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Article

Transcriptomic profiling identifies differential gene expression associated with childhood abuse

Darwin Li[1], Lynda Yearwood[2]

[1] Upper Canada College, Toronto, Ontario, Canada

[2] Design Department, Upper Canada College, Toronto, Ontario, Canada

SUMMARY

Childhood abuse is a pervasive global issue that has profound and lasting impacts on the psychological, physiological, and molecular levels of affected individuals. Here, we attempted to identify differentially expressed genes in individuals with a history of childhood abuse and determine enriched biological pathways from those genes that may explain the lasting physiological effects of childhood abuse. We hypothesized that gene groups associated with neuronal plasticity, stress response, and mood regulation would be upregulated in individuals with a history of childhood abuse. We analyzed an existing dataset reporting RNA-sequencing data from victims of childhood abuse (N=24) and healthy controls (N=21). We conducted a differential expression analysis between these two cohorts. Using the results, we entered the top 250 upregulated and downregulated genes into the STRING database to identify gene networks and relationships. We found that oxidative phosphorylation and ribosomal pathways were significantly upregulated, consisting of 7 and 6 genes, respectively. With applications in the field of personalized medicine, this study can guide therapeutic interventions and optimize treatment based on individual gene profiles.

INTRODUCTION

Childhood abuse is a collective term that refers to any form of physical, emotional, and sexual mistreatment or neglect that causes injury or emotional damage to a child (1). With detrimental effects ranging from brain impairment to severe injuries, a history of childhood abuse has great implications on healthy development and psychological functioning (2). Childhood abuse is also a primary factor for developing post-traumatic stress disorder (PTSD), which may occur as a result of a singular traumatic event, and Complex-PTSD, which is generally due to a series of events (3). A 1999 study found over 30% of children who suffered from childhood abuse met the DSM-III-R criteria for lifetime PTSD (1). With the World Health Organization estimating that up to a billion children aged 2–17 years have experienced childhood abuse in 2022, and with factors such as the COVID-19 pandemic exacerbating this issue, it is increasingly important to develop an effective and efficient process of diagnosing and treating individuals with a history of childhood abuse (2).

Previous studies have demonstrated the complexity of the pathophysiology associated with a history of childhood abuse (4, 5). There was great focus placed on analyzing the relationship between childhood abuse and DNA methylation, with results revealing greater methylation of *NR3C1* in adults exposed to childhood abuse and hypermethylation of *CRH* in maltreated Tanzanian children (6). The latter, hypermethylation, commonly leads to decreased gene expression. These genes help regulate stress response and their epigenetic changes suggest a possible mechanism for the effect of childhood abuse on PTSD and other disorders (7). Further, epigenetic changes may be intergenerational, as childhood trauma results in alterations in methylation patterns in human sperm (7). Current research has revealed alterations to the epigenome of prefrontal pyramidal neurons in victims of childhood abuse and early-life adversity (ELA) (8, 9). However, there is a lack of additional studies correlating gene expression with childhood abuse. In addition, this study will further analyze prefrontal pyramidal neurons using transcriptomic profiling. As a key component of the prefrontal cortex, pyramidal neurons are known for their critical role in complex cognitive behavior, personality expression, and decision-making (10, 11). Unraveling the transcriptomic signatures behind childhood abuse could greatly enhance our understanding of its biological sequelae, facilitating the development of objective diagnostic tools and paving the way for innovative, targeted therapeutic strategies. This endeavor holds the potential to alter treatments to long-term impacts of childhood abuse, ultimately enabling more effective interventions that could help victims reclaim their lives and thrive despite their traumatic pasts.

Considering the extensive neurobiology and mental health effects of childhood abuse, we hypothesized that gene groups associated with neuronal plasticity, stress response, and mood regulation would be upregulated in individuals with a history of childhood abuse. These genes are responsible for responding to environmental factors, such as chronic stress and other impacts that come with childhood abuse. Their upregulation may be considered a coping mechanism to maintain cellular homeostasis and energy balance under stress.

In order to find differentially expressed genes, we conducted a differential expression analysis using a dataset reporting read counts of individuals with a history of childhood abuse and healthy controls. We input the significant genes into the STRING database for protein interaction and pathway enrichment analysis. Through this process, we determined that the NDU and RPL gene groups, correlating to the oxidative phosphorylation (OXPHOS) and ribosomal pathways, were upregulated in individuals with a history of childhood abuse. While their roles of energy production and protein synthesis are not directly related to neuronal plasticity, stress response, or mood regulation, the OXPHOS and ribosomal

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pathways are responsible for nearly all cellular functions. Their upregulation greatly suggests a potential adaptation mechanism in response to environmental adversity.

