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Profiling of miRNAs in serum of children with attention-deficit hyperactivity disorder shows significant alterations

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ABSTRACT

Background: Attention-deficit/hyperactivity disorder (ADHD), a common psychiatric disorder, is identified by abnormal levels of impulsivity, inattention, and hyperactivity. MiRNAs play important roles in neural network development of the brain. Circulating miRNAs (cmiRNAs) are offered as promising noninvasive markers for psychiatric disorders. In this study, the expression level of neurologically relevant miRNAs was evaluated in serum samples of ADHD individuals.
Methods: RNA extraction was performed for 60 subjects with ADHD and 60 healthy controls, and the cDNAs were synthesized for all the miRNAs. The expression level of 84 cmiRNAs was then examined in 4 ADHD subjects and 4 controls. The altered expression of 10 cmiRNAs was further evaluated in validation cohort comprising 56 ADHD and 56 control samples by qPCR. The diagnostic power of the miRNAs was determined by use of Receiver-operating characteristic (ROC) analysis. The cmiRNAs target genes were predicted using DIANA mirPath software and gene ontology enrichment analysis was performed using Cytoscape CLUGO.
Results: Initially, 10 miRNAs showed differential expression in ADHD individuals. Further analysis confirmed four miRNAs (hsa-miR-101-3p, hsa-miR-130a-3p, hsa-miR-138-5p and hsa-miR-195-5p) upregulated and one miRNA (hsa-miR-106b-5p) downregulated. These miRNAs showed significant predictive values for discriminating ADHD individuals. Enrichment analysis highlighted the involvement of the deregulated cmiRNAs in many canonical neurobiological pathways and mechanisms.
Conclusions: Our report is the first comprehensive study on the expression profiling of miRNAs in serum of ADHD subjects. These findings suggest a set of cmiRNAs as potential noninvasive biomarkers for ADHD.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD), a very common psychiatric disorder, is developmentally identified by abnormal conditions of impulsivity, inattention, and hyperactivity. It has an average prevalence of 5% in children and juvenile worldwide (Angold et al., 2000; Edition, 2013; Tarver et al., 2014) The children with ADHD usually show cognitive disability and learning difficulty which hinder children's development and education. These symptoms can be prolonged to adulthood and exist during the lifespan which makes ADHD a lifelong condition (Barkley, 2002). Many individuals with ADHD are also affected by other psychiatric disorders such as conduct and anxiety disorders (Herguner and Herguner, 2012). Even though the etiological factors and pathogenic causes of ADHD still remain unclear, few studies have suggested that is it caused by either genetic or different environmental factors (Jarick et al., 2014; Silva et al., 2014; Williams et al., 2012). Previously reported studies of segregation analysis in families having close genetic relationships showed the strong role of genetics in ADHD with estimated heritability ranging from 60 to 90% indicating the substantial genetic impact on the disorder (Burton et al., 2018). However, ADHD is still heterogeneous in terms of etiology due to lack of specific and efficient evaluation approaches for both diagnosis and

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investigating the causes (Massat et al., 2018). Therefore, an efficient and specific molecular signature is urgently needed for ADHD diagnosis.

MicroRNAs (miRNAs), a small group of non-coding RNAs, post-transcriptionally modulate gene expression by specifically disruption and degradation of target mRNAs (Cristino et al., 2014). These molecules play a wide variety of regulatory roles in numerous processes, involving cell proliferation and differentiation, development, and programmed cell death (Greco et al., 2017). Lately, miRNAs have intriguingly demonstrated crucial regulatory roles in neural functions and activities, modulation of homeostasis at the neural junctions and axon transport (Xu et al., 2012). Moreover, they contribute to synaptic interactions in the central nervous system and play an important role in memory and intellectual disability (Cao et al., 2006). The involvement of miRNAs in almost every biological activity highlights the difficulties related to finding specific causative genes linked to the etiology of psychiatric disorders (Geaghan and Cairns, 2015). The association of numerous signaling pathways with psychiatric disorders underlies the complication of the efforts to investigate the underlying biological causes (Sarachana et al., 2010). Notwithstanding, deciphering the miRNAs roles in psychiatric disorders may give us a clue to the involved causative mechanisms and reach specific and efficient therapeutic and diagnostic approaches. However, analyzing the transcriptome and miRNA levels of the brain biopsies from individuals is invasive and has ethical issues. Intriguingly, miRNAs have shown stability in circulation and their circulation level may be associated with diseases. Interestingly, circulating miRNAs have already been detected in all body fluids; however it is not yet known about their purpose and whether they play any role in normal physiology. It is also unclear how diseases change the levels of specific circulating miRNAs (Reid et al., 2011). Several studies have suggested circulating miRNAs as efficient markers for the evaluation of neurodevelopmental diseases as they have demonstrated the alteration of miRNAs in subjects with schizophrenia disorder (Moreau et al., 2011), autism spectrum disorder (Vasu et al., 2014), Tourette syndrome (Rizzo et al., 2015), and anxiety disorder (Muiños-Gimeno et al., 2011). Also, a few miRNAs have been investigated in subjects with ADHD suggesting their potential role in the pathogenesis of many neurological disorders (Kandemir et al., 2014).

