Pantothenate kinase-associated neurodegeneration (PKAN): molecular confirmation of a Turkish patient with a rare frameshift mutation in the coding region of the *PANK2* gene

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SUMMARY: Cangül H, Özdemir Ö, Yakut T, Okan M, Morgan NV, Baytan B, Kurian MA, Spiegel R, Maher ER. Pantothenate kinase-associated neurodegeneration (PKAN): Molecular confirmation of a Turkish patient with a rare frameshift mutation in the coding region of the *PANK2* gene. Turk J Pediatr 2009; 51: 161-165.

Here we report the clinical, neuroimaging, and molecular findings of a classic pantothenate kinase-associated neurodegeneration (PKAN) patient of Turkish origin. Our patient is the first reported case of PKAN in Turkey with molecular genetic confirmation of the diagnosis. The frameshift mutation c.821_822delCT of the *PANK2* gene detected in our patient has only been described in such classic patients to date, and our case provides further evidence of the association of this mutation with the classic PKAN phenotype. Since this mutation is a rare disease-causing mutation in other populations, further studies of more Turkish PKAN patients will show if it is the result of a founder effect in this population. In our case, molecular diagnosis allowed accurate prenatal genetic testing and counseling for this family. This case report highlights the importance of magnetic resonance imaging and molecular investigation in children who have progressive neurodegenerative symptoms of parkinsonism, dystonia, pyramidal features, and dementia.

Key words: neurodegeneration with brain iron accumulation, pantothenate kinase-associated neurodegeneration, PKAN, PANK2, frameshift mutation, consanguineous, eye-of-the-tiger.

Pantothenate kinase-associated neurodegeneration (PKAN; OMIM # 234200) is the most prevalent form of a heterogeneous group of disorders called neurodegeneration with brain iron accumulation (NBIA). PKAN is autosomal recessively inherited and caused by mutations in the pantothenate kinase 2 (*PANK2*) gene on chromosome 20p13-p12.3[1,2]. There is clinical overlap between this disease and INAD (infantile neuroaxonal dystrophy), an infantile form of NBIA, which is caused by mutations in the *PLA2G6* gene[3]. PKAN is clinically characterized by parkinsonian features with progressive dystonia and rigidity, choreoathetosis, spasticity, retinitis pigmentosa, optic atrophy, seizures and a progressive dementia. The neuroradiological hallmark of PKAN is the "eye-of-the-tiger" sign. The BIA manifests as bilateral areas of hyperintensity within a region of hypointensity within the median globus pallidus on T2-weighted brain magnetic resonance imaging (MRI)[4].

Onset of this disease can be in different age groups and heterogeneous in clinical presentation. The classification commonly used is that made by Swaiman[5], who classified the patients as (a) Classic PKAN: early onset childhood type (symptom onset before 10 years of age) including both rapidly and slowly progressive types; (b) Atypical PKAN: late onset, slowly progressive type with symptom onset after 10 years and before 18 years of age, and (c) Adult onset: slowly progressive type with symptom onset after 18 years. Classic PKAN manifests in the first decade with severe

extrapyramidal signs and progresses rapidly with loss of ambulation within 15 years from onset. Retinitis pigmentosa may be associated with this form of the disease. 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. Atypical disease is clinically heterogeneous and encompasses several disorders[6,7].

Although a disease-causing gene in PKAN has been identified, there are no biochemical markers of the disease. This may be particularly problematic in developing countries where genetic testing is not widely available. In this situation, the diagnosis of PKAN is still based on clinical history, examination, and brain MRI.

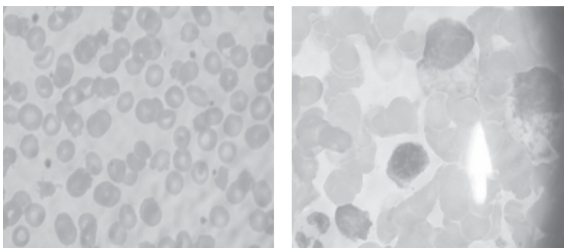
Here we report the clinical, radiological, and molecular findings of a classic PKAN patient of Turkish origin with a frameshift mutation in the coding region of the *PANK2* gene.

