Research Paper

Deciphering the role of precursor miR-12136 and miR-8485 in the progression of intellectual disability (ID)

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A R T I C L E   I N F O

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A B S T R A C T

The short, non-coding RNAs known as microRNAs modulate the expression of human protein-coding genes. About 90 % of genes in humans are controlled by the expression of microRNAs. The dysfunction of these microRNA target genes leads to many human diseases, including neurodevelopmental disorders as well. Intellectual disability (ID) is a neurodevelopmental disorder that is characterized by adaptive behavior and intellectual functioning which includes logical reasoning, ability in learning, practical intelligence, and verbal skills. Identification of microRNA involved in ID and their associated target genes can help in the identification of diagnostic biomarkers related to ID at a very early age. The present study is an attempt to identify microRNA and their associated target genes that play an important role in the development of intellectual disability patients through the meta-analysis of available transcriptome data. A total of 6 transcriptomic studies were retrieved from NCBI and were subjected to quality check and trimming before alignment. The normalization and identification of differentially expressed microRNA were carried out using the EdgeR package of R studio. Further, the gene targets of downregulated microRNA were identified using miRDB. The system biology approaches were also applied to the study to identify the hub target genes and the diseases associated with main microRNAs.

Introduction

MicroRNAs refer to non-coding, small RNAs that undergo degradation and protein level adjustment to regulate the expression of mRNA. microRNA regulates approximately 90 % of the human genes (Miranda et al., 2006). These microRNAs are highly conserved in nature and are involved in major human biological processes like cell proliferation, developmental timing, metabolism, apoptosis, morphogenesis, and cell differentiation (Ambros, 2004). These microRNAs regulate the expression of genes by binding to the UTR region of the target mRNA inhibiting the synthesis of proteins and may lead to the degradation of mRNAs (Guo et al., 2010). One microRNA regulates the expression of more than one mRNA. Similarly, One mRNA is targeted by more than one microRNA (Willemsen et al., 2011). Most of the microRNA is expressed in the brain of humans and is thus found to play a pivotal role in the progression of various neurological disorders (Gonçalves et al., 2019). Around 70 % of the diagnosed microRNAs are expressed in the human brain that is majorly specific to the brain tissues only (Hohjoh and Fukushima, 2007). The biological processes regulated by microRNA in the central nervous system include neuronal maturation and differentiation, synaptic plasticity, synaptogenesis, memory formation, the proliferation of neural stem cells and their progenitors, and non-neuronal transcript silencing (Bian and Sun, 2011).

MicroRNAs that are derived from the longer transcripts of hairpin precursor microRNAs (pre-microRNAs). The enzymatic cleavage of pre-microRNAs leads to microRNA formation. The identification of microRNAs is largely dependent on the predictive models for the characteristic features from pre-microRNAs. The major challenge with microRNA identification is the short length of microRNA genes and the lack of pronounced sequence features (Lopes et al., 2016). Thus, due to the intimate relevance of pre-microRNA with the biogenesis of microRNA and small interfering RNA design, the identification of precursor microRNA has gained utmost importance (Fu et al., 2019).

Intellectual disability (ID) is a neurodevelopmental disorder that involves disrupted adaptive behavior and intellectual functioning (American Psychiatric Association, 2013). Polygenes and multiple environmental factors both play a vital role in the development of intellectual disability thereby affecting a broad spectrum of clinical and behavioral characteristics. The mechanism that causes intellectual disability and the progression of the condition is still not well understood (Wei-Hong et al., 2013). The recent approaches used in

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neuroscience had identified the genetic defects and abnormalities that can lead to intellectual disability but the probable link between these identified abnormalities and the progression of ID is still not known. This makes it necessary to fill this gap in research and also to investigate the role of miRNA in the progression of the disease.

In the current study, we have aimed to identify the potent miRNAs that play a significant role in the progression of neurodevelopmental disorders specifically intellectual disability. The transcriptomic data collected from the publicly available database is analyzed through differential expression analysis and gene-miRNA network to identify the miRNA which plays a pivotal role in the progression of intellectual disability.

Materials and methods

An intense literature survey was done to formulate an innovative pipeline for this unique and novel study. The complete pipeline is illustrated in Fig. 1.