However, despite the existence of many miRNAs in different brain parts, their importance in the function and development of nervous system and etiology of psychiatric disorders such as ADHD is still unclear. Therefore, in this study, the aim was to comprehensively examine the expression profile of circulating miRNAs in children with ADHD.

2. Materials and methods

2.1. Subjects

This study included a group of 60 subjects with ADHD recruited from Emam Sajad Hospital and 60 control subjects (Fig. 1). All patients and controls were successfully matched in age and sex. All subjects had the same race and ethnicity and were collected from the same region (Yasuj, Iran). All patients with ADHD were randomly selected from a small community of children with ADHD and the control individuals were recruited voluntarily and had no clinical signs at the time of recruitment. The diagnosis of ADHD was performed by child psychiatrists with ADHD expertise, according to DSM-IV (the diagnostic and statistical manual of mental disorders, fourth edition, American Psychiatric Association, 2000) diagnostic criteria with a structured interview (First et al., 1997). The Conners' Parent Symptom Questionnaire (PSQ) and Conners' Teacher Rating Scale (TRS) were also applied to make the screening diagnosis. Subjects with other psychiatric disorders were excluded from the study by applying Structured Clinical Interview for DSM-IV (SCID). Furthermore, patients with a history of severe head injury, neurodevelopmental disorders, dysaudia, vision disorder, epilepsy or cardiovascular disorders were excluded. All the subjects had an intelligence quotient (IQ) of higher than 85. None of the subjects received drug treatment for ADHD. All enrolled participants prepared a written signed consent before sample conducting the experiments. The study procedures involving human participation were conducted according to the Declaration of Helsinki and ethical standards and approved by ethics committee of Yasuj University of Medical Sciences.

2.2. Serum separation

Whole blood samples were collected from ADHD patients and controls before the noon by use of phlebotomizing and transferred to collection tubes equipped with Clot activator (Sigma Aldrich).To separate serum from cellular components, samples were rotated end-over end at 25 °C for 20 min, then were placed at 25 °C for 25 min and centrifuged for 15 min at 3000 rpm (640 ×g) at 25 °C to isolate serum. To remove remaining circulating cells or debris r in the samples, supernatant was again centrifuged at 5000 rpm (1780 ×g). As the next step, the clear supernatant was isolated and carefully transferred into RNase-free microtubes in 300 μl aliquots for miRNA extraction and stored at −80 °C. Previously, it has been shown that the presence of lysed red blood cells in serum samples is the major source of variability in the level of miRNAs (Kirschner et al., 2011). Accordingly, all samples were then checked by a microplate reader spectrometer (Thermo Fisher Scientific) at a wavelength of 414 nm to distinguish non-hemolyzed sera.

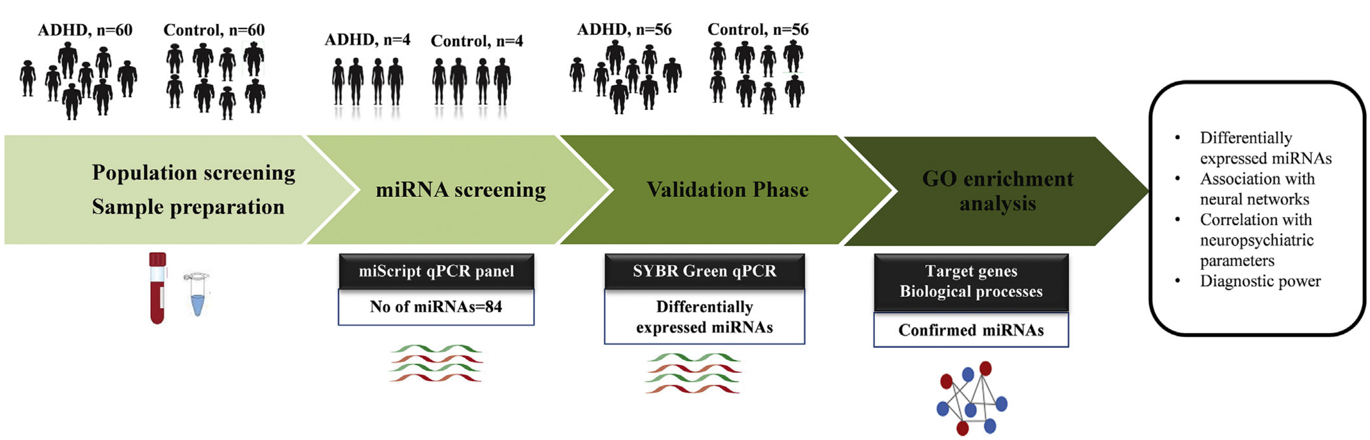

Fig. 1. A diagram showing different steps of the study. ADHD, attention deficit hyperactivity disorder.

