Clinica Chimica Acta 413 (2012) 126–130

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

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

# Non-invasive urinary screening for aromatic l-amino acid decarboxylase deficiency in high-prevalence areas: A pilot study

Han-Chih Hencher Lee [a,1], Chi-Kong Lai [a,1], Kin-Cheong Eric Yau [b], Tak-Shing Siu [c], Chloe Miu Mak [a], Yuet-Ping Yuen [a], Kwok-Yin Chan [b], Sidney Tam [c], Ching-Wan Lam [d,*], Albert Yan-Wo Chan [a]

[a] Department of Pathology, Princess Margaret Hospital, Hong Kong, China
[b] Department of Paediatrics and Adolescent Medicine, Princess Margaret Hospital, Hong Kong, China
[c] Division of Clinical Biochemistry, Queen Mary Hospital, Hong Kong, China
[d] Department of Pathology, The University of Hong Kong, Hong Kong, China

ARTICLE INFO

*Article history:*
Received 24 December 2010
Received in revised form 6 September 2011
Accepted 7 September 2011
Available online 21 September 2011

*Keywords:*
Aromatic L-amino acid decarboxylase deficiency
Chinese
Gas chromatography–mass spectrometry
Screening

ABSTRACT

*Background:* The diagnosis of aromatic L-amino acid decarboxylase (AADC) deficiency, one of the pediatric neurotransmitter disorders, is classically made with plasma enzyme level or cerebrospinal fluid (CSF) neurotransmitter profile, while both are technically demanding and the latter requires the invasive lumbar puncture. So far less than 100 cases have been reported worldwide with 20% from Taiwan. It was postulated that the condition might have been under-diagnosed among Chinese populations and a non-invasive screening tool should be developed in areas with high prevalence.
*Methods:* Urine metabolic profiles performed by gas chromatography–mass spectrometry (GC-MS) in a 31-month period were retrospectively reviewed: those with vanilmandelic acid concentration lower than one percentile plus the presence of 3-*o*-methyldopa were defined as positive and the patients were further evaluated.
*Results:* Among 1046 metabolic profiles (from 845 patients) reviewed, 3 profiles from 2 patients were screened positive: both cases had compatible CSF neurotransmitter profiles and the diagnosis was further confirmed by genetic analysis of *DDC* gene. 13 negative urinary metabolic profiles from 7 patients who had CSF neurotransmitters analyzed were identified as controls: all 7 CSF neurotransmitter profiles were not compatible for AADC deficiency.
*Conclusions:* The GC-MS-based urine metabolic profiling was shown to be a satisfactory screening tool for AADC deficiency. Further confirmation can be performed by mutation analysis in the *DDC* gene, thus avoiding risks of lumbar puncture. We advocate all ethnic Chinese patients presenting with dystonia have their urine organic acids analyzed before proceeding to CSF neurotransmitters analysis.



## 1. Introduction

Neurotransmitter disorders are an emerging group of inheritable neurometabolic syndromes caused by disturbances of neurotransmitter metabolism. These disorders involve defects in the enzymes either in the synthetic or degradative pathways of the neurotransmitters or in the synthesis of the cofactors for these pathways. So far disorders of the metabolism of monoamines, glycine and γ-amino butyric acid have been defined [1]. The presentations can be non-specific, which often include dystonia, developmental delay, truncal hypotonia, dyskinesia, spastic paraparesis, choreoathetosis, oculogyric crises, seizures, Parkinsonism and autonomic dysfunctions; and diurnal fluctuations are often observed in these patients [2]. A correct diagnosis for neurotransmitter disorders has important therapeutic implications when medications are readily available for some of these disorders and the response to treatment can be dramatic and impressive [3].

Aromatic L-amino acid decarboxylase (AADC) deficiency (MIM#608643), one of the neurotransmitter disorders, is a rare autosomal recessive disorder of biogenic amines metabolism first described by Hyland et al. in 1990 [4]. The homodimer enzyme dopa decarboxylase or AADC (EC 4.1.1.28), coded by *DDC* (MIM*107930), catalyzes the decarboxylation of levodopa and 5-hydroxytryptophan into dopamine and serotonin respectively, with pyridoxal 5′-phosphate as the enzyme cofactor [5]; supplementation of the cofactor, commonly in form of pyridoxine, has become the mainstay of treatment of AADC deficiency, in addition to dopamine agonists and monoamine oxidase inhibitors [5]. The condition is rare with so far less than 100 cases reported worldwide [6–9]; however around 20% of the patients were

*Abbreviations:* AADC, aromatic L-amino acid decarboxylase; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HTP, 5-hydroxytryptophan; HVA, homovanillic acid; 3-OMD, 3-ortho-methyldopa (vanilalanine); PNPO, pyridoxal 5′-phosphate; VMA, vanilmandelic acid.

* Corresponding author at: Department of Pathology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong, China. Tel.: +852 2255 5655; fax: +852 2255 9915.
*E-mail address:* ching-wanlam@pathology.hku.hk (C.-W. Lam).

[1] Han-Chih Hencher LEE and Chi-Kong LAI contributed equally to this study.

0009-8981/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2011.09.008

Taiwanese, with the relatively high incidence attributed to a common mutation in Taiwan [6].

Patients with AADC deficiency often have non-specific presentations, which can include truncal hypotonia, limb dystonia, hypokinesia, oculogyric crises, severe irritability, hypersalivation, hypotension, nasal congestion, excessive sweating and hypoglycemia [7]. The first step in diagnosing AADC deficiency and other neurotransmitter disorders is the investigation of neurotransmitters in cerebro-spinal fluid (CSF) [7,10]. The typical pattern shows reductions in homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), as well as elevations in levodopa, 5-hydroxytryptophan (5-HTP) and 3-ortho-methyldopa (3-OMD, or vanilalanine). Hyland et al. and Abdenur et al. have reported the urine pattern of AADC deficiency patients in their case reports, with reductions in vanilmandelic acid (VMA) and 5-HIAA and also increases in vanillactate, vanilpyruvate, *N*-acetyl-vanilalanine and *N*-acetyl-tyrosine, while HVA concentrations are normal [11,12].

