Novel mutation of PPOX gene in a patient with abdominal pain and syndrome of inappropriate antidiuresis.

Endocrine

NOVEL MUTATION OF PPOX GENE PRESENTING WITH SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

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	G>A (p.Cys459Tyr) in heterozygous state. The same variant was later found in one of her cousins with skin lesions and other three younger asymptomatic relatives. We provided evidence that this novel mutation is likely to be pathogenetic. Conclusions Our case highlights the importance of considering VP in the differential diagnosis of SIADH and underlines the role of genetic screening in the management of such patients. The finding of a novel mutation of PPOX gene in our index case has allowed to recognize an affected family.
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NOVEL MUTATION OF *PPOX* GENE PRESENTING WITH SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION.

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Abstract

Purpose Acute porphyrias are metabolic disorders of heme biosynthesis characterized by acute life-threatening attacks. The diagnosis is often missed since clinical presentation is aspecific mimicking other medical and surgical conditions. Variegate porphyria (VP) is an autosomal dominant inherited disease with incomplete penetrance due to decreased activity of the Protoporphyrinogen Oxydase (PPOX) gene; most VP mutations are family specific. We report the case of a 40 year-old woman who presented many times to the emergency department complaining of unexplained abdominal pain and laboratory investigations showed repeatedly hyponatremia. Inappropriate antidiuretic hormone secretion (SIADH) was confirmed and measurement of urine porphobilinogen and delta-aminolevulinic acid disclosed the diagnosis of acute porphyria. The genetic analysis of PPOX gene was performed.

Methods The entire coding sequence and exon/intron boundaries of PPOX gene were amplified in 5 different Polymerase Chain Reaction (PCR) fragments. In silico prediction of the pathogenicity of the mutation was determined by using different tools, Polyphen2, SNPs&GO, SNPs3D.

Results The genetic analysis of PPOX gene revealed a novel missense variant c.1376 G>A (p.Cys459Tyr) in heterozygous state. The same variant was later found in one of her cousins with skin lesions and other three younger asymptomatic relatives. We provided evidence that this novel mutation is likely to be pathogenetic.

Conclusions Our case highlights the importance of considering VP in the differential diagnosis of SIADH and underlines the role of genetic screening in the management of such patients. The finding of a novel mutation of PPOX gene in our index case has allowed to recognize an affected family.

Introduction

Acute porphyrias are 4 metabolic disorders resulting from a deficient activity of a distinct enzyme in the heme biosynthetic pathway causing life-threatening manifestations [1, 2]. Variegate porphyria (VP) is an autosomal dominant inherited disease with incomplete penetrance due to decreased activity of the Protoporphyrinogen Oxydase (PPOX) gene [3]. VP occurs worldwide, but its prevalence is not accurately known because many carriers of PPOX mutations remain asymptomatic; in Europe, the estimated prevalence is 3.2 cases per million while there is an exceptionally high frequency in South Africa [3]. In VP, approximately 60% of patients with overt porphyria have only skin symptoms, as blistering lesions on sun-exposed skin, 20-30% suffer from acute neurovisceral attacks and 10-20% have both manifestations [4]. The diagnosis is often delayed because clinical manifestations are unspecific, since neuropathic abdominal pain is the presenting manifestation in 85-90% of cases. Acute attacks are often triggered by administration of porphyrinogenic drugs. Delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) are almost always elevated in urine during acute attacks. Mild to severe hyponatremia may be found during an attack [5], resulting from SIADH due to the neurotoxicity of ALA [6,7].

