DOI: https://doi.org/10.2298/SARH190812005S
UDC: 616.83:575.224.2

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Novel *PANK2* mutation identified in a patient with pantothenate kinase-associated neurodegeneration

Marina Svetel[1,2], Ivana Novaković[3], Svetlana Tomić[4], Nikola Kresojević[1], Vladimir Kostić[1,2]

[1]Clinical Centre of Serbia, Neurology Clinic, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Medicine, Institute for Human Genetics, Belgrade, Serbia;
[4]Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek University Hospital Center, Clinical Department of Neurology, Osijek, Croatia

SUMMARY

Introduction Pantothenate kinase-associated neurodegeneration (PKAN) is a rare, recessively inherited disorder caused by mutations in the pantothenate kinase 2 (*PANK2*) gene on chromosome 20p13. The objective of this report is to present a patient with atypical PKAN with the novel heterozygous *PANK2* mutation.

Case outline We present a 32-year-old female who had disease onset at the age 20 (depression, speech, chewing problems and backward falls) with progressive course. Neurological examination revealed hypomimia, *risus sardonicus,* dysphagia, tachylalia and severe dystonic dysarthria, moderate arms, legs, and jaw-opening dystonia, postural instability, urge incontinence, and decreased visual acuity. Brain magnetic resonance imaging revealed iron accumulation in the bilateral globus pallidus and putamen ("eye-of-the-tiger"), a radiological finding pathognomonic for PKAN. Genetic analysis revealed known mutation p.T528M (c.1583C>T) in exon 6, and novel p.Y405D (c.1213T>G) in exon 3 of the *PANK2* gene. *In silico* analyses strongly suggested this mutation to be pathogenic.

Conclusion We report a patient with PKAN, and novel substitution p.Y405D (c.1213T>G) in *PANK2* that has not been previously described in PKAN patients.

Keywords: neurodegeneration with brain iron accumulation; pantothenate kinase-associated neurodegeneration; *PANK2*

INTRODUCTION

Pantothenate kinase-associated neurodegeneration (PKAN) is a recessively inherited disorder caused by bi-allelic mutations in the pantothenate kinase 2 (*PANK2*) gene on chromosome 20p13 [1]. Two most frequent mutations (c.1231G > A, c.1253C > T) account for about one-third of all cases; however, to date, 155 different mutations have been reported [2].

Typical PKAN presents in early childhood with gait difficulty (spastic/dystonic gait), in almost 90% of the patients, followed by generalized pyramidal and extrapyramidal features (mainly dystonia), neuropsychiatric involvement, and pigmentary retinopathy. Clinical course is progressive and affected children generally become wheelchair-bound within a few years [3, 4].

Atypical PKAN presents later with less pronounced motor involvement, but cognitive decline and psychiatric features may be prominent [5]. Disease progresses over the first five years, followed by a long-lasting, rather stable period of slower progression [6].

In this report, we present a patient with atypical PKAN with the novel heterozygous *PANK2* mutation.

CASE REPORT

A 32-year-old female, born from a non-consanguineous marriage, had unremarkable family history (her brother was diagnosed with spondylitis ankylopoietica). Delivery and developmental milestones were normal. At the age of 20, she was treated by a psychiatrist due to depression. At that time, she noticed speech and chewing problems, and frequent backward falls. Three years later, urge incontinence appeared and gradually worsened. The patient sought medical care at the Clinical Centres of Zagreb and Clinical Hospital in Osijek (Croatia) and Belgrade (Serbia). In the course of years, she experienced slow progression of symptoms and gradual but slight worsening of gait, speech, and postural stability.

