

# Tyrosine hydroxylase deficiency: a treatable disorder of brain catecholamine biosynthesis

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**Tyrosine hydroxylase deficiency is an autosomal recessive disorder resulting from cerebral catecholamine deficiency. Tyrosine hydroxylase deficiency has been reported in fewer than 40 patients worldwide. To recapitulate all available evidence on clinical phenotypes and rational diagnostic and therapeutic approaches for this devastating, but treatable, neurometabolic disorder, we studied 36 patients with tyrosine hydroxylase deficiency and reviewed the literature. Based on the presenting neurological features, tyrosine hydroxylase deficiency can be divided in two phenotypes: an infantile onset, progressive, hypokinetic-rigid syndrome with dystonia (type A), and a complex encephalopathy with neonatal onset (type B). Decreased cerebrospinal fluid concentrations of homovanillic acid and 3-methoxy-4-hydroxyphenylethylene glycol, with normal 5-hydroxyindoleacetic acid cerebrospinal fluid concentrations, are the biochemical hallmark of tyrosine hydroxylase deficiency. The homovanillic acid concentrations and homovanillic acid/5-hydroxyindoleacetic acid ratio in cerebrospinal fluid correlate with the severity of the phenotype. Tyrosine hydroxylase deficiency is almost exclusively caused by missense mutations in the *TH* gene and its promoter region, suggesting that mutations with more deleterious effects on the protein are incompatible with life. Genotype–phenotype correlations do not exist for the common c.698G>A and c.707T>C mutations. Carriership of at least one promoter mutation, however, apparently predicts type A tyrosine hydroxylase deficiency. Most patients with tyrosine hydroxylase deficiency can be successfully treated with L-dopa.**

**Keywords:** tyrosine hydroxylase; neurotransmitters; cerebrospinal fluid; dystonia; L-dopa

**Abbreviations:** 5HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylethylene glycol; THD = tyrosine hydroxylase deficiency

## Introduction

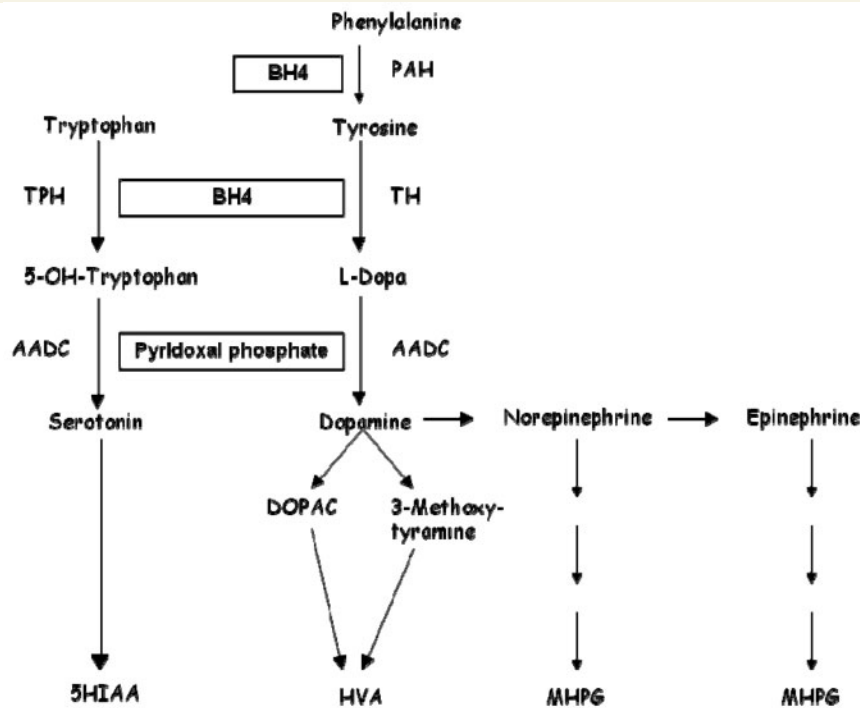
The enzyme tyrosine hydroxylase (EC 1.14.16.2) catalyzes the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-dopa), which is the rate-limiting step in the biosynthesis of the catecholamines dopamine, norepinephrine and epinephrine (Fig. 1). Catecholamines are produced in the brain and adrenal medulla, but also in non-neuronal, e.g. renal, intestinal and lymphoid tissues. Their vital functions as neurotransmitters and hormones, and the crucial role of tyrosine hydroxylase in their biosynthesis are demonstrated by the observation that complete loss of tyrosine hydroxylase activity is lethal in knock-out mice (Zhou *et al.*, 1995).

Human tyrosine hydroxylase deficiency [THD; Online Mendelian Inheritance in Man (OMIM) number 191290] is an autosomal recessive neurometabolic disorder due to mutations in the *tyrosine hydroxylase* (*TH*) gene on chromosome 11p15.5. The first reports of THD described patients with an early onset, progressive L-dopa-responsive dystonia (Castaigne *et al.*, 1971; Rondot and Ziegler, 1983; Rondot *et al.*, 1992). Later, neonates were recognized with a more severe phenotype described as progressive, L-dopa-non-responsive encephalopathy (Hoffmann *et al.*, 2003). THD can be diagnosed by demonstrating decreased CSF levels of the down-stream metabolites of the catecholamine degradation pathway (Fig. 1), i.e. homovanillic acid (HVA) and

3-methoxy-4-hydroxyphenylethylene glycol (MHPG) and by mutation analysis of the *TH* gene.

THD has been reported in fewer than 40 patients worldwide, reviewed in this article. This disorder is known under different names in the literature, namely 'Segawa syndrome', 'infantile parkinsonism' and 'L-dopa-responsive dystonia'. 'Segawa syndrome', however, is also used to indicate another defect in neurotransmitter biosynthesis, caused by *GTP cyclohydrolase 1* mutations. 'Infantile parkinsonism' and L-dopa-responsiveness are not found in all patients with THD. Furthermore, the L-dopa-responsive dystonias encompass a heterogeneous class of movement disorders, THD being only one of them (Muller *et al.*, 1998; Albanese *et al.*, 2006; Tarsy and Simon, 2006; Muller, 2009). Finally, the phenotype of THD can be so complex that it is not simply associated with an extrapyramidal movement disorder (Hoffmann *et al.*, 2003). Altogether, we prefer to name the disorder after its underlying enzymatic defect, as is common practice for inborn errors of metabolism.

In this article, we summarize the medical literature on human THD, and include detailed novel clinical, biochemical and genetic data on thus far unpublished patients. This large review on THD recapitulates all available evidence on clinical phenotypes and rational diagnostic and therapeutic approaches of this severe, but potentially treatable disorder.



**Figure 1** Simplified scheme of the biosynthesis and catabolism of serotonin and the catecholamines dopamine, norepinephrine and epinephrine. TPH = tryptophan hydroxylase; AADC = aromatic amino acid decarboxylase; PAH = phenylalanine hydroxylase; TH = tyrosine hydroxylase; BH4 = tetrahydrobiopterin; DOPAC = 3,4-dihydroxyphenylacetic acid.

## Methods

Our laboratory has a longstanding tradition of neurotransmitter analysis, and we have been performing *TH* gene mutation analysis since the genetic basis of THD was elucidated. This history supplies us with a unique database including patients with biochemically and genetically proven THD from many different countries.

The laboratory methodologies used for CSF neurotransmitter analysis have previously been reported in detail (Brautigam *et al.*, 1998; Verbeek *et al.*, 2008). Reference values for HVA and 5-hydroxyindoleacetic acid (5HIAA; end-product of serotonin degradation) in CSF decrease with age, and there is a rostrocaudal gradient for the concentrations of both metabolites, necessitating analysis of a standardized CSF fraction (Brautigam *et al.*, 1998). Mutation analysis of the *TH* gene was performed as previously described (van den Heuvel *et al.*, 1998). Numbering of coding sequence mutations was according to GenBank reference sequence NM\_199292.1 (tyrosine hydroxylase isoform A) in which the A of the ATG transcription initiation codon is designated position 1. Mutations were named according to the guidelines of the Human Genome Variation Society ([www.hgvs.org](http://www.hgvs.org)).

A questionnaire was sent to all physicians who have referred (samples from) THD patients to our centre. In this way we collected detailed information on demographic data, pregnancy and perinatal period, presenting clinical features, mode of treatment, follow-up during treatment and results of cerebral imaging studies. The results of CSF and mutation analysis were available in our database. In those few patients in whom CSF analysis was performed elsewhere, the results were obtained together with the appropriate reference values.

A Pubmed search was performed for reports in English, using the terms: 'tyrosine hydroxylase recessive' and 'tyrosine hydroxylase dystonia'. The reference lists of all relevant papers were checked for other citations, especially those reports from the era before THD was recognized as a separate disease entity.