Data retrieval

The current study signifies the importance of transcriptomic sequence analysis on patient and control samples. The GEO (Clough and Barrett, 2016), SRA (Leinonen et al., 2011), and BioProject (Barrett et al., 2012) databases from National Centre for Biotechnology Information (NCBI) were used as the major platform for data retrieval and collection of related information.
Degree and betweenness centrality of the hub genes obtained from Cytoscape.

| Study ID          | miRNA            | miRNA targets |
|-------------------|------------------|---------------|
| GSE77742          | hsa-miR-1260b    | 111           |
|                   | hsa-miR-1260a    | 8             |
| GSE108887(TAF2)   | hsa-miR-612      | 104           |
|                   | hsa-miR-8485     | 1056          |
| GSE145710         | hsa-miR-4507     | 39            |
| GSE98476 YY1Mut   | hsa-miR-151b     | 12            |
|                   | hsa-miR-4537     | 6             |
|                   | hsa-miR-4539     | 84            |
|                   | hsa-miR-5691     | 4             |
|                   | hsa-miR-663a     | 17            |
| GSE98476 HemiDel  | hsa-miR-3615     | 9             |
| GSE74263          | hsa-miR-1181     | 2             |
|                   | hsa-miR-12136    | 1030          |
|                   | hsa-miR-3176     | 74            |
|                   | hsa-miR-3618     | 104           |
|                   | hsa-miR-3665     | 82            |
|                   | hsa-miR-4442     | 6             |
|                   | hsa-miR-5047     | 120           |
|                   | hsa-miR-6125     | 10            |
|                   | hsa-miR-620      | 81            |

Table 4

Degree and betweenness centrality of the hub genes obtained from Cytoscape.

| Gene name | Degree | Betweenness centrality |
|-----------|--------|------------------------|
| EP300     | 44     | 0.275                  |
| SRC       | 42     | 0.196                  |
| STAT3     | 29     | 0.124                  |
| GRB2      | 28     | 0.058                  |
| PTEN1     | 27     | 0.025                  |
| MAPK1     | 24     | 0.081                  |
| SMAD2     | 24     | 0.078                  |
| PIK3CA    | 22     | 0.050                  |
| PIK3R1    | 20     | 0.015                  |
| JAK2      | 19     | 0.035                  |
| CDK1      | 19     | 0.101                  |
| VEGFA     | 18     | 0.094                  |
| MAPK14    | 18     | 0.032                  |
| CBL       | 18     | 0.029                  |
| RUNX2     | 17     | 0.131                  |
| CREB1     | 17     | 0.110                  |
| YAP1      | 16     | 0.056                  |
| EZH2      | 16     | 0.064                  |
| NOTCH1    | 15     | 0.043                  |
| EGF       | 15     | 0.017                  |
| FOXO1     | 15     | 0.030                  |
| ITGB1     | 13     | 0.028                  |
| PTEN1     | 13     | 0.006                  |
| BMP2      | 13     | 0.032                  |
| IRS1      | 13     | 0.005                  |
| PPP1CC    | 12     | 0.032                  |
| CCNA2     | 12     | 0.033                  |
| RNF2      | 12     | 0.017                  |
| MET       | 11     | 0.022                  |
| INSR      | 11     | 0.005                  |
| SMAD1     | 11     | 0.022                  |
| SUZ12     | 11     | 0.011                  |
| PPP1CCA   | 11     | 0.023                  |
| ERBB4     | 11     | 0.029                  |
| PPP2R1A   | 11     | 0.019                  |
| KAT2B     | 10     | 0.015                  |
| ITGB3     | 10     | 0.022                  |
| SIRT1     | 10     | 0.027                  |
| IGF1      | 10     | 0.001                  |
| STAT5A    | 10     | 0.018                  |
| STAT5B    | 10     | 0.013                  |
| WWTR1     | 10     | 0.016                  |
| CAV1      | 10     | 0.038                  |
| SPI       | 10     | 0.016                  |
| GNAQ      | 10     | 0.032                  |

Quality control and trimming

FASTQC was used for quality checking of each sample (Andrews, n.d.). FASTQC is an analysis tool that performs quality control checks on raw sequence data derived from high throughput sequencing pipelines. The low-quality reads and duplicated sequences were removed from the sample sequence using the trim galore tool. The trim galore tool automates quality and adapter trimming that removes biased methylation positions. FASTQC and Trim Galore were accessed from the GALAXY server (Jalili et al., 2020).

