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Original article

Mutational spectrum of *PTS* gene and *in silico* pathological assessment of a novel variant in Mexico

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Abstract

Background: Tetrahydrobiopterin (BH4) is the cofactor for 6-pyruvoyl-tetrahydropterin synthase (PTPS); it is involved in BH4 biosynthesis and is encoded by *PTS* gene. Its deficiency (PTPSD) is characterized by hyperphenylalaninemia (HPA) and deficit in central monoamine neurotransmitters. We describe the clinical and mutational spectrum of five patients with PTPSD, from four unrelated Mexican families. All patients had symptomatic diagnosis and presented severe early neurological manifestations and HPA.

Methods: Clinical and biochemical data from studied patients were recorded. Responsible PTPSD genotypes was determined by direct and bidirectional Sanger DNA sequencing of the six *PTS* coding exons and their exon-intron borders, and these were directly searched in the available relatives. The novel *PTS* missense variant [NM_3000317.2:331G > T, p.(Ala111Ser)] was subjected to *in silico,* to predict a possible deleterious effect.

Results: Diminished fetal movements were perceived as a uniform characteristic in the studied group. DNA sequencing showed two known p.(Arg25*) and p.(Val132TyrFs*19) and the novel missense p.(Ala111Ser) *PTS* variants, the latter representing potentially a frequent PTPSD-responsible allele (50%, 4/8) in Mexican patients. *In silico* protein modeling analysis of the p.(Ala111Ser) variant revealed loss of hydrophobic interactions between the alanine and neighboring valines, suggesting that these changes in polarity may be detrimental for enzyme function, structure and/or stability.

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Conclusions: This work contributes to the knowledge of PTPS molecular spectrum. The delayed diagnosis of these patients emphasizes the importance of considering BH4 metabolism defects in the differential diagnosis of HPA, especially for countries that are beginning their HPA newborn screening programs.

Keywords: Diminished fetal movements; Enterocolitis; Newborn screening; Tetrahydrobiopterin (BH4) deficiency

1. Introduction

The 6-pyruvoyl-tetrahydropterin synthase (PTPS, EC 4.6.1.10) is involved in the *de novo* biosynthesis of tetrahydrobiopterin (BH4), a cofactor of aromatic amino acid hydroxylases, alkylglycerol monooxygenase, and nitric oxide synthases (NOS) [1]. PTPS deficiency (PTPSD, MIM #261640) leads to an autosomal recessive disorder, mainly characterized by hyperphenylalaninemia (HPA) and deficits in central dopamine and serotonin neurotransmitters [2–4].

Except for sepiapterin reductase deficiency, BH4 disorders cause HPA, including autosomal recessive GTP cyclohydrolase I (GTPCH), PTPSD, pterin-4-alpha-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR) deficiencies, and thereby can be detected through neonatal screening programs (NBS). Therefore, neopterin and biopterin profiles, along with the evaluation of DHPR activity must be performed for the differential diagnosis of HPA [2,3].

PTPSD is the most common (65.3%) among BH4 disorders, with more than 690 reported cases to date (as of August 2017, BIODEF database at http://www.biopku.org). Affected patients exhibit neurological symptoms, characterized by truncal hypotonia, dystonia, seizures, movement disorders, extrapyramidal signs, and neurodevelopmental delays [3–5]. The biochemical hallmarks of PTPSD are HPA, high levels of neopterin and low levels of biopterin in blood, urine, and cerebrospinal fluid (CSF), and a low PTPS activity in e.g. primary skin fibroblasts (<15% of wild-type). Moreover, the neurotransmitter metabolites 5-hydroxyindoleacetic and homovanillic acids, reflecting low serotonin and dopamine levels, respectively, are decreased in the CSF. The life-long treatment of PTPSD with L-dopa/Carbidopa, 5-hydroxytryptophan, and BH4 supplementation should be initiated as early as possible [3].

The *PTS* gene (cytogenetic location:11q23.1 MIM*612719) comprises six exons and according to the PND database (http://www.biopku.org), over 110 variants responsible for PTPSD have been reported, mostly comprised by single nucleotide missense and nonsense changes (76%) [6,7]. The PTPS functional unit is a homohexamer, and its crystallographic structure is deposited in the Protein Data Bank (PDB, code 3I2B). The enzyme is arranged as two trimers, associated face-to-face by electrostatic interactions, with an overall barrel-like shape enclosing a hydrophilic core. The active site comprises the histidine 24, 49, 51 and 90, cysteine 43, and glutamic acid 134 residues. There is one active site per monomer, and each coordinates a zinc atom [8].

Here, we describe the clinical picture of five Mexican patients (including two affected siblings) with a delayed diagnosis of PTPSD, and report the responsible *PTS* mutational spectrum, including a novel variant, and with protein *in silico* modelling to infer the deleterious effect of the new and potentially frequent *PTS* pathogenic variant among the Mexican population.

2. Methods

2.1. Subjects

Three non-related PTPSD patients and two affected siblings were analyzed; all were diagnosed late (mean age 1.4 months), by urinary or blood pterins analysis when available, which were performed as previously described [9]. Their clinical and biochemical picture is summarized in Table 1. Clinical data was deposited in Phenome Central (https://phenomecentral.org).

2.2. Genotype analysis

Genomic DNA was isolated by standard methods from dried blood spots. Direct and bidirectional (automated) Sanger DNA sequencing was applied to the six *PTS* coding exons and their exon-intron borders (NM_000317.2, NG_008743.1) [10]. All missense *PTS* variants were assessed relative to the HGMD, dbSNP (http://www.ncbi.nlm.nih.gov/snp), the NHLBI Exome Sequencing Project at the Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS/) Genome Aggregation Data Base (genomAD Browser, http://gnomad.broadinstitute.org), the literature, and the PND database (http://www.biopku.org). The novel *PTS* missense variant was subjected to *in silico* analysis using online tools (SIFT, PolyPhen2, MutationTaster, PROVEAN, FoldX) and the pathogenicity scoring system recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) [11]. Furthermore, we

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