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Blood, urine and cerebrospinal fluid analysis in TH and AADC deficiency and the effect of treatment

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ABSTRACT

Background: Aromatic L-amino acid decarboxylase (AADC) deficiency and tyrosine hydroxylase (TH) deficiency are rare inherited disorders of monoamine neurotransmitter synthesis which are typically diagnosed using cerebrospinal fluid examination of monoamine neurotransmitter metabolites. Until now, it has not been systematically studied whether analysis of monamine neurotransmitter metabolites in blood or urine has diagnostic value as compared to cerebrospinal fluid examination, or whether monoamine neurotransmitter metabolites in these peripheral body fluids is useful to monitor treatment efficacy.

Methods: Assessment, both by literature review and retrospective analysis of our local university hospital database, of monoamine neurotransmitter metabolites in urine, blood and cerebrospinal fluid, and serum prolactin levels, before and during treatment in patients with AADC and TH deficiency.

Results: In AADC deficiency, 3-*O*-methyldopa in serum or dried blood spots was reported in 34 patients and found to be (strongly) increased in all, serotonin in serum was decreased in 7/7 patients. Serum prolactin was increased in 34/37 and normal in 3 untreated patients. In urine, dopamine was normal or increased in 21/24 patients, 5-hydroxyindoleacetic acid was decreased in 9/10 patients, and vanillactic acid was increased in 19/20 patients. No significant changes were seen in monoamine neurotransmitter metabolites after medical treatment, except for an increase of homovanillic acid in urine and cerebrospinal fluid after levodopa therapy, sometimes even in absence of a clinical response. After gene therapy, cerebrospinal fluid homovanillic acid increased in most patients (8/12), but 5-hydroxyindoleacetic acid remained unchanged in 9/12 patients.

In TH deficiency, serum prolactin was increased in 12/14 and normal in the remaining untreated patients. Urinary dopamine was decreased in 2/8 patients and normal in 6. Homovanillic acid concentrations in cerebrospinal fluid increased upon levodopa treatment, even in the absence of a clear treatment response.

Conclusions: This study confirms that cerebrospinal fluid is the most informative body fluid to measure monoamine neurotransmitter metabolites when AADC or TH deficiency is suspected, and that routine follow-up of cerebrospinal fluid measurements to estimate treatment response is not needed. 3-*O*-methyldopa in dried blood spots and vanillactic acid in urine are promising peripheral biomarkers for diagnosis of AADC deficiency.

Abbreviations: 3-OMD, 3-*O*-methyldopa; 5-HIAA, 5-Hydroxyindoleacetic acid; 5-HTP, 5-Hydroxytryptophan; AADC, Aromatic L-amino acid decarboxylase; CSF, Cerebrospinal fluid; HVA, Homovanillic acid; MHPG, 3-methoxy 4-hydroxyphenylglycol; TH, Tyrosine hydroxylase; TML, Translational Metabolic Laboratory; VMA, Vanillylmandelic acid; VLA, Vanillactic acid.

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However, in many patients with TH or AADC deficiency dopamine in urine is normal or increased thereby not reflecting the metabolic block. The value of serum prolactin for follow-up of AADC and TH deficiency should be further studied.

1. Introduction

Monoamine neurotransmitter deficiencies are rare, usually autosomal recessively inherited neurometabolic disorders in which an enzyme deficiency leads to impaired synthesis, metabolism, or transport of catecholamines (dopamine, norepinephrine and epinephrine) and/or serotonin, depending on the specific deficiency [1]. In aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.28) deficiency synthesis of serotonin and catecholamines [2,3] is affected, which leads to hypotonia, developmental delay, movement disorders (especially oculogyric crises), and autonomic symptoms [4].Tyrosine hydroxylase (TH; EC 1.14.16.2) deficiency only affects catecholamine synthesis and clinically leads to dopa-responsive dystonia or a more severe infantile onset complex encephalopathy [5]. AADC and TH deficiency can be diagnosed by quantification of monoamine neurotransmitter metabolites in cerebrospinal fluid (CSF) and molecular analysis of the *DDC* or *TH* gene [4,5]. In addition, AADC (but not TH) enzyme activity can be determined in plasma [4]. Treatment of AADC deficiency is complex and often little successful [4], although promising results of gene therapy have been obtained recently [6,7]. Treatment of TH deficiency is often more straightforward and consists primarily of L-dopa supplementation with a peripheral decarboxylase inhibitor like carbidopa [5].

