

# Inborn Errors of Proline Metabolism<sup>1–3</sup>

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## Abstract

L-Proline concentration is primarily related to the balance of enzymatic activities of proline dehydrogenase [proline oxidase (POX)] and  $\Delta$ -1-pyrroline-5-carboxylate (P5C) reductase. As a result, P5C plays a pivotal role in maintaining the concentration of proline in body fluids and inborn errors of P5C metabolism lead to disturbance of proline metabolism. Several inborn errors of proline metabolism have been described. Hyperprolinemia type I (HPI) is a result of a deficiency in POX. The POX gene (*PRODH*) is located on chromosome 22 (22q11.2) and this region is deleted in velo-cardio-facial syndrome, a congenital malformation syndrome. In addition, this gene locus is related to susceptibility to schizophrenia. The other type of hyperprolinemia is HPII. It is caused by a deficiency in P5C dehydrogenase activity. Hypoprolinemia, on the other hand, is found in the recently described deficiency of P5C synthetase. This enzyme defect leads to hyperammonemia associated with hypoorithinemia, hypocitrullinemia, and hypoargininemia other than hypoprolinemia. Hyperhydroxyprolinemia is an autosomal recessive inheritance disorder caused by the deficiency of hydroxyproline oxidase. There are no symptoms and it is believed to be a benign metabolic disorder. The deficiency of ornithine aminotransferase causes transient hyperammonemia during early infancy due to deficiency of ornithine in the urea cycle. In later life, gyrate atrophy of the retina occurs due to hyperornithinemia, a paradoxical phenomenon. Finally, prolidase deficiency is a rare autosomal recessive hereditary disease. Prolidase catalyzes hydrolysis of dipeptide or oligopeptide with a C-terminal proline or hydroxyproline and its deficiency can cause mental retardation and severe skin ulcers. J. Nutr. 138: 2016S–2020S, 2008.

## Introduction

Over 45 y ago, the first case of direct involvement of proline in human disease was reported by Schafer et al. (1) in a family with hyperprolinemia, cerebral dysfunction, renal anomalies, hereditary nephropathy, and deafness. Since then, many families with hyperprolinemia have been reported in the literature. Various phenotypes including asymptomatic members were found among these families. Various authors have discussed the probability of

coincidental association between these clinical features and hyperprolinemia.

Recently, our understanding of proline metabolism, its biological function, and disease association have increased dramatically. Now, at least 6 enzymes, 3 transporters, and 7 structural genes are known to be directly involved in the interconversions of proline and its immediate metabolites. The functional characteristics, tissue distribution, regulation, and subcellular location of these proteins have been determined and structural information is available for some (2).

### L-Proline metabolism and human disease

Monogenic human inborn errors of metabolism of L-proline are known for hyperprolinemia type I (HPI),<sup>4</sup> HPII,  $\Delta$ 1-pyrroline-5-carboxylic acid (P5C) synthetase deficiency, ornithine aminotransferase (OAT) deficiency, hydroxyprolinemia, and iminoglycinuria. Some neuropsychiatric disorders are also related to hyperprolinemia. Meanwhile, prolidase deficiency is characterized by massive imidodipeptiduria containing excessive amounts of proline. The characteristics and molecular biology of these enzymes in humans are summarized (Table 1).

Hyperprolinemia is present in 2 inherited metabolic disorders. These 2 disorders, HPI and HPII, are defined by distinct

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<sup>4</sup> Abbreviations used: HPI, hyperprolinemia type I; OAT, ornithine aminotransferase; 3OH-P5C,  $\Delta$ -1-pyrroline-3-hydroxy-5-carboxylic acid; P5C,  $\Delta$ -1-pyrroline-5-carboxylate; P5CDh,  $\Delta$ -1-pyrroline-5-carboxylic acid dehydrogenase; PEPD, peptidase D; POX, proline oxidase; VCFS, velo-cardio-facial syndrome.