AADC deficiency is considered to be under-recognized and underdiagnosed with its non-specific presentations and rarity [5]. Although the condition has never been reported in Hong Kong or Mainland China, we postulated that the mutation event could have happened in Mainland China, and patients could be found in Chinese populations residing near Taiwan. Although CSF neurotransmitter analysis is the classical investigation method of choice, it is not considered a good screening test when it requires invasive lumbar puncture to obtain the specimen. The test also has stringent specimen collection and storage requirements due to the rostro-caudal gradient and low stability of the neurotransmitters in CSF. On the other hand, qualitative urine metabolic profiling is a widely available test which only requires a random urine sample in plain bottle without the need of preservative.

We here report the results of our pilot study using gas chromatography–mass spectrometry-based urine metabolic profiling for screening of AADC deficiency in Hong Kong, a densely populated area with the majority of residents being Chinese and thus a potentially high prevalence of AADC deficiency.

## 2. Methods

All urine metabolic profiles generated by a routine qualitative GC-MS urine metabolic profiling in a 31-month period (from March 2007 to September 2009) in a single tertiary medical center were retrospectively reviewed. Two metabolites were specifically looked for: VMA and 3-OMD. Those urine metabolic profiles with VMA concentration lower than one percentile (previously determined locally in 593 normal urine controls) plus the presence of 3-OMD were defined as screened-positive. For the positive cases, the clinical history were retrieved and reviewed for the possibility of AADC deficiency. Patients with the clinical details compatible with AADC deficiency or other neurotransmitter disorders were arranged for CSF neurotransmitter analysis, if this had not been performed, and mutation analysis for *DDC*. Cases screened-negative by urine metabolic profiling that also had CSF neurotransmitter analysis performed for a clinical suspicion and/or other evidence of neurotransmitter disorders were identified as the negative controls; the CSF neurotransmitter profiles of these patients were reviewed.

### 2.1. GC-MS urine metabolic profiling

Spot urine samples were collected fresh from the patients in plain bottles and frozen at −20 °C until the time of analysis and all frozen urine were completely thawed prior to analysis. Ethyl acetate solvent extraction and trimethylsilyl derivatization were performed on a creatinine-adjusted volume of urine. Analyses were performed on a GC-MS (Model 6890 / 5975, Agilent Technologies, Santa Clara, CA), with an HP-5MS cross-linked 5% phenylmethylsilicone fused-silica capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness) and 1.0 ml/min constant flow helium as the carrier gas [13]. The oven program was 62–320 °C with a linear change of 8.05 °C/min. The mass detector was operated in scan mode in the range of 50–800 atomic mass units and the peaks on the chromatograms were identified through libraries according to the mass spectra.

### 2.2. CSF neurotransmitters analysis

Informed consent was obtained from the parents/legal guardians for lumbar puncture. Neurotransmitters and pterins were determined in CSF specimens that have been snap-frozen at bedside on pre-cooled ice rack with protection from light and transported to the chemical pathology laboratory without delay [14]. The second 1-mL was used for analysis due to the rostro-caudal concentration gradient. The CSF specimens were briefly centrifuged to remove any cellular content and frozen at −80 °C in dark until analysis. HVA and 5-HIAA were analyzed using high-performance liquid chromatography (HPLC) with amperometric electrochemical detector, while 3-OMD and 5-HTP were analyzed using HPLC with fluorescence detector.

### 2.3. Molecular studies

Informed consent was obtained from the parents/legal guardians for mutation analysis. Genomic DNA was extracted from peripheral whole blood of the patient and parents using the QIAamp blood kit (Qiagen, Hilden, Germany). For the patient, all coding exons with the flanking intronic regions of the *DDC* gene were amplified (primer sequences, PCR mixture components and thermocycling conditions available upon request). Both strands were sequenced using the amplification primers and BigDyeDeoxy™ terminator cycle sequencing reagents (Applied Biosystems, Foster City, CA, USA). The products of sequencing reactions were purified with Auto-Seq G-50 columns (General Electric Healthcare, Uppsala, Sweden) and detected on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Fster City, CA).

## 3. Results

All 1046 urine metabolic profiles from 845 patients performed in the 31-month period were retrieved and reviewed (Fig. 1 and Table 1). Among these, three urine metabolic profiles from 2 patients (0.24%) fulfilled the screening criteria and were determined positive. Both cases had presentations compatible with neurotransmitter disorders and CSF

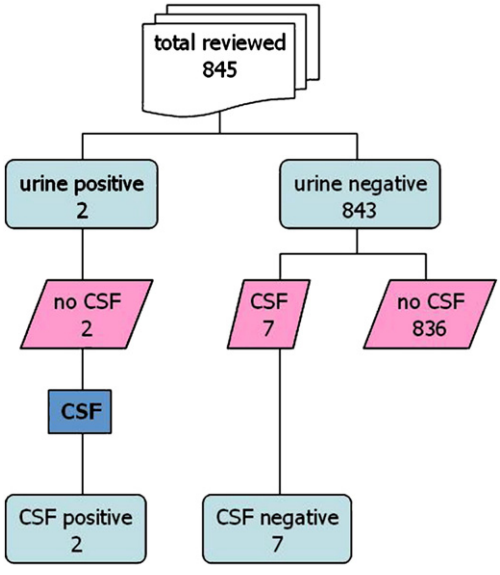


**Fig. 1.** Current study protocol and results.