Antioxidant therapy in a patient with Hyperprolinemia type I presented with mild neuromotor retardation and speech disturbance

Melike Ersoy (zeynepcey@hotmail.com)
Bakirkoy Dr Sadi Konuk Training and Research Hospital: Istanbul Bakirkoy Dr Sadi Konuk Egitim ve Arastirma Hastanesi

Semra Yılmaz
Bakırköy Prof Dr Mazhar Osman Ruh Sağlığı Sinir Hastalıkları Eğitim ve Araştırma Hastanesi: Istanbul Bakirkoy Prof Dr Mazhar Osman Ruh Sagligi ve Sinir Hastaliklari Egitim ve Arastirma Hastanesi

Serdar Ceylaner
Institute of Genetics

Case report

Keywords: Hyperprolinemia type I, mitochondrial dysfunction, antioxidant therapy

Posted Date: December 16th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-128629/v1

Abstract

Background: Hyperprolinemia type 1 (HPI) is an autosomal recessive inborn disease caused by mutations/deletions of PRODH gene, which is located on chromosome 22q11. The clinic spectrum involves mainly delayed psychomotor development, mild-to-severe mental retardation, neuropsychiatric symptoms and epilepsy. Although HPI can easily be diagnosed in patients undergoing metabolic screening tests, there is no effective therapy protocol in use. There are studies showing that it does most of its clinical findings by disrupting mitochondria function.

Case report: We present a long-term follow-up of a four-year-old girl with mild neuromotor retardation and speech disturbance, diagnosed with HPI and treated with antioxidant therapy (vitamin C, CoenzymeQ10, vitamin B complex and L-carnitine) for six years. It has been shown that antioxidant therapy decreases proline levels properly and provides clinical improvement.

1. Introduction

Hyperprolinemia type I (HPI) (#239500) is one of the two inherited metabolic disorders resulting from defects in the proline catabolic pathway [1]. The first step in proline catabolism is catalyzed by proline dehydrogenase (PRODH), a flavoenzyme localized at the inner mitochondrial membranes that converts proline to proline 5-carboxylate (P5C). In the second step, delta-1- proline-5-carboxylate dehydrogenase (P5CD) catalyzes the conversion of P5C to glutamate (Fig. 1). The deficiency of the P5C and P5CD enzymes leads to hyperprolinemia type I and type II, respectively. *PRODH* is located in the 22q11 chromosomal region, where microdeletions, which are responsible for DiGeorge (velocardiofacial) syndrome, can present with psychiatric disorders [2]. Clinical findings that develop due to HPI vary. Although genotype/phenotype evaluations have been determined for certain mutations, there is still little evidence [3]. Symptoms of HPI include delayed psychomotor development, mild-to-severe mental retardation, epilepsy, neuropsychiatric symptoms, neurosensory hearing loss, and renal abnormalities. Progressive speech deterioration, sleep disturbance, autistic features, stereotypic behaviors, hyperactivity and schizophrenia have also been reported [1,4,5]. In addition to the effect of high proline levels, the *PRODH* gene has direct and indirect regulatory effects on mitochondrial functions [6-9].

There is no definite treatment protocol yet, and symptomatic treatments are recommended.

Proline-restricted diet and supplementation with vitamin B6 are not effective. Here, we report the long-term clinical and laboratory outcomes of a girl diagnosed with HPI who was treated with antioxidant therapy.

2. Case

A four-year-old girl was admitted to the hospital with complaints of speech and neuromotor developmental delay, attention deficit and learning disability. She was born to a consanguineous parent by caesarean section without any perinatal complications. Her birth weight was 2900 grams.

