RESEARCH ARTICLE

Molecular Portrait of Potential Attention Deficit/ Hyperactivity Disorder Candidate Genes and Regulating Micrornas Expression in Normal Human Developing Brain Tissues

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Abstract

Attention-deficit/hyperactivity disorder (ADHD) is the most common neuropsychiatric disorder in childhood affecting 5-6% of children, and is a major global health concern, which seems to increase in magnitude. The etiology of ADHD is still poorly understood, however; there are indications of genetic as well as environmental and epigenetic factors contributing to the development of the disorder. The objectives of this study was i) to identify potential ADHD candidate genes; ii) to explore spatial and temporal transcriptional fluctuation of the identified ADHD candidate genes in normal developing human brain tissues, and iii) to identify miRNAs regulating the identified ADHD candidate genes and explore how these miRNAs are expressed in normal developing human brain tissues.

From search in literature and publicly available databases, we identified 103 shared potential ADHD candidate genes. These genes were expressed and enriched in several human brain regions and developmental stages. Clustering analysis of these genes based on their expression levels showed a clear difference between fetal stage and the other developmental stages. There was no clear gender or brain region differences between samples. Further, functional analysis of these genes revealed that they participate in a variety of different and widely distributed functional pathways implicated with ADHD.

From miRNA-target prediction analysis, we identified twenty miRNAs regulating the identified 103 genes, and the expression pattern of these miRNAs was developmental stage dependent. These miRNAs were enriched in functional pathways and disease ontologies relevant to neurodevelopment.

The knowledge of the expression pattern of potential ADHD candidate genes and miRNAs, which regulate these genes across different stages of brain development, is essential for understanding normal brain development and subsequent disease development of the brain. In addition, identification of miRNA-regulated ADHD candidate genes can be used to develop blood-based molecular markers to be investigated in future studies of ADHD patients.

Keywords: Attention-deficit/hyperactivity disorder, ADHD, microRNA, epigenetics, neurodevelopment, transcription

1. Background

Attention-deficit/hyperactivity disorder (ADHD) is the most common neuropsychiatric disorder in childhood affecting 5-6% of children, which often begins during childhood persisting into adulthood in the majority of patients(1, 2), and is associated with poor academic and social outcomes (3). The core areas of difficulties for subjects with ADHD are hyperactivity, impulsivity, and inattention over time and across situations (4),(5). The etiology of ADHD is still poorly understood, however; there are indications of genetic as well as environmental factors contributing to the development of the disorder, and epigenetic changes have been suggested to be involved. It also shares genetic risk factors with other neurodevelopmental disorders like autism, schizophrenia and epilepsy (6). Family studies have shown that ADHD runs in families (7), and twin studies indicate that the heritability of ADHD in children is 70-80% (8);(9). However, findings from molecular genetic studies thus far can only explain a small fraction of the heritability(10), indicating that ADHD risk variants will be of very small effect size and include multiple rare variants (8);(11). The disorder is associated with impaired social functioning, lower academic achievement, substance abuse and criminality. In addition, ADHD is associated with increased healthcare costs for patients and their family members. The disorder is thus of great societal concern, and increased knowledge of the etiology may lead to earlier diagnosis and improved treatment and health (12);(13);(14).

Candidate gene association studies have focused on genes related to catecholamines, and meta-analyses have indicated that the most consistent findings are related to the dopaminergic and serotonergic systems (15). A recent meta-analysis of ADHD Genome-wide association studies (GWAS) identified significant risk loci (12 genomic loci) located within or nearby genes involved in neurodevelopment processes (16). Accumulating evidence, however, indicate that rare mutations of larger effects may account for a substantial proportion of the heritability of complex disorders (17). Some of the missing heritability might be explained by gene-environment interaction and epigenetic mechanisms.

There is a probable interplay between genetics, epigenetics and environmental factors that is far from understood. More knowledge concerning this interplay is likely to contribute to a better understanding of early-life exposures, maternal or paternal, and consequences for the health of the child later in life. By means of mouse models it has been shown that in utero exposure leads to changes which are persistent through several generations and they were most probably due to epigenetic rather than genetic mechanisms (18);(19);(20). Epigenetic modifications like DNA methylation, histone modification, chromatin remodeling, and microRNAs, are influenced by nutritional and environmental factors, and may regulate gene expression downstream of both environmental and genetic risk factors. Epigenetic changes in early life may influence disease susceptibility in later life, and mediation of environmental factors on epigenetic mechanism may have a key role in the onset and course of common neurological conditions, including ADHD.

