Hyperprolinemia type I caused by homozygous p.T466M mutation in PRODH

Rina Hama1, Jun Kido1,2✉, Keishin Sugawara2, Toshiro Nakamura3 and Kimitoshi Nakamura1,2
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Hyperprolinemia type I (HPI) is an autosomal recessive metabolic disorder caused by defects in proline oxidase (POX, EC: 1.5.99.8), also called proline dehydrogenase (PRODH). The PRODH gene is located on chromosome 22 (22q11.21) and encodes the POX enzyme, which converts proline to pyrroline-5-carboxylate (P5C) in mitochondria.

The clinical phenotype of HPI has not been clearly characterized. Patients with HPI may present with seizures, intellectual disability, language delay, autism spectrum disorder (ASD), schizophrenia, and/or bipolar disorder. Conversely, others are asymptomatic1-3. Very few case reports of patients with HPI have been reported worldwide, with only two described in Japan4,5.

An 8-year-old boy was referred to our institution for further investigation of the cause of his short stature and suspected hypoglycemia. He was the third child of healthy nonconsanguineous parents. He was born without complications at 40 weeks of gestation, with a length of 50 cm (−0.9 SD) and a weight of 2932 g (−0.3 SD) and a weight of 3 months, the patient was doing well in a supported education class and did not require medication. Written informed consent was obtained from the patient’s parents for hyperprolinemia type II. At the age of 10 years and 3 months, the patient was doing well in a supported education class and did not require medication. Written informed consent was obtained from the patient’s parents for

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Table 1A. Plasma amino acid and PRODH genetic analysis in our patient and literature review. A. Analysis of plasma amino acids.

| Amino acid | Reference (µmol/L) | Age (8 years) | Age (10 years 2 months) |
|------------|--------------------|---------------|-------------------------|
| Hydroxyproline | ≤21.6              | 11.3          | 28.0                    |
| Threonine   | 66.5–188.9         | 81.8          | 101.2                   |
| Serine      | 72.4–164.5         | 106.9         | 122.3                   |
| Asparagine  | 44.7–96.8          | 43.5          | 60.4                    |
| Glutamic acid| 12.6–62.5          | 28.0          | 18.3                    |
| Glutamine   | 422.1–703.8        | 494.6         | 573.0                   |
| Proline     | 77.8–272.7         | 530.2         | 624.5                   |
| Glycine     | 151.0–351.0        | 203.4         | 184.9                   |
| Alanine     | 208.7–522.7        | 292.7         | 390.3                   |
| Citrulline  | 17.1–42.6          | 29.2          | 21.2                    |
| Valine      | 147.8–307.0        | 130.5         | 218.2                   |
| Cystine     | 13.7–28.3          | 9.1           | 11.9                    |
| Methionine  | 18.9–40.5          | 16.0          | 28.6                    |
| Isoleucine  | 43.0–112.8         | 34.9          | 65.5                    |
| Leucine     | 76.6–171.3         | 67.7          | 128.2                   |
| Tyrosine    | 40.4–90.3          | 57.9          | 91.6                    |
| Phenylalanine| 42.6–75.7         | 60.0          | 90.6                    |
| Histidine   | 59.0–92.0          | 68.0          | 78.4                    |
| Tryptophan  | 37.0–74.9          | 59.1          | 71.1                    |
| Ornithine   | 31.3–104.7         | 33.1          | 53.5                    |
| Lysine      | 108.7–242.2        | 94.2          | 149.6                   |
| Arginine    | 53.6–133.6         | 61.7          | 101.4                   |
| Total AA    | 2068.2–3510.3      | 2587.9        | 3248.7                  |
| NEAA        | 1381.6–2379.4      | 1966.7        | 2317.3                  |
| EAA         | 660.0–1222.3       | 612.2         | 931.4                   |
| BCAA        | 265.8–579.1        | 233.1         | 411.9                   |
| EAA/NEAA    | 0.40–0.63          | 0.31          | 0.4                     |
| BCAA/Total AA| 0.11–0.18         | 0.09          | 0.13                    |
| Fisher ratio| 2.43–4.40          | 1.98          | 2.26                    |

AA: amino acids, BCAA: branched-chain amino acids, EAA: essential amino acids, NEAA: non-essential amino acids. At the age of 8 years, he ate fish but had little pork and meat. At the age of 10 years and 2 months, he had consumed more meat and pork than before.

this report. This study was approved by the Institutional Ethics Committee of the Faculty of Life Science, Kumamoto University.