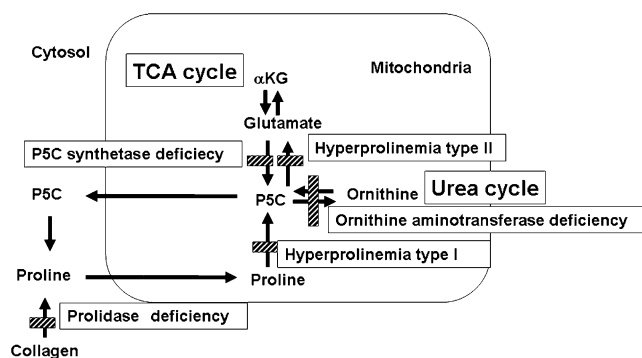
**TABLE 1** Characteristics and molecular biology of human enzymes in proline metabolism

	P5C synthase	P5C reductase	OAT	POX	P5C dehydrogenase	Prolidase
EC	None	EC1.5.1.2	EC2.6.1.13	None	EC1.5.1.12	EC3.4.13.9
Subcellular location	Mitochondrial Inner membrane	Cytoplasm? Membrane associated	Mitochondrial matrix	Mitochondrial inner membrane	Mitochondrial matrix	Cytoplasm
Subunit size, <i>kDa</i>	81	32/35	49	63	62	54.3
Structure	Hexamer	Homopolymer	Homohexamer	Unknown	Homodimer	Homodimer
Cofactors	ATP, NAD(P)H	NAD(P)H	Pyridoxal phosphate	Unknown	NAD+	Mn <sup>2+</sup> , Co <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>2+</sup>
Activity in tissues	Small intestine mucosa, Colon, pancreas, brain, thymus	Ubiquitous	Ubiquitous	Liver, kidney, brain	Ubiquitous	Ubiquitous
Disease association	Hypoprolinemia		Gyrate atrophy	HPI	HPII	Prolidase deficiency
Genbank accession	U76542/U68758	P32322		U79754AF120278.1	U24266	
Map location	10q24.3 to 24.6	17/1	10q26	22q11.2	1p36	19q12–13.11
mRNA, <i>kb</i>	3.6	1.8/1.85	2.0	2.4	3.2	2.1–2.2
ORF, <i>bp</i>	2385/2379	957/960	1317	1800	1689	1479
Comments	2 isoforms results from alternative splicing	2 isoforms encoded by separate genes				

biochemical and genetic deficiencies in the proline catabolic pathway (Fig. 1).

### HPI (MIM 239500)

HPI is caused by a deficiency in proline oxidase [also called proline dehydrogenase (POX)], a mitochondrial inner-membrane enzyme that converts proline to P5C, the first step in the conversion of proline to glutamate. HPI is biochemically diagnosed by high plasma proline levels without urinary excretion of P5C, whereas the presence of P5C in the urine is indicative of HPII. The POX gene, *PRODH*, is localized in the 22q11 chromosomal region, deleted in velo-cardio-facial syndrome (VCFS) (3,4). The clinical phenotype of HPI is still not well characterized. Prior to the identification of *PRODH*, >15 families had been reported in the literature with plasma proline levels ranging from 2 to 10 times above normal values (1,5–15). Various phenotypes were found in these families. Whereas some patients had neurological, renal, and/or auditory defects, others were asymptomatic. Various authors have discussed the probable coincidental association between these clinical features and HPI (9–12). Hyperprolinemia has also been reported in patients with a microdeletion in the 22q11 region (4,16). Since the identification of *PRODH*, various mutations (heterozygous and homozygous) have been identified in hyperprolinemic patients with various phenotypes (patients with schizophrenia, a 22q11 microdeletion, and/or early neurological impairment) (16–19).

**FIGURE 1** L-Proline metabolism and enzyme deficiencies.

Jacquet et al. (16) identified a complete homozygous deletion of *PRODH* located on chromosome 22q11 in a child with a severe form of HPI with severe psychomotor delay and status epilepticus associated with a very high level of plasma proline (2246  $\mu\text{mol/L}$ ). These studies show unambiguously that the severe form of HPI, characterized by neurological manifestations, results from homozygous inactivating alterations of *PRODH* (16).

A recent functional study divided the *PRODH* mutations into 3 groups: those leading to mild (< 30%), moderate (30–70%), and severe (>70%) reduction of POX activity. Serum proline levels seem to correlate with severity of POX deficiency (18,19). By other recent studies, reverse correlation was found between blood proline levels and intelligence quotients in the patients with 22q11 deletion (including the region of *PRODH*) syndrome. In addition, the relationship between *PRODH* and adult or early onset schizophrenia has become clear (18).