Patients and methods

Case report

We report the case of a 40 year-old woman with a history of idiopathic generalized epilepsy from childhood, on chronic treatment with phenobarbital for persistent EEG epileptiform activity. In February 2015, valproic acid was added to phenobarbital. From March, she complained of repeated episodes of malaise and diffuse abdominal pain requiring emergency evaluation. Abdominal pain was poorly localized without peritoneal signs, no fever or leukocytosis. Imaging studies (abdominal US, X-rays, CT) and colonoscopy were negative, while laboratory investigations showed persistent hyponatremia ranging form 129 to 131 mmol/L, which was never investigated. In April, the patient was admitted to the surgical department because of persistent abdominal pain, and an exploratory laparoscopy was performed. No lesions potentially responsible for the severe pain reported by the patient were found; at that time, sodium was 122 mmol/L. During hospitalization, hypertension was diagnosed and an ACE-inhibitor was initiated. Three days after discharge, she came back to hospital for diffuse abdominal pain: abdomen X-rays was negative, and laboratory tests were unrewarding except for a sodium value of 117 mmol/L. She was then transferred to our department. At admission, she was conscious, oriented, afebrile, blood pressure was 155/95 mmHg, pulse 100R, Glasgow Coma Scale 15/15, without signs of fluid overload or depletion. Abdominal examination revealed diffuse tenderness without peritoneal signs. Fluid restriction to correct hyponatremia was instituted and valproic acid was discontinued due to the suspected SIADH. Blood osmolarity was 250 mOsm/kg**,** urine osmolarity 266 mOsm/kg, urinary sodium concentration 32 mmol/L**,** blood sugar 5.44 mmol/L**,**

normal electrophoretic protein pattern and lipid profile; adrenal insufficiency and hypothyroidism were ruled out and SIADH was confirmed. Abdominal CT was negative. In the absence of explanation for abdominal pain, an acute porphyria was suspected and we observed the change of color of a urine specimen from yellow to reddish-brown after photo-exposure. The diagnosis was confirmed by very high levels of urinary porphobilinogen (42.7 mg/g creatinine; normal value <2) and urinary ALA (21 mg/g creatinine; n.v.<5) with a high total urinary porphyrins level (238 µg/24h, n.v. < 150). Plasma emission spectrum test showed a fluorescence peak at wavelength of 627 nm. We observed a progressive increase of serum sodium concentration, from 117 mmol/L to 134 mmol/L in 2 weeks, a spontaneous resolution of abdominal pain and normalization of blood pressure without any anti-hypertensive drug. Only phenobarbital was continued since no abdominal symptoms were reported previously. At discharge, the patient was asymptomatic and she had genetic counseling. Screening of Hydroxymethylbilane synthetase (HMBS, OMIM 609806) mutations for the acute intermittent porphyria was negative, thus the sequencing of the Protoporphyrinogen Oxydase gene (PPOX, OMIM 600923) responsible of variegate porphyria was performed.

Genetic analysis

PCR amplification of PPOX gene

Genetic analysis was performed after obtaining written informed consent from all subjects examined. Genomic DNA was extracted from blood using commercially available kits (QIAGEN). The entire coding sequence and exon/intron boundaries of PPOX gene were amplified in 5 different Polymerase Chain Reaction (PCR) fragments. All PCR products were checked for purity on agarose gel. PCR products intended for DNA sequencing were cleaned up with NucleoSpin kit (Macherey Nagel), checked on agarose gel for yield and subjected to direct sequencing for mutation screening [8].

DNA sequencing and mutation detection

DNA sequencing was performed by Eurofins Genomics SRL, Ebersberg, Germany, using the same primers as in PCR. Sequences data were analyzed using the software Chromas Pro Version 1.33. Once identified, mutation has been confirmed by sequencing on a second amplified fragment. Mutation identified is described as recommended by HGVS (Human Genome Variation Society, http://www.hgvs.org/). Nucleotide numbers are referred to coding DNA sequence (cDNA) derived from genomic *PPOX* sequence (GenBank accession NM_000309.3). In silico prediction of the pathogenicity of the mutation was determined by using different tools, Polyphen2, SNPs&GO, SNPs3D.

