Great Ormond Street Hospital for Children NHS Trust

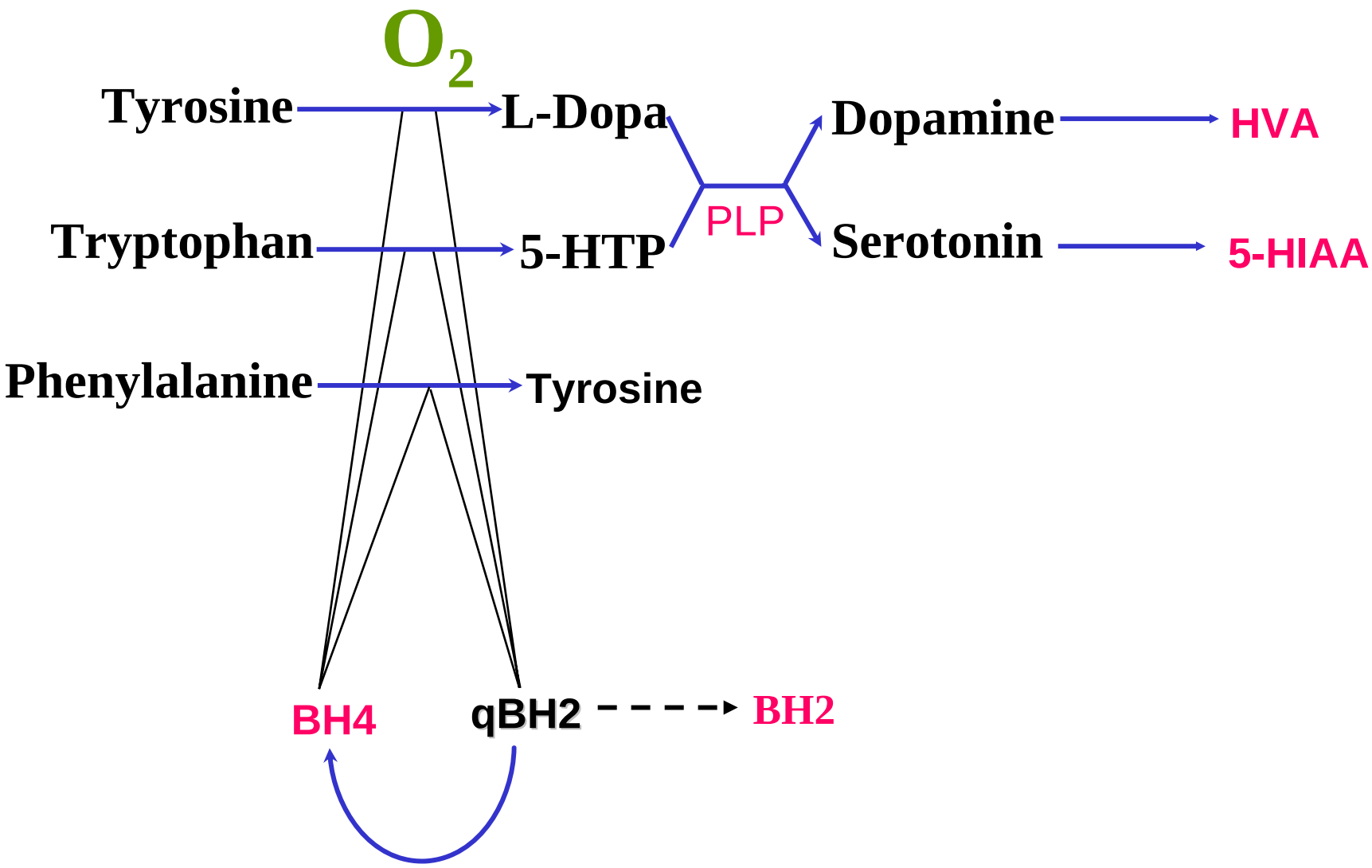
University College London Hospitals NHS Foundation Trust

# Analysis of "Neurotransmitters"

Simon Heales
HealeS@gosh.nhs.uk

UCL

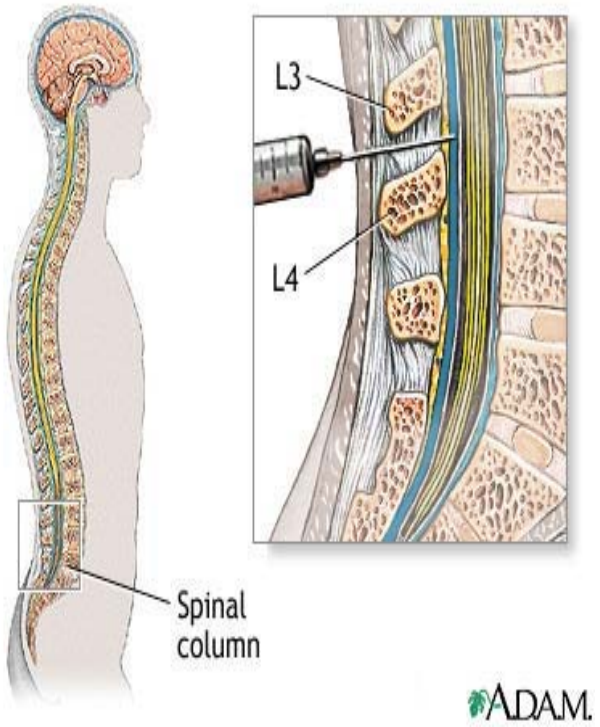
European Research Network ERNDIM Inherited Disorders of Metabolism


O2
Tyrosine
L-Dopa
Dopamine
HVA
Tryptophan
5-HTP
PLP
Serotonin
5-HIAA
Phenylalanine
Tyrosine
BH4
qBH2
BH2

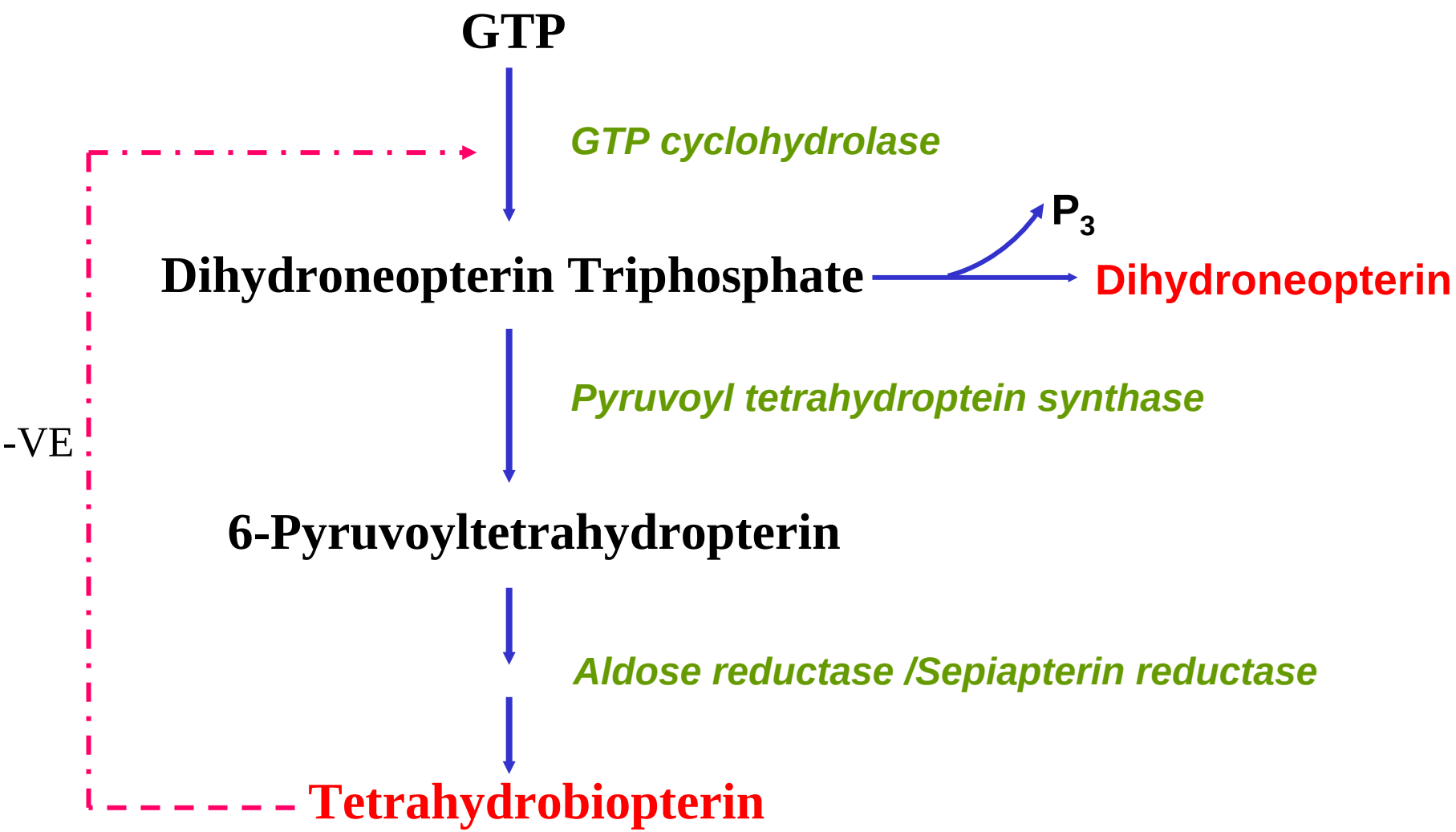
# CSF – Sample Requirements

- *Tube 1* 0.5ml **HVA & 5-HIAA**
- *Tube 2* 0.5ml **5-MTHF/PLP**
- *Tube 3* 1.0ml **Pterins**

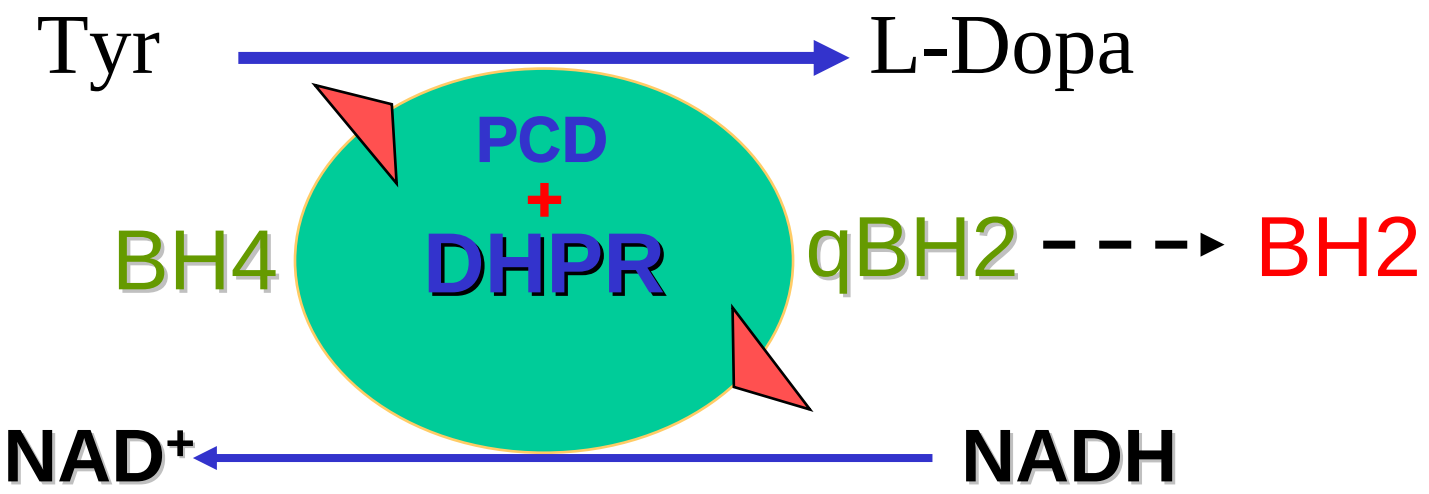
(DTE/DETAPAC)