2.3. RNA extraction and cDNA synthesis

RNA was totally extracted from 300 µl serum samples by use of a miRNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. Briefly, serum samples were lysed using QIAzol lysis reagent and Caenorhabditis elegans miR-39 as a synthetic spike-in control was then added as an internal normalizer to the tubes containing lysed samples. Chloroform was equally added and the samples were then centrifuged at 13,000 rpm for 12 min at 4 °C. Next, the clear supernatant was transferred to a new tube and mixed with absolute ethanol, centrifuged, washed, and eluted in 30 µl elution buffer in the collection tube. The quantity of extracted RNAs was analyzed by spectrophotometry. The quality of RNAs was checked by use of Agilent bioanalyzer 2100 (Agilent, CA, USA) based on RIN (RNA integrity). All the RNAs had a high quality (RIN > 8).

After RNA extraction, cDNA synthesis was performed using miScript II RT kit (QIAGEN) according to the manufacturer's protocol. The reaction mixture with a 20 µl final volume contained 2 µl RNA, RT mix, Hispec buffer, dNTPs, and RNase-free water. Next, the prepared mixture was incubated 30 min at 37 °C, 60 min at 42 °C, 5 min at 95 °C and then held at 4 °C. The synthesized cDNA samples were kept at −20 °C for the next step.

2.4. miRNA profiling

At the discovery phase, the screening of miRNA was performed by use of the Human Neurological Development & Disease miScript miRNA PCR Array (QIAGEN GmbH, Hilden, Germany), including 84 miRNAs which have previously been reported to be involved in the development of the brain and different neurological disorders and also neural stem cells differentiation (Stevanato and Sinden, 2014). Although the number of present miRNAs in the brain is very high, selected miRNAs were also present in circulating system (Camacho et al., 2013) and in association with several brain disorders in human and animals (Garza-Manero et al., 2015; Lukiw et al., 2008; Rippo et al., 2014; Rizzo et al., 2015; Trompeter et al., 2011; Wei et al., 2015). For example, Mahesh et al. have previously reported the association of some of these miRNAs with autism (Vasu et al., 2014). Accordingly, we decided to use this available miRNA PCR array. The array contained *C. elegans* miR-39 as an internal normalizer, snoRNA/snRNA (SNORD48, SNORD61, SNORD68, SNORD72, SNORD95, SNORD96A and RNU6-2) as normalization controls for the array data, miRNA reverse transcription control (miRTC) primer assays and positive PCR controls (PPC).At this stage, 4 samples from ADHD subjects and 4 healthy controls were randomly selected for the screening. Then, prepared cDNAs were used for the profiling assay. The quantification of miRNAs expression level was performed using SYBR Green qPCR on an ABI 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

C. elegans miR-39 primer assay was used as an internal calibrator for the data sets. To perform an accurate miRNA profiling, the Ct values from the data sets were normalized to the whole array Ct mean. Relative quantification, as recommended by the manufacturer, was obtained using the $2^{-\Delta CT}$ method. Differential expression of miRNAs was determined between the ADHD and control groups by multiple t-tests followed by Bonferroni correction. P value < 0.00059 was considered as statistical significant.

2.5. Validation by quantitative reverse-transcription PCR

In this validation phase, 56 subjects with ADHD and 56 healthy subjects were examined individually. For the verification of differentially expressed miRNAs, the synthesized cDNAs were quantified by SYBR Green qPCR. All the SYBR Green qPCR reactions were run in triplicate with conditions as follows: 15 min at 95 °C, 40 cycles of 15 s at 94 °C, 30 s at 57 °C and 30 s at 72 °C, on an ABI 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

2.6. Data analysis

All statistical analyses were done with GraphPad Prism 6 (GraphPad Software, Inc, La Jolla, Ca, USA) and R studio 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria). In order to test any difference in the age and sex between the two groups, Student's t-test and chi-square test were used, respectively. To determine differentially expressed circulating miRNAs, multiple comparison *t*-test followed by Bonferroni correction was applied. Pearson correlation test was used to determine any correlation between miRNAs expression and neuropsychiatric parameters. The diagnostic power of the miRNAs was determined by use of Receiver-operating characteristic (ROC) analysis to discriminate between ADHD patients and healthy individuals by using validation cohort samples (56 versus 56). We have used logistic regression model for all the miRNAs independently and also jointly as a unique cluster. We partitioned the data from validation phase into train and test group to make a model. A p-value of < 0.05 was considered statistically significant.

2.7. Gene ontology analysis

First, the differentially expressed cmiRNAs target genes were predicted by means of DIANA mirPath version 3.0 tools. The tool retrieves the experimentally verified targets from TarBase v6 (http://mirtarbase.mbc.nctu.edu.tw/). Then, enrichment in GO terms from the biological processes and KEGG pathways for the most relevant predicted genes was obtained using ClueGO plugin (Bindea et al., 2009) of Cytoscape v3.6 (Shannon et al., 2003), analyzed by multiple *t*-test followed by Bonferroni correction with a threshold p-value < 0.01.

3. Results

3.1. Demographic data

Sixty children with ADHD [age 9.97 ± 1.44, sex M = 41, F = 19] and 60 controls [age 10.06 ± 1.35, sex M = 44, F = 16] were enrolled in this study. The distribution of age was not different between the ADHD cohort and controls (p-value: 0.7338). Also, the difference of sex distribution was not statistically significant (p-value: 0.5468). Also, there was no difference in IQ score between patients and controls. Neuropsychiatric parameters showed a significant difference of impulsive-hyperactive score (p < 0.0001), hyperactive score (p < 0.0001) and the total score (p < 0.0001) between the groups (Table 1).