Results

PPOX catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX (the penultimate step of heme byosinthesis) in the presence of the coenzyme flavin adenine dinucleotide (FAD). Direct sequencing of the 13 exons and exon/intron

boundary regions of the PPOX gene from the patient revealed a novel missense variant c.1376 G>A, p.Cys459Tyr at heterozygous state (Figure 1). The same variant in PPOX gene was found later in one of her cousins who presented the typical skin lesions of VP on her hands, in particular after sun exposure and she was then diagnosed to have VP (Figure 1). The same mutation was found also in other three younger relatives (two sibs and the son of the proband's affected cousin) who were asymptomatic. The variant detected is a novel missense mutation, a single nucleotide substitution at position 1376 of the coding sequence causing the replacement of the Cysteine at the amino acid position 459 with a Tyrosine. This residue is located in the highly conserved FAD-binding domain [8], composed of 13 β-strands and 8 α-helices. Classical bioinformatic tools assigned pathogenetic scores to this variant: the HumVar of PolyPhen2 gave a score of 0.99; the SNPs&GO prediction gave a "disease" effect with a reliability index of 9 and SNPs3D gave a z score -2.80. We also tested 100 healthy controls and they were all negative for the presence of the variant c.1376 G>A.

Discussion

The examined patient presented several features of AP, such as unexplained recurrent abdominal pain, new-onset hypertension, hyponatremia, history of seizures, and recent use of porphyrinogenic drugs (valproic acid and phenobarbital). The present case underlines how VP diagnosis is typically delayed, because clinical manifestations commonly mimic abdominal pain of other origins or neurological conditions, and is only considered after extensive investigation, including unnecessary surgery [9]. Investigations done in our patient revealed severe euvolemic hyponatremia that met all criteria for SIADH [10]. Valproic acid probably triggered crises in our patient, since they were not seen before its use and have not been detected after drug discontinuation. Our findings are consistent with case reports of acute attacks of VP that remitted spontaneously after withdrawal of any harmful medication [3]. The mechanism underling the AP-induced SIADH is unclear but it may be due to hypothalamic damage by ALA accumulation that is neurotoxic. [1,6,7]. It is known that valproic acid may display a porphyrinogenic effect inducing ALA synthase enzyme [1] and the drug has also the potential for causing SIADH increasing hypothalamic antidiuretic hormone secretion [11]. Thus, in our patient valproic acid is likely to have played a role in promoting both VP attacks and SIADH. SIADH associated with AP is responsive to fluid restriction and high-carbohydrate loading [7,12], or hematin administration [6], but in our patient fluid restriction coupled with valproic acid withdrawal was sufficient to restore normality. The presence of a Cys459Tyr variant in both our proband and her affected cousin, the absence of this variant in 100 healthy controls, the location of the residue located in the highly conserved FAD-binding domain and the results of different in silico tools used to predict pathogenicity, all give to the c.1376 G>A (p.Cys459Tyr) variant a very likely pathogenetic role. The presence of healthy relatives carrying the same Cys459Tyr variant in the PPOX gene could be explained by the reduced penetrance of VP and by the younger age of some of the relatives. We have detected a novel mutation associated with VP

that is family-specific, as most VP mutations are [13] and we have provided evidence that this novel mutation is likely to be pathogenetic. Moreover the identification of asymptomatic carriers of the mutation is very important because they could receive counseling on methods to minimize the risk of acute porphyric attacks, such as avoidance of certain drugs or other environmental factors [7]. The present case is an archetype of the challenges encountered in recognizing VP and underlines the importance of a genetic screening in affected patients and their family members.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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FIGURE 1

A) Family pedigree: filled circles - affected subjects carriers of the PPOX c.1376G>A missense variant; empty symbols - unaffected subjects, not tested for the PPOX gene; empty symbols with black dots - apparently unaffected subjects carriers of the PPOX missense variant c.1376G>A ;arrow indicates the proband;

B) DNA sequencing result from the PPOX gene, showing the c.1376G>A missense variant

Figure 1.