

The diverse phenotype and genotype of pantothenate kinase-associated neurodegeneration

Abstract—Pantothenate kinase-associated neurodegeneration (PKAN) is a rare autosomal-recessive disorder caused by mutations in the *PANK2* gene. The authors report clinical and genetic findings of 16 patients with PKAN. The authors identified 12 mutations in the *PANK2* gene, five of which were new. Only nine patients could be classified as classic or atypical PKAN, and intermediate phenotypes are described. Two patients presented with motor tics and obsessive-compulsive behavior suggestive of Tourette syndrome.

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M.T. Pellicchia, MD*; E.M. Valente, MD, PhD*; L. Cif, MD; S. Salvi, BSc; A. Albanese, MD; V. Scarano, MD; U. Bonuccelli, MD; A.R. Bentivoglio, MD, PhD; A. D'Amico, MD; C. Marelli, MD; A. Di Giorgio, BSc; P. Coubes, MD; P. Barone, MD, PhD; and B. Dallapiccola, MD

Pantothenate kinase-associated neurodegeneration (PKAN [MIM 234200]) is a rare autosomal recessive disorder with iron accumulation in the basal ganglia, due to mutations in the *PANK2* gene. *PANK2* encodes a pantothenate kinase, the key regulatory enzyme in coenzyme-A synthesis.¹ The neuroradiologic hallmark of PKAN is the eye of the tiger sign, characterized by bilateral areas of hyperintensity within a region of hypointensity in the median globus pallidus on T2-weighted brain MRI.² An almost complete correlation exists between this neuroradiologic sign and the presence of *PANK2* mutations.³ Conversely, the PKAN phenotype is variable and overlaps with a wide spectrum of disorders characterized by neurodegeneration with brain iron accumulation (NBIA), formerly known as Hallervorden-Spatz syndrome.^{4,5}

Two main phenotypes have been identified so far. Classic PKAN manifests in the first decade with severe extrapyramidal signs and progresses rapidly with loss of ambulation within 15 years from onset. In atypical PKAN, the onset is in the second to third decade with less severe extrapyramidal signs, slower progression, and maintenance of independent ambulation well after 15 years of disease.³ To date, several distinct mutations have been identified in classic and

atypical cases, all carrying the eye of the tiger sign.^{1,3,4}

Methods. We identified nine Italian (nos. 1 through 9), three French (nos. 10 through 12), and four Portuguese (nos. 13 through 16) patients with probable PKAN (onset during the first three decades of life, progressive course, extrapyramidal dysfunction, and presence of the eye of the tiger sign). All cases were sporadic except two sibpairs (nos. 6–7 and 8–9). Patients underwent neurologic, neuropsychological, psychiatric, and ophthalmologic evaluation, including electroretinogram, and testing for red blood cell acanthocytosis. Neuropsychological evaluation included Mini-Mental State Examination, IQ testing on the Wechsler Intelligence Scale for Children–Revised scale, and Raven's Colored Progressive Matrices. All families received genetic counseling and blood samples were collected after obtaining informed consent.

The seven coding exons and exon-intron junctions of the *PANK2* gene were PCR-amplified and directly sequenced in both directions using the Big Dye Terminator chemistry and an ABI-PRISM 3100 automated DNA sequencer (Applied Biosystem, Foster City, CA). The frequency of all novel nucleotidic changes was assessed by sequencing 300 control chromosomes. All mutations were named according to the previous nomenclature.³

Results. Clinical findings. Clinical and molecular results are summarized in the table. All patients had onset in the first two decades (mean 8.2 ± 5.2 years; range 1 to 17 years), and mean disease duration was 16.9 ± 11.0 years (range 3 to 39 years). The presenting feature was gait disturbance in six patients, difficulty in writing or handling objects in five, behavioral problems or motor tics in three, and psychomotor delay in two. The most common symptoms were dysarthria, dystonia, rigidity, and corticospinal signs. Gait problems were present in 14 patients, but only 7 lost the ability to walk 2 to 27 years after onset. Cognitive decline was evident in 10 patients, and 8 showed psychiatric signs, such as behavioral disturbance, obsessive-compulsive disorder, hyperactivity, or depression. Ocular involvement and acanthocytosis were rare.

Only nine of our patients had a disease history compatible with the clinical classification of classic and atypical PKAN.³ Five patients (nos. 4, 10, 13, 14, 16) had classic disease with onset in the first decade and rapid progression to severe disability, with loss of independent ambulation 2 to 9 years after onset. Four patients (nos. 1, 8, 9, 15) had atypical course with onset in the second decade, slow progression, and maintenance of independent ambulation 15 to 39 years after onset. Four patients had intermediate phenotypes, presenting either with onset in the first decade but slow progression without early loss of ambulation

*These authors contributed equally to this work.

