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Dopaminergic and serotonergic alterations in plasma in three groups of dystonia patients

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ABSTRACT

Introduction: In dystonia, dopaminergic alterations are considered to be responsible for the motor symptoms. Recent attention for the highly prevalent non-motor symptoms suggest also a role for serotonin in the pathophysiology. In this study we investigated the dopaminergic, serotonergic and noradrenergic metabolism in blood samples of dystonia patients and its relation with (non-)motor manifestations.
Methods: Concentrations of metabolites of dopaminergic, serotonergic and noradrenergic pathways were measured in platelet-rich plasma in 41 myoclonus-dystonia (M-D), 25 dopa-responsive dystonia (DRD), 50 cervical dystonia (CD) patients and 55 healthy individuals. (Non-)motor symptoms were assessed using validated instruments, and correlated with concentrations of metabolites.
Results: A significantly higher concentration of 3-methoxytyramine (0.03 vs. 0.02 nmol/L, $p < 0.01$), a metabolite of dopamine, and a reduced concentration of tryptophan (50 vs. 53 μmol/L, $p = 0.03$), the precursor of serotonin was found in dystonia patients compared to controls. The dopamine/levodopa ratio was higher in CD patients compared to other dystonia groups ($p < 0.01$). Surprisingly, relatively high concentrations of levodopa were found in the untreated DRD patients. Low concentrations of levodopa were associated with severity of dystonia ($r_s = -0.3$, $p < 0.01$), depression ($r_s = -0.3$, $p < 0.01$) and fatigue ($r_s = -0.2$, $p = 0.04$).
Conclusion: This study shows alterations in the dopaminergic and serotonergic metabolism of patients with dystonia, with dystonia subtype specific changes. Low concentrations of levodopa, but not of serotonergic metabolites, were associated with both motor and non-motor symptoms. Further insight into the dopaminergic and serotonergic systems in dystonia with a special attention to the kinetics of enzymes involved in these pathways, might lead to better treatment options.

1. Introduction

Dystonia is a movement disorder characterized by involuntary muscle contractions causing repetitive twisting movements and/or abnormal postures [1]. Although the precise pathophysiology of dystonia is unknown, evidence suggest a central role for the basal ganglia, of which the functions are modulated by different neurotransmitters.

Dopamine is considered to be one of the main neurotransmitters

involved in dystonia, and alterations may cause an imbalance in the direct and indirect striato-pallidal pathways [2]. However, the causal relation between dopamine and dystonia is multifaceted and is as yet not fully understood in most forms of dystonia.

The serotonergic system is of special interest in dystonia pathogenesis since there is convincing evidence of non-motor manifestations, such as psychiatric disorders, sleep disturbances, fatigue and pain, to be part of the phenotype of dystonia [3]. Serotonin among other

Table 1
Clinical characteristics and non-motor symptoms.

