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Novel Mutations in the Tyrosine Hydroxylase Gene in the First Czech Patient with Tyrosine Hydroxylase Deficiency

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Abstract: Tyrosine hydroxylase deficiency manifests mainly in early childhood and includes two clinical phenotypes: an infantile progressive hypokinetic-rigid syndrome with dystonia (type A) and a neonatal complex encephalopathy (type B). The biochemical diagnostics is exclusively based on the quantitative determination of the neurotransmitters or their metabolites in cerebrospinal fluid (CSF). The implementation of neurotransmitter analysis in clinical praxis is necessary for early diagnosis and adequate treatment. Neurotransmitter metabolites in CSF were analyzed in 82 children (at the age 1 month to 17 years) with clinical suspicion for neurometabolic disorders using high performance liquid chromatography (HPLC) with electrochemical detection. The CSF level of homovanillic acid (HVA) was markedly decreased in three children (64, 79 and 94 nmol/l) in comparison to

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age related controls (lower limit 218–450 nmol/l). Neurological findings including severe psychomotor retardation, quadruspasticity and microcephaly accompanied with marked dystonia, excessive sweating in the first patient was compatible with the diagnosis of tyrosine hydroxylase (TH) deficiency (type B) and subsequent molecular analysis revealed two novel heterozygous mutations c.636A>C and c.1124G>C in the *TH* gene. The treatment with L-DOPA/carbidopa resulted in the improvement of dystonia. Magnetic resonance imaging studies in two other patients with microcephaly revealed postischaemic brain damage, therefore secondary HVA deficit was considered in these children. Diagnostic work-up in patients with neurometabolic disorders should include analysis of neurotransmitter metabolites in CSF.

Introduction

Paediatric neurotransmitter disorders refer to an inherited group of neurometabolic syndromes attributable to a disturbance of neurotransmitter metabolism. Ten enzyme deficiencies in the pathway of the biogenic amines metabolism have been described, until now (Pearl et al., 2007). The enzyme tyrosine hydroxylase (TH; EC 1.14.162) catalyzes the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-dopa), which is the rate-limiting step in the biosynthesis of the catecholamines dopamine, norepinephrine and epinephrine.

Human tyrosine hydroxylase deficiency (THD; OMIM 191290) is an autosomal recessive neurometabolic disorder due to mutations in the *TH* gene on chromosome 11p15.5 (Lüdecke et al., 1996). Many different features of THD (hypokinesia, bradykinesia, rigidity, dystonia, chorea, tremor, oculogyric crises, ptosis and hypersalivation, among others) are caused by cerebral dopamine and norepinephrine deficiency (Grattan-Smith et al., 2002). After careful evaluation of the detailed case histories in the literature, it was possible to class the different phenotypes at presentation into two major groups: an infantile progressive hypokinetic-rigid syndrome with dystonia (type A) and a neonatal complex encephalopathy (type B). In almost all patients with type A THD, treatment with L-dopa results in an excellent response, sometimes even a miraculous improvement of the neurological condition. In type B, L-dopa treatment does not improve all signs equally, and it may take months before all effects of treatment become clear (Willemsen et al., 2010).

Tyrosine hydroxylase deficiency can be diagnosed by demonstrating decreased CSF levels of the metabolites of the catecholamine degradation pathway (Figure 1), homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) and by mutation analysis of the *TH* gene. Since dopamine suppresses the release of prolactin, THD may lead to hyperprolactinaemia (Hyland, 1999). Disease in affected infants may remain undiagnosed because extrapyramidal or parkinsonian symptoms do not predominate in that age group. Likely misdiagnoses are suspicion of unexplained neonatal death, neuromuscular disorders, or cerebral palsy (CP)

(Hoffmann et al., 2003). Hence, the implementation of neurotransmitter analysis in routine clinical praxis is necessary for early diagnosis and adequate treatment.

Up to now THD has been reported in about 50 patients worldwide. Here we present the first Czech patient with TH deficiency confirmed on molecular level. Two novel mutations in the *TH* gene were found.