Alignment and read counts

Each sample included in the study was aligned to the reference genome of Homo sapiens (hg38) using a MiRDeep2 mapper (Friedlander et al., 2012). miRDeep2 is an improved algorithm that identifies the canonical and non-canonical miRNAs with 98.6–99.9 % accuracy and 71–90 % sensitivity. After completion of alignment procedure, the number of expressed miRNAs in the control and patient sample were counted using the miRDeep2 quantifier. GALAXY platform was preferred for accessing these software.

Identifying differentially expressed miRNA

The differentially expressed miRNA in the patient sample were analyzed using EdgeR (empirical analysis of DGE in R) (McCarthy et al., 2012) package of R (RStudio Team, 2020). The package is used for examining the differential expression of replicated count data. The TMM method, that is, ‘Trimmed Mean of M-values’ included in the EdgeR package was used for the normalization of raw gene count file. It uses the Poisson model for biological variability and the empirical Bayes method for moderating the degree of overdispersion across transcripts which helps in increasing the reliability of results (Robinson et al., 2010).

miRNA target identification

The miRNA that was identified to be either upregulated or downregulated in the patient sample as compared to the control sample was further used for the identification of miRNA targets. The miRNAs targets were identified through miRDB (Chen and Wang, 2020). miRDB is a significant online resource used for the prediction of miRNA targets and for further functional annotations. The database contains around 2.1 million predicted gene targets that are regulated by around 6709 miRNAs.

Gene target interaction network

The miRNA-gene interaction network was constructed to identify the hub. The hub genes are the ones which have maximum connections in the network. Thus, the abnormal expression of these genes can pose a significant impact on the progression of the disorder. Therefore, a network of miRNA-gene interactions was created using the STRING plugin of Cytoscape (Doncheva et al., 2019). Cytoscape software is a best suited offline tool for the construction and analysis of large networks. It is significant to state that it provides flexibility with respect to the import of data, network analysis, and visualization of the additional data related to the network.

Functional analysis of hub gene

The miRNAs whose most of the genes interacted at a 0.95 confidence level were further analyzed for their functional analysis. The functional enrichment of miRNAs was done using Metascape (Zhou et al., 2019). Metascape is a web-based portal that combines functional enrichment, gene annotation, and interactome analysis from 40 independent knowledge bases. It uses hypergeometric test and Benjamini-Hochberg
p-value correction algorithm for the functional enrichment analysis of the targeted genes.

**Disease-miRNA network**

The miRNAs which had the most number of gene targets interacting at a confidence level of 0.95 in the miRNA-gene interaction network were further taken into consideration for constructing a disease-miRNA network. This was done to predict the diseases associated with the target miRNA that may lead to its downregulation expression. The mammal ncRNA-disease repository (MNDR v3.0) (Ning et al., 2021) was used to identify the association of the filtered downregulated miRNA with other diseases. MNDR v3.0 is an online web-based tool that prepares an ncRNA-disease landscape using accurate and comprehensive data.

**Tissue expression analysis**

The expression of has-miR-8485 and has-miR-12136 precursor miRNAs in different human tissues was analyzed using miRNA-TissueAtlas2 (Keller et al., 2022). miRNA-TissueAtlas2 is a database of small noncoding RNA tissue atlas that determines the expression of 9 different types of sncRNAs. TissueAtlas determines its expression data for 21 different organs using 188 human samples. The RPMM normalization method was used for studying the tissue expression and the results were computed using log2 values of the collapsed tissues.

**Result and discussion**

**Data retrieved**

6 studies related to transcriptomic sequencing of intellectual disability patients were selected from NCBI. All samples of each study were included in our study for further analysis. Studies that dealt with multiple conditions were further broken down into different groups for analysis. Table 1 lists the details of the studies selected for the analysis.

**Identifying differentially expressed miRNA**

EdgeR package of R was used for the differential expression analysis. miRNA that had a false discovery rate less than 0.05 were considered to be significantly expressed in relation to the study. Out of these significantly expressed miRNA, ones that had a positive log2FoldChange were considered as upregulated miRNA and the ones with negative log2FoldChange were considered as downregulated (C. Li et al., 2020). The data studies that had multiple conditions were further divided into groups to avoid the normalization of data that could alter the results. Table 2 summarizes the differential miRNA expression as analyzed from the edgeR package.

