Neurodegeneration with brain iron accumulation

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Abstract

Neurodegeneration with brain iron accumulation (NBIA) describes a group of progressive extrapyramidal disorders with radiographic evidence of focal iron accumulation in the brain, usually in the basal ganglia. Patients previously diagnosed with Hallervorden-Spatz syndrome fall into this category. Mutations in the PANK2 gene account for the majority of NBIA cases and cause an autosomal recessive inborn error of coenzyme A metabolism called pantothenate kinase-associated neurodegeneration (PKAN). PKAN is characterized by dystonia and pigmentary retinopathy in children or speech and neuropsychiatric disorders in adults. In addition, a specific pattern on brain MRI, called the eye-of-the-tiger sign, is virtually pathognomonic for the disease. Pantothenate kinase is essential to coenzyme A biosynthesis, and the PANK2 protein is targeted to the mitochondria. Hypotheses of PKAN pathogenesis are based on the predictions of tissue-specific coenzyme A deficiency and the accumulation of cysteine-containing substrates. Identification of the major NBIA gene has led to more accurate clinical delineation of the diseases that comprise this group, a molecular diagnostic test for PKAN, and hypotheses for treatment.

Keywords

NBIA; Hallervorden-Spatz syndrome; pantothenate kinase-associated neurodegeneration; PKAN; PANK2

Introduction

Even prior to the discovery of a major causative gene for Hallervorden-Spatz syndrome, the diagnosis was suspected to encompass several related disorders characterized by basal ganglia iron accumulation. In 2001, the genetic basis for the majority of HSS cases was revealed through studying a subset of families with a clear and consistent phenotype. This form of HSS was named pantothenate kinase-associated neurodegeneration, or PKAN, after causative mutations in the pantothenate kinase 2 gene were found [68]. In the five years following the gene discovery, significant progress in delineating the clinical and molecular aspects of this disorder have contributed to the development of accurate diagnostic tests, more effective treatment options, and ongoing studies of rational therapies.

Background

Hallervorden-Spatz syndrome was originally described by Julius Hallervorden and Hugo Spatz in 1922 [18]. For decades most patients with radiographic or pathologic evidence of high iron in the basal ganglia were given this diagnosis, regardless of the observation of marked clinical heterogeneity. This category likely comprised several distinct disorders, but only in recent

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years did the delineation of clinical features and the accompanying discovery of a major gene [68] fuel the reclassification of this syndrome.

Within the pool of patients with HSS, a specific phenotype consisting of movement abnormalities, pigmentary retinopathy, and autosomal recessive inheritance was distinguished. Taylor et al [57] mapped the gene for this specific subtype of HSS to chromosome 20p13 using a large consanguineous family. Mutations in the gene encoding pantothenate kinase 2 (*PANK2*) were found in this family and in additional families with a similar phenotype [68]. In still other families diagnosed with HSS, *PANK2* mutations were not found, and linkage to 20p13 was excluded.

The first subtype of HSS, identified by both *PANK2* mutations and specific clinical and radiographic findings, was designated as pantothenate kinase-associated neurodegeneration, or PKAN [68]. In 2002 new disease nomenclature was proposed [22]. This was motivated by concern over the objectionable wartime activities of Hallervorden and Spatz, German neuropathologists who were involved in active euthanasia of "mental defectives" during World War II [49], with the new nomenclature based on the gene identity. Neurodegeneration with brain iron accumulation (NBIA) describes the group of progressive extrapyramidal disorders in which there is radiographic evidence of focal iron accumulation in the brain, usually in the basal ganglia. Patients previously diagnosed with Hallervorden-Spatz syndrome fall into this category, including those now known to have PKAN. Others for whom PKAN has been ruled out remain in this category and have NBIA, but the causative gene or genes have not yet been identified. In addition to PKAN, NBIA includes neuroferritinopathy, caused by mutations in the ferritin light chain [8] and aceruloplasminemia, caused by mutations in the ceruloplasmin gene [15].

PKAN accounts for 50-70% of cases of NBIA [23] and can be distinguished based on clinical, radiographic and molecular features. Historically, another rare variant called hypoprebetalipoproteinemia, acanthocytosis, retinopathy, and pallidal degeneration (HARP) was thought to be a clinically distinct form of NBIA. Unlike typical NBIA, HARP patients have decreased or absent prebetalipoproteins [24,40]. Once the PKAN genotype and phenotype were delineated, the classification of HARP as a separate disease entity came into question. In 2002 a *PANK2* mutation was found in the original HARP patient [7] and it is no longer distinguished from PKAN.

Clinical features

The clinical phenotype of PKAN generally falls into one of two categories based on the age of onset, presenting signs and symptoms, and the rate of progression. As is often the case, a spectrum of severity exists and some patients fall between the designated categories. Classic PKAN, however, is surprisingly homogeneous [23]. It is characterized by early onset, usually before six years of age, and rapid progression. The mean age of onset is three to four years based on data from our patient registry. Children commonly present with gait abnormalities and have often been considered clumsy prior to identification of obvious problems. Their main clinical features are dystonia, dysarthria, and rigidity, with corticospinal tract involvement resulting in spasticity, hyperreflexia, and extensor toe signs. Affected children generally lose the ability to ambulate 10 to 15 years after disease onset. Some children have developmental delay, which is primarily motor but sometimes global. Classical PKAN progresses at a nonuniform rate for reasons that are unclear. Patients tend to experience episodes of rapid decline for one to two months, interspersed with longer periods of relative stability. Infection or other common causes of catabolic stress do no seem to precipitate these episodes.

Atypical PKAN comprises a more heterogeneous group. By definition, onset is later and progression is slower. Based on patient registry data, the average age of onset is 13 to 14 years.

Speech difficulty, including palilalia and dysarthria, was either the sole presenting feature or an early sign of disease in nine of 23 atypical patients in one study [23]. Psychiatric symptoms are significantly more prominent than in classic disease [35,55,64] and include depression, emotional lability, impulsivity, and violent outbursts. These symptoms also occur early and can delay diagnosis. Motor involvement is also prominent in atypical disease, but it is generally less severe and has a slower progression. Patients do eventually develop gait disturbances due to dystonia, rigidity, and spasticity with loss of independent ambulation occurring 15 to 40 years after onset. Unique to atypical disease are repetitive actions, freezing, and palilalia, which reflect the underlying basal ganglia involvement [5,4]. Freezing during ambulation, particularly when turning corners or when surface variations are encountered, is remarkably similar to freezing in Parkinson disease [16].