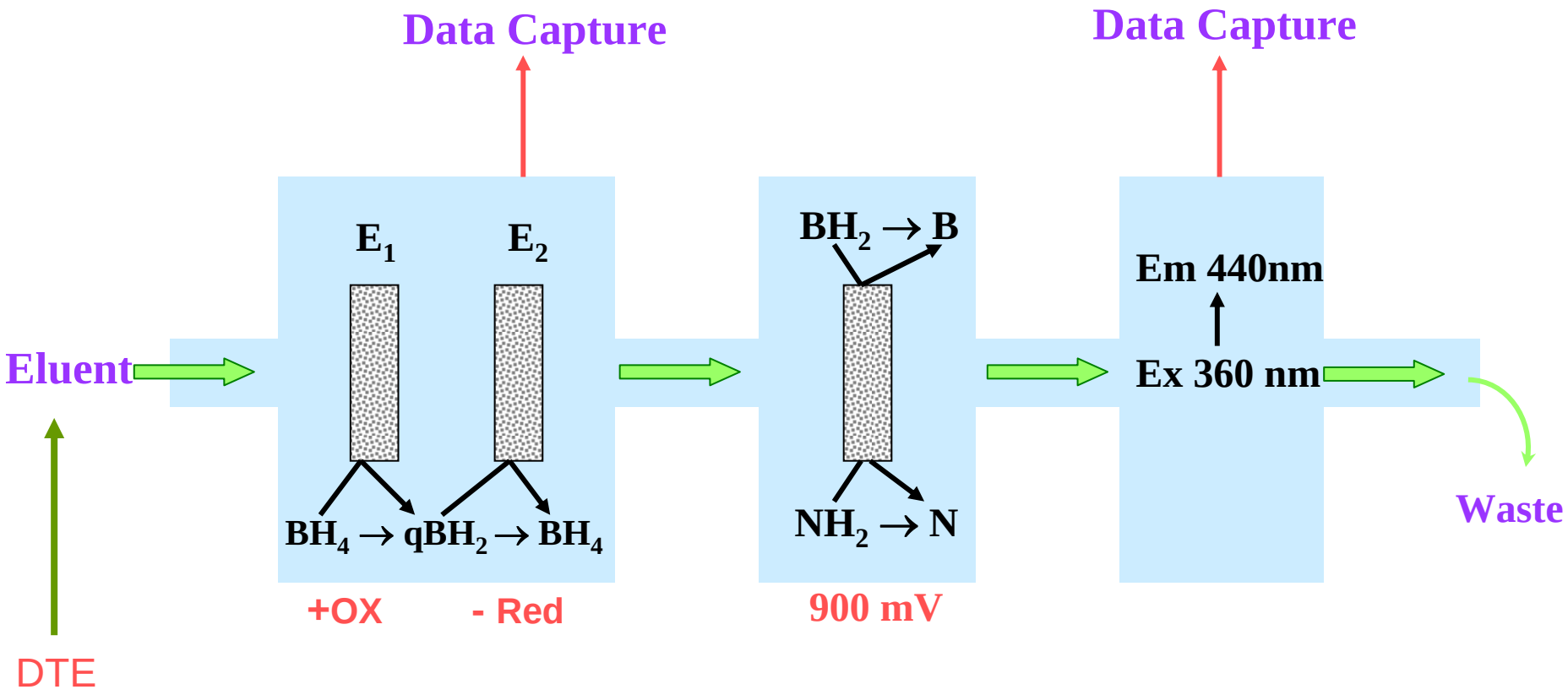

***Collect at bedside and freeze immediately (not the form !)***
***Date of Birth, Sample Date, Medication – essential***


GTP
GTP cyclohydrolase
P3
Dihydroneopterin Triphosphate
Dihydroneopterin
Pyruvoyl tetrahydroptein synthase
-VE
6-Pyruvoyltetrahydropterin
Aldose reductase /Sepiapterin reductase
Tetrahydrobiopterin

# BH4 Salvage




PCD = pterin carbinolamine dehydratase
DHPR = dihydropteridine reductase


Data Capture
Data Capture
E1
E2
BH2 → B
Em 440nm
Eluent
Ex 360 nm
Waste
BH4 → qBH2 → BH4
NH2 → N
+OX
- Red
900 mV
DTE

| | | nmol/L | |
|---|---|---|---|
| Metabolite | Age (years) | Mean | Range |
| **BH4** | 0 - 0.33 | 67 | 27-105 |
| | 0.34 - 0.66 | 37 | 23-55 |
| | 0.67 – 1.00 | 38 | 19-56 |
| | 1.10 – 5.00 | 33 | 8-57 |
| | 5.1- Adult | 23 | 9-39 |
| | | | |
| **BH2** | ALL | 5.6 | 0.4-13.9 |
| **NH2** | ALL | 19 | 7-65 |

*Pediatr Res (1993) 34, 10-14*

# Disorders of BH4 metabolism

**With Hyperphenylalaninemia**

GTP cyclohydrolase I (GTPCH) deficiency;
Phe = 90-1200 umol/L

*6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency;*
Phe = 240-2500 umol/L

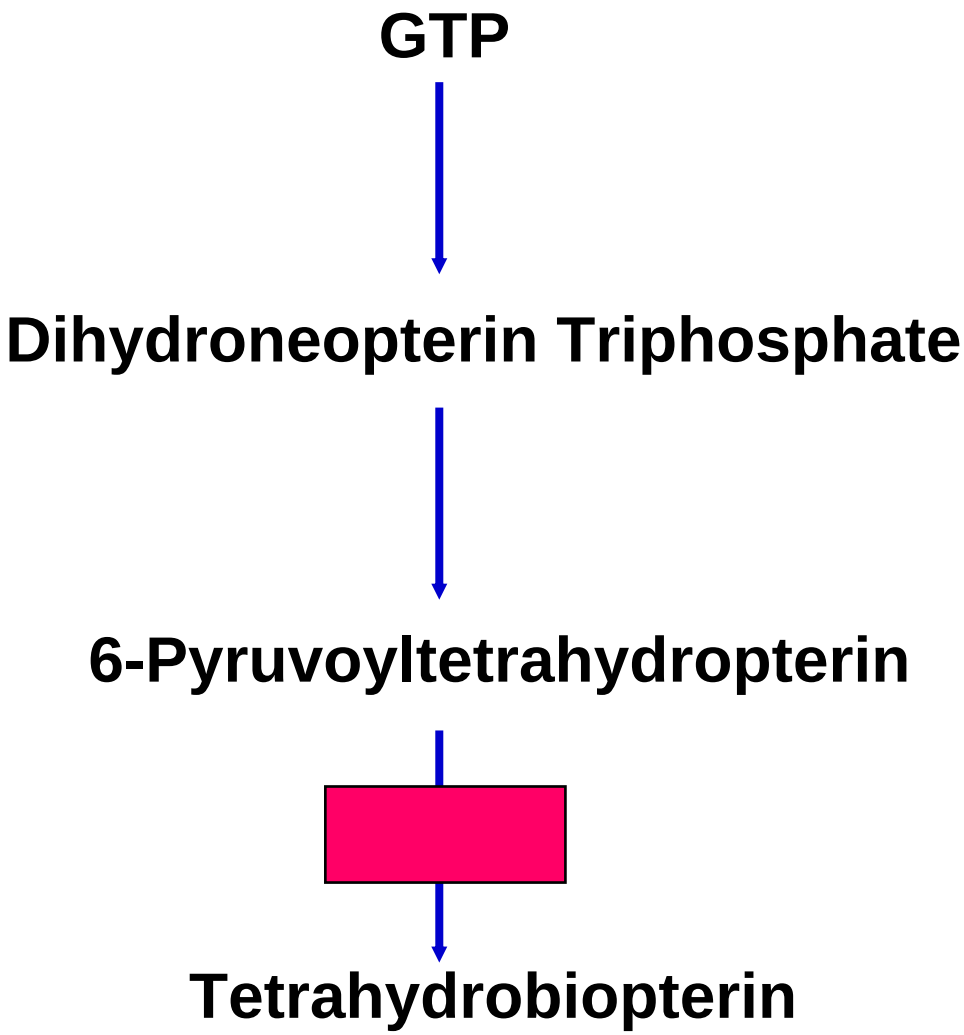
Dihydropteridine reductase (DHPR) deficiency;
Phe = 180-2500 umol/L

Pterin-4a-carbinolamine dehydratase (PCD) deficiency;
Phe = 180-1200 umol/l

**Without hyperphenylalaninemia**

*Sepiapterin reductase deficiency (SR).*
*Dopa-responsive dystonia (DRD) due to GTPCH deficiency*;