From the Department of Neurological Sciences (Drs. Pellicchia, Scarano, and Barone), University "Federico II," Naples; IRCCS CSS (Dr. Valente, S. Salvi, and A. Di Giorgio), Mendel Institute, Rome, Italy; Department of Neurosurgery (Drs. Cif and Coubes), Gui de Chauliac University Hospital, Montpellier, France; National Neurologic Institute "Carlo Besta" (Drs. Albanese and Marelli), Milan; Catholic University (Dr. Albanese), Milan; Department of Neuroscience (Dr. Bonuccelli), University of Pisa; Institute of Neurology (Dr. Bentivoglio), Catholic University, Rome; Unit of Molecular Medicine (Dr. D'Amico), Bambin Gesù Pediatric Hospital, Rome; Università degli Studi Milano Bicocca (Dr. Marelli), Milan; and Department of Experimental Medicine and Pathology (Dr. Dallapiccola), University "La Sapienza," Rome, Italy.

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Address correspondence and reprint requests to Dr. Maria Teresa Pellicchia, Department of Neurological Sciences, University "Federico II," Via Pansini 5, 80131, Naples, Italy; e-mail: pellec3@hotmail.com

Table Clinical and genetic findings in 16 patients with pantothenate kinase-associated neurodegeneration (PKAN)

Patient	Sex/ age, y	Mutation (exon)	Result	Age at onset, y	Signs at onset	Dyst	Dysar	Dysph	Rig	Trem	CS signs	Cogn decl	Psych	Gait/loss amb	PR OA	Acan	Clinical groups
4	M/12	<i>T883G (3)*</i>	<i>Y295D</i>	1	Pm delay	Gen	+	+	+	—	+	—	—	+/10	PR	+	C
10	M/14	<i>493_494del (2)</i> not found	<i>fs180X</i> —	1	Writ/handl	Gen	+	+	+	—	+	+	—	+/9	—	—	C
13	F/19	<i>G740C (3)</i> <i>G1231A (6)</i>	<i>R247P</i> <i>G411R</i>	6	Writ/handl	Gen	+	+	+	—	—	+	Behav	+/10	OA	—	C
14	F/13	<i>G1231A (6)</i> <i>C1253T (6)</i>	<i>G411R</i> <i>T418M</i>	10	Writ/handl	Gen	+	+	—	—	—	—	—	+/10	OA	—	C
16	F/14	<i>G1231A (6)</i> not found	<i>G411R</i> —	9	Gait	Gen	+	+	+	—	+	+	—	+/9	—	—	C
1	M/26	<i>C460T (2)</i> <i>A635G (2)</i>	<i>R154W</i> <i>E212G</i>	11	Motor tics, behav	—	+	—	+	—	+	+	Behav, OCD, hyper, motor-vocal tics	+/-	—	—	A
8	M/51	<i>C526T (2)*</i>	<i>R176C</i>	12	Gait	Gen	+	+	—	—	+	+	—	+/-	—	—	A
9	F/43	<i>C526T (2)*</i>	<i>R176C</i>	12	Gait	LL	+	—	—	—	+	+	—	+/-	—	—	A
15	M/33	<i>G740C (3)*</i>	<i>R247P</i>	16	Motor tics, behav, gait	Gen	+	—	+	—	+	+	Behav, OCD, motor tics	+/-	PR	—	A
2	F/22	<i>IVS1+5G>C (1)</i> <i>A635G (2)</i>	<i>splice</i> <i>E212G</i>	2	Gait	OM, T	+	—	+	—	+	—	OCD, hyper	+/-	—	—	I
5	F/42	<i>G461A (2)</i> <i>850 851del (3)</i>	<i>R154Q</i> <i>fs284X</i>	5	Gait	Gen	+	—	+	—	+	—	Hyper	+/32	PR	+	I
6	M/23	<i>C526T (2)*</i>	<i>R176C</i>	5	Behav	Gen	+	—	—	+	+	+	Behav, OCD	—	NPR	—	I
11	M/42	<i>G740C (3)*</i>	<i>R247P</i>	17	Writ/handl	Gen	+	+	+	+	+	—	Depr	+/29	—	—	I
3	F/6	<i>C1021T (4)</i> <i>G1231A (6)</i>	<i>R341X</i> <i>G411R</i>	2	Pm delay	LL	+	—	—	—	+	+	—	+/-	—	—	U
7	M/20	<i>C526T (2)*</i>	<i>R176C</i>	10	Gait	Gen	+	—	+	—	+	+	OCD	+/-	—	—	U
12	M/22	<i>G1231A (6)</i> <i>C1253T (6)</i>	<i>G411R</i> <i>T418M</i>	14	Writ/handl	Gen	+	—	—	—	+	—	—	—	—	—	U