TH and *DDC* genes are expressed throughout the body [8–10], and dopamine and serotonin not only act as neurotransmitters but also as endocrine, autocrine and paracrine substances [11–14]. However, it is generally accepted that – in contrast to what is seen in CSF – the quantity of monoamine neurotransmitter metabolites in urine do not reflect the metabolic defect in patients with primary monoamine neurotransmitter disorders. In AADC deficiency, monoamine neurotransmitter excretion patterns have been studied quite extensively and it has been shown that urinary dopamine is often paradoxically increased due to the combination of excessive availability of the precursor L-dopa and residual enzymatic capacity of AADC in the kidneys [10,15]. For monoamine neurotransmitter metabolites in blood, fewer data are available. Consensus in AADC deficiency is that urine and plasma measurements of monoamine neurotransmitter metabolites are not suitable for diagnostic purposes [4] with the exception of vanillactic acid (VLA) and 3-*O*-methyldopa (3-OMD) in plasma [4,16] and urine [17], which may serve as “red flags” that make AADC deficiency highly suspicious. In TH deficiency, urine and plasma are probably not useful for diagnostic purposes at all, but monoamine neurotransmitter metabolite patterns in both blood and urine have not been systematically studied.

Pituitary prolactin release is controlled by dopamine through a negative feedback [18]. Therefore, in disorders with low dopamine levels like TH and AADC deficiency, serum prolactin levels would be expected to be high. However, the diagnostic value of hyperprolactinemia in AADC deficiency is unclear [4] and its significance in TH deficiency has not been systematically studied before. The serum prolactin concentration is easily influenced by multiple factors, including stress of a venous puncture and diurnal variation, and references ranges are age- and gender specific [18]. Therefore, values should always be interpreted with caution. Nonetheless, serum prolactin could be an interesting biomarker to support diagnosis, and potentially also to monitor the efficacy of treatment in patients with a primary monoamine neurotransmitter disorder. The analysis of prolactin is widely available (in contrast to analysis of monoamine neurotransmitter metabolites in body fluids, which is restricted to specialized labs), relatively cheap, and does not require a lumbar puncture. Therefore, in this study we also reviewed available data of prolactin measurements in AADC and TH deficiency before and during treatment.

The value of CSF monoamine neurotransmitter analysis to diagnose AADC and TH deficiency and many other primary monoamine disorders is solid [1,4,5], and needs no confirmation. Routine follow-up CSF measurements are generally neither used nor needed in the management of stable patients with primary monoamine neurotransmitter deficiencies [4], and therefore it is not well known how CSF profiles change due to treatment in these disorders. Therefore, we evaluated available data on CSF monoamine neurotransmitter metabolites before and during treatment.

In this study, we combined a literature review of laboratory measurements in AADC and TH deficiency with a study of the database of the Translational Metabolic Laboratory (TML) of our hospital, to analyze patterns of monoamine neurotransmitter metabolism in urine, blood, and CSF before and during treatment, and prolactin levels in blood before and during treatment. With this, we aim to further expand the knowledge on central and peripheral patterns of dopamine and serotonin metabolites, expose knowledge gaps to guide further research, and provide practical advice concerning body fluid examination in the management of these disorders.

2. Materials and methods

2.1. Data collection

We searched the PubMed database using the following search terms: dopa responsive dystonia, tyrosine hydroxylase deficiency, TH-deficiency, aromatic L-amino acid decarboxylase deficiency, AADC-deficiency, and dopa decarboxylase. Language filters used were English, French, and Dutch. The last search was performed May 2020. Reference lists of all relevant papers were checked for missing articles. The TML database was searched to include all patients with TH and AADC deficiency diagnosed and/or followed in our laboratory from January 1997 until May 2020. This database contains all records of all neurochemical investigations that were performed in body fluids collected form patients for whom these ancillary laboratory investigations were indicated as part of the diagnostic process. Patients (both from the literature and TML database) were included if (1) any of the monoamine neurotransmitter metabolites (dopamine, norepinephrine, epinephrine, serotonin, homovanillic acid (HVA), 5-hydroxyindole acid (5-HIAA), ratio HVA/5-HIAA, 3-methoxy 4-hydroxyphenylglycol (MHPG), 3-*O*-methyldopa (3-OMD), L-Dopa, 5-hydroxytryptophan (5-HTP), vanillylmandelic acid (VMA), and vanillactic acid (VLA)) were reported in urine and/or blood, and/or (2) prolactin levels were reported in serum/plasma, and/or (3) monoamine neurotransmitter metabolites in CSF were reported before and during medical treatment.

