Clinical/Scientific Notes

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A CASE OF ALS-FTD IN A LARGE FALS PEDIGREE WITH A K17I *ANG* MUTATION

Approximately 90% of amyotrophic lateral sclerosis (ALS) cases are sporadic (SALS), but 10% are familial (FALS). Mutations in *SOD1, Alsin*, *Dynactin*, *SETX*, *DJ-1*, *VAPB*, and TDP-43[1] have been reported (table e-1 on the *Neurology*® Web site at www.neurology.org). After the identification of sequence variation *VEGF* in patients with ALS, mutations in another angiogenic gene (*ANG*) were identified in SALS and FALS.[2,3] Studies in other populations have identified *ANG* mutations in patients with ALS, but also in healthy controls. This suggests that not all mutations are pathogenic.[3,4]

Methods. A total of 39 unrelated FALS patients, negative for *SOD1* mutations, were screened for *ANG* mutations. This study was approved by the local ethics committee and participants provided informed consent. DNA was isolated from venous blood and *ANG* mutation analysis was performed as described in appendix e-1. A total of 275 unrelated, healthy controls were taken from a prospective population-based study on ALS in The Netherlands and were also screened.[5] PMut (http://mmb2.pcb.ub.es:8080/PMut/) was used to predict the impact of an amino acid substitution on the structure and function of the protein.

Results. We identified one mutation in one patient (122 A>T) (figure, A), leading to an amino acid substitution of lysine to isoleucine (K17I) (figure, B). PMut analysis predicted this mutation to be pathogenic. Sequence alignments of *ANG* in different species demonstrated high conservation (figure, C).

Analysis of this pedigree revealed an autosomal dominant inheritance of the mutation (male to male transmission) (figure, D). DNA was available from 44 out of 62 family members (five affected individuals). All affected family members carried the K17I mutation.

Ten carriers were identified, but all were under 50 years of age (except one who was 75 years old without symptoms or signs of ALS). The K17I mutation was not found in 275 control samples.

