



# Biomarcadors del metabolisme de la dopamina en alteracions neurològiques en la infància

Marta Molero Luis

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**BIOMARCADORS DEL METABOLISME DE LA DOPAMINA  
EN ALTERACIONS NEUROLÒGIQUES EN LA INFÀNCIA**

**Marta Molero Luis  
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# Homovanillic acid in cerebrospinal fluid of 1388 children with neurological disorders

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## PUBLICATION DATA

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## ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-MTHF	5-methyltetrahydrofolate
CSF	Cerebrospinal fluid
HVA	Homovanillic acid
L-DOPA	L-3,4-dihydroxyphenylalanine
MAO	Monoamino oxidase

**AIM** To determine the prevalence of dopaminergic abnormalities in 1388 children with neurological disorders, and to analyse their clinical, neuroradiological, and electrophysiological characteristics.

**METHOD** We studied biogenic amines in 1388 cerebrospinal fluid (CSF) samples from children with neurological disorders (mean age 3y 10mo, SD 4y 5mo; 712 males, 676 females). Correlations among CSF homovanillic acid (HVA) values and other biochemical, clinical, neuroradiological, and electrophysiological parameters were analysed.

**RESULTS** Twenty-one patients with primary dopaminergic deficiencies were identified. Of the whole sample, 20% showed altered HVA. We report neurological diseases with abnormal CSF HVA values such as pontocerebellar hypoplasia, perinatal asphyxia, central nervous system infections, mitochondrial disorders, and other genetic diseases. Overlapping HVA levels between primary and secondary dopamine deficiencies were observed. Prevalence of low CSF HVA levels was significantly higher in neonatal patients ( $\chi^2=84.8$ ,  $p<0.001$ ). Abnormalities in white matter were associated with low CSF HVA (odds ratio 2.3, 95% confidence interval 1.5–3.5).

**INTERPRETATION** HVA abnormalities are observed in various neurological diseases, but some are probably an unspecific finding. No clear limits for CSF HVA values pointing towards primary diseases can be stated. We report several neurological diseases showing HVA alterations. No neuroimaging traits were associated with low HVA values, except for white matter abnormalities.

Dopamine and serotonin are neurotransmitters involved in several neurological and systemic functions. Their turnover in the central nervous system (CNS) can be assessed by biochemical analysis of several biogenic amines in cerebrospinal fluid (CSF) at are closely related to them.<sup>1–4</sup> Homovanillic acid (HVA), which is the final product in dopamine catabolism, can be used as a marker of dopamine metabolism. Dopamine is synthesized from tyrosine by tyrosine hydroxylase (EC1.14.16.2), which converts tyrosine into L-3,4-dihydroxyphenylalanine (L-DOPA) with tetrahydrobiopterin as a cofactor. Aromatic L-amino-acid decarboxylase (EC4.1.1.28) transforms L-DOPA into dopamine using pyridoxal-5'-phosphate as a cofactor. Dopamine is catabolised by monoamino oxidase (MAO, EC1.4.3.4) and catechol-*O*-methyltransferase (EC2.1.1.6), yielding HVA as the stable final product (Fig. S1, online supporting information). Acquired and genetic neurological diseases associated with dopaminergic dysfunction are known to cause movement disorders and neuropsychiatric disorders.<sup>1</sup> There are several genetic disorders caused by defects in the synthesis of dopamine in which the CSF HVA values

are decreased, including tyrosine hydroxylase (OMIM# 191290), L-amino-acid decarboxylase (OMIM# 107930), guanosine triphosphate cyclohydrolase (OMIM# 128230), sepiapterin reductase (OMIM# 182125), and pyridox(am)ine 5'-phosphate oxidase (OMIM# 603287) deficiencies. Other diseases that alter the normal function of dopamine in the synapses may increase the CSF HVA values, such as the recently reported dopamine transporter deficiency (OMIM# 126455).<sup>4</sup>

There is scientific evidence suggesting that impaired HVA values could be a biomarker for dopamine dysfunction in several neurological diseases in children.<sup>1,2,5–9</sup> In this study, we explored the prevalence of dopaminergic abnormalities in a large cohort of children with neurological disorders and correlated their HVA levels with clinical, radiological, and electrophysiological features.

