The effect of maternal methyl-DOPA and other exo- and endogenous interfering substances on the results of 3-*O*-methyl-DOPA determinations and therefore, the screening for AADC deficiency

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MASDIAG DIAGNOSTIC LABORATORY

Scope of Activities:

- **medical diagnostics, including:**
  - endocrinological diagnostics (steroid hormones, biogenic amines, vitamin D metabolites)
  - diagnostics of inherited metabolic disorders (amino acids, acylcarnitines, purines, pyrimidines, organic acids) – over 1.1 million samples since 2012 (~8.3 thousand samples/month).
  - diagnostics based on **microsampling methods**, particularly DBS (Dried Blood Spot) and VAMS (Volumetric Absorptive Microsampling, Capitainer) (vitamin D metabolites, aminoacids, homocysteine, acylcarnitines, fatsoluble vitamins and CoQ10, thyroid hormones) - over 270 thousand samples since 2018 (~4.5 thousand samples/month).
- **toxicology**
- **scientific research mainly in the field of metabolomic studies** (from 2018 to 2023, a total of 27 original scientific publications with an average impact factor (IF) = 4.9)

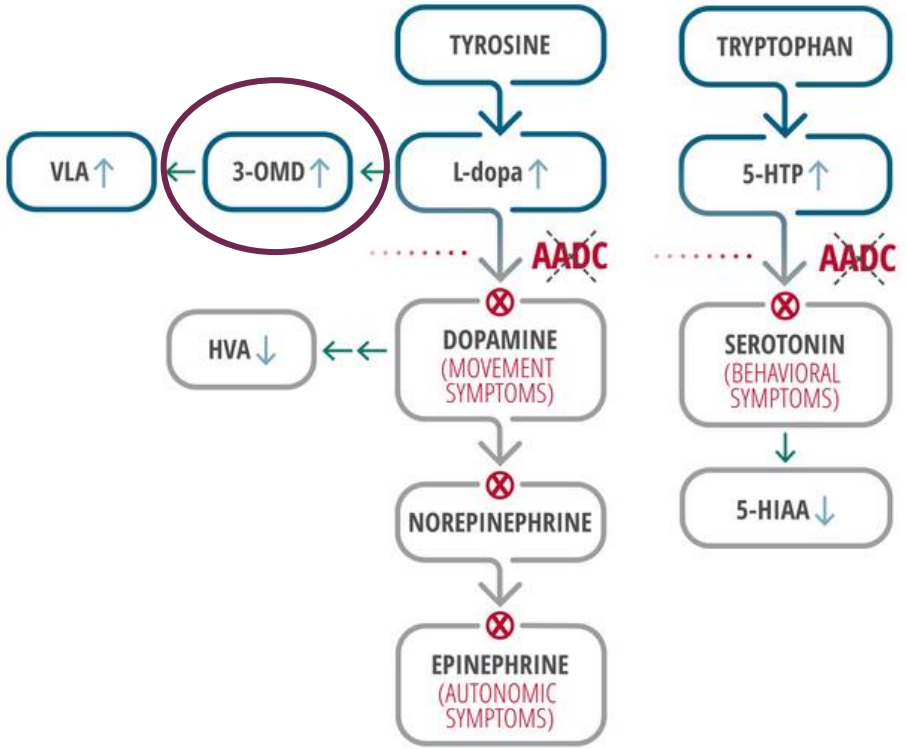
Equipment:

- 7 LC-MS/MS systems combining HPLC/UHPLC Shimadzu pumps with CTC PAL autosamplers and QQQ or QTRAP Sciex mass spectrometers (with varied sensitivity from API3200 to QTRAP®5500+), 1 DART-MS system (Bruker), 1 LC-MS/MS system combining HPLC/UHPLC Shimadzu pumps with CTC PAL autosampler and QQQ mass spectrometer (Bruker)

Staff:

- 22 staff members including 7 with a scientific degree of PhD, laboratory diagnosticians, and chemists

AROMATIC L-AMINO ACID DECARBOXYLASE DEFICIENCY (AADCd)



DIAGNOSTIC PATHWAY FOR SUSPECTED

HYPOTONIA, HYPERTONIA
AND
DELAYED MOTOR DEVELOPMENT
AND
STRUCTURALLY NORMAL MRI

+

MOVEMENT DISORDERS	AND/OR	AUTONOMIC SYMPTOMS
Oculogyric crisis (can be mistaken for seizures)		Ptosis
Dystonia		Excessive sweating
Hypokinesia and/or bradykinesia		Nasal congestion
		Temperature instability

Patients with AADC deficiency may present with one or a combination of symptoms.[2]