Diagnostic criteria for HSS were first proposed by Dooling et al. [10] based on 42 cases that formed a distinct clinicopathological entity. Swaiman revised these after brain MRI became a valuable diagnostic tool [53]. Following the identification of *PANK2* and the development of a molecular diagnostic test, we proposed revised PKAN obligate and corroborative features (table I) that may be useful for determining which patients may benefit from for molecular testing [22].

Since PKAN cases can now be reliably distinguished from other forms of NBIA, the ophthalmological phenotype has been better characterized. Pigmentary retinal degeneration was previously recognized as a prominent symptom in patients with early-onset NBIA [10, 33,38,44]. Sixty-eight percent of classic PKAN patients had clinical or electroretinographic evidence of retinopathy in a study of 66 individuals, while it was less common in atypical patients [23].

We previously reported neuro-ophthalmological examinations and electroretinograms in a cohort of 16 PKAN patients who covered the spectrum from classic to atypical disease [12]. Although only four patients showed a pigmentary retinopathy, 11 had abnormal electroretinography. Ten of the patients had neuro-ophthalmologic exams and of these, eight had sluggish pupillary reactions with sectoral iris paralysis and patchy loss of the pupillary ruff similar to bilateral Adie's pupils. This was a completely novel finding. Ocular motility studies showed hypometric and slowed saccadic pursuits in all patients studied and eight of 10 had hypometric and slowed vertical saccades. These findings suggest that midbrain deposition of iron in or near the substantia nigra may induce a proximal degeneration in the fibers leading to the iris and cause pupillary changes similar to Adie's pupils. Iron accumulation in the brainstem would also explain the majority of the other neuro-ophthalmologic findings in the study, as opposed to local iron accumulation in the short cilliary nerves, although the latter is also a possibility [12].

During the same study we evaluated intellectual and adaptive behavior functioning (Freeman, unpublished data). Historically, NBIA has been associated with intellectual impairments [10, 53]. These observations were mainly based on clinical impression and studies of one or two patients using specific neuropsychological tools. The severe limitations of movement and speech that occur with disease progression likely impact both formal testing results and informal observations and make intellectual function difficult to assess. By carefully choosing psychometric tools that required limited or no verbal and motor skills, we hoped to more thoroughly determine intellectual functioning in PKAN.

In this study of a large sample of individuals with PKAN, we found a varied cognitive phenotype. Estimates of general cognitive functioning, using either the Wechsler Abbreviated Scale of Intelligence [58] or Leiter International Performance Scale – Revised [43], indicated global intellectual functioning ranging from high average to markedly below average. Similar

diversity in adaptive behavior functioning was found. A significant negative correlation was detected when IQ scores by either test were compared against a global disease severity scale, meaning that those most severely affected by PKAN scored the lowest (Freeman, unpublished data).

Neuroimaging

Magnetic resonance imaging (MRI) has contributed to our understanding of PKAN and is particularly useful in separating PKAN cases from other forms of NBIA. Brain MRI has become a standard component in the diagnostic evaluation for this group of disorders. Newer neuroimaging technologies, including magnetic resonance spectroscopy (MRS), may also prove useful, although their utility has not yet been established.

The primary radiographic change associated with NBIA is high iron in the basal ganglia, appreciated as hypointense lesions in the globus pallidus and substantia nigra pars reticulata on T2-weighted images [11,36,48,56]. In PKAN, a central region of hyperintensity with surrounding hypointensity of the globus pallidus on T2-weighted images is virtually pathognomonic for this form of the disease (Figure 1). The hyperintense central region indicates a primary tissue insult leading to edema or necrosis, while the surrounding hypointense region indicates high iron, which we believe to accumulate as a secondary effect.

To date our research group has found an absolute correlation between this pattern, called the eye-of-the-tiger sign, and the presence of mutations in *PANK2* [23]. In presymptomatic patients the hyperintense lesions predominate. With disease progression, the hypointensities appear and eventually dominate [21]. There is some evidence that the central hyperintensity continues to diminish and may ultimately disappear [3,13,23]. For this reason it is prudent to perform *PANK2* mutation screening on NBIA patients without the eye-of-the-tiger sign to more definitively rule out the PKAN form of the disease.

Magnetic resonance spectroscopy (MRS) has been of interest for PKAN because specific biochemical perturbations are likely to occur based on current knowledge of the disease. Sener reported results of proton MRS in a 15 year-old PKAN patient. Markedly decreased levels of N-acetylaspartate were found in the globus pallidus, thought to represent neuronal loss in this region [47]. Hajek et al. had similar findings in a study of three PKAN patients with NAA levels decreased relative to controls [17]. MR relaxometry was also used to estimate the concentration of iron in the globus pallidus, which they found to be approximately three times greater than in controls. Efforts to quantify analytes using MRS and to characterize regional iron deposition across the globus pallidus are underway in the principle investigator's laboratory.

Neuropathology

The heterogeneity of the entities grouped as NBIA has been a limiting factor in the evaluation of neuropathological data [20]. Since the recognition of PKAN as a genetically homogeneous disease, however, it has been possible to retrospectively identify reports of patients likely to have had PKAN based on clinical and radiographic data. This has supported the delineation of neuropathologic findings in PKAN.

NBIA was a postmortem diagnosis prior to the availability of MRI as a diagnostic tool. On gross sectioning, the globus pallidus and reticular zone of the substantia nigra show rust-brown pigmentation mainly composed of iron [19]. Routine iron stains detect the metal mainly in the microglia and macrophages, but scattered neurons are also reactive. Iron is also detected extracellularly, frequently concentrated around blood vessels.

Iron accumulates abnormally in the brain regions that are typically iron-rich. In normal human brain, iron is regionally distributed and highest in the globus pallidus, substantia nigra pars reticulata, dentate nucleus, and red nucleus [25,26]. In PKAN the accumulation of iron is specific to the globus pallidus and substantia nigra; a global increase in brain iron is not seen [50]. Older neuropathological studies of tissue from NBIA patients showed that the globus pallidus and substantia nigra contain approximately three times the normal amount of iron, yet iron content is normal in other regions of the brain and in the retina and optic nerve [10,31, 61,62]. A more recent estimate specific to PKAN patients found a similar three-fold increase using T2 relaxometry [17]. Systemic iron metabolism is normal as well [52,55]. Iron-uptake studies in NBIA patients suggest that accumulation of iron in the basal ganglia is secondary to increased iron uptake with normal turnover [52,55,61].

