Microarray Analysis Uncovers Immune-Specific Gene Downregulation and Regulatory Pathway Changes in Children and Adults with Friedreich's Ataxia

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Abstract

There is no cure for the inherited condition known as Friedreich's Ataxia (FRDA). In order to develop FRDA, new biomarkers and crucial mechanisms must be identified as soon as feasible. A hereditary disorder of the spinal cord and cerebellum known as Friedreich's ataxia (FRDA) is mostly brought on by homozygous repeated amplification of the Guanine-Adenine-Adenine (GAA) triplet in the frataxin gene. Repeat amplification and mutation lead to a decrease in the expression level of functional Frataxin.

Frataxin deficiency can lead to ferroptosis and oxidative stress, which in turn can lead to mitochondrial dysregulation. Children's initial symptoms frequently include ataxia that worsens and loss of balance. As the condition worsens, patients may have dysarthria and loss of tendon reflex; in many cases, these symptoms are followed by myocardial infarction and diabetes. FRDA progression cannot currently be stopped with an effective therapy; instead, most therapies are symptomatic. Therefore, a deeper comprehension of the underlying pathophysiology and the creation of more potent therapeutic strategies are essential. Recently, some serum biomarkers have been found to be potential crucial indicators in the aetiology of FRDA. For instance, Friedreich's ataxia patients have significantly higher levels of neurofilament light and heavy chains, and these levels decrease with age. Additionally, it has been demonstrated that serum hsTnT, NTproBNP, and miRNAs are related to the progression of cardiomyopathy in adult FRDA patients. However, the clinical utility of these biomarkers has not yet been established in prospective cohorts, and it is unclear how these indicators interact clinically. Furthermore, the clinical diagnosis of FRDA has not yet used these biomarkers.

Therefore, finding more biomarkers may offer vital details on the diagnosis and therapy of FRDA. In a variety of illnesses, such as cancer, heart disease, and neurodegenerative disease, key biomarkers closely linked to disease prognosis are currently being discovered via bioinformatics analysis. Additionally, the knowledge of the unique mechanism of transcriptional regulatory networks in disease progression will be aided by competitive endogenous RNA (ceRNA) networks.

Keywords: Friedreich's Ataxia • Guanine-Adenine-Adenine • GSEA

Introduction

Despite current research concentrating on FRDA-induced transcriptome alterations, very few studies have examined the association between Differentially Expressed Genes (DEGs) in children and adult FRDA. We identified co-expressed Differentially Expressed Genes (co-DEGs) by intersecting the up-regulated and down-regulated DEGs in child and adult FRDA data. Then, using a PPI network and various enrichment studies, we were able to pinpoint the main pathways and hub genes linked to the development of FRDA in both children and adults. We also discovered hub gene target miRNAs and validated the diagnostic value of selected hub genes using the GSE30933 dataset. Finally, using interactions between mRNAs, miRNAs, and long non-coding RNAs (lncRNAs), we constructed ceRNA networks connected to FRDA [1]. Our study provides a new perspective on the pathophysiology of FRDA progression at the transcriptome level and suggests potential targets for FRDA diagnosis and treatment in both adolescents and adults.

Immune infiltration analysis and GSEA

We used GSEA to discover that the genes in children were significantly enriched in antigen receptor mediated signaling, defense response to virus, NF-B signaling, regulation of immune response signaling pathways, and T cell receptor signaling pathways. This helped us better understand the biological process and immune cell subtype involved in the child and adult samples [2, 3]. The majority of the enriched gene sets in adults were related to interferon-gamma response, virus response, virus response and viral gene expression. After that, we performed immune infiltration analysis to determine that there were differences in the proportions of immune cell subtypes between groups. Compared to the Control children group, the FRDA children group showed more resting memory CD4+ T cells and neutrophils. In addition, the FRDA adult group significantly increased the number of CD8+ T cells and activated NK cells in comparison to the Control adult group, while memory B cells, resting memory CD4+ T cells, activated memory CD4+ T cells, M1 Macrophages, resting Dendritic cells, activated Dendritic cells, and resting Mast cells decreased.

Functional Enrichment Analysis and Co-DEG Identification

29 co-up-regulated and 59 co-down-regulated DEGs were found when the DEGs from the child and adult datasets were combined. We performed GO enrichment analysis on the DAVID website to learn more about the enrichment pathways connected to these co-DEGs. These co-DEGs participated in a variety of biological processes, including hemopoiesis, the immunological effector process, viral defense, immune system process, and defense response to other species. Additionally, KEGG pathway enrichment analysis showed that the main functions of these co-DEGs in the intestinal immune network were IgA synthesis, autoimmune thyroid disease, measles, lysosome, necroptosis, and influenza A. Reactome enrichment study revealed that the immune system, inflammatory response, necrosis, and signal transduction were these co-DEGs' main areas of enrichment. We examine immune infiltration of co-DEGs in the child and adult datasets. The proportions of immune cell subtypes in the groups were quite obvious. Plasma cells, M0 Macrophages, and Neutrophils significantly outnumbered resting memory CD4+ T cells, active memory CD4+ T cells, monocytes, M1 Macrophages, and M2 Macrophages in the FRDA children group. The FRDA adult group significantly differed from the Control adult group in that it had significantly more naive B cells, plasma cells, M0 macrophages, and neutrophils, while having significantly less naive T cells CD4, M1 macrophages, M2 macrophages, and resting mast cells [4].