Developmental history revealed that her neurodevelopmental milestones were delayed; she began to walk at 23 months of age and spoke her first words at the age of 32 months. In clinical examination, her fine motor development was below the normal level for her age, and her speech development was poor (i.e., she only spoke 2-word sentences, and she was talking with incomprehensible words). However, she could understand basic instructions. There was no evident autistic symptom in her behavioral examination or on parental reports. The score of the IQ test (Stanford-Binet Intelligence Scale) requested for the evaluation of her mental capacity was 68, which pointed to mild mental retardation. There was no additional systemic finding. Routine biochemical tests were normal, but amino acid analysis revealed high serum proline 892 µmol/L and 789 µmol/L (n: 59-369), respectively. While the lactate level was within the normal range, the alanine level was slightly elevated by 638 µmol/L (n: 152-547). Brain magnetic resonance imaging (MRI) was normal. *PRODH* gene sequence analysis was performed by using the MiSeq next generation sequencing (NGS) platform, an FDA-approved diagnostic system (Illumina, San Diego, CA, USA). We found that NM_016335.4:c.1357C>T (p. Arg453Cys) (rs3970559) homozygous mutation in the *PRODH* gene. In the evaluation of the family members, it was found that the mother of the case was being followed up with the diagnosis of multiple sclerosis, and she had demyelinated plaques in her brain MRI. Further examinations of the mother showed that she had borderline intelligence with 78 IQ, and her blood proline level was as high as 793 µmol/l. Genetic screening of the family revealed that the mother was homozygous and the father was heterozygous for the same mutation. In her sister and father, the plasma proline levels were in the normal range, and there were no clinical symptoms.

We initially supported a low-protein diet, but no biochemical or clinical improvement was observed. After obtaining informed consent from the patient and her family, we started antioxidant therapy with 100 mg/day coenzyme Q10, B complex (B1+B2+B6+B12), 500 mg/day vitamin C, and 500 mg/day L-carnitine. The proline values in the third and sixth months of treatment were 273 and 400 µmol/l, respectively, almost falling to normal limits. Alanine also reached the normal limit and never rose again. In the follow-up period, proline levels were observed to increase twice in short periods of discontinuation of treatment (Fig. 2). To observe the probable effect of this treatment approach on the serum proline level, we ceased antioxidant therapy for one month and observed that the proline levels gradually increased to the levels of diagnosis range. It was found that speech impairment increased and fine motor skills (eg writing) was impaired dramatically during periods of cessation of treatment. The proline levels of our patient, with and without antioxidant treatment were evaluated and a significant difference was observed ($p<0.005$) (Table). At 69 months of treatment, when she was 9 years and 9 months of age, IQ assessment with the Wechsler Intelligence Scale for Children (WISC-R) showed a verbal score of 70, a performance score of 95, and a full-scale IQ score of 81. Her speech disturbance was improved. No side effects of the drugs were observed during the treatment.

3. Discussion

We presented a long-term follow-up of a patient with a homozygous *PRODH* gene mutation and mild clinical manifestations who showed an important decline in plasma proline levels and a slight improvement in clinical symptoms with an increase in intelligence scores after antioxidant therapy.

The type of the mutation or the length of deletion can determine the clinical variability and severity of the disease and the levels of serum proline. High serum proline levels are related to serious clinical findings, such as epilepsy, severe neuromotor retardation, and behavioral problems [10]. The presence of mild and atypical clinical symptoms, such as speech disturbance, mild neuromotor development delay, attention deficit, and learning disability, can be related to mildly elevated proline levels. The proline levels of our patient are moderately high and may be associated with mild clinical findings and even good therapy response.

NM_016335.4:c.1357C>T (p. Arg453Cys) (rs3970559) mutation is a frequent variant in the population (gnomAD Exomes: 0.01065). The UniProt database classifies this variant as a disease-causing mutation for both HPI and schizophrenia. The Dann Score (0.9985), Mutation taster, FATHMM-MKL, LRT, Mutation Assessor, SIFT and Provean tools predict this variant as a pathogenic variant, while others predict it as a benign variant (FATHMM, MetaSVM, MetaIR). There are conflicting reports in the ClinVar database from benign variant to pathogenic [3]. As our case does not have a classical clinical phenotype, unlike previous reports, this variant is most likely patological variant but does not cause severe clinical symptoms. The mother of our patient also has mild developmental delay, learning difficulties, and cranial MRI with white matter involvement and the same genotype as her daughter. We see that the older sister and the father, who have no clinical findings and whose proline levels are within normal limits, are heterozygous.