In regions of massive iron accumulation, spheroid bodies, many positive for iron, are also seen [30]. Axonal spheroids are posited to represent swollen or bloated axons, possibly secondary to defects in axonal transport. They are seen in normal aging brains and in a number of other neurodegenerative disorders, including the neuroaxonal dystrophies. Other neuropathologic findings include demyelination, neuronal loss, and gliosis, which occur predominantly within the globus pallidus and substantia nigra, where focal, symmetrical destruction may be grossly evident. In addition to iron deposition and spheroid formation in the brains of people with NBIA, ceroid-lipfuscin and neuromelanin accumulate both intra and extraneuronally.

Numerous papers on NBIA report the presence of Lewy bodies and neurofibrillary tangles with accumulations of tau and alpha-synuclein [2,14,37-39,45,51,60,63]. Yet, not a single case in the literature is likely to have been PKAN, based on the clinical and radiographic information available. This observation underscores the need to carefully reexamine our current knowledge of the neuropathology in NBIA in order to determine what can be applied to PKAN. Confirmed PKAN brain tissue has only recently become available and plans for further studies are underway.

Nonspecific systemic cytologic abnormalities reported in NBIA include bone marrow macrophages containing ceroid-lipofuscin and circulating lymphocytes with vacuoles and cytosomic inclusion bodies, similar to those seen in ceroid-lipofuscin storage disorders [52]. The patients in whom these "sea-blue histiocytes" were reported likely represent the non-PKAN form of NBIA. Acanthocytes have been reported in a subset of patients with NBIA [24,33,44,54,59], many of whom probably had PKAN. Lipofuscin and acanthocytes can both result from lipid peroxidation, a process stimulated by iron [9,41]. These cytologic abnormalities, while not prominent in the pathology of this disorder, may shed light on the underlying pathophysiology of PKAN.

Since retinopathy is characteristic of PKAN, the pathology literature on this feature is likely to be specific to this disorder. Ophthalmoscopic examination in patients diagnosed with NBIA showed prominent bone spicule pigmentation and accumulation beneath the sensory retina of numerous yellowish-white globular masses [33,38,44,59]. These findings were recently confirmed in a clinical study by Egan et al. of 10 patients with confirmed PKAN. Ophthalmoscopic examination showed bone spicule accumulation in four of 10 patients of varying age and disease severity. These four also had severe rod and cone function abnormalities as measured by electroretinogram (ERG). Several additional patients with normal ophthalmoscopic exams had mild or moderate ERG changes [12]. It is unknown whether early-stage patients such as these may have sub-clinical changes detectable by pathologic examination.

On light microscopic study, there is a total loss of the outer segment of the photoreceptor cells and near total loss of the inner segment. The outer nuclear and outer plexiform layers are thinned

or absent. The retinal pigment epithelium comprises a population of enlarged epithelial cells that contain both individual pigment granules and large, round pigment aggregates, a change seen primarily in the equatorial and pre-equatorial regions. Similar pigment-laden cells are present in the outer retinal layers peripheral to the perimacular area. The pigment granule clusters are melanolipofuscin complexes and represent the pathologic correlate of the yellowish-white globular masses seen on funduscopic examination [59]. There is migration of the retinal pigment epithelium into the inner retinal layers in perivascular regions [33], which likely accounts for the ophthalmoscopic appearance of bone spicule pigmentation. No stainable iron is seen in any part of the eye; however, focal axonal degeneration and cytoid bodies (spheroids) are noted [59]. Abnormal accumulation of lipfuscin is reported in conjunctival fibroblasts, retinal vessel pericytes, and macrophages [33]. Glial proliferation occurs throughout the retina and around blood vessels.

Since male mice defective for Pank2 have azoospermia [32], we analyzed semen samples from two adult males with atypical PKAN as part of our recent clinical study. Both sperm samples were found to have similar morphologic and motility abnormalities. These included an increased frequency of tail abnormalities and amorphous sperm heads. The percentages of motile sperm with normal morphology were reduced relative to the WHO standards [65]. Prior to knowledge of azoospermia in the mice, fertility had not been questioned or explored in the PKAN population because most patients do not reproduce due to the severity of their movement disorder and shortened lifespan.

Genetics and abnormal gene function

PKAN is an autosomal recessive inborn error of coenzyme A metabolism. It is caused by mutations in *PANK2*, the gene encoding pantothenate kinase 2 [68]. Pantothenate kinase is a key regulatory enzyme in the biosynthesis of coenzyme A (CoA). CoA is critical to energy metabolism, fatty acid synthesis and degradation, and neurotransmitter and glutathione metabolism, among others. In humans, three additional genes are predicted to encode related proteins, based on homology studies, and are designated *PANK1* [29,68], *PANK3*, and *PANK4* [68]. The degree of genomic redundancy between these four genes underscores the importance of the CoA biosynthetic pathway for survival. In contrast to the other pantothenate kinases, only *PANK2* is targeted to the mitochondria [28]. The PANK2 gene encodes a 1.85-kb transcript that is derived from seven exons spanning just over 35 kb of genomic DNA. 5′ RACE and EST data provide evidence for at least five initiating exons, but only one of these, exon 1C, has an open reading frame with potential initiation codons that splice in-frame to exon two [68].

Null mutations lead to early-onset, rapidly progressive PKAN. More mild disease with a later onset generally occurs in patients with mutations that preserve partial enzyme function. Deleterious mutations have been found in all seven exons of the gene; several splice site mutations have also been identified. Two common mutations account for about one-third of disease alleles. The most frequent is 1231 G->A, which accounts for 25% of disease alleles. Patients homozygous for this mutation have classic PKAN. 1253 C->T accounts for an additional 8%. The remainder of identified mutations are generally private to each affected family [23]. Molecular diagnostic testing is currently available at two laboratories in the U.S. (http://www.genetests.org) and in several other countries. The authors maintain a database of all known *PANK2* sequence variants.

PKAN is the first known inborn error of coenzyme A metabolism (figure 2). Mutations in *PANK2* are predicted to result in deficient enzyme activity leading to product deficit. Secondary metabolite accumulation may also contribute to disease pathogenesis. Since pantothenate is a water-soluble compound with no known toxicity, its accumulation is not likely to contribute

to disease. Two other substrates for pantothenate kinase, N-pantothenoyl-cysteine and pantetheine, are predicted to accumulate and are more likely to cause toxicity to cells.