## METHOD STUDY GROUP

Between January 2001 and September 2010, 1713 CSF samples were analysed in the Sant Joan de Déu Hospital

laboratory. The samples were collected from patients with several neurological disorders for which a diagnostic lumbar puncture was indicated. The samples originated from several hospitals in Spain, Portugal, Greece, Turkey, Argentina, and India. Three hundred and twenty-five samples were excluded for one of the following reasons: patient older than 16 years, use of dopaminergic drugs, traumatic (haematic) puncture, or inadequate collection and preservation of the samples (such as lack of rostral-caudal gradient sampling and light protection, and inappropriate storage temperatures).

Of the final 1388 samples evaluated (patients' mean age 3y 10mo, SD 4y 5mo; age range 1d–16y; 712 males, 676 females), we were able to review the clinical data from 696 cases, and most patients were followed up at the Paediatric Neurology Department in the Sant Joan de Déu Hospital (Fig. 1). The complete clinical data of 696 patients were included in a database for further analysis. The patients' medical records were reviewed to determine an aetiological diagnosis, head circumference, electroencephalogram (EEG), and neuroimaging findings. A total of 256 patients had a specific aetiological diagnosis, and a syndromic diagnosis was determined for the other 440 patients. For descriptive purposes, the 696 patients were divided into nine different clinical groups (Appendix S1, online supporting information).

Samples from patients were obtained in accordance with the 2000 revised Helsinki Declaration of 1975. The study was approved by the local ethics committee of Sant Joan de Déu Hospital. For the genetic studies, written informed consent was obtained from the patients or their parents in the different centres involved in the study.

### SAMPLE SIZE

Previous work in the same area established a prevalence of HVA alterations in CSF of 6.7% in a sample of 446 people<sup>5</sup> and of 17.9% in a sample of 56 individuals.<sup>7</sup> We based our calculation on our primary outcome, the prevalence of HVA alterations in CSF, and found that our final sample of 1367 people had a bilateral precision of 1.3% for the estimation of a proportion of 6% of abnormalities with a confidence interval of 95%.

### LABORATORY STUDIES

Screening laboratory tests for the diagnosis of metabolic and genetic diseases (e.g. lactate, ammonium, amino acids, organic acids, acylcarnitines, glycosaminoglycans, sialotransferrin, fatty acids, and karyotype) and prolactin were performed using standard procedures. Enzymatic and molecular studies were performed in several reference centres for the diagnosis of rare diseases.

### CEREBROSPINAL FLUID ANALYSIS

CSF samples were collected by lumbar puncture as previously reported.<sup>10</sup> Biogenic amine metabolites (HVA for dopamine and 5-hydroxyindoleacetic acid [5-HIAA] for serotonin), pterins, and 5-methyltetrahydrofolate