Cluster module analysis, ppi network analysis, and hub gene identification

The PPI network of coDEGs was constructed using the STRING website and viewed using Cytoscape. It included 45 nodes and 56 edges. Then, we removed a cluster module including six genes that were down-regulated using the MCODE plugin (Figure 5B). The results of five cytoHubba plugin algorithms (Degree, MNC, Closeness, Stress, and Radiality) were then combined to reveal a total of ten hub genes [5]. The majority of the hub genes were involved in immune system functions and responses to other species, and all of them had significantly lower levels of expression in FRDA samples. These results indicate that FRDA's pathophysiology is significantly influenced by the hub genes' reduced expression [6].

Target miRNA functional enrichment analysis, interaction network design, and target miRNA mining

MiRNAs play a crucial role in inducing gene degradation by binding to the 3'UTR of mRNAs, acting as a negative regulator. We discovered 156 mRNA-miRNA pairs, 150 target miRNAs, and eight hub genes. Additionally, based on the prediction outcomes, an mRNA-miRNA interaction network with 158 nodes and 156 edges was built and displayed using the Cytoscape software. Two miRNAs were identified as having the most cross-linked genes.

Furthermore, protein serine/threonine kinase activity, transcription factor activity, GTPase activity, ubiquitin-specific protease activity, receptor binding, and receptor signaling protein serine/threonine kinase activity were found to be significantly enriched in molecular functions by miRNA functional analysis [7]. The main biological processes implicated were the glypican pathway, syndecan-1-mediated signaling, proteoglycan syndecan-mediated signaling, IFN-gamma route, ErbB receptor signaling, and c-Metmediated signaling. LncRNAs have the ability to regulate the biological function of miRNAs because they are upstream molecules of miRNAs. We therefore predicted the target lncRNAs of the miRNAs for the CD28, FAS, and IFIT5 genes. In contrast to the FAS-miRNA and IFIT5-miRNA interaction networks, which produced 3 and 12 target lncRNAs, respectively, the CD28-miRNA interaction network produced 5 target lncRNAs. Three ceRNA networks were generated using the Cytoscape programme and presented. After that, we searched the literature and found that miR-24-3p had only been reported in FRDA. Therefore, we postulated that NEAT1-hsa-miR-24-3p-CD28 is a potential RNA regulatory mechanism involved in the development of FRDA in children and adults.

Discussion

An autosomal recessive hereditary condition called FRDA damages numerous organ systems. In recent years, bioinformatics analysis has been extensively developed and used to a variety of diseases, revealing the pathophysiology at its core and uncovering key biomarkers for disease diagnosis and prognosis. Despite this, there hasn't been any published comprehensive investigation on the relationship between child and adult FRDA based on bioinformatics. 88 co-DEGs were found by intersecting the up-regulated and down-regulated DEGs in the kid and adult datasets. These genes were primarily enriched in the immune response in both the child and adult datasets, according to GSEA and immune infiltration analyses. GO and KEGG pathway enrichment analysis of co-DEGs revealed a much higher immunological response, as measured by immune cell activation and innate immune response regulation, in FRDA samples. Activation of the immune system, necrosis, and signal transduction were all connected to the development of FRDA in both children and adults, according to reactome analysis. For the prognosis of many illnesses, including FRDA, immune system dysfunction is crucial. Bioinformatics has recently been utilised to demonstrate that FRDA patients have significantly less Natural Killer (NK) cells than control, carrier, or FRDA group members. Further evidence that macrophage activation may be implicated in the neuropathology of FRDA comes from the discovery of higher levels of the cytokine IL-6, which is produced by macrophages, in the blood plasma of FRDA patients.

There was a notable decrease in macrophages and a spectacular increase in activated NK cells in the FRDA adult group, but there was no statistically significant difference in the number of natural killer cells and macrophages between the FRDA kid and Control child groups. The following details could be to blame for this discrepancy: To begin with, using several datasets for analysis creates batch effects, which can lead to a variety of results. Second, the outcomes may vary if the subjects come from different regions or racial groups. Another possible explanation for the contradictory results is that we separated FRDA into adult and child categories and examined the connections between FRDA and immune cell types in adults and children. Additionally, we used the Cibersort algorithm rather than the quadratic programming approach in our work to analyse immune infiltration, indicating that the influence of various analytic methodologies on the outcomes should not be disregarded.

Conclusion

In conclusion, our work discovered that the course of FRDA in both children and adults may be influenced by the downregulation of three immune-specific hub genes, CD28, FAS, and IFIT5. In addition, the RNA regulatory pathway NEAT1-hsa-miR-24-3p-CD28 might be connected to the pathophysiology of both child and adult FRDA. These results offer a fresh perspective for investigating the pathophysiological mechanisms underlying the transcriptome-level evolution of FRDA.

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