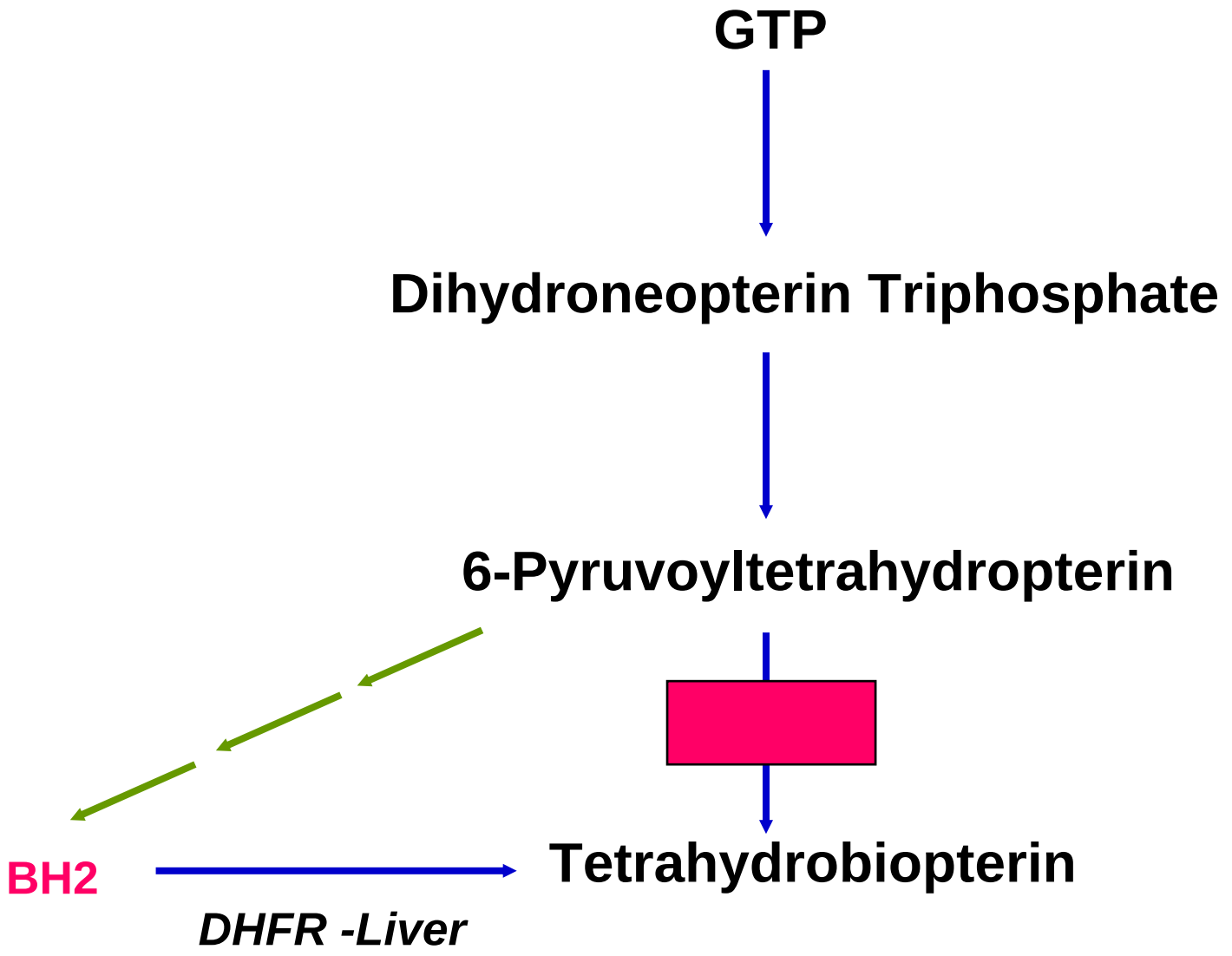
# Sepiapterin Reductase Deficiency

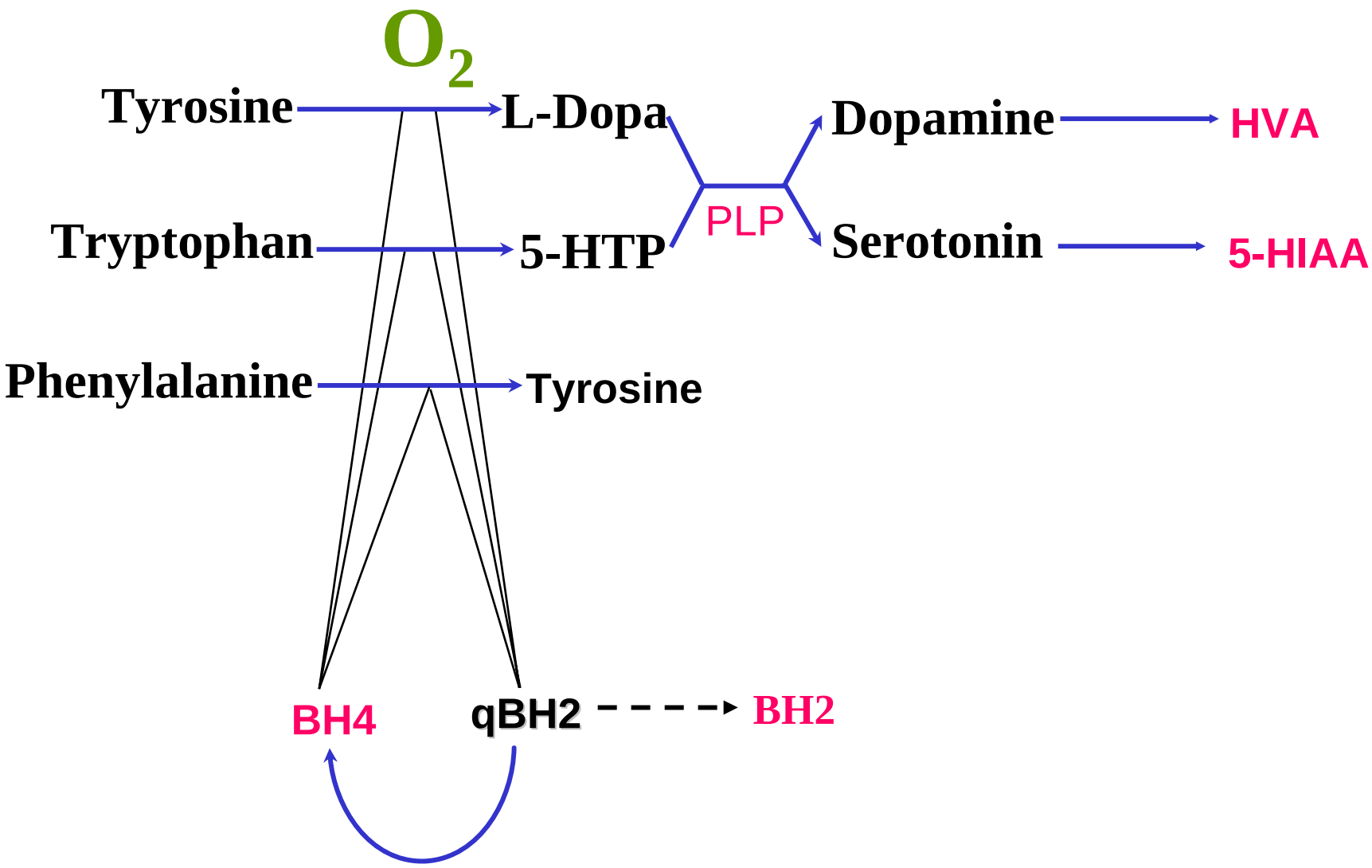


# Sepiapterin Reductase Deficiency

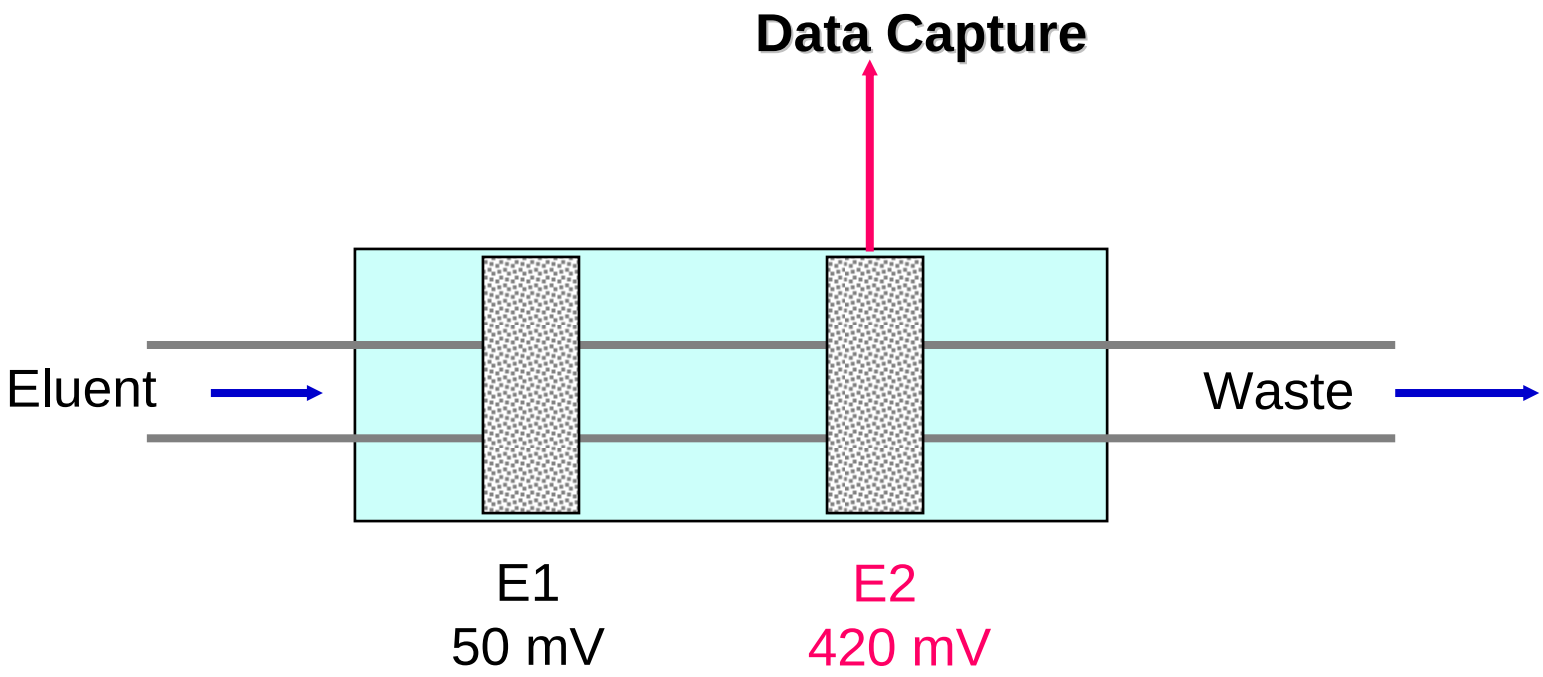
Sex; Male. Dob; 31/12/1987. Sample; 09/05/2003. Dystonia responsive to L-DOPA. No hyperphenylalaninaemia. DHPR normal.

| | | |
|---|---|---|
| **HVA:** | **23** | **(71- 565 nmol/L)** |
| **5-HIAA:** | **2** | **(58- 220 nmol/L)** |
| **BH4:** | **11** | **(9- 39 nmol/L)** |
| **BH2:** | **64** | **(0.4- 13.9 nmol/L)** |
| **Total Neopterin:** | **19** | **(7- 65 nmol/L)** |


GTP
Dihydroneopterin Triphosphate
6-Pyruvoyltetrahydropterin
BH2
Tetrahydrobiopterin
DHFR -Liver


O2
Tyrosine
L-Dopa
Dopamine
HVA
Tryptophan
5-HTP
PLP
Serotonin
5-HIAA
Phenylalanine
Tyrosine
BH4
qBH2
BH2

# Electrochemical Detection



| | | nmol/L | |
|---|---|---|---|
| Metabolite | Age (years) | Mean | Range |
| **HVA** | 0 - 0.33 | 714 | 324-1098 |
| | 0.34 - 0.66 | 587 | 362-955 |
| | 0.67 – 1.00 | 508 | 176-851 |
| | 1.10 – 5.00 | 465 | 154-867 |
| | 5.1- Adult | 281 | 71-565 |
| | | | |
| **5-HIAA** | 0 - 0.33 | 417 | 199-608 |
| | 0.34 - 0.66 | 271 | 63-503 |
| | 0.67 – 1.00 | 250 | 68-451 |
| | 1.10 – 5.00 | 185 | 89-367 |
| | 5.1- Adult | 98 | 58-220 |