This study was approved by the ethics committee of the Radboud University Nijmegen Medical Centre, The Netherlands. The requirement for additional local ethical approval differed between participating countries and was obtained if required.

## Results

We had the names of 36 patients with THD in our database. Questionnaires were completed by the referring physicians of all patients.

## Literature

Besides reports concerning patients who had been diagnosed in our laboratory (Table 1) (Castaigne *et al.*, 1971; Rondot and Ziegler, 1983; Rondot *et al.*, 1992; van den Heuvel *et al.*, 1998; Brautigam *et al.*, 1999; Wevers *et al.*, 1999; de Lonlay *et al.*, 2000; de Rijk-Van Andel *et al.*, 2000; Dionisi-Vici *et al.*, 2000; Janssen *et al.*, 2000; Swaans *et al.*, 2000; Haussler *et al.*, 2001; Grattan-Smith *et al.*, 2002; Hoffmann *et al.*, 2003; Schiller *et al.*, 2004; Verbeek *et al.*, 2007; Zafeiriou *et al.*, 2009), we only found 14 other THD patients from 12 families in whom the diagnosis was genetically proven (Ludecke *et al.*, 1995, 1996; Knappskog

Table 1 Clinical characteristics, demographic data and results of mutation analysis in 36 patients with THD

Patient (family) birth	Year of birth	Origin	Phenotype	Age at onset	I-dopa-response	Note	Allele 1	Allele 2	References <sup>a</sup>
1 (1)	1962	French	A	5 years	Good		c.1010G>A/p.Arg337His	c.1481C>T/p.Thr494Met	Castaigne et al., 1971 (Case 2); Rondot and Ziegler, 1983 (Case 1); Rondot et al., 1992 (Case 2); Swaans et al., 2000 (Case 2)
2 (2)	1950	Swiss	A	3 years	Good		c.1127C>T/p.Ala376Val	c.1493A>G/p.Asp498Gly	Schiller et al., 2004 (Case 1)
3 (2)	1961	Swiss	A	3 years	Good		c.1127C>T/p.Ala376Val	c.1493A>G/p.Asp498Gly	Schiller et al., 2004 (Case 2)
4 (1)	1965	French	A	2 years	Good		c.1010G>A/p.Arg337His	c.1481C>T/p.Thr494Met	Castaigne et al., 1971 (Case 1); Rondot and Ziegler, 1983 (Case 2); Rondot et al., 1992 (Case 1); Swaans et al., 2000 (Case 1)
5 (3)	1965	Belgian	A	1.5 year	Good		c.826A>C/p.Thr276Pro	c.941C>T/p.Thr314Met	Swaans et al., 2000 (Case 3)
6 (4)	1989	Turkish	A	> 1 year	Good		c.-70G>A	c.-70G>A	Verbeek et al., 2007
7 (5)	2002	Lebanese	A	<12 months	Good		c.-70G>A	c.-70G>A	Verbeek et al., 2007
8 (6)	2004	German	A	9 months	Good		c.680A>G/p.Asp227Gly	c.698G>A/p.Arg233His	
9 (7)	1990	Lebanese	A	8 months	Moderate		c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	
10 (8)	1993	Italian	A	8 months	Good		c.-69T>A	c.-69T>A	Verbeek et al., 2007
11 (9)	2004	Pakistan	A	8 months	Good	Sudden onset	c.1181T>C/p.Ile394Thr	c.1181T>C/p.Ile394Thr	
12 (4)	1999	Turkish	A	6 months	Good		c.-70G>A	c.-70G>A	Verbeek et al., 2007
13 (10)	1997	Irish	A	6 months	Good		c.620G>A/p.Cys207Tyr	c.698G>A/p.Arg233His	
14 (11)	1992	Dutch	A	6 months	Good		c.295delC/p.Leu99fs <sup>b</sup>	c.698G>A/p.Arg233His	
15 (12)	1989	Dutch	A	6 months	Good		c.-71C>T	c.1159C>A/p.Leu387Met	Brautigam et al., 1998; Wevers et al., 1999; de Rijk-Van Andel et al., 2000
16 (13)	1993	Dutch	A	5 months	Good		c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	Verbeek et al., 2007
17 (7)	1987	Lebanese	A	4 months	None		c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	Brautigam et al., 1998; Wevers et al., 1999; de Rijk-Van Andel et al., 2000
18 (14)	2003	Fijian	A	4 months	Good		c.-70G>A	c.1475C>T/p.Pro492Leu	Verbeek et al., 2007
19 (5)	2004	Lebanese	A	4 months	Good		c.-70G>A	c.-70G>A	Verbeek et al., 2007
20 (15)	2000	Greek	A	3 months	Unknown	Lost from follow-up	c.707T>C/p.Leu236Pro	c.707T>C/p.Leu236Pro	
21 (16)	2003	Brazilian	A	3 months	Good		c.698G>A/p.Arg233His	c.721G>A/p.Ala241Thr	
22 (17)	1992	Dutch	A	3 months	Good		c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	Brautigam et al., 1998; van den Heuvel et al., 1998; Wevers et al., 1999; de Rijk-Van Andel et al., 2000
23 (18)	1993	Dutch	A	3 months	Good		c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	Brautigam et al., 1998; Wevers et al., 1999; de Rijk-Van Andel et al., 2000
24 (19)	1998	Lebanese	A	2 months	Good		c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	Brautigam et al., 1998; van den Heuvel et al., 1998; Wevers et al., 1999; de Rijk-Van Andel et al., 2000
25 (20)	2005	Italian	A	2 months	Moderate		c.776A>G/p.Glu259Gly	c.1529T>A/p.Leu510Gln	Grattan-Smith et al., 2002
26 (21)	1997	Dutch	B	3 months	Good	Sudden onset at 3 months and sudden deterioration at 22 months during infection	c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	

(Continued)

Table 1. Continued

Patient (Family)	Year of birth	Origin	Phenotype	Age at onset	L-dopa-response	Note	Allele 1	Allele 2	References <sup>a</sup>
27 (16)	1990	Brazilian	B	3 months	Moderate		c.698G>A/p.Arg233His	c.721G>A/p.Ala241Thr	Hoffmann <i>et al.</i> , 2003 (Case II)
28 (22)	2001	Dutch	B	2 months	Good		c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	Hoffmann <i>et al.</i> , 2003 (Case II)
29 (23)	1984	German	B	Neon (5 months)	None	Died at 9 years of age	c.1198-24T>A / p.?	c.698G>A/p.Arg233His	Hoffmann <i>et al.</i> , 2003 (Case II)
30 (24)	2000	Greek	B	Neon (4 months)	Moderate		c.1375C>T/p.Gln459X	c.1475C>T/p.Pro492Leu	Brautigam <i>et al.</i> , 1999; Hoffmann <i>et al.</i> , 2003 (Case I); Dionisi-Vici <i>et al.</i> , 2000
31 (25)	1995	Italian	B	Neon (4 months)	Moderate		c.1076G>T/p.Cys359Phe	c.1076G>T/p.Cys359Phe	Brautigam <i>et al.</i> , 1999; Hoffmann <i>et al.</i> , 2003 (Case I); Dionisi-Vici <i>et al.</i> , 2000
32 (23)	1990	German	B	Neon (4 months)	Moderate		c.1198-24T>A/p.?	c.698G>A/p.Arg233His	Janssen <i>et al.</i> , 2000; Hausler <i>et al.</i> , 2001; Hoffmann <i>et al.</i> , 2003 (Case III)
33 (26)	1994	French	B	Neon (3 months)	None	Died at 2.5 years of age	c.707T>C/p.Leu236Pro	c.707T>C/p.Leu236Pro	Hoffmann <i>et al.</i> , 2003 (Case IV)
34 (27)	2001	Greek	B	Neon (2 months)	None		c.707T>C/p.Leu236Pro	c.707T>C/p.Leu236Pro	Zafeiriou <i>et al.</i> , 2009
35 (28)	2004	Belgian	B	Neon	Moderate		c.698G>A/p.Arg233His	c.698G>A/p.Arg233His	Hoffmann <i>et al.</i> , 2003 (Case IV)
36 (29)	1994	Turkish	B	Neon	None		c.926T>C/p.Phe309Ser	c.926T>C/p.Phe309Ser	De Lonlay <i>et al.</i> , 2000

Phenotype A = 'progressive extrapyramidal movement disorder (hypokinetic-rigid syndrome with dystonia) with onset in infancy or childhood'; B = 'complex encephalopathy with onset in the neonatal period or early infancy'; Neon = neonatal. Response to L-dopa: 'none' means that there was no beneficial response at all, often reflecting the occurrence of such severe dyskinesia that treatment was impossible.

