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Analysis of lncRNA, miRNA , mRNA associated ceRNA networks in Autism

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Abstract

Autism is a multifactorial behavioral disease, which is caused by different environmental and genetic alterations. In this disease, various molecular pathways such as inflammation and cell-cell connections are affected. In this study, by analyzing RNA-seq data and bioinformatics approaches, candidate genes were selected for further analysis, and the expression of candidate genes in the blood samples of autistic people was compared to normal. Finally, the candidate genes showed potential asbiological markers for prognosis in autism. Through the analysis of these candidate genes, the researchers were able to identify changes in the expression of certain genes that were associated with the disease. By understanding how these genes are involved in the disease, researchers can develop better treatments and diagnose the disease earlier.

Key words: Autism, Bioinformatics, LncRNA, miRNA

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder and a complex syndrome consisting of a wide range of symptoms caused by abnormalities in the structure and function of the brain. This condition is characterized by difficulties communicating and social skills, delayed speech and motility, abnormal reactions to sound and other stimuli, repetitive behaviors and interests, and digestive problems. Autism is inherited in 80% of cases but environmental factors, random mutations and epigenetics can also play a role in its occurrence[1, 2].

The prevalence of autism around the world has increased in recent years due to advances in screening and diagnostic methods. In the United States in 2022, the prevalence of autism spectrum disorderswas about 1 in 44 children . Moreover, it was similar between races and there was no difference between black and white children. ASD is potentially affected by gender, with boys being about 4 times more likely to be diagnosed than girls.

Autism symptoms usually appear in the first three years of life and it can be diagnosed at the age of 18 months or even earlier, although the diagnosis is more reliable at 2 years of age[3]. However, some people are not diagnosed until adolescence. Early detection of autism is a vital step, for it has a significant impact on improving symptoms and raising the life quality of ASD children.

. According to science, ASD gender-dependence stems from the fact that women have two x chromosomes and alterations to one chromosome can be compensated by another healthy chromosome.But for men, having only one x chromosome, defects on the x chromosome can lead to an unhealthy phenotype. For instance, a defect in the *NLGN4* gene, which acts to create and maintain synaptic structures and is located on two sex chromosomes (one on the x chromosome and one on the y chromosome), has been shown to be involved in autism. *NLGN4Y* protein does not have the required function due to having a different single amino acid and the main function of this protein is related to *NLGN4X* protein. The region around this amino acid in the *NLGN4X* gene is a mutation-sensitive region, and different autism-associated variants have been seen in people with autism[4].

Genomic studies since 2005 have led to the identification of numerous CNVs, SNVs and SNPs, with CNVs accounting for about 15% and SNVs for 7% of autism cases.However,their association with autism is limited to a small number of people and has a very low prevalence in society[5, 6]. In addition, more than 1,000 autism-related genes are candidates in case-control studies. Most of these genes encode proteins involved in synaptic structure and function, and the others have functions in cell proliferation, chromatin rearrangement, and transcriptional regulation (released 20 July 2022, gene.sfari.org).

In humans, almost 80% of the genome is transcribed, with only 2% of these transcripts encoding proteins and the rest including non-coding RNA[7]. Two main groups of ncRNAs include microRNAs (miRNA) and long noncoding RNAs (lncRNAs), which have a regulatory role in various cellular processes.Changes in them are related to the occurrence of diseases and abnormalities[8].

To date, a large number of ncRNAs are known to be associated with neurodevelopment. Changes in their expression level are related to neurodevelopmental diseases, including autism[9]. Analysis of circulating serum samples from children with autism has led to the identification of a number of differentially expressed miRNAs[10]. The interaction between these miRNAs and the critical ASD genes indicates a potential role for the ncRNAs in the molecular pathways involved in ASD. Moreover, examining prefrontal cortex and cerebellum tissues of Postmortem brain, identified more than 200 autism-related lncRNAs with regions containing genes associated with neurodevelopmental disease[11]. LncRNAs can affect the regulation of gene expression through sponging miRNAs[12]. The study of cross-talk between miRNA-lncRNA-mRNA as a ceRNA network analysis has provided a way to identify biomarkers and molecular pathways associated with autism.

In this study we aimed to identify and screen potential biomarkers for ASD.We also studied the interactions between differentially expressed RNAs in the autistic brain through the construction and analysis of a CERNA network.. Based on the connections between DEmRNAs, DEmiRNAs and DElncRNAs we were able to identify a biomarker for ASD.