Case Report

An 11-year-old boy was referred to Uludağ University Hospital for progressive gait disturbance and speech difficulties. His parents were healthy consanguineous first cousins. His 19-year-old sister was also fit and well. The pregnancy was uncomplicated, and the delivery was spontaneous at term. He had normal achievement of early developmental milestones in infancy. However, at the age of two his parents noticed his unsteady gait and frequent falls. The disease progressed rapidly and around the age of eight, he developed difficulty in walking, use of his hands and speaking. At the age of 11 years, he was unable to walk without support.

Neurological examination revealed spastic tetraparesis, particularly affecting the lower limbs, with dystonic posturing of the extremities, dysarthria, risus sardonicus, choreoathetosis in both hands, hyperreflexia, and bilateral positive Babinski sign. His motor power was 3-4/5 in both upper and lower extremities. Systemic examination was otherwise unremarkable. Ophthalmological examination revealed pigmentary retinopathy and slit lamp ophthalmological examination was normal.

On biochemical investigation, serum ceruloplasmin and copper levels were normal. Nerve conduction studies were normal. Visual evoked potential responses showed mild delay. Peripheral blood smear test revealed acanthocytes and hypergranulation in lymphocytes (Fig. 1a). Bone marrow aspiration showed sea-blue histiocytes (Fig. 1b). MRI of the brain demonstrated the "eye of the tiger" sign (Fig. 2). The clinical findings are summarized in Table I.



(a) (b)

Fig. 1. (a) Peripheral blood smear showing acanthocytes, and (b) bone marrow aspiration sample showing sea-blue histiocytes.

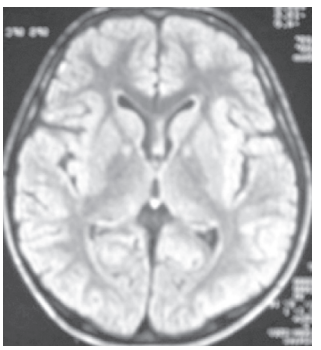


Fig. 2. Axial T_2 magnetic resonance image, revealing the classic "eye of the tiger" sign characteristic of pantothenate kinase-associated neurodegeneration.

Table I. Clinical Findings of the Case

Symptom or sign	Status
Dystonia	P
Rigidity	P
Tremor	A
Bradykinesia	U
Choreoathetosis	P
Dysarthria	P
Dysphagia	P
Dementia	A
Tics	P
Parkinsonism	A
Gait disturbance	P
Seizures or epilepsy	A
Emotional lability	A
Personality change	A
Depression	U
Retinal degeneration	P
Optic atrophy	A
Hypoprebetalipoproteinemia	A
Acanthocytosis	P
MRI: iron in globus pallidus	P
MRI: eye-of-the-tiger sign	P

P: Present. A: Absent. U: Unknown.

A diagnosis of classic PKAN was made based on clinical and radiological findings.

Genetic Studies

Genetic investigations established linkage at the *PANK2* locus (Table II). Mutational analysis was then undertaken. The polymerase chain reaction (PCR) amplification and subsequent direct sequencing of the patient's DNA revealed that he had homozygous mutation c.821_822del (nt493-494del) in exon2 of the *PANK2* gene (GenBank accession number BK000010; Fig. 3). This deletion of two nucleotides (CT) in codon Leu164 causes a frameshift (fs) resulting in substitution of leucine at amino acid position 164 of the PANK2 protein into valine, and also leading to a preliminary stop codon 16 codons downstream (p.Leu164ValfsX16). This result confirmed the clinical diagnosis of PKAN at the DNA level. We also confirmed that both his parents and his sister carried the same mutation in heterozygous state.

Table II. Linkage Analysis with the Markers Flanking the *PANK2* Gene

Marker	Physical position (bp)	Father		Mother		Affected		Unaffected	
D20S889	3.894.952	98	100	100	114	100	100	98	100
D20S482	4.454.247	157	144	144	144	144	144	157	144
D20S115	7.607.866	240	244	244	242	244	244	240	244
D20S186	8.471.794	128	134	134	128	134	134	128	134