<sup>a</sup> References: papers in which (clinical, biochemical or genetic) data of the patient have been published previously.

<sup>b</sup> The c.295delC mutation was previously designated c.291delC, but renamed according to the guidelines of the Human Genome Variation Society ([www.hgvs.org](http://www.hgvs.org)).

*et al.*, 1995; Surtees and Clayton, 1998; Furukawa *et al.*, 2001; Diepold *et al.*, 2005; Moller *et al.*, 2005; Yeung *et al.*, 2006; Giovanniello *et al.*, 2007; Ribases *et al.*, 2007; Wu *et al.*, 2008; Clot *et al.*, 2009; Doummar *et al.*, 2009). The key data on these patients, and type A/B classification (see next paragraph) based on available clinical descriptions, are summarized in Table 2.

## Clinical features

After careful evaluation of the detailed case histories in the literature and the questionnaires used in this study, it was possible to class the different phenotypes at presentation into two major groups. Most patients ( $n=25$ ) suffered from a disorder that can be summarized as a progressive hypokinetic-rigid syndrome with dystonia. The onset of symptoms was generally in the first year of life (age range: 2 months and 5 years). This is the first phenotype described in the literature, further referred to as type A in this article (Castaigne *et al.*, 1971; Rondot and Ziegler, 1983; Rondot *et al.*, 1992; Knappskog *et al.*, 1995; Ludecke *et al.*, 1995; Swaans *et al.*, 2000; Furukawa *et al.*, 2001; Diepold *et al.*, 2005; Yeung *et al.*, 2006; Giovanniello *et al.*, 2007; Ribases *et al.*, 2007; Wu *et al.*, 2008; Clot *et al.*, 2009). The other eleven patients suffered from a more 'complex encephalopathy' with earlier onset (age range: 0–3 months), as described by Hoffmann *et al.* (2003), designated type B. These two phenotypes are defined in detail in Boxes 1 and 2. Although, we found no difficulties in designating individual patients into type A or B THD, it was obvious that the phenotype of THD is a spectrum with overlap of clinical features between both groups. Patient 24, previously described by Grattan-Smith *et al.* (2002), is a good example of a type A patient with a phenotype very close to type B.

Table 1 summarizes the key demographic data, and clinical characteristics, as well as the results of mutation analysis. The 36 patients came from 29 families with their roots in 13 different countries. THD is a movement disorder with very early onset: all type B patients had an age at onset within the first months of life, and 19 out of 25 (76%) of type A patients presented in the first year of life. The majority of patients (69%) suffered from type A THD. While responsiveness to L-dopa was absent (36%), moderate (45%) or good (18%) in the type B patients, it was good in 84% of the type A patients. Table 3 provides further details on the prevalence of other clinical features as well as the response to L-dopa treatment in type A and B patients as a group. Most additional clinical features are not unique to either type A or B patients. Nevertheless, there is a clear predominance of extra features in type B patients, especially with regard to perinatal abnormalities, diurnal fluctuations, autonomic disturbances and body length and weight at presentation. None of the patients in this series had clinical features suggesting systemic deficiency of catecholamines, such as abnormalities in the maintenance of blood pressure. These systemic phenomena were not, however, formally studied.

Type A patients generally showed a beneficial L-dopa response within the first 2 weeks of treatment, while positive effects in type B patients always occurred later. Selegiline, an inhibitor of dopamine degradation, as well as the dopamine-agonists bromocriptine and pramipexole, were prescribed in one type A and five type B



Table 2 Patients reported in the literature (not diagnosed in our laboratory)

Family, Patient	Pheno-type	Age at onset	Cognitive impairment	L-dopa-response	Presenting clinical features	Allele 1	Allele 2	References
I	A	5 years	N.r.	Good	Spastic paraplegia	c.296delT	c.1493A>G	Furukawa <i>et al.</i> , 2001
II	A	3 years	N.r.	Good	Delayed walking and rigidity, wheelchair bound at 10 years	c.698G>A	c.739C>A	Wu <i>et al.</i> , 2008
III	A	Childhood	Mild	Good	Progressive dystonia	c.956G>C	c.1240G>A	Clot <i>et al.</i> , 2009
IV	A	2 years	Mild	Good	Spastic paraplegia from age 1 year, deterioration at 11 years	c.1240G>A	c.1529T>A	Giovanniello <i>et al.</i> , 2007
V	A	14 months	Mild	Good	Normal until 14 months, then deterioration	c.736C>T	c.1493A>G	Diepold <i>et al.</i> , 2005
VI-1	A	N.r.	N.r.	N.r.	Progressive L-dopa-responsive dystonia	c.1141C>A	c.1141C>A	Ludecke <i>et al.</i> , 1995; Knappskog <i>et al.</i> , 1995
VI-2	A	N.r.	N.r.	N.r.	Progressive L-dopa-responsive dystonia	c.1141C>A	c.1141C>A	Ludecke <i>et al.</i> , 1995; Knappskog <i>et al.</i> , 1995
VII	A	6 months	N.r.	Moderate	L-dopa-responsive encephalopathy	c.-71C>T	c.-71C>T	Ribases <i>et al.</i> , 2007
VIII-1	A	4 months	Yes	Moderate	Mental retardation, 'mixed type cerebral palsy', galactorrhea	(p.Arg169X) <sup>b</sup>	c.698G>A	Yeung <i>et al.</i> , 2006
VIII-2	A	N.r.	Yes	Moderate	As sibling but without galactorrhea	(p.Arg169X) <sup>b</sup>	c.698G>A	Yeung <i>et al.</i> , 2006
IX	B	9 months	No	Good	Hypokinesia, hypotonia, dystonia, ptosis; adopted child	c.1125C>G	c.1399A>G	Clot <i>et al.</i> , 2009; Doummar <i>et al.</i> , 2009
X	B	5 months	Yes	Good	Complex movement disorder, abnormal eye movements, diurnal fluctuation	c.901C>G	c.901C>G	Clot <i>et al.</i> , 2009
XI	B	<5 months	Yes	Moderate	Complex movement disorder, ptosis, irritability	c.982C>T	c.1196C>T	Moller <i>et al.</i> , 2005
XII	B	3 months	No	Good	Complex movement disorder, ptosis	c.614T>C	c.614T>C	Ludecke <i>et al.</i> , 1996; Surtees and Clayton, 1998

a References: papers in which (clinical, biochemical or genetic) data of the patient have been published previously.

b The cDNA mutation was not reported by the authors, only the protein change was given. N.r. = not reported.

patients, with some, although limited, additional effects. Treatment with L-dopa dramatically improved motor outcome in type A patients: one patient was lost from follow-up, Patients 17 and 25 remained wheelchair bound, and all others (22 out of 25, 88%) were able to walk independently during follow-up. Type B patients 26, 28 and 35 finally learned to walk independently, although the initial response to L-dopa had been less impressive than in most type A cases. The majority (67%) of type A patients had normal cognitive capacities during follow-up, while 10 out of 11 (91%) type B patients in our series were mentally retarded. Two type B patients (cases 29 and 33) died during follow-up due to infectious and respiratory complications.

## Cerebral imaging

Most subjects (29 out of 36) underwent cerebral MRI studies. The images were not systematically reviewed for the purpose of this study. Non-specific, mild white matter signal changes and increased volume of extra-cerebral CSF spaces were reported in nine patients, while the majority of patients ( $n=20$ ) had normal images. The number of patients with abnormalities on MRI was 4 out of 21 (19%) and 5 out of 8 (63%) in Types A and B patients, respectively. Importantly, gross structural abnormalities of the brain and signal changes in the basal ganglia were never observed.

## Biochemical findings

Based on the metabolic pathway involved, it can be predicted that CSF levels of the down-stream metabolites of the dopamine degradation pathway will be low, while the serotonin pathway is not affected (Fig. 1). Age-dependent reference values for the different metabolites have been published in detail (Brautigam *et al.*, 1998). As an example, we give here the reference values (P2.5–P97.5) in childhood (2–5 years): HVA 384–769 nmol/l, 5HIAA 110–265 nmol/l, MHPG 35–64 nmol/l and HVA/5HIAA ratio 1.8–4.4.