TEST FOR AADC DEFICIENCY

Core diagnostic tests	Results
Single gene or genetic panel	Mutation(s) in the *DDC* gene
Plasma enzyme activity assay	**LOW** plasma AADC enzyme activity
AND/OR	
CSF neurotransmitter metabolite panel	**REDUCED** HVA, 5-HIAA, and MHPG **ELEVATED** 3-OMD, L-dopa, and 5-HTP **NORMAL** pterins

3-OMD = 3-O-methyldopa, 5-HIAA = 5-hydroxyindoleacetic acid, HVA = homovanillic acid,
L-dopa = L-3,4-dihydroxyphenylalanine, VLA = vanillactic acid,
MHPG = 3-methoxy-4-hydroxyphenylglycol

https://aadcinsights.com/diagnosis/

CHALLENGES

- Clinical overlap and misdiagnosis
- Analytical limitations and cut-off setting

CLINICAL OVERLAP AND MISDIAGNOSIS

Since the clinical presentation of AADC deficiency shares certain similarities with other disorders (e.g., cerebral palsy or epilepsy), AADCd often remains undiagnosed or is misdiagnosed.

Symptoms of AADC deficiency

1. Himmelreich N, et al. Mol Genet Metab. 2019;127:12–22; 2. Pearson TS, et al. Mov Disord. 2019;34:625–636; 3. Manegold C, et al. J Inherit Metab Dis. 2009;32:371–380; 4. Wassenberg T, et al. Orphanet J Rare Dis. 2017;12:12; 5. DeFilippis M and Wagner KD. Psychopharm Bull. 2016;46:18–41.