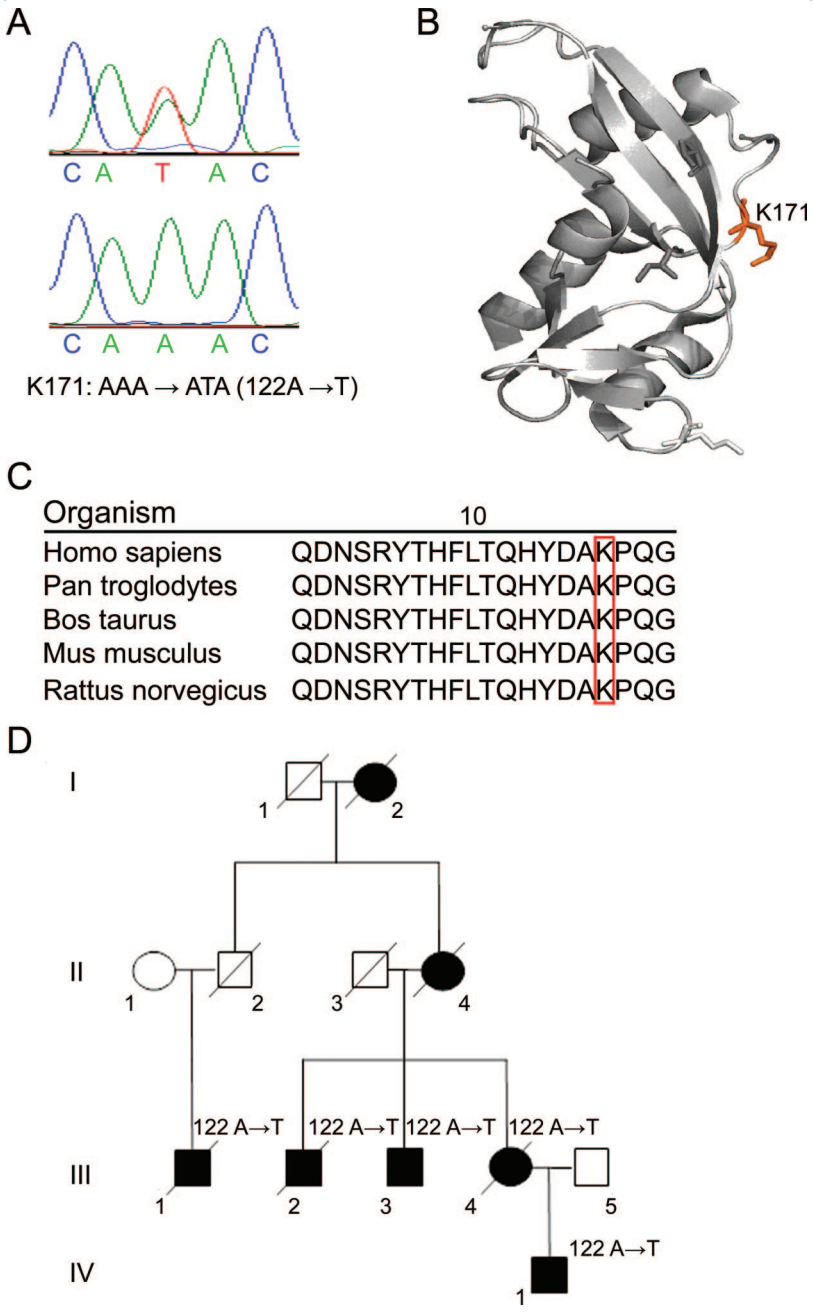
Cases III-3, III-4, and IV-1 all presented with progressive upper and lower motor neuron loss of limbs. Case III-1 rapidly developed weakness in both arms with atrophy, fasciculations, and dyspnea, but no upper motor neuron signs. The patient died after 6 months from onset. Case III-2 initially presented with parkinsonism (bradykinesia, diminished postural reflexes, cogwheel rigidity [right arm], shuffling, short-stepped gait, and decreased spontaneous eye blink rate). There was no autonomic dysfunction and eye movements were intact. Dopaminergic treatment had little effect. After 5 years, the patient developed progressive weakness of the arms and legs with atrophy, fasciculations, and hyperreflexia. Interestingly, the patient also demonstrated symptoms characteristic of frontotemporal dementia (FTD), such as loss of interest in social contacts and family, short attention span, logopenia, verbal apraxia, perseveration, decreased personal hygiene, hyperorality, reckless behavior in traffic, sexual disinhibition, and apathy. Case I-2 and II-4 also appear to have been affected. However, no medical records were available. Patient I-2 developed limb weakness at age 70, leading to paralysis and death within 3 years. Patient II-4 developed speech impairment at age 60 and also died within 3 years. Patient II-2 (obligate carrier) died at age 50 from cardiovascular disease. Detailed clinical characteristics are provided in table e-2.

Discussion. Several *ANG* mutations in FALS have been reported, but clear segregation of mutations with the disease has not been shown. Here, we report the K17I mutation segregating with disease in a large pedigree. The fact that II-2 and a carrier (75 years of age) were without symptoms of ALS suggests incomplete penetrance of the mutation. This might explain why mutations in this codon have only been found in SALS. The K17I mutation was previously reported in three cases and K17E in two cases.[3,6]

This study provides a report of a patient with an *ANG* mutation and ALS, FTD, and parkinsonism. Five percent of patients with ALS also have FTD and up to 50% demonstrated mild cognitive impairment. Similarly, relatives of patients with ALS have an increased risk for developing PD. Therefore, genes involved in ALS are also considered candidate genes for