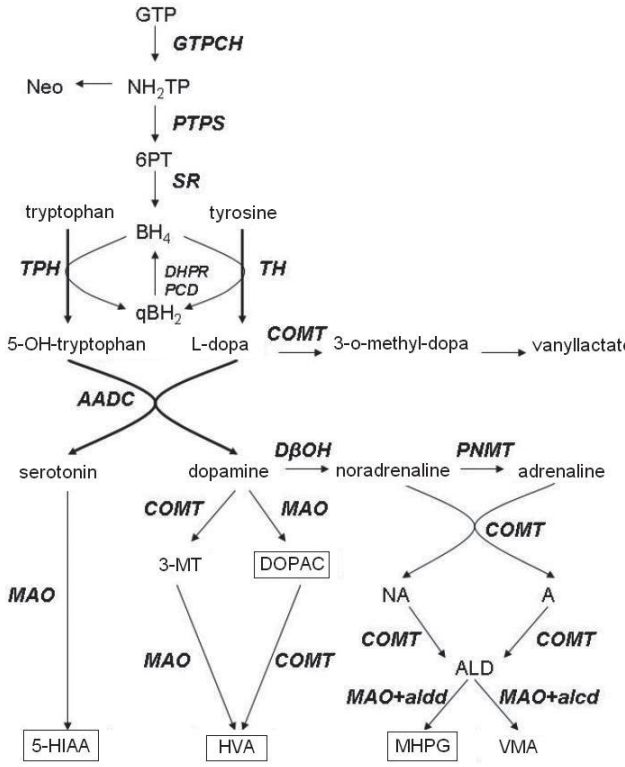

Figure 1 – Metabolism of biogenic amines (adjusted from Hoffmann et al., 2003). Enzymatic defects indicated in bold and italic type. The metabolites in boxes can be measured in our laboratory.
Neo – neopterin; GTP – guanosine triphosphate; NH2TP – dihydroneopterintriphosphate; DHPR – dihydropteridine reductase; BH4 – tetrahydrobiopterin; qBH2 – quinoid dihydrobiopterin; GTPCH – GTP cyclohydrolase I; PTPS – 6-pyruvoyltetrahydropterinsynthase; 6-PT – 6-pyruvoyltetrahydropterin; SR – sepiapterin reductase; TH – tyrosine hydroxylase; TPH – tryptophan hydroxylase; AADC – aromatic L-amino acid decarboxylase; NA – noradrenaline; A – adrenaline; COMT – catechol-ortho-methyltransferase; MAO – monoamine oxidase; 3-MT – 3-methoxytyramine; DβOH – dopamine-β-hydroxylase; PNMT – phenylethanolamine N-methyltransferase; DOPAC – 3,4-dihydroxyphenylacetic acid; HVA – homovanillic acid; 5-HIAA – 5-hydroxyindolacetic acid; MHPG – 3-methoxy-4-hydroxy-phenylglycol; ALD – intermediate aldehyde (3-methoxy-4-hydroxyphenyl-hydroxyacetat-aldehyde); VMA – vanillylmandelic acid; aldd – aldehyd dehydrogenase (CNS); alcd – alcohol dehydrogenase (periphery)

Material and Methods

Patients studied group

Altogether 82 children (girls/boys: 43/39) suspected from neurometabolic disorder entered in this study aged from one months to 17 years (median 1.3 years). The CSF level of homovanillic acid (HVA) was markedly decreased in three children (64, 79 and 94 nmol/l) in comparison to age related controls (lower limit 218–450). In only one patient the clinical presentation was consistent with the suspected diagnosis of THD. MRI studies in two other patients with microcephaly revealed postischaemic brain damage, therefore secondary HVA deficit was considered in these children. Informed consent was obtained from parents.