Although the exact pathophysiologic mechanism is not yet known, several mechanisms have been suggested to explain how high proline concentrations can affect brain functions. In the literature, the most commonly emphasized and proposed mechanism is the alteration in glutamatergic homeostasis. Glutamate is an excitatory neurotransmitter that is important in normal brain function. Glutamate excitotoxicity has been shown to be linked with mitochondrial dysfunction, with energy impairment and subsequent partial membrane depolarization with resultant relief of magnesium blockage of the N-methyl-D-aspartate channel, which results in excessive influx of Ca+2. It has been suggested that hyperprolinemia by causing a reduction in glutamate reuptake and Na+, K+ ATPase activity leads to glutamatergic excitotoxity, which leads to pathological signaling and in turn contributes to cell injury and death via the production of free radicals [11]. This cell death may lead to cellular damage in the brain.

It has been shown that increased proline levels induce oxidative stress in rat brains [12] as well as alter glutamatergic transmission at hippocampal synapses [13]. Crabtree et al. found that L-proline is a GABA (gamma aminobutyric acid)-mimetic and can act at multiple GABAergic targets, but disease-relevant concentrations lead to disturbances in GABAergic production by blocking GAD blockage. Researchers suggested that this disturbance in the GABAergic system leads to the accumulation of neuroactive metabolites that cause molecular and synaptic dysfunction and finally psychotic disorders. Abnormalities in the GABAergic system have been shown in many neurodevelopmental disorders, such as attention-deficient hyperactivity disorder and schizophrenia [14].

Recent studies mentioned that low PRODH activity not only causes hyperprolinemia but also has additional effects on the electron transport chain (ETC). PRODH directly and indirectly regulates the ETC

[10]. Hancock et al found that PRODH binds directly to coenzyme Q1 and that CoQ1-dependent PRODH activity requires functional Complex III and Complex IV, so PRODH supports respiration independent of Complex I and II activity. It has been shown that in the presence of increasing CoQ1, PRODH activity increases in living cell cultures [15]. We have no additional laboratory findings of mitochondrial dysfunction in our case. A negative correlation with proline levels and antioxidant therapy was demonstrated. In addition to laboratory improvements, it can be accepted that there is objective progress in speech disturbance and intellectual disability.

We may speculate that antioxidant therapy (including coenzyme Q, carnitine, B complex) has an important influence on the activity of PRODH, and this treatment approach decreases proline levels and provides optimal acting ETC. Further studies with larger sample sizes are needed to evaluate the clinical and laboratory efficiency of antioxidant therapy in patients with HPI.

Abbreviations

COMT: Catechol-O-methyltransferase

ETC: Electron transport chain

GABA: Gamma aminobutyric asid

HPI: Hyperprolinemia type 1

MDS: Mitochondrial Disease Score

NGS: Next generation sequencing

P5C: Proline 5-carboxylate

P5CD: Proline-5-carboxylate dehydrogenase

PRODH: Proline dehydrogenase

Declarations

Acknowledgment:

I would like to thank the family of our patient for their wonderful collaboration

Disclosure:

The authors declare no conflict of interest.