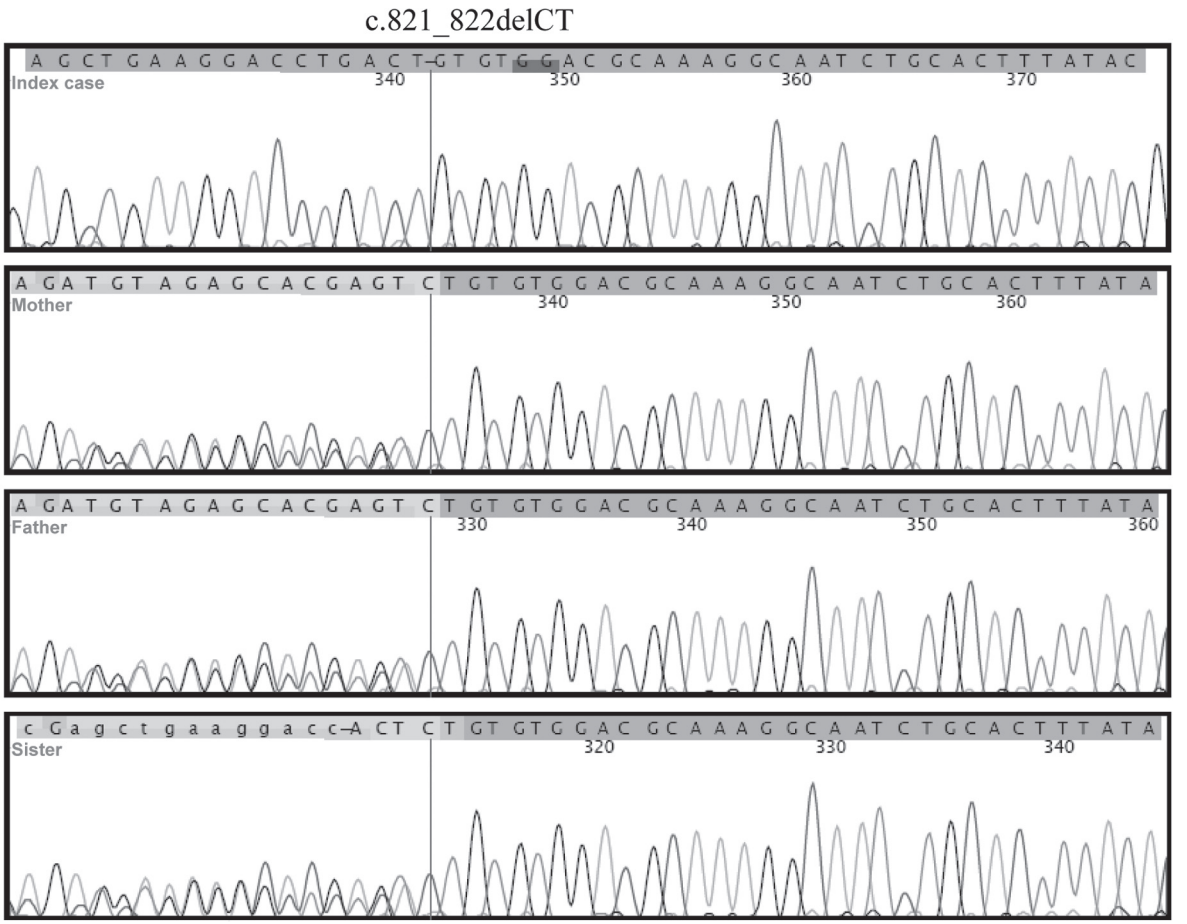


Fig. 3. Electropherograms showing the mutation c.821_822delCT in homozygous state in the index case (line 1), and in heterozygous state in his mother (line 2), father (line 3), and sister (line 4).

Prenatal Diagnosis

In a subsequent pregnancy, the parents requested antenatal *PANK2* testing. Amniocentesis was performed in the 15th week of pregnancy. The sample was divided into two volumes and then DNA isolation was performed from one part and cell culture from the other. The *PANK2* gene amplification and sequencing of the fetus's DNA revealed heterozygous mutation c.821_822del (nt493-494del) in exon2 of the *PANK2* gene, and maternal cell contamination was excluded by microsatellite analysis. Parental history was negative for radiation or drug exposure that might have caused a chromosomal breakage or aneuploidies. Amniotic fluid volume was normal. Amniocyte cell culture was performed according to standard methods, and chromosomal analyses were then performed after GTG banding in two flasks. Cytogenetic analysis of amniocentesis revealed normal karyotype of the fetus. Ultrasonographic examination of the fetus during the pregnancy did not show any phenotypic abnormality. Genetic counseling was given according to these results. The pregnancy was uncomplicated, and the delivery was spontaneous at term. The post-natal development of the child has been normal and reflects the normality in her genetic carrier status.

