Great Ormond Street Hospital for Children NHS Foundation Trust

University College London Hospitals NHS Foundation Trust

Biomarkers for Neurotransmitter Disorders (Dopamine and Serotonin)

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Financial Disclosure Statement

Simon Heales

Speaker fees from PTC

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Simon Pope

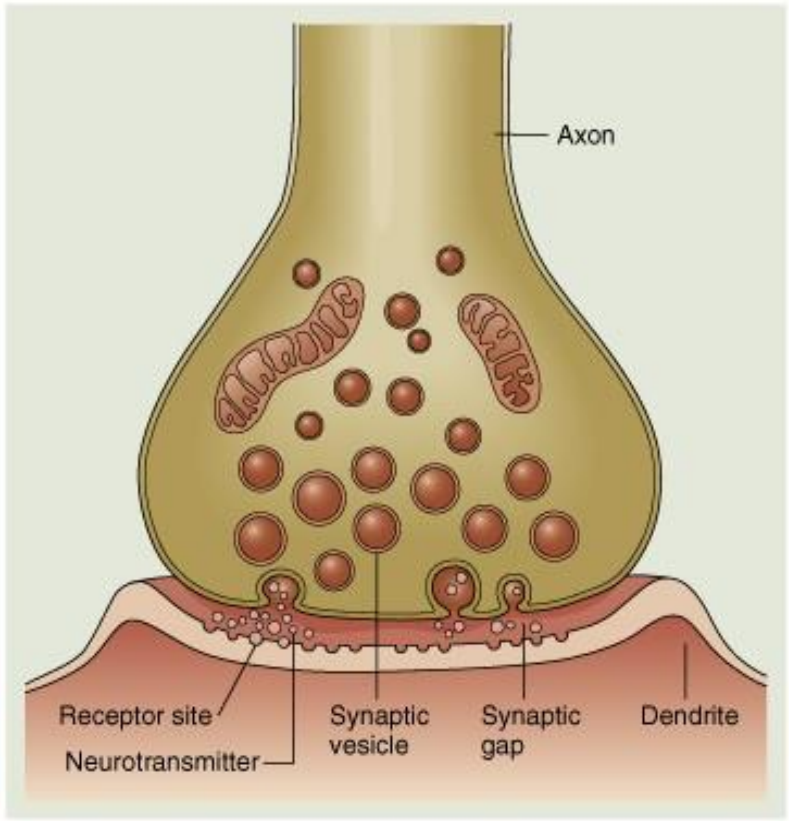
None

Plan

- Dopamine and Serotonin Metabolism (SH)
- Current approach (SH)
  - -CSF biomarkers
  - -Peripheral “biomarkers/tests”
- Overview of current methods and biomarkers (SP)
- Emerging methods and biomarkers (SP)

Chemical Neurotransmission

- Neurotransmitters – *Substances that upon release from nerve terminals, act on receptor sites at post-synaptic membranes to produce either excitation or inhibition of the target cell*



Clinical Symptoms

DOPAMINE DEFICIENCY

- Movement disorders
- Dystonia
- Parkinsonism
- Chorea, hyperkinesia
- Myoclonus
- Ocular symptoms
- Abnormal peripheral tone
- Abnormal neurodevelopment
- Microcephaly
- Bulbar dysfunction
- Epilepsy