*Pediatr Res (1993) 34, 10-14*

# Pilot External QC

*J. Inherit. Metab. Dis.* 25 (2002) 287–298


# Neurotransmitter metabolites in CSF: An external quality control scheme

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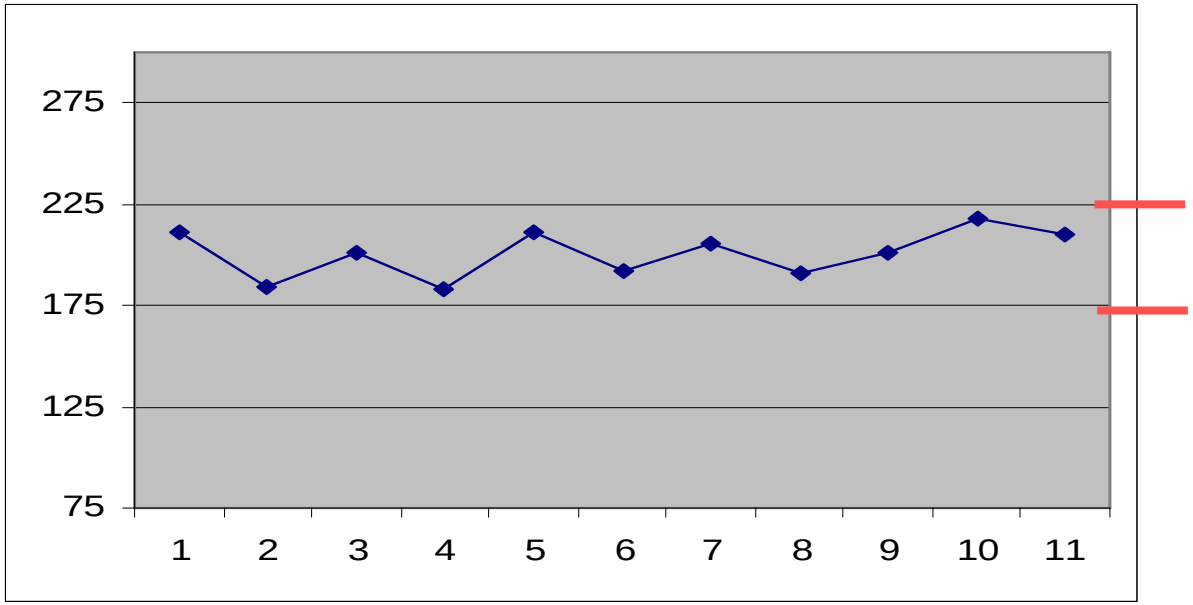
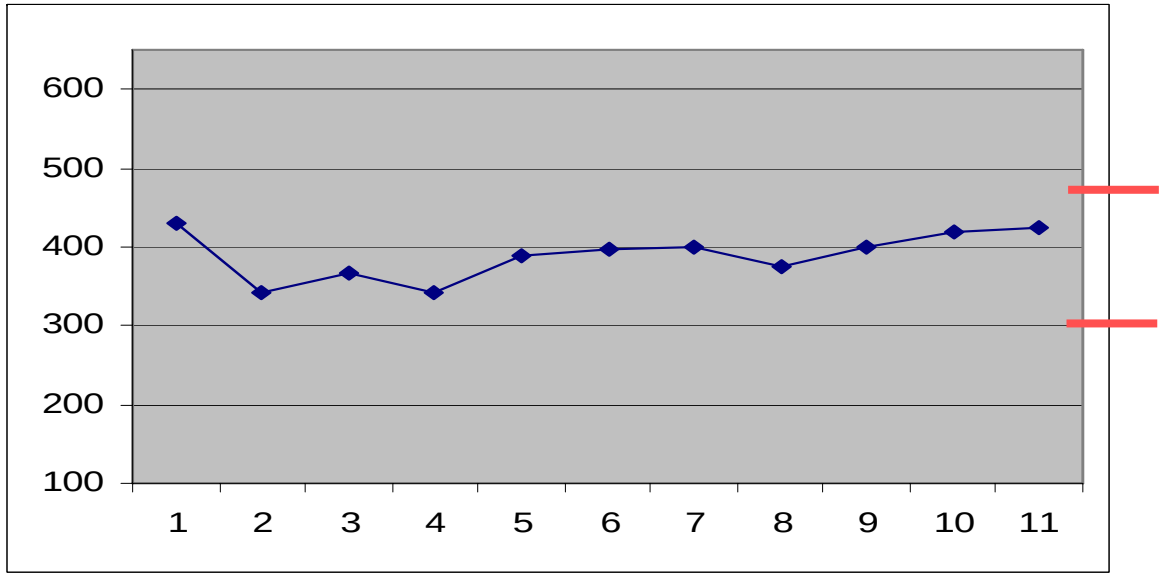
*MS received 22.01.02 Accepted 12.03.02*

# CSF “in house” QC scheme

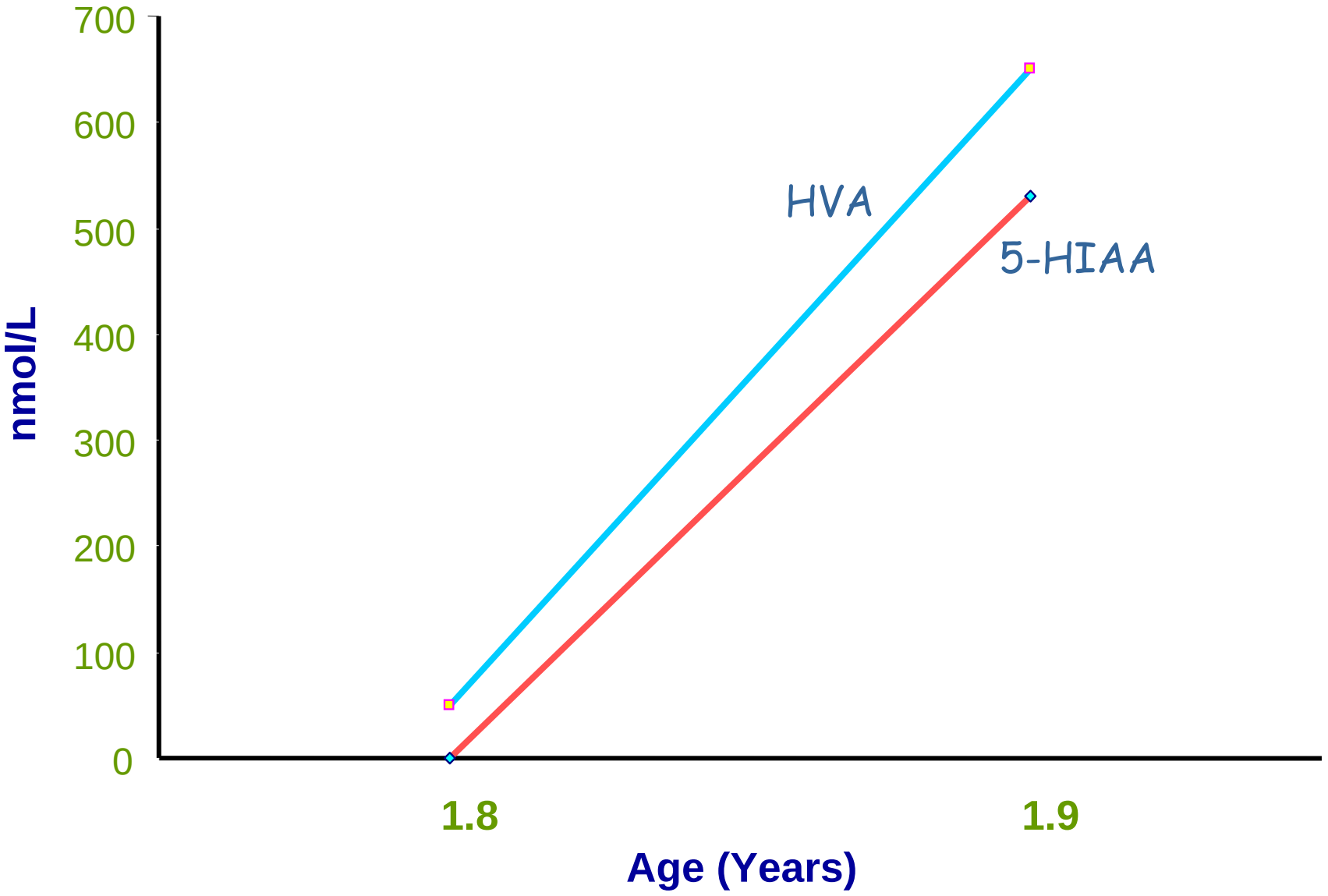
5-HIAA nmol/L




HVA nmol/L



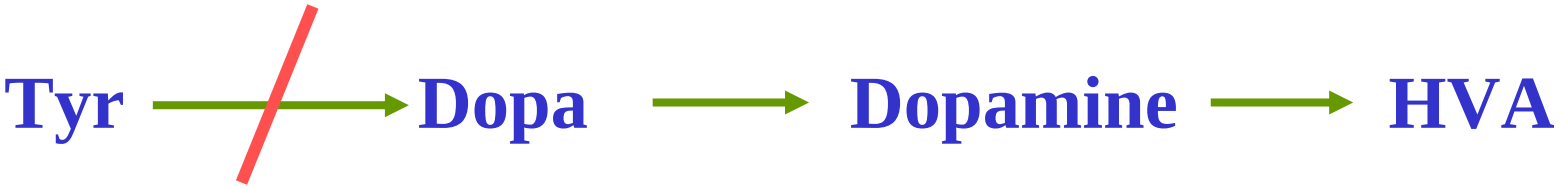
# DHPR Deficiency – Response to Treatment



# Other Neurotransmitter Disorders

- Tyrosine Hydroxylase Deficiency
- Dopamine Transporter Defect
- Aromatic Amino Acid Decarboxyalse Deficiency

# Tyrosine Hydroxylase Deficiency




- **Parkinsonian, ptosis, drooling, myoclonic jerks, severe head lag and trunkal hypotonia.**
- **L-Dopa → marked and sustained improvement in hypokinesia and parkinsonian symptoms.**
- **Identified from *CSF analysis*; *Normal pterin & 5-HIAA concentration. Very low HVA. Mutation analysis also available.***

# Tyrosine Hydoxylase Deficiency

**Sex; Male. Dob; 17/05/2007.**
**Sample; 27/02/2008**

| | | |
|---|---|---|
| **HVA:** | **<10** | **(154-867 nmol/L)** |
| **5-HIAA:** | **137** | **(68 -451 nmol/L)** |
| **BH4:** | **36** | **(19-56 nmol/L)** |
| **BH2:** | **8** | **(0.4-13.9 nmol/L)** |
| **Total Neopterin:** | **9** | **(7-65 nmol/L)** |
| **Serum Prolactin** | **706** | **(86 – 324 mU/ml)** |

# Infantile parkinsonism–dystonia and elevated dopamine metabolites in CSF

B.E. Assmann, MD; R.O. Robinson, FRCP; R.A.H. Surtees, PhD; C. Bräutigam, PhD; S.J.R. Heales, PhD; R.A. Wevers, PhD; J. Zschocke, PhD; K. Hyland, PhD; R. Sharma, PhD; and G.F. Hoffmann, MD

**Abstract**—Two girls and one boy are described, with severe infantile parkinsonism–dystonia. This syndrome is usually caused by endogenous dopamine deficiency but in these patients was associated with elevated dopamine metabolites in CSF and an unusual eye movement disorder: ocular flutter together with saccade initiation failure. Pyramidal tract signs also emerged in the course of the disease in two patients. This combination of symptoms and biochemical findings suggests a unique pathogenic mechanism.