### What this paper adds

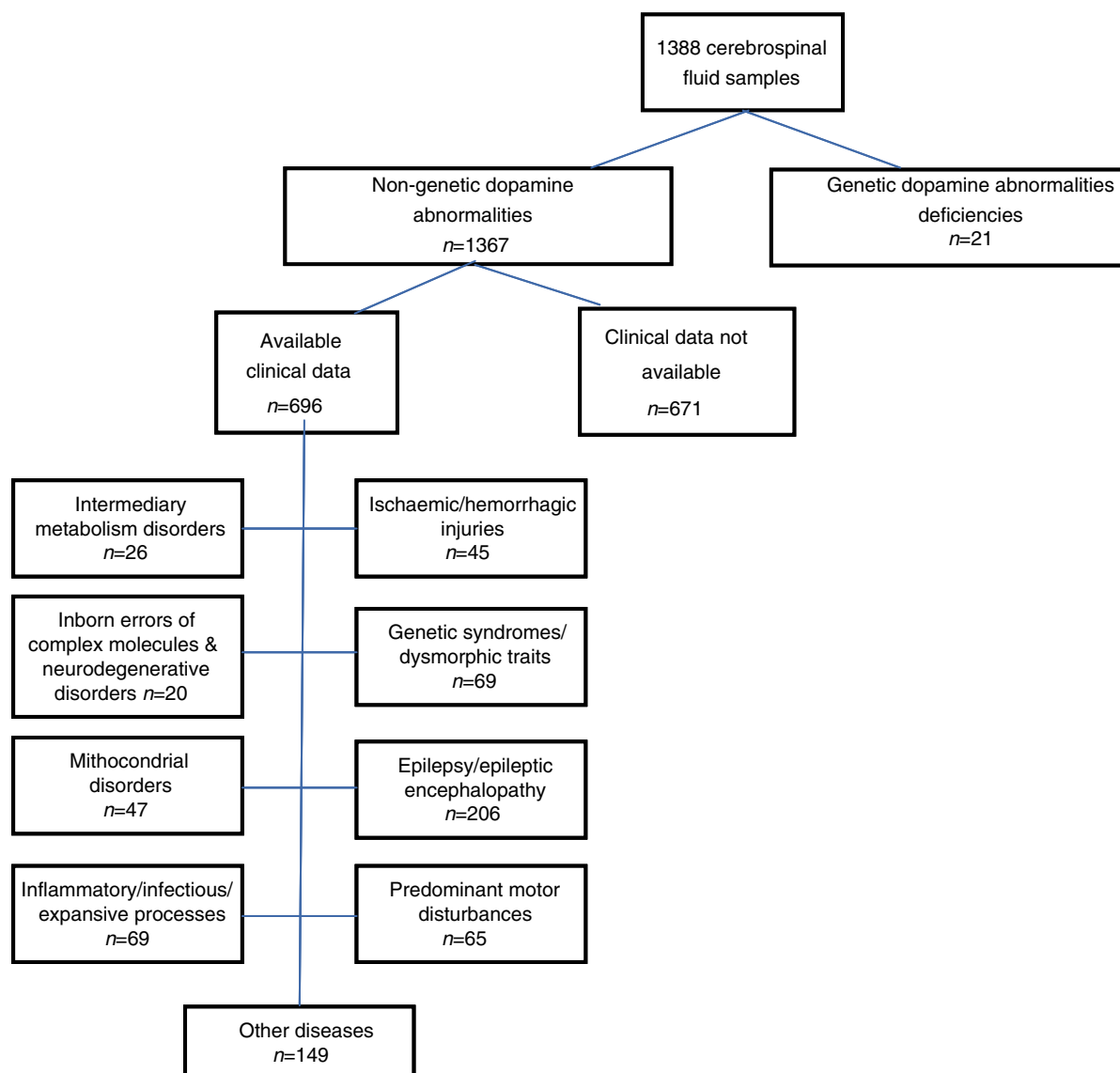
- Assessment of CSF HVA was performed in the largest cohort of neuropaediatric patients to date.
- Altered HVA values can overlap in primary and secondary diseases.
- A statistically significant relation was observed between impaired HVA values and white matter abnormalities.

(5-MTHF) were analysed using high-performance liquid chromatography with electrochemical and fluorescence detection respectively.<sup>1,2,10–12</sup> The results were compared with the reference values established by our laboratory, which were determined based on data from 127 individuals from our geographical area (mean age 3y 10mo; range 11d–16y;) whose CSF samples were submitted for possible viral or bacterial meningitis and encephalitis. Additional details about obtaining, storing, and transporting samples have been previously described by our group.<sup>10</sup> The biogenic amine analysis complies with the ERNDIM external quality control programme (available on request).

Genetic defects in dopamine metabolism were studied in patients with suggestive clinical and CSF biochemical features. In selected cases, molecular analysis of tyrosine hydroxylase, L-amino-acid decarboxylase, guanosine triphosphate cyclohydrolase, sepiapterin reductase, and MAO-A/B deficiencies was performed (MAO-A: OMIM# 309850; MAO-B: OMIM# 309860).

### STATISTICAL ANALYSIS

In the whole sample ( $n=1367$ ), once the genetic dopaminergic deficiencies were excluded, we analysed HVA as a categorical and as a continuous variable. To describe the prevalence of HVA abnormalities, first we classified HVA continuous values as low, normal, or high considering the age group according to our own CSF HVA reference intervals.<sup>10</sup> Then we reported the proportion of abnormalities for the whole sample and for each age group. We also applied Pearson's  $\chi^2$  test and computed the odds ratio (OR) to explore which were the age groups with significantly more patients with impaired HVA status. Correlations between continuous values of HVA concentration and age, pterins, 5-HIAA, 5-MTHF, and plasma prolactin were analysed. Histograms of the data were checked to assess normality. If the plot did not show normal distribution, some basic mathematical transformations were studied and applied to make the data normally distributed; then a series of Pearson's correlation coefficients were computed. To assess for an independent association among continuous HVA values and the rest of variables, a multiple linear regression model was considered. The multivariable study was performed to adjust regression coefficients for possible confounding effects between variables. Normality of residuals was checked by analysing their histogram. All confidence intervals were computed by the bootstrap method over 10 000 samples with replacement of the same size as the original sample. The  $\chi^2$  test, Fisher's exact test, and odds ratios were computed to search for associations between altered CSF HVA results and



**Figure 1:** Algorithm of the distribution of patients.

altered 5-MTHF values, abnormal plasma prolactin levels, clinical groups, neurophysiological, and neuroradiological data. All analyses used SPSS version 19.0 (SPSS Inc, Chicago, IL, USA) and R 2.15.0 (R Development Core Team; <http://www.R-project.org>);  $p < 0.05$  was considered as statistically significant.