Data were excluded if it was not described if the samples were taken before or during treatment and/or no information on the method by which the patient was diagnosed (genetic analysis, CSF studies, enzyme activity) was available. For each included patient, it was recorded which core diagnostic characteristics (CSF monoamine neurotransmitter profile and/or molecular analysis in TH and AADC deficiency, and additionally AADC activity in AADC deficiency) were available. Because it

was not described in most case reports if samples from different body compartments were taken on the same day – this could only be confirmed for data included in the TML database- no direct comparisons between monoamine neurotransmitter metabolites in different body compartments were made. All patients that were included in the TML database have been reported previously (Supplement A and B).

2.2. Laboratory methodologies

The laboratory methodologies used for monoamine neurotransmitter analysis in TML have previously been described [19,20]. In other laboratories, local methodologies may apply. For literature references of included articles, see supplemental material (Supplement A and B).

2.3. Data analysis

Reference values of monoamine neurotransmitter metabolites are age dependent and differ between different laboratories. In order to be able to compare data, we classified values as decreased, normal, or increased according to the reference range in the corresponding laboratory. We defined mildly decreased as 50–99% of lower limit of reference range, moderately decreased as 10–49% of lower limit of reference range, strongly decreased as <10% of lower limit of reference range, mildly increased as 101–199% of upper limit of reference range, moderately increased as 200–499% of upper limit of reference range, and strongly increased as ≥500% of upper limit of reference range. To compare monoamine neurotransmitter metabolites in CSF before and during treatment, 1-tailed dependent *t*-test was used for all measurements except 3-OMD (1-tailed independent *t*-test). These basic statistical analyses were performed using Microsoft Windows Excel 2007. For prolactin, measurements were classified as decreased, normal, or increased compared to reported reference value

2.4. Ethical considerations

This study was in accordance with the International Declaration of Helsinki and consisted of purely retrospective and anonymous use of data. The retrospective use of data from the TML database was approved by the local ethical committee of Radboud university medical center (registration number: 2016-3011).

3. Results

Supplements A (AADC deficiency) and B (TH deficiency) give an overview of included patients regarding reference (TML and/or literature), core diagnostic tests which were reported to ascertain diagnosis, and which data was available for inclusion to answer our research questions. In TH deficiency, we found no reports of monoamine neurotransmitter metabolites in blood.

3.1.1. AADC deficiency: monoamine neurotransmitter metabolites in plasma en DBS in untreated patients

The profile of monoamine neurotransmitter metabolites in untreated patients is summarized in Table 1, and resembles the profile found in CSF with high levels of metabolites upstream from the metabolic defect (L-dopa, 3-OMD and 5-HTP), and decreased levels of metabolites downstream from the metabolic defect (HVA, VMA, MHPG, NE, E, 5-HIAA, 5-HTP and serotonin), except that in one patient a normal 5-HIAA level was reported. Measurements in more than five patients were present for serotonin ($n = 7$) and 3-OMD ($n = 34$) only. 3-OMD was increased in all patients. When a precise value was reported, 3-OMD was always very strongly increased (766–13.350% of upper limit of reference range, median 2312%).

3.1.2. AADC deficiency: monoamine neurotransmitter metabolites in plasma during treatment

In three patients, monoamine neurotransmitter metabolites in plasma could be compared before and during two treatment strategies. Under vitamin B6 monotherapy, which gave no clear clinical response in these three patients, 5-HTP, 3-OMD, L-DOPA, 5-HT and NE remained unchanged. Epinephrine was reported in two patients and remained below detection limit [21,22]. Treatment with vitamin B6 and tranylcypromine (a MAO inhibitor) yielded some positive effects in these patients with improvement in muscle tone and oculogyric crises. Biochemically, MHPG increased from 15% to 86% of lower limit of reference range in one patient, norepinephrine normalized in two patients, epinephrine remained below detection limit in two patients, and serotonin increased from strongly to moderately decreased in two patients, but did not change in another patient. L-DOPA, 3-OMD and 5-HTP remained strongly elevated in all three patients, although 3-OMD levels diminished by about 50% [21,22].

3.1.3. AADC deficiency: monoamine neurotransmitter metabolites in urine

The profile of monoamine neurotransmitter metabolites in urine of untreated patients is summarized in Table 2a. Although urine measurements were recorded in 49 patients with AADC deficiency (Supplement 1), simultaneously reported multiple metabolites were available for only 15 patients, of which for four only measurements during treatment were available.

