Quantification of 3-O-methyl-dopa in dried blood spots for the biochemical screening of aromatic L-amino acid decarboxylase deficiency

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Introduction
Aromatic L-amino acid decarboxylase (AADC) deficiency is an autosomal recessive disorder that leads to increased levels of the L-dopa metabolite 3-O-methyl-dopa (3-OMD). Diagnosis has been based on the sequencing of the DDC gene and/or in the assay of AADC in plasma, which may limit the diagnosis of the disease due to high cost and/or unavailability. Levels of 3-OMD have been described as elevated in plasma, urine, and dried blood spots of AADC patients, and the assay of this metabolite has been used for the newborn screening (NBS) of AADC patients.

Material and methods
3-O-methyl-dopa (3-OMD) (M4255) was from Sigma-Aldrich (Saint Louis, USA). The deuterated internal standard L-3-(4-Hydroxy-3-methoxy-d3-phenyl)alanine (D-6782) was from C/D/N isotopes (Quebec, Canada), formic acid (F0507) (reagent grade), and methanol (1.08018) (LC grade) were from Sigma-Aldrich (Saint Louis, USA). Sterile clear 96-well filter plates 0.45 μM (MAIPS4510) were from Millipore-Sigma (Burlington, USA). 96-well polypropylene sample collection plates (186002643) were from Waters (Milford, USA). Working internal standard (3-OMD-D3 7.5 ng/mL) and the calibration curve (20–4380 ng/mL) were prepared as described by Chen et al., 2014. Blood was collected with EDTA by venipuncture and 150 μL of blood was spotted onto filter paper. DBS from 8 patients with a prior diagnosis (biochemical and molecular analysis) of AADC, 35 non-AADC controls, and 22 general newborns were collected. Informed consent was obtained at each Institute for all the patients and control samples according to IRB approval. All samples were shipped (with desiccant, but without refrigeration) and stored at ~20 °C after arrival at the laboratory. Samples were prepared according to the method previously described by Chen et al., 2014 with slight modifications. Briefly, a single 3.2 mm disc was punched into a 96-well polypropylene plate with 200 μL of 7.5 ng/mL 3-OMD-D3 and the plate was incubated at 37 °C with shaker for 3 h, as well as the spiked DBS used for the calibration curve. The supernatant was then transferred to the 0.45 μM filter plate coupled with clean 96-well plates and centrifuged at 2000 RPM for 3 min. The filtrate was collected in the receiver plate and 1.5 μL was injected into the liquid chromatography tandem mass spectrometer (Chen et a., 2011).

Results
We have analyzed DBS from 8 patients with confirmed diagnosis of AADC, and 38 age-matched controls. All eight patients had very high levels of 3-OMD (average = 3,073 ng/mL, range: 1,367-12,302 ng/mL). All controls had low levels of 3-OMD with an average of 34 ng/mL (range: 20 to 331 ng/mL) (Figure 1 A, B).

Figure 1A. Distribution of 3-OMD levels in AADCD patients, general newborns and non-AADCD controls according to age. Figure 1B. Average levels of 3-OMD in AADCD patients, general newborns and non-AADCD controls. The levels of 3-OMD were significantly higher compared to newborns** (p < 0.0005) and to controls ** (p < 0.0001). Adapted from: Kubaski et al., 2021 MGMT 27, 100744.

It is also interesting to point out that one homozygote for the p.Arg347Gln variant treated with L-dopa had the highest level of 3-OMD (12,302 ng/mL), while another homozygote untreated had a much lower 3-OMD level (1,504 ng/mL) (Table 1).

Table 1: Distribution of AADC patients according to gender, age, 3-OMD concentrations and genotype. Adapted from: Kubaski et al., 2021 MGMT 27, 100744.

Conclusions
The validation of the LC/MS-MS 3-OMD assay in DBS enables its use for the screening of AADC in suspected patients (diagnosis to be confirmed by DDC sequencing and/or AADC enzyme activity measurement) and can also be considered for NBS for this condition.

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