**miRNA target identification**

The miRNA that was identified as downregulated, that is, had a p-value less than 0.05, and a log2foldchange value negative were taken into consideration for further studies. The gene targets for each downregulated miRNA were identified using miRDB. Only those targets were...
taken into consideration that had a target score greater than or equal to 80. The number of targets identified for each available miRNA using miRDB is summarized in Table 3.

miRNA-gene interaction

The gene targets of all the downregulated miRNA were used for network construction. The STRING plugin of Cytoscape software was used for the construction of miRNA-gene interaction of all 2559 gene targets. The confidence level of the network was set to 0.95 to get a strongly correlated network. The network was further confined to only those nodes with a degree equal to or greater than 10. Out of these 2559 genes, only 45 genes had a degree greater than or equal to 10. The resultant network contained 45 nodes and 1319 edges. Most of the hub genes involved in the network are regulated by hsa-miR-12136 and hsa-miR-8485, therefore, these miRNAs were taken into consideration for future study. Table 4 lists the degree and betweenness centrality of each node. Fig. 2 shows the interaction network designed on Cytoscape.

Functional enrichment of target genes

Metascape was used to analyze the functional characteristics of the gene targets which interacted with each other at a 0.95 confidence level and a degree greater than 10 in Cytoscape. The functional role of these genes was studied to know biological processes which can get affected by hsa-miR-8485 and hsa-miR-12136. It was observed that these target genes play a key role in important biological pathways like negative regulation of cell differentiation, prolactin signaling pathway, gland development, and androgen receptor signaling pathway. Fig. 3 shows the enrichment cluster network of the hub genes obtained from Metascape. The clustering is done on the basis of Kappa scores and is named after the most statistically significant term. Table 5 lists the adjusted p-value of the top biological pathways. The p-value is calculated using cumulative hypergeometric distribution and the Log10(q) value is calculated using the Benjamini-Hochberg procedure for multiple tests. Log10(q) value represents the multi-adjusted p-value.

Identifying disease-miRNA relation

The gene targets of the downregulated miRNAs hsa-miR-8485 and hsa-miR-12136 were maximum when the miRNA-gene network was constructed at a 0.95 confidence level. The association of these miRNAs with other diseases was further analyzed to evaluate the role of hsa-miR-12136 and hsa-miR-8485 in the progression of diseases that may act as a triggering factor for the development of intellectual disability in the future generations. The disease-miRNA relation was identified using MNDR online tool. Table 6 lists the association of these miRNAs with different diseases.

Tissue expression analysis

The expression of has-miR-8485 and has-miR-12136 was studied through miRNATissueAtlas2 to confirm that the concerned miRNAs express in the brain region. The RPMM normalization method was used for studying the tissue expression. The graph was constructed using log2 values of the collapsed tissues. The expression of has-miR-8485 ranges from 0.71 in the pituitary gland to 3.78 in the nucleus caudatus region of the brain. The expression of has-miR-12136 ranged from 6.68 in the occipital lobe to 12.01 in the dura mater region of the brain. Figs. 4 and 5 depict the expression of hsa-miR-8485 and hsa-miR-12136 in brain.

Conclusion

Development of Neurodevelopmental disorders occurs prominently at the foetal stage itself. Therefore, it becomes challenging to diagnose these disorders at an early stage and treat them subsequently. Till now, prenatal screening is available for trisomy 21, 13, and 18 only. With the increasing cases of neurodevelopmental disorders, it has become essential to study the mechanism of diagnosis of such neurodevelopmental disorders so that their early treatment or prevention can be made possible. miRNAs are one of the most studied regulatory elements owing to the fact that the majority of the miRNAs discovered till now are highly expressed in the brain region (Cao et al., 2006). These miRNAs exert their control at transcriptional as well as...
post-transcriptional levels in capable of integrating different intracellular signals and coordinating various signaling pathways as well. Thus, miRNAs are highly studied elements to identify the pathophysiology and etiology of neurodevelopmental disorders (Xu et al., 2010). Previous studies reported with reference to intellectual disability has found strong correlation between the expression of miRNA and development of intellectual disability (Gonçalves et al., 2019). Willemse et al., demonstrated in his study that miR-137 is associated with microdeletions in intellectual disability (Gonçalves et al., 2019). Willemsen et al., demonstrated hsa-miR-137 and hsa-miR-12136 as the key miRNAs that play an important role in the progression of intellectual disability (Willemsen et al., 2011). Qiao et al., also studied the function of miRNAs and their gene targets in copy number variations (CNV) occurring in patients with intellectual disability disorder (Qiao et al., 2013).