Urine before treatment did not reflect the typical CSF profile in most patients, with normal HVA in 6/7 patients and normal or increased dopamine in 21/24 patients. Urinary epinephrine was decreased in all patients. 5-HIAA was decreased in most patients (9/10), and normal in one. VLA in urine was reported in 20 patients and was increased in 19 of them. The patient with normal VLA levels in urine had a severe classical phenotype of AADC deficiency complicated by epilepsy and was genetically confirmed (p.R347Q homozygous), but neither CSF analysis nor AADC enzyme activity was available [17]. 3-OMD in urine was reported in 6 patients, and was normal in one patient and increased in five. There was insufficient data available for other monoamine neurotransmitter metabolites.

Table 1
Profile of monoamine neurotransmitter metabolites in plasma in untreated patients with AADC deficiency.

N	L-dopa	3-OMD	HVA	VMA	MHPG	NE	E	5-HTP	5-HIAA		5-HT
	5	34	3	3	4	3	2	3	2	1	7
Result	↑↑↑	↑↑↑*	↓↓	↓↓	↓↓	↓↓	↓↓↓	↑↑↑	↓↓↓	n	↓↓-↓↓↓

Description of monoamine neurotransmitter metabolites in plasma in untreated patients with AADC deficiency. ↓: mildly decreased (50–99% of lower limit of reference range) ↓↓: moderately decreased (10–49% of lower limit of reference range), ↓↓↓: strongly decreased (<10% of lower limit of reference range), ↑: mildly increased (101–199% of upper limit of reference range); ↑↑: moderately increased (200–499% of upper limit of reference range), ↑↑↑: strongly increased (≥500% of upper limit of reference range), n = normal.
*Only in 13 patients a precise value was given, the others were described as 'increased'. 3-OMD was measured in DBS in 29 patients and in plasma in 5 patients.
Abbreviations: 3-OMD: 3-*O*-methyldopa, DBS: dried blood spot; HVA: homovanillic acid; VMA: vanillylmandelic acid; NE: norepinephrine, E: epinephrine, 5HTP: 5-hydroxytryptophane, 5HIAA: 5-hydroxyindoleacetic acid; 5HT: serotonin.

Table 2
Profile of monoamine neurotransmitter metabolites in urine in untreated AADC and TH deficiency.

2a. AADC deficiency

	VLA		3-OMD		5HIAA		HVA		VMA		DA			NE		E
N	1	21	1	5	9	1	1	6	7	3	3	9	12	4	2	5
Result	n	↑↑↑*	n	↑↑↑	↓↓↓-↓	n	↓	n	↓↓-↓	n	↓	n	↑↑↑-↑	↓↓↓-↓↓	n	↓↓↓-↓↓

2b. TH deficiency

	VLA	3-OMD	5HIAA		HVA		VMA		DA		NE		E		
N	–	–	7	1	8	3	8	1	2	6	5	3	1	6	1
Result			n	↑	↓↓↓-↓	n	↓↓↓-↓	n	↓↓	n	↓↓-↓	n	↓↓↓	n	↑↑

Description of monoamine neurotransmitter metabolites in urine in untreated patients with AADC (2a) and TH deficiency (2b).
↓: mildly decreased (50–99% of lower limit of reference range) ↓↓: moderately decreased (10–49% of lower limit of reference range), ↓↓↓: strongly decreased (<10% of lower limit of reference range), ↑: mildly increased (101–199% of upper limit of reference range); ↑↑: moderately increased (200–499% of upper limit of reference range), ↑↑↑: strongly increased (≥500% of upper limit of reference range), n = normal.
*In 16/21 patients, no value was given, only a description (increased).
Abbreviations: AADCD: AADC deficiency, N: number of patients in which data was recorded. THD: tyrosine hydroxylase deficiency. VLA: vanillactic acid, 3-OMD: 3-*O*-methyldopa, 5HIAA: 5-hydroxyindoleacetic acid, HVA: homovanillic acid; VMA: vanillylmandelic acid; DA: dopamine; NE: norepinephrine, E: epinephrine.

Upon treatment with L-dopa there was a strong increase of HVA and dopamine. Epinephrine did not clearly increase in response to different treatment regimens and remained decreased in all patients.

3.1.4. AADC deficiency: serum prolactin

Serum prolactin was reported in 37 untreated patients with AADC deficiency. Concentrations were increased in 34 patients and normal in three. In two patients, the treatment effect of rotigotine (a dopamine agonist) and vitamin B6 was reported, with normalization of prolactin levels [23]. In two patients, only measurements during treatment (vitamin B6 in one, DA-agonist, vitamin B6 and MAO-inhibitor in the other) were given, and also showed normal prolactin levels.