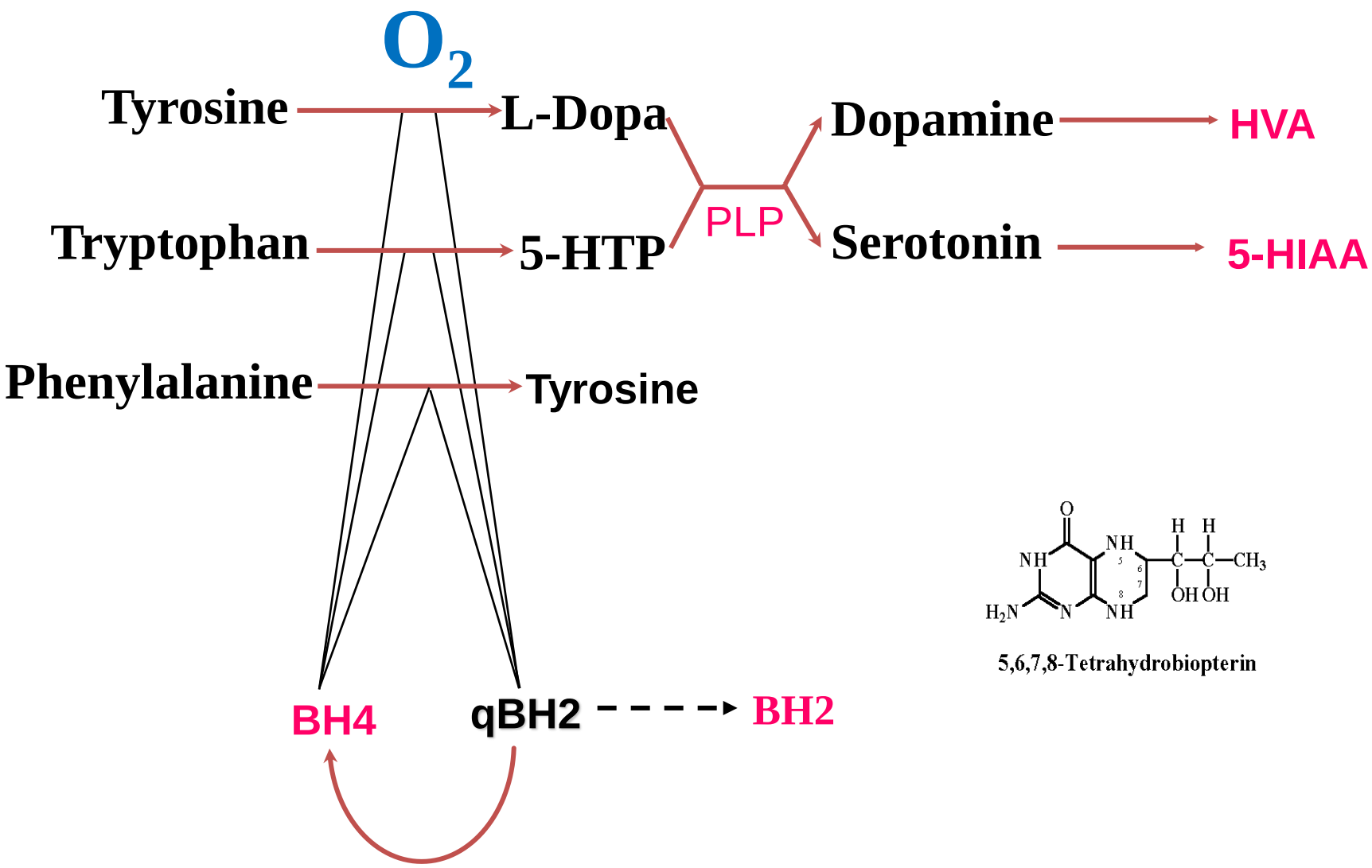
NOREPINEPHRINE/EPINEPHRINE

- Autonomic dysfunction
- Sleep disturbance
- Temperature instability

SEROTONIN DEFICIENCY

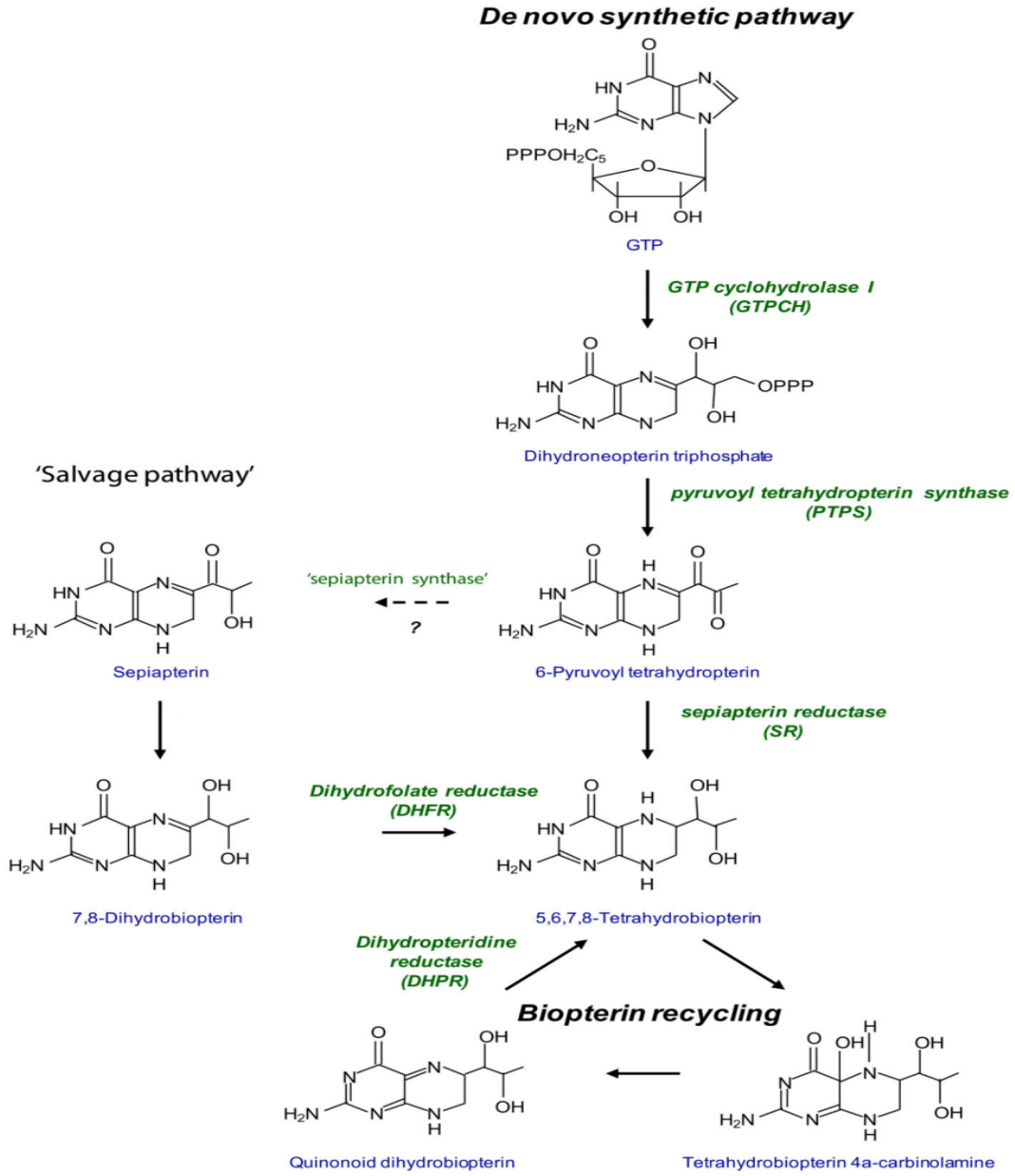
- Temperature instability
- Sweating
- Mood
- Movement disorders

Kurian et al 2011



5,6,7,8-Tetrahydrobiopterin

Tetrahydrobiopterin Metabolism



Nitric Oxide. 2011 August 01; 25(2): 81–88. doi:10.1016/j.niox.2011.04.004.

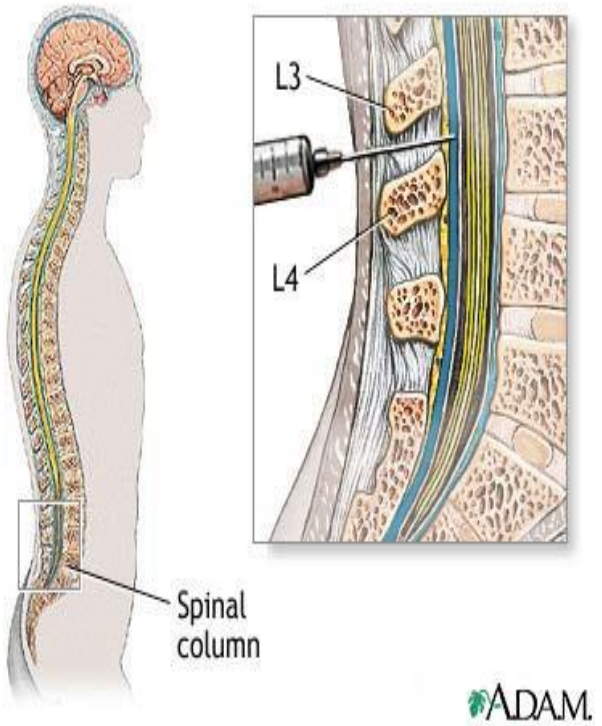
CSF – Sample Requirements

(Lab Specific – Check)

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

(DTE/DETAPAC)

Rostro-caudal Gradient



Collect at bedside and freeze immediately (not the form !)