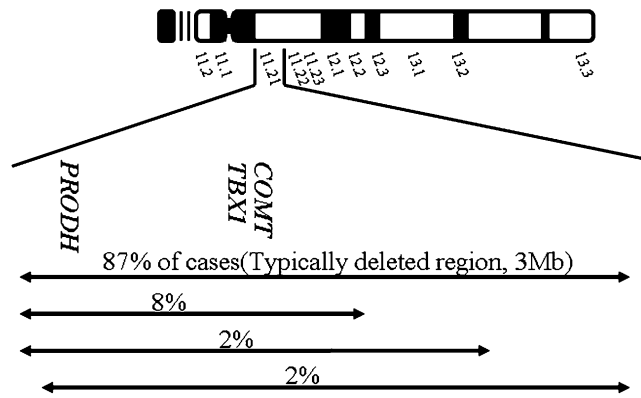
For treatment of HPI, dietary therapies such as restriction of proline have been attempted. However, only modest control of plasma proline was achieved and no impact was made on the clinical phenotype. Some reviewers suggest that dietary therapy is unnecessary (2).

### VCFS

A 22q11 microdeletion causes VCFS, a genetic, autosomal dominant condition defined by Shprintzen et al. (20). They reported 39 patients with a syndrome characterized by some of the following more frequent features: cleft palate, cardiac anomalies, typical facies, and learning disabilities. Less frequent features include microcephaly, mental retardation, short stature, slender hands and digits, minor auricular anomalies, and inguinal hernia. Its frequency is estimated at 1/4000 live births. Most of these deletions occur spontaneously. The typical deleted region in VCFS includes the locus of *PRODH* (4,16,18,20) (location of VCFS deletions in chromosome 22 presented in Fig. 2).

### Neuropsychiatric disorders associated with HPI

The increased prevalence of schizophrenia among patients with 22q11 interstitial deletion associated with DiGeorge syndrome has suggested the existence of a susceptibility gene for schizophrenia within the DiGeorge syndrome chromosomal region on 22q11. This deletion was associated with hyperprolinemia in



**FIGURE 2** Chromosome 22 and the location of VCFS deletions. The positions of candidate genes within the typically deleted region and the frequencies of the deleted regions are also indicated. TBX1, T-BOX transcription factor gene; COMT, catechol-*o*-methyl-transferase gene.

schizophrenic patients. In addition, 2 heterozygous *PRODH* missense mutations (L441P and L289M), detected in 3 of 63 schizophrenic patients but none among 68 controls, were also associated with increased plasma proline levels (16,17).

Bender et al. (21) identified at least 16 *PRODH* missense mutations in studies of HPI and schizophrenia, 10 of which are present at polymorphic frequencies. Although there is limited information on plasma proline levels in individuals of known *PRODH* genotypes, existing data suggest that severe hyperprolinemia (>800  $\mu\text{mol/L}$ ) occurs in individuals with large deletions and/or *PRODH* missense mutations with the most severe effect on function (L441P and R453C), whereas modest hyperprolinemia (300–500  $\mu\text{mol/L}$ ) is associated with *PRODH* alleles with a moderate reduction in activity. Interestingly, 3 of the 4 alleles associated with or found in schizophrenia (V427M, L441P, and R453C) resulted in severe reduction of POX activity leading to hyperprolinemia.

### HPII (MIM 239510)

HPII is due to absence of  $\Delta$ -1-pyrroline-5-carboxylic acid dehydrogenase (P5CDh; EC 1.5.1.12) activity. Valle et. al (22) measured P5CDh activity radioisotopically in normal and HPII fibroblasts. The HPII cells had no detectable activity over a range of reaction conditions, whereas normal cells had easily measurable activity. This enzymatic defect accounts for the biochemical abnormalities in HPII. HPII is usually characterized by mental retardation and convulsions (22).

P5CDh is a mitochondrial matrix NAD<sup>(+)</sup>-dependent dehydrogenase and catalyzes the second step of the proline degradation pathway. Deficiency of this enzyme causes accumulation of P5C and proline (Fig. 1).