METHODS

Differential expression of lncRNAs, mRNAs, and microRNA

In order to investigate and find differentially expressed RNA, including mRNA, microRNA and LncRNA in autism, the raw data of sequencing (*SRP174367*) was taken from the SRA database (http://www.ncbi.nlm.nih.gov/sra). The selection of raw data was done based on a search for the word autism. Information files related to phenotype and expression level were extracted from primary data. Genes were annotated using HTSeq-Count and then 16 numbers of autism samples and 14 healthy samples were compared in terms of expression levels. Using the R package edgeR with the threshold of P-value < 0.05 and |log2-fold change (FC)| > 2 dRNAs were determined and then used for further analysis.

lncRNA-miRNA-mRNA related network

Mircode database (Version 11; http://www.mircode.org/mircode/, accessed on 25 June 2022), targetscan (Version 8.0; http://www.mircode.org/mircode/, accessed on 25 June 2022) and starbase were used to investigate the interaction between demRNA, demiRNA and lncRNA and to predict the direct relationship between them. The mircode database makes it possible to check the interactions and binding sites of miRNAs among the complete GENCODE annotated transcriptome. Through it, the binding of mir lncRNAs can also be checked [8]. The interactions between Mir and mRNA were explored using the Mirbase, TargetScan and Starbase databases. Starbase explores Mir targets with data obtained from CLIP-Seq and Degradome-Seq[9]. The possible targets for miR were predicted based on the complementary relationship and connection between the miR seed region and the 3UTR region of mRNA.

The ceRNA regulatory network

The interactive relations between the differentially expressed miRNAs, mRNAs lncRNAs were drived from pre-named databases. Then the predicted miRNA-lncRNA interactions and miRNA-mRNA interactions were visualized as a novel ceRNA network related to autism using Cytoscape software.

Enrichment analysis

To classify the genes based on their function either in molecular function(MF), biological processes(BP) and cellular components(CC) the Gene Ontology(GO) analysis and to map genes in pathways Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were made by the use of R.

Patient samples & q-RT-PCR

A total of 10 autism blood samples and 10 healthy controls were collected from Baqitullah Hospital.

The samples were children between 5 and 10 years old with autism.

In this study, there was no accompanying syndrome. With autism in patients.

Gender distribution: 7 boys and 3 girls

The level of autism disease is level one and two, and level 3 has been excluded from the study.

Blood samples were taken from patients, blood cells were separated, RNA was extracted and expression was performed on them.

My healthy people were in the age range of 8 to 12 years.The The samples were children between 5 and 10 years old with autism.

In this study, there was no accompanying syndrome, that is, it was not investigated

Gender distribution was : 7 boys and 3 girls.

The disease was leveling from one to two, and level three has been excluded from the study. Blood samples were taken from patients, blood cells were separated, RNA was extracted and expression was performed on them.

Healthy people were in the age range of 8 to 12 years.characteristics of each sample are listed in the table. Blood cells were precipitated and RNA extraction was done with Trizol reagent according to the manufacturer's instructions, then RNAs quality and quantity were evaluated. RNA purity and concentration were measured by nanodrop. In order to remove DNA from the sample, RNA was treated with DNAse. 1 µg of treated RNA was reverse transcribed by … enzyme. In the next step, in order to measure the expression level of the studied genes, real-time PCR was performed with Cybergreen. Gapdh was used as an internal control. The list of primers used in this study is given in the table.

Results

Differential expression analysis of lncRNAs, mRNAs, and miRNAs from public SRA data

Differential expression analysis led to 499 DEGs 150 DEMs and 550 DELs, from which 225 mRNA 75 miRNA 260 lncRNA were upregulated and 274 mRNA 75 miRNA 290 lncRNA were downregulated (table1&2). All were evaluated with the criteria of logfc< 0.5 or >2 and pv <0.05. expression level of differentially expressed mRNA and lncRNAs is shown in heatmap and volcano plot .(fig1)

Prediction and construction of the lncRNAs-miRNAs-mRNAs network

A lncRNA–miRNA–mRNA regulatory network was constructed based on the links between miRNA and mRNA and also between miRNA and lncRNA . Possible interactions were predicted using starbase, micode and targetscan. A competitive endogenous RNA network is built to describe lncRNA functions in competing with miRNAs in binding to mirs and also sponging them in order to regulating target genes expression (fig2).