## RESULTS

### CSF HVA findings in the study group

#### **Prevalence of CSF HVA abnormalities in the whole sample**

Through the analysis of the 1388 CSF samples, 21 patients (7 males, 14 females) with genetic dopamine deficiencies were identified: 11 with tyrosine hydroxylase deficiencies, four with guanosine triphosphate cyclohydrolase deficiencies, three with L-amino-acid decarboxylase deficiencies,

and one each with a sepiapterin reductase deficiency, MAO-A and MAO-B deficiency, and NPO deficiency (Table I<sup>13–18</sup>). After excluding these deficiencies ( $n=1367$ ), when compared with our age-related reference values, CSF HVA values were low in 15.4% (95% confidence interval [CI] 13.5–17.4) of patients, high in 4.6% (95% CI 3.6–5.9), and normal in 80.0% (95% CI 77.8–82.1).

#### **Prevalence of CSF HVA abnormalities by age group**

Distribution of patients and abnormal HVA proportions among age groups is shown in Figure S2 (online supporting information). Association between age groups and HVA alteration proportion was statistically significant ( $\chi^2=84.8$ ,  $p < 0.001$ ). The proportion of low HVA values was significantly higher in patients under 1 month of age compared with the oldest sample (OR 1.78, 95% CI 1.08–2.98).

**Table I:** Biochemical and molecular findings from 21 individuals with primary dopamine deficiencies

	Defect	Age	HVA (nmol/l)	HVA decrease (%)	Molecular findings
1 M	PNPO	1d	654	-0.6	p.A174X/p.A174X
	Rf values	0-30d	658-1434		
2 F	TH	5mo	31	-91.2	p.L236P/p.L236P
3 F	TH	5mo	18	-99.8	p.L236P/p.L236P
	Rf values	1-5mo	354-1328		
4 F	TH	7mo	18	-94.8	p.L236P/p.L236P
5 F	AADC	11mo	108	-68.6	p.G123R/p.T245I
6 M	GTPCH	1y	268	-22.1	p.Q89X
7 M	TH	1y	45	-87.0	p.R233H/p.R233H
8 F	AADC	1y	6	-98	On course
9 M	MAO-A/B	1y	Not detectable	-100	Xp11.3 del 43.2-43.7 (hemizygous)
10 F	TH	2y	74	-78.5	g-70C>T/g-70C>T
11 F	TH	2y	50	-85.5	p.L236P/p.L236P
	Rf values	6 mo-2y	344-906		
12 F	TH	3y	151	-50.3	g-70C>T/c.[1197 + 25_1391del]
13 M	TH	3y	20	-93.4	p.238W/p.T399W
14 F	AADC	4y	44	-85.5	p.R347Q/p.R347Q
	Rf values	3-6y	304-658		
15 F	GTPCH	7y	318	No decrease	Still on course <sup>a</sup>
16 F	TH	8y	5	-97.5	p.L236P/p.L236P
	Rf values	7-10y	202-596		
17 F	SR	11y	48	-69.2	c.448A>G(p.R150G)/c.304G>T (p.G102C)
18 F	GTPCH	15y	104	-33.3	c.595C>G
19 F	GTPCH	15y	92	-41.0	c.595C>G
20 M	TH	16y	53	-66.0	p.R233H/p.R233H
21 M	TH	25y <sup>b</sup>	23	-85.2	p.R233H/p.R233H
	Rf values	>11y	156-410		

<sup>a</sup>The molecular analysis revealed no abnormalities, including direct sequencing of exonic regions and intronic boundaries, and multiplex ligation-dependent probe amplification of the *GCH1* gene. Other analyses are still on course. <sup>b</sup>One adult patient was added because he was the brother of patient number 20. The percentage homovanillic acid (HVA) decrease represents the decrease in HVA levels compared with the lower limit of the reference values in each age group.<sup>10</sup> M, male; F, female; PNPO, pyridox(am)ine 5'-phosphate oxidase; Rf values: reference values; TH, tyrosine hydroxylase; AADC, L-amino-acid decarboxylase; MAO-A/B, monoamine oxidase-A, -B; GTPCH, guanosine triphosphate cyclohydrolase; SR, sepiapterin reductase. Data from some of the patients have been previously published.<sup>13-18</sup>

To compare the degree of CSF HVA deficiency in genetic dopaminergic disturbances (primary dopamine deficiency) with other diseases (secondary dopamine deficiency), CSF HVA values in both groups of patients were analysed and are represented in Table II and Figure 2. An overlapping in HVA concentration was observed between these two groups, especially in older patients (Fig. 2)

#### **Association between CSF HVA continuous values and other biochemical parameters**

Because continuous data were highly dispersed and histograms did not show normality, to make data normally dis-

tributed the common logarithm in base 10 was found to be the most appropriate transformation to apply to the response HVA and to all other explanatory variables in the study before performing association analyses.