Laboratory findings examined in the course of her illness (since the onset of the symptoms until present) included normal serum ferritin, ceruloplasmin, albumin, liver tests, copper (workup for Wilson's disease), and lipoprotein levels. The blood smear was negative for acanthocytes. After several years of the disease, brain magnetic resonance imaging (MRI) revealed iron accumulation in the bilateral globus pallidus and putamen ("eye-of-the-tiger") (Figure 1). We examined her after obtaining brain MRI, and due to the typical "eye-of-the-tiger"

Received • Примљено: August 12, 2019
Revised • Ревизија: December 13, 2019
Accepted • Прихваћено: January 19, 2020
Online first: January 21, 2020

Correspondence to:
Vladimir KOSTIĆ
Dr Subotića starijeg 6
11000 Belgrade, Serbia
vladimir.s.kostic@gmail.com

finding, we diagnosed her with neurodegeneration with brain iron accumulation (NBIA) – PKAN.

Personal neurological examination (neurological reports were also used for the follow-up of the patient's status) 12 years after disease onset, revealed hypomimia, *risus sardonicus*, dysphagia, tachylalia and severe dystonic dysarthria, moderate arms, legs, and jaw-opening dystonia, postural instability, and decreased visual acuity. Tendon reflexes were brisk, Babinski sign negative. Her gait was unstable. Cerebellar signs and Romberg test were negative. The Mini Mental State Examination score was 30/30. Twelve years after the symptoms onset she was still able to walk unassisted and to take care of herself.

At the moment of examination, her psychological status was within the normal range, without symptoms of depression.

DNA was extracted using a commercial kit. After PCR amplification of the *PANK2* exons 5 and 6 and surrounding regions, direct Sanger sequencing was performed using BigDye Terminator v.3.1 Cycle Sequencing kit (Thermo Fisher Scientific – Life TEchnology, Waltham, MA, USA) on ABS 3500 Genetic Analyzer (ABS Global, Inc., DeForest, WI, USA). For data analysis, Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA) was used. After detection of only one heterozygous *PANK2* mutation in exon 6, the analysis was continued by next generation sequencing (NGS) of DNA. We used TruSight One Panel (Illumina, Inc., San Diego, CA, USA) and MiSeq NGS platform (Illumina, Inc.). Data analysis was performed by the Variant Studio provided for Illumina users. *In silico* characterization of the detected gene variants was performed by PolyPhen, Shift, MetaLR, REVEL, and MutationTaster software. Confirmation of NGS-detected *PANK2* mutation was done by Sanger sequencing after PCR amplification of the target region, as described above.

Initial targeted sequencing of selected *PANK2* gene exons reveled known mutation p. T528M (c.1583C>T) in exon 6, in heterozygous state. In addition, NGS analysis detected substitution p.Y405D (c.1213T>G) in exon 3, also as a heterozygous change.

This change was confirmed in our patient by another targeted Sanger sequencing. Substitution c.1213T>G at transcript NM_153638.2 is the missense mutation leading to replacement of tyrosine to aspartic acid at amino acid position 405. This change was not detected previously in population databases ExAC and 1000G and it is also absent from disease-related bases ClinVar, LVOD, and HGMD. The variant is located in exon 3 of the *PANK2* gene that corresponds to catalytic domain of the protein, and this nucleotide and amino acid position is evolutionary highly conserved. According to the *in silico* prediction, p.Y405D (c.1213T>G) is ranged as deleterious (by Sift), probably damaging (by PolyPhen), or damaging (by MetaLR), likely disease-causing (by REVEL), and disease-causing (by MutationTaster). Aforementioned features are sufficient to classify this variant as (likely) pathogenic [7].

DNA analysis revealed that the proband's neurologically healthy father and brother were heterozygous carriers of the known p.T528M (c.1583C>T), while healthy mother was a heterozygous carrier of the newly described p.Y405D (c.1213T>G) mutation.

DISCUSSION

Clinical presentation of our patient was consistent with atypical PKAN based on time of the disease onset, neurological features, the presence of behavioral and psychiatric abnormalities, and the mode of disease progression. In addition to characteristic MRI scans, mutational analysis confirmed the diagnosis.

Initial complaints were psychiatric, in accordance with the previous findings that psychiatric symptoms (depression, anxiety, emotional lability, tics, obsessive-compulsive disorder, and psychosis) were common in the atypical PKAN, often preceding motor features [6, 8, 9].