Based on the association of PANK2 with mitochondria [28], we hypothesize that defects in the protein lead to CoA deficiency and a range of metabolic consequences, the most relevant being energy and lipid dyshomeostasis. In this model, insufficient energy production leads to the generation of reactive oxygen species, which damage membrane phospholipids via peroxidation and lead to apoptosis. The basal ganglia and retina are among the tissues most sensitive to oxidative stress, and their destruction in PKAN may occur due to either increased oxidative damage or a defect in lipid metabolism. We also speculate that the cardinal feature of basal ganglia iron deposition may be explained by the accumulation of cysteine, which chelates iron.

Based on predictions of the metabolic impact of defective PANK2 and on the observation of elevated brain levels of cysteine in patients with probable PKAN [42], we proposed a hypothesis for iron accumulation [68]. In a normal brain, nonheme iron accumulates regionally and is highest in the medial globus pallidus and the substantia nigra pars reticulata, the two regions most severely affected in PKAN. Phosphopantothenate, the product of pantothenate kinase, normally condenses with cysteine in the next step in CoA synthesis. In PKAN, phosphopantothenate is deficient, theoretically leading to cysteine accumulation. N-pantothenoyl-cysteine and pantetheine, also substrates of phosphorylation by pantothenate kinase and both containing cysteine, are also predicted to accumulate. A high cysteine concentration has been found in the globus pallidus in PKAN [42], and cysteine effectively binds iron. Cysteine, itself cytotoxic, undergoes rapid auto-oxidation in the presence of iron, which results in free radical production [67]. Furthermore, iron-induced lipid peroxidation, a likely mechanism of secondary pathogenesis in PKAN [41,59], is enhanced by free cysteine [46,66], further stressing the cell that is predicted to have an impaired capacity to repair membranes [27]. In contrast to PKAN, but also involving basal ganglia iron deposition and pigmentary retinopathy, aceruloplasminemia involves a primary defect in iron homeostasis, which leads to the generation of highly reactive oxygen species and to secondary lipid peroxidation [34]. Thus, iron dyshomeostasis is very likely to contribute to disease pathogenesis in PKAN.

Animal models

A *Drosophila melanogaster* hypomorphic mutant with deficient pantothenate kinase function was identified over a decade ago. The mutant has a cell division defect leading to sterility, for which it has primarily been studied [1]. The fly is also described as uncoordinated, resulting in impaired ability to climb, fly, and mate [1]. This model is currently being utilized to test potential drug treatments for PKAN.

A murine model for PKAN was developed by our team and our collaborators at the University of California San Francisco. We expected the knock-out mouse to be a model for the human disease given the high degree of identity of *PANK2* gene homologs humans and mice along with evolutionary conservation of the entire pantothenate kinase gene family. To some extent this has held true, although the mice do not display most of the hallmark neurologic features of PKAN.

Over time, the homozygous mice manifest retinal degeneration, as evidenced by electroretinography, light microscopy, and pupillometry response. Homozygous male mutants are infertile due to azoospermia, a condition has not been previously appreciated in humans, although the *fumble* flies are also sterile. Arrest occurs during spermiogenesis, with complete absence of elongating and mature spermatids [32]. Female murine reproduction is also impaired, possibly due to in utero effects of maternal Pank2 protein deficiency. In contrast to

humans with PKAN, no changes have been observed in the basal ganglia of the mice by MRI or histological examination, nor are there signs of dystonia, even after following the mice for over one year. The knock-out mice have a 20% decrease in weight compared to their wild-type littermates, but dysphagia is not present [32]. Since the mutant mice failed to exhibit the movement disorder and pathology of the globus pallidus characteristic of PKAN, we have embarked on several strategies to provoke the neurological phenotype in the mutant animals.

Treatment

Pharmacologic and surgical interventions are aimed at palliation of symptoms. For many of the interventions that offer improvement of clinical symptoms, the period of benefit is limited. Even with these limitations, it is possible for clinicians to work closely with families and make periodic adjustments to maintain as high a quality of life as possible. Baclofen and trihexyphenidyl remain the most effective drugs for disabling dystonia and spasticity. As a rule, patients with PKAN do not benefit from L-DOPA, although patients with non-PKAN NBIA and parkinsonism will sometimes respond to this treatment. Botulinum toxin can be helpful for many patients, especially those whose quality of life is improved by treating a limited body region. For example, injections in the facial or orobucculingual muscles can greatly improve speech and eating abilities.

When oral baclofen is no longer able to adequately control the movement disorder, placement of a continuous intrathecal baclofen pump may be considered. The risks associated with a baclofen pump, including the need to refill the pump periodically and the potential for programming errors, often make it a treatment that is elected only after other options have been exhausted. Deep brain stimulation (DBS) is also an option for relieving some symptoms. Anecdotal cases suggest that the benefit from these more invasive treatments is relatively short-lived, but they can provide short-term relief to patients experiencing extreme dystonia and spasticity. A recent report by Castelnau et al [6] on the use of DBS in six PKAN patients suggests that it may hold more promise than previously recognized. The patients treated with DBS showed overall improvements in writing, speech, walking, and global measures of motor skills. The length of follow-up time, however, varied from six to 42 months at the time of publication [6]. For those who decide to undergo DBS, the chance for a good outcome will be increased by working with an experienced DBS team that specializes in this procedure.

Families of patients with PKAN frequently also try a number of vitamins and other dietary supplements in a quest to improve function or slow disease progression. None of these have been studied formally in a clinical setting and only anecdotal information is available on their various effects or lack thereof. Based on the disease mechanism, we speculated early on that loading with pantothenate may drive any residual enzyme activity by substrate overload. This would probably benefit only those patients with low levels of enzyme, who are likely to be later onset or "atypical" patients. However, since there is evidence that high levels can be taken without any apparent toxicity, we have suggested a trial of high dose B5 in patients with PKAN. Some patients have reported improvements in speech and balance, though no trials of this compound have yet been developed.

Conclusions

The discovery of the *PANK2* gene and ensuing investigations revealed the first known inborn error of CoA metabolism. The involvement of the CoA pathway in PKAN, a neurodegenerative disease, suggests potential involvement in other forms of NBIA and more common disorders of oxidative stress, such as Parkinson disease. Since the gene discovery, the clinical delineation of PKAN has been established, animal models have been developed and exploration of novel therapeutics is underway.