CLINICAL OVERLAP AND MISDIAGNOSIS

Despite symptom onset during infancy, diagnosis is typically delayed

Mean age of symptom onset

2.7 months

Mean age of diagnosis

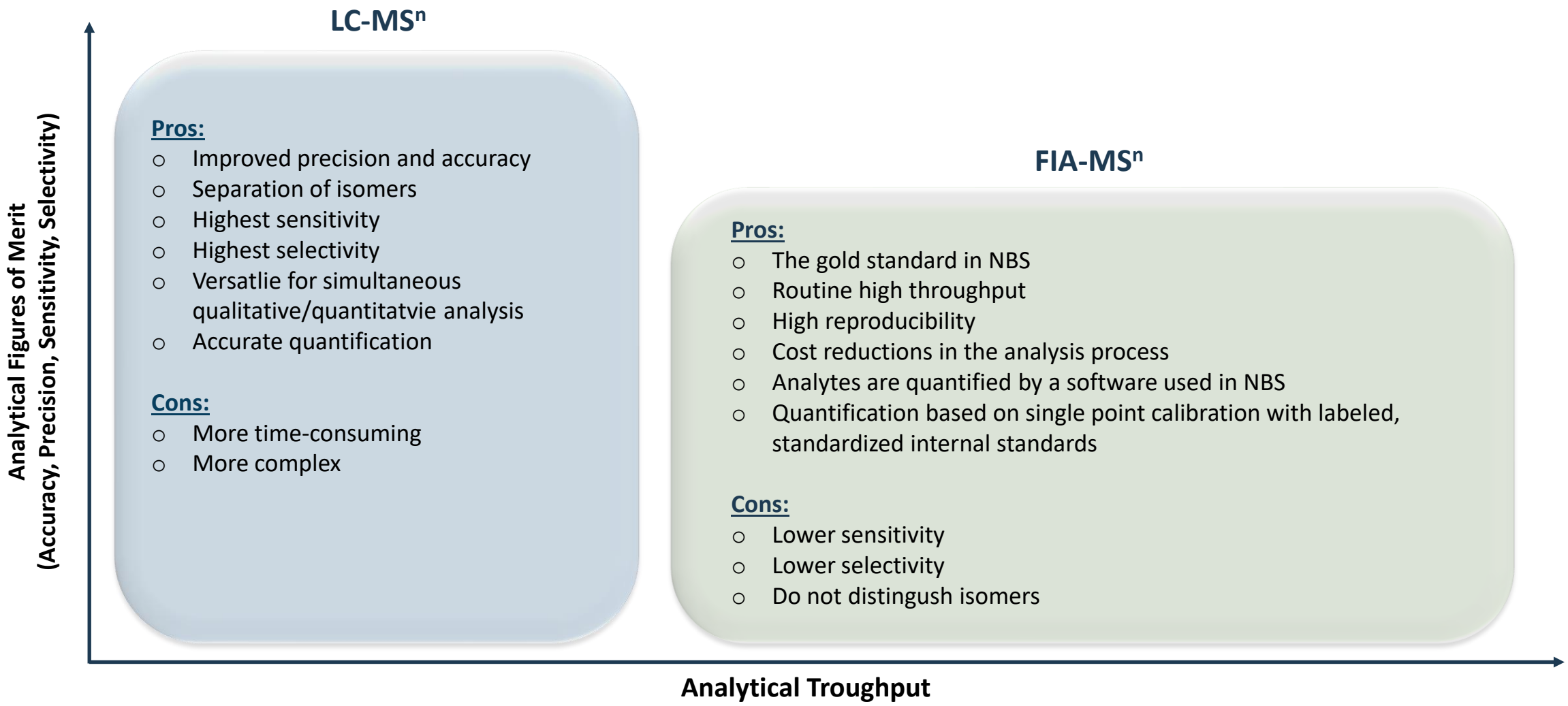
3.5 years

Age range of diagnosis

2 months to 23 years

1. Himmelreich N, et al. Mol Genet Metab. 2019;127:12–22; 2. Pearson TS, et al. Mov Disord. 2019;34:625–636; 3. Manegold C, et al. J Inherit Metab Dis. 2009;32:371–380; 4. Wassenberg T, et al. Orphanet J Rare Dis. 2017;12:12;
5. DeFilippis M and Wagner KD. Psychopharm Bull. 2016;46:18–41.

ANALYTICAL LIMITATIONS: FIA-MS/MS vs LC-MS/MS



LC-MS^n

Pros:
- Improved precision and accuracy
- Separation of isomers
- Highest sensitivity
- Highest selectivity
- Versatlie for simultaneous qualitative/quantitatvie analysis
- Accurate quantification

Cons:
- More time-consuming
- More complex

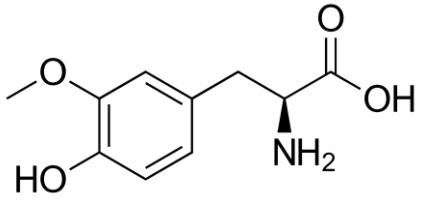
FIA-MS^n

Pros:
- The gold standard in NBS
- Routine high throughput
- High reproducibility
- Cost reductions in the analysis process
- Analytes are quantified by a software used in NBS
- Quantification based on single point calibration with labeled, standardized internal standards

Cons:
- Lower sensitivity
- Lower selectivity
- Do not distingush isomers

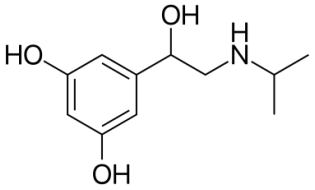
Analytical and Bioanalytical Chemistry, 408, 23–33 (2016)

ANALYTICAL LIMITATIONS: INRERFERENCES

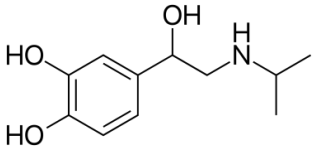


3-OMD

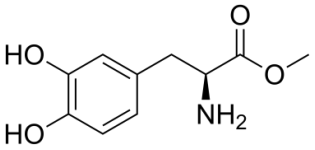
Same mass



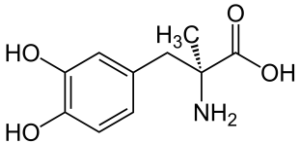
Orciprenaline (metaproterenol)
treatment of asthma



Isoprenaline (isoproterenol)
increase heart rate and relax airways.



Melevodopa
prodrug of L-DOPA



L-methyldopa (Dopegyt)
treatment of high blood pressure, especially for pregnant women.

Different mass
Same metabolite

L-DOPA
treatment of pediatric dystonia and extrapyramidal disorders

FROM SAMPLE PREPARATION TO INTERFERENCE-FREE ANALYSIS

Dried blood spot (DBS)
2 x ø 3 mm discs
(~6.2 µl blood)

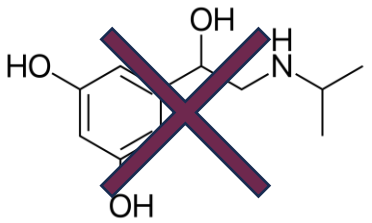
Cutting on 2 x 3 mm DBS discs to 96-well plate;
Extraction, protein precipitation, and **derivatization with n-butanol**, as in the standard FIA-MS NBS protocol.

Chromatography:
Reversed-phase chromatography, total runtime 4.5 min

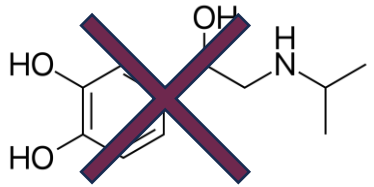
Mass spectrometry:
Sciex 4500QTRAP, ESI; MRM mode, positive ionization

Online survey: asked about L-DOPA use → still included L-DOPA in method for reliability.