In Table 1C, we summarize nine cases, including ours, of the POX p.T466M variant. The PRODH gene, which is located in the 22q11 chromosomal region, is hemideleted in 22q11 deletion syndrome, also known asvelo-cardio-facial syndrome (VCFS). VCFS shares some clinical features with HPI.

A combination of mutations and polymorphisms in the PRODH gene cause dysregulation of POX enzyme activity and may lead to a variety of phenotypes in HPI, including neuronal function disorders. The proline metabolic pathway leads cellular proline levels with glutamate and glutamine levels in neurons, and POX has been proposed to play a regulatory role in glutamatergic neurotransmission by affecting the cellular concentration of glutamate. Moreover, proline is thought to induce oxidative stress in the rat brain. Hyperprolinemia induces significant oxidative damage to proteins, lipids, and DNA, decreases the activities of antioxidant enzymes, and induces lipid peroxidation in the blood of rats.

Mouse models of POX deficiency, which is also present in individuals with schizophrenia, exhibit increases in neurotransmitter release at glutamatergic synapses as well as deficits in associative learning and response to psychomimetic drugs. Furthermore, hyperprolinemia has been suggested to be a risk factor for schizophrenia, and polymorphisms in PRODH, such as rs372055, which this case harbored, are thought to correlate with schizophrenia.

Some pharmacological, biochemical, and behavioral studies have suggested the involvement of the glutamatergic system in ASD pathology and ASD has been considered a common clinical manifestation of HPI. Poor social, adaptive, and academic skills are often evident in patients with HPI. Our patient displayed a complex learning disorder, including deficits in reading, writing, and math, and required specific educational training. Although this case and other reports suggest a correlation between intelligence and plasma proline levels, further studies are required for confirmation.

Bender et al. proposed the following classification of PRODH mutations based on POX activity reduction: mild (<30%), moderate (30–70%), and severe (>70%). PRODH uses flavin adenine dinucleotide (FAD) as a cofactor. The homozygous c.1397 C > T (p. T466M) mutation results in reduced enzyme activity to less than 20% of the control. The T466M in POX interacts with the adenine moiety of FAD to stabilize noncovalent binding of the cofactor to the POX apoenzyme, and the T466M mutation alters the affinity of the POX apoenzyme for FAD. Genotype-phenotype correlations in HPI have been suggested. For example, Rosa et al. reported two patients with the same PRODH genotype and the same range of plasma proline levels (376 and 493 µmol/L); these patients presented with a similar neurobehavorial profile, including aggressiveness and sexual disinhibition. Our patient first presented with short stature and mild hyperprolinemia but without obvious intellectual disability, though developmental delay became more noticeable with age. Alexandre et al. described a patient with the p.T466M variant who presented with clinical manifestations and blood proline levels similar to those in our case. Although p.T466M is predicted to be damaging (0.943) in Polyphen 2, Ford et al. reported persistent short stature persisting from infancy (Fig. 1C). Harries et al. reported persistent short stature until the age of 27 months in a patient with HPI receiving a low-proline diet. Moreover, van de Ven et al. described a patient with HPI presenting with a variable eating behavior pattern that is consistent with that of our patient. Indeed, our patient preferred a carbohydrate-rich diet and disliked protein-rich foods. The manifestations of short stature and food preference may be derived from HPI, but more case reports are needed to clarify this association.

In conclusion, we encountered a case of HPI that was first detected through plasma amino acid analysis performed during the detailed evaluation of short stature in a child. Mild intellectual disability, mild learning disorders, autism tendencies, and attention-deficit hyperactivity disorder (ADHD) tendencies are considered phenotypes related to HPI. The patient's unique dietary habit is also thought to be one of the phenotypes of HPI, and blood proline levels vary depending on the dietary content. As very few cases of HPI have been reported to date, other patients may display as-yet-unidentified phenotypes. The accumulation of more cases is essential to further our understanding of the clinical characteristics of HPI.
**Fig. 1** Clinical manifestations in our case. 

A. Brain MRI (T2-weighted image).

B. Sanger sequencing. The patient’s father and mother carried a heterozygous mutation of c.1397 C>T (p.T466M) in the PRODH gene.

