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Analysis of amino acids in autism spectrum disorders

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ABSTRACT

The relationship between comorbidities associated with amino acids (AAs) and autism spectrum disorder (ASD) is the subject of much research. The diagnosis of these co-occurring disorders based on measurements of levels of AAs in clinical samples poses a challenge. The development of a variety of analytical platforms, particularly chromatographic methods, mass spectrometry and magnetic resonance spectroscopy, together with chemometric statistical methods, enables sensitive detection of abnormalities related to AAs. We present the application of analytical methods to the study of AAs in ASD. Moreover, this review highlights the role of some AAs as potential biomarkers for ASD.

Contents

Abbreviations: AA, Amino acid; Ala, Alanine; Asn, Asparagine; ASD, Autism spectrum disorders; CE, Capillary electrophoresis; Cit, Citrulline; CSF, Cerebrospinal fluid; CSI, Chemical shift imaging; Cys, Cysteine; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th edition; EC, Electrochemical detection; FLD, Fluorescence detection; GABA, Gammaaminobutyric acid; GC, Gas chromatography; GC-MS, Gas chromatography–mass spectrometry; Gln, Glutamine; Glu, Glutamic acid; Glx, Combined glutamate and glutamine signal; Gly, Glycine; ^{1}H–^{13}C NMR, Proton and carbon-13 nuclear magnetic resonance spectroscopy; Hcy, Homocysteine; His, Histidine; HILIC, Hydrophilic interaction chromatography; ^{1}H MRS, Proton magnetic resonance spectroscopy; ^{1}H NMR, Proton nuclear magnetic resonance spectroscopy; HPLC, High performance liquid chromatography; Hyp, Hydroxyproline; IEC, Ion-exchange chromatography; Ile, Isoleucine; LC, Liquid chromatography; LC-MS, Liquid chromatography–mass spectrometry; LC-MS/MS, Liquid chromatography–tandem mass spectrometry; Leu, Leucine; LLE, Liquid–liquid extraction; Lys, Lysine; MCE, Microchip electrophoresis; Met, Methionine; MRSI, Magnetic resonance spectroscopic imaging; MS, Mass spectrometry; NAA, N-acetyl aspartate; NMR, Nuclear magnetic resonance spectroscopy; O-PLS-DA, Orthogonal partial least square discriminant analysis; PCA, Principal component analysis; PDA, Photodiode-array detection; Phe, Phenylalanine; PLS-DA, Partial least squares discriminant analysis; RPC, Reversed-phase chromatography; SAH, S-Adenosylhomocysteine; SAM, S-Adenosylmethionine; Ser, Serine; Tau, Taurine; Thr, Threonine; Trp, Tryptophan; Tyr, Tyrosine; UPLC, Ultra-performance liquid chromatography; UPLC-MS/MS, Ultra-performance liquid chromatography–tandem mass spectrometry; UV, Ultraviolet detection; UV-Vis, Ultraviolet–visible detection; Val, Valine.

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1. Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), autism spectrum disorder (ASD) is a neurodevelopmental disorder with onset early in life. It is characterized by varying deficits in social communication, interaction and restricted and repetitive behaviors [1]. Estimated prevalence of ASD is 11.3 per 1000 children aged 8 [2]. ASD was recently considered a multifactorial disorder caused by the interaction with genetic, environmental and immunological factors, and other factors (e.g., age of parents or maternal infections during pregnancy). Many of the cognitive and behavioral features of ASD result from the dysfunction of the central nervous system (CNS), and from multiple non-CNS physiological abnormalities associated with ASD. This may indicate that conditions in ASD are caused by systemic rather than organ-specific abnormalities [3]. Among biomedical abnormalities occurring in ASD, a number of disorders are associated with amino acids (AAs), such as oxidative stress, decreased detoxification capacity, disorder of AA transporters, and impaired methylation and sulfation [4].

The diagnosis of ASD is based on qualitative impairments in social function and communication accompanied by rigid or stereotyped behaviors, detected according to the accepted criteria, usually the Diagnostic and Statistical Manual of Mental Disorders criteria. Specialists also recommend other diagnostic tests, such as genetic studies, electroencephalography, structural neuroimaging, and functional neuroimaging, and metabolic studies [5,6]. Metabolic studies are recommended for the children who, apart from the autistic symptoms, show signs of metabolic disorders (e.g., lethargy, cyclic vomiting or failure to thrive). Recommendations also include metabolic tests for AAs [6]. Clinical tests in ASD are performed multistage and the source of information is usually body fluids (Fig. 1). Some of the AAs are also considered potential biomarkers for ASD. Their importance is expected to grow and should even become one of the priorities in the investigation of ASD [7].