References

1. Afshar K, Gonczy P, DiNardo S, Wasserman SA. Fumble encodes a pantothenate kinase homolog required for proper mitosis and meiosis in Drosophila melanogaster. Genetics 2001;157:1267–1276. [PubMed: 11238410]

2. Arawaka S, Saito Y, Murayama S, Mori H. Lewy body in neurodegeneration with brain iron accumulation type 1 is immunoreactive for alpha-synuclein. Neurology 1998;51:887–889. [PubMed: 9748051]

3. Baumeister FA, Auer DP, Hortnagel K, Freisinger P, Meitinger T. The eye-of-the-tiger sign is not a reliable disease marker for Hallervorden-Spatz syndrome. Neuropediatrics 2005;36:221–222. [PubMed: 15944911]

4. Benke T, Hohenstein C, Poewe W, Butterworth B. Repetitive speech phenomena in Parkinson's disease. J Neurol Neurosurg Psychiatry 2000;69:319–324. [PubMed: 10945806]

5. Benke T, Butterworth B. Palilalia and repetitive speech: two case studies. Brain Lang 2001;78:62–81. [PubMed: 11412016]

6. Castelnau P, Cif L, Valente EM, Vayssiere N, Hemm S, Gannau A, Digiorgio A, Coubes P. Pallidal stimulation improves pantothenate kinase-associated neurodegeneration. Ann Neurol 2005;57:738–741. [PubMed: 15852393]

7. Ching KH, Westaway SK, Gitschier J, Higgins JJ, Hayflick SJ. HARP syndrome is allelic with pantothenate kinase-associated neurodegeneration. Neurology 2002;58:1673–1674. [PubMed: 12058097]

8. Curtis AR, Fey C, Morris CM, Bindoff LA, Ince PG, Chinnery PF, Coulthard A, Jackson MJ, Jackson AP, McHale DP, Hay D, Barker WA, Markham AF, Bates D, Curtis A, Burn J. Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. Nat Genet 2001;28:350–354. [PubMed: 11438811]

9. Defendini R, Markesbery WR, Mastri AR, Duffy PE. Hallervorden-Spatz disease and infantile neuroaxonal dystrophy. Ultrastructural observations, anatomical pathology and nosology. J Neurol Sci 1973;20:7–23. [PubMed: 4355342]

10. Dooling EC, Schoene WC, Richardson EP Jr. Hallervorden-Spatz syndrome. Archives of Neurology 1974;30:70–83. [PubMed: 4808495]

11. Drayer B. Magnetic resonance imaging and brain iron: implications in the diagnosis and pathochemistry of movement disorders and dementia. Barrow Neurol Inst Q 1987:15–30.

12. Egan RA, Weleber RG, Hogarth P, Gregory A, Coryell J, Westaway SK, Gitschier J, Das S, Hayflick SJ. Neuro-ophthalmologic and electroretinographic findings in pantothenate kinase-associated neurodegeneration (formerly Hallervorden-Spatz syndrome). Am J Ophthalmol 2005;140:267–274. [PubMed: 16023068]

13. Gallucci M, Cardona F, Arachi M, Splendiani A, Bozzao A, Passariello R. Follow-up MR studies in Hallervorden-Spatz disease. J Comput Assist Tomogr 1990;14:118–120. [PubMed: 2298974]

14. Galvin JE, Giasson B, Hurtig HI, Lee VM, Trojanowski JQ. Neurodegeneration with brain iron accumulation, type 1 is characterized by alpha-, beta-, and gamma-synuclein neuropathology. Am J Pathol 2000;157:361–368. [PubMed: 10934140]

15. Gitlin JD. Aceruloplasminemia. Pediatr Res 1998;44:271–276. [PubMed: 9727700]

16. Guimaraes J, Santos JV. Generalized freezing in Hallervorden-Spatz syndrome: case report. Eur J Neurol 1999;6:509–513. [PubMed: 10362909]

17. Hajek M, Adamovicova M, Herynek V, Skoch A, Jiru F, Krepelova A, Dezortova M. MR relaxometry and 1H MR spectroscopy for the determination of iron and metabolite concentrations in PKAN patients. Eur Radiol 2005;15:1060–1068. [PubMed: 15565311]

18. Hallervorden J, Spatz H. Eigenartige Erkrankung im extrapyramidalen System mit besonderer Beteiligung des Globus pallidus und der Substantia nigra. Z Ges Neurol Psychiatr 1922;79:254–302.

19. Hallervorden J. Uber eine familiare Erkrankung im extrapyramidalen System. Dtsch Z Nervenheilkd 1924:204–210.

20. Halliday W. The nosology of Hallervorden-Spatz disease. J Neurol Sci 1995;134:84–91. [PubMed: 8847549]

21. Hayflick SJ, Penzien JM, Michl W, Sharif UM, Rosman NP, Wheeler PG. Cranial MRI changes may precede symptoms in Hallervorden-Spatz syndrome. Pediatr Neurol 2001;25:166–169. [PubMed: 11551748]

22. Hayflick, SJ. Pantothenate kinase-associated neurodegeneration. In: Pulst, S., editor. Genetics of Movement Disorders. Academic Press; San Diego: 2002.

23. Hayflick SJ, Westaway SK, Levinson B, Zhou B, Johnson MA, Ching KH, Gitschier J. Genetic, clinical and radiographic delineation of Hallervorden-Spatz syndrome. N Engl J Med 2003;348:33–40. [PubMed: 12510040]

24. iggins JJ, Patterson MC, Papadopoulos NM, Brady RO, Pentchev PG, Barton NW. Hypoprebetalipoproteinemia, acanthocytosis, retinitis pigmentosa, and pallidal degeneration (HARP syndrome). Neurology 1992;42:194–198. [PubMed: 1734303]

25. Hill JM, Switzer RC III. The regional distribution and cellular localization of iron in the rat brain. Neuroscience 1984;11:595–603. [PubMed: 6717804]

26. Hill, JM. Brain Iron: Neurochemical and Behavioural Aspects. Youdim, MB., editor. Taylor & Francis; London, New York and Philadelphia: 1988.