References

1. Afenjar A, Moutard ML, Doummar D, Guët A, Rabier D, Vermersch AI, et al. Early neurological phenotype in 4 children with biallelic PRODH mutations. Brain Dev. 2007;29:547-52.
2. Duarte M, Afonso J, MoreiraA, Antunes D, Ferreira C, CorreiaH, et al. Hyperprolinemia as a clue in the diagnosis of a patient with psychiatric manifestations. Brain Dev. 2017;39:539-41
3. Guilmatre A, Legallic S, Steel G, Willis A, Di Rosa G, GoldenberG A, et al. Type I Hyperprolinemia: Genotype/Phenotype Correlations. Hum Mutat. 2010;31:961-65.
4. Jacquet H, Berthelot J, Bonnemains C, Simard G, Saugier-Veber P, Raux G, et al. The severe form of type I hyperprolinaemia results from homozygous inactivation of the PRODH gene. J Med Genet. 2003;40:e7.
5. Di Rosa G, Pustorino G, Spano M, Campion D, Calabrò M Aguennouz, M, et al. Type I hyperprolinemia and proline dehydrogenase (PRODH) mutations in four Italian children with epilepsy and mental retardation. Psychiatr Genet. 2008;18:40-42.
6. Savio LE, Vuaden FC, Rosemberg DB, Bogo MR, Bonan CD, Wyse AT. Long-term proline exposure alters nucleotide catabolism and ectonucleotidase gene expression in zebrafish brain. Metab Brain Dis. 2012;27:541-9.
7. Clelland CL, Read LL, Baraldi AN, Bart CP, Pappas CA, Panek LJ, et al. Evidence for association of hyperprolinemia with schizophrenia and a measure of clinical outcome. Schizophr Res. 2011;131:139-45.
8. Ferreira AG, Lima DD, Delwing D, Mackedanz V, Tagliari B, Kolling J, et al. Proline impairs energy metabolism in cerebral cortex of young rats. Metab Brain Dis. 2010;25:161-8.
9. Srivastava D, Singh RK, Moxley MA, Henzl MT, Becker DF, Tanner JJ. The three-dimensional structural basis of type II hyperprolinemia. J Mol Biol. 2012;420:176-89.
10. van de Ven S, Gardeitchik T, Kouwenberg D, Kluijtmans L, Wevers R, Morava E. Long-term clinical outcome, therapy and mild mitochondrial dysfunction in hyperprolinemia. J Inherit Metab Dis. 2014;37:383-90.
11. Nakamura T, Lipton SA. Redox regulation of mitochondrial fission, protein misfolding, synaptic damage, and neuronal cell death: potential implications for Alzheimer's and Parkinson's diseases. Apoptosis. 2010;15:1354-63.
12. Delwing D, Bavaresco CS, Chiarani F, Wannmacher CM, Wajner M, Dutra-Filho CS, et al. In vivo and in vitro effects of proline on some parameters of oxidative stress in rat brain. Brain Res.2003;991:180-6.
13. Paterlini M, Zakharenko SS, Lai WS, Qin J, Zhang H, Mukai J, et al. Transcriptional and behavioral interaction between 22q11.2 orthologs modulates schizophrenia-related phenotypes in mice. Nat Neurosci. 2005;8:1586-94.
14. Crabtree GW, Park AJ, Gordon JA, Gogos JA. Cytosolic Accumulation of L-Proline Disrupts GABA-Ergic Transmission through GAD Blockade. Cell Rep. 2016;17:570-82.
15. Hancock CN, Liu W, Alvord WG, Phang JM. Co-regulation of mitochondrial respiration by proline dehydrogenase/oxidase and succinate. Amino Acids.2016;48:859-72.

Tables

Table Proline levels of patient with and without antioxidant therapy

Proline levels (µmol/L)	**N**	**Mean**	**SD**	**p**	**r***
With Antioxidant	16	271.5	59.49		
Without Antioxidant	12	801.25	90.45	**0,00000004**	**-0.249**