RESULTS

We input dataset GSE157197, which reported read counts of transcriptomic alterations to prefrontal deep-layer pyramidal neurons in individuals with a history of childhood abuse compared to controls consisting of individuals who died with no psychiatric history, into GEO2R, a tool used to conduct a differential expression analysis (12). This identified the top 250 upregulated and downregulated genes in individuals with a history of childhood abuse by adjusted (adj.) p-value. Upregulation and downregulation were determined from the log2 fold change value (Log2FC), where positive and negative values indicate upregulation and downregulation, respectively. In addition, the Log2FC value quantifies the magnitude of a gene's expression change between cases and controls. The Log2FC values of downregulated genes ranged from -2.145 to -0.724 while Log2FC values of upregulated genes ranged from 0.436 to 2.303.

We plotted genes with significant adj. p-values (Padj < 0.05) (**Figure 1**). The genes with greatest Log2FC all have adj. p-values of 0.0129 and are classified as most significant in terms of differential gene expression between cases and controls. However, no notable relationships between these genes were identified. Specifically, there were no gene clusters, neighborhoods, or families consisting of four or more genes and relating to the same pathway. As a result, we loosened the threshold and investigated the top 250 downregulated genes (Padj < 0.1648).

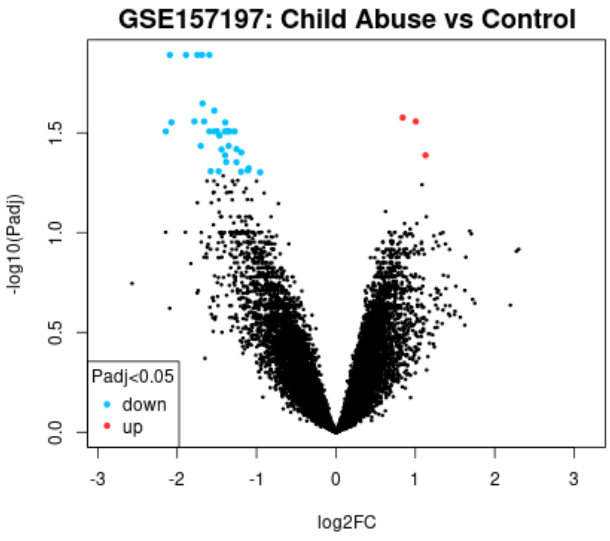

Figure 1: Differentially expressed genes between individuals with a history of childhood abuse and healthy controls, Padj < 0.05. This volcano plot is a result of a differential expression analysis conducted using GEO2R and displays differentially expressed genes in individuals with a history of childhood abuse compared controls consisting of individuals with no psychiatric history. Each dot represents a different gene. Genes colored red are upregulated in individuals with a history of childhood abuse, while genes colored blue are downregulated. Genes colored black are statistically insignificant (Padj >= 0.05).

Pathway ID	Description	Count in network	P-value
hsa00190	Oxidative phosphorylation	7	0.0075
hsa05012	Parkinson's disease	9	0.0075
hsa05020	Prion disease	9	0.0097
hsa05016	Huntington's disease	9	0.0182
hsa03010	Ribosome	6	0.0197
hsa04932	Non-alcoholic fatty liver disease	6	0.0326

Table 1: Top 6 most significantly upregulated KEGG pathways from STRING database. The first column indicates KEGG pathway IDs for identification and reference purposes. The third column refers to the number of genes belonging to that pathway.

We employed the STRING database, which reports interactions amongst proteins, to investigate connections between the protein products of the genes we identified (13). To begin, we entered the top 250 downregulated genes into STRING for further analysis (**Figure 2**). We observed a small gene group near the bottom involved in RNA processing, consisting of *DHX34*, *SRRM3*, and *U2AF2*. The green edges show that this is a gene neighborhood and the pink edges mean that these connections were experimentally determined. However, being only 3 genes with low Log2FC values (|Log2FC| < 0.817), we deemed this unworthy of further analysis. As a result, gene interactions observed between the top 250 downregulated genes were not further analyzed.

Next, we entered the top 250 upregulated genes (Padj < 0.1648) into STRING (**Figure 3**). We found two important clusters and gene groups. We identified the nodes in red as part of the L (RPL) and S (RPS) ribosomal protein groups in addition to two mitochondrial ribosomal proteins (MRP). The nodes in blue, on the other hand, are Complex I NADH:dehydrogenase [ubiquinone] proteins (NDU), as identified by STRING.