The determination of AAs in biological samples causes many difficulties due to their high water solubility, range of ionic characteristics, the lack of a selective ultraviolet spectrum [8], and the complexity of sample matrices. Thus, to make the studies successful, the ability to select appropriate analytical techniques and appropriate methodologies for sample preparation and analysis is fundamental. In order to determine AAs in body fluids, generally, there is a need for both derivatization and extraction methods as well as separation methods, including ion-exchange chromatography (IEC), reversed-phase chromatography (RPC), hydrophilic interaction chromatography (HILIC), gas chromatography (GC), capillary electrophoresis (CE), and microchip electrophoresis (MCE) [9]. Among a number of such diverse methods, a standard for AA analysis is still high-performance liquid chromatography (HPLC) with fluorescence detection (FLD) or ultraviolet–visible (UV-Vis) detection [8], which offers relatively short analysis times, low instrumentation costs and maintenance, and higher sensitivity and flexibility [10]. A new aspect of AA analysis is using ultra-performance liquid chromatography (UPLC), which comes from HPLC and helps to improve chromatographic resolution, speed and sensitivity of analysis. Additionally, UPLC, used in combination with mass spectrometry (MS), is especially suitable for the profile analysis of physiological AAs in metabolomics [11]. AA analysis in metabolomic studies seems to be particularly important in search for biomarkers for ASD. In this approach, LC-MS [12], GC-MS [13] and nuclear magnetic resonance spectroscopy (NMR) [14,15] play a particularly important role. In the studies of AAs in ASD, magnetic resonance spectroscopy (MRS) also plays an important role. MRS allows non-invasive *in vivo* evaluation of various chemical metabolites in tissues [16]. MRS can be applied using a single voxel technique or multiple voxels. Multiple-voxel MRS is also known as magnetic resonance spectroscopic imaging (MRSI) or chemical-shift imaging (CSI) [17]. Biochemical studies of the brain in ASD may be the first step in explaining the involvement of the limbic system and cerebellum in the expression of aggression by participants with ASD [18].

In this review, we present the current status of the analytical methods applied in the field of metabolic studies of AAs in ASD, and the role of these compounds as potential biomarkers for ASD.

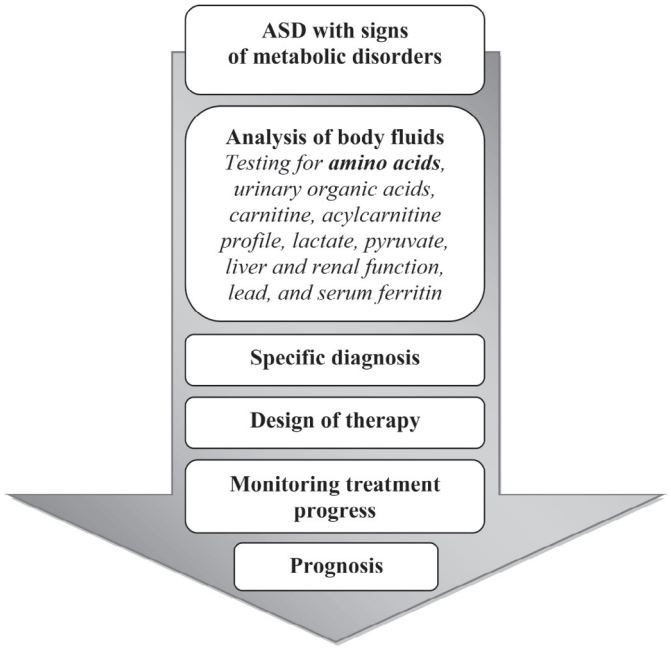

Fig. 1. Metabolic studies in diagnosis and therapy of comorbid disorders in autism spectrum disorder (ASD).

2. Analytical methods in the study of amino acids in ASD

Table 1 shows a summary of literature references that describe the applications of analytical methods for the study of AAs in ASD used in this work.

2.1. Biological material and sample preparation

Despite the invasiveness of the sampling, plasma is the most commonly used clinical specimen in the study of AAs in ASD. It provides important information on the health status of a child with ASD, including dietary intake and absorption, neurotransmitter-AA metabolism, gut dysfunction and oxidative stress. In the study of the levels of AAs in plasma, morning fasting specimens are preferred. This results from the reduced influence of diet under typical dietary patterns. Nevertheless, a plasma-free AA profile measured after overnight fasting can be affected after a high-protein meal in the evening [47]. However, the fasting period should be controlled because, if prolonged over 8 hours, it may lead to spurious elevations of plasma-AA concentrations [48]. Analysis of plasma is recommended when the concentration of AAs in blood cannot be deduced from urine results or, in the case of differential diagnosis, when the knowledge of plasma results is required. A major advantage of using urine in AA analysis is the possibility of collecting information on the set of end-products of metabolism and non-invasive sampling, which is especially beneficial and important in ASD.

Due to the high diurnal variation of urine production in clinical studies of urinary metabolite levels, the selection of the method of sampling is important. To assess the solute excretion, 24-h urine