Table 1
Clinical and demographic variables of subjects.

Clinical/demographic	ADHD (n = 60)	Control (n = 60)	P value
Sex: male/female (n)	41/19	44/16	0.5468 (x2 = 0.3630)
Age: mean ± SD (year)	9.97 ± 1.44	10.06 ± 1.35	0.7338
ADHD subtypes (n)			
Attention deficit	6		
Hyperactivity/ impulsivity	3		
Combined	51		
IQ score (mean ± SD)	102.5 ± 11.06	99.75 ± 7.69	0.11
Impulsive-hyperactive score (mean ± SD)	1.56 ± 0.83	0.66 ± 0.47	< 0.0001
Hyperactive score (mean ± SD)	1.68 ± 1.11	0.56 ± 0.82	< 0.0001
Total score (mean ± SD)	31.8 ± 4.44	23.8 ± 5.17	< 0.0001

ADHD, attention-deficit hyperactivity disorder.

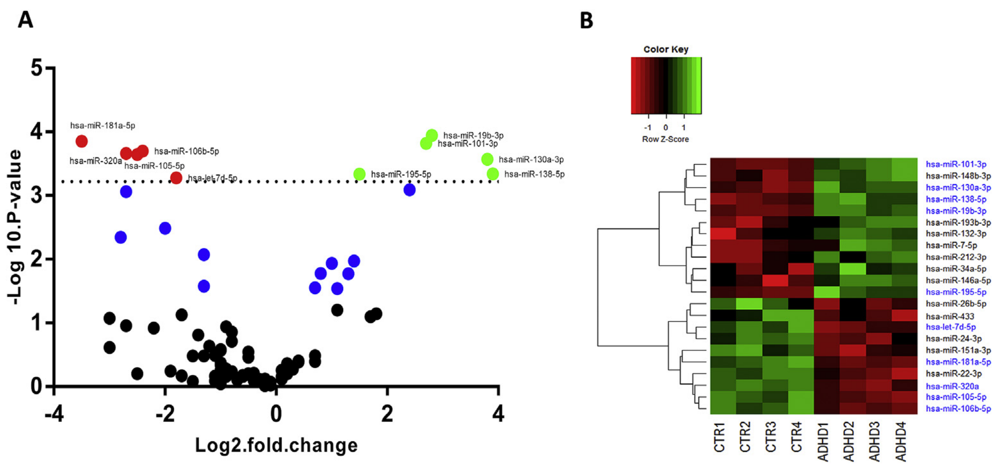

Fig. 2. Initial screening of miRNAs in ADHD and control samples. (A) Volcano plot shows p values in a negative Log10 scale and fold changes in a log2 scale. Dotted line shows the significance threshold (-log 10 p-value = 3.22) Green dots and red dots represent significantly upregulated miRNAs and downregulated miRNAs, respectively, after correction with Bonferroni method. Blue dots show miRNAs that were significantly deregulated with *t*-test but did not pass Bonferroni correction (B) The heat map shows altered expression of cmiRNAs as log2 values. Green = upregulation; red = downregulation (ADHD, Attention-deficit hyperactivity disorder; CTR, control). Significantly deregulated miRNAs are highlighted in blue. P value < 0.00059 was considered as significant (-log 10 p-value > 3.22). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. miRNA screening

In the preliminary array screening, 4 samples with ADHD were randomly selected and 4 matched controls were chosen. Altered expression of 10 circulating miRNAs in the ADHD samples were observed in comparison with those of controls (Fig. 2). Of these, 5 miRNAs (hsa-miR-101-3p, hsa-miR-130a-3p, hsa-miR-138-5p, hsa-miR-195-5p, and hsa-miR-19b-3p) were upregulated (Fig. 2A green dots) and 5 miRNAs (hsa-let-7d-5p, hsa-miR-105-5p, hsa-miR-106b-5p, hsa-miR-181a-5p and hsa-miR-320a) were downregulated (Fig. 2A red dots). Twelve miRNAs were significantly deregulated with *t*-test but they showed no significance difference after Bonferroni correction (Fig. 2A blue dots). A p-value of < 0.00059 (-log10 p-value > 3.22) was considered as significant. Fig. 2B shows a heat map of log2 fold change of significantly altered miRNAs in ADHD and control samples in screening phase. (See also supplementary data 1).

3.3. Verification with quantitative PCR

The differentially expressed cmiRNAs were further analyzed by SYBR Green qPCR in 56 samples with ADHD and 56 controls. Of these, four miRNAs (hsa-miR-101-3p, hsa-miR-130a-3p, hsa-miR-138-5p, and hsa-miR-195-5p) were confirmed upregulated and one miRNA (hsa-miR-106b-5p) was verified downregulated (Fig. 3).

