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Case Report / Приказ болесника

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

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

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

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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. A brain MRI 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 *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*

САЖЕТАК

Увод Неуродегенерација удружена са пантотенат киназом (ПКАН) је ретко, аутозомно рецесивно обољење узроковано мутацијама у гену за пантотенат киназу 2 (*ПАНК2*) на хромозому 20п13.

Циљ овог приказа је презентација болесника са атипичним обликом ПКАН 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 уочена је 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), радиолошки налаз патогномоничан за ПКАН. Генетском анализом откривена је од раније позната мутација *p.T528M* (*c.1583C>T*) у егзону 6, и нова мутација p.*Y405D* (*c.1213T>G*) у егзону 3 гена *ПАНК2*. *In silico* анализа указује да је новооткривена мутација патогена.

Закључак Приказали смо болесницу са ПКАН и новом мутацијом *p.Y405D* (*c.1213T>G*) у *ПАНК2,* која никада раније није описана код болесника са ПКАН.

Кључне речи: неуродегенерација са таложењем гвожђа; неуродегенерација удружена са пантотенат киназом; *ПАНК2*

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, but 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 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 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 (brother was diagnosed as spondylitis ancylopoetica). Delivery and developmental milestones were normal. At the age of 20 she was treated by psychiatrist due to depression. At that time, she noticed speech, chewing problems and frequent backward falls. Three years later urge incontinence appeared and gradually worsened. 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 beginning of symptoms until nowadays) included normal serum ferritin, ceruloplasmin, albumin, liver tests, copper (work up for Wilson s disease), and lipoprotein levels. The blood smear was negative for acanthocytes. After few years of disease, a brain MRI revealed onset iron accumulation in the bilateral globus pallidus and putamen (Fig. 1) (“eye-of-the-tiger”). We saw her after getting brain MRI, and due to typical “eye-of-the-tiger” finding we diagnosed her as NBIA (PKAN).

Personal neurological examination (neurological reports were also used for follow up of patients’ 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. Gait was unstable. Cerebellar signs and Romberg test were negative. The Mini Mental State Examination score was 30/30. Twelve years after symptoms onset she was still able to walk unassisted and to take care of herself.

Her psychological status was at the moment of examination within normal range without symptoms of depression.

DNA was extracted using commercial kit. After PCR amplification of *PANK2* exon 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, USA) on ABS 3500 Genetic Analyzer (ABS, USA). For data analysis Sequencher software has been used. After detection of only one heterozygous *PANK2* mutation in exon 6, analysis was continued by Next Generation Sequencing (NGS) of DNA. We used TruSight One Panel (Illumina, USA) and MiSeq NGS platform (Illumina, USA). Data analysis was performed by Variant Studio provided for Illunina's users. *In-silico* characterization of detected gene variants was performed by PolyPhen, Shift, MeataLR, REVEL and Mutation Taster softwares. Confirmation of NGS detected *PANK2* mutation was done by Sanger sequencing after PCR amplification of 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 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 Mutation Taster). Features mentioned above are sufficient to classify this variant as (likely) pathogenic [7].

DNA analysis revealed that proband's neurologically healthy father and brother were a heterozygous carriers of 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 disease onset, neurological feature, presence of behavioural and psychiatric abnormalities and mode of disease progression. Besides 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 *PANK2* gene. Substitution c.1583C>T (p.T528M) is one of the most common mutations in European NBIA patients, and confirmed founder mutation in Serbian population [10]. This variant affects catalytic domain of the enzyme; frequently it is associated with 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 population databases 1000 Genomes and ExAC nor in disease related databases such as ClnVar, LVOD and HGMD. Several in silico predictions indicate this variant is damaging. In addition, segregation analysis confirmed p.Y405D (c.1213T>G) is in trans with 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 patients with atypical form of the disease may have a 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 combination and concentration of the mutant proteins were the features that mainly affected 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

Conflict of interest: None declared.

Ethical compliance statement: We confirm that we have read the Journal's position on issues involved in 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.

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