3.1.5. AADC deficiency: CSF monoamine neurotransmitter metabolites before and during medical treatment

CSF HVA and 5-HIAA measurements could be compared in untreated and treated condition in 14 patients.

As expected, HVA and 5-HIAA were decreased in all these untreated patients (mean HVA 25.5% of lower limit of reference range, range 2.3–54.5%; mean 5-HIAA 12.5% of lower limit of reference range, range 0.0–41.6%). The CSF MHPG concentration in untreated patients was decreased in nine patients (mean percentage of lower limit of reference range 19.5%, range 4.1–43.3%) but normal in one.

Treatment conditions were variable and consisted of dopamine agonists, MAO-inhibitors, vitamin B6, L-dopa, or selective serotonin

Table 3
Summary of CSF findings before and during medical treatment in AADC and TH-deficiency.

	AADC deficiency Untreated	AADC deficiency Treated (L-dopa excluded)	AADC deficiency Treated (total, L-dopa included	TH deficiency Untreated	TH deficiency Treated
	Mean (range) Number of patients	Mean (range) p-value Number of patients	Mean (range) p-value Number of patients	Mean (range) Number of patients	Mean (range) p-value Number of patients
HVA	↓↓ (↓↓↓-↓) n = 14	↓↓ (↓-↓↓) p = 0.14 n = 9	↓ (↓↓-n) p = 0.03 n = 14	↓↓ (↓↓↓-↓) n = 16	↓ (↓↓↓-↑) p < 0.001 n = 16
5-HIAA	↓↓ (↓↓↓-↓↓) n = 14	↓↓ (↓↓↓-↓) p = 0.22 n = 9	↓↓ (↓↓↓-↓) p = 0.07 n = 14	n (↓-↑) n = 16	n (↓-↑) p = 0.06 n = 16
MHPG	↓↓ (↓↓↓-n) n = 10	↓↓↓ (↓↓↓- ↓↓) p = 0.05 n = 6	↓↓ (↓↓↓-n) p = 0.47 n = 10	↓↓ (↓↓↓-↓) n = 9	↓ (↓↓↓ - n) p = 0.01 n = 9
3-OMD	↑↑↑ (↑↑-↑↑↑) n = 9	↑↑↑ (↑↑-↑↑↑) p = 0.29 n = 7	↑↑↑ (↑↑-↑↑↑) p = 0.06 n = 9	n (↓↓↓ - m) n = 14*	↑↑↑ (↑↑-↑↑↑) p < 0.001 n = 13*
L-dopa	↑↑↑ (↑-↑↑↑) n = 10	↑↑ (↑↑-↑↑↑) p = 0.03 n = 7	↑↑↑ (↑↑-↑↑↑) p = 0.13 n = 10	No data	↑↑ (n - ↑↑↑) n = 6
5-http	↑↑↑ (↑↑-↑↑↑) n = 10	↑↑↑ (↑-↑↑↑) p = 0.12 n = 6	↑↑ (↑-↑↑↑) p = 0.04 n = 10		

↓: mildly decreased (50–99% of lower limit of reference range) ↓↓: moderately decreased (10–49% of lower limit of reference range), ↓↓↓: strongly decreased (<10% of lower limit of reference range), n: normal, ↑: mildly increased (101–199% of upper limit of reference range), ↑↑: moderately increased (200–499% of upper limit of reference range), ↑↑↑: strongly increased (>500% of upper limit of reference range). *p*-value refers to the significance level of the change in metabolite levels in untreated vs. treated conditions (1-tailed dependent *t*-test). *no direct comparison of patients, independent *t*-test performed.

reuptake inhibitors in various combinations. HVA levels in CSF could increase, remain stable or decrease upon treatment. Significant HVA increase was only reached when treatments included L-dopa, a drug which is only effective in a small subset of patients with AADC deficiency due to L-dopa responsive mutations. In three of these responsive patients (siblings), normalization of the HVA level was reached upon treatment with L-dopa and vitamin B6. One other, clinically non-responsive, patient also had normalization of HVA level under treatment with L-dopa, vitamin B6 and a MAO-inhibitor. 5-HIAA and MHPG levels did not change significantly upon treatment, regardless of whether L-dopa was used or not.

Table 3 summarizes CSF findings in medically untreated and treated TH and AADC deficiency.

