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Hypertryptophanemia due to tryptophan 2,3-dioxygenase deficiency

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ABSTRACT

In this report we describe the first human case of hypertryptophanemia confirmed to be due to tryptophan 2,3-dioxygenase deficiency. The underlying etiology was established by sequencing the *TDO2* gene, in which there was compound heterozygosity for two rare variants: c.324G > C, p.Met108Ile and c.491dup, p.Ile165Aspfs*12. The pathogenicity of these variants was confirmed by molecular-level studies, which showed that c.491dup does not produce soluble protein and c.324G > C results in a catalytically less efficient Met108Ile enzyme that is prone to proteolytic degradation. The biochemical phenotype of hypertryptophanemia and hyperserotoninemia does not appear to have significant clinical consequences.

1. Introduction

L-Tryptophan (L-Trp) is an essential aromatic amino acid. It is a precursor to the neurotransmitters serotonin and melatonin, as well as nicotinamide which is also supplied from niacin in the diet. The overall metabolic pathway is summarized in Fig. 1: about 95% is catabolized via the kynurenine pathway. The first rate-limiting step in this to *N*-formylkynurenine is catalyzed by two heme-containing enzymes: L-tryptophan 2,3-dioxygenase (TDO, EC 1.13.11.11) and indoleamine 2,3-dioxygenase (IDO, EC1.13.11.52). TDO, encoded by the *TDO2* gene, is mainly expressed in the liver, has high substrate specificity for L-tryptophan, and its activity is increased by the substrate, whereas IDO, encoded by the *IDO1* gene, has more widespread tissue distribution, less substrate specificity, and its activity is increased by inflammation [1]. A third enzyme, *IDO2*, encoded by the *IDO2* gene catalyzes the same reaction; although its relative activity is less than IDO, recent work suggests that it has an important immunoregulatory role [2]. TDO activity is thought to be quantitatively more important than IDO activity under normal physiological conditions: using knockout mouse models, it was estimated to account for 75% of tryptophan oxidation [3], and $TDO2^{-/-}$ mice had plasma tryptophan concentrations 9.3 times controls [4], compared with only 1.3 times for $IDO1^{-/-}$ [5].

Hypertryptophanemia has rarely been reported in the medical literature. Tada et al. described a 9-year-old girl with significant hypertryptophanuria and parental consanguinity [6]. She had growth and developmental delay, ataxia, and photosensitive pellagra-like rash with hyperpigmentation, all features clinically suggestive of Hartnup disease. However, unlike that disorder, her fasting plasma tryptophan was high rather than low, at 91 μmol/L (controls 51–81 μmol/L, N = 10), and after tryptophan loading became higher, and for a longer time, than controls, with less kynurenine excretion. There was also no accompanying neutral aminoaciduria. The suggested explanation was a block in the conversion of tryptophan to kynurenine. A 7-year-old boy with somewhat similar clinical and biochemical findings was described by Wong et al., though the fasting tryptophan level was more normal [7]. In this case the renal clearance of tryptophan was documented as being normal, and the photosensitive skin rash was responsive to nicotinamide therapy. Much higher plasma tryptophan levels were observed in two adult siblings, who also had marked hypertryptophanuria, but without other significant aminoaciduria [8,9,10]. Fasting plasma tryptophan in the brother at age 23 was 476.7 μmol/L (mean of 3 measurements), and 406.9 μmol/L at follow

Abbreviations: TDO, tryptophan 2,3-dioxygenase; ITC, isothermal titration calorimetry; L-Trp, L-tryptophan; 5-HIAA, 5-hydroxyindoleacetic acid; αMTrp, α-methyltryptophan.

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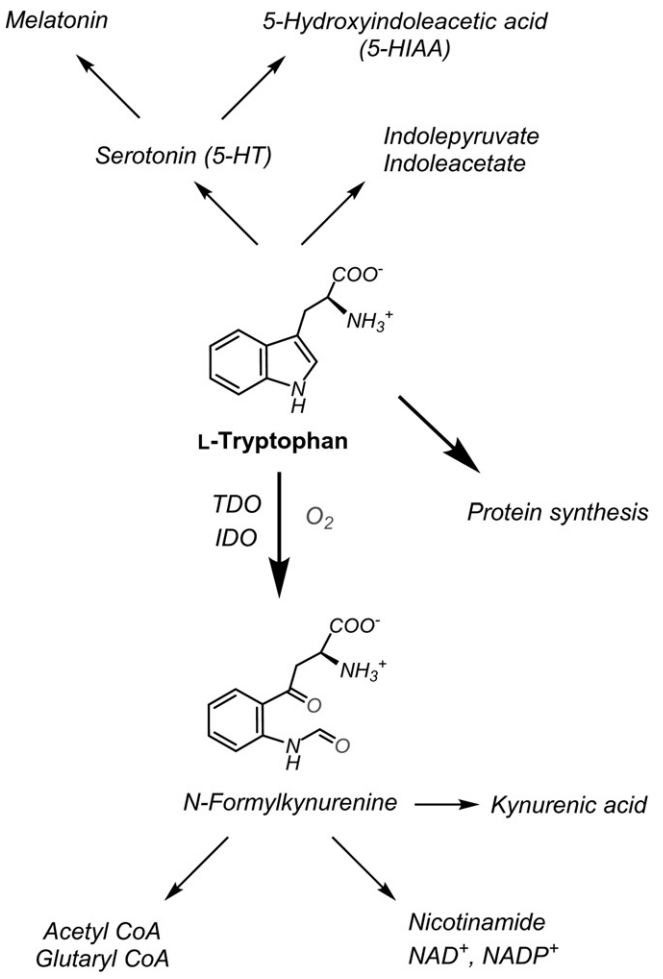