DNA analysis showed one known mutation and one newly described variant in the *PANK2* gene. Substitution c.1583C>T (p.T528M) is one of the most common mutations in European NBIA patients, and confirmed founder mutation in the Serbian population [10]. This variant affects catalytic domain of the enzyme; frequently it is associated with an atypical form of PKAN, supporting biochemical data of residual enzyme activity.

Substitution p.Y405D (c.1213T>G) has not been previously described in NBIA patients. Also, this variant was not found in 1000 Genomes and ExAC population databases nor in disease-related databases such as ClnVar, LVOD, and HGMD. Several *in silico* predictions indicate that this variant is damaging. In addition, segregation analysis confirmed p.Y405D (c.1213T>G) is in trans with an already known disease-related mutation p.T528M, which all support its own pathogenicity.

Previous reports have demonstrated that in patients with two loss-of function alleles, symptoms were always presented at an early stage of life, while those in atypical patients often resulted in amino acid changes. This indicated that many of the patients with an atypical form of the disease may have residual *PANK2* activities. It is believed that in the presence of missense mutations, residual activity of the *PANK2* determines the age of onset, without playing a role in the progression of the disorder [11]. Although variable expressivity of alleles, as well as the combination and the concentration of the mutant proteins, were the features that mainly affected the PKAN phenotype, there were also other genetic and non-genetic modifiers that might alter *PANK2* catalytic activity [12–15].

Although we were unable to determine the enzymatic activity of *PANK2* in our case, these compound heterozygous mutations may have been responsible for the adult onset and delayed progressive nature of the disease. Our novel *PANK2* mutation may probably add to understanding the clinic–genetic correlations in atypical PKAN.

NOTE

Ethical compliance statement: We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines.

Ethical standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

Funding sources: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (project no. 175090 to Vladimir Kostić).

Financial disclosures for the previous 12 months:

Marina Svetel has received speaker's honoraria from Actavis.

Ivana Novaković, Svetlana Tomić, and Nikola Kresojević report no sources of funding and no conflicts of interest.

Vladimir Kostić has received research grants from the Ministry of Education, Science and Technological Development of the Republic of Serbia and the Serbian Academy of Sciences and Arts, and speaker honoraria from Actavis and Salveo.

Conflict of interest: None declared.