In order to analyze the biological pathways associated with these genes, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) (14). According to STRING, the most significant KEGG pathways were OXPHOS, Parkinson's disease, Prion disease, Huntington's disease, ribosome, and non-alcoholic fatty liver disease (**Table 1**). The pathways selected for further discussion are the OXPHOS (ID: 00190) and ribosomal (ID: 03010) pathways, containing 7 and 6 upregulated genes, respectively. This selection was made considering each pathway's p-value and whether belonging genes were connected by STRING. It follows that the remaining pathways consist of disjoint genes with no profound relationships (no evidence of common connections determined by STRING, such as belonging to the same gene neighborhood or family). Therefore, the genes part of the oxidative phosphorylation and ribosomal pathways are of interest in this study as potential subjects of gene expression changes due to the lasting physiological effects of childhood abuse. While the OXPHOS and ribosomal pathways are not directly linked to neuronal plasticity, stress response, and mood regulation, they are responsible for energy production and protein synthesis. Since these make up the basis of all cellular functions, it is important to determine whether their

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upregulation can be specifically correlated to childhood abuse.

DISCUSSION

The results have demonstrated the upregulation of the OXPHOS and ribosomal pathway genes in individuals with a history of childhood abuse. This demonstrates the potential of research attempting to unveil differential gene expression associated with the lasting physiological effects of childhood abuse.

In this study, the most significantly upregulated pathway was OXPHOS (Padj = 0.0075), containing 7 differentially expressed genes. We confirmed all, except for *ATP5J*, to be accessory subunits of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I) with roles essential for its assembly and stability (15). Complex I is an integral component of the mitochondrial electron transport chain, which is responsible for the transfer of electrons from NADH to the respiratory chain. This transfer of electrons is a key step in the production of adenosine triphosphate (ATP), the cell's primary energy currency. In individuals with a history of childhood abuse, the upregulation of OXPHOS may suggest an altered metabolic state. This altered metabolic state could reflect a compensatory mechanism where the cells are producing more of these subunits to maintain energy balance under stress, possibly due to increased reactive oxygen species (ROS) production and inflammation commonly associated with stress and trauma. In cases of acute and chronic DNA damage, increased OXPHOS was found to be a beneficial adaptive response, showing a link between genotoxic stress and energy metabolism (16). Similarly, upregulation of OXPHOS as a result of a history of childhood abuse can trigger a cellular stress response that leads to alterations in the energy metabolism of cells (17). This could be to sustain ATP production and cellular energy levels during periods of increased stress. Additionally, increased oxidative stress, a well-established cause of DNA damage, can also result from chronic psychological stress, such as that experienced by survivors of child abuse (18). The upregulation of OXPHOS in response to stress provides the necessary energy to repair damaged molecules and maintain ionic balances disrupted by damage to membranes (17). However, chronic upregulation could potentially lead to imbalances in mitochondrial function and contribute to the pathophysiology of various health conditions, including neurodegenerative diseases, cardiovascular diseases, and metabolic disorders (19).

The second pathway of significance is the ribosomal pathway (Padj = 0.0197), containing 6 differentially expressed genes that encode for large (RPL), small (RPS), and mitochondrial (MRP) ribosomal proteins. The ribosomal pathway is responsible for the assembly of proteins and the translation of mRNA.. Given the central role of the ribosomal pathway, any dysfunction can have profound cellular and systemic implications. Among its impacts, ribosomal dysfunction affects serotonergic activity in the brain (20). This may lead to psychiatric disorders, such as PTSD, which frequently develop following an incident of childhood abuse (20). In addition, ribosome dysfunction is an early indicator of Alzheimer's Disease (21). Autopsies have presented impairments in protein synthesis within Alzheimer's Disease subjects (22). This is relevant in individuals with a history of childhood abuse as these traumatic events may leave chronic wounds, including a decline in executive function that exacerbates the development of neurological disorders (23).

Furthermore, the ribosomal pathway also has a critical role in cellular resilience or vulnerability mechanisms. In response to traumatic experiences such as incidents of childhood abuse, cells rely on stress response pathways to maintain cellular homeostasis and mitigate the adverse effects of harmful environmental stimuli (23). Among response pathways, the ribotoxic stress response (RSR) is activated when ribosomes encounter certain stressors or toxins. This response involves signaling pathways that can lead to various cellular outcomes, including the activation of stress-related kinases, inflammatory signaling, and in severe cases, cell death (21). Additionally, in individuals with a long history of traumatic experiences, consistent activation of the RSR may leave a molecular signature in cells (25). Further research is needed to clarify the influence of the RSR on gene expression and its implication in psychiatric disorders.

