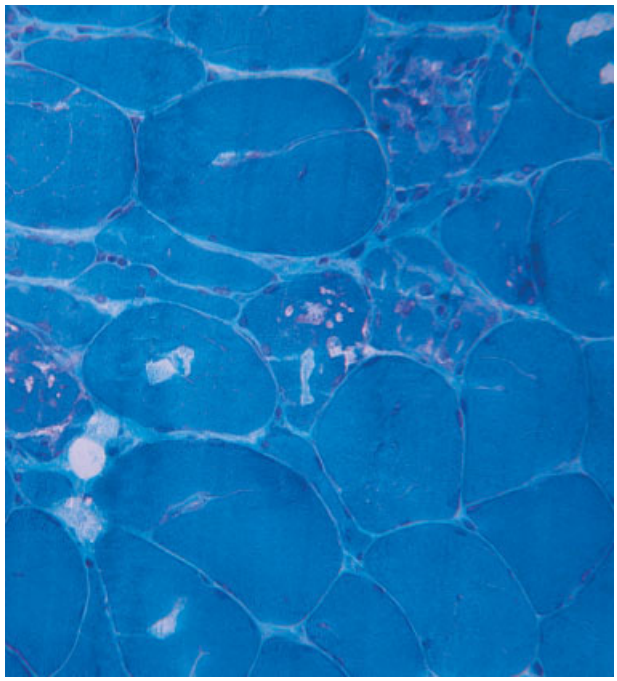

FIGURE 3. Biopsy from the left quadriceps femoris muscle. Light microscopic section of a biopsy taken from the left quadriceps femoris muscle of Patient III.4 shows angulated fibers suggesting group atrophy of neurogenic origin. Vacuolization is noted in several muscle fibers.

ors, vastus lateralis, vastus medialis, and gastrocnemius, were rated at grade 4 using the MRC scale. The flexor hallucis brevis, tibialis anterior, and plantar flexors were grade 5. Muscle tone was normal. Tendon reflexes in the legs were absent. The initial working diagnosis was myopathy due to HypoPP.

Nerve conduction studies, including compound muscle action potential amplitudes were normal. However, EMG examination performed in proximal and distal muscles in the upper and lower limbs revealed signs of denervation and polyphasic motor unit potentials only in the vastus lateralis and vastus medialis muscles. There were mixed neurogenic–myogenic changes. Fibrillation potentials and positive sharp waves, with complex repetitive discharges and fasciculation potentials, were evident in these muscles. A biopsy taken from the left quadriceps muscle revealed severe vacuolar myopathy characterized by single or multiple centrally placed vacuoles. Furthermore, several atrophic groups (6 fibers; <20 μm) were found (Fig. 3). NADH activity was increased in atrophic fibers and in some of the vacuolar fibers. ATPase reactivity revealed atrophy of both type 1 and type 2 fibers. Motor evoked potentials recorded in the distal extremity muscles following transcranial magnetic stimulation were normal. MRI of the brain and spinal cord and blood and CSF examinations were also unremarkable. The clinical diagnosis was hypokalemic periodic paralysis, vacuolar myopathy, and lower motor neuron disease.

Family History. The father of the index patient (I:1, Fig. 1) experienced attacks of generalized paralysis. He developed a progressive disabling tetraparesis and died in the fourth decade of life. The sister of the index patient (II:2, Fig. 1) had paralytic episodes. Subsequently, she developed proximal fixed weakness of the lower limbs. She did not consent to clinical evaluation. One of her sons (III:2, Fig. 1) experienced occasional weakness of the limb muscles; he died at age 20, purportedly from acute alcohol intoxication and drug overdose. One of the two grandsons (IV:2, Fig. 1) of the index patient (II:4) was reported to suffer from mild episodes of periodic paralysis.

Molecular Analysis. Individuals diagnosed with HypoPP were evaluated for mutations within the *CACNA1S* gene. Genomic DNA from the probands (Fig. 1) was used to screen the following known molecular defects: c.3716G>A (p.R1239H), c.3715C>G (p.R1239G), c.1582C>G (p.R528G) and c.1583G>A (p.R528H). Primer sequences used in polymerase chain reaction (PCR) amplification have been reported previously.[6] In the index patient, mutations in the gene for superoxide dismutase 1 (*SOD1*) were excluded.

Haplotype Analysis. Haplotype analysis was carried out using five highly polymorphic microsatellite markers (*D1S2622, D1S373, D1S2738, D1S1723* and *D1S2615*) flanking the *CACNA1S* gene. The primer sequences and genotyping method have been previously reported.[10]

RESULTS

Mutation analysis of the *CACNA1S* gene identified a heterozygous transition c.1583G>A (p.R528H). Haplotype analysis using adjacent DNA markers is compatible with the cosegregation of the disease-associated allele within the family. All patients carrying the R528H mutations (Fig. 1) had symptoms of HypoPP.