In the present study, an integrated approach of transcriptomic data analysis and network biology was applied to identify the miRNAs that play a crucial role in the development and progression of intellectual disability. The statistical test and approaches applied in the study identified hsa-miR-8485 and hsa-miR-12136 as the key miRNAs that play an important role in the progression of intellectual disability.

hsa-miR-8485 is a precursor miRNA that contains 8 repeats of CA (cytosine-adenosine) which is the most common class of microsatellite in the human genome. CA repeat microsatellite is known to regulate the stability and splicing of RNA. hsa-miR-8485 is reported to regulate the expression of gene NRXN1 (Fan et al., 2014). NRXN1 or neurexin1 is a well-reported gene involved in various neurodevelopmental disorders (Ishizuka et al., 2020). The exonic deletions overlapping the NRXN1 gene have been reported to play a role in the development of intellectual disability (Zahir et al., 2007) and autism spectrum disorder (Marshall et al., 2008). NRXN1 is a neuronal cell adhesion protein present in the presynaptic membrane. It binds the presynaptic neurotransmitter machinery with the postsynaptic transmembrane proteins like neurotransmitter receptors and neuraligins. This gene also regulates the presynaptic calcium channels and the release of neurotransmitters (Mozhui et al., 2011). Therefore, hsa-miR-8485 downregulation affects the functioning of NRXN1 gene paving way for intellectual disability.

hsa-miR-12136 is a precursor miRNA that is known to be involved in the process of translation. The recent studies done on miRNAs have observed that abnormal expression of hsa-miR-12136 can lead to developmental deformities including neurodevelopmental delay and intellectual disability (Salloum-Asfar et al., 2021; Morgan et al., 2020).

The involvement of the target genes of these two miRNAs has been seen in various important biological processes like negative regulation of cell differentiation, prolactin signaling pathway, gland development (Lombardo et al., 2020; La Rosa et al., 2021) which play an important role in the development and progression of neurodevelopmental disorders.

Further, the results of MNDR (Mammalian ncRNA-disease repository) suspect that the cases of intellectual disability have more chances to occur in couples who have had severe or prolonged COVID-19 infection (Vastrad et al., 2020a, 2020b; Wu et al., 2021; Fernández-Pato et al., 2022; Demirci et al., 2021; Chow and Salmena, 2020), or HIV infection (Valle-Millares et al., 2022). The intellectual disability may also occur in babies who are born to a family that has a history of any metabolic syndrome (Y. Li et al., 2020) like type II diabetes (Lan et al., 2022), progressive supranuclear palsy (Nonaka et al., 2022),...
Parkinson’s disease (Schulze et al., 2018), oral lichens planus (Gholizadeh et al., 2020), psoriasis (Lin et al., 2021), penile squamous cell carcinoma (Ayoubian et al., 2021), and pre-eclampsia (Pan et al., 2022) because of the association of these diseases with hsa-miR-8485 and hsa-miR-12136.

Thus, the current study reveals that hsa-miR-8485 and hsa-miR-12136 play an important role in the development of the nervous system. If any stress, environmental factor, or genetic factor causes an abnormal expression of these miRNA, it can lead to poor development of the brain causing intellectual disability in the growing fetus. Any mutation or exprssional abnormality in these miRNAs can lead to neuro-developmental delay. Thus, in the future attempts can be made to target these miRNAs for therapeutic interventions, so that they can be used as a biomarker for the early diagnosis of the disease. These miRNAs, their gene targets and associated biological pathways can further be analysed and validated so that they can be targeted for the treatment of intellectual disability.

Ethical Standards

I have read and have abided by the statement of ethical standards for manuscripts submitted to Neuroscience.

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CRediT authorship contribution statement

Prekshi Garg: Methodology, Formal analysis, Visualization, Data curation, Writing – original draft. Farrukh Jamal: Writing – review & editing, Supervision. Prachi Srivastava: Conceptualization, Writing – review & editing, Supervision.

Conflicts of Interest

The authors declare that no conflicting financial interests exist.
Data Availability

The datasets analyzed during the current study are available in the National Center for Biotechnology Information (NCBI) repository, [https://www.ncbi.nlm.nih.gov/].

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P. Garg et al.

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