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HPLC-HRMS AS A TOOL FOR AUTISM SPECTRUM DISORDER BIOMARKER SEARCH IN BLOOD SAMPLES

G. Ventura[1], C.D. Calvano[1,2], V. Porcelli[3], L. Palmieri[3,4], F. Palmisano[1,2],T.R.I. Cataldi[1,2]

[1]*Department of Chemistry, University of Bari Aldo Moro, Bari, Italy*
[2]*SMART Center, University of Bari Aldo Moro, Italy*
[3]*Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari Aldo Moro, Bari, Italy*
[4]*Institute of Biomembranes and Bioenergetics, CNR, Bari Italy*

It is recognized that dyslipidemia plays a role in neurodevelopmental syndromes including the so called Autism spectrum disorder (ASD), a broad and heterogeneous group of neurological developmental disorders classified according to Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Since ASD involves mainly problems with social skills, communication and repetitive pattern of behaviors to date, it is diagnosed by psychologists because of the absence of reliable chemical biomarkers [1]. The aim of this preliminary work is to search for putative biomarkers in blood easily collected in non-invasive way from young ASD patients. After blood treatment on a ficol gradient, blood mononucleates (lymphocytes and monocytes predominantly) are separated from plasma. So, the composition of lipid species in plasma and lymphocytes has been examined by using hydrophilic interaction liquid chromatography (HILIC) coupled with electrospray ionization and Fourier-transform mass spectrometry (ESI-FTMS) [2]. The study has been carried out on samples obtained from kids (age ranged between 3 and 16 years) affected by ASD with severity degree from 1 to 3 without any pharmacological treatment and from their unaffected brothers or sisters considered as healthy subjects.

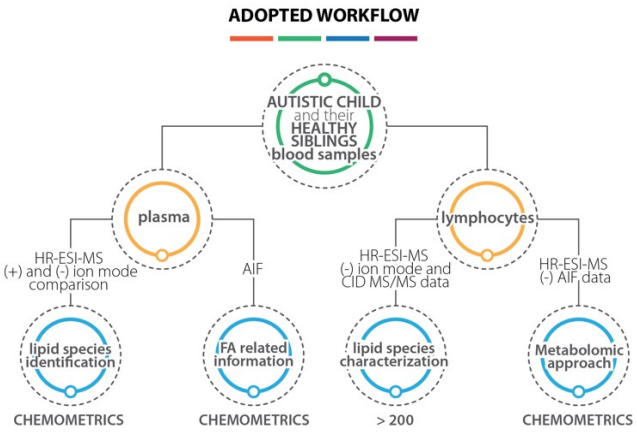

Figure 1. Adopted Workflow for lipid biomarker discovering in ASD patients

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The LC separation by HILIC column allowed phospholipids separation according to their polar head; in this way, lipid species from different classes having the same nominal mass, often almost co-eluent due to their side chains structural similarity in reversed phase chromatographic (RPC) separations, are well separated and non-ambiguous identification are possible by using high resolution mass spectrometry [3]. To manage the large amount of data obtained using this untargeted lipidomic approach (i.e. the comprehensive analysis of all the measurable lipids in a sample and lipid abundance inferred from the arbitrary intensity values usually normalized to each class), Alex ^{123} [4] software was employed.
For plasma samples, comparison between positive and negative ion mode spectra, together with orthogonal information provided by lipid class retention time, allowed a confident assignment of extracted lipids. The application in positive ion mode of the orbital-trap exclusive fragmentation process, called “all ion fragmentation” (AIF) where all ions are fragmented without precursor ion isolation allowed lipid class confirmation, while in negative ion mode information on fatty acid composition can be retrieved; so, phospholipids and fatty acids levels were compared among siblings using paired t-test.
In the case of lymphocytes, the regiochemical characterization was accomplished on more than 200 lipid species, including phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols and their lyso-forms, sphingomyelins and glycolipids. Despite metabolomic approach is quite common in biomarker discovery [5], there are very few examples in literature of this approach using AIF data [6]. Upon implementing a proper database, Alex^{123} software was exploited and chemometric examinations were applied for biomarker discovery. Here we demonstrated the potentiality of this combined data mining in highlighting small differences hidden with classical metabolomic approach, but which would be fundamental for biomarker discovery in so complex and still enigmatic pathology.

References

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