C. The growth curve of the patient from birth. Filled circles, height measurements; open circles, weight measurements. These circles are superimposed onto the Cross-sectional Growth Chart for Boys (0–18 y) provided by the 2000 National Growth Survey on Preschool Children & School Health Statistics Research. The height of our patient was −2.0 SD of the mean height for his age among Japanese male children. The dotted lines at −2.5 SD and −3.0 SD of height indicate the criteria for starting growth hormone (GH) treatment for GH insufficiency and achondroplasia. SD: standard deviation.
Table 1B. PRODH genetic variants, detected in our patient.

| RefSNP ID  | Nucleic acid | Amino acid | Location | Allele frequency in controls (%) | ClinVar       | PolyPhen-2 (Score) | Human Splicing Finder |
|------------|--------------|------------|----------|----------------------------------|---------------|-------------------|-----------------------|
| rs2008720  | c.56C>A      | p.P19Q     | Exon 2   | 83.68±5ab, 29c                  | Benign        | −                 | −                     |
| rs4819756  | c.553T>C     | p.W185R    | Exon 5   | 96.72±5ab, 48, 33.14d           | Benign        | −                 | −                     |
| rs1808320  | c.991T>C     | p.L331=    | Exon 9   | 83.74±5ab                       | −             | −                 | −                     |
| rs1076466  | c.1105→14C>T | p.T466M    | Exon 12  | 2±5.36±5ab, 1.40±5d             | Conflcting interpretations of pathognicity | Possibly damaging (0.943) |
| rs2870984  | c.1397C>T    | p.T466M    | Exon 12  | 96.11±5ab, 5.37±5d              | Benign        | −                 | −                     |
| rs455072   | c.1515T>C    | p.F505=    | Exon 13  | 92.06±5ab                       | −             | −                 | −                     |
| rs1808320  | c.991T>C     | p.L331=    | Exon 9   | 83.74±5ab                       | −             | −                 | −                     |
| rs450046   | c.1562G>A    | p.R521Q    | Exon 14  | 96.11±5ab, 5.37±5d              | Benign        | −                 | −                     |
| rs372055   | c.1741C>T    | p.L581=    | Exon 15  | 79.59±5ab, 28.49±5              | −             | −                 | −                     |

The variants which detected in our patient were all of homozygous. The c.1562 G > A (p.R521Q) was found in family members including the patient’s father, mother, brother, and sister as homozygous. They did not present with hyperprolinemia.

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Table 1C. Reported cases of HPI with p.T466M variant.

| Patient No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-------------|---|---|---|---|---|---|---|---|---|
| Sex (age at diagnosis) | M (9) | M (6.5) | M (7) | F (13) | M (3) | M (3) | M (9) | M (13) | M |
| Autism      | + | + | + | + | + | + | + | + | N.A |
| Seizure     | − | − | + | + | + | + | + | + | N.A |
| Psychomotor delay | − | − | − | − | − | − | − | − | − |
| Hypotonia   | − | − | − | − | − | − | − | − | − |
| Unbalanced diet | + | N.A | N.A | N.A | N.A | N.A | N.A | N.A | N.A |
| Language disorder | − | Few words | Short sentence | − | − | − | + | − | N.A |
| Intellectual disability | + | + | + | + | + | + | + | + | + |
| Aggressiveness | − | − | − | − | − | − | − | − | − |
| Plasma proline level (μmol/L) | 530–625 | 939–1000 | 595–715 | 637–1,667 | 1,200 | 414–804 | 679 | 605 | N.A |
| MRI         | Normal | CC enlargement | Mild CC enlargement | Normal | Normal | Abnormal | Normal | Normal | N.A |
| 22q11 microdeletion | − | − | − | − | − | − | − | − | − |
| Variants    | T466M/T466M + R521Q/R521Q | T466M + W185R | T466M/T466M + R453C | T466M + R453C/R431H | T466M/R453C + R431H | T466M/R453C + R431H/Q19P | T466M + R453C + W185R + Q19P + P30S/R431H | T466M + Q19P + W185R/R431H | T466M |
| Reference   | This study | Afenjar et al. (2007) | Guilmatre et al. (2010) | Chérot et al. (2018) |

CC: corpus callosum, N.A: not available
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CONFLICT OF INTEREST

All authors declare no competing interests.

ADDITIONAL INFORMATION

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