NEUROLOGY 2004;62:1872–1874

# Increased Dopamine Turnover

First female child of consanguineous parents. 36 week gestation. Feeding difficulties from birth. 6 months reduced movements and failure to achieve milestones. 9 months able to smile but general paucity of movements. Rigidity of all limbs suggestive of dopamine deficiency. Left convergent squint but no abnormal eye movements detected.

**HVA:** **1704** **(154–867 nmol/L)**

**5-HIAA:** **250** **(89-367 nmol/L)**

**Pterin profile and 5-MTHF status unremarkable**

**Elevated urinary HVA**

**Serum Prolactin; 915 (<500 mU/ml)**

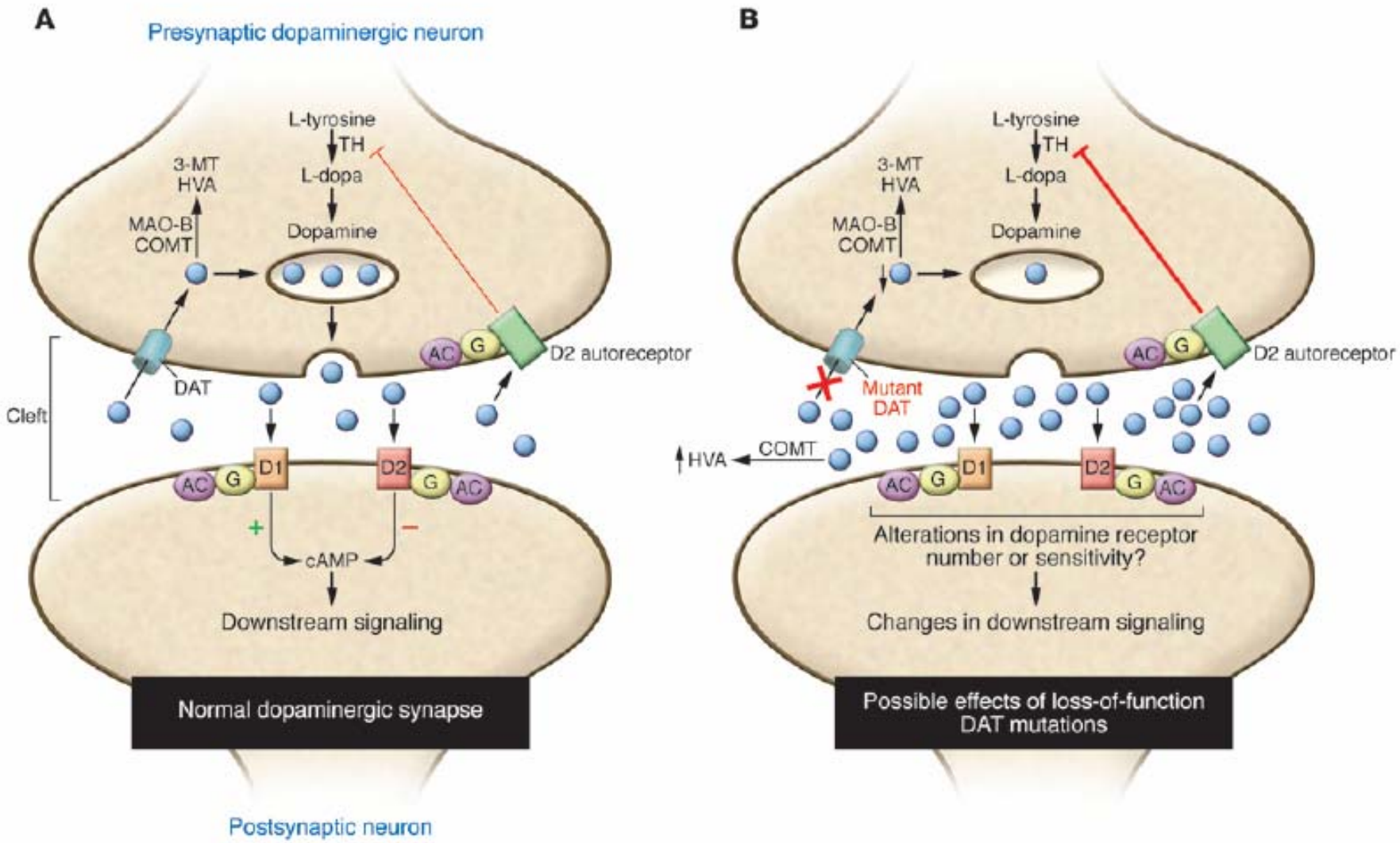
# Dopamine Transporter Defect

Related Commentary, page 1455 Research article

## Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia

Manju A. Kurian,[1,2] Juan Zhen,[3] Shu-Yuan Cheng,[3] Yan Li,[3] Santosh R. Mordekar,[4] Philip Jardine,[5] Neil V. Morgan,[1] Esther Meyer,[1] Louise Tee,[1] Shanaz Pasha,[1] Evangeline Wassmer,[2] Simon J.R. Heales,[6] Paul Gissen,[1] Maarten E.A. Reith,[3,7] and Eamonn R. Maher[1,8]

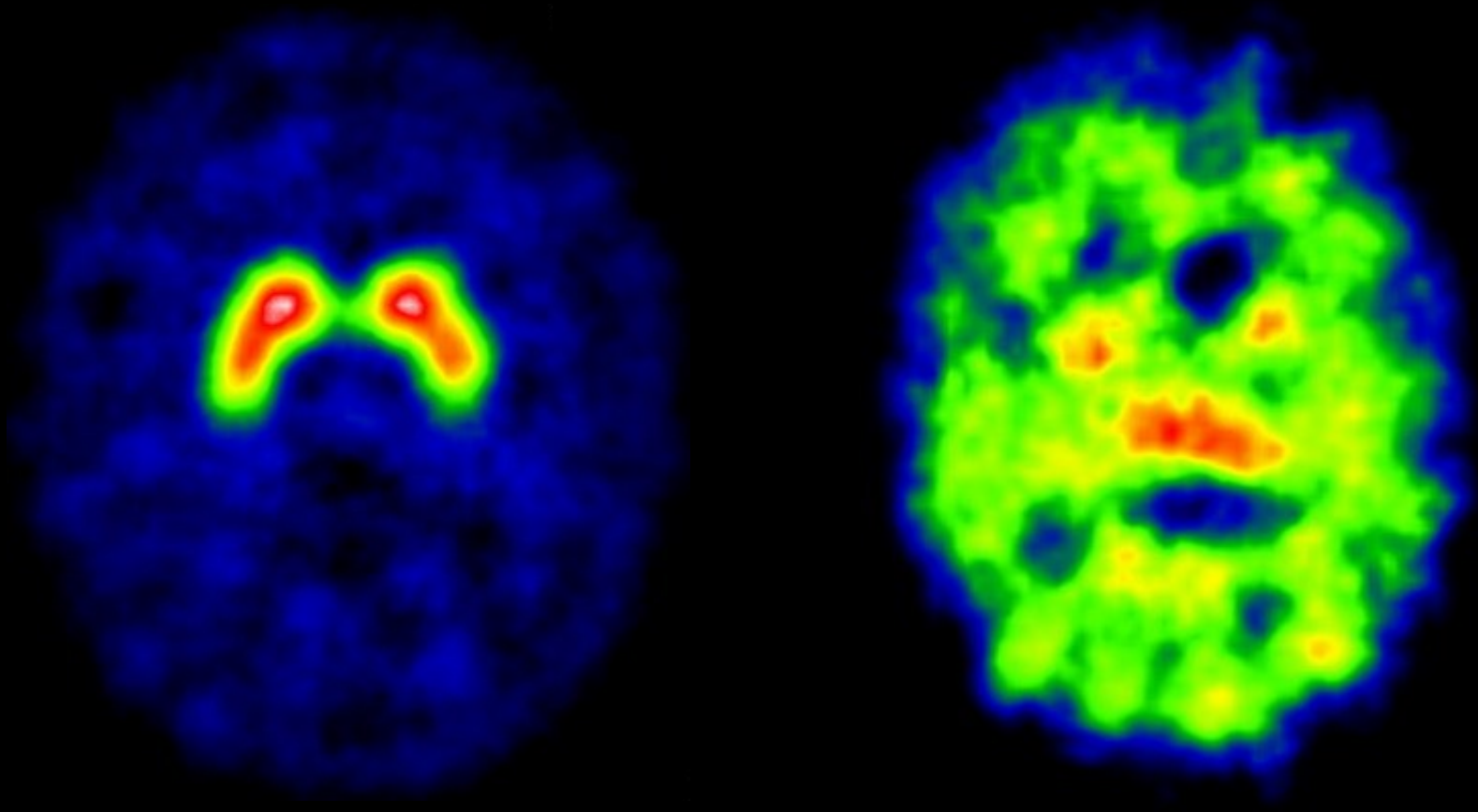
The Journal of Clinical Investigation http://www.jci.org Volume 119 Number 6 June 2009


A
Presynaptic dopaminergic neuron
L-tyrosine
TH
L-dopa
Dopamine
3-MT
HVA
MAO-B
COMT
DAT
Cleft
AC
G
D2 autoreceptor
D1
D2
+
−
cAMP
Downstream signaling
Normal dopaminergic synapse
Postsynaptic neuron
B
L-tyrosine
TH
L-dopa
Dopamine
3-MT
HVA
MAO-B
COMT
Mutant
DAT
HVA
COMT
D2 autoreceptor
Alterations in dopamine receptor
number or sensitivity?
Changes in downstream signaling
Possible effects of loss-of-function
DAT mutations

# DAT Deficiency

| | 1 | 2 | 3 | |
|---|---|---|---|---|
| **CSF** | | | | |
| **HVA** | 1,873 | 1,704 | 1,135 | (154-867nmol/L) |
| 5-HIAA | 141 | 250 | 91 | (89-367 nmol/L) |
| HVA:5-HIAA | 13.2 | 6.8 | 12.5 | (1.0-3.7) |
| Urine | | | | |
| HVA:Cr | 22 | 27 | - | (2-15umol/mmol) |
| Serum | | | | |
| Prolactin | 150 | 915 | 688 | (93-630 mU/ml) |