Supplemental data at www.neurology.org

Figure Mutation analysis and partial pedigree



(A) Wild type sequence and the K17I mutation. (B) Three-dimensional structure of ANG modeling the K17I mutation in ball-and-stick representation. The figure was created using the program PyMOL (DeLano Scientific). (C) Sequence alignments of *ANG* in different species. Sequences were multiply aligned using Homologene (http://www.ncbi.nlm.nih.gov/sites/entrez/query.fcgi?db = homologene). The numbering on top of the alignments correlates with the human amino acid sequence. Amino acid 17, which is the site of K17I, is indicated in red. (D) A simplified version of the pedigree is depicted to protect the privacy of the family. A partial four-generation pedigree is shown. All individuals marked in black have amyotrophic lateral sclerosis. All individuals carrying the122 A>T (K17I) mutation are marked in the pedigree. The obligate carrier (II-2) died at 50 years of age due to cardiovascular disease. No DNA was available from this individual. The spouse of II-2 tested negative for the mutation.

other neurodegenerative disorders. Indeed, an Italian study reported a SALS patient with a 132C→T mutation and frontal lobe dysfunction.[4]

ANG is highly conserved between species, suggesting it has an important biologic function. Modeling of the K17I mutation using PMut predicted this to be pathogenic. Two functional studies demonstrated that the K17I mutation results in loss of function, possibly leading to insufficient ribosomes synthesis, decreased protein translation, and ultimately decreased motor neuron viability.[6,7]

We report segregation of the K17I mutation with FALS and a patient with FALS, FTD, and parkinsonism, which possibly implicates *ANG* in these diseases.

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INFLAMMATORY PSEUDOTUMOR ASSOCIATED WITH HIV, JCV, AND IMMUNE RECONSTITUTION SYNDROME

A 37-year-old HIV-positive African woman developed severe chronic diarrhea. Her $CD4^+$ T cell count was 25 cells/mm^3. Within 1 month of initiation of highly active antiretroviral therapy (HAART) her plasma HIV viral load became undetectable and $CD4^+$ T cell count rose to 96 cells/mm^3, and continued to rise over the following months.

Two months after the initiation of HAART she developed vertigo, loss of balance, incoordination, slurred speech, and tremor of the neck and limbs. Neurological examination revealed ocular abnormalities, dysarthria, and monotonic speech. She had bilateral limb dysmetria, past-pointing and endpoint tremor, impaired heel-knee-shin testing, head tremor, and a wide based, ataxic gait.

Initial brain MRI revealed a confluent, nonenhancing area of signal abnormality predominantly involving the inferior right cerebellar hemisphere and extending to the posterior vermis, right cerebellar peduncle, and inferomedial aspect of the left cerebellar hemisphere. Six months later, MRI revealed progression of the cerebellar lesion, with nodular enhancement along the inferomedial aspect of the right cerebellar hemisphere. The patient remained clinically stable. MRI 8 months later revealed a large cystic ring-enhancing lesion in the location of the previously noted high signal intensity lesions of the cerebellum, with compression of the posterior fourth ventricle (figure, A).

CSF revealed WBC 1, Prot 64, Gluc 49, and negative cytomegalovirus DNA PCR, Cysticercosis IgG Ab, Epstein-Barr virus DNA PCR, Venereal Disease Research Laboratory, and Cryptococcus Ag. Bacterial, viral, and fungal cultures were negative. JCV PCR was positive. Stereotactic biopsy of the cerebellar lesion, performed 17 months after the onset of neurologic symptoms, revealed giant cells with pleomorphic hyperchromatic nuclei, often multiple, surrounded by a dense infiltrate of lymphocytes and plasma cells (figure, B). The bizarre, pleomorphic cells were GFAP positive, demonstrated diffuse nuclear reactivity for p53 antigen, a high MIB-1 (Ki-67) index, and focal, faint reactivity for polyoma virus T antigen (figure e-1 on the *Neurology*® Web site at www.neurology.org). Inflammatory infiltrates marked both for T and B cells (CD3, CD43, CD20, CD79a). The adjacent cerebellar folia were atrophic, with total loss of granular cell neurons, preservation of Purkinje cells, and infiltrates of lymphocytes and histiocytes. Four 10-μm-thick sections of the mass were cut and utilized for DNA extraction; PCR was performed and demonstrated a 173 base pair band diagnostic of polyoma virus; its identity as JCV was further confirmed with a *Bam*HI digest which produces 2 DNA fragments of 120 and 53 base pairs (JCV, but not BKV or SV40 has this restriction site in the amplicon)[1] (figure, C). A JCV-associated inflammatory pseudotumor was diagnosed. The patient has a stable pancerebellar syndrome 24 months after onset of neurologic symptoms.