Biochemical investigations

Serum prolactin was estimated by chemiluminescence immunoanalysis on Centaur analyzer (CLIA Centaur, Siemens). CSF specimens were collected according to previously described protocol (Hyland, 2008). Neurotransmitter metabolites in CSF were measured by isocratic HPLC system using Phenomenex reversed-phase column (C18, 250 mm × 2 mm; 4 µm). Mobile phase (pH 5.1) consisted of 28 mmol/l citrate acid, 83 mmol/l sodium acetate, 100 µmol/l EDTA and methanol (85/15 v/v). Detection was performed by amperometric electrochemical detector Gilson 712 (850 mV) at a flow of 0.3 ml/min. Data were automatically collected and processed using software Gilson Appl. 712 (Figure 2A and 2B).

Molecular investigations

Total genomic DNA was isolated from blood lymphocytes by phenol-chloroform extraction. All 12 exons and adjacent intronic regions of the *TH* gene were amplified by PCR and analyzed by direct sequencing at genetic analyzer ABI3100 Avant (Applied Biosystems, USA). PCR primers were designed with use of Primer3 software. The presence of mutation c.636A>C (p.Gln212Pro) was confirmed by PCR-RFLP with use of specific endonuclease *Bsrl*. The presence of mutation c.1124G>C (p.Glu375Gln) was confirmed by HRM method at LightScanner instrument Idaho Technology, Inc. (Rozen and Skaletsky, 2000).

Case report

Six-year-old boy was born prematurely to healthy non-consanguineous parents after 31 weeks of gestation by caesarean section because of foetal distress. His birth weight (1,100 g) and birth length (34 cm) fulfilled the criteria for IUGR (–2 SDS). Perinatal respiratory distress with apnoea claimed ventilatory support for a few days. Postnatal brain ultrasonography was normal. Moderate muscular hypotonia and feeding difficulties were considered as a sequel of perinatal events. Muscle hypotonia persisted and at the age of three months he was diagnosed with psychomotor delay. Neurological picture during the first year of life developed into quadruspasticity, hypokinesia, facial hypomimia, dystonia, ptosis, microcephaly and

severe psychomotor retardation although thoroughgoing rehabilitation. Spasticity was prominent on lower limbs, where the contractures of hip adductors were present. Marked hypertonia due to spasticity was emphasized by generalized dystonia. The ability to walk or even crawl was not achieved. He was followed up with the diagnosis of cerebral palsy. The child remained alert and euphoric, although he had prolonged periods of lethargy with increased sweating, drooling,