REFERENCES

1. Taylor TD, Litt M, Kramer P, Pandolfo M, Angelini L, Nardocci N, et al. Homozygosity mapping of Hallervorden-Spatz syndrome to chromosome 20p12.3-p13. Nat Genet.1996;14(4):479–81.
2. Akcakaya NH, Iseri SU, Bilir B, Battaloglu E, Tekturk P, Gultekin M, et al. Clinical and genetic features of PKAN patients in a tertiary centre in Turkey. Clin Neurol Neurosurg. 2017;154:34–42.
3. Hayflick SJ, Westaway SK, Levinson B, Zhou B, Johnson MA, Ching KH, et al. Genetic, clinical, and radiographic delineation of Hallervorden-Spatz syndrome. N Engl J Med. 2003;348(1):33–40.
4. Hayflick SJ. Neurodegeneration With Brain Iron Accumulation: From Genes to Pathogenesis. Semin Pediatr Neurol. 2006;13(3):182–5.
5. Gregory A, Polster BJ, Hayflick SJ. Clinical and genetic delineation of neurodegeneration with brain iron accumulation. J Med Genet. 2009;46(2):73–80.
6. Tomić A, Petrović I, Svetel M, Dobričić V, Dragašević Mišković N, Kostić VS. Pattern of disease progression in atypical form of pantothenate-kinase-associated neurodegeneration (PKAN) - Prospective study. Parkinsonism Relat Disord. 2015;21(5):521–4.
7. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
8. Diaz N. Late onset atypical pantothenate-kinase-associated neurodegeneration. Case Rep Neurol Med. 2013;2013:860201.
9. Pellecchia MT, Valente EM, Cif L, Salvi S, Albanese A, Scarano V, et al. The diverse phenotype and genotype of pantothenate kinase-associated neurodegeneration. Neurology. 2005;64(10):1810–2.
10. Svetel M, Hartig M, Cvetković D, Beaubois C, Maksić J, Novaković I, et al. Phenotypic expression and founder effect of PANK2 c.1583C>T (p.T528M) mutation in Serbian Pantothenate kinase-associated neurodegeneration patients. Arch Biol Sci. 2019;71(2):275–80.
11. Hartig MB, Hörtnagel K, Garavaglia B, Zorzi G, Kmiec T, Klopstock T, et al. Genotypic and phenotypic spectrum of PANK2 mutations in patients with neurodegeneration with brain iron accumulation. Ann Neurol. 2006;59(2):248–56.
12. Morales-Briceño H, Chacón-Camacho OF, Pérez-González EA, Arteaga-Vázquez J, Rodríguez-Violante M, Cervantes-Arriaga A, et al. Clinical, imaging, and molecular findings in a sample of Mexican families with pantothenate kinase-associated neurodegeneration. Clin Genet. 2015;87(3):259–65.
13. Polster BJ, Yoon MY, Hayflick SJ. Characterization of the human PANK2 promoter. Gene. 2010;465(1–2):53–60.
14. Wilfred BR, Wang WX, Nelson PT. Energizing miRNA research: A review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. Mol Genet Metab. 2007;91(3):209–17.
15. Siudeja K, Srinivasan B, Xu L, de Jong J, Nollen EA, Jackowski S, et al. Impaired Coenzyme A metabolism affects histone and tubulin acetylation in Drosophila and human cell models of pantothenate kinase associated neurodegeneration. EMBO Mol Med. 2011;3(12):755–66.

Нова мутација у гену *PANK2* код болесника са неуродегенерацијом удруженом са пантотенат-киназом

Марина Светел[1,2], Ивана Новаковић[3], Светлана Томић[4], Никола Кресојевић[1], Владимир Костић[1,2]

[1]Клинички центар Србије, Клиника за неурологију, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Универзитет у Београду, Медицински факултет, Институт за хуману генетику, Београд, Србија;
[4]Свеучилиште Јосипа Јурја Штросмајера у Осијеку, Медицински факултет, КБЦ Осијек, Клиника за неурологију, Осијек, Хрватска

САЖЕТАК

Увод Неуродегенерација удружена са пантотенат-киназом (*PKAN*) ретко је, аутозомно рецесивно обољење узроковано мутацијама у гену за пантотенат-киназу 2 (*PANK2*) на хромозому *20p13*.

Циљ овог рада је приказивање болесника са атипичним обликом *PKAN* који је носилац новооткривене хетерозиготне мутације.

Приказ болесника Приказујемо жену стару 32 године чија је болест почела у двадесетој години (депресија, проблем са говором и гутањем и падови уназад) и има прогресиван ток. Неуролошким прегледом уочени су хипомимија, *risus sardonicus*, дисфагија, тахилалија и тешка дистоничка дизартрија, умерена дистонија руку, ногу и дистонија отварања вилице, постурална нестабилност, ургенција микције и смањена оштрина вида. Преглед мозга магнетном резонанцом указао је на таложење гвожђа у глобусу палидусу и путамену обострано (знак тигровог ока), а радиолошки налаз је био патогномоничан за *PKAN*. Генетском анализом откривена је одраније позната мутација *p.T528M* (*c.1583C>T*) у егзону 6, и нова мутација *p.Y405D* (*c.1213T>G*) у егзону 3 гена *PANK2*. Анализа *in silico* указује да је новооткривена мутација патогена.

Закључак Приказали смо болесницу са *PKAN* и новом мутацијом *p.Y405D* (*c.1213T>G*) у *PANK2,* која никада раније није описана код болесника са *PKAN*.

Кључне речи: неуродегенерација са таложењем гвожђа; неуродегенерација удружена са пантотенат-киназом; *PANK2*