DISCUSSION

Motor neuron degeneration in conjunction with HypoPP has been recognized as distinct from HypoPP-related myopathy.[1,3] Several reports have described

familial cases of HypoPP that developed a disabling motor neuron disorder resembling spinal muscle atrophy or PMA.[1,3] At the time of those reports, the molecular basis of HypoPP was unknown. We report on HypoPP that arose from a known mutation in the *CACNA1S* gene. The index patient demonstrated a clinical syndrome of HypoPP in conjunction with PMA. Permanent weakness began with a proximal and symmetric pattern suggestive of myopathy. Subsequently, he developed asymmetric and distal pareses as typical for motor neuron disease. Interestingly, postmortem studies showed Bunina bodies, which are common morphological features of PMA and the classic form of ALS.[5,9] Given the rarity of HypoPP and PMA, a chance association of the *CACNA1S* mutation and this phenotype was unlikely. Furthermore, the daughter of the index patient (III:4, Fig. 1) showed vacuolar myopathy in combination with electromyographic and histological features of incipient motor neuron degeneration. In this patient, further development of PMA is not excluded. A single case of HypoPP with PMA within the genetic trait of *CACNA1S*-linked HypoPP does not permit the conclusion that the PMA phenotype is caused by the calcium-channel mutation. Nevertheless, it is possible that a closely linked locus near that of *CACNA1S* may be altered, resulting in motor neuron degeneration. In this case, the *CACNA1S* mutation is the genetic cause of periodic paralysis, whereas the mutation in the tightly linked locus may be responsible for the motor neuron disorder. Alternatively, the *CACNA1S* gene may represent a previously unknown genetic risk factor for PMA. For most PMA patients, the genetic cause is unknown. In 10%–15% of familial cases, mutations in the *SOD1* gene have been found. *SOD1* mutations were excluded in the index patient.

Our observations may support the idea that most cases of PMA represent a complex genetic disorder in which the phenotype arises from multiple genes and yet unknown exogenous factors. The functional impact of the *CACNA1S* mutation on the genotype–phenotype correlation is unknown. The gene product is found in the membrane of the transverse tubular system and appears to be involved in excitation–contraction coupling of the muscle.[2] Expression of *CACNA1S* RNA in motor neurons has recently been shown (GEO Profiles, Access. No. GDS1455). The ability to access the normal function of the Cav1.1 protein and the pathogenic consequences of its mutations have been hampered by the poor expression and altered properties of this L-type channel in nonmuscle cells.[2] It is conceivable, though, that genetic variants of the *CACNA1S* gene may contribute to a common pathway in HypoPP, vacuolar myopathy, and motor neuron disease. Its delineation may provide further insight into the mechanisms of motor unit disorders, including ALS.

Supported by the Immendorff-Fonds for ALS Research (to T.M., J.S.D., P.L.) and the Deutsche Forschungsgemeinschaft (Ju 470/1-2). A.H. and S.S. are members of the German Muscular Dystrophy Network MD-NET funded by the Bundesministerium für Bildung und Forschung (BMBF, 01GM0601), Germany.

REFERENCES

1. Biemond A, Daniels A. Familial periodic paralysis and its transition into spinal muscle atrophy. Brain 1934;57:91–108.
2. Cannon SC. Pathomechanisms in channelopathies of skeletal muscle and brain. Annu Rev Neurosci 2006;29:387–415.
3. Delaney JF, Jozeczyk PB. Progressive muscle atrophy in a case of hypokalemic periodic paralysis. Dis Nerv Syst 1975;36:678–680.
4. Fontaine B, Vale-Santos J, Jurkat-Rott K, Reboul J, Plassart E, Rime CS, et al. Mapping of the hypokalaemic periodic paralysis (HypoPP) locus to chromosome 1q31–32 in three European families. Nat Genet 1994;6:267–272.
5. Ince PG, Lowe J, Shaw PJ. Amyotrophic lateral sclerosis: current issues in classification, pathogenesis and molecular pathology. Neuropathol Appl Neurobiol 1998;24:104–117.
6. Jurkat-Rott K, Lehmann-Horn F, Elbaz A, Heine R, Gregg RG, Hogan K, et al. A calcium channel mutation causing hypokalemic periodic paralysis. Hum Mol Genet 1994;3:1415–1419.
7. Miller TM, Dias da Silva MR, Miller HA, Kwiecinski H, Mendell JR, Tawil R, et al. Correlating phenotype and genotype in the periodic paralyses. Neurology 2004;63:1647–1655.
8. Stevens JR. Familial periodic paralysis, myotonia, progressive amyotrophy, and pes cavus in members of a single family. AMA Arch Neurol Psychiatry 1954;72:726–741.
9. Van Den Berg-Vos RM, Van Den Berg LH, Visser J, de Visser M, Franssen H, Wokke JH. The spectrum of lower motor neuron syndromes. J Neurol 2003;250:1279–1292.
10. von der Hagen M, Schallner J, Kaindl AM, Koehler K, Mitzscherling P, Abicht A, et al. Facing the genetic heterogeneity in neuromuscular disorders: linkage-analyses as economic diagnostic approach towards the molecular diagnosis. Neuromuscular Disord 2006;16:4–13.