	M-D (n = 41)	DRD (n = 25)	CD (n = 50)	Controls (n = 55)	p-value
Age	43.8 (7–75)	37.8 (12–77)	54.2 (20–80)	50.1 (15–83)	**0.005**[a]
Gender m/f	17/24	9/16	15/35	16/39	0.573[b]
Smoking	5 (12%)	2 (8%)	14 (28%)	10 (18%)	0.062[b]
SSRI/SNRI/TCA	4/2/1	2/0/2	0	0	
Levodopa	0	19 (76%)	0	0	
Motor severity scale (CGI-S)	2 (1–5)	2 (1–2)	4 (2–7)	*NA*	
Psychiatry	*N=41*	*N=25*	*N=50*	*N=55*	*p-value*[b]
Any psychiatric diagnosis	24 (59%)	16 (64%)	33 (66%)	17 (31%)	**0.001**
Depression	13 (32%)	9 (36%)	16 (32%)	8 (15%)	0.072
Panic disorder	12 (29%)	4 (16%)	6 (12%)	2 (4%)	**0.004**
Agoraphobia	9 (22%)	7 (28%)	12 (24%)	2 (4%)	**0.003**
Social phobia	5 (12%)	4 (16%)	9 (18%)	0 (0%)	**0.003**
Specific phobia	7 (17%)	3 (12%)	8 (16%)	2 (4%)	0.095
OCD	10 (24%)	2 (8%)	1 (2%)	1 (2%)	**0.000**
Alcohol abuse	3 (7%)	1 (4%)	2 (4%)	3 (6%)	0.962
Generalized anxiety	2 (5%)	7 (28%)	6 (12%)	1 (2%)	**0.002**
Severity scales					
Psychiatry adults only	*N=33*	*N=15*	*N=50*	*N=50*	*p-value*[a]
BDI	8 (0–30)	5 (0–18)	8 (0–28)	2,5 (0–19)	**0.000**
BAI	8 (0–42)	6 (0–12)	8 (1–31)	3 (0–21)	**0.000**
Psychiatry adults and children	*N=40*	*N=24*	*N=50*	*N=55*	*p-value*[a]
z-score BDI/CDI	0.1 (-1-5.5)	0.1 (-1-2.9)	0.8 (-1-5.1)	−0.5 (-1-3.1)	**0.000**
z-score BAI/SCARED	1.0 (−1.5-9.6)	0.1 (−2.6-2.1)	1.1 (−0.7-6.8)	−0.2 (−1.2-4.3)	**0.000**
YBOCS	0 (0–12)	0 (0–18)	0 (0–12)	0 (0–9)	0.104
Sleep and fatigue	*N=35*	*N = 20*	*N=43*	*N=47*	*p-value*[c]
ESS	7.9 (5.5)	11.2 (5.7)	8.8 (6.9)	6.2 (4.7)	**0.012**
FSS	35.1 (15.5)	34.1 (15.6)	39.6 (15.8)	26.0 (13.1)	**0.000**
PSQI	5.9 (3.1)	8.1 (4.5)	7.4 (4.0)	5.6 (4.4)	**0.036**

Data is shown as median (range), number of participants (%), mean (SD). [a] Kruskal-Wallis test, [b]Fisher-Freeman-Halton test or [c]ANOVA were used to compute p-values. M-D: Myoclonus-Dystonia; DRD: Dopa Responsive Dystonia; CD: Cervical Dystonia; SSRI: Selective Reuptake Inhibitor; TCA: Tricyclic Antidepressant; CGI-S: Clinical Global Impression Scale for dystonic symptoms; OCD: Obsessive Compulsive Disorder; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; CDI: Child Depression Inventory; SCARED: Screen for Child Anxiety Related Disorders; YBOCS: Yale Brown Obsessive Compulsive Scale; ESS: Epworth Sleepiness Scale; FSS: Fatigue Severity Scale; PSQI: Pittsburgh Sleep Quality Index.

neurotransmitters as dopamine and noradrenaline are suggested to be involved in these non-motor symptoms and their role is extensively studied in psychiatric disorders [4,5]. Furthermore, in mechanisms regulating sleep, again dopamine and serotonin are also both involved [6].

The serotonergic system has mainly an inhibitory effect on the dopaminergic neurons in the substantia nigra, which might play a role in the pathophysiology of dystonia. Next to this indirect effect it has been suggested that the serotoninergic metabolism itself might be involved in the dysfunctional basal ganglia network resulting in dystonia [7]. Treatment with drugs affecting the serotonergic system can potentially induce dystonia, and in different subtypes of dystonia low concentrations of 5-hydroxyindolacetic acid (5-HIAA), the main metabolite of serotonin, in the cerebral spinal fluid (CSF) have been reported [7].
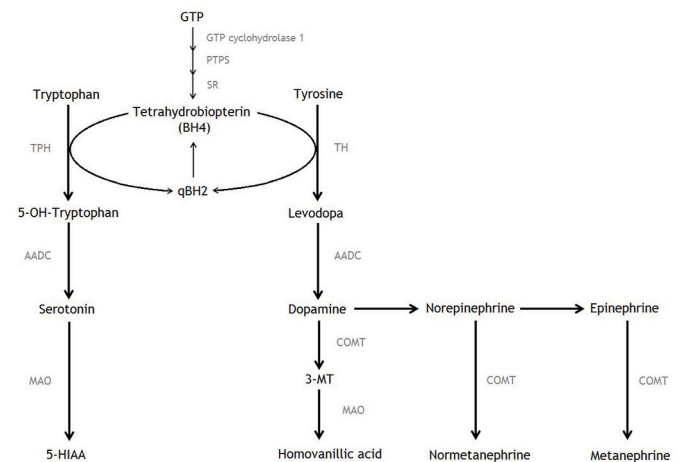

