

Heterozygosity at Polymorphic Codon 219 in Variant Creutzfeldt-Jakob Disease

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Background: Genetic variants of the prion protein gene (*PRNP*) strongly determine susceptibility to prion diseases. All tested patients with definite variant Creutzfeldt-Jakob disease (vCJD) are homozygous for methionine at a common polymorphism at codon 129. A further genetic polymorphism at codon 219, a common variant in several Asian populations, is considered protective against sporadic CJD.

Objective: To report a finding of heterozygosity at codon 219 in 2 patients with vCJD.

Design: Case reports.

Setting: MRC (Medical Research Council) Prion Unit and Department of Neurodegenerative Disease, University College London Institute of Neurology, and National Prion Clinic, National Hospital for Neurology and Neurosurgery.

Patients: Two patients with clinical and investigation findings consistent with the diagnoses of probable vCJD.

Main Outcome Measures: Clinical and genetic findings.

Results: A 34-year-old man had a 15-month history of behavioral change progressing to ataxia, dysarthria, involuntary choreiform movements, and severe cognitive impairment. Cerebrospinal fluid analysis was positive for 14-3-3 protein, electroencephalography showed generalized slowing, and magnetic resonance imaging revealed thalamic high signal bilaterally, typical of vCJD. A 31-year-old woman had a 16-month history of cognitive decline, ataxia, involuntary choreiform movements, and myoclonic jerks. Magnetic resonance imaging showed bilateral pulvinar high signal. The diagnosis was confirmed by a tonsillar biopsy demonstrating abnormal prion protein deposition in a typical pattern for vCJD. *PRNP* sequencing showed a methionine homozygous codon 129 genotype and an E219K polymorphism in both patients.

Conclusions: The E219K polymorphism is neutral or may even confer susceptibility to vCJD. The observations are interpretable in the context of the conformational selection model of prion replication. A barrier to prion disease transmission depends on the degree to which permitted pathologic conformations of the prion protein overlap between the inoculum and the host.

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PRIONS ARE LETHAL HUMAN pathogens that cause neurodegenerative diseases with acquired, inherited, or unknown etiologies.¹ Variant Creutzfeldt-Jakob disease (vCJD), an acquired prion disease transmitted by exposure to bovine spongiform encephalopathy prions, was first recognized in the mid-1990s.² More than 200 cases have been identified worldwide, most in the United Kingdom.³ In neuropathologically confirmed vCJD, every patient who has undergone genotyping to date has been homozygous for methionine at a common polymorphism of the prion protein gene (*PRNP*) at codon 129. This genetic variant has profound susceptibility effects across all categories of prion disease.³ Studies^{4,5} modeling human susceptibility to bovine spongiform encephalopathy

prion infection in transgenic mice have suggested that different clinicopathologic phenotypes may occur in individuals with other *PRNP* codon 129 genotypes.

A further genetic polymorphism has been reported at codon 219 in which guanine (G) is replaced by adenine (A) in the first position of codon 219, resulting in substitution from glutamic acid (E) to lysine (K). This polymorphism is common in Japan, with an allele frequency of 0.06 in the healthy population. It has not been reported in patients with sporadic CJD (sCJD) in the Korean and Japanese populations and is, therefore, considered strongly protective against this disease.^{6,7} Herein we report heterozygosity at codon 219 in 2 patients with diagnoses of vCJD and their clinical and investigation findings.

PATIENT 1

A 34-year-old British man presented in 2004 with a few months' history of personality change and mild episodic memory impairment. He became obsessive, socially withdrawn, and apathetic, and he began to experience difficulty with managing finances. Several months later his gait was noted to be unsteady, and he was intermittently incontinent of urine. Subsequently, he developed clumsiness in the upper limbs and difficulty manipulating fine objects, and he could no longer use a knife and fork. His gait became increasingly unsteady, and he started to fall. His mood deteriorated significantly, and he began to demonstrate suicidal ideation. He was soon unable to perform his usual daily activities. He subsequently became doubly incontinent, he started to experience choreiform movements in the upper limbs, and he spoke less. He seemed to respond to visual hallucinations and experienced a disordered sleep-wake cycle. Physical examination revealed a bilateral grasp reflex. Involuntary choreiform limb movements were present, in addition to purposeful rubbing of his chest and hair. Spontaneous myoclonus of the lower limbs was present. His speech was hypophonic, mumbling, and monosyllabic. His gait was ataxic, with reduced arm swing, and he was unsteady on turning. There was increased tone and preserved power in all 4 limbs. His reflexes were symmetrically brisk, and the plantar responses were extensor bilaterally.