Consequently, mutations in the P5CDh gene cause HPII. Geraghty et al. (23) surveyed P5CDh genes from 4 patients with HPII. They found 4 mutant alleles, 2 with frameshift mutations [A7fs(–1) and G521fs(+1)] and 2 with missense mutations (S352L and P16L). To test the functional consequences of 3 of these, they expressed them in a P5CDh-deficient strain of *Saccharomyces cerevisiae*. In contrast to wild-type human P5CDh, yeast expressing S352L and G521fs(+1) did not grow on proline and had no detectable P5CDh activity. Interestingly, the G521fs(+1) allele segregates in the large pedigree of Irish travelers (nomads) used to define the HPII phenotype (23). Flynn et al. (24) reported HPII in another pedigree. They described a

study of 312 subjects in 71 families closely related to a proband with HPII. The subjects were also Irish travelers, among whom consanguineous marriage and high fertility are common. Thirteen additional cases of HPII were discovered. A further 50 subjects were found to have mild hyperprolinemia. They found a strong association between HPII and seizures during childhood but no significant association with mental retardation. Most adults with HPII enjoy normal health (24).

### Vitamin B-6 deficiency and seizures in HPII

P5C is a unique endogenous vitamin antagonist. Vitamin B-6 deactivation may contribute to seizures in HPII, which are so far unexplained and may be preventable with long-term vitamin B-6 supplementation (25). HPII is present with seizures in childhood, usually precipitated by infection. A diagnosis of HPII and vitamin B-6 deficiency has been made in a well-nourished child with seizures. Pyridoxine deficiency is believed to be involved in these seizures, the result of inactivation of vitamin B-6 by the proline metabolite, P5C (26).

### Hydroxyproline metabolism

Hydroxyproline is produced in humans by a post-translational hydroxylation of proline in nascent collagen peptides. A peptidyl-proline hydroxylase catalyzes the reaction. Free hydroxyproline is derived from both endogenous collagen turnover and breakdown of dietary collagen. The hydroxyproline degradation pathway resembles that of proline (2).

The initial step in the degradation pathways of proline and hydroxyproline is catalyzed by POX and hydroxyproline oxidase, yielding P5C and  $\Delta$ -1-pyrroline-3-hydroxy-5-carboxylic acid (3OH-P5C), respectively. The second step is the oxidation of P5C to glutamate and 3OH-P5C to  $\gamma$ -hydroxy-glutamate. A common P5CDh catalyzes the oxidation of both P5C and 3OH-P5C, deficient in HPII. Hydroxyproline metabolism was studied in 2 patients with HPII using oral loadings of hydroxyproline or hydroxyproline-ornithine. Both 3OH-P5C and P5C can be identified in urine. However, the urinary excretion of both 3OH-P5C and P5C increased in HPII patients but not in healthy controls with oral loading of hydroxyproline or hydroxyproline-ornithine. Conversely, plasma levels of proline in patients with HPII are extremely high, but hydroxyproline concentration is normal or only slightly increased. Therefore, one can assume that hydroxyproline is converted to pyrrole-2-carboxylic acid, which is excreted in urine as a glycine conjugate (27). Dooley and Applegarth (28) reported the identification of 3OH-P5C in a previously reported patient with HPII.

### Hyperhydroxyprolinemia (MIM 237000)

Hydroxyproline metabolism was evaluated in 2 sisters with hydroxyprolinemia and their mother. The sisters excreted 33% and 21% of an oral hydroxyproline load (200 mg/kg), 5.4% by the mother, and 1.3% by normal subjects. Plasma and erythrocyte values in the sisters and their mother were elevated, indicating that extra- and intracellular hydroxyproline pools were increased. A deficiency of hydroxyproline oxidase in the 2 sisters was indicated by the lack of 3OH-P5C (29).

### P5C synthetase deficiency (MIM 138250)

Deficiency of P5C synthetase was newly recognized as an inborn error in 2 siblings with progressive neurodegeneration, joint laxity, skin hyperelasticity, and bilateral subcapsular cataracts. Their metabolic phenotype includes hyperammonemia, hypo-ornithinemia, hypocitrullinemia, hypoargininemia, and hypo-prolinemia. Both were homozygous for a missense mutation,