3.4. ROC curve analysis

To discriminate between ADHD patients and healthy individuals, the diagnostic power of the differentially expressed miRNAs was determined by use of Receiver-operating characteristic (ROC) analysis. The data from validation cohort (56 controls versus 56 patients) were used here. The analysis was performed by partitioning the data into train and test groups to make a model and then logistic regression model was applied. First we analyzed the ROC curve for each miRNA independently to see which miRNA shows higher predictive power. The independent analysis showed significant diagnostic values for all the differentially expressed miRNAs for ADHD (p < 0001). Of these, higher predictive values of the area under the curve (AUC), sensitivity and specificity were observed for four miRNAs: hsa-miR101-3p, hsa-miR-106b-5p, hsa-miR-130a-3p and hsa-miR-138-5p (Fig. 4). Also, we performed multiple ROC curve test to compare the ROC curve results between the miRNAs (Fig. S1). Interestingly, hsa-miR-195-5p showed significant difference compared with other four miRNAs (data not shown) which may be due to its lower fold change as we see the higher ROC curve values for those with higher fold change. In addition, we collapsed all 5 miRNAs data to a single cluster and re-analyzed the data. We observed significant AUC (0.68), sensitivity (67.5%) and specificity (71.4%) values (p < 0.0001) (Fig. 4). This is probably due to the highly significant fold change of the differentially expressed miRNA between ADHD and control group.

3.5. Enrichment pathway analysis

As illustrated in Fig. 5, the differentially expressed cmiRNAs target genes predicted by DIANA mirpath version 3.0 were found to be involved in several neurological pathways. The number of predicted genes with highest score for hsa-miR-101-3p, hsa-miR-130a-3p, hsa-miR-138-5p, hsa-miR-195-5p, and hsa-miR-106b-5p was 1482, 2265, 1591, 1459, and 2590, respectively. Then, all the predicted genes were used for enrichment analysis by Cytoscape ClueGO. The most relevant neurological pathways were: regulation of dendrite development, neuron recognition, regulation of axon regeneration, central nervous system neuron axonogenesis, regulation synapse structure or activity, central nervous system neuron development, cellular senescence, Wnt signaling pathway, and cell cycle G1/S phase transition. In addition, we searched for the common genes targeted by the miRNAs. To do so, we selected all predicted genes and draw Venn diagrams using FunRich V.3.0. The results showed many genes in common between these five miRNAs (Fig. 6) (See supplementary data 2).

3.6. Correlation between neuropsychiatric parameters and miRNAs expression

To test the correlation between the expression of validated cmiRNAs and clinical characteristics of ADHD patients, their expression levels were tested against different neuropsychiatric parameters including IQ score, impulsive-hyperactive score, hyperactive score and total score. There was only a poor correlation between total score and the expression level of hsa-miR-138-5p (P = 0.039, r = −0.27).The expression level of other miRNAs did not show any correlation with these parameters (see Table S1).

4. Discussion

MiRNAs have important roles in many biological processes and organ development (Huntzinger and Izaurralde, 2011). The alteration

