Epigenetic Effects of Developmental Alcohol: A Pilot Study

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Abstract

One of the most pervasive effects of developmental alcohol exposure in children includes impaired memory abilities that do not seem to disappear with age. Previous studies have used both human and animal models to examine these effects at the behavioral, anatomical, functional and genetic levels, but very few have examined developmental alcohol's effect at the epigenetic level. For example, previous research in adolescent rats show a significant effect of neonatal alcohol exposure, equivalent to the 3rd trimester of human pregnancy, on DNA methylation in the hippocampus and prefrontal cortex (Otero et al., 2012). The current research focused on further investigating this effect on gene expression in the hippocampus of rats exposed to 4.5g/kg alcohol on neonatal days 4 through 6. Rat subjects were grouped by gender (male and female), age (adolescent and older adults) and treatment (prenatal alcohol exposure and no prenatal alcohol exposure). The hippocampi were excised from each specimen and processed to observe level of DNA methylation according to manufactures' protocols for DNA isolation, digestion and methylation measurements. After the process of DNA isolation multiple methods were utilized to determine the successful isolation of DNA, however each of the techniques showed little to no usable amount of DNA in the sample and therefore did not allow the research to progress any further. Possible reasons for failure to isolate DNA include contamination, destruction of DNA due to initial method of fixation or user error. Further research will serve to address these limitations regarding appropriate fixation methods and proper storage of brain tissue suitable for efficient DNA isolation.

Keywords

Epigenetics

DNA Methylation

Fetal Alcohol Syndrome

Gene Expression

Brain Development

Introduction

Fetal alcohol spectrum disorder is a condition wherein a developing fetus is exposed to alcohol which then presents physiological and psychological abnormalities in the child once born. While it is not always a debilitating condition, it has a lifelong consequence on the affected children. Currently, most research into the disorder has focused on the physiological changes that show clearly in the most extreme cases as well as basic psychological evaluations such as behavior observance and intelligence questioning. Only within the past few decades has research in this field changed from observing the effects of prenatal alcohol exposure to attempts at discovering the mechanisms behind the condition. This has led to a great deal of more information about the actual effects of the exposure on the brain, such as clear changes in the integrity of the brain and its substructures. Furthermore, some of this research has uncovered a few treatments for FASD children, however they are not significantly effective and their impact does not cause long term changes. It is therefore vital that FASD research look at the deepest possible levels of brain structure, gene expression, to find a basic causal effect of developmental alcohol.

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The original research into what is now known as Fetal Alcohol Spectrum Disorder was based upon obvious phenotypical differences in children that had been exposed to alcohol during their development within the womb (Jones & Smith, 1973). Much of this research noted common aberrations among such children including webbed skin around the eyes, lack of impression above the lip, and many other facial irregularities. The condition was first labeled and more commonly known to the public as Fetal Alcohol Syndrome. While the initial research did note changes in mental faculties in the affected children, it did conduct rigorous testing to determine the severity of the change. Within a few years of classifying the disorder, more research looked into the mental impact of the alcohol exposure with various tests and measurements. Such research noticed significant changes in typical behavior and intellectual capabilities in FAS affected children (Landesman-Dwyer, 1978; Abel, 1979). The most prominent number gained from such literature is that children diagnosed with Fetal Alcohol Syndrome had a significantly lower I.Q. and distinct difficulties with memory.

At the turn of the century, the research into Fetal Alcohol Syndrome changed dramatically. This change is marked by the reclassification of Fetal Alcohol Syndrome to the condition Fetal Alcohol Spectrum Disorder (Streissguth & O'Malley, 2000). The reason for this reclassification came from research showing not all children exposed to alcohol during prenatal development had the same dramatic symptoms used to diagnose in the past. Data shows that despite the abnormalities to facial structure, almost all alcohol exposed children showed deficits in the cognitive abilities examined in past studies. The nature of the research went a different course as well during this time. Most of the scientific inquiry assessed the brains of alcohol exposed subjects and resulted in important information. The brains of FASD subjects, both

animal and human, showed clear differences at a topographical level (Archibald et al., 2001). Such information directed the field to examine the brain even more in-depth and uncovered more culprits for the condition. Chief among the issues of learning and memory is changes in the size and activity within the hippocampus substructure of the brain (Willoughby et al., 2008). The hippocampus is a brain structure directly tied to learning and memory capacities, which are the two reported measures that differ in FASD children.

The most current studies have attempted to look even farther into the brain and have based their measurements off of gene expression. Gene expression is a measure of how active genes in a DNA sequence are. Using rodent models of FASD, contemporary studies have produced data showing a significant decrease in gene expression due to an inhibition caused by alcohol (Garro et al., 1991; Perkins et al., 2013). A reduction in gene expression is also linked to the natural decline in mental capabilities due to aging and is markedly apparent in the hippocampus (Liu et al., 2009). While these investigations are promising to the field of FASD research, they have had conflicting information and have not all focused on substructures of the brain tied to the effects of FASD. One study met both requirements and looked into a purported alleviant of the condition and resulted in information that is decisive for the sake of the discussion (Otero et al., 2012). This research showed significant changes between the exposed groups and found an interesting interaction between alcohol exposure and the supposed treatment for the condition on gene expression.

