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Metabolic screening and metabolomics analysis in the Intellectual Developmental Disorders Mexico Study

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Abstract
Objective. Inborn errors of metabolism (IEM) are genetic conditions that are sometimes associated with intellectual developmental disorders (IDD). The aim of this study is to contribute to the metabolic characterization of IDD of unknown etiology in Mexico. Materials and methods. Metabolic screening using tandem mass spectrometry and fluorometry will be performed to rule out IEM. In addition, target metabolomic analysis will be done to characterize the metabolomic profile of patients with IDD. Conclusion. Identification of new metabolomic profiles associated with IDD of unknown etiology and comorbidities will contribute to the development of novel diagnostic and therapeutic schemes for the prevention and treatment of IDD in Mexico.

Keywords: Intellectual development disorders; screening; inborn errors metabolism; metabolomics

Resumen
Objetivo. Los errores innatos del metabolismo (EIM) son condiciones genéticas que pueden asociarse con trastornos del desarrollo intelectual (TDI). El objetivo de este estudio es contribuir a la caracterización metabólica de los pacientes con TDI de etiología desconocida. Material y métodos. Se realizará un tamiz metabólico mediante espectrometría de masas-tándem y fluorometría para descartar EIM; además, se analizará el perfil metabolómico de los pacientes con TDI. Conclusión. La identificación de perfiles metabolómicos asociados con los TDI de etiología desconocida contribuirá al desarrollo de nuevos esquemas diagnósticos y terapéuticos para la prevención y tratamiento de los TDI en México.

Keywords: trastornos del desarrollo intelectual; tamiz; errores innatos del metabolismo; metabolómica

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Inborn errors of metabolism (IEM) represent a group of about 500 rare genetic diseases, closely associated with intellectual developmental disorders (IDD), where the diversity of metabolic pathways involved explains the difficulties in making an accurate and early diagnosis.\textsuperscript{1,2} Basic biochemical procedures, including quantification of amino acids (AA) and acylcarnitines (AC), should be systematically performed whenever an IDD is suspected,\textsuperscript{3} to identify treatable congenital metabolic disorders (table I). Routine metabolic screening is especially important in countries like Mexico, where newborn screening (NBS) programs are still limited to only a few diseases\textsuperscript{4,5} and where IDD affect close to 2 million people under 18 years of age.\textsuperscript{6}

IEM are a complex group of monogenic disorders leading to the accumulation of toxic compounds, cellular

### Table 1

<table>
<thead>
<tr>
<th>IEM associated with IDD that could be detected through tandem-mass spectrometry metabolic screening</th>
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<tbody>
<tr>
<td>Disease name</td>
</tr>
<tr>
<td>Argininemia</td>
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<tr>
<td>Arginosuccinic acidemia</td>
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<tr>
<td>Defect of biotinidase biosynthesis</td>
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<tr>
<td>Defects of biotinidase regeneration</td>
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<tr>
<td>CPS I</td>
</tr>
<tr>
<td>Phenylketonuria</td>
</tr>
<tr>
<td>Citrullinemia type I</td>
</tr>
<tr>
<td>Citrullinemia type II</td>
</tr>
<tr>
<td>Homocystinuria</td>
</tr>
<tr>
<td>Hypermethioninemia</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
</tr>
<tr>
<td>Orotic aciduria</td>
</tr>
<tr>
<td>Tyrosinemia type I</td>
</tr>
<tr>
<td>Ketohyoasparagine deficiency</td>
</tr>
<tr>
<td>Ethylmalonic encephalopathy</td>
</tr>
<tr>
<td>Glutaric acidemia type I</td>
</tr>
<tr>
<td>Glutaric acidemia type II</td>
</tr>
<tr>
<td>3-Hydroxy-3-methylglutaric aciduria</td>
</tr>
<tr>
<td>Isovaleric aciduria</td>
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<tr>
<td>Isovaleric acidemia</td>
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<tr>
<td>Methylmalonic acidemia</td>
</tr>
<tr>
<td>Methylmalonic acidemia &amp; hypermethioninemia</td>
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<tr>
<td>Multiple carboxylase deficiency</td>
</tr>
<tr>
<td>Propionic acidemia</td>
</tr>
<tr>
<td>(\beta)-fatty acids oxidation defect</td>
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<td>(\beta)-fatty acids oxidation defect</td>
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</table>

Source: references 5, 7 and 9
energy deficiency, or a lack of the substrates necessary for important biochemical processes. Although individually rare, collectively IEM represent the causes of devastating disturbances of the developing nervous system, including brain formation abnormalities and mild to severe mental disability. Opportune detection of IEM is essential because specific treatments may be available, metabolic decompensation could be avoided, and accurate genetic counseling can be provided, thereby offering the possibility of preventing the effects of IEM on brain development and function or reverting to some extent the consequences on mental health.