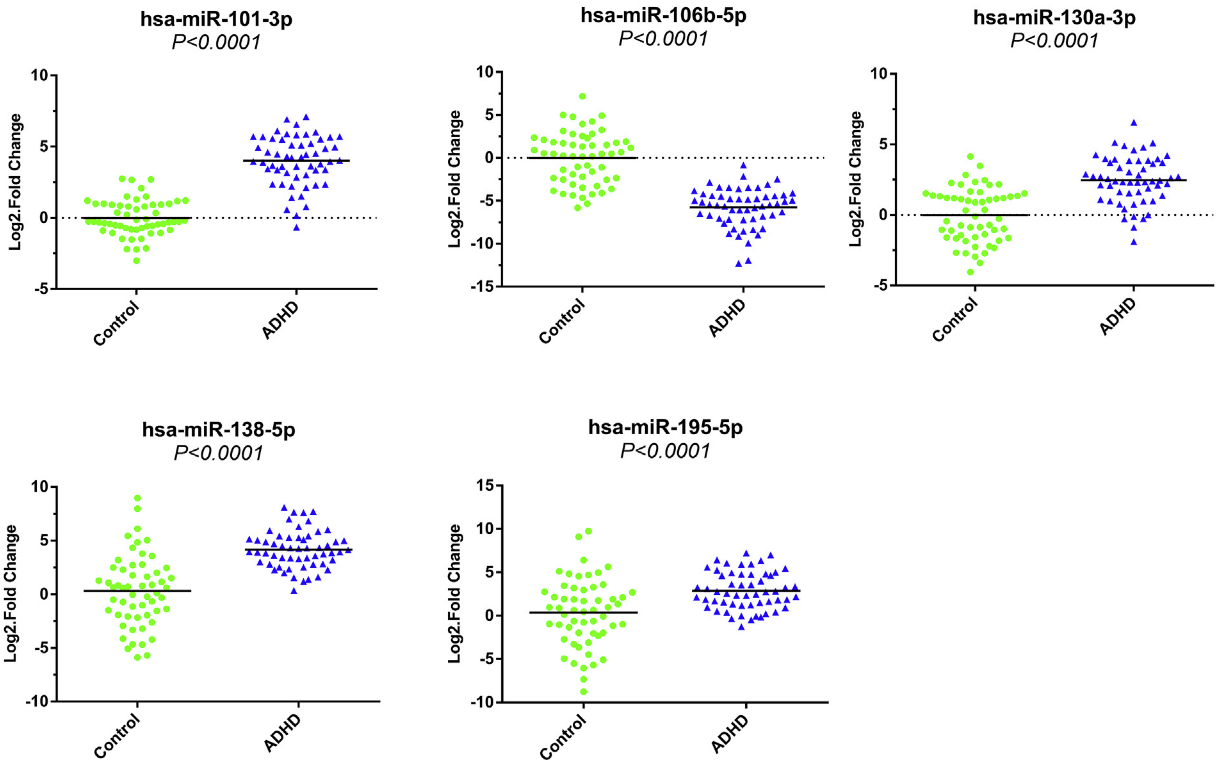

Fig. 3. Scatter plot shows validation of the differential expression of verified miRNAs in ADHD individuals. Values are represented as mean ± SD. A p-value of < 0.005 was considered statistically significant.

of their mechanism may affect downstream pathways and cause vital side effects (Fabian et al., 2010). The mechanism of many miRNAs has been previously reported to be deregulated in neurological pathways involved in the development of different brain parts including the prefrontal cortex, midbrain, and hindbrain, neural differentiation, synapse formation causing psychiatric disorders (Xu et al., 2012). Li et al. have previously reported the expression alteration of Let-7d in serum of ADHD patients. However, they have evaluated the expression of only one miRNA in 35 patients while we investigated a more comprehensive set of miRNAs (84 miRNAs) in a larger study cohort (60 patients and 60 controls) which may lead to more precise profiling or miRNAs. Differential expression of 5 cmiRNAs (four miRNAs upregulated; one miRNA

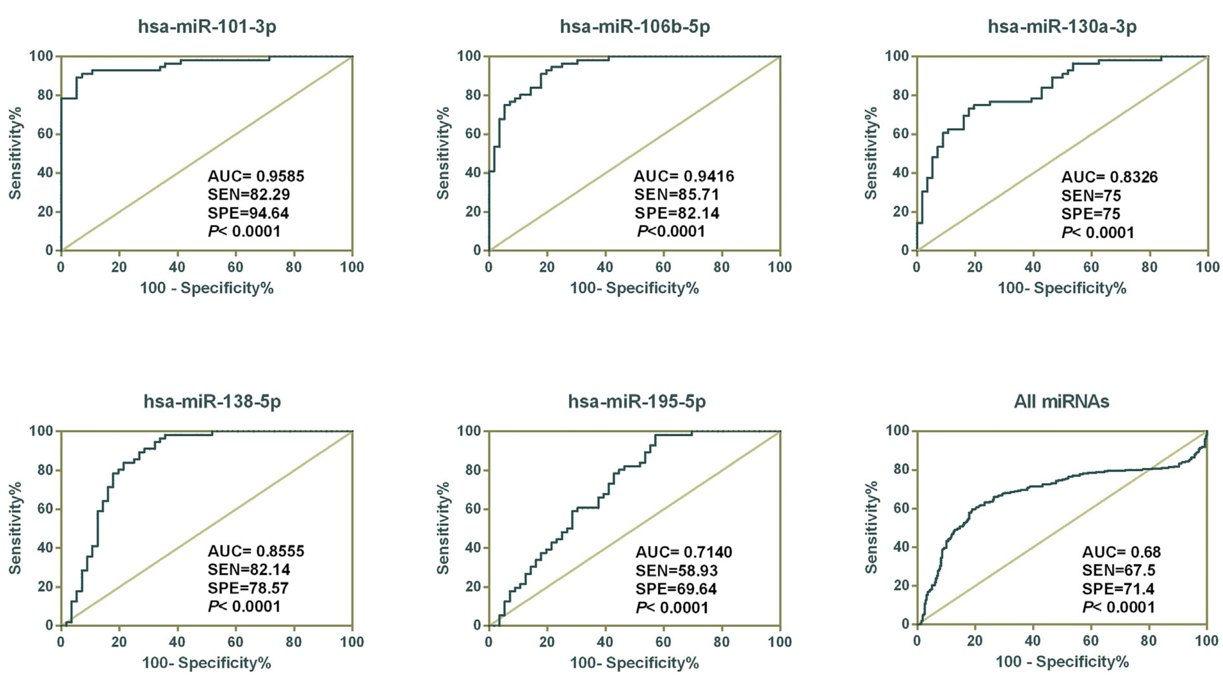

Fig. 4. Receiver operating characteristic (ROC) curve of differentially expressed miRNAs in ADHD subjects. In independent ROC curve analysis using logistic regression model, hsa-miR101-3p, hsa-miR-106b-5p, hsa-miR-130a-3p and hsa-miR-138-5p show higher predictive values. We have also used the data from all five miRNA as a unique feature using logistic regression model and the results showed significant predictive values. AUC, area under the ROC curve; SEN, sensitivity; SPE, specificity (p-value < 0.05 was considered as significant).