Pre-treatment CSF results were lacking from seven patients [1–5, 17 (Family 7) and 29 (Family 23)]. Patients 1–5 were initially clinically diagnosed as having a L-dopa-responsive movement disorder in the 1970s, and never underwent lumbar puncture. In Families 7 and 23, a lumbar puncture was performed in only one of the two affected siblings. Decreased CSF concentrations of HVA and MHPG, with decreased HVA/5HIAA ratios were demonstrated in all patients in whom CSF was analysed (Fig. 2). To enable comparison of patients of different ages, with different reference values, CSF HVA concentrations and HVA/5HIAA ratios were expressed as percentage of the lower reference limit (2.5th percentile) (Brautigam *et al.*, 1998). The mean CSF HVA concentration in type B patients was significantly lower ( $P<0.005$ ) than in type A patients, namely 8.8% (SD 8.9) and 32.7% (SD 19.8), respectively. As a group, type B patients also had a significantly lower ( $P<0.005$ ) mean CSF HVA/5HIAA ratio than type A patients, namely 5.6 (SD 4.7) and 36.7 (SD 18.9), respectively. After ranking the patients in order of age of onset of the disease (as in Table 1), we were able to show a positive correlation between age of onset and CSF HVA concentrations as well as HVA/5HIAA ratio (Fig. 3). Urinary concentrations of catecholamines and their

**Box 1. THD type A: 'Progressive extrapyramidal movement disorder (hypokinetic-rigid syndrome with dystonia) with onset in infancy or childhood'**

These patients are born after uncomplicated pregnancies and develop normally during the first months of life. In rather exceptional cases (including the first patients described with THD), psychomotor development is even normal or only slightly delayed during the first 2 to 5 years of life (Castaigne *et al.*, 1971; Rondot and Ziegler, 1983; Rondot *et al.*, 1992). Thereafter, however, progressive motor signs appear. Affected individuals become hypokinetic and rigid, and dystonia develops. In early stages, generally only one leg is involved, but with time both legs and also the arms, trunk, face and oropharyngeal musculature become affected. Initial complaints thus encompass abnormal posturing and walking difficulties, or frequent falls in those who already learned to walk before onset of symptoms. These children become wheelchair bound within some years.

Most patients with this type A THD are younger than those described above. In these infants, hypokinesia, bradykinesia and rigidity may dominate the neurological picture while dystonia tends to be less prominent. Initial motor symptoms are generally symmetric and involve arms as well as legs. The ability to walk is not achieved unless children are treated. Severity of dystonia may fluctuate during the day (generally worse in the afternoon), but can also fluctuate within days, giving the impression of a paroxysmal dystonia especially in the early stages of the disease. Mild, non-progressive mental retardation can be found in patients with relatively early onset of motor symptoms, while cognitive functions appear unaffected in patients who develop symptoms after the first year of life. Besides the hypokinetic-rigid syndrome with dystonia, other features like tremor, chorea, oculogyric crises and ptosis, as well as behavioural or autonomic disturbances are absent or—if present—are found as a mild feature and in a minority of patients.

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. During follow-up, all patients continue to be asymptomatic or display only mild motor or cognitive impairment while on a low dose of L-dopa. They show no evidence of progressive disease, and tolerate L-dopa well during many years. Extensive, very readable clinical descriptions of type A THD were, for example, provided by Castaigne *et al.* (1971), Rondot and Ziegler, (1983), Rondot *et al.* (1992), de Rijk-Van Andel *et al.* (2000) and Schiller *et al.* (2004).

**Box 2. THD type B: 'Complex encephalopathy with onset in the neonatal period or early infancy'**

Immediately after birth, or after a symptom-free interval of only weeks, these patients rapidly develop a complex disorder. In most patients, the presenting signs are initially contributed to their complicated perinatal history, which makes estimation of age of onset difficult (Table 1; Patients 29–34). The initial signs may differ between infants, but they all develop a varied neurological disorder that generally includes marked hypokinesia, bradykinesia and hypotonia, mixed with focal or generalized dystonic features and (often excessive) jerky movements like tremor and myoclonus, and that can also encompass bilateral ptosis and oculogyric crises. Diurnal fluctuation of symptoms may be present to a minor degree but is generally absent. However, especially in the most severely affected infants dystonic crises occur within regular intervals of 4–5 days. Mental retardation is generally present, but—as far as can be judged in these severely handicapped children—cognitive functions seem stable over time. Autonomic functions are often disturbed, especially during periods of dystonia or so-called 'lethargy-irritability crises', leading to excessive drooling, sweating, body temperature instability and marked periods of 'pyrexia of unknown origin'. True epileptic seizures and non-epileptic paroxysms may further complicate the clinical picture. L-dopa treatment does not improve all signs equally, and it may take months before all effects of treatment become clear. Hypersensitivity to L-dopa is an important management problem in many of these patients, necessitating (extremely) low L-dopa doses at start, divided over four to six doses per day, and only increased over periods of weeks or months. Compared to type A THD, prognosis with regard to final outcome is worse for motor as well as cognitive functions. Very readable case histories and videotapes have been provided on patients with this type B phenotype, for example by Surtees and Clayton (1998), de Lonlay *et al.* (2000), Hoffmann *et al.* (2003) and Zafeiriou *et al.* (2009).

degradation products were available in a minority of the patients (data not shown) and were generally found to be non-informative.

## Mutation analysis

We identified 24 different *TH* gene mutations (Tables 1 and 4; Fig. 4), located in the promoter sequence, exons 3, 5–14, and in intron 11. Six mutations were not reported previously. The novel c.1375C>T mutation was predicted to lead to a stop codon (p.Gln459X). The other five novel mutations (c.620G>A, c.680A>G, c.721G>A, c.776A>G and c.1181T>C) were all considered pathogenic since they were (i) not encountered in 200 control alleles; (ii) not reported as polymorphisms in databases and the literature (Haavik *et al.*, 2008); and (iii) affecting amino acids within the tyrosine hydroxylase protein that are highly conserved among various species. Additionally, the program SIFT (Sorting Intolerant From Tolerant) (Ng and Henikoff, 2001) predicted 'not tolerated', i.e. a deleterious effect for all five mutations.

The total number of mutated alleles reported in THD is 100 (Table 4). Five out of these 100 alleles harboured (four different) mutations that lead to protein truncation [c.295delC, c.296delT,

p.Arg169X (c.DNA change not reported) and c.1375C>T], while all other 95 alleles were affected by less deleterious missense mutations. Homozygosity for the common c.698G>A mutation was found in six type A and three type B patients. Homozygosity for the c.707T>C mutation occurred in one type A and two type B patients. Promoter mutations were only encountered in type A THD with good L-dopa responsiveness.

## Discussion

In general, the results of the present study and available data in the literature are in perfect agreement. In total, reports on 50 THD patients (from 41 families) are now available in the literature, and are reviewed in this article. Type A THD ( $n=35$ ) is more often diagnosed than type B THD ( $n=15$ ).

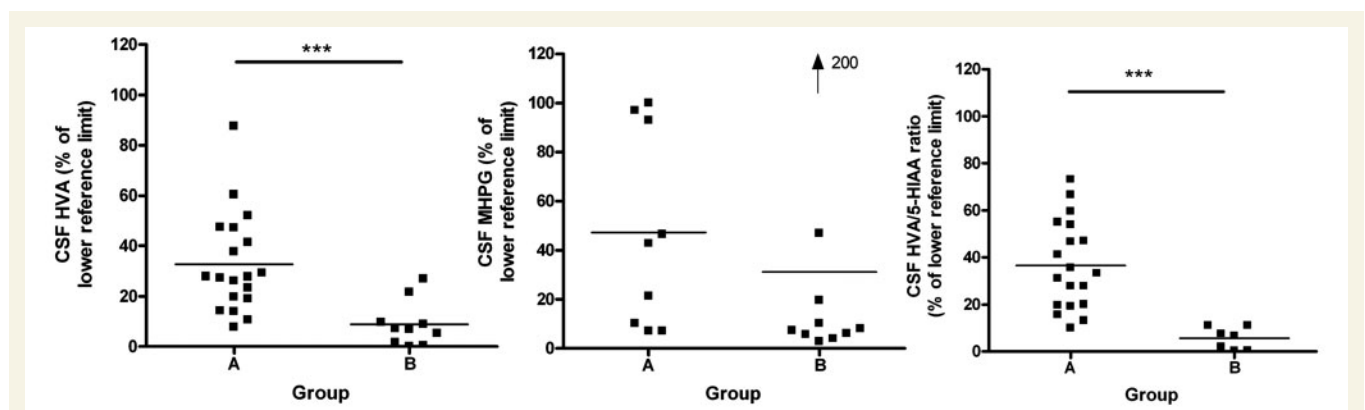
## Clinical features at presentation

The many different neurological features of THD (hypokinesia, bradykinesia, rigidity, dystonia, chorea, tremor, oculogyric crises, ptosis and hypersalivation, among others) are caused by cerebral