Fig. 1. Monoamine neurotransmitter pathway This figure shows a simplified version of the monoamine neurotransmitter pathway. *3-MT: 3-Methoxytyramine; 5-HIAA: 5-Hydroxyindoleacetic acid; AADC: aromatic-ʟ-amino acid decarboxylase; COMT: Catechol-O-methyltransferase; GTP: Guanosine triphosphate; MAO: Monoamino-oxidase; PTPS: 6-pyruvoyl-tetrahydropterin synthase; SR: Sepiapterin reductase; TH: Tyrosine hydroxylase; TPH: Tryptophan hydroxylase.*

Other neurotransmitters are likely to play a role in dystonia as well as exemplified by the complex interactions of the noradrenergic, cholinergic, serotonergic and dopaminergic systems.

To obtain a comprehensive overview of the metabolism of the monoamine neurotransmitters in dystonia, and to study dystonia subtype specific changes, we included three subtypes of dystonia that are of special interest, myoclonus-dystonia (M-D), dopa-responsive dystonia (DRD) and idiopathic cervical dystonia (CD). In a previous study we reported a high prevalence of non-motor manifestations and a distinctive phenotype in these three different subtypes of dystonia [8]. Furthermore, disruptions in dopaminergic, serotonergic and noradrenergic metabolism in these dystonia subtypes have been reported in literature [7,9] [–] [11].

We hypothesized that alterations in the dopaminergic, serotonergic and noradrenergic metabolism in dystonia patients are involved in the pathophysiology of dystonia, with dopamine mainly related to the motor and serotonin to the non-motor symptoms. Therefore, the main aim of this study was to investigate the concentrations of metabolites of monoamine neurotransmitters in platelets and plasma of dystonia patients and to correlate this to the motor and non-motor symptoms.

2. Methods

41 M-D patients with a *SGCE* mutation; 25 DRD patients with a *GCH-1* mutation; 50 CD patients and 55 healthy individuals without a movement disorder were included. Both adults and children (age >6 year) were eligible to participate. Demographic information was collected by means of a standardized interview. Informed consent was obtained from all participants and the study was approved by the medical ethics committee of the University Medical Center Groningen (METc 2014/034).

2.1. (Non-)motor symptoms

In all patients severity of dystonia was assessed with the Clinical Global Impression Scale (CGI-S). Presence and severity of psychiatric disorders in adults were assessed with respectively the Mini International Neuropsychiatric Interview –PLUS (MINI-PLUS), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI) and Yale Brown Obsessive Compulsive Scale (YBOCS). Impaired sleep quality, excessive daytime sleepiness and fatigue were assessed with respectively the

Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS) and Fatigue Severity Scale (FSS). For children comparable and age appropriate validated questionnaires were used (Supplementary Table 1).

2.2. Monoamine neurotransmitters

Concentrations of tryptophan, serotonin, 5-HIAA, levodopa, dopamine, 3-methoxytyramine (3-MT), norepinephrine, normetanephrine, epinephrine and metanephrine were measured in EDTA anticoagulated blood. Blood samples were obtained in non-fasted state in the morning, and participants were instructed not to consume serotonin-rich products (kiwi, nuts, pineapple or bananas) from the evening before. Samples were stored at −80 °C until analyzed in batches. Tryptophan, serotonin and 5-HIAA were measured in platelet-rich plasma (PRP), which was isolated from EDTA anticoagulated blood samples. To obtain PRP, blood tubes were centrifuged at 120g for 30 min at room temperature; 0.5-mL PRP was used for the platelet count (Sysmex XE-2100). Remaining PRP was stabilized with glutathione. The PRP sample was analyzed using an online solid-phase extraction (SPE)–liquid chromatographic method with tandem mass spectrometric detection (LC-MS/MS) as reported before [12]. Free (non-conjugated) fractions of levodopa, catecholamines and metanephrines were also analyzed in the PRP sample using stable isotope labeled standards for each analysis in combination with online SPE and LC-MS/MS as previously described [13].

