Overexpressions of Fatty Acid-Related Genes During Neurodevelopment in a FASD Model

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Abstract

Fetal Alcohol Spectrum Disorders (FASD), caused by prenatal alcohol exposure (PAE), is characterized by congenital central nervous system dysfunction resulting in impaired learning and motor skill deficits. FASD affects 2-5% of children in the U.S., with similar or higher rates reported worldwide.1 It is estimated that for every one child diagnosed with neurological and physical symptoms of FASD, three other children are born with only the neurological consequences of the condition.2 Moreover, fatty acids are critical for regulating neuronal structure and function, and interferences in fatty acid metabolism are associated with neurodevelopmental disorders like autism and ADHD. Changes of fatty acid contents suggestively serve as peripheral biomarkers of FASD. In this presentation, we will discuss how the expressions of fatty acid-related genes are altered due to the effects of PAE in the brain.

Introduction

Previous research identified that moderate expression of Heat Shock Factor 1 (HSF1), a transcription factor of stress signaling, plays an important role in protecting developing brains against environmental insults, including alcohol.1 Using a mouse model that recapitulated the normal conditions of a human patient, it was found that knockout HSF1 mice showed more severe structural abnormalities in the brain and increased risk for developing neuropsychiatric symptoms.4

Subsequent single-cell RNA-sequencing in cortex of PAE mice showed variable gene expressions of individual neurons that persisted throughout life. Markedly, high expressions of fatty acid elongase 4 (ELOVL4) and fatty acid synthase (FASN), analogous genes involved in fatty acid biosynthesis, were observed in a specific neuronal population in the PAE mouse cortex. FASN encodes a multi-enzyme protein involved in synthesis of palmitate into long-chain saturated fatty acids, and ELOVL4 encodes a membrane-bound protein involved in elongation of very long chain saturated and polyunsaturated fatty acids in the brain.3,5 However, functions of fatty acids synthesized by ELOVL4 and FASN remain unknown, and pathological mechanisms due to disturbed fatty acid contents in the brain are elusive.

Our working hypothesis is that epigenetic influences introduced by environmental stress, such as alcohol, are associated with an increase of these fatty acid-related gene expressions in the cerebral cortex that are involved in the pathophysiology of FASD.

Methods

Experimental Timelines for FASD Mouse Model:

<table>
<thead>
<tr>
<th>Event</th>
<th>Time</th>
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<tbody>
<tr>
<td>In Utero Electroporation</td>
<td>E15</td>
</tr>
<tr>
<td>PBS/EIOH</td>
<td>E16-17</td>
</tr>
<tr>
<td>Collection</td>
<td>P30</td>
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<td>Birth/P0</td>
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Results

- The single immunohistochemistry (IHC) staining schematic was performed to measure ELOVL4 expression in non-electroporated control vs PAE brain tissue.
- The triple IHC staining schematic was performed to measure co-localization of ELOVL4 and HSF1 expression in control and PAE brain tissue previously in utero electroporated at E14 with the pHS-E-RFP plasmid; enables detection of HSF1 expression under cellular induced stress (i.e. by alcohol).7 (Below image).

Conclusion and Discussion

- Upregulation of fetal ELOVL4 expression in the PAE group as compared to the control in the primary motor cortex layer III/III.
- ELOVL4 expression is highly increased in stressed positive cells, meaning that the cells that show robust activation of Heat Shock signaling in response to PAE may show significant disturbances in fatty acid biogenesis.
- Increase of ELOVL4 mRNA defined in single cell RNA seq was further confirmed at protein level.
- The data reflects preliminary findings, and goals of longitudinal research include continuing IHC experiments, as well as determining if behavioral data (i.e. learning and motor deficits) corresponds to the level of ELOVL4 increase.
- Current research shows promising insights on potential genes of interest related to neurological effects of children afflicted by FASD. This model serves as a platform for further modification and research by others to address related health concerns (i.e. cognitive learning impairment due to substance abuse, old age, genetic congenital disabilities).

References and Acknowledgements


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