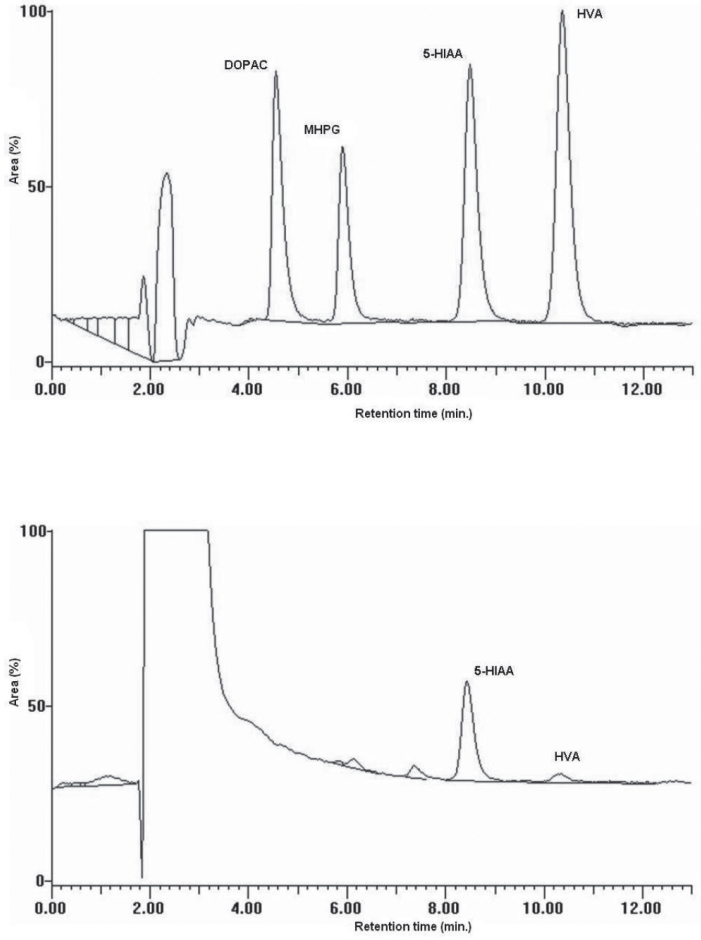

Figure 2 – Chromatograms of neurotransmitter metabolites.
A: Chromatogram of a mixture of standard compounds. B: Chromatogram of cerebrospinal fluid from patient with tyrosine hydroxylase deficiency. Note the normal concentration of 5-hydroxyindoleacetic acid and the very low concentration of homovanillic acid. DOPAC – 3,4-dihydroxyphenylacetic acid; MHPG – 3-methoxy-4-hydroxyphenylglycol; HVA – homovanillic acid; 5-HIAA – 5-hydroxyindolacetic acid

nasal and oropharyngeal secretions alternating with irritability, sporadic dystonic movements and oculogyric crisis accompanied with failure to thrive. At the age of 24 months magnetic resonance imaging of the brain, electroencephalographic and neurophysiologic examinations were unremarkable. At six years of age his anthropometric parameters were severely reduced (height 94.5 cm, weight 10.2 kg, head circumference 47.5 cm; all parameters below 3rd percentile, <–3 SD). The presence of marked generalized dystonia with diurnal fluctuation accompanied with disturbed autonomic functions and findings of elevated serum prolactin level led us to indicate CSF neurotransmitter metabolites analysis. This revealed severe impairment in dopamine biosynthesis, suggesting THD, which was confirmed by molecular analysis. Therapy with low-dose L-dopa (1 mg/kg body weight and day) together with the decarboxylase inhibitor carbidopa was initiated. After six month of therapy we could only very slightly increase the initial dose of L-dopa to 1.85 mg/body weight and day in five doses. Higher doses were not tolerated because of increasing hyperkinesia and irritability as a side effect of medication. Over this period, there was a slow overall improvement. The severity of dystonia, lethargy, drooling and sweating was substantially reduced. Psychosocial interaction improved. The boy developed more vocalization and facial motility. However, the motor impairment of the child is still severe.

Results

Clinical findings in our patient fulfilled criteria for THD type B "complex encephalopathy with onset in the neonatal period". The presenting clinical features and response to treatment in up to date described patients with THD type B (Willemsen et al., 2010) is compared with our patients. Data are summarized in Table 1.

Biochemistry

The biochemical analyses showed increased level of serum prolactin (37 μg/l; controls <12 μg/l). At diagnosis, CSF neurotransmitter determination revealed very low level of HVA (64 nmol/l; ref. range 233–928 nmol/l) and normal 5-HIAA (209 nmol/l; ref. range 74–345 nmol/l) (Figure 2B). After six months of treatment with L-dopa CSF HVA concentration increased twice to 115 nmol/l. Phenylalanine and tyrosine in serum and CSF were normal. Lactate and alanine in both serum and CSF were not elevated.

Mutation analysis

The sequencing of all exons of *TH* gene revealed two novel heterozygous mutations c.636A>C and c.1124G>C. The *healthy parents* were shown to be *heterozygous* for different *mutation.* The father is a *healthy carrier* with the mutation c.636A>C and the mother is a healthy carrier with the mutation c.1124G>C. None of both mutations were found in 200 control samples.