Age related reference ranges

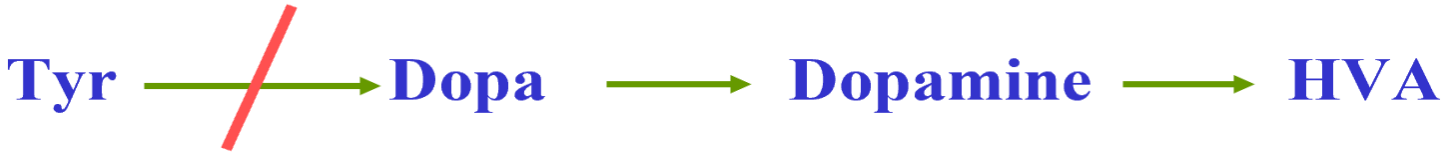
Clinical Details and Drugs

ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

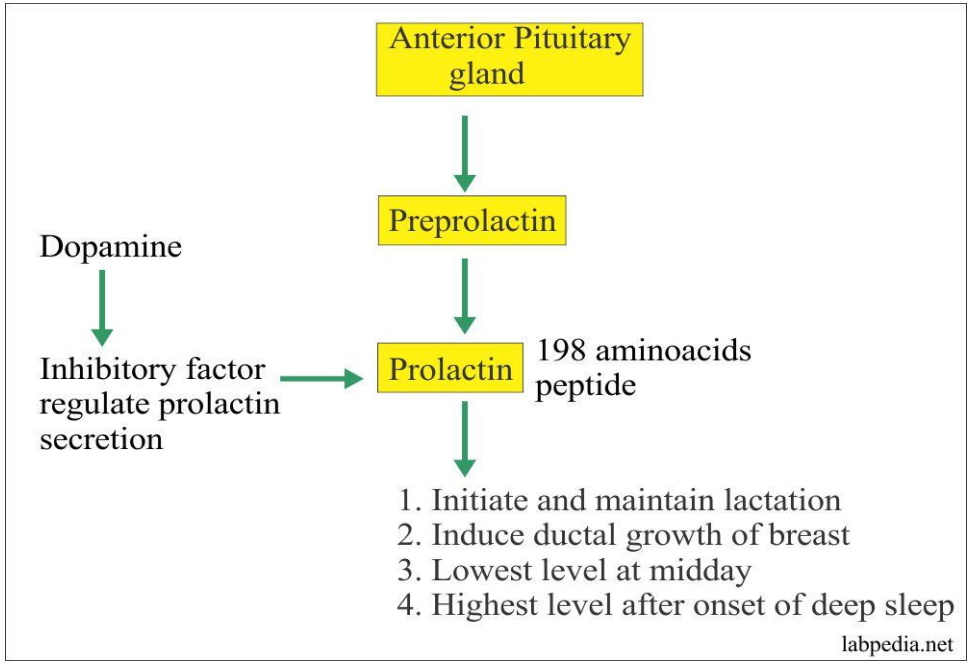
Case

- Parkinsonian, ptosis, drooling, myoclonic jerks, severe head lag and truncal hypotonia.

CSF	Value nmol/L (Reference Range)
HVA	**<10** (154-867)
HIAA	**137** (68-451)
Neopterin	**9** (7-61)
BH2	8 (0.4-13.9)
BH4	**36** (8-57)
5-MTHF	**126** (52-178)



Serum Prolactin 706 (86 – 324 mU/ml)



Case

- MRI brain scan normal
- very floppy
- Long episodes of arching and eye deviation
- Generalised hypotonia,
- mixed complex movement disorder

Normal phenylalanine

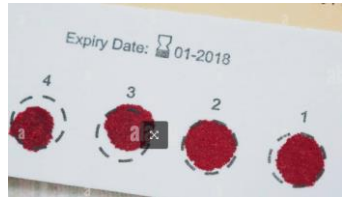
- Serum prolactin **900** (85 – 250 mU/ml)
- CSF neurotransmitters

CSF	Value (Reference Range)
HVA	**48** (176-851 nmol/L)
5-HIAA	**13** (68- 451 nmol/L)
Neopterin	11 (7-65 nmol/L)
BH2	12 (0.4-13.9 nmol/L)
BH4	43 (19-56 nmol/L)
3-O-methyl-dopa	**1543** (<100 nmol/L)
5-MTHF	60 (72-305)

- Urine organic acids

Peak of vanillylactate

Vanillactic acid



3-Methyldopa

Tyrosine → L-Dopa → Dopamine → HVA

Tryptophan → 5-HTP → Serotonin → 5-HIAA

BH4

AADC

PLP

Case

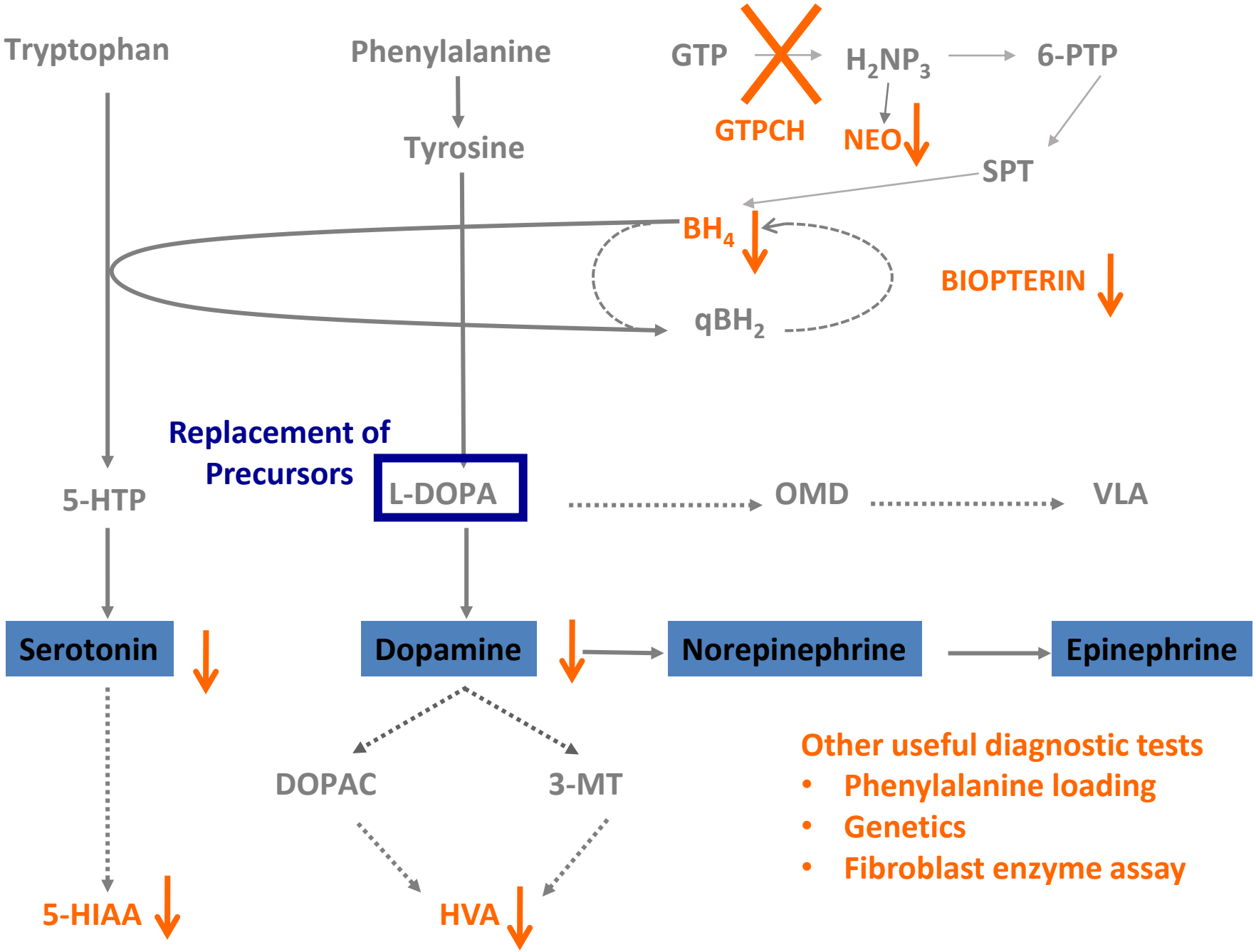
- From beginning of ambulation, walked on toes
- Gradually got worse over early childhood
- Some hand cramping when writing
- Cognition normal
- Normal speech
- Given a diagnosis of ‘cerebral palsy’
- Therapeutic trial of L-dopa