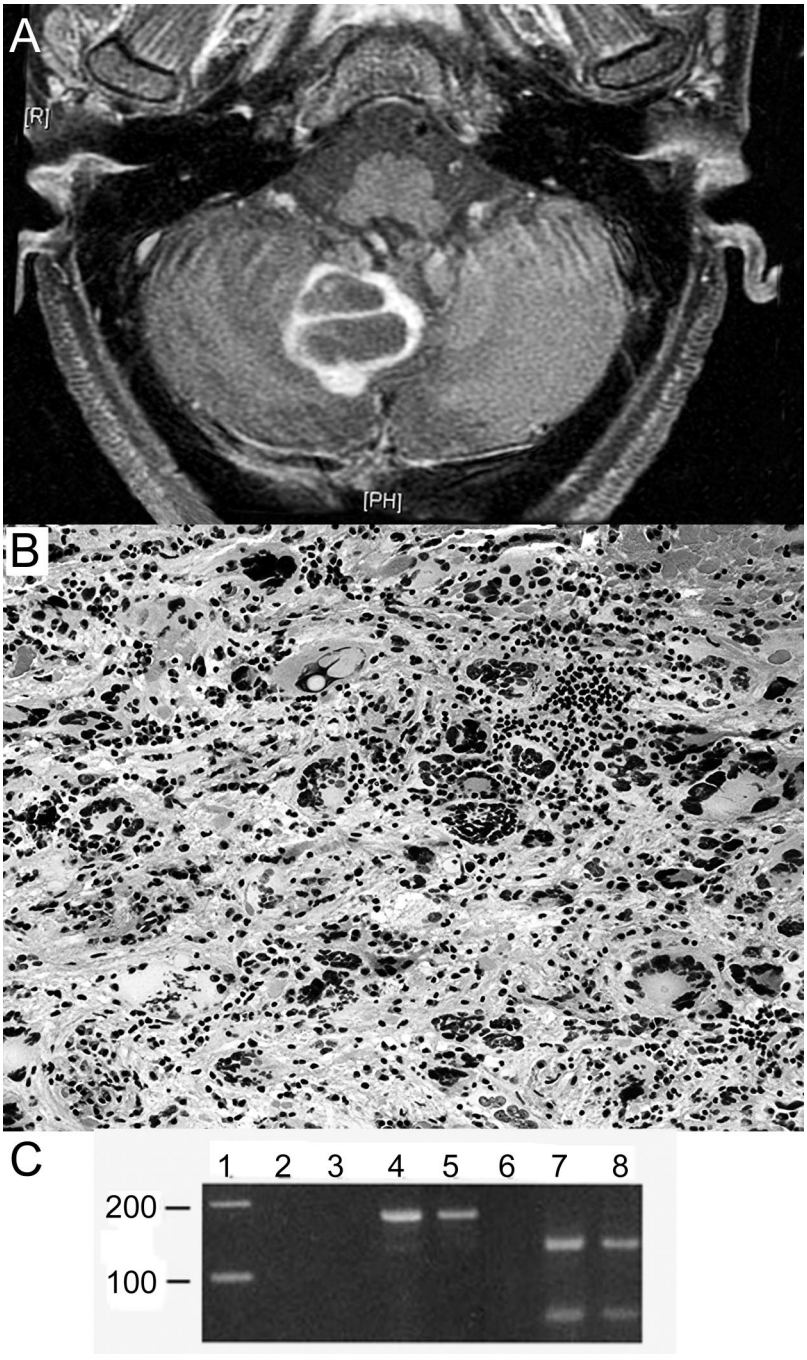
Supplemental data at www.neurology.org

Discussion. The immune reconstitution inflammatory syndrome (IRIS) in HIV-infected patients receiving HAART is characterized by paradoxical clinical or radiologic deterioration despite an increasing $CD4^+$ T cell count and decreasing HIV viral load.[2] Foreign organisms become unmasked and trigger a disproportionate immune response.[2] IRIS has been reported in association with JCV infection, the cause of progressive multifocal leukoencephalopathy (PML).[3] The radiologic features of PML are generally characterized by focal high-signal lesions predominantly affecting white matter structures; in the cerebellum, it has been associated with atrophy of the folia, with or without white matter involvement. While PML generally shows minimal contrast enhancement, this is more frequent in IRIS-associated PML. The radiologic features in the current patient, demonstrating a cystic lesion with nodular enhancement were unusual, raised the possibility of a secondary neoplastic or infectious non–JCV-related lesion, and led to the eventual performance of a brain biopsy.

PML is histologically characterized by the triad of oligodendroglial inclusions, demyelination, and bizarre, atypical astrocytes.[4] In the cerebellum, selective loss of granular cell neurons, as seen in the present case, is common. PML may be associated with variable host inflammatory response. In the case of IRIS-associated PML, there are appreciable inflammatory infiltrates, with a preponderance of T cells.[4,5] Oligodendroglial nuclei with characteristic viral inclusions may be rare or absent, and bizarre pleomorphic or multinucleated cells may have astrocytic or histiocytic origins.