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 23. A *p*-value <0.05 was considered statistically significant.

Fisher-Freeman-Halton exact tests, one way ANOVA or Kruskal-Wallis test were performed to compare clinical characteristics and non-motor symptoms between the four groups. To compare concentrations of metabolites between the groups Mann-Whitney U tests or Kruskal-Wallis tests were performed. All analyses were done with and without patients who were using levodopa and/or antidepressants. A ranked ANCOVA was performed with age as covariate. For variables which showed a significant difference post hoc analysis was performed with Bonferroni correction.

We performed univariate correlation analyses in the dystonia group (excl. patients who were using levodopa and/or antidepressants) to assess associations between the (non-)motor symptoms and concentrations of metabolites, which were significantly different in the previous analysis. Mann Whitney U tests were performed for dichotomous variables which showed significant correlations.

3. Results

The median age was significantly different between the four groups ($p = 0.01$) due to younger participants in the MD and DRD group, but not between the whole dystonia group and controls ($p = 0.3$). Significantly more patients in the dystonia group had a psychiatric diagnosis compared to controls (63% vs 31%, $p = 0.00$). Eleven dystonia patients were treated with antidepressants; six patients used a selective serotonin reuptake inhibitor, two patients a serotonin-norepinephrine reuptake inhibitor and three patients a tricyclic antidepressant. Most DRD patients (19/25) were on levodopa therapy and one DRD patient used 5-hydroxytryptophan as additional treatment. Four of the DRD patients who were not treated with levodopa were asymptomatic mutation carriers. Of the remaining two symptomatic patients one ceased treatment because of side-effects (dyskinesia and blurry vision) and the other patient stopped because the benefits no longer outweighed the effort of daily medication taking. Three M-D patients used the anticholinergic drug trihexyphenidyl, which might also influence striatal dopamine release [14]. See Table 1 for clinical characteristics and non-motor symptoms.
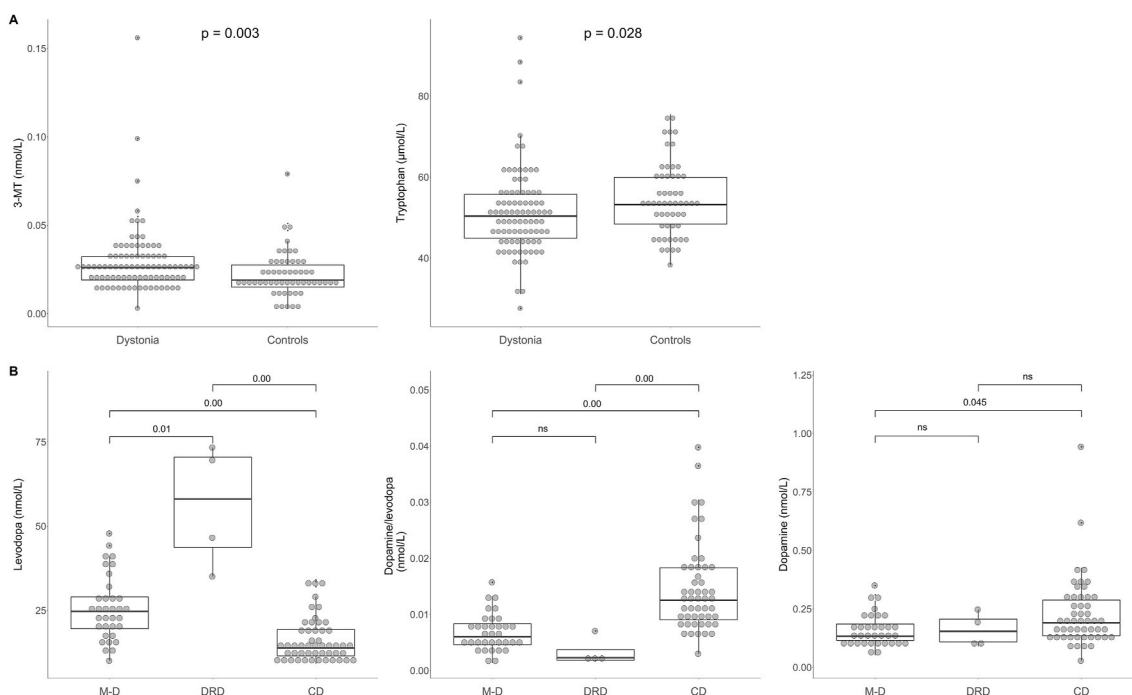