In current literature, the simultaneous enrichment of the ribosomal and OXPHOS pathways is associated with the development of cognitive and psychiatric disorders (18, 26). The relationship between these two pathways is symbiotic. The OXPHOS pathway requires proteins for its function, which are

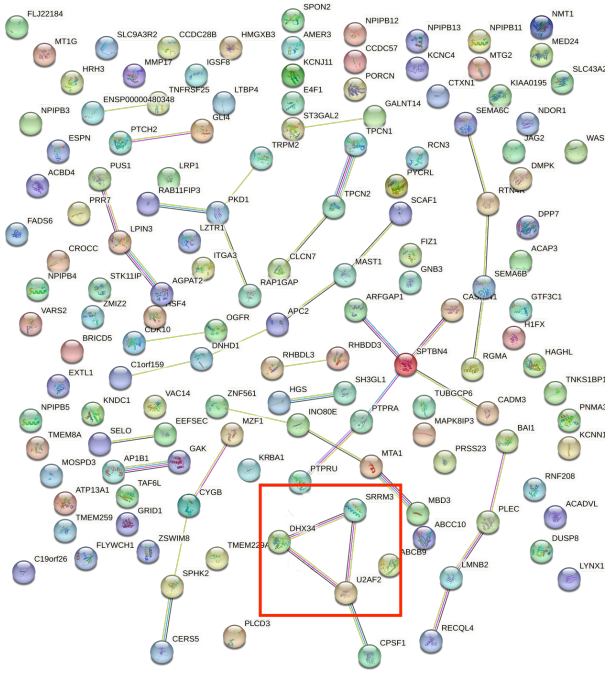

Figure 2: Gene interaction network of top 250 downregulated genes in individuals with a history of childhood abuse in STRING database. Each node represents a gene while edges represent correlations between genes. Light blue and pink connections show known interactions established by curated databases and experiments, respectively. Green, red, and blue connections show predicted interactions established by gene neighborhoods, fusions, and co-occurrence, respectively. Yellow, black, and purple connections show interactions established by text mining, co-expression, and protein homology, respectively. The gene group consisting of *DHX34*, *SRRM3*, and *U2AF2* are highlighted in the red box.

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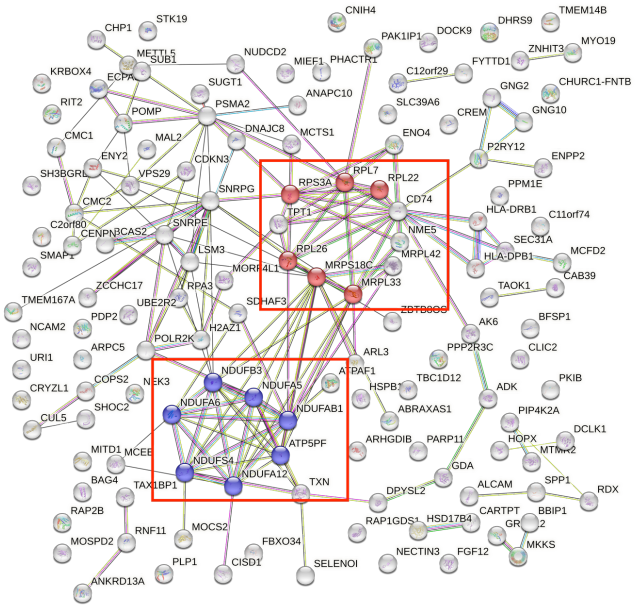

Figure 3: Gene interaction network of top 250 upregulated genes in individuals with a history of childhood abuse in STRING database. Each node represents a gene while edges represent correlations between genes. Light blue and pink connections show known interactions established by curated databases and experiments, respectively. Green, red, and blue connections show predicted interactions established by gene neighborhoods, fusions, and co-occurrence, respectively. Yellow, black, and purple connections show interactions established by text mining, co-expression, and protein homology, respectively. Red nodes represent genes part of the ribosomal pathway and blue nodes represent genes part of the oxidative phosphorylation pathway. Genes corresponding to ribosomal and oxidative phosphorylation are highlighted in the red boxes.