27. Jackowski S. Cell cycle regulation of membrane phospholipid metabolism. J Biol Chem 1996;271:20219–20222. [PubMed: 8702749]

28. Johnson MA, Kuo YM, Westaway SK, Parker SM, Ching KH, Gitschier J, Hayflick SJ. Mitochondrial localization of human PANK2 and hypotheses of secondary iron accumulation in pantothenate kinase-associated neurodegeneration. Ann N Y Acad Sci 2004;1012:282–298. [PubMed: 15105273]

29. Karim VV. Human pantothenate kinase 1 (PANK1) gene: characterization of the cDNAs, structural organization and mapping of the locus to chromosome 10q23.2-23.31. Am J Hum Genet 2000;67:A984.

30. Koeppen AH, Dickson AC. Iron in the Hallervorden-Spatz syndrome. Pediatr Neurol 2001;25:148–155. [PubMed: 11551745]

31. Kornyey S. Die Stoffwechechselstorungen bei der Hallervorden-Spatzschen Krankheit. Zeitschrift Neurol 1964:178–183.

32. Kuo YM, Duncan JL, Westaway SK, Yang H, Nune G, Xu EY, Hayflick SJ, Gitschier J. Deficiency of pantothenate kinase 2 (Pank2) in mice leads to retinal degeneration and azoospermia. Hum Mol Genet 2005;14:49–57. [PubMed: 15525657]

33. Luckenbach MW, Green WR, Miller NR, Moser HW, Clark AW, Tennekoon G. Ocular clinicopathologic correlation of Hallervorden-Spatz syndrome with acanthocytosis and pigmentary retinopathy. American Journal of Ophthalmology 1983;95:369–382. [PubMed: 6829683]

34. Miyajima H, Adachi J, Tatsuno Y, Takahashi Y, Fujimoto M, Kaneko E, Gitlin JD. Increased very long-chain fatty acids in erythrocyte membranes of patients with aceruloplasminemia. Neurology 1998;50:130–136. [PubMed: 9443469]

35. Morphy MA, Feldman JA, Kilburn G. Hallervorden-Spatz disease in a psychiatric setting. J Clin Psychiatry 1989;50:66–68. [PubMed: 2914882]see comments

36. Mutoh K, Okuno T, Ito M, Nakano S, Mikawa H, Fujisawa I, Asato R. MR imaging of a group I case of Hallervorden-Spatz disease. J Comput Assist Tomogr 1988;12:851–853. [PubMed: 3170846]

37. Neumann M, Adler S, Schluter O, Kremmer E, Benecke R, Kretzschmar HA. Alpha-synuclein accumulation in a case of neurodegeneration with brain iron accumulation type 1 (NBIA-1, formerly Hallervorden-Spatz syndrome) with widespread cortical and brainstem-type Lewy bodies. Acta Neuropathol (Berl) 2000;100:568–574. [PubMed: 11045680]

38. Newell FW, Johnson RO, Huttenlocher PR. Pigmentary degeneration of the retina in the Hallervorden-Spatz syndrome. Am J Ophthalmol 1979;88:467–471. [PubMed: 573556]

39. Odawara T, Iseki E, Yagishita S, Amano N, Kosaka K, Hasegawa K, Matsuda Y, Kowa H. An autopsied case of juvenile parkinsonism and dementia, with a widespread occurrence of Lewy bodies and spheroids. Clinical Neuropathology 1992;11:131–134. [PubMed: 1319291]

40. Orrell RW, Amrolia PJ, Heald A, Cleland PG, Owen JS, Morgan-Hughes JA, Harding AE, Marsden CD. Acanthocytosis, retinitis pigmentosa, and pallidal degeneration: a report of three patients, including the second reported case with hypoprebetalipoproteinemia (HARP syndrome). Neurology 1995;45:487–492. [PubMed: 7898702]

41. Park BE, Netsky MG, Betsill WL Jr. Pathogenesis of pigment and spheroid formation in Hallervorden-Spatz syndrome and related disorders. Neurology 1975;25:1172–1178. [PubMed: 1238961]

42. Perry TL, Norman MG, Yong VW, Whiting S, Crichton JU, Hansen S, Kish SJ. Hallervorden-Spatz disease: cysteine accumulation and cysteine dioxygenase deficiency in the globus pallidus. Ann Neurol 1985;18:482–489. [PubMed: 4073841]

43. Roid, G.; Miller, L. Leiter International Performance Scale – Revised. Psychological Assessment Resources; Lutz: 1997.

44. Roth AM, Hepler RS, Mukoyama M, Cancilla PA, Foos RY. Pigmentary retinal dystrophy in Hallervorden-Spatz disease: clinicopathological report of a case. Surv Ophthalmol 1971;16:24–35.

45. Saito Y, Kawai M, Inoue K, Sasaki R, Arai H, Nanba E, Kuzuhara S, Ihara Y, Kanazawa I, Murayama S. Widespread expression of alpha-synuclein and tau immunoreactivity in Hallervorden-Spatz syndrome with protracted clinical course. J Neurol Sci 2000;177:48–59. [PubMed: 10967182]

46. Searle AJ, Willson RL. Stimulation of microsomal lipid peroxidation by iron and cysteine. Characterization and the role of free radicals. Biochem J 1983;212:549–554. [PubMed: 6882383]

47. Sener RN. Pantothenate kinase-associated neurodegeneration: MR imaging, proton MR spectroscopy, and diffusion MR imaging findings. AJNR Am J Neuroradiol 2003;24:1690–1693. [PubMed: 13679293]

48. Sethi KD, Adams RJ, Loring DW, el Gammal T. Hallervorden-Spatz syndrome: clinical and magnetic resonance imaging correlations. Ann Neurol 1988;24:692–694. [PubMed: 3202617]

49. Shevell M. Racial hygiene, active euthanasia, and Julius Hallervorden. Neurology 1992;42:2214–2219. [PubMed: 1436542]see comments

50. Spatz H. Uber des Eisenmachweiss im Gehirn besonders in Zentren des extra-pyramidalmotorischen Systems. Z Gesamte Neurol Psychiatr 1922;77:261.

51. Sugiyama H, Hainfellner JA, Schmid-Siegel B, Budka H. Neuroaxonal dystrophy combined with diffuse Lewy body disease in a young adult. Clin Neuropathol 1993;12:147–152. [PubMed: 8391956]

52. Swaiman KF, Smith SA, Trock GL, Siddiiqui AR. Sea-blue histiocytes, lymphocytic cytosomes, movement disorder and 59Fe-uptake in basal ganglia: Hallervorden-Spatz disease or ceroid storage disease with abnormal isotope scan? Neurology 1983;33:301–305. [PubMed: 6681873]

53. Swaiman KF. Hallervorden-Spatz syndrome and brain iron metabolism. Archives of Neurology 1991;48:1285–1293. [PubMed: 1845035]

54. Swisher CN, Menkes JH, Cancilla PA, Dodge PR. Coexistence of Hallervorden-Spatz disease with acanthocytosis. Trans Am Neurol Assoc 1972;97:212.