Fig. 2. Concentrations of metabolites of monoamine neurotransmitters *This figure shows the concentrations of metabolites in which we found significant differences between the groups (excl. patients who used antidepressants/levodopa), for results of all metabolites see* Supplementary Tables 1 and 2 *The plots in part A display the concentration of 3-Methoxytyramine (*3-MT*) and tryptophan in dystonia patients compared to controls. Mann withney U test was used to compute p-values. Part B shows three plots with the concentration of levodopa, dopamine/levodopa ratio and dopamine in the four groups. Mann Withney U test with bonferonni correction was used to compute p-values. M-D: myoclonus-dystonia; DRD: dopa-responsive dystonia; CD: cervical dystonia.*
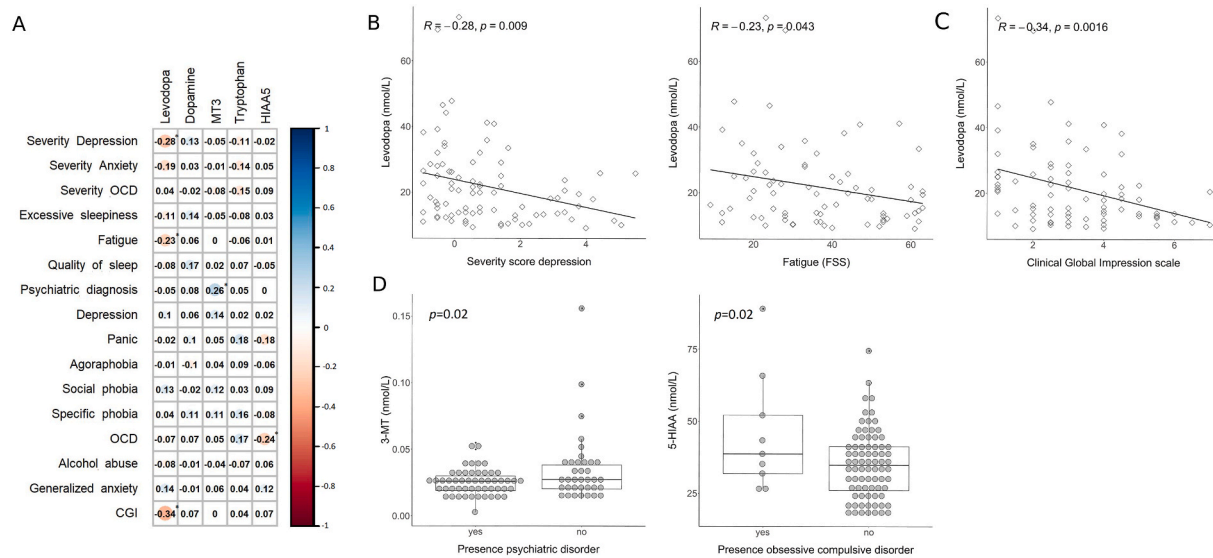
Fig. 3. Correlation between metabolites and non-motor symptoms in dystonia group

This figure shows the correlations between the non-motor symptoms and concentrations of metabolites in the dystonia group (excl. patients who used antidepressants/levodopa). Part A shows a correlation matrix with Pearson's correlation coefficients, significant correlations are depicted by an asterisk. In part B the significant correlation between levodopa and severity of depression and fatigue is shown. Pearson's correlation coefficient (R) and p-value are shown. In part C the significant Pearson's correlation coefficient (R) between levodopa and severity of dystonia, measured with the Clinical Global Impression Scale (CGI), is shown. In part D the significant relationships between respectively 3-methoxytyramine (3-MT) and the presence of a psychiatric disorder, and 5-hydroxyindolacetic acid (5-HIAA) and presence of obsessive compulsive disorder (OCD) are shown. P-values were calculated with Mann Whitney U test. The significant difference between 3-MT and presence of a psychiatric disorder is depicted by two outliers in the group without a psychiatric disorder.