His Mini-Mental State Examination score was 12 of 30 at the time of investigation, 12 months after symptom onset. Investigation findings were positive for cerebrospinal fluid 14-3-3 protein and a raised S-100B protein level of 1.36 ng/mL (reference range, <0.38 ng/mL), with a cerebrospinal fluid protein concentration of 0.07 g/dL (reference range, 0.02-0.05 g/dL [to convert to grams per liter, multiply by 10]) and otherwise normal cerebrospinal fluid constituents and no oligoclonal bands. Electroencephalography revealed nonspecific slow wave activity. Diffusion-weighted magnetic resonance imaging showed increased thalamic signal bilaterally, consistent with the pulvinar sign, with an additional increase in the left occipital cortex, and was thought to be most compatible with vCJD. Findings from autoimmune, inflammatory, and paraneoplastic screening were negative. Results of treponemal and human immunodeficiency virus serologic analyses were negative. Results of screening tests for Wilson disease were negative. Mitochondrial DNA analysis showed no evidence of the MELAS (mitochondrial, encephalopathy, lactic acidosis, and stroke-like episodes) 3243 mutation. DNA analysis showed both Huntington gene alleles to be within the reference range for CAG repeats.

A diagnosis of probable vCJD was made. PRNP sequencing was performed and showed a methionine homozygous codon 129 genotype and an E219K polymorphism. Twelve months after the illness began, the patient deteriorated further in terms of speech, swallowing, mobility, and cognitive function. He became bed bound and mute; he died 3 months later. Neither diagnostic tonsil biopsy⁸ nor autopsy was performed.

A 31-year-old British woman of Afro-Caribbean descent presented in 2001 with episodic memory impairment noticed by her husband. She was 34 weeks pregnant when the symptoms started. Her son was born at 36 weeks by cesarean delivery owing to antepartum hemorrhage. She had no problems caring for him initially, but she subsequently started to experience short-term memory problems and low mood, so the husband became more involved with caring for their baby. The patient became increasingly apathetic, and when she tried to return to work 6 months later she was unable to function at her previously high level. She developed gait ataxia and an involuntary choreiform movement disorder. Her husband described her as having difficulty with speech and language. At this stage, she was noted to have myoclonic jerks of both upper and lower limbs.

Physical examination revealed a Mini-Mental State Examination score of 16 of 30. Her gait was broad based and unsteady, and her speech was slurred. She had a positive grasp reflex bilaterally and brisk finger jerks. The patient had abnormal movements of her upper and lower limbs and a rocking movement of the body, which were suppressible only for a short period. Tone and power were normal. Her reflexes were symmetrical, and the plantar response was flexor. She was disorientated in time and place. She had evidence of frontotemporal and parietal lobe cognitive dysfunction demonstrated by dyspraxia with difficulty miming, substitution of body parts as tools, and problems with motor sequencing. Investigation findings were positive for cerebrospinal fluid 14-3-3 protein. Magnetic resonance imaging showed bilateral pulvinar high signal. Electroencephalography was unremarkable. A tonsillar biopsy demonstrated abnormal prion protein (PrP) typical of vCJD on Western blotting (type 4t PrP^{Sc} using the London classification) (**Figure**).^{8,9} PRNP sequencing revealed a methionine homozygous codon 129 genotype and an E219K polymorphism. The patient deteriorated further and became bed bound and mute. She died 16 months after the onset of symptoms. An autopsy was not performed.

COMMENT

Heterozygosity at codon 219 is a common polymorphism in the healthy Japanese population and in populations in Oceania, South Asia, and the Middle East, but it has not been detected in Africans or Europeans.¹⁰⁻¹² Eleven patients and controls with E219K all had Asian ethnicity or ancestry based on a heterogenous diagnostic referral series totaling more than 1800 samples at the MRC (Medical Research Council) Prion Unit.