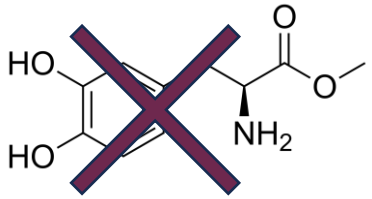
DERIVATIZATION WITH N-BUTANOL



Orciprenaline



Isoprenaline



Melevodopa

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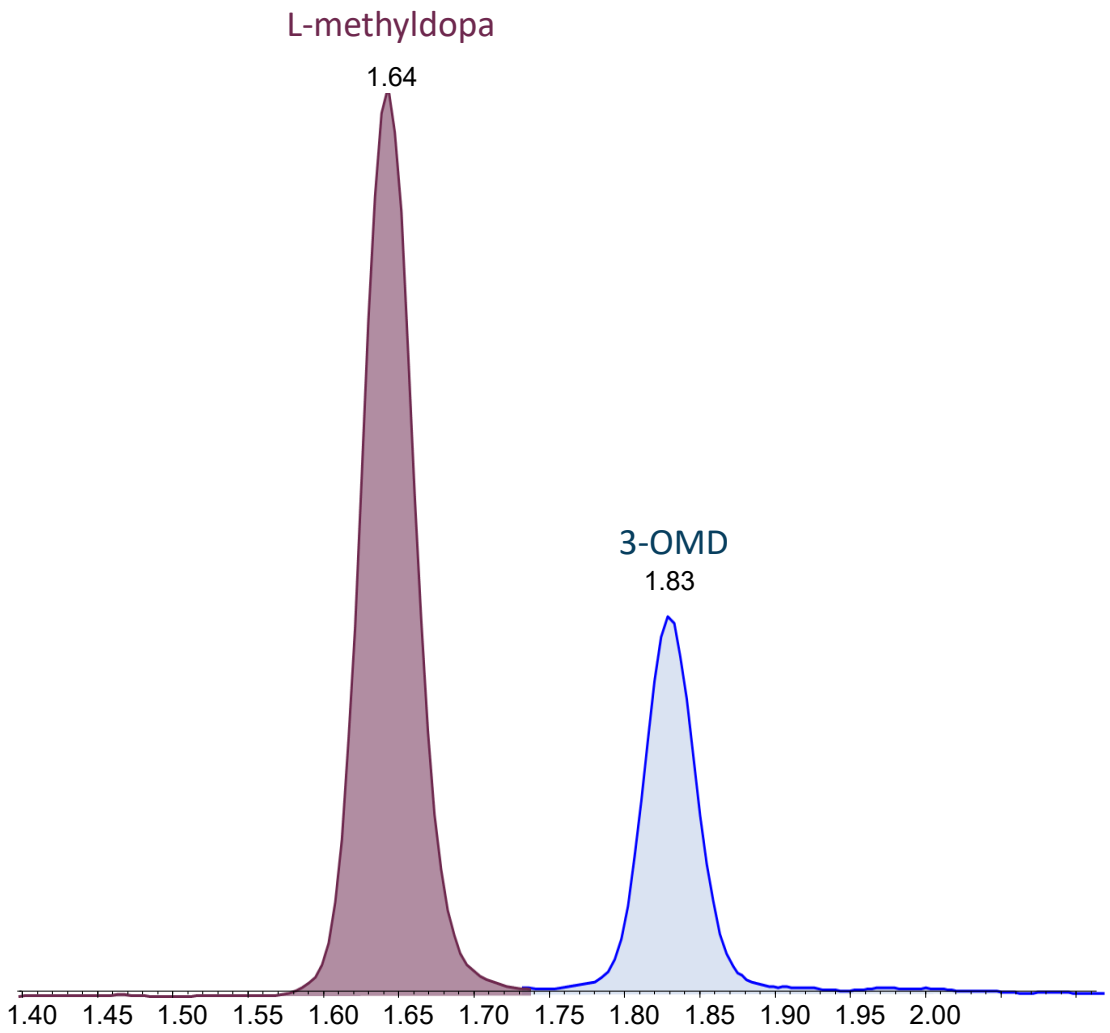
CHROMATOGRAHPIC SEPARATION



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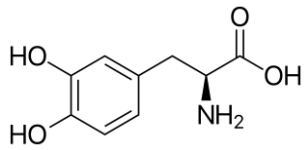
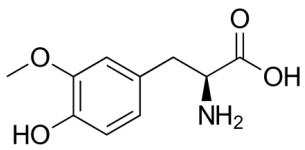
ONLINE SURVEY