[6] Pathology in the current patient was unusual, as the combination of dense inflammation and bizarre glial cells resulted in a pseudotumor formation, heretofore unreported in JCV-associated IRIS. In our patient, oligodendroglial inclusions were not evident, although the abnormal morphology of giant cells was typical of JCV-transformed astrocytes, and the granular cell loss was characteristic of PML. The juxtaposition of the intense predominantly lymphoplasmacytic infiltrate surrounding these transformed cells is likely to represent an IRIS-induced inflammatory response.[7]

This JCV-associated pseudotumor is an unusual manifestation of the spectrum of IRIS neuropathologies. Given its clinical and radiologic overlap with

Figure MRI and histologic examination of the cerebellum



(A) Gadolinium-enhanced T1 FLAIR MR image demonstrating a large cystic ring-enhancing lesion in the context of high signal intensity lesions of the cerebellum. (B) Excisional biopsy of the cerebellum demonstrating bizarre multinucleated giant cells surrounded by an inflammatory cell infiltrate (hematoxylin-eosin, original magnification 100×). (C) Ethidium-bromide stained gel demonstrating presence of JCV in the patient's pseudotumor. Formalin-fixed, paraffin-embedded sections of the patient's lesion (lanes 4 and 7) and autopsy-derived progressive multifocal leukoencephalopathy (PML) (lanes 5 and 8) and normal brain (lanes 3 and 6) were used to extract DNA for PCR. Lanes 2, 3, 4, and 5 show the results of PCR amplification of a 173 base pair segment of polyoma virus in a reaction run without template DNA (lane 2), with normal brain DNA (lane 3), DNA from the patient's lesion (lane 4), and from an unrelated case of PML (lane 5). Lanes 6, 7, and 8 display *Bam*HI digests of the PCR products run in lanes 3, 4, and 5, respectively. Both the patient's lesion and the case of PML show specific, 120 and 53 base pair fragments that occur only with JCV, which has a *Bam*HI restriction site in the amplicon (SV40 and BKV do not share this restriction site). Lane 1 contains a 100 bp DNA ladder.

other tumoral and infectious entities, clinicians must be alert to the differential diagnosis.

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