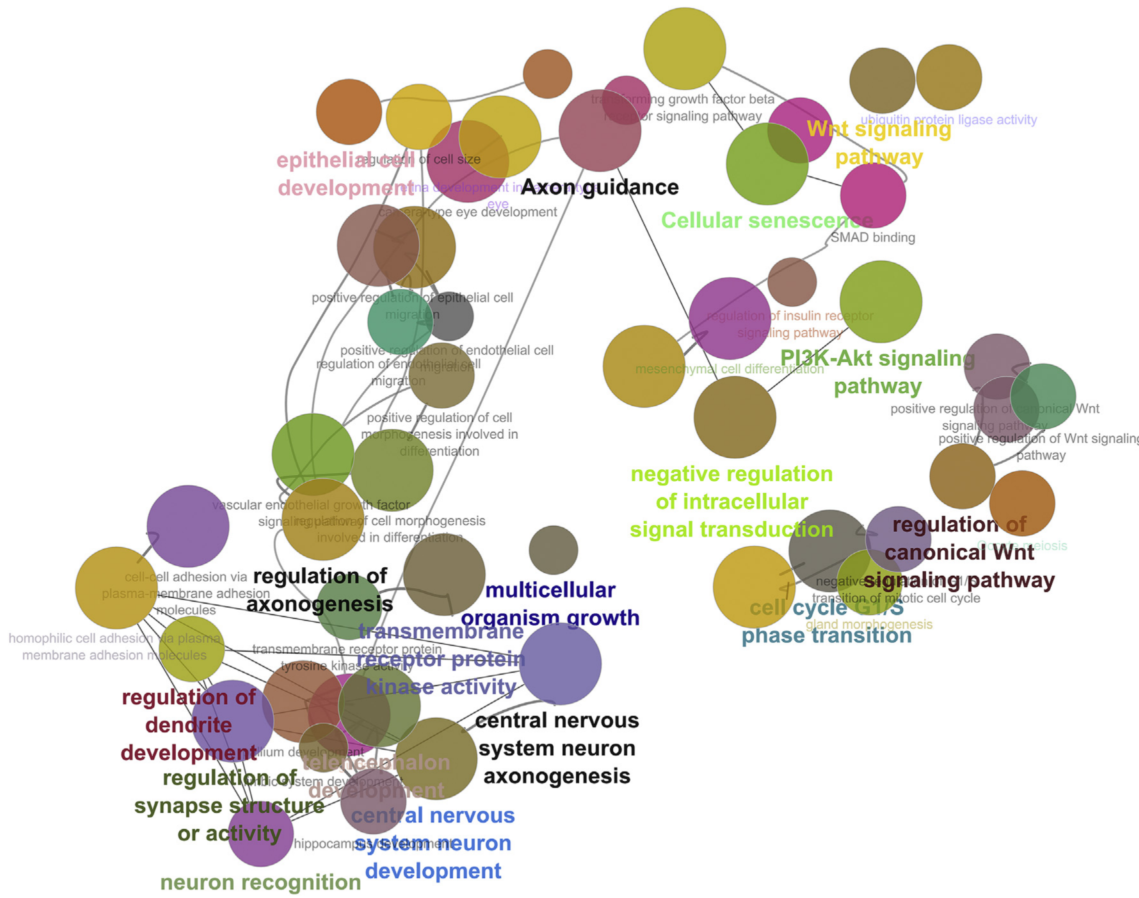

Fig. 5. Gene Ontology enrichment analysis of the differentially expressed cmiRNAs in ADHD samples. Nodes represent ontology terms or KEGG pathways that were significantly enriched for the predicted target genes. Edges represent connections within the ontology tree and colors highlight terms correlated in meaning. The most significant terms of the group showing a biological meaning in association with the system under analysis are captioned. A p-value of < 0.01 was considered statistically significant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

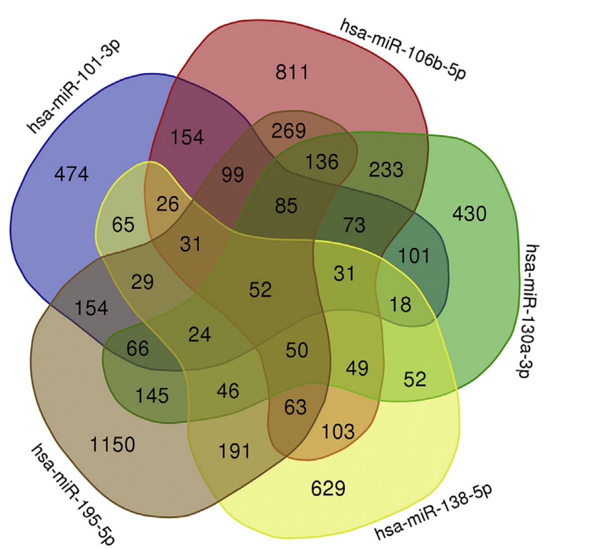

Fig. 6. Venn diagram of miRNAs targets. All predicted genes for the differentially expressed miRNAs were selected for checking gene targets overlap. Results show most of the miRNAs target common genes.