55. Szanto J, Gallyas F. A study of iron metabolism in neuropsychiatric patients. Hallervorden-Spatz disease. Arch Neurol 1966;14:438–442. [PubMed: 5948398]

56. Tanfani G, Mascalchi M, Dal Pozzo GC, Taverni N, Saia A, Trevisan C. MR imaging in a case of Hallervorden-Spatz disease. J Comput Assist Tomogr 1987;11:1057–1058. [PubMed: 3680689]

57. Taylor TD, Litt M, Kramer P, Pandolfo M, Angelini L, Nardocci N, Davis S, Pineda M, Hattori H, Flett PJ, Cilio MR, Bertini E, Hayflick SJ. Homozygosity mapping of Hallervorden-Spatz syndrome to chromosome 20p12.3-P13. Nature Genetics 1996;14:479–481. [PubMed: 8944032]

58. The Psychological Corporation. Wechsler Abbreviated Scale of Intelligence. Harcourt; San Antonio: 1999.

59. Tripathi RC, Tripathi BJ, Bauserman SC, Park JK. Clinicopathologic correlation and pathogenesis of ocular and central nervous system manifestations in Hallervorden-Spatz syndrome. Acta Neuropathologica 1992;83:113–119. [PubMed: 1557943]

60. Tuite PJ, Provias JP, Lang AE. Atypical dopa responsive parkinsonism in a patient with megalencephaly, midbrain Lewy body disease, and some pathological features of Hallervorden-Spatz disease. J Neurol Neurosurg Psychiatry 1996;61:523–7. [PubMed: 8937352]

61. Vakili S, Drew A, Von Schuching S, Becker D, Zeman W. Hallervorden-Spatz syndrome. Arch Neurol 1977;34:729–738. [PubMed: 201232]

62. Volkl A, Ule G. Trace elements in human brain: Iron concentrations of 13 brain areas as a function of age. Neurology 1972;202:449–454.

63. Wakabayashi K, Yoshimoto M, Fukushima T, Koide R, Horikawa Y, Morita T, Takahashi H. Widespread occurrence of alpha-synuclein/NACP-immunoreactive neuronal inclusions in juvenile and adult-onset Hallervorden-Spatz disease with Lewy bodies. Neuropathol Appl Neurobiol 1999;25:363–368. [PubMed: 10564525]

64. Williamson K, Sima AAF, Curry B, Ludwin SK. Neuroaxonal dystrophy in young adults: a clinicopathological study of two unrelated cases. Ann Neurol 1982;11:335–343. [PubMed: 7103414]

65. World Healh Organization. WHO Laboratory Manual for the Examination of Human Sperm and Sperm-Cervical Interaction. 4. Cambridge: 1999. p. 138

66. Yang EY, Campbell A, Bondy SC. Configuration of thiols dictates their ability to promote iron-induced reactive oxygen species generation. Redox Rep 2000;5:371–375. [PubMed: 11140748]

67. Yoon SJ, Koh YH, Floyd RA, Park JW. Copper, zinc superoxide dismutase enhances DNA damage and mutagenicity induced by cysteine/iron. Mutat Res 2000;448:97–104. [PubMed: 10751627]