3.1. Dopaminergic and adrenergic pathway

Levodopa, dopamine and 3-MT concentrations were significantly higher in the dystonia group compared to controls ($p = 0.01$, $p = 0.04$ and $p = 0.00$). However, when patients who used levodopa and/or antidepressants were excluded only a significant difference between 3-MT concentrations was found ($p = 0.00$) (Fig. 2A, Supplementary Table 2).

All measured dopaminergic and adrenergic metabolites, except for epinephrine were significantly different between the three dystonia groups (Supplementary Table 3). When patients who used levodopa and/or antidepressants were excluded, still a significant difference in levodopa, dopamine and dopamine/levodopa ratio was observed ($p = 0.00$, $p = 0.02$ and $p = 0.00$) (Fig. 2B, Supplementary Table 3). Post hoc tests, with Bonferroni correction, showed a lower concentration of levodopa in the CD group compared to the other two patient groups (CD vs M-D $p = 0.00$, CD vs DRD $p = 0.00$). The concentration of levodopa was also significantly different between the DRD and the M-D group, with surprisingly higher concentrations of levodopa in the untreated DRD group ($p = 0.01$). Dopamine concentrations were significantly higher in the CD compared to the M-D patients ($p = 0.045$). The dopamine/levodopa ratio was also higher in the CD group compared to M-D and DRD (CD vs M-D $p = 0.00$, CD vs DRD $p = 0.00$). The results of the ranked ANCOVA with age as covariate did not influence the results, except that concentrations of dopamine were no longer significantly different between the three groups (Supplementary Table 4).

3.2. Serotonergic pathway

Concentrations of 5-HIAA were significantly lower in the dystonia group compared to controls ($p = 0.01$). Similarly, the concentrations of tryptophan were lower in the dystonia group, but this did not reach statistical significance ($p = 0.06$). When patients who used levodopa and/or antidepressants were excluded, 5-HIAA concentrations were still lower in the dystonia group, but did not reach statistical significance ($p = 0.05$). Concentrations of tryptophan were significantly lower in the dystonia group compared to the controls when patients who used

levodopa/antidepressants were excluded ($p = 0.03$) (Fig. 2A), correction for age did not change significance (Supplementary Table 5). No differences were found in concentrations of serotonin in platelets between dystonia patients and controls.

Remarkably, between the three dystonia groups no differences in any of the serotonergic metabolites were found. When patients who used levodopa and/or antidepressants were excluded, DRD and M-D patients had lower concentrations of 5-HIAA (31 and 32 nmol/L) compared to CD patients and controls (38 and 39 nmol/L), however this was not significantly different ($p = 0.2$) (Supplementary Table 3).

3.3. Correlation between (non-)motor symptoms and metabolites in dystonia patients

Severity of the motor symptoms on the GCI score was associated with lower concentrations of levodopa ($p=0.00$). Another negative correlation was found between levodopa concentrations and severity of depression ($p = 0.01$) and fatigue ($p = 0.04$), indicating that more severe depressive symptoms and more fatigue were associated with lower levodopa concentrations (Fig. 3). Having any psychiatric diagnosis was associated with lower 3-MT concentrations ($p = 0.02$). However, this association was based on two outliers in the group without any psychiatric disorder. Having the diagnosis of obsessive compulsive disorder (OCD) was associated with higher concentrations of 5-HIAA ($p = 0.02$).

4. Discussion

This study showed alterations in the dopaminergic and, to a lesser extent, serotonergic metabolism in plasma in dystonia patients. A significantly higher concentration of 3-MT, one of the metabolites of dopamine, was found in dystonia patients compared to controls. Next, the dopamine/levodopa ratio in CD patients was higher compared to the other dystonia groups. Surprisingly, relatively high concentrations of levodopa were found in the untreated DRD patients. We found that low concentrations of levodopa, but not of serotonergic metabolites, were associated with more severe motor symptoms of dystonia, depression

and fatigue in the dystonia group. Finally, a significantly reduced concentration of tryptophan, the precursor of serotonin was found in dystonia patients compared to controls.