    Cessation of toe walking

    Able to run

    Virtually normalised

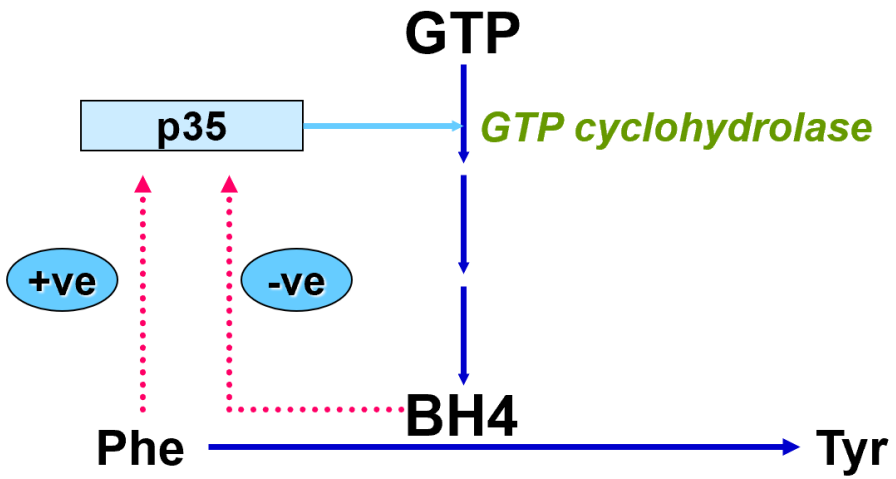
CSF	Value nmol/L (Reference Range)
HVA	**155** (154-867)
HIAA	**85** (89-367)
Neopterin	**6** (7-61)
BH2	6.4 (0.4-13.9)
BH4	**8** (8-57)
5-MTHF	126 (52-178)

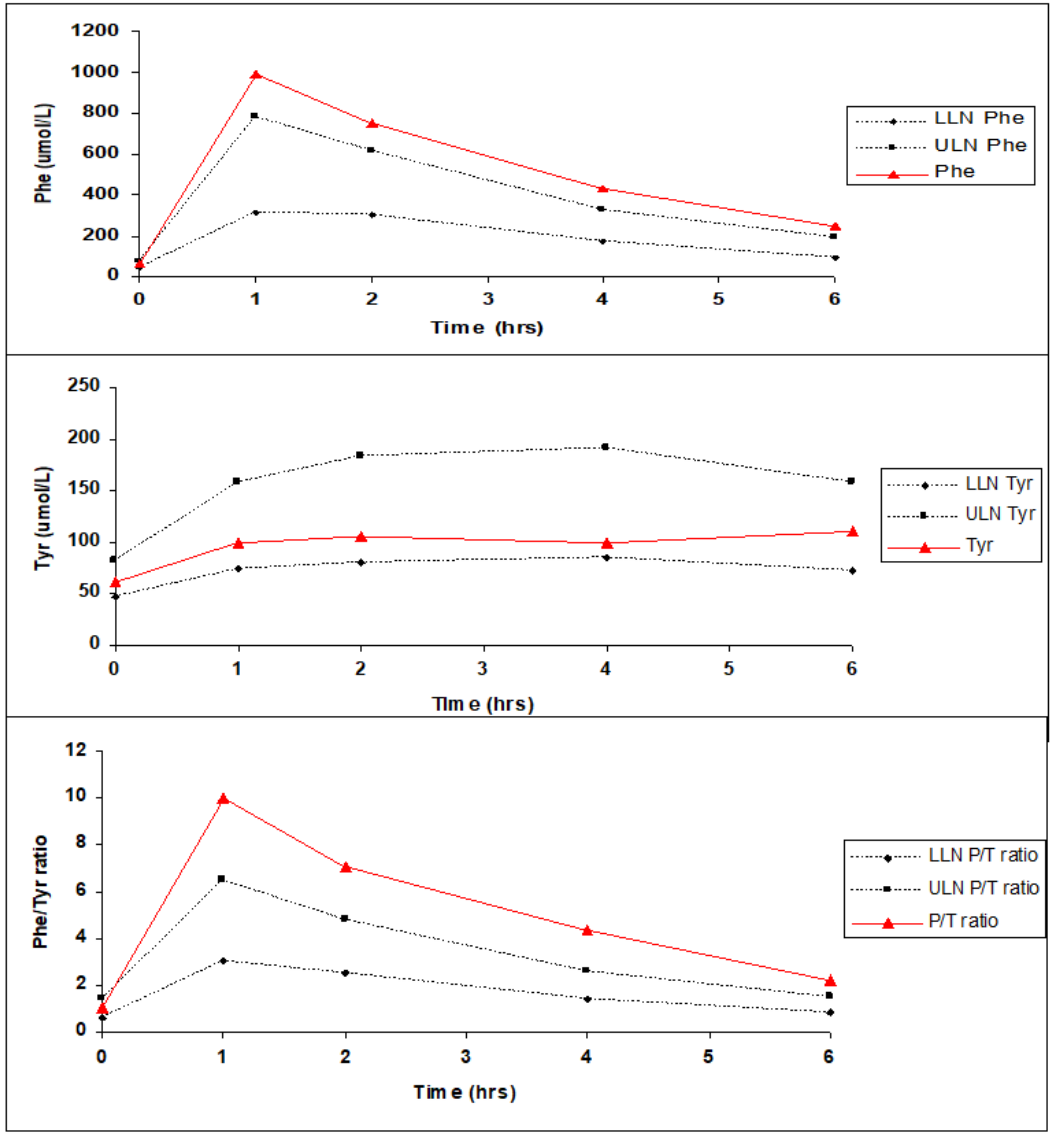


Other useful diagnostic tests

- **Phenylalanine loading**
- **Genetics**
- **Fibroblast enzyme assay**

Phenylalanine Loading Test





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.