Metabolic screening comprises biochemical testing of blood, urine, or cerebrospinal fluid samples, to be used in the diagnosis of an IEM. In developed countries, the detection of IEM has been focused on newborns, so as to obtain the earliest diagnosis possible and receive prompt treatment. However, some studies highlight the importance of IEM detection in adults with IDD. NBS was first applied to massive detection of phenylketonuria, an IEM of amino acids. Initially, screening was done using a simple bacterial inhibition assay, but over time, technological advances have enabled the detection of many other metabolic disorders. Tandem mass spectrometry (MS/MS) is a powerful analytical tool that can be applied to metabolic screening both in neonates and in people of other ages. MS/MS methodology analyzes biological samples for both amino acids and acylcarnitines, among other metabolites. In a systematic literature review update in 2013, 89 treatable IEM that cause IDD have been identified, many of which can be detected by MS/MS (table I). Worldwide, there are important variations in the number and type of disorders detected through NBS. Congenital hypothyroidism is the most frequently screened disease, but mandatory screening for IEM only exists in developed countries. In Mexico, the NBS disease panel has also varied. Currently, the Ministry of Health only includes five diseases in the screening program: congenital hypothyroidism, congenital adrenal hyperplasia, cystic fibrosis, phenylketonuria, and galactosemia. Therefore, the number of IEM screened remains very low, which results in the high likelihood to find undiagnosed patients between the IDD Mexican population.

Recently, metabolomics has been used for the study of pediatric neurologic and psychiatric conditions such as Down syndrome, schizophrenia, and autism. Metabolomics could be used to identify unreported metabolic alterations associated with IDD. Metabolomics refers to the comprehensive measurement of small molecules, typically <1500 daltons (e.g., sugars, amino acids, organic acids, nucleotides, acylcarnitines, and lipids), called metabolites, which are present in biological samples. Metabolomics is a potent tool for the study of human metabolism in health and disease. Comparative statistical analysis can reveal perturbations of metabolite levels in disease conditions and thus has the potential to identify novel biomarkers for diagnosis, prognosis, and treatment response. Metabolomics is complementary to genomics, transcriptomics, and proteomics. Metabolites represent the end products of the genome and proteome and can therefore be helpful in providing a holistic physiologic phenotype of a system or metabolic pathway. Metabolome profiling can be useful in disease heterogeneity for evaluating the underlying biological state of individuals through assessment of metabolite levels, thus providing a better understanding of disease mechanisms.

To identify novel metabolic diseases associated with IDD of unknown etiology and comorbidities, the objective of this study is to carry out an extensive metabolic analysis, including standard metabolic screening and metabolomics analysis, as part of the Intellectual Developmental Disorders Mexico Study (IDD Mexico Study).

Materials and methods

This methodology paper is part of the IDD Mexico Study, which has been reviewed and approved by the Ethics in Research Committee of the National Institute of Public Health, with number CI 1456. The diagnostic algorithm shown in figure 1 will be followed to identify individuals with IDD of unknown etiology and comorbidities so as to conduct the metabolic screening and metabolomics analysis described herein, as well as the complementary genomics characterization previously presented.

Metabolic screening test

After clinical and clinical genetic diagnoses (first and second selection filters previously discussed by Lazcano and colleagues), metabolic screening (third selection filter) will be done at the Laboratory of Inborn Errors of Metabolism and Screening of the National Institute of Pediatrics, as follows:

1. Quantification of AA, AC, and succinylacetone (SA). Dried blood spots (DBS) will be analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS), using a Quattro micro API tandem MS with a commercial kit (NeoBase Non-derivatized MSMS Kit; PerkinElmer, Waltham, MA, USA). Quantification of the metabolites is achieved using appropriate internal standards as reference. This method has
**Figure 1. Diagnostic algorithm for the evaluation of subjects with IDD of unknown etiology**

### Study population
- Recruitment in hospital, reference center and school
- Primary care
- Clinical referral (pediatrics, psychology, neurology, psychiatry, others)
- Infant care professionals

### Clinical diagnosis
**First selection filter**
- Clinical evaluation
  - Medical history
  - Psychiatric clinical diagnosis
  - Formal diagnostic assessment
  -Neurologic examination
- Excluded from the study:
  - Cases without confirmed diagnosis or do not meet inclusion criteria
- Continuation of pre-existing clinical regime

### Clinical genetic diagnosis
**Second selection filter**
- Medical genetic assessment
  - Clinical/dysmorphological examination
  - Family history of IDD, ASD, ADHD
- Excluded from the study:
  - Cases with suspected syndromic types
  - Cases with familial aggregation

### Metabolic screening
**Third selection filter**
- Metabolic screening test
  - CGG repeats (fragile X)
  - Excluded from the study:
    - Cases with specific genetic diagnosis (fragile X syndrome)

### Fragile X screening
**Fourth selection filter**
- CGG repeats (fragile X)
- Exome analysis
- Excluded from the study:
  - Cases with specific genetic diagnosis (fragile X syndrome)

### Genomic and metabolomic analysis
- (Father-mother-child triad)
- (Child) Exome analysis
- (Child) Metabolomic analysis

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* Recruiting and evaluation of triads with children and adolescents 6-15 years of age will be carried out at the Dr. Juan N. Navarro Children’s Psychiatric Hospital (HPIDJNN) in Mexico City and recruitment and evaluation of triads of people over 18 years old will be done at the Integral training and development center (CADI A.C.) in Mexico

† Formal diagnostic evaluation will be done through application of tools described in the section on clinical diagnosis

‡ Metabolic screening will be done at National Institute of Pediatrics (INP) in Mexico City. Children and adolescents with IDD or autism spectrum disorders will be evaluated, to exclude cases with inborn metabolism errors.


Algorithm modified from reference 6