3.1.6. AADC deficiency: CSF metabolites before and during gene therapy

To date, intraparenchymal gene therapy has been reported in 16 patients [6,7]. In 12 patients, HVA and 5-HIAA in CSF has been reported in treatment naïve condition and after gene therapy. HVA increased in 8 patients, but remained strongly decreased in 5 of these 8 patients. In one patient, who had moderately decreased HVA before gene therapy, normal HVA levels after gene therapy were reached. HVA remained unchanged in four patients (strongly decreased in three, moderately decreased in one). All patients showed a favourable motor response after gene therapy.

5-HIAA remained strongly decreased in 9 patients. In three patients, 5-HIAA increased, to moderately ($n = 1$) or to mildly ($n = 2$) decreased levels. 3-OMD was reported in 6 patients before treatment and after gene therapy, but remained moderately to strongly increased without a significant change ($p = 0.17$).

3.1.7. TH deficiency: monoamine neurotransmitter metabolites in urine

The profile of monoamine neurotransmitter metabolites in urine (HVA, 5-HIAA, VMA, dopamine, norepinephrine and epinephrine) in untreated TH deficiency is summarized in Table 2b. There were insufficient data for the other monoamine neurotransmitter metabolites, or for urinary monoamine neurotransmitter metabolites on medical treatment (reported in one patient only).

Although in most patients before treatment urinary HVA and VMA were decreased and 5-HIAA was normal (in line with the neurometabolic block), this was not found in all patients. Three patients had normal HVA, one patient had normal VMA and one patient had increased 5-HIAA levels.

Urinary dopamine was normal in 6/8 patients and decreased in the other two patients. Norepinephrine was normal in 3/8 patients and decreased in the other five patients. Epinephrine was decreased in only one patient, normal in 6, but increased in one. Normal epinephrine concentrations were found in patients with decreased dopamine ($n = 2$) and/or decreased norepinephrine ($n = 4$).

3.1.8. TH deficiency: serum prolactin

In untreated TH deficiency, prolactin was increased in 12 and normal in two patients. In two patients, only measurements under L-dopa treatment were available, which showed normal levels. In two patients under L-dopa and selegiline treatment, prolactin levels did not change. In one male patient, multiple measurements of prolactin were reported from age 1.8 until the age of 15 years under different treatment regimes. He had very high levels at the time of diagnosis and clinically was a very slow responder to medication. On follow-up, prolactin could be normal ($n = 1$), decreased ($n = 3$) or increased ($n = 6$). There was no clear correlation between treatment regime, clinical response, and prolactin levels (see Leuzzi et al. 2017 for a detailed description [24]).

3.1.9. TH deficiency: CSF monoamine neurotransmitter metabolites before and during treatment

In 16 patients, CSF measurements before and during treatment were available. The summary of results is shown in Table 3.

In all untreated patients, HVA was decreased (mean percentage of lower limit of reference range 16.8%, range 0.00–63.0%), 5-HIAA was normal in 13/16 patients, mildly increased in two and mildly decreased in one patient. CSF HVA/5-HIAA ratio was decreased in all untreated patients.

Upon treatment, which always included L-dopa/carbidopa, there were no significant changes in 5-HIAA levels. In contrast, there was a clear significant increase in HVA to a mean of 81.3% of lower limit of reference range. Normal levels of HVA were reached in five patients; three on a combination therapy of L-dopa and a MAO-inhibitor, two on an L-dopa monotherapy. However, in two patients with a severe, early onset phenotype that exhibited normal HVA levels, clinical response was described as only partial or minimal [25,26]. In one patient, mildly increased HVA levels (133% of upper limit of reference range) occurred on L-dopa monotherapy. The clinical response in this patient was not described.

A comparison of MHPG concentrations before and during treatment could be made in 9 patients. MHPG was decreased in all untreated patients (mean percentage of lower limit of reference range 32.8%, range 5.71–85.0%) and increased upon treatment to a mean of 56.1% of lower limit of reference range, with a normal level reached in three patients, all on L-dopa/carbidopa monotherapy. HVA in two of these patients remained decreased, while in one patient it reached mildly increased levels (clinical response not described).

Insufficient data for CSF 3-OMD prohibited a direct intra-individual comparison. 3-OMD in untreated patients was normal in 11 patients, and mildly, moderately, or strongly decreased in one patient each. Levels in treated condition (L-dopa with or without dopamine agonist) were increased in all patients.