68. Zhou B, Westaway SK, Levinson B, Johnson MA, Gitschier J, Hayflick SJ. A novel pantothenate kinase gene (PANK2) is defective in Hallervorden-Spatz syndrome. Nat Genet 2001;28:345–349. [PubMed: 11479594]
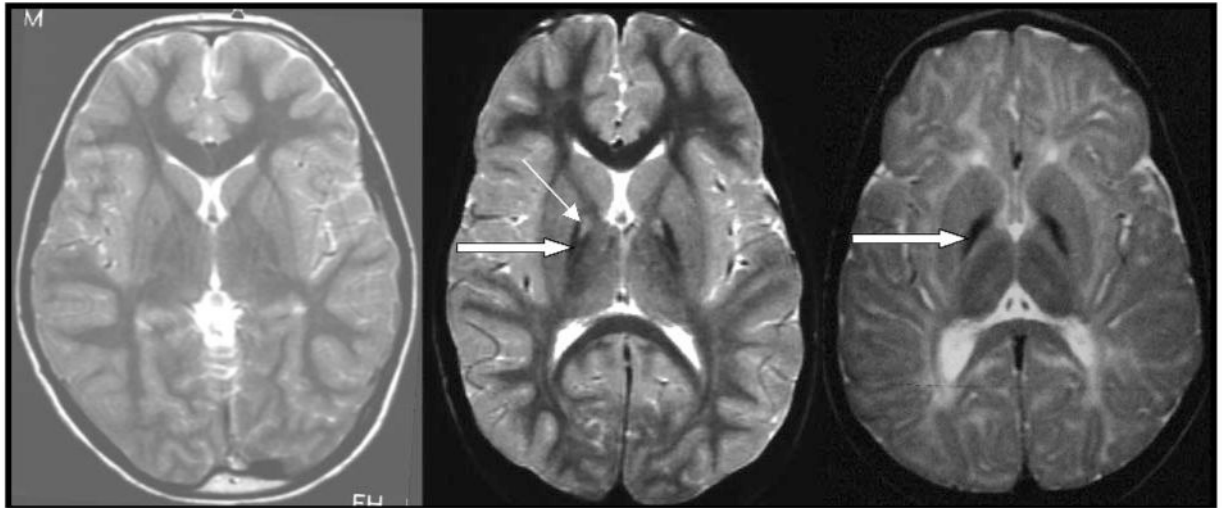
Fig. 1.
Patterns on T2-weighted brain magnetic resonance imaging. The image on the left is from a
normal patient. The center image from a *PANK2* mutation-positive patient shows hypointensity
(thick arrow) with a central region of hyperintensity (thin arrow) in the medial globus pallidus
(eye-of-the-tiger sign). The right image from a *PANK2*-negative patient with NBIA shows only
a region of hypointensity (arrow) in the medial globus pallidus. Image reprinted with
permission from the Massachusetts Medical Society (copyright 2003): Hayflick SJ, Westaway
SK, Levinson B, Zhou B, A. JM, Ching KHL and Gitschier J. Genetic, clinical and radiographic
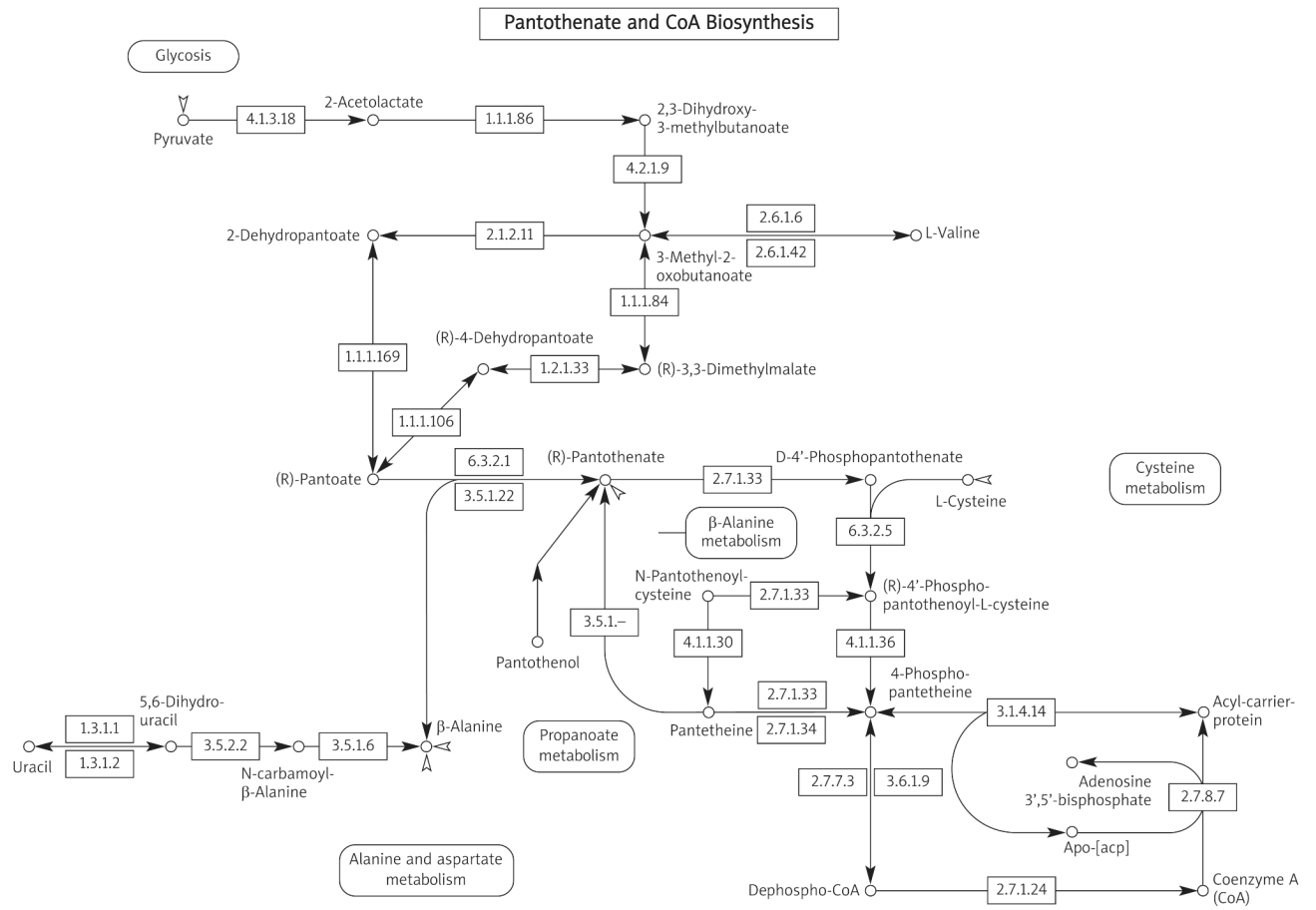delineation of Hallervorden-Spatz syndrome. N Engl J Med 2003; 348: 33-40

Pantothenate and CoA Biosynthesis

Glycosis

Pyruvate — 4.1.3.18 → 2-Acetolactate — 1.1.1.86 → 2,3-Dihydroxy-3-methylbutanoate

4.2.1.9

2-Dehydropantoate ← 2.1.2.11 ← 3-Methyl-2-oxobutanoate

2.6.1.6 / 2.6.1.42 → L-Valine

1.1.1.84

1.1.1.169

(R)-4-Dehydropantoate — 1.2.1.33 → (R)-3,3-Dimethylmalate

1.1.1.106

(R)-Pantoate — 6.3.2.1 / 3.5.1.22 → (R)-Pantothenate — 2.7.1.33 → D-4'-Phosphopantothenate

L-Cysteine

β-Alanine metabolism

6.3.2.5

N-Pantothenoyl-cysteine — 2.7.1.33 → (R)-4'-Phospho-pantothenoyl-L-cysteine

3.5.1.–

Pantothenol

4.1.1.30

4.1.1.36

5,6-Dihydro-uracil

1.3.1.1 / 1.3.1.2

Uracil — 3.5.2.2 → N-carbamoyl-β-Alanine — 3.5.1.6 → β-Alanine

Propanoate metabolism

Pantetheine — 2.7.1.33 / 2.7.1.34 → 4-Phospho-pantetheine

3.1.4.14 → Acyl-carrier-protein

2.7.7.3 / 3.6.1.9

Adenosine 3',5'-bisphosphate — 2.7.8.7

Apo-[acp]

Alanine and aspartate metabolism

Dephospho-CoA — 2.7.1.24 → Coenzyme A (CoA)

Cysteine metabolism

Fig. 2.
Pantothenate forms the chemical core of coenzyme A (from Kyoto Encyclopedia of Genes and Genomes, http://www.genome.ad.jp/kegg/)

Table I

Diagnostic Criteria for PKAN

Obligate features	Corroborative features
onset in the first three decades	corticospinal tract involvement
progression of signs and symptoms	pigmentary retinopathy or optic atrophy
evidence of extrapyramidal dysfunction	family history suggestive of autosomal recessive inheritance
on T2-weighted brain MRI: bilateral anteriomedial hyperintensity surrounded by a region of hypointensity in the medial globus pallidus (eye-of-the-tiger sign)	acanthocytosis