Functional and pathway enrichment analysis

By using enrichers, GO analysis containing Cellular Components (CC), Biological Functions (BF) and Molecular Processes (MP) and pathway analysis was performed to evaluate DEGs functions.The KEGG database was also examined to indicate the involvement of DELs in different pathways. Accordingly, 14 MF, BP, CC and pathways were associated with DEGs ($p<0.05$). Top GO analysis and KEGG pathways are shown in (fig3).

LncRNAs-microRNAs-mRNAs expression by qRT-PCR in human samples

The qRT-PCR assessments on 10 autism and 10 control blood samples revealed changes in expression level of genes and lncRNAs of interest(fig.4).

Discussion

Autism spectrum disorder is a type of neurodevelopmental spectrum disorder that is characterized by abnormal communication and verbal behaviors[13]. The symptoms of this disorder usually appear before the age of 1 to 3 years and its cause is still unknown, but it is believed that genetic factors and the environment are involved in it. The social, economic, lifestyle, and education of parents do not affect this disorder[14].

In recent studies, it has been found that the molecular pathways of the inflammatory response[15] and PI3K[16], MAP Kinase[17], and TGFb play significant roles in the development of autism[18]. In addition, it has been found that LncRNAs and MicroRNAs, which are ncRNAs[19], play a significant role in physiological processes[20]. Cellular and also in causing or preventing various diseases including cancers, diabetes, cardiovascular diseases, and nerve diseases[21]. For example, lncRNAGAS5 causes autism by affecting mir-221 on the PI3K signaling pathway[22].

Our bioinformatics analysis revealed a number of lncRNAs, microRNAs, and mRNAs involved in pathways such as inflammation, WNT, TGFB, and PI3K, which are significant pathways in this disease. Such as the genes *LIN28A*[23], *CLDN6*[24], *COL6A3*[25], *FBLN5*[25], and LncRNAs *HOXA-A3*, *DANCR*, *LINC00461*, *LINC00261*, and microRNAs *miR-103*, *miR-494*, *miR-520*, *miR-141*, *miR-214*, *miR-33*.

Conclusion

Several genes are regulated by these factors through the influence they have on each other, affecting multiple signaling pathways.As a final conclusion, it can be suggested that the genes identified in this study may serve as biomarkers for the diagnosis of autism. The identified genes may provide insights into how environmental factors interact with the genetic makeup of an individual, and how the resulting combination may lead to the development of autism. This could potentially help to create more accurate diagnostic tests that can be used to identify autism in its early stages. Furthermore, this research may open the door to new treatment and prevention strategies for autism, enabling individuals to better manage and live with the condition.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical/Scientific Committee of Tarbiat Modares University (IR.MODARES.REC.1400.122).

Competing interests

The authors declare that they have no competing interests.

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Author contributions

ASTA,MM,YZ&AN performed the experiments. ARJ &AKM designed the experiments and supervised the study. AS helped with lab works.

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Reference

1. Bai, D., et al., *Association of genetic and environmental factors with autism in a 5-country cohort.* JAMA psychiatry, 2019. **76**(10): p. 1035-1043.
2. Bölte, S., S. Girdler, and P.B. Marschik, *The contribution of environmental exposure to the etiology of autism spectrum disorder.* Cellular and Molecular Life Sciences, 2019. **76**(7): p. 1275-1297.
3. Lord, C., et al., *Autism from 2 to 9 years of age.* Archives of general psychiatry, 2006. **63**(6): p. 694-701.
4. Nguyen, T.A., et al., *A cluster of autism-associated variants on X-linked NLGN4X functionally resemble NLGN4Y.* Neuron, 2020. **106**(5): p. 759-768. e7.
5. Lupski, J.R. and P. Stankiewicz, *Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes.* PLoS genetics, 2005. **1**(6): p. e49.
6. Masini, E., et al., *An overview of the main genetic, epigenetic and environmental factors involved in autism spectrum disorder focusing on synaptic activity.* International journal of molecular sciences, 2020. **21**(21): p. 8290.
7. Mattick, J.S., *Non-coding RNAs: the architects of eukaryotic complexity.* EMBO reports, 2001. **2**(11): p. 986-991.
8. Xue, M., Y. Zhuo, and B. Shan, *MicroRNAs, long noncoding RNAs, and their functions in human disease.* Bioinformatics in MicroRNA Research, 2017: p. 1-25.