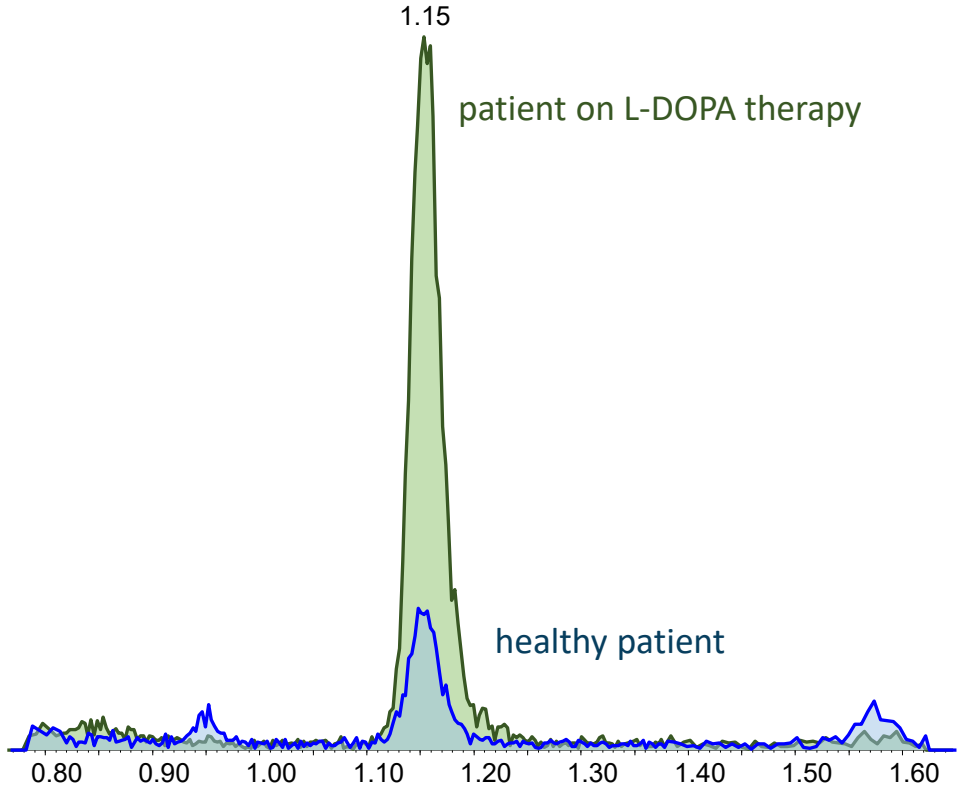


L-DOPA

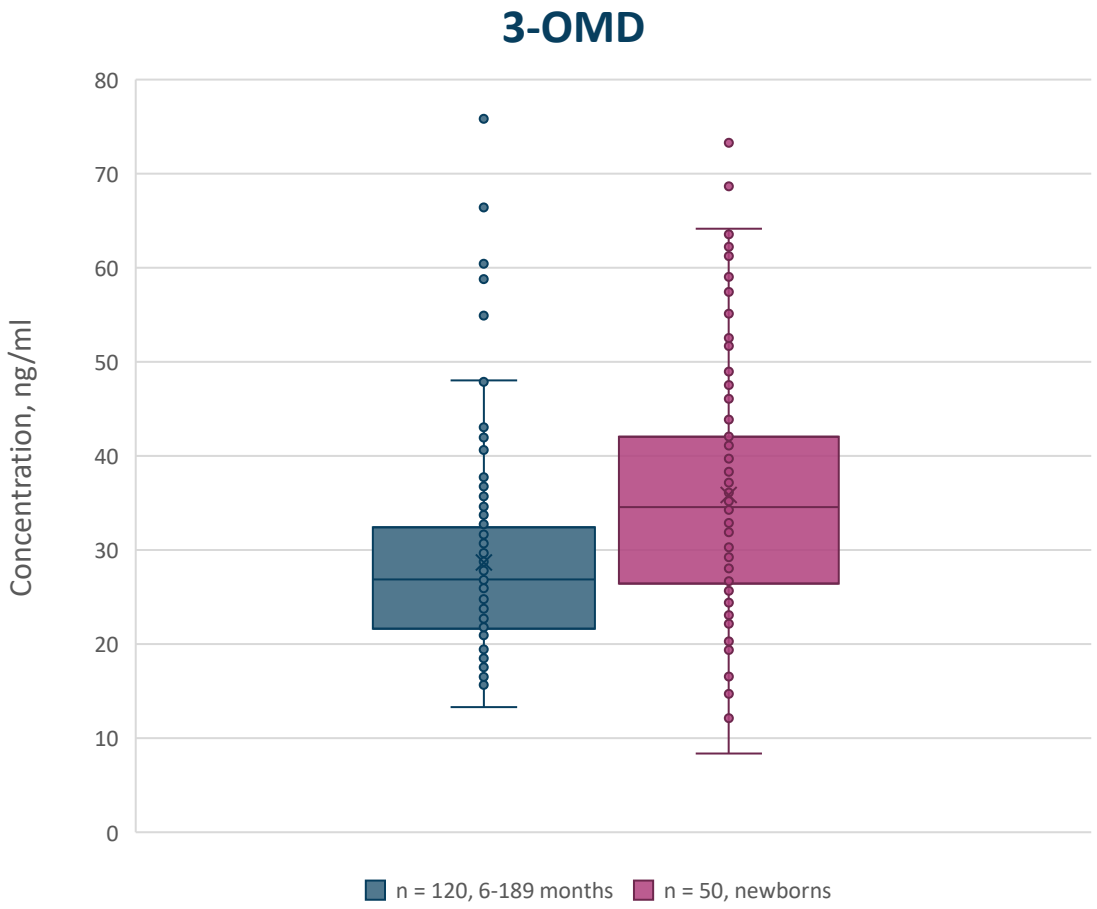
COMT



3-OMD



CLINICAL VALIDATION: CUT-OFF SETTING



Mean	29 ng/mL
Median	27 ng/mL
Percentile 2.5	17 ng/mL
Percentile 97.5	60 ng/mL

Mean	34 ng/mL
Median	32 ng/mL
Percentile 2.5	12 ng/mL
Percentile 97.5	69 ng/mL

Our LC-MS/MS results:

Children: avg 29 ng/mL; cut-off 60 ng/mL (percentile 97.5)

Newborns: avg 34 ng/mL; cut-off 69 ng/mL (percentile 97.5)

† Brennenstuhl, Heiko et al. *Journal of inherited metabolic disease* (2020): 602-610; Burlina, Alberto et al. *Molecular genetics and metabolism* (2021): 