The asterisks indicate homozygous mutations; novel mutations are in italics.

dyst = dystonia; dysar = dysarthria; dysph = dysphonia; rig = rigidity; trem = tremor; CS signs = corticospinal signs; cogn decl = cognitive decline; psych = psychiatric problems; gait = gait difficulties; loss amb = age at loss of ambulation (years); PR = pigmentary retinopathy; OA = optic atrophy; acan = acanthocytosis; pm = psychomotor; gen = generalized; C = classic PKAN; fs = frameshift mutation; writ/handl = difficulty in writing or handling objects; behav = behavioral disturbances; OCD = obsessive-compulsive disorder; hyper = hyperactivity; A = atypical PKAN; LL = lower limbs; OM = oromandibular; T = trunk; I = intermediate PKAN; depr = depression; NPR = nonpigmentary retinopathy; U = undefined (disease duration too short to assess the rate of progression and loss of independent ambulation).

(nos. 2, 5, 6), or with onset in the second decade but rapid progression and ambulation loss few years after onset (no. 11). The remaining three patients (nos. 3, 7, 12) had a disease duration too short (4 to 10 years) to predict the severity of progression.

Mutation analysis. We identified mutations in the *PANK2* gene in all patients, confirming the clinical and neuroradiologic diagnosis of PKAN. Mutations included missense, nonsense, small deletions, and splice site mutations. Out of 12 mutations, 5 (*R154Q*, *Y295D*, *R247P*, *fs284X*, and *IVS1 + 5G>C*) are novel and were not detected in 300 control chromosomes. For *R247P* and *R154Q* mutations, different amino acid changes at the same resi-

due were previously reported.^{1,3} Seven patients from six families carried homozygous mutations, although none of the families were aware of parental consanguinity. Seven patients were compound heterozygotes, while in two patients only one mutation could be identified. Five mutations were found in more than one patient. Of these, only *G411R* occurred in patients from all three countries. There was no clear correlation between *PANK2* mutations and PKAN phenotypes.

Discussion. The identification of the *PANK2* gene and subsequent testing of a large number of patients

with NBIA allowed defining a novel clinical entity, PKAN, which was further subclassified into classic and atypical PKAN.³ Yet phenotypic heterogeneity is wide and some patients have been described not falling within either of these two categories. Two siblings had onset in the second decade with stuttering and then evolved to a clinical picture of pure akinesia,⁶ while another patient had onset in the fourth decade with dysarthria and bulbar involvement resembling bulbar amyotrophic lateral sclerosis.⁷ Other peculiar presentations included early-onset parkinsonism and severe intermittent dystonia.¹

Of our 16 patients with PKAN, only nine could be clearly classified as classic or atypical PKAN. Two patients (nos. 1, 15) presented in the first or second decade with motor tics and obsessive-compulsive behavior suggestive of Tourette syndrome, which still represented the main clinical features after several years, although additional clinical signs emerged over time. None of them had lost the ability to walk 16 to 18 years after onset. Patients with NBIA and similar phenotypes at onset have been described but *PANK2* molecular testing was not available at the time.⁸⁻¹⁰ Cases with stuttering and facial tics have been reported; however, further details are unknown.¹

In our patients, the frequencies of major neurologic features, such as extrapyramidal involvement and gait abnormalities, were in line with those previously reported,^{3,4} while corticospinal tract involvement was much more common in our series (87% vs 18 to 25%). The frequency of pigmentary retinopathy in our classic PKAN cases was only 20%, much lower than the previously reported value of 68%.³ Psychiatric disturbances, namely obsessive-compulsive traits, behavioral abnormalities, and depression, were found in about half of our patients and seemed to correlate more with a slow disease progression than with age at onset. Psychiatric symptoms had been previously reported in about 30% of atypical cases, and it was suggested that these could be a marker of atypical disease together with speech-related symptoms. All our patients had the eye of the tiger sign,

confirming the striking correlation between this neuroimaging finding and *PANK2* mutations.

We could not draw genotype–phenotype correlations and were not able to confirm that classic PKAN is usually associated with truncating mutations.³ Three of five classic cases had missense mutations while in the remaining two cases only one mutation could be found, the fs180X truncating mutation (no. 10) and the G411R missense mutation (no. 16). G411R was already found as the single heterozygous mutation in several patients and was suspected to act in a semidominant fashion.^{1,3} Only 12 mutations were identified in 30 mutated alleles and seven patients had homozygous mutations without any obvious consanguinity. These findings suggest that a limited pool of mutations accounts for the vast majority of PKAN cases in different countries.

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