**Table 3** Prevalence of additional clinical features and response to treatment in type A and B patients as a group

Clinical data	Type A	Type B	P-value
Sex, pregnancy, delivery, neonatal period	<i>n</i> = 25	<i>n</i> = 11	
Male/female	12/13	8/3	N.S.
Preterm birth (<37 weeks)	0	4	<0.01
Foetal distress (meconium, heart rate abnormalities)	0	6	<0.01
Perinatal asphyxia	0	3	<0.05
Presenting symptoms/signs	<i>n</i> = 25	<i>n</i> = 11	
Age at onset	2 month–5 years	Neonatal–3 months	
Age at onset >12 months	6	0	N.S.
Diurnal fluctuation	2	5	<0.05
Oculogyric crisis	8	6	N.S.
Ptosis	8	5	N.S.
Autonomic disturbances	3	6	<0.05
Lethargy-irritability crises	3	4	N.S.
Sleep disturbances	3	3	N.S.
Seizures	0	2	N.S.
Length <−2SD	1	4	<0.05
Weight <−2SD	0	3	<0.05
Head circumference <−2SD	3	4	N.S.
Follow-up with medication	<i>n</i> = 24	<i>n</i> = 11	
Age at start L-dopa	6 months–15 years	6 months–7 years	
Chronic L-dopa dose (mg/kg/dy)	3.0–10	0.5–20	
L-dopa response: good/moderate/none	21/2/1	2/5/4	
L-dopa response: within 1 week/within 2 weeks/after 2 weeks	12/2/9	0/0/7	
Selegiline trial	1	5	
Effects of selegiline	Positive	Positive but limited	
Other drugs (dopamine agonists) tried	None	Bromocriptine, pramipexole	
Long term follow-up	<i>n</i> = 24	<i>n</i> = 11	
Mental retardation (IQ <70) <sup>a</sup>	8	10	<0.01
Independent walking	22	3	<0.001
Died	0	2	N.S.

The differences in the occurrence of clinical features in the two patient categories were studied by using Fisher's exact test. N.S. = not significant. a See 'Discussion' section for details.



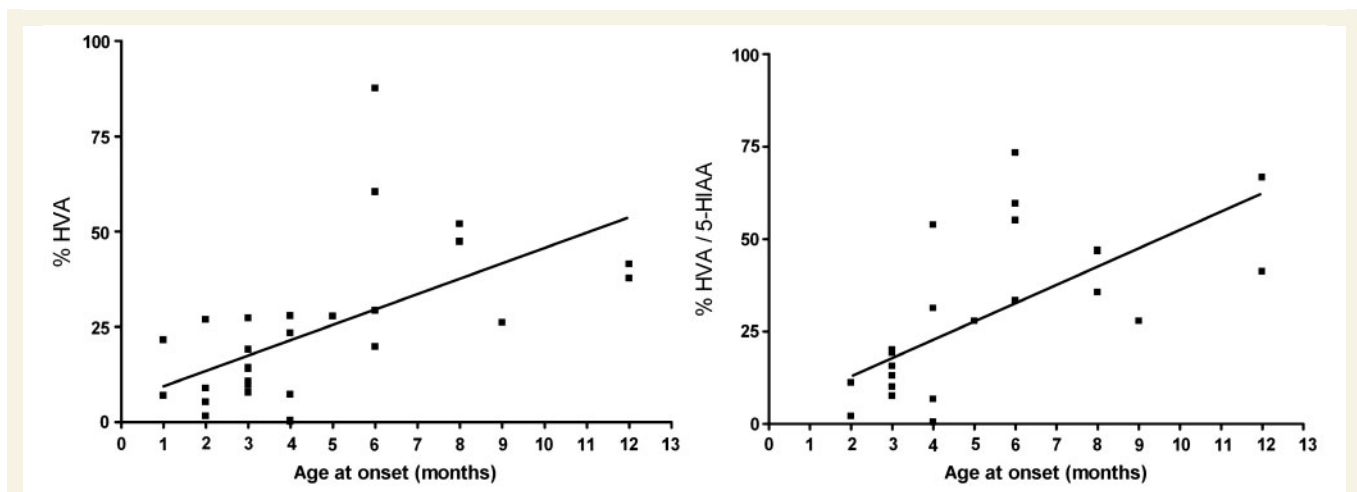
**Figure 2** Concentrations of HVA (left panel) and MHPG (middle panel), and HVA/5HIAA ratio (right panel) in CSF of patients with THD at diagnosis (i.e. without treatment) according to THD subtype. Concentrations are given in percentage of the lower reference limit (2.5th percentile) in controls (see text). For comparisons between the two groups the Student's *t*-test was used. \*\*\**P* < 0.005.

dopamine and norepinephrine deficiency, as nicely explained and discussed previously (Grattan-Smith *et al.*, 2002). THD leads to symptoms early in life, generally in infancy, but sometimes as early as the neonatal period. Presentation in childhood was very

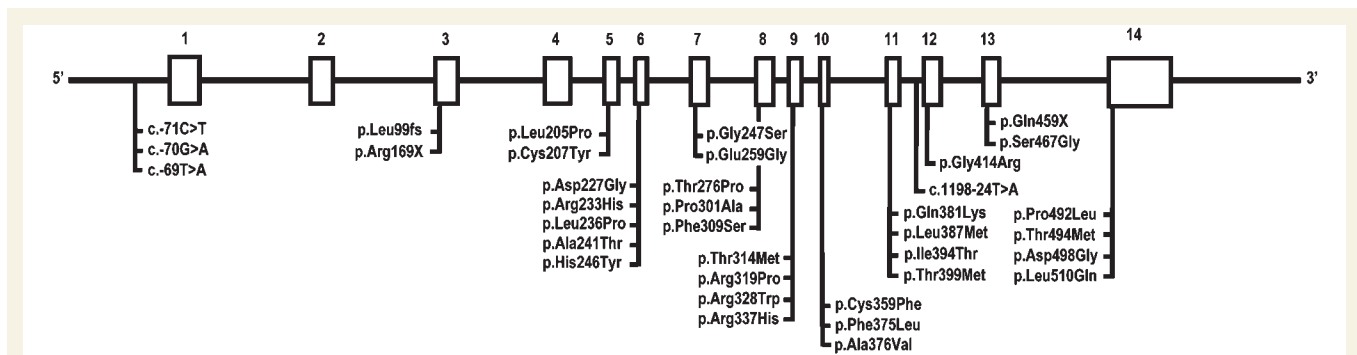
rare, and no patients identified to date have presented in adolescence or adulthood.

Since THD is rare and its features overlap with many other neurological disorders, the diagnosis will generally not be made





**Figure 3** HVA concentration (left panel) and HVA/5HIAA ratio (right panel) concentrations in CSF of THD patients at diagnosis (i.e. without treatment), in relation to age at onset of disease. Concentrations are given in percentage of the lower reference limit (2.5th percentile) in controls. Regression coefficients are: HVA  $r^2=0.52$  ( $P<0.0001$ ), HVA/5HIAA ratio  $r^2=0.66$  ( $P<0.0001$ ).



**Figure 4** Overview of all known pathogenic mutations in the *TH* gene. The cyclic adenosine monophosphate response element of the *TH* promoter resides between residues  $-67$  and  $-74$  upstream of the ATG initiation codon.

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. Type B THD is often accompanied by perinatal complications (Table 3), which may further distract the attention in the direction of common infectious or hypoxic-ischaemic encephalopathies. Type B THD can also mimic genetic disorders like catastrophic epileptic encephalopathies or mitochondrial disorders. 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 cerebral palsy, which may lead to serious diagnostic delay. Besides cerebral palsy, the differential diagnosis of 'juvenile parkinsonism' also includes various other acquired as well as genetic disorders, among which GTP cyclohydrolase deficiency and other defects (like sepiapterine reductase deficiency) in the synthesis of the tyrosine

hydroxylase co-factor tetrahydrobiopterin (BH4) (Muller *et al.*, 1998; Albanese *et al.*, 2006; Tarsy and Simon, 2006; Muller, 2009). 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.

In GTP cyclohydrolase deficiency, the most common defect of tetrahydrobiopterin biosynthesis, diurnal fluctuation of dystonia, can be a prominent hallmark. Both THD and GTP cyclohydrolase deficiency are considered L-dopa-responsive dystonias, and have been named Segawa syndrome and DYT5 in the past (see 'Introduction') (Muller *et al.*, 1998; Albanese *et al.*, 2006; Tarsy and Simon, 2006; Muller, 2009). THD, however, is generally more severe and characterized by an earlier onset of symptoms. Furthermore, the CSF profile of neurotransmitter and pterin metabolites discriminates between the two disorders. It has recently been proposed to designate these conditions into GTP cyclohydrolase deficiency and THD 'dystonia 5a' and 'dystonia 5b', respectively, illustrating their clinical and biochemical relationship and reflecting their different molecular basis (Muller *et al.*, 1998).