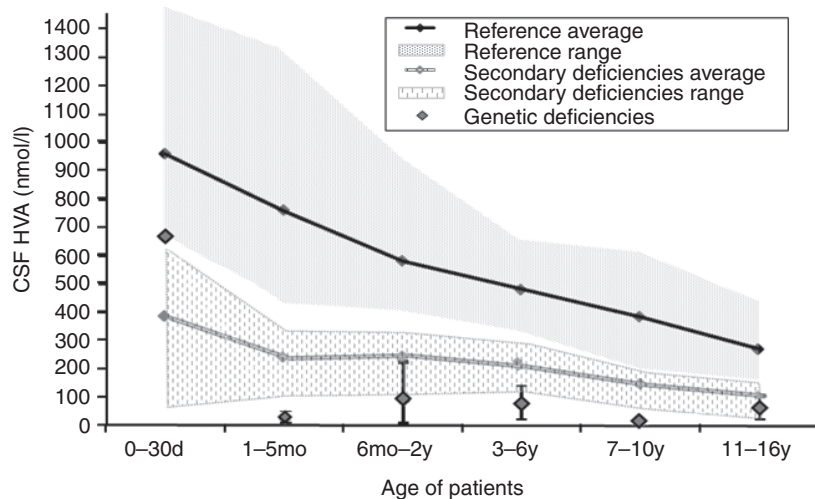
Pearson's linear correlation coefficients showed a high association between CSF HVA and CSF 5-HIAA ( $r=0.75$ ; 95% CI 0.72-0.77) and age ( $r=-0.51$ , 95% CI -0.55 to -0.47), moderate with CSF biopterin ( $r=0.38$ ; 95% CI 0.34-0.43), and low with CSF neopterin ( $r=0.15$ ; 95% CI 0.10-0.20). HVA was the dependent variable and 5-HIAA, age, biopterin, and neopterin the independent variables. For this model,  $r^2$  was 0.59. The coefficients and bootstrap CIs were

**Table II:** Cerebrospinal fluid homovanillic acid values (ranges, mean [SD]) in the comparison and secondary and primary dopamine deficiency groups

Age	0-30d	1-5mo	6mo-2y	3-6y	7-10y	11-16y
Reference values <sup>10</sup>	<i>n</i> =11	<i>n</i> =22	<i>n</i> =38	<i>n</i> =24	<i>n</i> =20	<i>n</i> =12
Reference range	658-1434	354-1328	344-906	304-658	202-596	156-410
Mean (SD)	955 (236)	757 (231)	579 (131)	478 (90)	383 (174)	269 (73)
Primary deficiencies	<i>n</i> =1	<i>n</i> =2	<i>n</i> =8	<i>n</i> =3	<i>n</i> =1 <sup>a</sup>	<i>n</i> =5
Range	654	19-31	1-268	20-151		23-104
Mean (SD)		25 (9)	71 (87)	72 (70)	5	64 (33)
Secondary alterations	<i>n</i> =49	<i>n</i> =15	<i>n</i> =52	<i>n</i> =45	<i>n</i> =18	<i>n</i> =31
Range	61-615	102-336	74-332	101-285	52-184	23-143
Mean (SD)	383 (158)	241 (66)	249 (70)	212 (54)	148 (35)	106 (31)

<sup>a</sup>One patient with a dopaminergic metabolism defect (guanosine triphosphate cyclohydrolase deficiency) did not have a homovanillic acid deficiency and is not included.





**Figure 2:** Graphical representation of cerebrospinal fluid homovanillic acid (CSF HVA) levels of Table II. Different averages and ranges of each group are stated in Table II.

as follows: for 5-HIAA,  $\beta=0.65$  (95% CI 0.60–0.70); for age,  $\beta=0.03$  (95% CI 0.01–0.05); for biopterin,  $\beta=0.18$  (95% CI % (0.13–0.23); and for neopterin,  $\beta=-0.06$  (95% CI  $-0.09$  to  $-0.02$ ), with a significant constant of 0.96 (95% CI 0.82–1.1). Because these coefficients were calculated for logarithmic-scale response and predictor variables,  $\beta$  values must be interpreted in exponential form for each independent variable (so  $HVA \sim 2.6 \times HIAA^{0.651} \times biopterin^{0.183} \times [1/neopterin^{0.057}] \times age^{0.028}$ ).