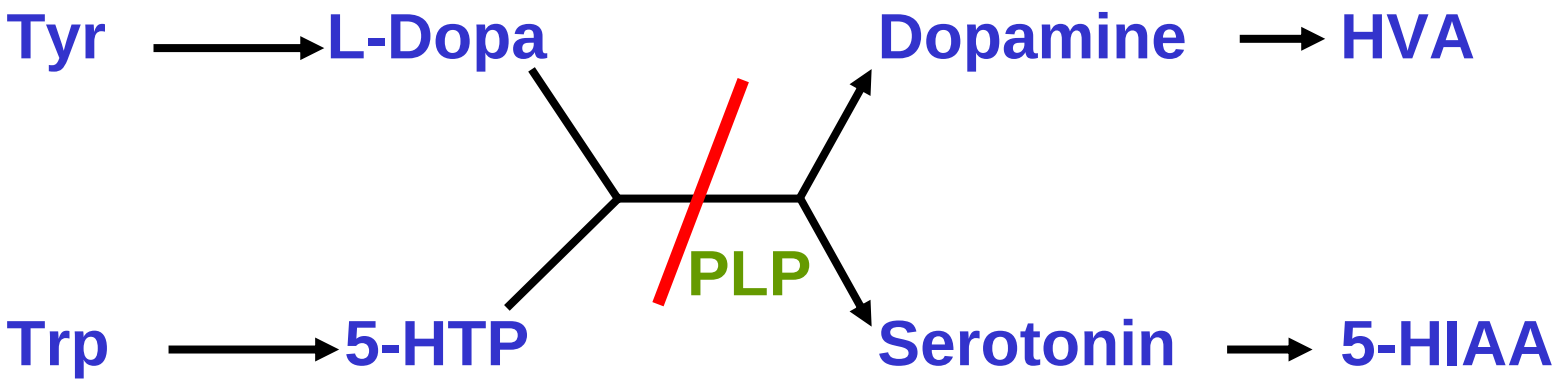
# In Vivo Evidence of Loss of Function in DAT



CONTROL

PATIENT 3

# Aromatic Amino Acid Decarboxylase Deficiency



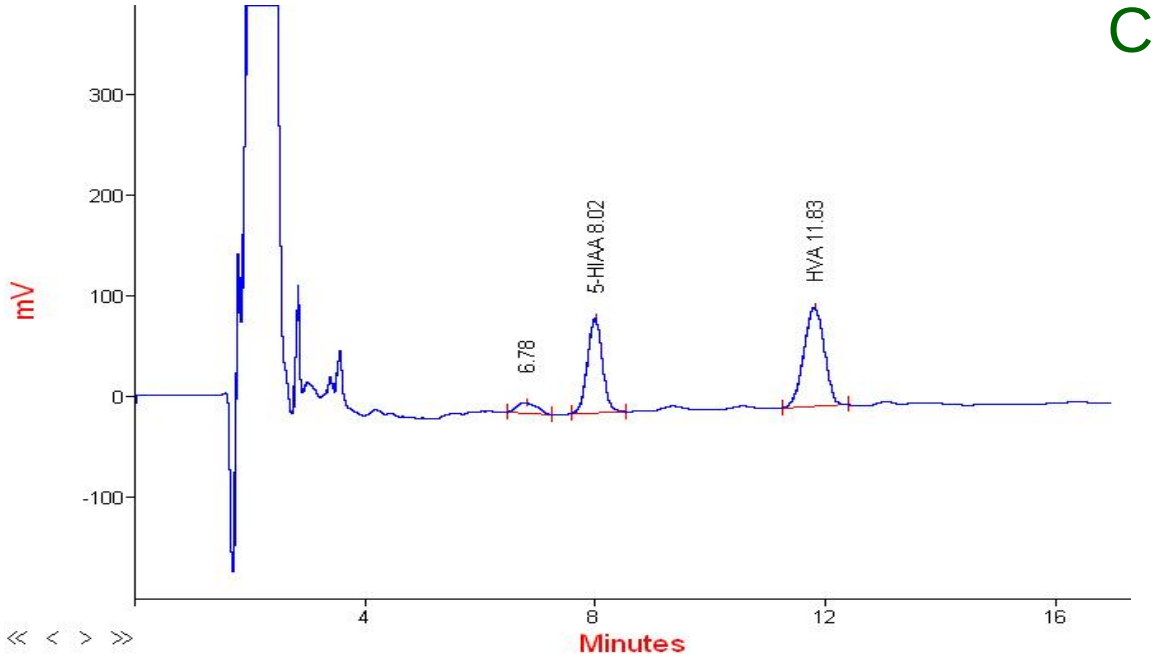

**Clinical features resemble those of recessive BH4 deficiency; hypotonia, occulogyric crises, ptosis and paucity of spontaneous movement. Can be fatal**

**Urine: Vanillactic acid**

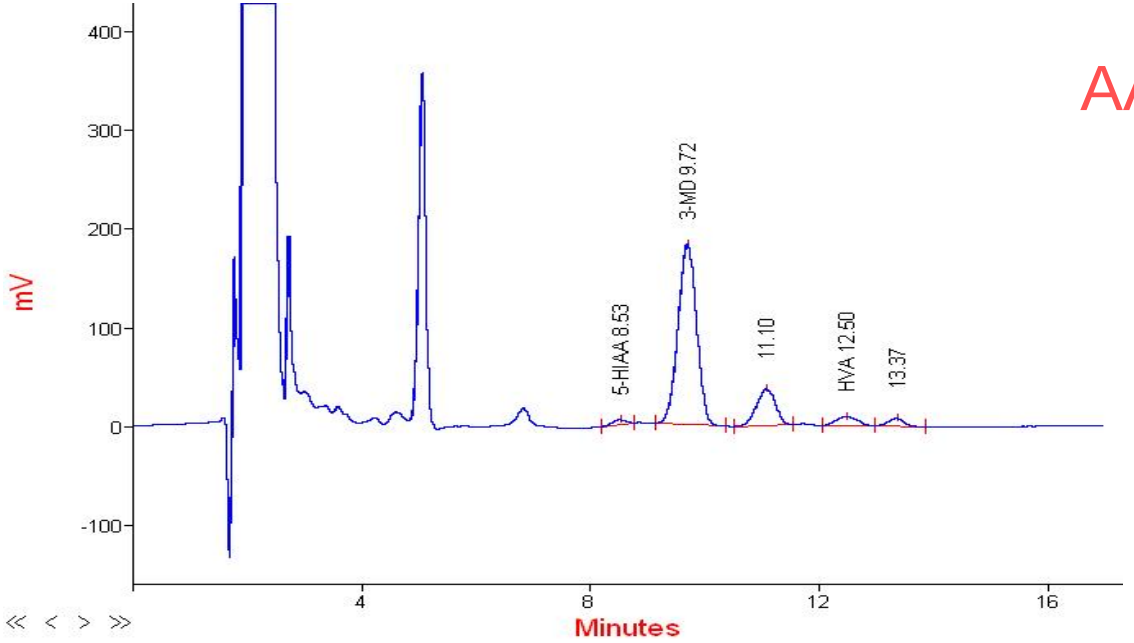
***CSF*: Low HVA + 5-HIAA, but *normal* pterin profile and accumulation of *3-O-methyldopa*. Enzymatic analysis possible on plasma.**

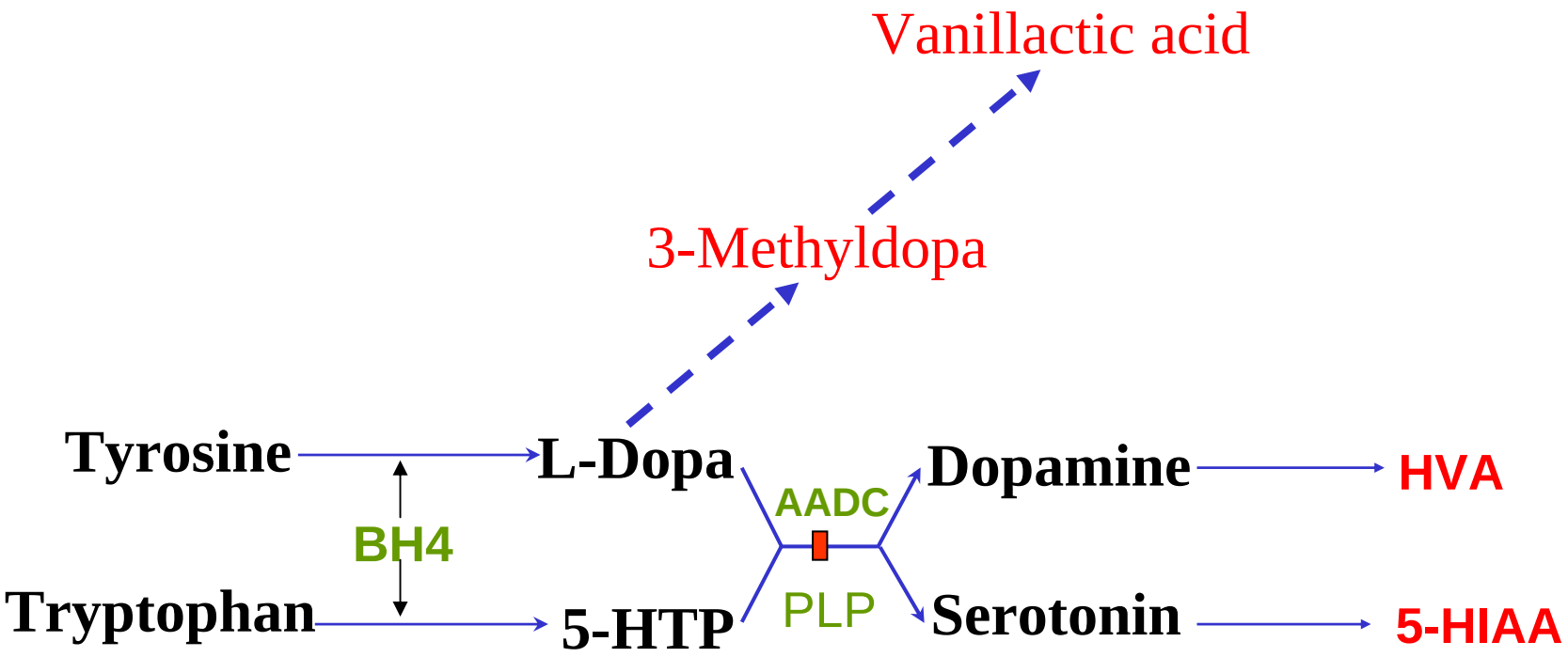
**Treatment; B6, MAOI & dopamine agonists.**