**Table 4** Mutations in the tyrosine hydroxylase gene that lead to THD

Number	Exon	Mutation	Protein change	Number of alleles affected
1	Promotor	c.–71C>T		3
2	Promotor	c.–70G>A		9
3	Promotor	c.–69T>A		2
4	3	c.295delC	p.Leu99fs	1
5	3	c.296delT	p.Leu99fs	1
6	3	n.r.	p.Arg169X	2
7	5	c.614T>C	p.Leu205Pro	2
<b>8</b>	<b>5</b>	<b>c.620G&gt;A</b>	<b>p.Cys207Tyr</b>	<b>1</b>
<b>9</b>	<b>6</b>	<b>c.680A&gt;G</b>	<b>p.Asp227Gly</b>	<b>1</b>
10	6	c.698G>A	p.Arg233His	28
11	6	c.707T>C	p.Leu236Pro	6
<b>12</b>	<b>6</b>	<b>c.721G&gt;A</b>	<b>p.Ala241Thr</b>	<b>2</b>
13	6	c.736C>T	p.His246Tyr	1
14	7	c.739G>A	p.Gly247Ser	1
<b>15</b>	<b>7</b>	<b>c.776A&gt;G</b>	<b>p.Glu259Gly</b>	<b>1</b>
16	8	c.826A>C	p.Thr276Pro	1
17	8	c.901C>G	p.Pro301Ala	2
18	8	c.926T>C	p.Phe309Ser	2
19	9	c.941C>T	p.Thr314Met	1
20	9	c.956G>C	p.Arg319Pro	1
21	9	c.982C>T	p.Arg328Trp	1
22	9	c.1010G>A	p.Arg337His	2
23	10	c.1076G>T	p.Cys359Phe	2
24	10	c.1125C>G	p.Phe375Leu	1
25	10	c.1127C>T	p.Ala376Val	2
26	11	c.1141C>A	p.Gln381Lys	4
27	11	c.1159C>A	p.Leu387Met	1
<b>28</b>	<b>11</b>	<b>c.1181T&gt;C</b>	<b>p.Ile394Thr</b>	<b>2</b>
29	11	c.1196C>T	p.Thr399Met	1
30	Intron 11	c.1198–24T>A	p.?	2
31	12	c.1240G>A	p.Gly414Arg	2
<b>32</b>	<b>13</b>	<b>c.1375C&gt;T</b>	<b>p.Gln459X</b>	<b>1</b>
33	13	c.1399A>G	p.Ser467Gly	1
34	14	c.1475C>T	p.Pro492Leu	2
35	14	c.1481C>T	p.Thr494Met	2
36	14	c.1493A>G	p.Asp498Gly	4
37	14	c.1529T>A	p.Leu510Gln	2

This table includes all mutations reported to date in THD, and six novel mutations (in bold) reported in this article. In case of affected sib-pairs, both patients are included. The total number of mutated alleles is 100. For all mutations: see Tables 1 (our series of patients) and 3 (all patients reported by others). n.r. = not reported.

Dopamine plays an important regulatory role in the neuro-endocrine system. Since dopamine suppresses the release of prolactin, THD may lead to hyperprolactinaemia. Indeed, serum prolactin may be increased in THD. In the literature, one patient with THD has been described who presented with galactorrhoea due to hyperprolactinaemia before the neurological features appeared (Yeung *et al.*, 2006). Dopamine is also known to play an important role in growth hormone secretion. Nevertheless, height and pubertal development are generally normal in THD.

## Treatment and clinical course

The natural course of THD is—in *sensu strictu*—unknown. However, the observations in all patients reported here and in the literature indicate that the severe neurological features will never reverse as long as patients are not treated properly.

Since THD leads to dopamine deficiency in the CNS, treatment with L-dopa is by far the strategy of first choice. In fact, the lacking metabolite can simply be supplemented (Fig. 1). Drugs containing L-dopa generally also contain a peripheral L-dopa decarboxylase inhibitor (benserazide or carbidopa) to prevent loss of L-dopa in the circulation. Without further specification, we write 'treatment with L-dopa', meaning L-dopa combined with a decarboxylase inhibitor.

L-dopa dosages commonly used in paediatric neurology range from 3 to 10 mg/kg bodyweight per day, given in three doses. Although L-dopa-responsive THD patients were usually treated with an ongoing L-dopa dose in this range, many types A and B patients started with lower doses, tolerating only very gradual increases in dosage over weeks or even months (see below). Type B patients especially were often extremely sensitive to L-dopa. This hypersensitivity necessitated initial dosages below 0.5 mg/kg bodyweight per day, in four (or even six) divided doses, and impeded some patients from being treated with L-dopa at all (Brautigam *et al.*, 1999; de Lonlay *et al.*, 2000; Dionisi-Vici *et al.*, 2000; Janssen *et al.*, 2000; Haussler *et al.*, 2001; Grattan-Smith *et al.*, 2002; Hoffmann *et al.*, 2003; Zafeiriou *et al.*, 2009).

Alternatively and in addition to L-dopa, THD patients can rationally be treated with inhibitors of dopamine degradation like selegiline, assuming that some dopamine is still formed in tyrosine hydroxylase-deficient neurons. Selegiline as well as dopamine agonists, anticholinergic drugs and benzodiazepines available for the treatment of dystonia (Albanese *et al.*, 2006; Tarsy and Simon, 2006) were, however, hardly ever used in this series.

Patients who tolerated L-dopa well generally showed a good or at least moderate response with dramatic improvement of movements due to disappearance of hypokinesia, tremor, rigidity and dystonia, and subsequent impressive gain in motor functions. Children who had been in a wheelchair for years started to walk again. Good responders generally also responded early (Table 3). These patients only developed L-dopa induced dyskinesia when dosages were increased too quickly or in too great increments. To date, only one subject (Patient 23), developed dyskinesia probably as the consequence of the long-term, i.e. 13 year, treatment with L-dopa. All others tolerated L-dopa very well and remained without serious side effects after more than one (Cases 6, 9, 10, 13, 14, 16, 22, 24, 25, 27, 31) or even two or three (Cases 1–5) decades of treatment. In a limited number of L-dopa-responsive THD patients, lumbar punctures were performed to monitor treatment effects at the biochemical level. In those cases, clinical responses were good irrespective to the fact that HVA never reached normal levels.

Despite appropriate treatment, long-term cognitive development was subnormal in many patients with THD. We did not systematically study cognitive profiles in this series but based on available test reports, school performance, social functioning and

clinical impression by experienced neurologists, it was concluded that mild to moderate mental retardation occurred in 33 and 91% of Types A and B patients, respectively (Table 3). In none of the patients we encountered signs of cognitive decline under treatment.

Based on shared experiences, but impossible to prove due to the low number of patients, we think that early diagnosis and treatment of THD improves the final outcome with regard to motor as well as cognitive functions. On the other hand, it has extensively been demonstrated in animal studies that dopamine plays a vital role in foetal brain development (Zhou *et al.*, 1995; Araki *et al.*, 2007). Dopamine deficiency might therefore cause, already before birth, irreversible structural brain abnormalities at the microscopic level and leading to mental retardation.

Taking all data together, THD can best be treated with an initial L-dopa dose of 0.5–1 (for type B patients) to 3 (for type A patients) mg/kg bodyweight per day, divided over three or four doses. When tolerated well, the dose can slowly be increased until the desired clinical response is observed or the occurrence of adverse effects forces dose reduction. It should be kept in mind that some patients respond only in the course of months: one should thus wait long enough to see the final effects in apparent moderate or non-responders. We think that type B patients with extreme L-dopa hypersensitivity might be ideal candidates for continuous duodenal administration of a soluble formulation of L-dopa (duodopa), although we did not encounter this approach in our series and the literature.

## Biochemistry and genetics

Catecholamine biosynthesis was long thought to occur only in specific neuronal cell populations in the CNS, sympathetic peripheral nervous system, adrenal glands and the kidneys. Interestingly, however, the presence of the tyrosine hydroxylase protein, its mRNA and its enzymatic activity, have been demonstrated in other non-neuronal tissues capable of catecholamine biosynthesis, such as lymphoid tissues, exocrine pancreas and the gastrointestinal tract (Mezey *et al.*, 1996; Eisenhofer *et al.*, 1997). Human THD leads to a neurological disorder, apparently leaving the other organs unaffected. An explanation for this observation might be that the brain is the most vulnerable organ, already severely affected by relatively minor changes in the *TH* gene (see below). A second explanation might be found in other enzymes, expressed in non-neuronal tissues, which can hydroxylate tyrosine. Tyrosinase (E.C. 1.14.18.1), for example, is expressed in the epidermis and plays a crucial role in melanin biosynthesis from tyrosine (Rios *et al.*, 1999). In humans, tyrosinase deficiency leads to oculocutaneous albinism type 1 (OMIM 606933) (Oetting *et al.*, 2003). In mice, tyrosinase substantially contributes to peripheral, tyrosine hydroxylase-independent dopamine production (Eisenhofer *et al.*, 2003).