For this model, a histogram of residuals shows data conforming to the assumptions of linear regression (Fig. S3, online supporting information).

For those patients with data available for plasma prolactin ( $n=245$ ), we found that abnormal levels of plasma prolactin and HVA showed no association (OR 1.5, 95% CI 0.4–6.6).

#### **Association between CSF HVA and clinical data**

Concerning the 696 samples obtained from patients for whom complete clinical data were available, HVA values were low in 15.8% and high in 5.0% of the patients. These results were very similar to those observed in the group of patients as a whole.

Among nine different clinical groups, there was statistical difference in the hypoxic–ischemic and haemorrhagic injuries group and the presence of altered HVA (OR 2.1, 95% CI 1.0–4.1). For other clinical groups, OR did not show significance (intermediary metabolism disorders: OR 1.3, 95% CI 0.5–3.5; mitochondrial diseases: OR 1.1, 95% CI 0.5–2.4; inflammatory/infectious/expansive processes: OR 1.1, 95% CI 0.6–2.2; genetic syndromes: OR 1.4, 95% CI 0.8–2.6; epilepsy and epileptic encephalopathies: OR 0.8, 95% CI 0.5–1.2; complex encephalopathies with prominent motor disturbances: OR 0.8, 95% CI 0.4–1.8; other diseases, including mainly patients with predominant

intellectual disability:\* OR 0.7, 95% CI 0.4–1.2; accumulate complex molecules/neurodegenerative disorders OR 1.3, 95% CI 0.4–4.1).

Altered EEG and epilepsy were present in 64.3% and 53.3% of the patients respectively, and no significance was observed between CSF HVA abnormalities and either of both conditions (OR 0.8, 95% CI 0.5–1.2, and OR 0.7, 95% CI 0.5–1.1). For cranial growth, 29.9% presented with microcephaly and 4.5% presented with macrocephaly. There was no statistical association between CSF HVA abnormalities and head circumference.

Abnormalities in white matter were observed in 38.5% of patients and we found a statistically significant relation with low HVA values (OR 2.3, 95% CI 1.5–3.5). Low CSF HVA values were statistically associated with the presence of calcifications (OR 7.5, 95% CI 2.3–24.0) and with the presence of migration disturbances (OR 6.9, 95% CI 1.5–31.3). These findings may be explained by the low number of patients per group (12 and seven patients respectively). We found no statistically significant association between low HVA values and deep grey matter (OR 1.6, 95% CI 0.9–2.9), cerebellum (OR 1.3, 95% CI 0.5–3.2), brainstem abnormalities (OR 1.1, 95% CI 0.2–5.2), or brain atrophy (OR 1.5, 95% CI 0.9–2.3). In those patients with white matter abnormalities on magnetic resonance imaging (MRI;  $n=217$ ), there was a statistically significant relation between the presence of low CSF HVA values and low CSF 5-MTHF values (OR 2.8, 95% CI 1.2–6.3). When white matter disturbances were reclassified to find differences among delayed myelination, demyelination, dysmyelination, hypomyelination, and known leukodystrophies and low HVA or low 5-MTHF, no statistical differences were found. No significant statis-

\*North American usage: mental retardation

tical relation was found between abnormalities in white matter and the presence of low 5-HIAA. Neuroimaging abnormalities were not related to high levels of CSF HVA.

### HVA values in patients with an aetiological diagnosis

The prevalence of altered HVA and additional biochemical data (HVA and 5-HIAA values) are detailed in Table SI (online supporting information), for the patients with a definitive aetiological diagnosis ( $n=256$ ). Of the 70 patients with altered CSF HVA concentrations, 49 had low and 21 had high HVA levels. Low HVA was frequent in the patients with CNS infectious processes ( $n=6/38$ ), perinatal hypoxic ischemic encephalopathy ( $n=10/27$ ), mitochondrial disorders ( $n=8/47$ ), and pontocerebellar hypoplasia type 2 ( $n=4/6$ ). High HVA levels were primarily detected in the patients with mitochondrial diseases ( $n=7/47$ ) and in preterm infants ( $n=4/7$ ).