56-62; Chen, Pin-Wen et al. *Clinica chimica acta; international journal of clinical chemistry* (2014): 19-22; Chen, Pin-Wen et al. *Molecular genetics and metabolism* (2023): 107687; Chien, Yin-Hsiu et al. *Molecular genetics and metabolism* (2016): 259-63; Di Carlo, Emanuele et al. *Journal of chromatography. B* (2021): 122999; Kubaski, Francyne et al. *Molecular genetics and metabolism reports* (2021): 100744; Reischl-Hajiabadi, Anna T et al. *Molecular genetics and metabolism* (2024): 108148.

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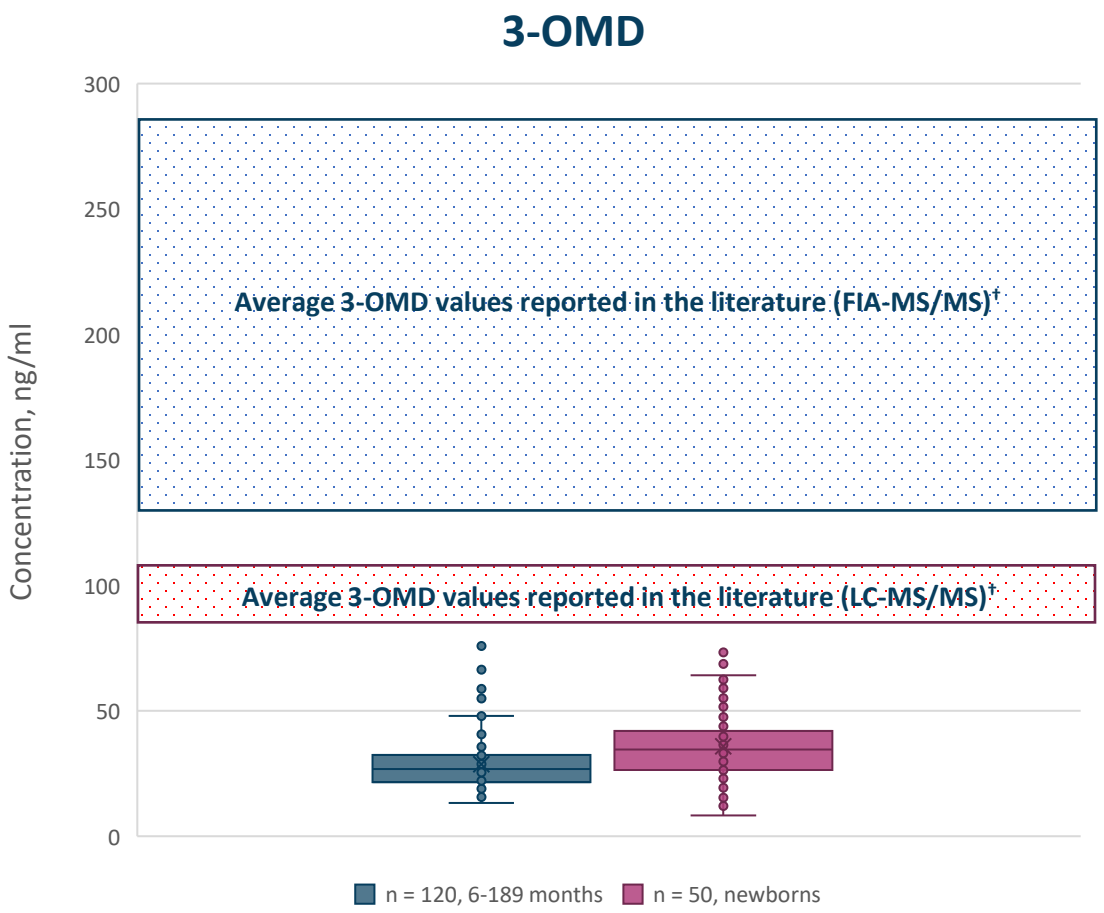