SD: Standart Deviation; * Pearson correlation

Figures

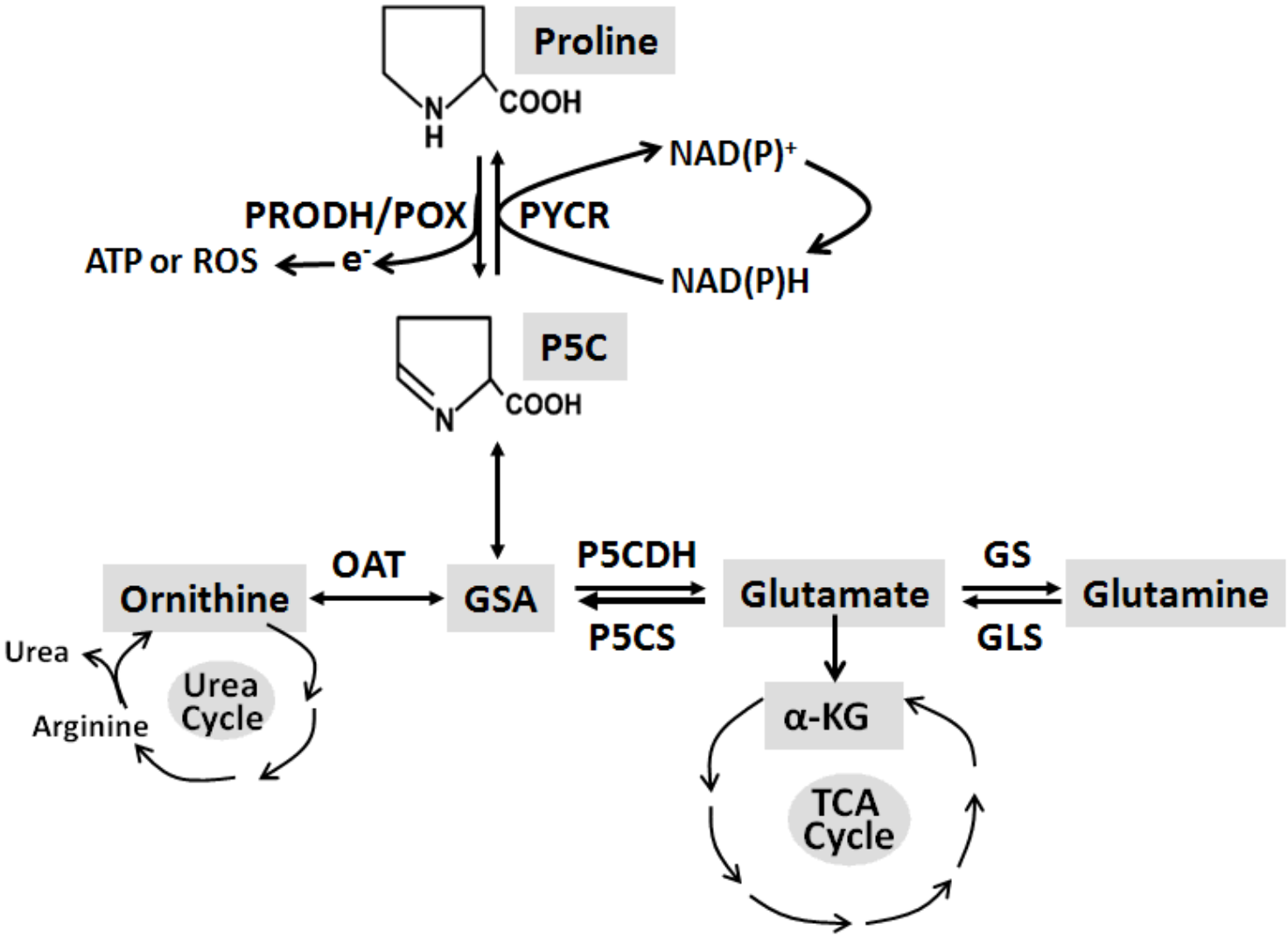

Figure 1

Proline related pathways. ATP:Adenosine triphosphate; α-KG:alpha-Ketoglutaric acid; GLS:Glutaminase; GS:Glutamine synthetase; GSA: Glutamate-5-semi-aldehyde; OAT:Ornithine Aminotransferase; P5C: 1-pyrroline-5-carboxylate; P5CDH:Pyrroline-5-carboxylate dehydrogenase; PRODH:Proline dehydrogenase; PYRC:Pyrroline-5-carboxylate reductase; ROS:Reactive oxygen species; TCA: Tricarboxylic acid