### DISCUSSION

To our knowledge, this is the largest cohort of children with neurological disorders in whom CSF biogenic amines have been assessed in combination with clinical data. Lumbar puncture and CSF analysis are essential for the diagnosis of certain neurotransmitter disorders. As for other biological fluids, analyses of catecholamines and their metabolites may show false negative results due to drugs or diet.<sup>10–12</sup>

From 2001 to 2010, we analysed 1388 CSF samples and diagnosed 21 genetic primary dopamine deficiencies, representing 1.4% of all of the samples evaluated. This finding suggests that an accurate selection of patients based on their clinical manifestations and other complementary data is needed.

Reference intervals are critical for data interpretation. Considering geographical and interlaboratory variations for biogenic amines, the available reference intervals in the literature are quite similar, but differences may be related to the age of the patients.<sup>1,10</sup> This reinforces the idea that establishment of own reference intervals in laboratories that commonly perform neurotransmitter studies is mandatory, as occurs for other biochemical parameters.

It has been postulated that the magnitude of reduction in CSF HVA levels may suggest the diagnosis of a primary genetic defect involving dopamine metabolism. However, clear limits for values that indicate such diseases have not been identified so far. In patients with low CSF HVA values, other possibilities should be considered before the diagnosis of a primary neurotransmitter disorder is made, particularly when the clinical manifestations are not suggestive. In our series, the CSF HVA values were sometimes lower for secondary defects than for the primary genetic conditions (Table II). It was remarkable that the degree of primary and secondary HVA deficiency appeared to be closer, particularly in the

oldest group (11–16y; Fig. 2). This finding may be explained by the fact that two patients with dominant guanosine triphosphate cyclohydrolase deficiency were included in this age group and patients with this disorder are known to have moderately low CSF HVA levels.<sup>19</sup>

We detected the lowest CSF HVA values in patients with tyrosine hydroxylase and L-amino-acid decarboxylase deficiencies; the lowest values in the group of patients with secondary HVA deficiencies were detected in cases of pontocerebellar hypoplasia type 2, CNS neoplasia, Steinert disease, Miller Fisher syndrome, methylentetrahydrofolate reductase deficiency, and several motor disturbances and epileptic encephalopathies with unknown aetiological diagnoses. However, not all of the diseases mentioned in this study showed a consistent reduction in HVA values, suggesting that HVA deficiency might be a non-specific finding in several of the patients that is not likely to be involved in the primary pathophysiology of the disease. Interestingly, the youngest group of patients showed the highest percentage of HVA deficiencies, suggesting that the dopamine system is more sensitive to different neurological conditions during early postnatal life. In general terms, patients with secondary deficiencies did not show clinical features that were strongly suggestive of a dopamine deficiency, such as movement disorders, oculogyric crises, or autonomic features. Parkinsonism features have been observed in some patients with mitochondrial disorders.<sup>8</sup> This group of diseases may present with both low and high HVA values.<sup>8,20</sup> A previous report showed clinical features of Parkinsonism in a group of patients with mitochondrial disorders, and their responses to L-DOPA were limited.<sup>8</sup> HVA alterations may have different pathophysiological mechanisms in mitochondrial disorders, and an explanation for these findings currently remains elusive.<sup>8,20</sup>

A significant correlation was observed between HVA and the other biochemical variables, with 5-HIAA being the variable with the strongest association according to the  $\beta$  coefficient of the linear regression analysis. This observation supports the idea that there is a close relation between dopamine and serotonin turnover, despite the fact that their metabolic pathways occur in different areas of the brain.<sup>2,21</sup> As shown in Table SI, approximately 43% of patients also presented with impaired 5-HIAA values, indicating that there is probably an alteration in both of the pathways in several conditions and that the underlying mechanisms in those diseases may be non-specific. Disturbances in 5-HIAA have been reported to be even more frequent than HVA defects,<sup>6,21</sup> suggesting that serotonin is easily impaired in a broad spectrum of diseases, perhaps owing to its widespread connectivity in different areas of the brain.

Hyperprolactinaemia is often detected in genetic disorders involving dopamine metabolism because of the role of dopamine in inhibiting prolactin secretion. In our series, all of the patients with a tyrosine hydroxylase deficiency initially presented with high plasma prolactin



values (range 1046–2151 mU/l; reference values in the paediatric population 79–325 mU/l in males and 108–466 mU/l in females). In other primary deficiencies of dopamine metabolism, such as guanosine triphosphate cyclohydrolase deficiencies, this finding was variable (i.e. one patient presented with normal values, whereas the other had slightly increased values). Therefore, we did not find any association between CSF HVA values and prolactin levels. We hypothesize that plasma prolactin reference values should be re-evaluated in paediatric patients to detect variations when dopaminergic disturbances are suspected.