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Our LC-MS/MS results:
Children: avg 29 ng/mL; cut-off 60 ng/mL (percentile 97.5)
Newborns: avg 34 ng/mL; cut-off 69 ng/mL (percentile 97.5)

Reference ranges FIA-MS/MS (literature)†: 127 - 285 ng/mL
Reference ranges LC-MS/MS (literature)†: 92 - 107 ng/mL

Literature FIA-MS/MS values far above our measurements
High FIA-MS/MS cutoffs → affected newborns may be missed

Proper separation is essential to avoid underdiagnosis of AADCd

† Brennenstuhl, Heiko et al. *Journal of inherited metabolic disease* (2020): 602-610; Burlina, Alberto et al. *Molecular genetics and metabolism* (2021): 56-62; Chen, Pin-Wen et al. *Clinica chimica acta; international journal of clinical chemistry* (2014): 19-22; Chen, Pin-Wen et al. *Molecular genetics and metabolism* (2023): 107687; Chien, Yin-Hsiu et al. *Molecular genetics and metabolism* (2016): 259-63; Di Carlo, Emanuele et al. *Journal of chromatography. B* (2021): 122999; Kubaski, Francyne et al. *Molecular genetics and metabolism reports* (2021): 100744; Reischl-Hajiabadi, Anna T et al. *Molecular genetics and metabolism* (2024): 108148.