4. Discussion

In this study of patients with TH and AADC deficiency, we retrospectively evaluated the literature and our laboratory database for monoamine neurotransmitter metabolite patterns in blood and urine and prolactin levels in blood, and analyzed the influence of medical treatment on monoamine neurotransmitter metabolites in CSF. This was the first structured study in both TH and AADC deficiency to evaluate this, yielding both expected and unexpected findings which are outlined below.

It is considered good practice to use CSF, but not blood or urine, to diagnose primary monoamine neurotransmitter disorders, and our study supports this habit. Although in patients with AADC deficiency the monoamine neurotransmitter profile in blood seems to reflect the metabolic block and the profile found in CSF, this was only reported in a very limited number of patients and 5-HIAA in blood was normal in one of these patients. In TH deficiency, no reports of monoamine neurotransmitter metabolites in blood were found at all. In urine, we confirm that the monoamine neurotransmitter profile does not reflect the metabolic block in many patients with AADC deficiency, in whom normal to high levels of dopamine metabolites are often found. We show here that also when TH is deficient, normal levels of dopamine and HVA can be found. Overall, we confirm that quantities of monoamine neurotransmitter metabolites in urine in patients with AADC and TH deficiency should be interpreted with extreme caution and are of very limited diagnostic value.

An exception to the rule above regards the metabolites that accumulate in AADC deficiency, namely 3-OMD, L-dopa, and VLA, and possibly 5-HTP. These metabolites are not only highly elevated in CSF, but also in blood and urine, a finding we could confirm here in a relatively large group of patients. The work that is done to develop newborn screening for AADC deficiency measuring 3-OMD in DBS [16,27,28] is therefore interesting and promising to decrease the long diagnostic delay that is often present in this disorder [3]. However, some caution regarding these accumulating metabolites is warranted. Firstly, in patients with AADC deficiency normal urinary VLA levels have been reported [17], although recently it was shown that this might be overcome

by measuring VLA/VMA ratios in patients with AADC deficiency [29]. Secondly, other conditions in which 3-OMD, L-dopa and VLA accumulate do exist. Besides due to treatment with L-dopa [30,31], accumulation can occur in the case of 'secondary AADC deficiency' due to deficiency of its co-factor pyridoxin, e.g. in some of the primary vitamin B6 responsive disorders like pyridoxamine 5′- phosphate oxidase (PNPO) deficiency [32,33], or pyridoxal phosphate binding protein (PLPBP) deficiency [34], which in fact can give a monoamine neurotransmitter metabolite profile indistinguishable from AADC deficiency. Clinically, these disorders are usually different from AADC deficiency because of prominent neonatal seizures [33]. Recently however, one patient with PLPBP deficiency was described who did not suffer from seizures but displayed a movement disorder compatible with AADC deficiency. Also in this patient, increased levels of VLA were found in urine [34]. This highlights the fact that molecular confirmation of a specific genetic diagnosis is crucial because treatment strategies of vitamin B6 responsive disorders and AADC deficiency are different.

In TH deficiency, neither accumulated metabolites in CSF, urine or blood are known nor identified with our study. At this moment, newborn screening for TH-deficiency using metabolites in blood therefore seems beyond the horizon. However, innovative techniques, e.g. using metabolomics or complexomics, might identify new biomarkers of TH deficiency in the future [35,36].

Although it can be argued that nowadays the use of biomarkers in CSF, blood and urine is losing its relevance since patients with a suspected neurometabolic disorders will first be investigated using a genetic approach [37], we believe that it is still important to delineate and understand the profile of monoamine neurotransmitter metabolites in body fluids. Not only can this help to establish or reject a diagnosis in patients in which genetic variants of uncertain significance are found, it is also relevant to the new 'omics' techniques mentioned above [35].

We found that serum prolactin is often increased in patients with AADC and TH deficiency, but that it can be normal as well. We cannot extrapolate these findings to all patients with TH and AADC deficiency because of the retrospective nature of this study with lack of information on withdrawal conditions which is important for correct interpretation of prolactin levels [18]. Although increased prolactin might serve as a possible clue towards monoamine neurotransmitter deficiencies, especially in low-resource countries [38], normal prolactin does not exclude TH or AADC deficiency. However, the usefulness of prolactin for diagnosis and treatment monitoring of primary monoamine deficiencies is an interesting topic for further prospective studies.