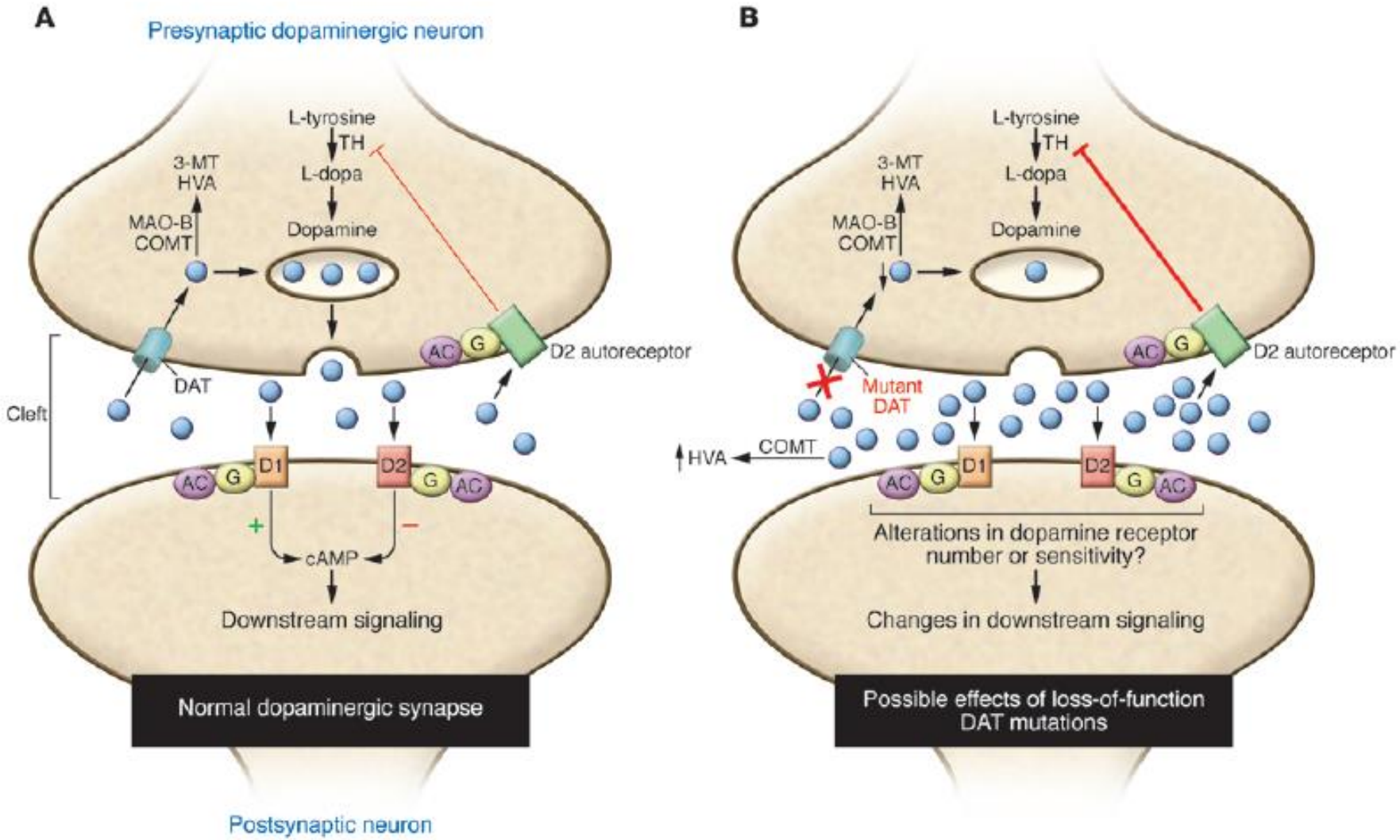
HVA: **1705** **(154–867 nmol/L)**

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

Pterin profile and 5-MTHF status unremarkable

Elevated urinary HVA

Serum Prolactin; 915 (<500 mU/ml)


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
Mutant
DAT
HVA
COMT
Alterations in dopamine receptor
number or sensitivity?
Changes in downstream signaling
Possible effects of loss-of-function
DAT mutations

Overview of current biomarkers and methods

- Summary of current peripheral and CSF biomarkers and the most popular methods currently being used
- Development of mass spectrometry methods
- Future directions

Peripheral markers

- Peripheral markers – initial tests
  - Newborn screening – Bloodspot Phenylalanine by mass spectrometry – pterins defects with hyperphe
  - Urine pterins by HPLC or LC-MS
  - Urine organic acids by GC-MS – raised vanillactic acid – possible AADC or PNPO deficiency
  - Serum prolactin – non-specific but can be elevated in dopamine deficiencies
  - Bloodspot 3-O-methyl dopa – elevated in AADC deficiency
  - Plasma vitamin B6 by HPLC with fluorescence detection – may pick up vitamin B6 metabolic disorders
  - Urine AASA by LC-MS – a marker of antiquitin deficiency/pyridoxine-dependent epilepsy
  - Peripheral folate – can be low in folate transport deficiencies as well as secondary to medications
  - Whole blood serotonin by HPLC with fluorescence
  - Plasma AADC enzyme analysis – HPLC with ECD or LC-MS
  - Phenylalanine loading test – useful for identifying/confirming more subtle pterin defects

CSF Biomarkers

- If a disorders of monoamine metabolism is suspected, these tests are typically performed

- CSF markers
  - CSF monoamine metabolites – useful for initial **diagnosis** and treatment **monitoring**

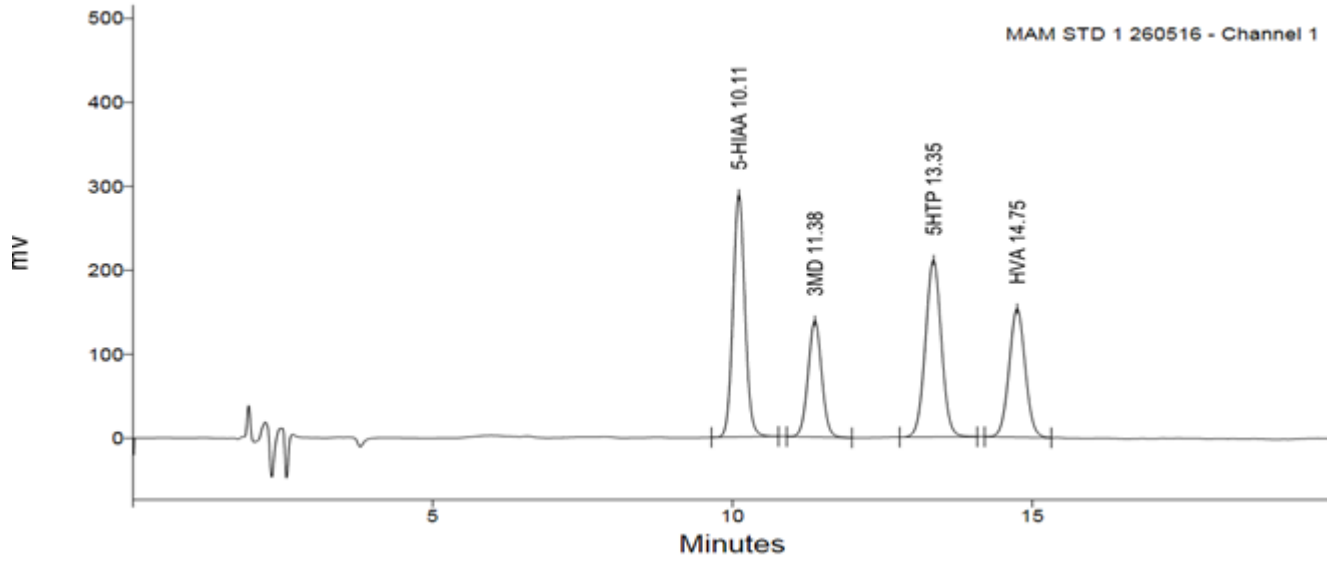
  - CSF pterins - is a pterin defect responsible for impaired monoamine metabolism. Not all pterin defects have hyperphe.