Table 1 – Clinical characteristics and response to treatment in the first Czech patient with tyrosine hydroxylase deficiency (THD) type B in comparison to patients from literature (Willemsen et al., 2010)

Clinical data	Czech patient	THD type B
Sex, pregnancy, delivery, neonatal period	n=1	n=11
male/female	male	8/3
preterm birth (<37 weeks)	+	4
foetal distress (meconium, heart rate abnormalities)	+	6
perinatal asphyxia	+	3
Presenting symptoms/signs		
age at onset	+	neonatal–3 mon.
age at onset >12 months	–	0
diurnal fluctuation	+	5
oculogyric crisis	+	6
ptosis	+	5
autonomic disturbances	+	6
lethargy-irritability crises	+	4
sleep disturbances	+	3
seizures	–	2
length <–2 SD	+	4
weight <–2 SD	+	3
head circumference <–2 SD	+	4
Follow-up with medication		
age at start L-dopa	6 years	6 mon.–7 years
chronic L-dopa dose (mg/kg/day)	1.85	0.5–20
L-dopa response: good/moderate/none	moderate	2/5/4
L-dopa response: within 1 week/within 2 weeks/after 2 weeks	after 2 weeks	0/0/7

Discussion

Clinical course

The first Czech patient with THD is described. The boy presented in newborn period after complicated perinatal history with hypotonia progressing into infantile encephalopathy dominated by severe psychomotor retardation, hypokinesia, facial hypomimia, generalized dystonia, ptosis, disturbed autonomic functions and oculogyric crisis. Patient showed marked hypersensitivity to L-dopa. Dosage could be only increased over months. After weeks of therapy the first improvement was observed. Patient is to be clearly classified as type B THD.

Indication of CSF neurotransmitter metabolites analysis

THD leads to symptoms developing in early life, generally in infancy, but sometimes immediately after birth or in neonatal period. No patients identified to date have

presented in adolescence or adulthood. Since THD is rare and its features overlap with many other neurological disorders, the diagnosis will generally not be made on clinical grounds alone. The differential diagnosis of THD in neonates or very young infants with type B presentation initially encompasses a long list of progressive as well as stable, hereditary as well as acquired disorders (Willemsen et al., 2010).

Type B THD is often accompanied by perinatal complications, which may further distract the attention in the direction of common infectious or hypoxic-ischaemic encephalopathies. Type B THD can mimic several genetic and congenital disorders, first of all mitochondrial disorders (García-Cazorla et al., 2008). Only extensive work-up, including cerebral imaging and screening for inborn errors of metabolism, including CSF analysis, will lead to the correct diagnosis. In type A patients on the other end of the spectrum, the children with "parkinsonian" features and L-dopa-responsive dystonia, clinical recognition of the diagnosis might be easier. Importantly, THD with a relatively mild course can strongly mimic dyskinetic cerebral palsy, which may lead to serious diagnostic delay (Willemsen et al., 2010). In our patient the diagnostic delay was 6 years. In the past decade, the prevalence of cerebral palsy in Europe has remained at rate of approximately two in every 1,000 live births (Surveillance of Cerebral Palsy in Europe, 2002). Dyskinetic CP is one of the most disabling forms of CP accounting for 3–15% of children with CP (Himmelmann et al., 2007). In addition, from the recent study, it can be concluded that prevalence of dyskinetic cerebral palsy, which occurs mostly in near-term children with birth weight of ≥2,500 g, appears to be increasing. Perinatal adverse events tend to be more common in dyskinetic cerebral palsy than in other types of cerebral palsy (Himmelmann et al., 2009). This substantial similarity in clinical course of especially dyskinetic cerebral palsy with type B THD makes the indication of CSF neurotransmitter metabolites considerable and to be recommended, because early diagnosis and treatment of THD could improve the final outcome with regard to motor as well as cognitive functions (Willemsen et al., 2010). There is clear accordance with the suggestion from Willemsen et al., as they strongly advise performance of a lumbar puncture in children with otherwise unexplained (simple and complex) movement disorders, to diagnose or rule out potentially treatable conditions like THD, while the lack of abnormalities on cerebral imaging studies and a marked responsiveness to L-dopa are clues to the disorders of neurotransmitter biosynthesis as a group.