The biochemical diagnosis of THD would ideally rely on direct measurement of enzyme activity in tissue samples, blood cells or cultured fibroblasts. Since it was traditionally thought that tyrosine hydroxylase was only expressed in tissues that would not be available for enzymatic analyses, we and others have always relied on CSF metabolites (Fig. 1) and *TH* gene

mutation analysis to diagnose THD. The collected data of the large series of patients described here clearly demonstrate that the most severely affected patients have the lowest concentrations of neurotransmitter metabolites in CSF (Fig. 3). The HVA concentration and HVA/5HIAA ratio in CSF showed overlap between Types A and B patients, however this overlap was only minor for the HVA/5HIAA ratio (Fig. 2). These parameters (especially the HVA/5HIAA ratio in CSF), most probably reflecting the degree of residual tyrosine hydroxylase activity in the brain, may thus be used to predict L-dopa responsiveness and overall outcome.

Measurements of phenylalanine and tyrosine in body fluids, and urinary concentrations of catecholamines, HVA and MHPG, are non-informative in patients with THD (Brautigam *et al.*, 1998; Wevers *et al.*, 1999; Hoffmann *et al.*, 2003). The surprisingly often normal urinary dopamine excretion in THD is hypothetically attributed to residual tyrosine hydroxylase enzyme activity in peripheral non-neuronal tissues or alternative enzymes with the capacity to hydroxylate tyrosine, as discussed above. The fact that profound cerebral dopamine deficiency due to defective dopamine biosynthesis can be accompanied by normal urinary dopamine excretion makes analysis of urine unreliable, even potentially misleading, in the diagnostic work-up for dopamine biosynthesis disorders.

The human *TH* gene (mRNA type 1) contains 14 exons with an open reading frame of 1491 bp encoding for a protein with 497 amino acids that is highly conserved among various species (Nagatsu and Ichinose, 1991) Tables 1, 2 and 4, and Fig. 4, give an overview of the 37 different *TH* mutations in THD patients. Out of 100 alleles, 95 were affected by missense mutations leading to amino acid substitutions in the protein with subsequent partial loss of enzyme activity. The pathogenic effects of some missense mutations on the protein have been confirmed, (Ludecke *et al.*, 1995, 1996; Knappskog *et al.*, 1995; Royo *et al.*, 2005; Haavik *et al.*, 2008) but most can only be designated pathogenic based on indirect evidence. Interestingly, we and others have recently identified pathogenic mutations in the promoter region of the *TH* gene as a rare underlying genetic mechanism (Ribases *et al.*, 2007; Verbeek *et al.*, 2007). A founder effect has been described for the c.698G>A mutation in the Dutch population (van den Heuvel *et al.*, 1998). Except for this and two other mutations (c.-70G>A and c.707T>C), almost all other pathogenic changes in the *TH* gene are so-called 'private' mutations (Table 4). Based on the latter finding, it can be expected that THD occurs in all parts of the world, irrespective of the fact that up to now most patients were diagnosed in Western Europe.

The relatively high number of patients in this paper allows us to discuss possible genotype–phenotype correlations in THD. Only five patients, from four families, harboured deleterious mutations that lead to protein truncation: namely c.295delC and c.296delT, which both lead to p.Leu99fs; p.Arg169X (the c.DNA change was not reported in the two affected siblings); and c.1375C>T, which causes p.Gln459X. Heterozygosity for each of these mutations was found in type A as well as type B patients. Until now, no patient has been reported with homozygosity or compound heterozygosity for two truncating *TH* mutations. This observation most

probably indicates that complete loss of tyrosine hydroxylase activity is incompatible with human life, comparable with the findings in knockout mice (Zhou *et al.*, 1995). Homozygosity for the common c.698G>A mutation and homozygosity for the c.707T>C mutation was found in both THD phenotypes (Table 1), leaving these genotypes without predictive value with regard to THD phenotype. Importantly, however, all patients carrying at least one promoter mutation ( $n=8$ , Tables 1 and 2) suffered from type A THD with good L-dopa responsiveness. As discussed previously, reduced *TH* gene transcription apparently leaves significant residual tyrosine hydroxylase enzyme activity, likely corresponding to a relatively mild type A phenotype (Ribases *et al.*, 2007; Verbeek *et al.*, 2007).

The differential diagnosis in children and adults with movement disorders is essentially different. This explains the remarkable diversity in the diagnostic approach depending on the patient's age. Adult patients with dystonia can be investigated at the genetic level after dedicated neurological classification (Muller *et al.*, 1998; Albanese *et al.*, 2006; Tarsy and Simon, 2006; Wu *et al.*, 2008; Clot *et al.*, 2009; Muller, 2009), while children often have to be exposed to more extensive and invasive diagnostic procedures to illuminate the underlying cause of their disorder (Assmann *et al.*, 2003). In contrast to what is advocated for adult patients, we 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.

In conclusion, THD is a severe but often very treatable neurometabolic disorder resulting from cerebral catecholamine deficiency. The diagnosis of THD relies on clinical suspicion and the analysis of CSF metabolites. Importantly, the CSF concentration of HVA and HVA/5HIAA ratio correlate with the severity of the clinical phenotype. THD can be proven by demonstrating mutations in the *TH* gene or its promoter region. The disorder is almost exclusively caused by missense mutations, suggesting that mutations with more deleterious effects on the protein will be incompatible with life. Genotype–phenotype correlations do not exist for the common c.698G>A and c.707T>C mutations. Carriership of at least one promoter mutation, however, apparently predicts type A THD. Most patients with THD, but not all, can successfully be treated with L-dopa.

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## References

- Albanese A, Barnes MP, Bhatia KP, Fernandez-Alvarez E, Filippini G, Gasser T, et al. A systematic review on the diagnosis and treatment of primary (idiopathic) dystonia and dystonia plus syndromes: report of an EFNS/MDS-ES Task Force. *Eur J Neurol* 2006; 13: 433–44.
- Araki KY, Sims JR, Bhide PG. Dopamine receptor mRNA and protein expression in the mouse corpus striatum and cerebral cortex during pre- and postnatal development. *Brain Res* 2007; 1156: 31–45.
- Assmann B, Surtees R, Hoffmann GF. Approach to the diagnosis of neurotransmitter diseases exemplified by the differential diagnosis of childhood-onset dystonia. *Ann Neurol* 2003; 54(Suppl 6): S18–24.
- Brautigam C, Steenbergen-Spanjers GC, Hoffmann GF, Dionisi-Vici C, van den Heuvel LP, Smeitink JA, et al. Biochemical and molecular genetic characteristics of the severe form of tyrosine hydroxylase deficiency. *Clin Chem* 1999; 45: 2073–8.
- Brautigam C, Wevers RA, Jansen RJ, Smeitink JA, de Rijk-van An del JF, Gabreëls FJ, et al. Biochemical hallmarks of tyrosine hydroxylase deficiency. *Clin Chem* 1998; 44: 1897–904.
- Castaigne P, Rondot P, Ribadeau-Dumas JL, Said G. Progressive extrapyramidal disorder in 2 young brothers. Remarkable effects of treatment with L-dopa. *Rev Neurol (Paris)* 1971; 124: 162–6.
- Clot F, Grabli D, Cazeneuve C, Roze E, Castelnau P, Chabrol B, et al. Exhaustive analysis of BH4 and dopamine biosynthesis genes in patients with Dopa-responsive dystonia. *Brain* 2009; 132: 1753–63.
- de Lonlay P, Nassogne MC, van Gennip AH, van Cruchten AC, Billatte de Villemeur T, Cretz M, et al. Tyrosine hydroxylase deficiency unresponsive to L-dopa treatment with unusual clinical and biochemical presentation. *J Inher Metab Dis* 2000; 23: 819–25.
- de Rijk-Van An del JF, Gabreëls FJ, Geurtz B, Steenbergen-Spanjers GC, van Den Heuvel LP, Smeitink JA, et al. L-dopa-responsive infantile hypokinetic rigid parkinsonism due to tyrosine hydroxylase deficiency. *Neurology* 2000; 55: 1926–8.
- Diepold K, Schutz B, Rostasy K, Wilken B, Hougaard P, Güttler F, et al. Levodopa-responsive infantile parkinsonism due to a novel mutation in the tyrosine hydroxylase gene and exacerbation by viral infections. *Mov Disord* 2005; 20: 764–7.
- Dionisi-Vici C, Hoffmann GF, Leuzzi V, Hoffken H, Bräutigam C, Rizzo C, et al. Tyrosine hydroxylase deficiency with severe clinical course: clinical and biochemical investigations and optimization of therapy. *J Pediatr* 2000; 136: 560–2.
- Doummar D, Clot F, Vidailhet M, Afenjar A, Durr A, Brice A, et al. Infantile hypokinetic-hypotonic syndrome due to two novel mutations of the tyrosine hydroxylase gene. *Mov Disord* 2009; 24: 943–5.
- Eisenhofer G, Aneman A, Friberg P, Hooper D, Fändriks L, Lonroth H, et al. Substantial production of dopamine in the human gastrointestinal tract. *J Clin Endocrinol Metab* 1997; 82: 3864–71.
- Eisenhofer G, Tian H, Holmes C, Matsunaga J, Roffler-Tarlov S, Hearing VJ. Tyrosinase: a developmentally specific major determinant of peripheral dopamine. *FASEB J* 2003; 17: 1248–55.
- Furukawa Y, Graf WD, Wong H, Shimadzu M, Kish SJ. Dopa-responsive dystonia simulating spastic paraplegia due to tyrosine hydroxylase (TH) gene mutations. *Neurology* 2001; 56: 260–3.
- Giovanniello T, Leuzzi V, Carducci C, Carducci C, Sabato ML, Artiola C, et al. Tyrosine hydroxylase deficiency presenting with a biphasic clinical course. *Neuropediatrics* 2007; 38: 213–5.
- Grattan-Smith PJ, Wevers RA, Steenbergen-Spanjers GC, Fung VS, Earl J, Wilcken B. Tyrosine hydroxylase deficiency: clinical manifestations of catecholamine insufficiency in infancy. *Mov Disord* 2002; 17: 354–9.
- Haavik J, Blau N, Thony B. Mutations in human monoamine-related neurotransmitter pathway genes. *Hum Mutat* 2008; 29: 891–902.
- Häussler M, Hoffmann GF, Wevers RA. L-dopa and selegiline for tyrosine hydroxylase deficiency. *J Pediatr* 2001; 138: 451–2.
- Hoffmann GF, Assmann B, Brautigam C, Dionisi-Vici C, Häussler M, de Klerk JB, et al. Tyrosine hydroxylase deficiency causes progressive encephalopathy and dopa-nonresponsive dystonia. *Ann Neurol* 2003; 54(Suppl 6): S56–65.