9. Roberts, T.C., K.V. Morris, and M.J. Wood, *The role of long non-coding RNAs in neurodevelopment, brain function and neurological disease.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2014. **369**(1652): p. 20130507.
10. Mundalil Vasu, M., et al., *Serum microRNA profiles in children with autism.* Molecular autism, 2014. **5**(1): p. 1-9.
11. Ziats, M.N. and O.M. Rennert, *Aberrant expression of long noncoding RNAs in autistic brain.* Journal of molecular neuroscience, 2013. **49**(3): p. 589-593.
12. Paraskevopoulou, M.D. and A.G. Hatzigeorgiou, *Analyzing miRNA–lncRNA interactions*, in *Long non-coding RNAs*. 2016, Springer. p. 271-286.
13. Baron-Cohen, S., *Autism: the empathizing–systemizing (E-S) theory.* Annals of the New York Academy of Sciences, 2009. **1156**(1): p. 68-80.
14. Van Heijst, B.F. and H.M. Geurts, *Quality of life in autism across the lifespan: A meta-analysis.* Autism, 2015. **19**(2): p. 158-167.
15. Depino, A.M., *Peripheral and central inflammation in autism spectrum disorders.* Molecular and Cellular Neuroscience, 2013. **53**: p. 69-76.
16. Sharma, A. and S. Mehan, *Targeting PI3K-AKT/mTOR signaling in the prevention of autism.* Neurochemistry International, 2021. **147**: p. 105067.
17. Hörnberg, H., et al., *Rescue of oxytocin response and social behaviour in a mouse model of autism.* Nature, 2020. **584**(7820): p. 252-256.
18. Khulood, A.K. and N.I. Wijdan, *GENE POLYMORPHISIM OF TRANSFORMING GROWTH FACTOR Beta 1 (TGF-β1) IN AUTISM SPECTRUM DISORDER ASD IN BASRAH.*
19. Panwar, B., A. Arora, and G.P. Raghava, *Prediction and classification of ncRNAs using structural information.* BMC genomics, 2014. **15**(1): p. 1-13.
20. Ramón y Cajal, S., M.F. Segura, and S. Hümmer, *Interplay between ncRNAs and cellular communication: a proposal for understanding cell-specific signaling pathways.* Frontiers in genetics, 2019. **10**: p. 281.
21. Yan, H., et al., *Eusocial insects as emerging models for behavioural epigenetics.* Nature Reviews Genetics, 2014. **15**(10): p. 677-688.
22. Zhou, X.-B., et al., *LncRNA GAS5 sponges miRNA-221 to promote neurons apoptosis by up-regulated PUMA under hypoxia condition.* Neurological Research, 2020. **42**(1): p. 8-16.
23. Cho, J., et al., *LIN28A is a suppressor of ER-associated translation in embryonic stem cells.* Cell, 2012. **151**(4): p. 765-777.
24. Lu, Y., et al., *SMAD2 inactivation inhibits CLDN6 methylation to suppress migration and invasion of breast cancer cells.* International journal of molecular sciences, 2017. **18**(9): p. 1863.
25. Lam, M., et al., *Single cell analysis of autism patient with bi-allelic NRXN1-alpha deletion reveals skewed fate choice in neural progenitors and impaired neuronal functionality.* Experimental Cell Research, 2019. **383**(1): p. 111469.

Legends

Fig1) PCA plot and heat map diagram for dysregulated genes. A) The PCA diagram shows that the autism samples are completely normal. B) The Heatmap diagram shows that the autism samples are completely normal. C) The volcano plot shows the distribution of dysregulated mRNAs. D) The PCA diagram shows that the autism samples are completely normal. E) The Heatmap diagram shows that the autism samples are completely normal. F) The volcano plot shows the distribution of dysregulated LncRNAs.

Fig2) The lncRNA-miRNA-mRNA ceRNA network analysis in Autism. A&B) The lncRNA-miRNA-mRNA ceRNA network analysis in Autism. C&D) The main ceRNA networks in Autism.

Fig3) Predicted cellular and molecular pathways involved in autism. A&B) KEGG analysis predicted pathways involved in autism.

Fig4) qPCR of gene expression in Autism and Normal patients.