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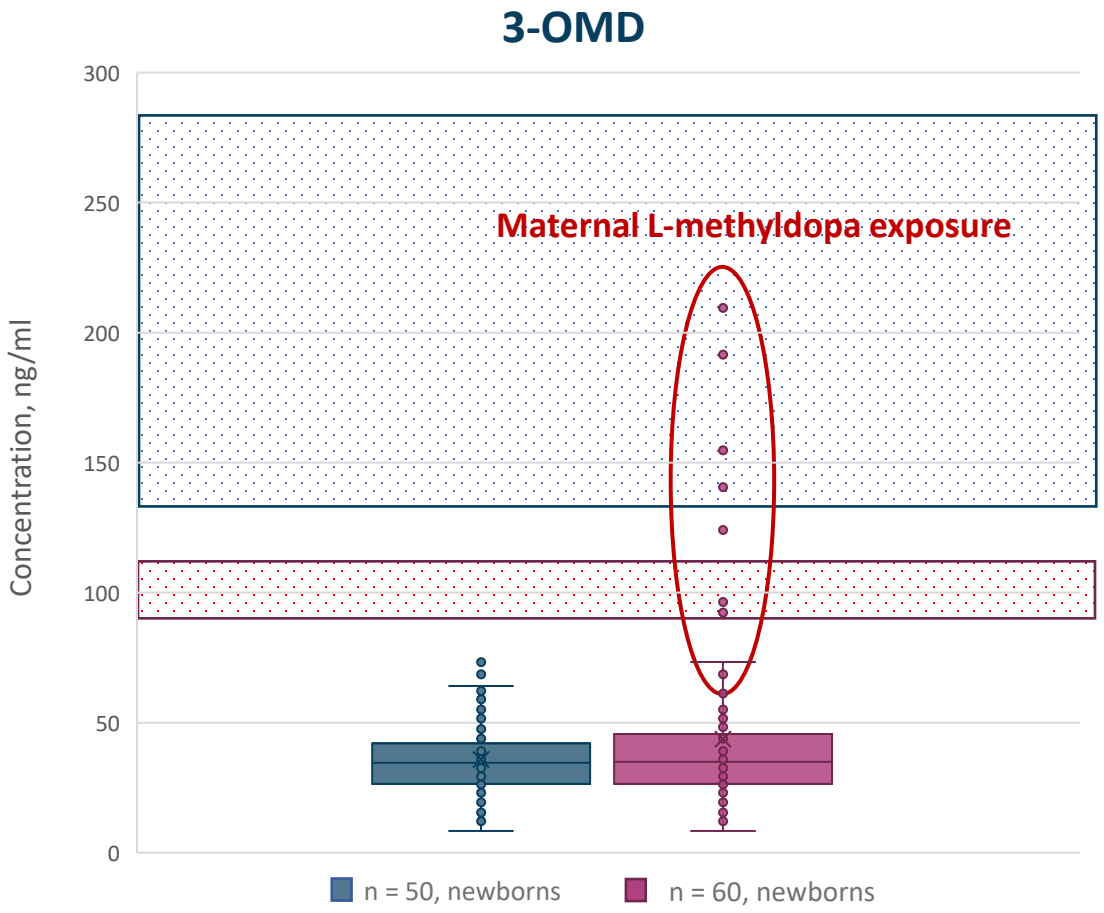
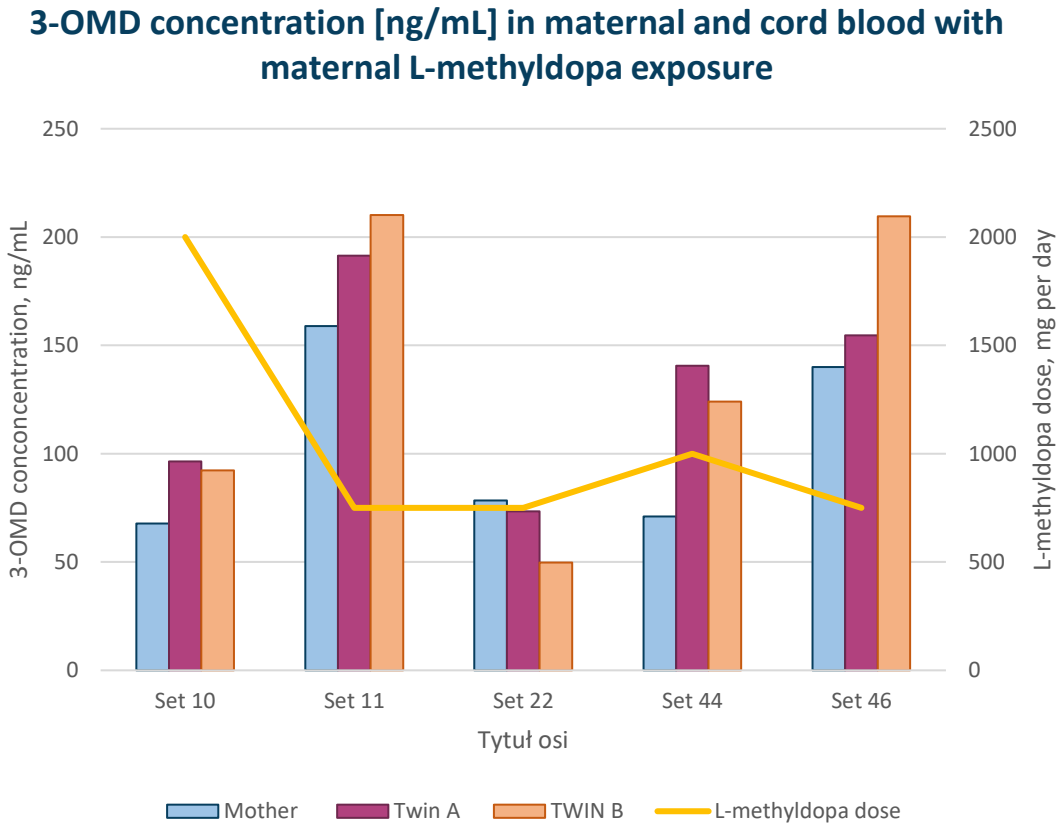


Mean	29 ng/mL
Median	27 ng/mL
Percentile 2.5	17 ng/mL
Percentile 97.5	60 ng/mL

Mean	39 ng/mL
Median	32 ng/mL
Percentile 2.5	12 ng/mL
Percentile 97.5	154 ng/mL



Set 10: 3x250 mg (2 days), 3x500mg (7 days), 4x500mg (2 days),
Set 11: 3x250 mg (19 weeks)
Set 22: 3x250 mg (7 days)
Set 44: 4x250mg (7 days)
Set 46: 3x250 mg (3 days)

CONCLUSION

Clinical overlap:
Symptoms often mimic more common conditions (e.g., cerebral palsy, epilepsy).

Analytical limitations:
FIA-MS/MS may produce many **false positives.**
Reference ranges can be **biased upward** due to interferences (e.g., L-maternal methyldopa in ~10% of pregnancies).

Risk: False negatives possible if screening cut-offs are set too high, reassessment of cut-off values and methods is warranted.

If global prevalence is ~1:100 000 (or ~1:30,000 in Taiwan), but **in Poland we know of only a few diagnosed patients** (~2) → this suggests **underdiagnosis**.

Early detection is crucial, because AADC deficiency is now treatable (gene therapy), so identifying affected newborns before symptom onset allows timely intervention.

Thank you for your attention!

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