Our finding of high concentrations of 3-MT in dystonia patients compared to controls and the higher dopamine/levodopa ratio in CD patients suggests an imbalance in the dopaminergic system. However, the precise mechanism in which dopamine is involved in the pathophysiology of dystonia is unclear [10]. In some subtypes, like CD, a hyperdopaminergic state is suggested, which is in line with our findings [9]. In many other dystonia subtypes a hypodopaminergic system is more likely, with DRD, caused by a deficiency of an enzyme in the dopamine synthesis pathway, serving as an good example [15].

Alterations in the activity of enzymes or the flux through the dopaminergic pathway might be responsible for this higher dopamine/levodopa ratio and a higher concentration of 3-MT. The most important enzymes involved are aromatic ʟ-amino acid decarboxylase (AADC), catechol-O-methyltransferase (COMT) and monoamino-oxidase (MAO) (Fig. 1). A previous study in CD patients did not reveal any differences in common variations in genes encoding for these enzymes [16]. However, the activity of these enzymes is regulated in several ways. AADC, which converts levodopa into dopamine in both brain and periphery, is regulated on the long-term by different polymorphisms in the promoter region and alternative splicing of the *DDC* gene encoding for AADC. In the short-term its activity is regulated by phosphorylation and DA receptor activation, the latter is regulated by DA concentrations [17]. Likewise, the activity of COMT and MAO, involved in the conversion of dopamine into 3-MT and 3-MT into homovanillic acid respectively, are also regulated in a complex manner [18,19]. Higher activity of COMT or lower activity of MAO might explain the high concentrations of 3-MT we found in dystonia patients. However, only a rather dated study did not show any alterations in activity of COMT and MAO in skin fibroblasts in patients with generalized dystonia compared to controls [20]. To the best of our knowledge no recent studies have been performed to assess the activity of the dopaminergic enzymes in dystonia and further studies are recommended.

We found that a lower concentration of levodopa in dystonia patients was associated with more severe motor symptoms of dystonia, depression and fatigue. Lower concentrations of levodopa might be due to an increased conversion into dopamine, consistent with the increased dopamine/levodopa ratio and increased concentrations of 3-MT we found, or alternatively, due to a decreased production of levodopa. These associations are complex to interpret, but the association between levodopa and motor symptoms confirms involvement of the dopamine metabolism in the etiology of dystonia. Moreover, previous studies also showed a negative association between severity of depression and dopamine concentrations in plasma and homovanillic acid, one of the main metabolites of dopamine, concentrations in CSF [21]. Studies suggest that treatment of depressed patients with pro-dopaminergic medication is beneficial for alleviating symptoms as fatigue and sleepiness [22]. Furthermore, a study in CD patients showed an association between lower striatal DAT and D2/3 receptor binding and depression [9]. Also in Parkinson's disease in which depression and fatigue are common non-motor symptoms, treatment with levodopa and dopamine agonist is proven to be beneficial for fatigue [23]. Our findings suggest that in dystonia patients alterations in the dopaminergic metabolism are related to the pathophysiology of both the motor as the non-motor symptoms.

Another surprising finding was the relatively high concentration of levodopa in the untreated DRD patients. These six patients were either asymptomatic (n = 4) or had little symptoms and stopped levodopa treatment in the past. We have no clear explanation for these increased concentrations of levodopa, and cannot completely be sure if these patients were (secretly) taking any levodopa medication, but the results were very consistent. Increased concentrations of levodopa in asymptomatic DRD patients have not been described before, but there is evidence that activity of GCH1 enzyme and the level of expression of mRNA

in asymptomatic carriers are not as severely affected as in symptomatic patients [24,25]. In contrast, in a postmortem study of the putamen of an asymptomatic carrier concentrations of BH4 were as low as in symptomatic patients [26]. However, concentrations of dopamine and tyrosine hydroxylase in the putamen were not severely affected, indicating a role for the activity of tyrosine hydroxylase in determining the severity of the symptoms in DRD [26]. Tyrosine hydroxylase is the enzyme converting tyrosine into levodopa in the brain and adrenal medulla and enhanced activity might be the explanation for the relatively few symptoms and high concentrations of levodopa in our untreated DRD patients.