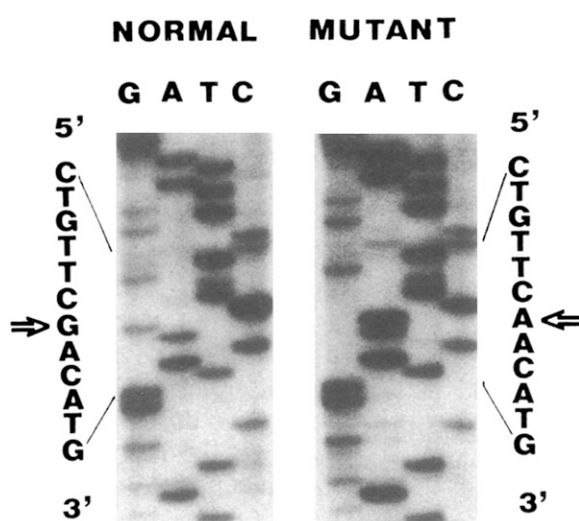
R84Q, in the P5C synthetase gene, which alters a conserved residue in the P5C synthetase  $\gamma$ -glutamyl kinase domain. This was newly recognized as an inborn error of P5C synthetase and should be considered in the differential diagnosis of patients with neurodegeneration and/or cataracts (30).

### OAT deficiency (MIA 258870)

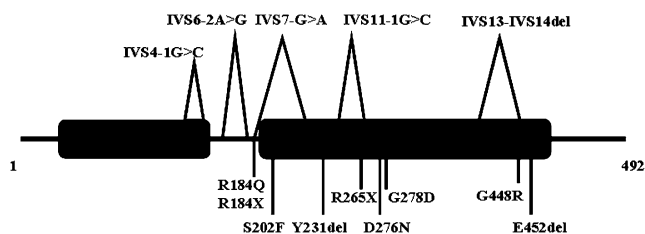
The deficiency of OAT causes transient hyperammonemia during early infancy due to deficiency of ornithine in the urea cycle (Fig. 1). In later life, gyrate atrophy of the retina is due to hyperornithinemia, a paradoxical phenomenon (31).

### Prolidase deficiency (MIM 170100)

Prolidase [imidodipeptidase, peptidase D (PEPD); EC3.4.13.9] deficiency is an autosomal recessive disorder characterized by massive imidodipeptiduria and elevated proline-containing dipeptides in plasma with variable symptoms, including skin lesions, recurrent infections, mental retardation, and abnormalities of collagenous tissues. Prolidase is a ubiquitous cytosolic enzyme that catalyzes hydrolysis of dipeptides with C-terminal proline or hydroxyproline (Fig. 1) (32). The enzyme is encoded by the PEPD gene located at 19q12-q13.11. PEPD spans 130 kb and has 15 exons. The 2.1- to 2.2-kb mRNA is translated into a 493-amino acid protein with a predicted molecular weight of 54.3 kDa. Native prolidase is a homodimer (33–36). We analyzed PEPD in patients with prolidase deficiency. In Japanese female siblings with polypeptide negative prolidase deficiency, we noted an abnormal mRNA with the skipping of a 192-bp sequence corresponding to exon 14 in lymphoblastoid cells taken from these patients (37). Transfection and expression analyses using the mutant prolidase cDNA revealed that a mutant protein translated from the abnormal mRNA had a Mr of 49,000 and was enzymatically inactive. The same mutation caused a wide range of clinical phenotypes of prolidase deficiency in this family, leading us to the conclusion that factor(s) unrelated to the PEPD product also contribute to the development of clinical symptoms. In 2 unrelated patients with polypeptide-positive (cross-reacting material-positive) prolidase deficiency, we identified a G-to-A



**FIGURE 3** A single nucleotide change in the prolidase gene in fibroblasts from patients with prolidase deficiency. A G-to-A substitution at nucleotide 826 in exon 12, resulting in the replacement of aspartic acid by asparagine at amino acid residue 276, was demonstrated.



**FIGURE 4** Mutations in prolidase genes.

substitution at nucleotide 826 in exon 12, resulting in replacement of aspartic acid by asparagine at amino acid residue 276. Both patients were homozygous for this mutation (Fig. 3). Analysis of structural mutation associated with prolidase deficiency in Japanese and other races have been recently reported (Fig. 4) (37–40).

Dietary supplementation with L-proline and essential amino acids, along with ointment containing L-glycine and L-proline as well as erythrocyte transfusions, have been given for treatment of prolidase deficiency. However, effective treatment of this disease has yet to be discovered (32).

Other articles in this supplement include references (41–51).

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