Concerning the 696 patients with complete clinical data, we failed to find statistical associations between CSF HVA abnormalities and EEG characteristics, presence of epilepsy, or abnormalities in cranial growth. The literature on HVA in CSF and epilepsy is scarce and often controversial. HVA values may increase soon after seizures.<sup>22</sup> However, epilepsy as a chronic condition may not alter HVA.<sup>23</sup> In light of our findings and reports from the literature, the lack of changes in HVA values could be attributable to epilepsy as a chronic condition.<sup>22,23</sup> For the neuroimaging results, although the white matter abnormalities on MRI were associated with low HVA in CSF, abnormalities in the deep grey matter were not. These results are difficult to interpret because 85% of dopaminergic neurons constitute the nigrostriatal pathway, and we would expect to find low HVA values when any of these areas are altered. We found a significant association between changes in HVA and 5-MTHF in the group of patients with abnormalities in white matter (the risk of presenting with low HVA values was 2.78-fold higher in patients with low CSF 5-MTHF concentrations). A normal brain folate status is needed for normal white matter and adequate HVA production,<sup>24</sup> which would explain the association mentioned.

HVA analysis may reveal dopamine deficiencies associated with unexpected diseases and provide insight into new treatments. We have detailed an exhaustive list of metabolic and non-metabolic conditions in which HVA may be altered (Table SI). Several of the diseases associated with low HVA values for the first time, even anecdotally, include the following: pontocerebellar hypoplasia type 2, non-ketotic hyperglycaemia, oligosaccharidosis, Niemann–Pick type C, occipital-horn syndrome, serine deficit, thiamine transporter 2 deficiency, vanishing white matter disease, Miller Fisher syndrome, and acute disseminated encephalomyelitis. Diseases associated with high HVA values include Angelman syndrome, preterm infants with varying degrees of intraventricular haemorrhage, and one report of carbamyl phosphate synthetase deficiency (urea cycle disorder). Regarding clinical groups of diseases, hypoxic–ischemic and haemorrhagic injuries showed the highest risk of association with impaired HVA values, supporting the previous observations that this group of patients may present a special risk of impaired dopaminergic status.<sup>6</sup>

Recent reports have reinforced the presence of secondary alterations in biogenic amines in the CSF of neurological patients, most of whom suffer from severe chronic diseases that are intractable from an aetiological point of view.<sup>2,5–9,20</sup> A therapeutic assay with L-DOPA/carbidopa could be justified in patients with very low CSF HVA values and clinical features compatible with dopaminergic deficiencies, which might offer new therapeutic options. However, a cautious approach is important for L-DOPA therapeutic trials because this treatment may also cause significant side effects.<sup>25</sup> In our experience (data not shown), the clinical response to L-DOPA is variable and does not always produce satisfactory results.

One of the limitations of this study is the potential variation in the CSF HVA reference intervals according to different geographical areas. The reference intervals reported by other groups are similar (but not identical) to those values reported in this study,<sup>26</sup> and therefore, results of some external patients should be cautiously interpreted. Another limitation is that in our cohort of patients, there is potential selection bias towards patients presenting rare neurological diseases (such as mitochondrial disorders, Rett syndrome, primary dopamine deficiencies). Finally, the CSF HVA values should be interpreted with caution, as HVA concentrations may not always reflect a disturbance in dopaminergic function.

## CONCLUSION

HVA abnormalities were frequently observed in children with variable neurological diseases. In genetic conditions affecting dopamine turnover, CSF HVA values may overlap with the values detected in other diseases, particularly in older paediatric patients. Several neurological diseases show consistent alterations in HVA levels, which are often associated with changes in 5-HIAA. The possibility of treating these patients with L-DOPA/carbidopa in a therapeutic assay should be considered. For neurological features, only non-specific white matter abnormalities on MRI, but not other neurological and neuroimaging signs, were associated with low HVA values.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

**Fig S1:** Dopamine and serotonin biochemical pathways.

**Fig S2:** Cerebrospinal fluid homovanillic acid values in different age groups.

**Fig S3:** Residual histogram of the study.

**Table S1:** Diseases with decreased (3a) or increased (3b) homovanillic acid levels by clinical group

**Appendix S1:** Different diagnosis included in the nine clinical groups of Figure 1.

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