synthesized by ribosomes. Conversely, the energy required for ribosomal activities, including protein synthesis, is supplied by ATP produced through oxidative phosphorylation. This mutual dependency ensures that cells maintain a balance between energy production and consumption, especially during times of stress. In this way, stress and trauma caused by childhood abuse could lead to the upregulation of both the ribosomal and OXPHOS pathways. Additionally, chronic stress, such as that experienced by individuals with a history of childhood abuse, can lead to oxidative stress, which damages cellular components, including DNA (27). To cope with this damage, cell energy production, via OXPHOS, and protein repair mechanisms, via the ribosomal pathway, are increased. Given the central roles of the OXPHOS and ribosomal pathways in energy metabolism and cellular function, their upregulation could reflect the specific individual's adaptive response to chronic stress and trauma.

One of the major limitations of this study revolves around its exclusive focus on prefrontal deep-layer pyramidal neurons. These neurons, while pivotal in cognitive functions, represent a subset of the diverse neuronal and glial cells in the brain. Childhood abuse, a complex and multifaceted form of trauma, is likely to exert widespread effects across various brain regions and cells. By concentrating solely on deep-layer pyramidal neurons, crucial transcriptomic changes occurring in other areas, which could be equally or even more relevant to the long-term effects of abuse, may be overlooked. Moreover, the interconnected nature of neural circuits means that alterations in one cell type could influence, or be influenced by, changes in other cells. Thus, future studies will be needed to investigate gene expression changes in other cell types in the brain. An additional limitation of this study arises from a lack of control of critical factors that may impact the results, such as age, post-mortem interval, sex, and diverse environmental influences. Lastly, varying environmental influences, from subsequent life stressors to socio-economic conditions, can further modulate the epigenetic changes associated with the lasting physiological effects of childhood abuse (28). These factors interact in complex ways; the lack of control of these factors warrants more detailed investigations of individuals with a history of childhood abuse.

The study has applications primarily in the field of personalized medicine. The differentially expressed genes and pathways identified offer potential therapeutic targets for mitigating the adverse consequences associated with a history of childhood abuse. Understanding the role of these genes at a molecular and cellular level can pinpoint molecular targets for potential therapeutic agents, allowing treatment tailored to individual gene profiles.

Future research should delve deeper into the interplay between genetic, epigenetic, and environmental factors in shaping gene expression profiles. Understanding the temporal dynamics of gene expression, from early developmental stages to adulthood, could offer insights into critical windows of vulnerability or resilience. This study could be enhanced by conducting additional analysis to pinpoint the exact locations in electron transport chain or the ribosomal subunit where the proteins coded for by the significant genes reside. Such an investigation would provide deeper insights into the functional roles these genes play within the cellular environment.

MATERIALS AND METHODS

Dataset GSE157197 was obtained from the NCBI Gene Expression Omnibus to investigate the correlation between 58,126 genes and a history of childhood abuse (12). The dataset reports read counts of transcriptomic alterations to prefrontal deep-layer pyramidal neurons in individuals who died suddenly without prolonged agonal state or medical illness with no psychiatric histories (N=21) and those who died with a history of childhood abuse (N=24) (29). Data originates from the usage of a laser capture microdissection followed by RNA sequencing in a study conducted by the Department of Psychiatry of McGill University, submitted in 2020.

The first form of analysis was a differential gene expression analysis, conducted using GEO2R. Arranged by adjusted p-value, the 250 upregulated and downregulated genes were recorded for further analysis along with the Log2FC statistic, which quantified gene expression. GEO2R takes in read count matrices as input and uses the DESeq2 package in R to identify differentially expressed genes in data originating from RNA-sequencing (30). DESeq2 is a highly specialized package with built-in functionalities including differential expression analysis. The results from the DESeq2 differential expression analysis were calculated using the Benjamini-Hochberg procedure, which serves to decrease the false discovery rate and ensure the statistical significance of the differences in gene expression (31).

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Next, in order to find gene networks and relationships between the 250 up or downregulated genes, the STRING Consortium Database of Protein-to-Protein Interaction Networks was used (13). STRING offered a visual representation of the data in addition to thorough explanations of each protein and interaction. Significant gene groups were defined as any gene cluster, neighborhood, or family consisting of four or more genes and pertaining to a common pathway. Pathways of interest were also identified through STRING and referenced with the Kyoto Encyclopedia of Genes (KEGG), which provided extensive information on complex biological systems and pathways (14).

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