  - CSF 5-methyltetrahydrofolate – can be low in DHPR and AADC deficiency as well as in patients taking L-dopa

  - CSF pyridoxal phosphate – Low CSF pyridoxal phosphate, as in PNPO deficiency, can impair monoamine metabolism. It is a co-factor for the AADC enzyme.

Common techniques - HPLC with electrochemical or fluorescence detection

- The most common technique for monoamines and their co-factors is still HPLC
- Labs have years of experience of these techniques and they are robust, selective, sensitive and reliable
- Below is an electrochemical chromatogram of monoamine metabolite standards
- Very important to separate components for accurate quantitation.



The move from HPLC to LC-MS

- HPLC methods tend to be robust, sensitive and reliable

- However HPLC requires complete separation of all analytes and the detection techniques (fluorescence, electrochemical detection) are only suitable for analytes with specific chemical properties Different methods required for different sets of analytes.

- Therefore, there has been a move to mass spectrometry methods in analytical labs as these methods allow a larger numbers of analytes to be measured simultaneously. Chromatographic separation is not always required as identity can be confirmed by mass and fragment ions.

- Speed, sensitivity and adaptability of LC-MS methods opens up new possibilities – quicker diagnosis and new biomarkers for treatment monitoring

Combining analysis using LC-MS

Combining separate HPLC methods into a single LC-MS analysis will save time and give a more comprehensive overview of metabolism

This is one example of combining monoamine and pterins analysis. Other examples with other co-factors and related amino acids.

Journal of Chromatography A 1635 (2021) 461775

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

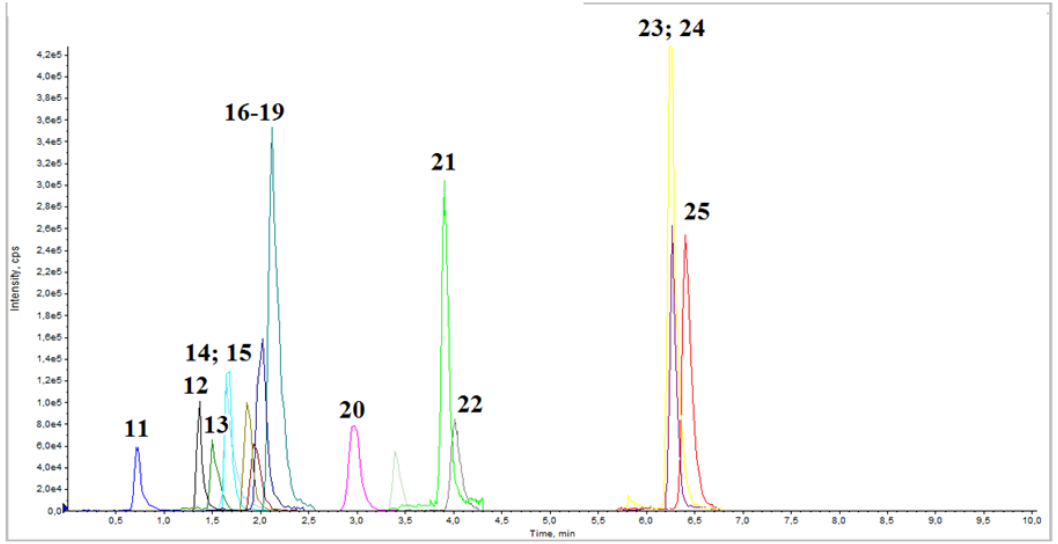
Simultaneous determination of 30 neurologically and metabolically important molecules: A sensitive and selective way to measure tyrosine and tryptophan pathway metabolites and other biomarkers in human serum and cerebrospinal fluid

Zsolt Galla[a,*], Cecília Rajda[b], Gábor Rácz[a], Nóra Grecsó[a], Ákos Baráth[a], László Vécsei[b,c], Csaba Bereczki[a], Péter Monostori[a]

[a] Metabolic and Newborn Screening Laboratory, Department of Paediatrics, University of Szeged
[b] Department of Neurology, University of Szeged, Hungary
[c] Department of Neurology, MTA-SZTE Neuroscience Research Group, Interdisciplinary Excellence Centre, Faculty of Medicine, Universtiy of Szeged, Hungary

SFigure 2

Representative chromatogram of a standard sample, Part 2 (11: HA R_t=0.71; 12: NEO R_t=1.36; 13: DA R_t=1.50; 14: QA R_t=1.65; 15: DOPA R_t=1.66; 16: BH2 R_t=1.87; 17: BIO R_t=1.94; 18: 3OHK R_t=2.02; 19: TYR R_t=2.12; 20: 3-MT R_t=2.96; 21: 3-*O*-MD R_t=3.40; 22: 5-HTP R_t=3.90; 23: 3-OHAA R_t=4.02; 24: XA R_t=6.25; 25: 5-HIAA R_t=6.27)



Expansion of current methods

- So far the most important biomarkers in these disorders are the monoamine metabolites and their co-factors

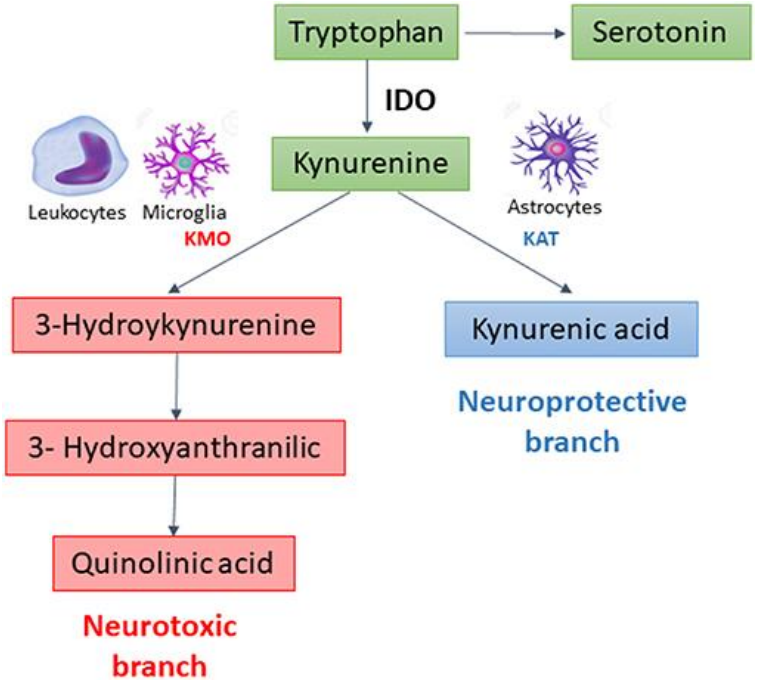
- However, we know there is a wide variation in response to treatment.