Rationale

Previous studies suggest that prenatal exposure to alcohol can have an effect on genomic DNA. A basic condition for the FASD diagnosis is deficits in learning and memory, the hippocampus is the related brain structure for these functions. If there is a significant difference in the amount of genetic activation measured by gDNA methylation between subjects administered alcohol and subjects left unexposed, we can infer that prenatal alcohol exposure has an effect on the genomic DNA and development of the hippocampus. Such findings can lead to promising and more permanent treatment options to combat developmental alcohol's effect on the cognitive abilities of exposed children.

Methods

This study utilized 72 Sprague-Dawley rat brains provided by the University of Arkansas Medical School. These samples were used in separate alcohol exposure research which did not require the entirety of the brain, leaving us with everything besides the cerebellum and olfactory bulbs. Half of the rat subjects were exposed to 4.5g/kg of ethanol on neonatal days 4 through 6. The rest of the subjects were exposed to a saline solution during the same period and served as controls. Developmentally, this postnatal period is equivalent to the third trimester of human fetal development. This amount of exposure leads to a blood alcohol content of 0.3 to 0.4 grams per deciliter, which is comparable to binge drinking in a human. The subjects were appropriately proportioned between male and female as well as age at which the brains were collected, 25 days or 150 days. Upon receiving the brains, all were inspected for any possible issues with their integrity or quality. Those that were not suitable for the research were noted and not utilized. The hippocampi of the brains were extracted according to common techniques for removal, and were placed back in the fixative solution until the process of DNA isolation could be conducted.

An initial batch of ten subject hippocampi were ran through the Promega ReliaPrep™ FFPE gDNA Miniprep System according to protocol. These were used to determine if the process was successful by measuring the presence of gDNA through a spectrophotometer. The measurements from this group were not promising, and as such continued to process the rest of the brains. Upon retrieval of the remaining hippocampi from their samples, some form of slime like mold had grown around some of the samples, indicative of possible contamination or improper storage. Subjects that were affected were set aside and not used for the second batch, which contained 34 usable samples. After the second batch was processed according to the same protocol, we utilized a gel electrophoresis test as a method to visibly determine the presence of

gDNA. After this process provided inconclusive results, the samples were run through the Promega Wizard® SV Gel and PCR Clean-Up System as an attempt to purify and concentrate any present gDNA in the sample solutions. After this, the samples were examined again by a spectrophotometer to provide conclusive proof the presence of gDNA. Were the samples to contain a useable concentration of gDNA, they would be processed via the Promega DNA Methylation Analyses Kit which provides a measure for gene expression. This measurement would be quantified through the use of a GENios microplate reader.

Results

The gel electrophoresis test ran before the purification attempt also did not show clear signs of gDNA present in the solutions. After the measure of the second batch in the spectrophotometer, it was determined that none of the samples contained a significant amount of gDNA to continue the research. It was not possible to run the DNA Methylation Analysis Kit and therefore no substantive measurements could be obtained regarding a difference in gene expression between the two treatment groups of the rat subjects.

Discussion

A number of possible reasons for failure of gDNA isolation have been determined after the results of our test proved inconclusive. Due to the presence of degraded and rotten samples upon initial inspection, it is highly likely that some issue took place before we obtained the brains. In this respect, it could be due to the age of the brains, as some had been removed and stored for years, as well as the fixative solution being too diluted to properly preserve the specimens. In review of possible culprits, some literature has suggested that DNA left in a fixative solution at room temperature could very well breakdown and become unusable (Koshiba et.al, 1993). It is possible that between the removal of the hippocampi from the brain and the process of DNA isolation that the gDNA has degraded to the point of being undetectable. The appearance of mold in the sample vials after the extraction and replacement of the hippocampi points to an issue of contamination that could have interfered with attempted measurements. Of course, amongst all this is the ever present possibility of user error which went unnoticed and resulted in a failure to isolate the gDNA.

The research was ultimately hindered by certain other aspects. We could only utilized the samples we were provided, which became a problem when the mold left many unusable. We also were not utilizing facilities that our research team had full admittance to, which required us to work within the times of the faculty who had access. This caused some issues when inclement weather shut down the university on days which could have been used to work. Also worth mentioning is that some of the equipment utilized was not what was required by the protocol information. Despite the lack of results here, the manner of the research is a worthwhile study that could very well lead to a solution to the issue of FASD. A nutrient found in some foods called Choline has showed promising application to alleviate the impact prenatal alcohol

exposure has on behavior and memory. Future research could examine the genetic sequences that are affected by this exposure, which could then lead on to a method for undoing the changes.

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