Discussion

We describe a rare case of PKAN associated with a frameshift mutation in the *PANK2* gene. The *PANK2* gene causing PKAN codes for the enzyme of the initial and the rate-limiting step in coenzyme A (CoA) biosynthesis, the phosphorylation of pantothenate[2]. PANK2 kinase has a mitochondrial targeting sequence that directs its transport into the organelle as demonstrated by an *in vitro* import assay and green fluorescent protein (GFP) fusion proteins[8]. 48 kDa mature PANK2 protein is generated after sequential cleavage at two sites by the mitochondrial processing peptidase and localizes to mitochondria in the human brain[9]. Altered mitochondrial CoA synthesis due to loss of the function of the enzyme is predicted to cause mitochondrial dysfunction with subsequent degeneration of susceptible neuronal tissues, mainly basal ganglia, the optic nerve, and the retina. The distinct phenotypes in different PKAN patients are considered to be the function of the residual enzyme activity left by distinct mutations.

A mouse model created by knocking out the murine *PANK2* gene showed that homozygous null mice gradually developed retinal degeneration with progressive photoreceptor decline, significantly lower scotopic a- and b-wave amplitudes, decreased cell number and disruption of the outer segment, and reduced pupillary constriction response[10]. Homozygous male mutants were infertile due to azoospermia, a condition that was not appreciated in affected humans with PKAN. In contrast to humans, homozygous null mice exhibited no basal ganglia changes or dystonia. According to immunohistochemistry, PANK2 protein was localized to mitochondria in both the retina and spermatozoa.

Comprehensive studies aiming to delineate the genotype-phenotype relationship in PKAN[11-13] have been undertaken. Hayflick et al.[11] subdivided the disease phenotype into classical and atypical forms. All classic patients in the study carried *PANK2* mutations whereas some atypical patients did not. Atypical patients with mutations showed a more severe phenotype than mutation-negative patients. The same study[11] also showed that some patients with clinical features of PKAN did not have PANK2 mutations. Valentino et al.[14] reported a case of classic PKAN who, despite having the "eye of the tiger" pattern, did not have a *PANK2* mutation. Conversely, patients presenting with classical clinical PKAN (*PANK2* mutation- positive) without the eye-of the tiger-sign have also been reported[15]. This is suggestive of the clinical, radiological, and genetic heterogeneity in PKAN. In fact, this clinical variation is further demonstrated by the observation that the HARP syndrome (hypoprebetalipoproteinemia, acanthocytes and retinitis pigmentosa) is also within the PKAN spectrum and associated with (often compound heterozygote) *PANK2* mutations[16].

Our patient had a clinical picture consistent with classic PKAN according to his clinical features: dystonia, rigidity, choreoathetosis, dysarthria, dysphagia and gait disturbance, which began when he was two years of age and progressed rapidly thereafter. The differential diagnosis of such a clinical picture could also include disorders such as INAD, infantile ceroid lipofuscinosis, infantile parkinsonism, other disorders of neuronal BIA (without a genetic basis), metabolic disorders (such as mitochondriocytopathies) and the leukodystrophies. However, the presence

of retinal degeneration, acanthocytosis and the eye-of-the-tiger sign on MRI are much more indicative of a diagnosis of PKAN. The frameshift mutation c.821_822del detected in our patient was exclusively found in classic patients[11-13], further confirming the clinical classification of our patient. However, this mutation is not a common disease-causing mutation, contributing to only 1/123, 2/72, and 1/16 of the cases in the genotype-phenotype studies mentioned above[11-13], respectively. Further studies of more Turkish PKAN patients will show if this mutation is the result of a founder effect in this population.

In our case, molecular diagnosis allowed accurate prenatal genetic testing and counseling for this family. Given the fact that this patient is the first Turkish PKAN patient with molecular confirmation of a *PANK2* gene mutation, it is possible that PKAN may be underdiagnosed. Our case report highlights the importance of MRI and molecular investigation in children who have progressive neurodegenerative symptoms of parkinsonism, dystonia, pyramidal features, and dementia.

Acknowledgements

Dr. Hakan Cangül was supported by grants from EMBO (ASTF 121.00-2007) and ESF (Activity: 'Frontiers of Functional Genomics'-Exchange Grant 2008).

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