Are there other biomarkers which may help explain response to therapies and modify treatment regimes

- Some pterin and TH deficiencies do not respond well to treatment with L-dopa and 5HTP even though they have active AADC. Why is this?

- Is a lack of dopamine and serotonin the only cause of clinical symptoms? Or are altered substrate specificities/alternative pathways causing an increase in toxic metabolites e.g. kynurenine pathway and trace amines?

Is the kynurenine pathway important in monoamine deficiencies?



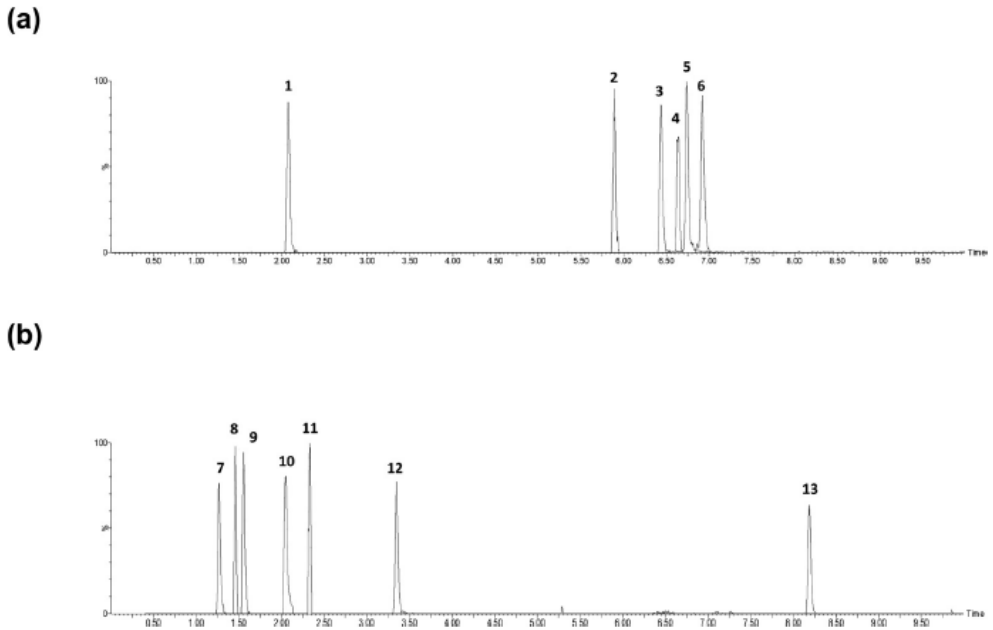
The kynurenine pathway is known to be stimulated by cytokines

Is it also increased if tryptophan conversion to serotonin is blocked?

What is the balance of neurotoxic and neuroprotective metabolites in disorders of monoamine metabolism?

Development of a translational inflammation panel for the quantification of cerebrospinal fluid Pterin, Tryptophan-Kynurenine and Nitric oxide pathway metabolites

Jingya Yan,[a,b] Velda X. Han,[a,c] Benjamin Heng,[d] Gilles J. Guillemin,[d] Sushil Bandodkar,[b,e,,#] and Russell C. Dale [a,e,#]*



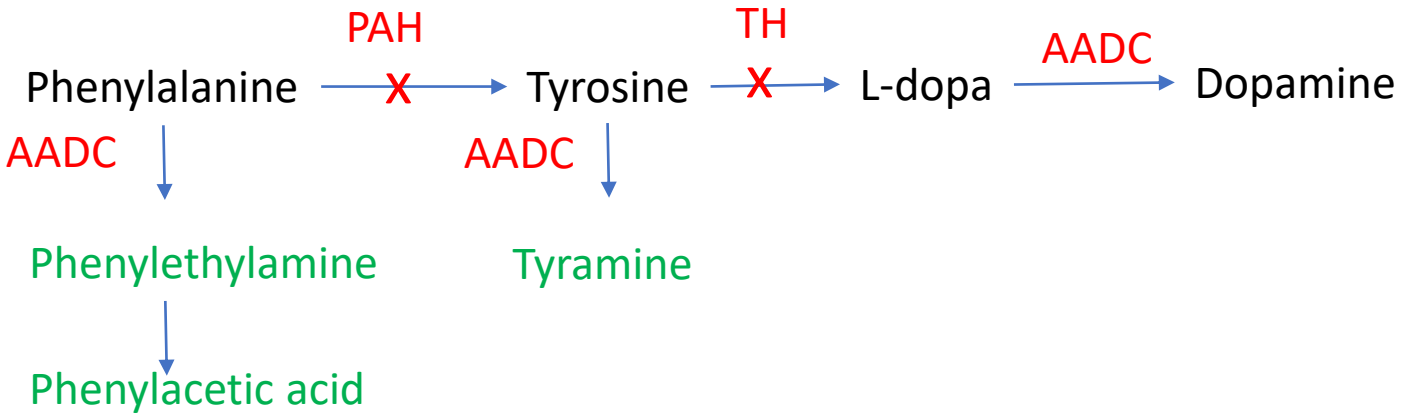
eBioMedicine 2022;77: 103917
Published online xxx
https://doi.org/10.1016/j.ebiom.2022.103917

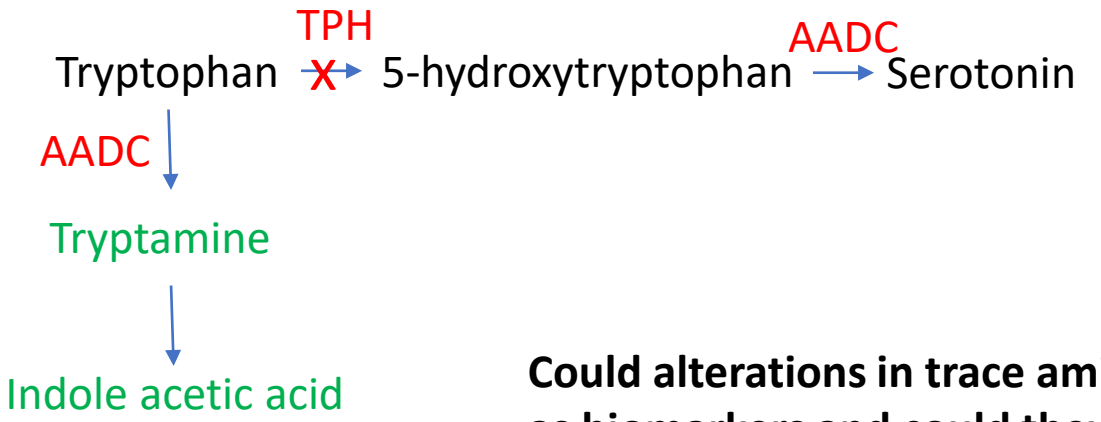
Figure 2. Representative extracted ion chromatograms of the targeted metabolites in human cerebrospinal fluid. (a) Metabolites prepared using the MPA/EDTA solution sample preparation method:[1] quinolinic acid,[2] kynurenine,[3] xanthurenic acid[4] 3-hydroxyanthranilic acid[5] tryptophan and[6] kynurenic acid. (b) Metabolites prepared by adding 20 μL of diluted mixed IS solution sample preparation method:[7] methylhistamine,[8] arginine,[9] citrulline,[10] picolinic acid,[11] neopterin (c)[12] 3-hydroxykynurenine and[13] anthranilic acid.