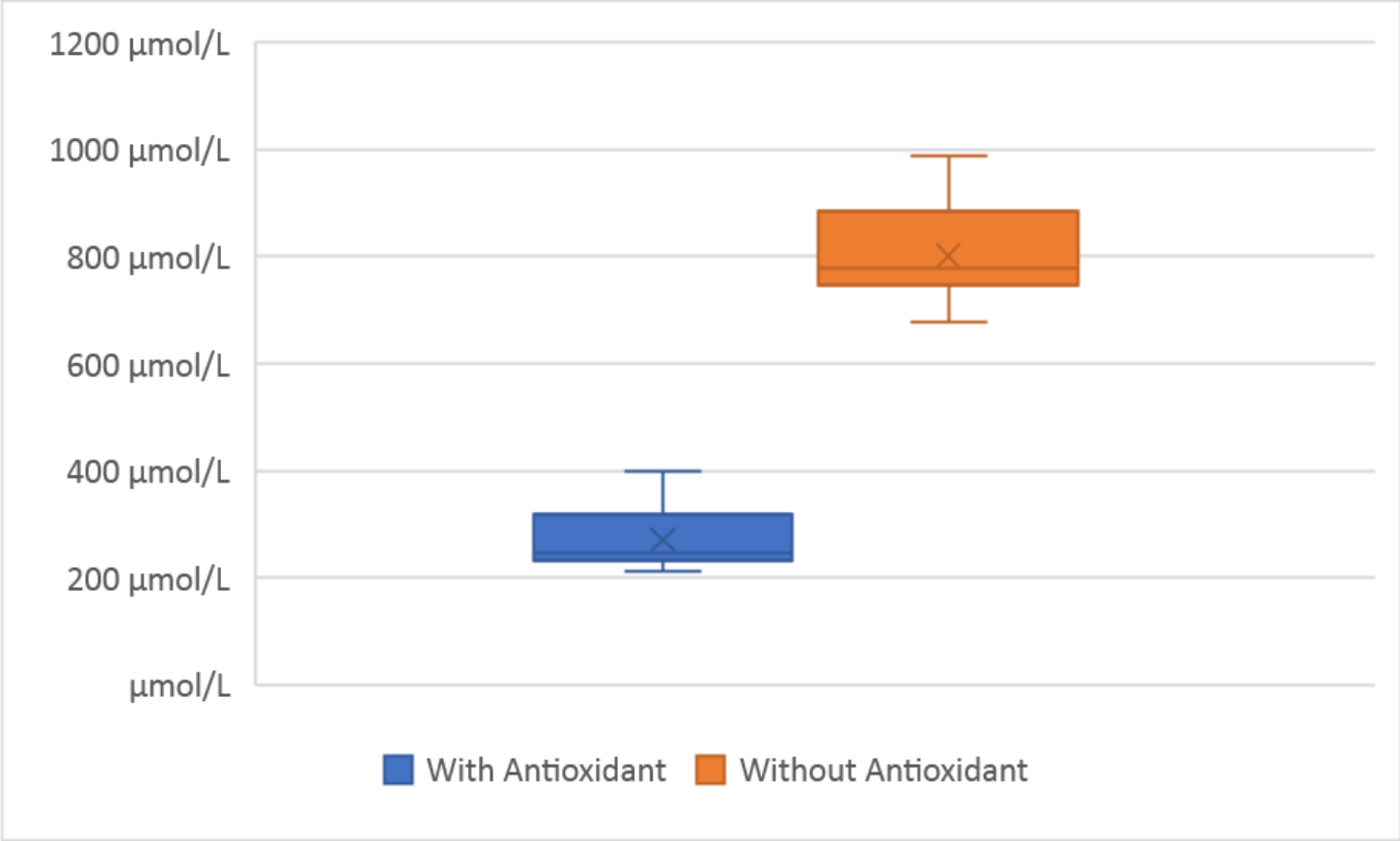

Figure 2

Proline levels of patient with and without antioxidant therapy