Besides cerebral palsy, the differential diagnosis among genetic disorders includes GTP cyclohydrolase deficiency and other defects (Muller et al., 1998; Albanese et al., 2006; Tarsy and Simon, 2006; Muller, 2009).

Sample collection and processing

Many factors have been suggested to affect concentration of neurotransmitter metabolites in CSF (Bertilsson and Asberg, 1984; Chotai et al., 2006). One of the most important factors is the rostrocaudal gradient for HVA and 5-HIAA within

the spinal cord. Values double with approximately every 5–10 ml of CSF drawn (Brautigam et al., 1998). For this reason, it is essential that patient data is compared to reference intervals obtained using the same fraction of CSF (Hyland, 2008).

Neurotransmitter metabolites are relatively stable in CSF, but blood contamination during collection of CSF can lead to oxidation of metabolites if the red blood cells are allowed to haemolyse. Blood contaminated samples should, therefore, be centrifuged as soon as possible after sample collection and the clear CSF should be transferred to a new container before freezing. Metabolite concentrations during analysis are stable for at least 24 hours if kept at 4 °C (Brautigam et al., 1998) and samples can be frozen and thawed several times without changes in 5-HIAA and HVA (Strawn et al., 2001).

Among laboratory procedures for biogenic amines analysis, HPLC with ECD detection is probably optimal, since it combines simplicity with good separation and detection limits (Hyland et al., 1993; Candito et al., 1994; Ormazabal et al., 2005; Marín-Valencia et al., 2008).

Secondary abnormalities of biogenic amines metabolism

In our studied group the CSF level of homovanillic acid (HVA) was markedly decreased in three children in comparison to age related controls. MRI studies in two patients with microcephaly revealed postischaemic brain damage, therefore secondary HVA deficit was considered.

The changes in CSF serotonin and catecholamine metabolites can occur as a consequence of problems in other areas of metabolism (Hyland, 2008). Young children with diverse neurological manifestations may have reduced synthesis of brain biogenic amines. Newborns, patients with severe motor disturbances, or disorders causing MRI abnormalities are more likely to have these alterations. Careful studies with selected and homogenous groups of patients are needed to define which pathological entities or clinical and neuroimaging factors could be related to low biogenic amine metabolites (García-Cazorla et al., 2007). Earlier observations suggested that a decrease of HVA is a secondary or epiphenomenon in a number of clinical disorders (Van Der Heyden et al., 2003; Marín-Valencia et al., 2008). There is growing evidence suggesting a relationship between mitochondria and the neurotransmitter system. It seems possible, that abnormal neurotransmission occurs in respiratory chain defects, as sustained neurotransmission is an energetically demanding process, moreover, mitochondria are critical in maintaining normal levels of neurotransmitter release during intense activity (Chang et al., 2006; Ly and Verstreken, 2006; García-Cazorla et al., 2008). Disturbation in other neurotranmitter metabolites was observed as a common biochemical finding among patients with congenital and genetic neurological conditions. Regarding metabolic diseases, mitochondrial disorders seem especially vulnerable, as well as those involving the cerebellum, white matter, and organic acidurias (De Grandis et al., 2010).

Conclusion

Here we present the first Czech patient with TH deficiency (type B) confirmed on molecular level. Two novel mutations in the *TH* gene were found. The follow up-diagnosis of atypical dyskinetic cerebral palsy with almost normal brain imaging and disturbed autonomic functions led us to investigate for paediatric neurotransmitter disorders. The treatment (L-dopa/carbidopa) was commenced immediately with obvious improvement concerning the alleviation of painful dystonia. Diagnostic work-up in children with atypical (especially incoherent findings in patients with cerebral palsy) or otherwise unexplained movement disorder should include analysis of neurotransmitter metabolites in CSF. Only early diagnosis and adequate treatment can provide benefit for patients and improve their prognosis.

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