Besides alterations in the dopaminergic metabolism we also found differences in the serotonergic metabolism of dystonia patients. Concentrations of tryptophan, the precursor of serotonin, were significantly lower in the dystonia group and in line with this also the concentration of 5-HIAA, the main downstream metabolite of serotonin, was lower, albeit that this did not reach statistical significance.

Tryptophan, the precursor of serotonin is an essential amino acid, meaning that synthesis of serotonin is dependent on supply of tryptophan from our diet. After tryptophan is absorbed in the gut and via the circulation arrives at the central nervous system, it is converted into serotonin. Serotonin itself cannot cross the blood-brain barrier, so production of serotonin in the brain depends on the plasma concentration of tryptophan [27]. It is hypothesized that low concentrations of tryptophan influence mood and acute tryptophan depletion in vulnerable populations can cause depressive symptoms [28,29]. The gut microbiota appears to be involved in regulating both tryptophan and serotonin metabolism [29] and the gut-brain-axis likely plays a role in various neuropsychiatric disorders, but has also suggested to be involved in movement disorders such as Parkinson's disease [30,31]. However, we could not find any correlations between psychiatric disorders and low concentrations of tryptophan in our cohort, probably due to our relatively small sample size and the exclusion of depressive patients using anti-depressants. We did found an association between presence of OCD (n = 9) and high concentrations of 5-HIAA, the main metabolite of serotonin. However, the pathophysiology of OCD is not clear and some studies reported, in line with our findings, increased concentrations of 5-HIAA in the CSF while others suggest a hyposerotonergic state [32]. Concluding, our results confirm the hypothesis of involvement of the serotonergic metabolism in dystonia patients. This involvement of the serotonergic system is further supported by a recent study showing that blockage of the 5HT-2A receptor reduced onset of dystonia in a mouse model of stress induced dystonia [33].

Another recent metabolomic study by Liu et al. shows interesting differences in specific metabolites between controls and CD patients [34]. However, monoamines and amino acids were not reported and therefore it is difficult to compare the results observed in our study and theirs. It does show that differences exist in patients with dystonia and warrants further study.

Although, we did find some very interesting findings in the metabolism of monoamine neurotransmitters our study has some limitations. Results of this study should be interpreted with caution since we measured the serotonergic and dopaminergic metabolites in blood instead of directly in the brain. Some but not all of the metabolites we measured, such as serotonin and dopamine, are able to cross the blood-brain-barrier [17,27]. A more ideal way would be to measure concentrations in CSF, which requires an invasive lumbar puncture. On top of that, sample collection and handling of CSF have to be stringently regulated, because there is a rostrocaudal gradient in the concentrations and contamination with blood can cause degradation of the metabolites [35]. The small numbers in some groups are yet another limitation of this study, however, this is inevitable given the relatively rare nature of DRD and M-D. Although we statistically corrected for age, the wide age range might still have influenced our results. Next, we did not measure renal function or dietary habits, which could be possible confounders [36] [–] [38]. However, none of the participants was known to have a

history of kidney disease.

In conclusion, results of this study show alterations in the dopaminergic and serotonergic metabolism of patients with dystonia compared to controls. Also, dystonia subtype specific changes were found. Low concentrations of levodopa were associated with both motor as non-motor symptoms. Further research on dopaminergic and serotonergic systems in dystonia, with a special attention to the kinetics of the enzymes involved in these pathways, is necessary to gain more insight in the pathophysiology of dystonia. In the end, this will lead to more adequate treatment of both the motor as non-motor symptoms in dystonia patients.

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Authors' roles

ET, MF, MS, AK, IK, MT, KN and TK contributed to the conception and organization of the research project. ET, MS, AK collected the data, MF, IH performed the laboratory analysis. ET designed and executed the statistical analysis, TK reviewed the analysis. ET and TK wrote the first draft of the article. All authors reviewed, edited and approved the final manuscript.

Data statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declaration of competing interest

None.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.08.019.

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