In patients with TH deficiency and AADC deficiency who are treated rationally (targeting the metabolic defect), CSF metabolite concentrations tend to become less deviant, but their characteristic diagnostic profile mostly remains present. Treatment with L-dopa leads to strongly increased 3-OMD levels, not only in patients with AADC deficiency who already have strongly increased levels, but also in patients with TH-deficiency. Interestingly, this is also found in patients with Parkinson's disease upon L-dopa treatment [39], and even in healthy volunteers who receive a single dose of L-dopa [30]. In our small and retrospective series, there is no clear correlation between alterations in CSF monoamine neurotransmitter metabolites and clinical response to medical treatment in TH or AADC deficiency. Normalization of CSF HVA levels may follow L-dopa supplementation in both disorders, but does not always reflect the clinical response. Remarkably, after intraputaminal gene therapy - a new treatment available for patients with AADC-deficiency - HVA in CSF shows a modest increase in most patients, but can remain strongly decreased even when there is a clear clinical response [6,7]. Therefore, we think it wiser and more patient friendly to use clinical parameters rather than biochemical measurements to target treatment response in the follow-up of AADC and TH deficiency.

Some findings we cannot easily explain relate to monoamine neurotransmitter metabolites in urine. Interestingly, epinephrine was consistently found to be decreased in AADC deficiency (5/5 patients), but was mostly normal or even increased in patients with TH deficiency (7/8 patients). In contrast, in both disorders urinary norepinephrine was decreased in most patients. There was no clear intra-individual correlation between the levels of urinary dopamine and epinephrine, and medical treatment did not change the level of urinary epinephrine in patients with AADC deficiency. For TH deficiency, there was insufficient data available to evaluate the treatment effect on urinary monoamine neurotransmitter metabolites. Possibly, urinary epinephrine is decreased in AADC but not TH deficiency due to the highly accumulated levels of L-dopa that are present in AADC deficiency only. L-dopa is metabolized to 3-OMD by COMT in a methylation reaction that uses s-adenosylmethionine as methyl donor [40]. If s-adenosylmethionine is depleted, the same methylation reaction that is needed to metabolize norepinephrine to epinephrine by phenylethanolamine *N*-methyltransferase might be hindered.

Another remarkable finding in urine metabolites in patients with AADC-deficiency is that 5-HIAA was most often decreased (9/10 patients). In line with our previous explanation for the often normal or even increased urinary dopamine concentration in patients with this disorder, namely the combination of excessive availability of the precursor L-dopa and sufficient residual capacity of AADC enzyme activity in the kidneys [10,15], one might expect that also 5-HIAA would be normal or increased because of redundant availability of the precursor 5-HTP. A possible explanation why this is not seen is that AADC has a higher affinity for L-dopa than for 5-HTP [20]. Indeed, it has been shown in rats that chronic L-dopa supplementation leads to increased excretion of dopamine but decreased excretion of serotonin [31]. However, further research is needed to exclude sampling error and better understand this observation.

We used purely retrospective data mainly derived from case reports with non-uniform sampling methods. Correct sampling is essential, especially for monoamine neurotransmitter metabolites in CSF because of a rostrocaudal gradient that is present for HVA, 5-HIAA and MHPG, but it was not always stated whether this was indeed taken into account. Furthermore, for most observations only a limited number was available. However, we think we can draw some valuable conclusions. Firstly, CSF should be regarded as the main body fluid to test monoamine neurotransmitter metabolites when there is a suspicion of TH or AADC deficiency. Secondly, accumulated metabolites in AADC deficiency (VLA, 3-OMD, L-Dopa) are promising biomarkers for neonatal screening, but some hurdles still need to be overcome before it can be implemented in clinical practice. Thirdly, the applicability of serum prolactin in diagnosis and follow-up of primary monoamine neurotransmitter disorders should be further studied in a standardized and prospective manner, ideally using international collaboration initiatives like the international working group on neurotransmitter related disorders (iNTD) [41]. Fourthly, regular follow-up of CSF measurements to target treatment response is not needed in these disorders. Furthermore, we show that, just like in AADC deficiency, in TH deficiency monoamine neurotransmitter metabolites in urine do not completely reflect the metabolic block and HVA and dopamine can be normal in both disorders. An interesting observation includes that in TH deficiency normal levels of urinary epinephrine are often reported, whereas these are generally decreased in AADC-deficiency. This finding highlights the complex and incompletely understood physiology of monoamine metabolism outside the central nervous system, and calls for further research on this topic. These rare primary monoamine neurotransmitter disorders may serve as unique models for this.

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Competing interest statement

The authors declare no competing interests. TW declares having

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Appendix A. Supplementary data

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