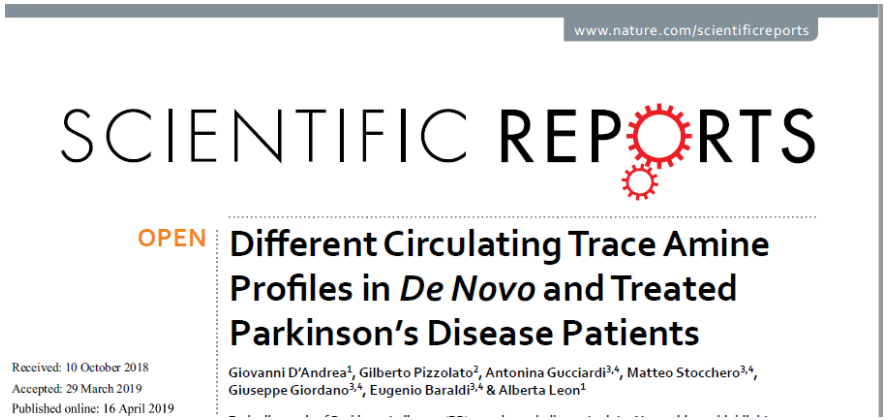
Are trace amines important in deficiencies of hydroxylases?

Trace amines bind to trace amine receptors (TAARs) which are found in periphery and brain

Low levels of trace amines and their metabolites in plasma and CSF can be measured by LC-MS






www.nature.com/scientificreports
SCIENTIFIC REPORTS
OPEN

Different Circulating Trace Amine Profiles in *De Novo* and Treated Parkinson's Disease Patients

Received: 10 October 2018
Accepted: 29 March 2019
Published online: 16 April 2019

Giovanni D'Andrea[1], Gilberto Pizzolato[2], Antonina Gucciardi[3,4], Matteo Stocchero[3,4], Giuseppe Giordano[3,4], Eugenio Baraldi[3,4] & Alberta Leon[1]

Could alterations in trace amines act as biomarkers and could they be responsible for some clinical symptoms?

Possible future uses of LC-MS methods

Review of current biomarkers and the future

Molecular Genetics and Metabolism Reports 27 (2021) 100762

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

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

Tessa Wassenberg [a,b], Ben P.H. Geurtz [c], Leo Monnens [d], Ron A. Wevers [c], Michèl A. Willemsen [e], Marcel M. Verbeek [a,c,*]

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DOI: 10.1002/jimd.12253

ORIGINAL ARTICLE

Confirmation of neurometabolic diagnoses using age-dependent cerebrospinal fluid metabolomic profiles

Tessa M. A. Peters[1,2] | Udo F. H. Engelke[1] | Siebolt de Boer[1] | Ed van der Heeft[1] | Cynthia Pritsch[3] | Purva Kulkarni[1] | Ron A. Wevers[1] | Michèl A. A. P. Willemsen[3] | Marcel M. Verbeek[1,2] | Karlien L. M. Coene[1]

Retrospective review of patient results
Conclusions 1. CSF still best matrix for diagnosis
2. VLA, 3-OMD and L-dopa promising biomarker for screening of AADC.
3. Serum prolactin measurement needs to be standardised and further studied for usefulness. Normal prolactin does not exclude TH or AADC def.
4. CSF monoamine levels do not always agree with clinical response. CSF analysis is not required for treatment monitoring?

Proof of principle: Metabolomics of CSF able to confirm neurometabolic diagnoses.

The future – neonatal diagnosis of AADC in bloodspots?

Molecular Genetics and Metabolism 133 (2021) 56–62

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Detection of 3-O-methyldopa in dried blood spots for neonatal diagnosis of aromatic L-amino-acid decarboxylase deficiency: The northeastern Italian experience

Alberto Burlina [a,*], Antonella Giuliani [a], Giulia Polo [a], Daniela Gueraldi [a], Vincenza Gragnaniello [a], Chiara Cazzorla [a], Thomas Opladen [b], Georg Hoffmann [b], Nenad Blau [c], Alessandro P. Burlina [d]

Proof of concept: Method able to measure 3-OMD in bloodspots in ‘controls’ (mean 1µM) from those taking L-dopa (mean 14µM) and patient with AADC deficiency (11µM)

The future – combining enzyme analysis and mass spectrometry to determine residual activity and specificity?

Examples:
Monoamine oxidase activity in fibroblasts

Pyridoxal kinase activity in bloodspots

PNPO activity in bloodspots

AADC activity in plasma

Received: 8 December 2020 | Accepted: 15 December 2020
DOI: 10.1002/jmd2.12194

RESEARCH REPORT

JIMD REPORTS SSIEM WILEY

Monoamine oxidase A activity in fibroblasts as a functional confirmation of *MAOA* variants

Tessa M. A. Peters[1,2] | Irma Lammerts van Bueren[2] | Ben P.B.H. Geurtz[2] | Karlien L. M. Coene[2] | Nicole de Leeuw[3] | Han G. Brunner[3,4,5,6] | Jón J. Jónsson[7,8] | Michèl A. A. P. Willemsen[9] | Ron A. Wevers[2] | Marcel M. Verbeek[1,2]

Molecular Genetics and Metabolism Reports 32 (2022) 100888

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

ELSEVIER

MGM Reports

Biochemical diagnosis of aromatic-L-amino acid decarboxylase deficiency (AADCD) by assay of AADC activity in plasma using liquid chromatography/tandem mass spectrometry

Gabriel Civallero[a,b,*], Francyne Kubaski[a,c], Danilo Pereira[d,e], Gabriel Rübensam[f], Zackary M. Herbst[g], Camilo Silva[d], Franciele B. Trapp[a], Edina Poletto[a,c], Larissa Faqueti[a], Gabrielle Iop[a], Juliano Soares[a], Vanessa van der Linden[h], Helio van der Linden[h], Charles M. Lourenço[i], Roberto Giugliani[a,b,j,k,l]

Conclusions

- There are currently many good and reliable markers for monoamine defects
- Many of these markers can be measured together using modern LC-MS techniques. This will allow faster diagnosis.
- Mass spectrometry assays are adaptable and sensitive so can be used for different matrices and different studies e.g. CSF neurotransmitters and bloodspot AADC enzyme analysis.
- Expand repertoire and develop new assays. Peripheral markers for treatment monitoring will be important to prevent need for repeated lumbar punctures
- It is important to find markers to better understand response to treatment – trace amines, kynurenine pathway etc.

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Viorica Chelban
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Mita Bertoldi (Italy)
ERNDIM (International Quality Assurance)

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AADC Trust
making a rare child healthy