Recent data from a transgenic animal knock-in model suggest that the 219E and 219K proteins can convert to PrP^{Sc} when challenged with sCJD or vCJD prions.¹³ Human PrP 219K-expressing mice succumbed to vCJD with a shorter incubation time compared with human PrP 219E-expressing mice. The E219K heterozygous mice had a longer incubation time to vCJD than did either homozygous mouse. However, the present patients suggest that the heterozygous genotype at codon 219 is not protective against vCJD and may even confer increased

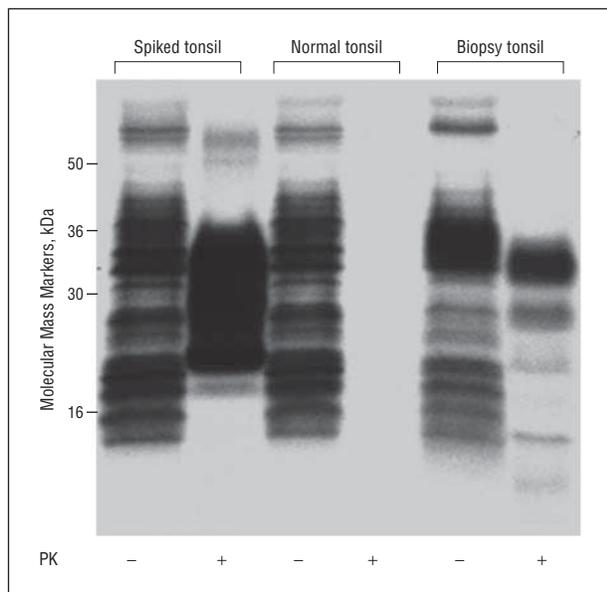


Figure. Using sodium phosphotungstic acid precipitation,⁹ 0.5-mL aliquots of 10% (wt/vol) tonsil biopsy homogenate from patient 2 (biopsy tonsil) or 10% (wt/vol) normal human tonsil homogenate, either lacking (normal tonsil) or containing a spike of 1- μ L of 10% (wt/vol) variant Creutzfeldt-Jakob disease brain homogenate (spiked tonsil), were analyzed. Twenty-microliter aliquots of whole samples isolated before centrifugation were analyzed in the absence (-) of proteinase K (PK) digestion and compared with PK digestion products (+) derived from the entire sodium phosphotungstic acid pellets. The immunoblot was analyzed with anti-prion protein monoclonal antibody 3F4 and high-sensitivity-enhanced chemiluminescence.⁹

risk. We do not know whether the PrP^{Sc} seen on Western blot of tonsillar tissue in patient 2 includes PrP with 219K or whether this is solely derived from the 219E PrP. The E219K polymorphism occurs as a result of a substitution of negatively charged E by the positively charged K. Further studies are necessary to establish whether this charge change may be critical in permitting the conversion of PrP to its abnormal isoform in vCJD but not sCJD. Our seemingly paradoxical observations may be interpreted in the context of the conformational selection model of prion transmission proposed by Collinge¹⁴ in 1999 and Collinge and Clark¹⁵ in 2007. This model proposes that prion transmission occurs efficiently when there is an overlap between the infecting prion and the permissible conformations of the host PrP. In this circumstance, the 219K protein may not adopt the molecular conformations found in sCJD, resulting in strong resistance to this disease. However, in vCJD, the model proposes that the 219K protein permits or even favors the bovine spongiform encephalopathy strain, resulting in no resistance to disease or even increased susceptibility.

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the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Lukic. *Acquisition of data:* Lukic, Beck, Joiner, Fearnley, Sturman, and Wadsworth. *Analysis and interpretation of data:* Lukic, Beck, Joiner, Brandner, Wadsworth, Collinge, and Mead. *Drafting of the manuscript:* Lukic, Joiner, and Wadsworth. *Critical revision of the manuscript for important intellectual content:* Beck, Fearnley, Sturman, Brandner, Wadsworth, Collinge, and Mead. *Administrative, technical, and material support:* Lukic, Beck, Joiner, and Wadsworth. *Study supervision:* Wadsworth, Collinge, and Mead.

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