downregulated) was observed in ADHD individuals. Previous investigations have shown the altered expression of hsa-miR101-3p, hsa-miR-130a-3p, and hsa-miR-195-5p autism (Hu et al., 2017; Vasu et al., 2014), hsa-miR-138-5p in anxiety (Muiños-Gimeno et al., 2011), hsa-miR-195-5p in schizophrenia (Wei et al., 2015), and hsa-miR-106b-5p in ADHD (Kandemir et al., 2014). The potential role of these cmiRNAs in different neuropsychiatric disorders is intriguing, as the researchers suggest that neuropsychiatric disorders including AHD, autism, schizophrenia and anxiety disorder have overlapping neurological features and common genetic background (Doherty and Owen, 2014). Hsa-miR-101orchestrates many neural networks and functions such as neural excitation, synapse formation and dendritic growth by targeting NKCC1, Kif1a and Ank2 which control GABA signaling. Perturbation of this axis displayed induction of hyper-excitability and constant memory impairment (Lippi et al., 2016). Also, hsa-miR101 regulates the metabolism of amyloid precursor protein in hippocampus neurons by the controlling the expression of Ran-binding protein 9 (RanBP9) which is important in memory loss in Alzheimer's disease (Barbato et al., 2014). These findings, in consistence with our results, suggest common neuronal mechanisms involved in the pathogenesis of ADHD and Alzheimer's. MiR-106b acts in concert with some other miRNAs to control cell cycle arrest during neuronal lineage differentiation by targeting cyclinD1 (CCND1), E2F1, CDKN1A (p21), PTEN, RB1, RBL1 (p107), RBL2 (p130) (Trompeter et al., 2011). Moreover, Toma et al. have previously reported an association between an SNP in the hsa-miR-106b gene and autism using whole exome sequencing which supports our finding concerning the involvement of this gene in brain disorder (Toma et al., 2015). Hsa-miR-130a modulates neurite outgrowth and dendritic density via downregulation of MECP2 gene which its dysfunction has already demonstrated association with Rett syndrome and autism (Zhang et al., 2016). This is in line with our results from GO, suggesting the molecular mechanisms involved in ADHD are in common with autism.

In addition, our data revealed a statistically significant correlation between the hsa-miR-138-5p expression and neuropsychiatric total score parameter, suggesting a potential association between circulating miRNAs expression and biological processes involved in attention and hyperactivity control such as dopaminergic pathways. Hsa-miR-138-5p is highly expressed in different parts of the brain such as prefrontal cortex, midbrain, and hippocampus (Betel et al., 2008). Moreover, hsa-miR-138-5p is found in dendritic cells and is suggested to regulate the dendritic spines formation in hippocampus neurons (Siegel et al., 2009). It was confirmed that hsa-miR-138-5p activates glycogen synthase kinase-3β (GSK-3β) and suppresses retinoic acid receptor alpha (RARA), leading to increased tau phosphorylation which controls synaptic plasticity and the formation of memory (Schröder et al., 2014; Wang et al., 2015). Also, hsa-miR-195-5p has been shown that down-regulates APP, death receptor 6 (DR-6) and BACE1 proteins in the hippocampus and cortex of rats protecting them against dementia by preventing dendritic degeneration and neural death (Ai et al., 2013; Chen et al., 2017). Our gene ontology enrichment analysis revealed a number of biological pathways and target genes for the differentially expressed cmiRNAs. Most of the genes were involved in neurobiological pathways such as regulation of dendrite development, neuron recognition, regulation of axon regeneration, central nervous system neuron axonogenesis, regulation synapse structure or activity, and central nervous system neuron development which highlights the association of these miRNAs with psychiatric disorders such as ADHD. Our results consistent with previous studies suggest ADHD in common with many other brain disorders share similar molecular pathways and pathogenesis.

Finally, significant predictive values were obtained by ROC curve analysis for four cmiRNAs with differential expression (Fig. 4). Four cmiRNAs including hsa-miR101-3p, hsa-miR-106b-5p, hsa-miR-130a-3p, and hsa-miR-138-5p revealed higher sensitivity, specificity and area under the curve (AUC) values. Therefore, these cmiRNAs may be considered as potential candidates for discriminating ADHD from unaffected healthy controls.

The discovery of noninvasive biomarkers in clinical experiments that could provide early detection of ADHD is presently the spotlight of neuropsychiatric research. Currently, clinical diagnosis of ADHD is based on DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, fourth edition). Therefore, investigation of cmiRNAs expression profile could be offered as a potentially important molecular diagnostic tool for detection of ADHD. To reach this goal, our findings, as an initial study, suggest that cmiRNAs could be considered as potentially important biomarkers of ADHD. Although the number of studied subjects was limited in this study, there the cmiRNAs expression showed high diagnostic values and correlation with neurological pathways.

5. Conclusions

In the current noninvasive study, statistically significant alterations of five cmiRNAs were observed in the sera of ADHD individuals. The computational enrichment analysis displayed that the targets of these miRNAs function in multiple neurological pathways and functions. Also, these miRNAs have shown significant predictive power which are proposed as promising biomarkers to distinguish ADHD patients from healthy individuals.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2018.12.013.

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