Control




AADC Def

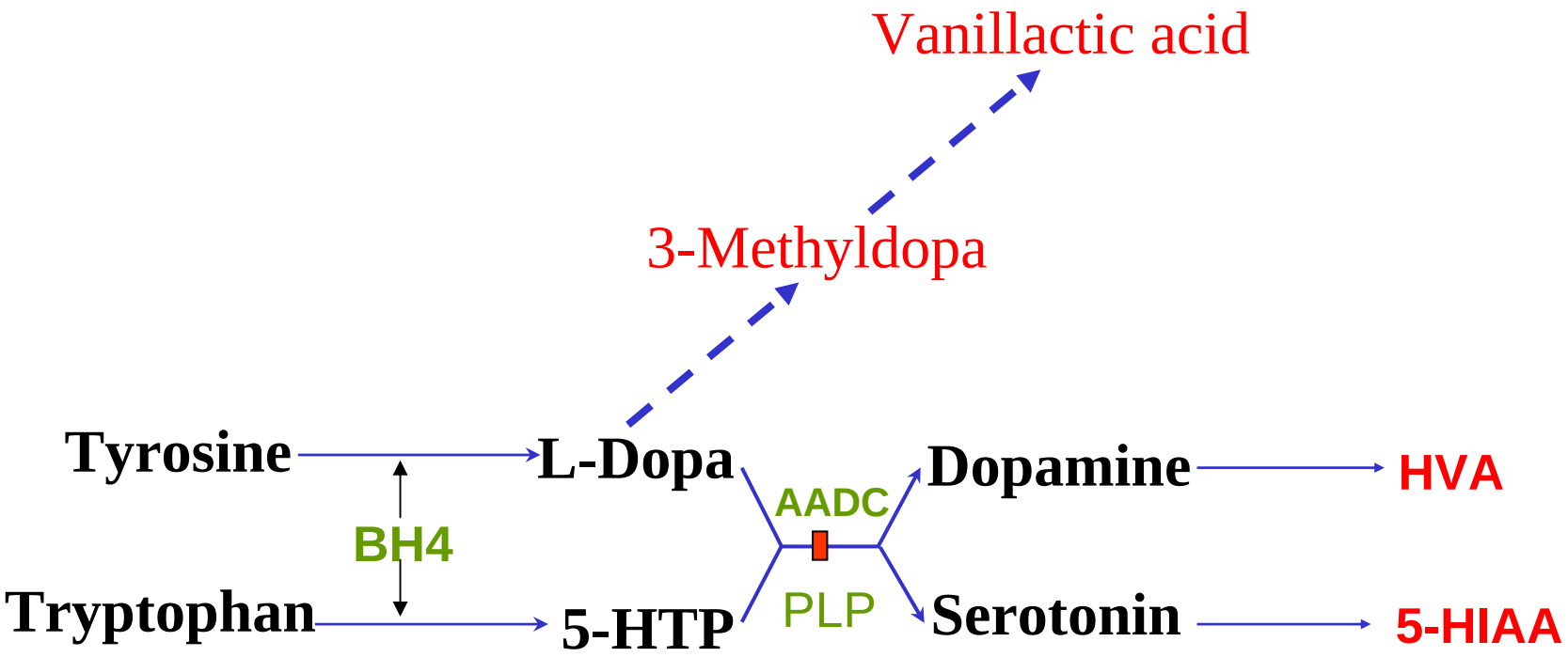



Vanillactic acid
3-Methyldopa
Tyrosine
L-Dopa
Dopamine
HVA
AADC
BH4
Tryptophan
5-HTP
PLP
Serotonin
5-HIAA

# Aromatic Amino Acid Decarboxylase Deficiency

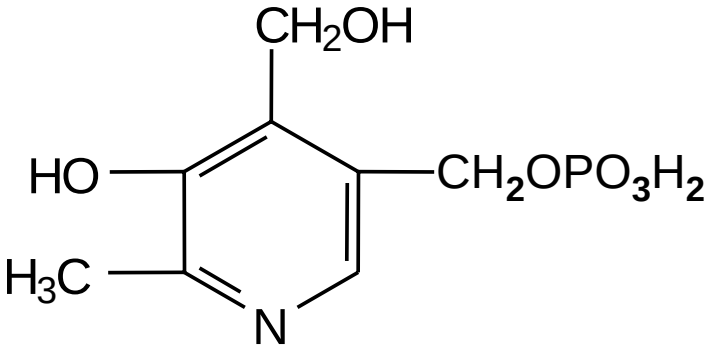
**Male. Dob; 28/08/2007.**
**Sample; 10/01/2008**
**Floppy, episodes of dystonia, developmental delay**

| | | |
|---|---|---|
| HVA | 47 | (362-955 nmol/L) |
| 5-HIAA | 14 | (63- 503 nmol/L) |
| 3-Methyldopa | 1170 | (<300 nmol/L) |
| PLP | 32 | (23-87 nmol/L) |
| **Serum Prolactin** | **900** | **(85 – 250 mU/ml)** |
| **Plasma AADC Activity** | **0.7** | **(36 -129 pmol/min/ml)** |

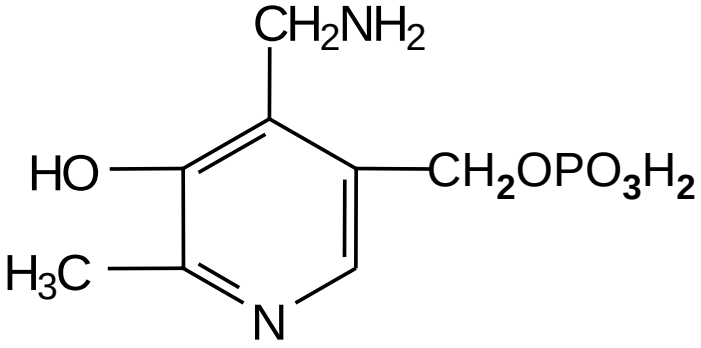

Vanillactic acid
3-Methyldopa
Tyrosine
L-Dopa
Dopamine
HVA
AADC
BH4
Tryptophan
5-HTP
PLP
Serotonin
5-HIAA

# Vitamin $B_6$

***Pyridoxine-5'- phosphate***



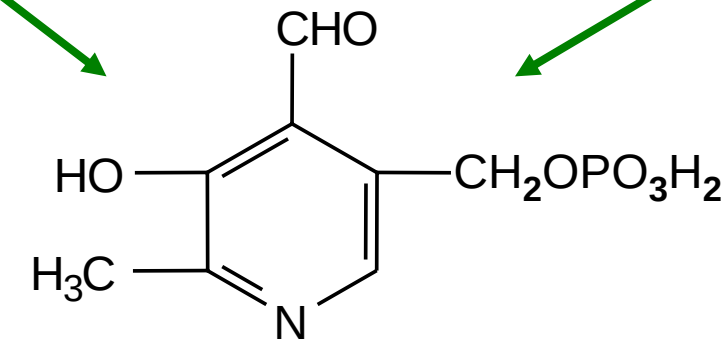

***Pyridoxamine-5'- phosphate***




***Pyridoxal-5'- phosphate***

PNPO

PNPO




PNPO = Pyridox(am)ine-5'-oxidase

# PNPO Deficiency

- **Neonatal epileptic encephalopathy**
- **Fetal distress, prenatal seizures, low Apgar**
- **Pseudo AADC deficiency – Not consistent**
- **↑ Glycine & Threonine – Not consistent**
- **↑ Vanillactate excretion – Consistent ?**

# PNPO Deficiency

J Inherit Metab Dis (2007) 30:96–99
DOI 10.1007/s10545-006-0508-4

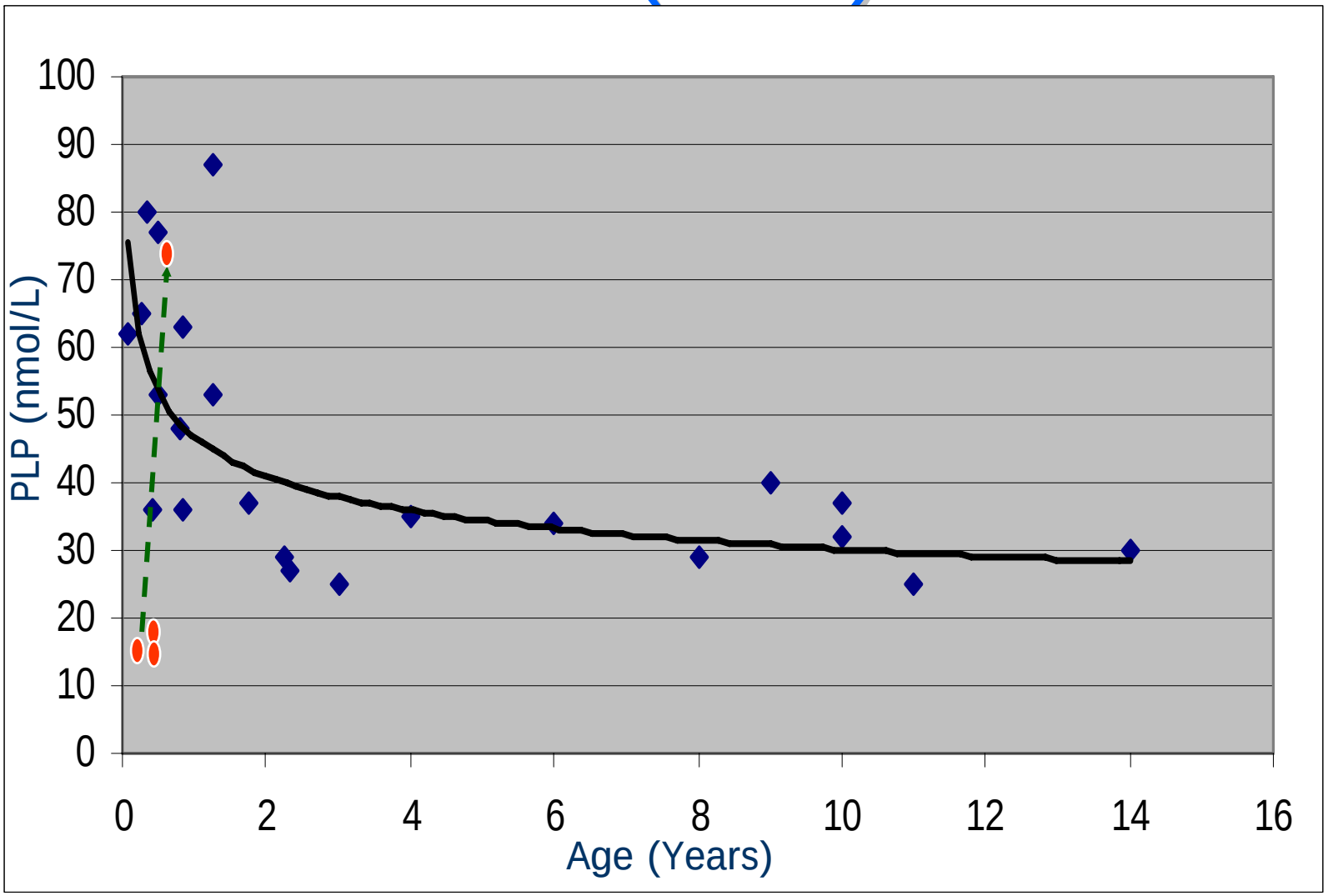
ORIGINAL ARTICLE

## Pyridoxal 5′-phosphate may be curative in early-onset epileptic encephalopathy

G. F. Hoffmann · B. Schmitt · M. Windfuhr · N. Wagner · H. Strehl · S. Bagci · A. R. Franz · P. B. Mills · P. T. Clayton · M. R. Baumgartner · B. Steinmann · T. Bast · N. I. Wolf · J. Zschocke

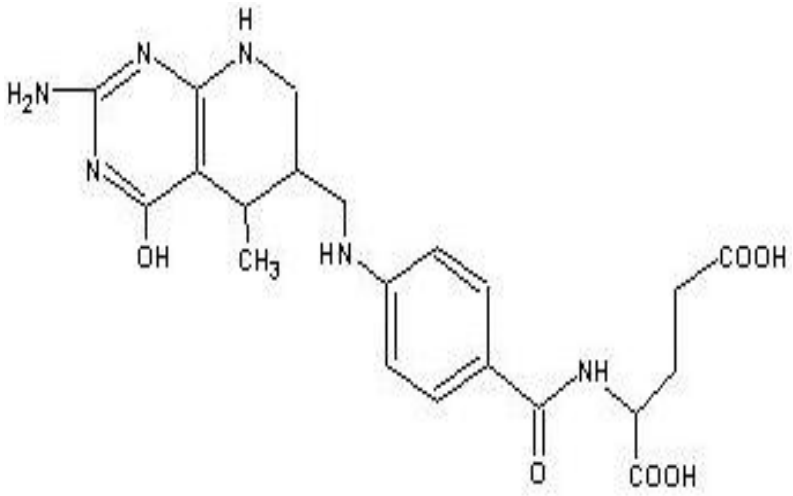
Received: 14 November 2006 / Submitted in revised form: 4 December 2006/ Accepted: 5 December 2006 / Published online: 23 December 2006