Fig. 1. Fate of L-tryptophan: TDO/IDO-mediated kynurenine pathway is the principle route of catabolism.

up 10 years later. The sister at age 22 had similar levels: the mean of two measurements was 472.5 μmol/L. Both had markedly increased levels of urinary indoles (indolelactate, indolepyruvate, indoleacetate and 5-HIAA), but normal or low kynurenine, 3-hydroxykynurenine, kynurenic acid and xanthurenic acid. They had some similar psychiatric features including descriptions of emotional lability, and hypersexuality, but otherwise their clinical descriptions were very different; a complicating factor in the sister was possible congenital rubella. She had more severe intellectual disability throughout childhood as well as profound sensorineural hearing loss. The brother's growth and development was apparently normal until adolescence when he developed hyperacusis and hyperalgesia, and joint issues including ulnar drift of fingers and limited elbow extension as well hyperflexibility, which may have been a familial trait. He was also noted to have high myopia, strabismus, and ocular hypertelorism. Neither sibling had ataxia or skin rashes.

Tryptophan is not routinely measured or reported in most neonatal metabolic screening programs, though an exception was in Manchester UK where paper chromatography of amino acids was used from 1961 to 1990. Persistently elevated plasma tryptophan (>49 μmol/L) was observed in 12/1,196,113 infants screened (1:99,743), but no consistent adverse effects were apparent, and it was concluded that it was a benign condition [11]. Tryptophanuria, as part of the characteristic pattern of urinary neutral amino acids seen in the renal transport defect Hartnup disease, was reported in 56/2,469,929 (1:44,106) infants in the Quebec urine newborn screening program, but it was never found to be isolated, or secondary to hypertryptophanemia [12].

In this report we describe an apparently asymptomatic adult who has significant chronic hypertryptophanemia and hyperserotoninemia, and we provide evidence that it is due to deficient activity of the TDO enzyme.

2. Methods

2.1. TDO2 sequencing

Genomic DNA was extracted from EDTA anti-coagulant peripheral blood using an Autopure LS System and Gentra Puregene Blood Kit reagents (Quiagen, Toronto, Canada). Bi-directional Sanger sequencing of *TDO2* was undertaken with primers designed by NCBI Primer-BLAST software (sequences are available upon request), the HotStar Plus amplification system (Qiagen, Toronto, ON), and resolution using a 3130xL Genetic Analyzer (Life Technologies, Burlington, ON). Sequence subtraction was performed using Mutation Surveyor V4.05 software (SoftGenetics, State College, PA) and variants analyzed using Alamut Software (Interactive Biosoftware, San Diego, CA).

2.2. Cloning, expression and purification of human TDO

The human TDO gene was purchased from DNASU. The N- and C-terminal truncated variant of TDO (40-390) was amplified by PCR using site specific primers (forward primer, 5′-GGAATTCCATATGCTTATCTATGGGAACTACCTG-3′; reverse primer, 5′-AACCGCTCGAGTTAATATAGAAATTTGTGAATGGTTG-3′ and was introduced into pET28a expression vector (Novagen) which was modified to have a TEV cleavage site behind the N-terminal polyhistidine tag using restriction enzymes *Nde*I and *Xho*I. c.324G > C and c.491dup variants were generated by quick change method (Stratagene). All the constructs generated were verified by DNA sequencing.

Escherichia coli BL21(DE3) cells which were transformed with each construct were grown in Luria-Bertani medium at 37 °C until OD600 reached 0.3, 160 mg of δ-aminolevulinic acid and 14 mg of ferrous ammonium sulfate (Mohr's salt) per liter of culture volume were supplemented. Temperature was lowered down to 20 °C and cell culture was continued for 18 h. Cells were harvested by centrifugation at 8000 ×*g* for 20 min, suspended in 50 mM Tris-HCl, 200 mM NaCl (pH 7.4) and disrupted by an LM20 cell disruptor from Microfluidizer. After centrifugation at 30,000 ×*g* for 1 h, the supernatant was loaded onto a Ni-charged affinity chromatography column (GE Healthcare) and purified by gradient elution with 50 mM Tris-HCl, 200 mM NaCl, 500 mM imidazole (pH 7.4). The elution fraction was desalted using Sephadex G25 column (GE Healthcare) with 50 mM Tris-HCl, 200 mM NaCl, 10% (v/v) glycerol. The purified proteins were frozen by liquid nitrogen or dry ice with ethanol bath and stored at −80 °C for further use.

2.3. HeLa cell expression and qPCR

The native TDO in HeLa cells was knocked down by siRNA. Then, the full-length wild-type TDO and the two variants c.491dup and c.324G > C, were transfected into the HeLa cell line by using lipofectamine 2000. Quantitation of the gene expression was achieved by qPCR analysis.

2.4. Enzyme characterization and activity assays

The catalytically active form of ferrous TDO was prepared from the ferric enzyme by adding sodium dithionite under O_2-free conditions and used for the Michaelis-Menten kinetics analyses on an Aglient 8453 spectrophotometer. The steady-state kinetics experiments were carried out as previously described [13,14,15]. The initial rate was obtained by monitoring the formation of *N*-formylkynurenine at 321 nm with the known extinction coefficient 3150 M^{-1} cm^{-1} [16]. Schlenk line and a gloveless anaerobic chamber (COY Laboratory Products)