- Janssen RJ, Wevers RA, Haussler M, Luyten JA, Steenbergen-Spanjers GC, Hoffmann GF, et al. A branch site mutation leading to aberrant splicing of the human tyrosine hydroxylase gene in a child with a severe extrapyramidal movement disorder. *Ann Hum Genet* 2000; 64: 375–82.
- Knappskog PM, Flatmark T, Mallet J, Ludecke B, Bartholome K. Recessively inherited L-DOPA-responsive dystonia caused by a point mutation (Q381K) in the tyrosine hydroxylase gene. *Hum Mol Genet* 1995; 4: 1209–12.
- Ludecke B, Dworniczak B, Bartholome K. A point mutation in the tyrosine hydroxylase gene associated with Segawa's syndrome. *Hum Genet* 1995; 95: 123–5.
- Ludecke B, Knappskog PM, Clayton PT, Surtees RA, Clelland JD, Heales SJ, et al. Recessively inherited L-DOPA-responsive parkinsonism in infancy caused by a point mutation (L205P) in the tyrosine hydroxylase gene. *Hum Mol Genet* 1996; 5: 1023–8.
- Mezey E, Eisenhofer G, Harta G, Hansson S, Gould L, Hunyady B, et al. A novel nonneuronal catecholaminergic system: exocrine pancreas synthesizes and releases dopamine. *Proc Natl Acad Sci USA* 1996; 93: 10377–82.
- Moller LB, Romstad A, Paulsen M, Hougaard P, Ormazabal A, Pineda M, et al. Pre- and postnatal diagnosis of tyrosine hydroxylase deficiency. *Prenat Diagn* 2005; 25: 671–5.
- Muller U. The monogenic primary dystonias. *Brain* 2009; 132: 2005–25.
- Muller U, Steinberger D, Nemeth AH. Clinical and molecular genetics of primary dystonias. *Neurogenetics* 1998; 1: 165–77.
- Nagatsu T, Ichinose H. Comparative studies on the structure of human tyrosine hydroxylase with those of the enzyme of various mammals. *Comp Biochem Physiol C* 1991; 98: 203–10.
- Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res* 2001; 11: 863–74.
- Oetting WS, Fryer JP, Shriram S, King RA. Oculocutaneous albinism type 1: the last 100 years. *Pigment Cell Res* 2003; 16: 307–11.
- Ribases M, Serrano M, Fernandez-Alvarez E, Pahisa S, Ormazabal A, Garcia-Cazorla A, et al. A homozygous tyrosine hydroxylase gene promoter mutation in a patient with dopa-responsive encephalopathy: clinical, biochemical and genetic analysis. *Mol Genet Metab* 2007; 92: 274–7.
- Rios M, Habecker B, Sasaoka T, Eisenhofer G, Tian H, Landis S, et al. Catecholamine synthesis is mediated by tyrosinase in the absence of tyrosine hydroxylase. *J Neurosci* 1999; 19: 3519–26.
- Rondot P, Ziegler M. Dystonia–L-dopa responsive or juvenile parkinsonism? *J Neural Transm Suppl* 1983; 19: 273–81.
- Rondot P, Aicardi J, Goutieres F, Ziegler M. [Dopa-sensitive dystonia]. *Rev Neurol (Paris)* 1992; 148: 680–6.
- Royo M, Daubner SC, Fitzpatrick PF. Effects of mutations in tyrosine hydroxylase associated with progressive dystonia on the activity and stability of the protein. *Proteins* 2005; 58: 14–21.
- Schiller A, Wevers RA, Steenbergen GC, Blau N, Jung HH. Long-term course of L-dopa-responsive dystonia caused by tyrosine hydroxylase deficiency. *Neurology* 2004; 63: 1524–1526.
- Surtees R, Clayton P. Infantile parkinsonism-dystonia: tyrosine hydroxylase deficiency. *Mov Disord* 1998; 13: 350.
- Swaans RJ, Rondot P, Renier WO, van den Heuvel LP, Steenbergen-Spanjers GC, Wevers RA. Four novel mutations in the tyrosine hydroxylase gene in patients with infantile parkinsonism. *Ann Hum Genet* 2000; 64: 25–31.
- Tarsy D, Simon DK. Dystonia. *N Engl J Med* 2006; 355: 818–29.
- van den Heuvel LP, Luiten B, Smeitink JA, de Rijk-van An del JF, Hyland K, Steenbergen-Spanjers GC, et al. A common point mutation in the tyrosine hydroxylase gene in autosomal recessive L-DOPA-responsive dystonia in the Dutch population. *Hum Genet* 1998; 102: 644–6.
- Verbeek MM, Blom AM, Wevers RA, Lagerwerf AJ, van de GJ, Willemsen MA. Technical and biochemical factors affecting cerebrospinal fluid 5-MTHF, biopterin and neopterin concentrations. *Mol Genet Metab* 2008; 95: 127–32.
- Verbeek MM, Steenbergen-Spanjers GC, Willemsen MA, Hol FA, Smeitink J, Seeger J, et al. Mutations in the cyclic adenosine monophosphate response element of the tyrosine hydroxylase gene. *Ann Neurol* 2007; 62: 422–6.
- Wevers RA, de Rijk-Van An del JF, Brautigam C, Geurtz B, van den Heuvel LP, Steenbergen-Spanjers GC, et al. A review of biochemical and molecular genetic aspects of tyrosine hydroxylase deficiency including a novel mutation (291delC). *J Inherit Metab Dis* 1999; 22: 364–73.
- Wu ZY, Lin Y, Chen WJ, Zhao GX, Xie H, Murong SX, et al. Molecular analyses of GCH-1, TH and parkin genes in Chinese dopa-responsive dystonia families. *Clin Genet* 2008; 74: 513–21.
- Yeung WL, Lam CW, Hui J, Tong SF, Wu SP. Galactorrhea—a strong clinical clue towards the diagnosis of neurotransmitter disease. *Brain Dev* 2006; 28: 389–91.
- Zafeiriou DI, Willemsen MA, Verbeek MM, Vargiami E, Ververi A, Wevers R. Tyrosine hydroxylase deficiency with severe clinical course. *Mol Genet Metab* 2009; 97: 18–20.
- Zhou QY, Quaife CJ, Palmiter RD. Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. *Nature* 1995; 374: 640–3.