# CSF (PLP)




*Mol. Genet. Metab. 94. (2008). 173-177*

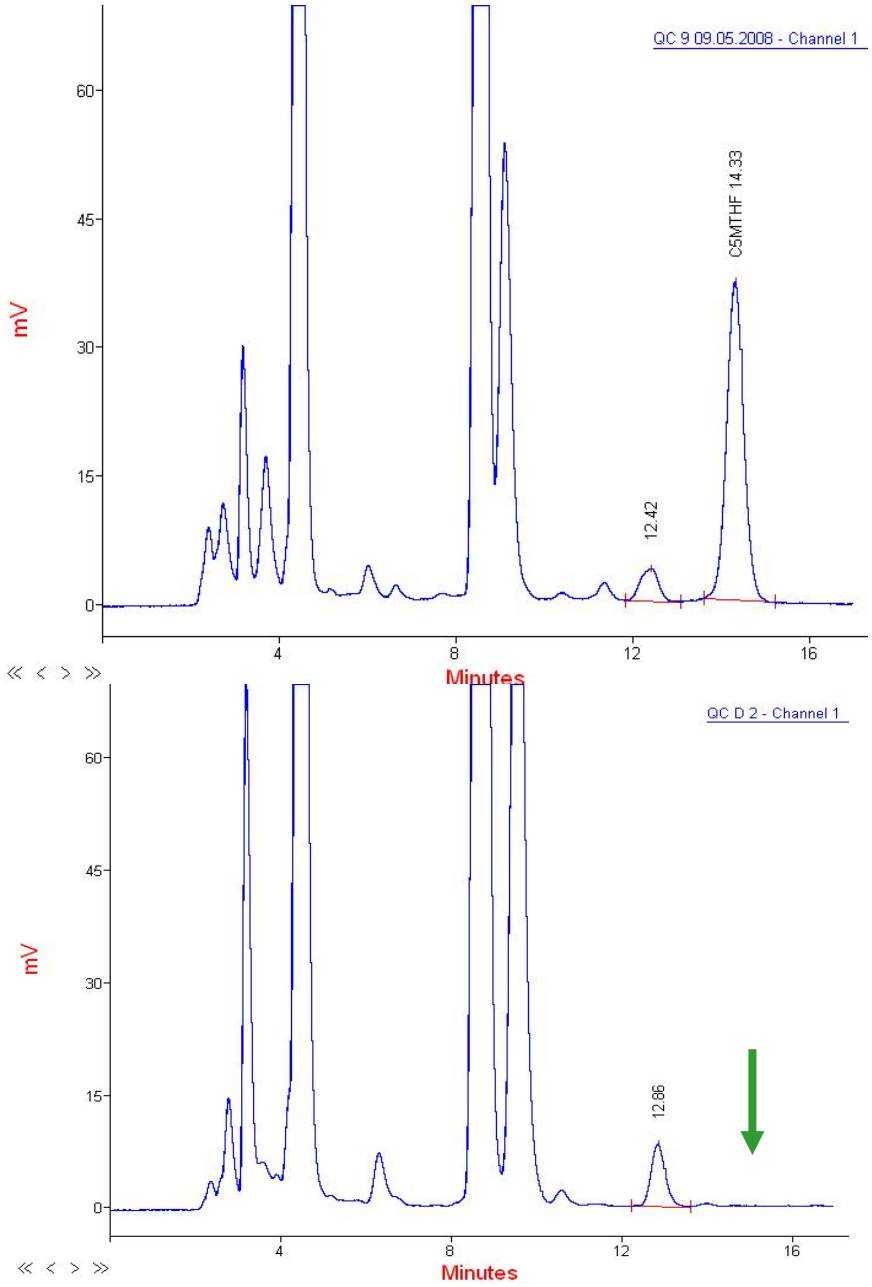
# CSF 5-MTHF Deficiency




5-methyl tetrahydrofolate

- DHPR deficiency
- MTHFR deficiency
- AADC deficiency
- 3-Phosphoglycerate dehydrogenase def
- Rett syndrome
- Aicardi Goutieres
- Mitochondrial disorders
- L-dopa treatment
- Methotrexate
- Anticonvulsants
- Steroids
- Co-trimoxazole

Cerebral Folate Deficiency - Neurological syndrome associated with low CSF 5-MTHF and normal peripheral folate.



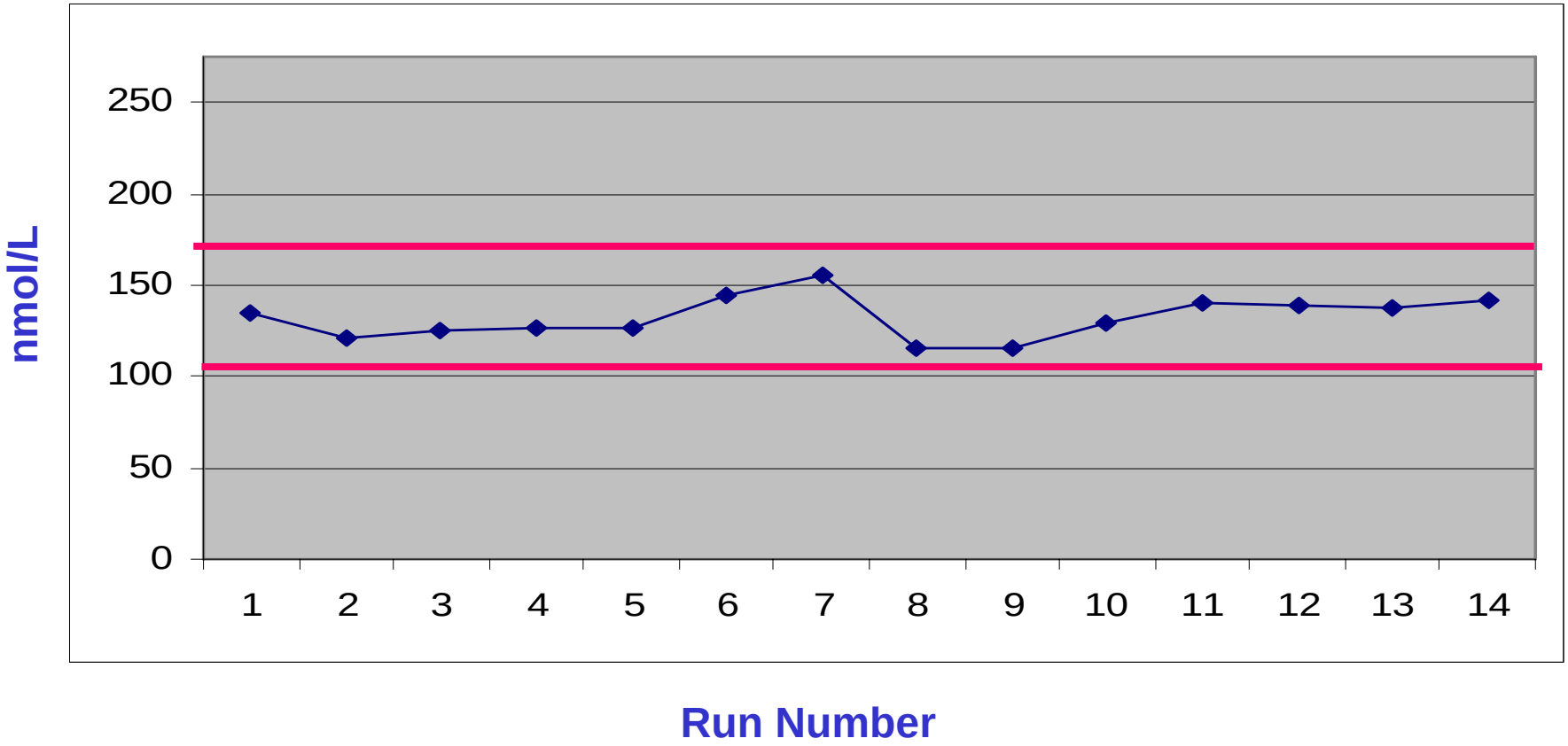

Control CSF

MTHFR Def CSF

*Food Chemistry, (1995), 53, 329-338.*

HPLC +Fluorescence
(290, 358 nm)

# QC8 5-MTHF

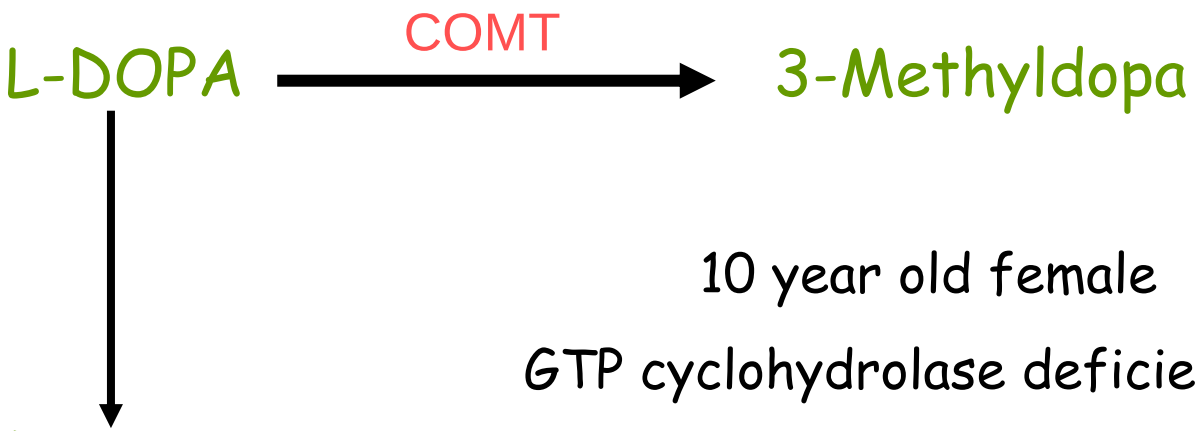


# CSF 5-MTHF Deficiency & Mitochondrial Disorders

| | 5-MTHF | |
|---|---|---|
| F. 15 yrs | 29 | (46 - 160 nmol/L) |
| M. 9 yrs | 5 | (72 – 172 nmol/L) |
| M. 8 yrs | 44 | (72 – 172 nmol/L) |
| F. 2 yrs | 17 | (52 - 178 nmol/L) |
| F. 6 yrs | 7 | (72 – 172 nmol/L) |

# CSF 5-Methyltetrahydrofolate

- DHPR deficiency
- Long term L-dopa administration




10 year old female

GTP cyclohydrolase deficiency

62 (72-172 nmol/L)

# Secondary Causes

- Hypoxia
- Neurodegeneration
- Epilepsy
- Mitochondrial Disease 
- Gaucher
- Drugs
- Sample Processing