As being a part of the epigenetic modulators, miRNAs are abundant in the nervous system, where they are involved in neural development and are likely an important mediator of neuronal plasticity. MiRNAs' role in neurodevelopmental diseases, both as diagnostic biomarkers as well as explaining basic disease etiology has come into focus. Aberrant miRNA function has been linked to the etiology of several neurological disorders, including fragile X syndrome, schizophrenia, autism spectrum

disorders (ASD), and Alzheimer disease (21);(22);(23);(24);(25);(26). The relationship between microRNA dysfunctions and neurological diseases is illustrated with fragile X mental retardation(21), and the absence of Fragile X mental retardation 1 protein (FMRP) impairs Dicer and RISC functions required for miRNA-mediated synaptic plasticity and dendritic development (21). Further, miRNA-mediated transcriptional regulation is dynamic and they play a role in the fine-tuning of protein translation, contributing to the molecular pathogenesis of neurodevelopmental disorders. A comprehensive understanding of how miRNA transcriptional response during human neurodevelopment is regulated is important. In recent years, there are studies investigating the role of miRNAs in ADHD (27);(28, 29);(30);(31);(32);(33);(34). These studies provide preliminary evidence; however, most of these studies are underpowered and there is so far little overlap between the identified ADHD linked miRNAs.

In order to understand pathogenesis mediated gene expression modulations, it is important to get a complete picture of gene expression regulation during normal human brain development, and how dysregulation of these processes contribute to the molecular pathogenesis of neurodevelopmental disorders, including ADHD. Thus, getting deeper insight into transcriptional regulation mediated by miRNAs that occur under normal neurodevelopment is essential for understanding the abnormal changes that may occur in the onset and course of common neurological conditions, including ADHD.

The primary objectives of this study was i) to identify potential ADHD candidate genes by searching the literature and publicly available disease-gene databases; ii) to explore spatial and temporal transcriptional fluctuation of already identified ADHD candidate genes in normal developing human brain tissues, and iii) to identify miRNAs regulating these ADHD candidate genes and explore how these miRNAs are expressed in normal developing human brain tissues. Secondary objectives were to identify miRNAs that regulate ADHD associated genes, and the role of these miRNA regulators as potential early biomarkers of ADHD. The identified miRNAs will be investigated in an ongoing ADHD project.

2. Methods

2.1 Selection of potential ADHD candidate gene set and functional analysis

To identify potential ADHD candidate genes, we downloaded all genes related to ADHD from the ADHDgene database (35), DisGeNET (36), and GeneCards (www.genecards.org) (37), and ADHD gene list from recent summary statistic of ADHD GWAS meta-analysis of European ancestry was obtained from the supplementary data of recent studies (38). We then compared the four gene lists and calculated intersecting genes. Shared genes among the four gene lists were selected as potential ADHD candidate gene set, and these genes were used in downstream analysis. Functional analysis of the identified ADHD gene set was performed using the gene set enrichments analysis tool GENEASE (39) to determine functional pathways relevant to the identified ADHD gene set.

2.2 Expression pattern of ADHD candidate gene set

The expression pattern of the selected ADHD candidate gene set was extracted from BrainSnap (https://www.brainspan.org/), an atlas of the developing human brain (40), and the details of tissue acquisition, processing, and RNA-sequencing can be found on the website. BrainSnap contains transcriptomic data categorized into different major brain developmental stages (RNA-seq data) from postmortem brains collected from individuals

ranging from 5-7 post-conceptional weeks (pcw) up to 40 years of age. RNA-Seq expression data are available for 13 stages of development over 16 brain regions. First, the RNA-seq data of human brain region- and developmental stage samples was downloaded (Developmental Transcriptome: RNA-Seq Gencode v10 summarized to genes), and then the RPKM (reads per kilobase per million) of the selected ADHD candidate gene set was extracted from the RNA-seq data. We regrouped the original samples into five developmental stages (fetal (8-38 pcw), infancy (birth-12 month), childhood (1-11 year), adolescence (12-19 year) and adulthood (21-40 year), respectively). The limma Bioconductor package (41) was used to compare these five groups and to identify genes significantly differentially expressed between the five developmental stages.

2.3 ADHD gene set targeting miRNA prediction and functional network analysis.

The Mienturnet-tool (microRNA-target enrichment and network-based analysis) (42) was used to predict the miRNAs targeting the identified ADHD gene set. We selected only experimentally validated miRNA-target interactions with strong evidence from the miRTarBase (43). The computed topological properties for each node in the miRNA-target interaction network was imported into the CytoScape v3.7.1 (44) to display the miRNA-target interaction network. The identified miRNA-target pairs was further analyzed with miRmapper R-package (45) in order to identify the most dominant miRNAs or mRNAs in the miRNA-target network to find similarities between miRNAs based on commonly regulated genes. Further, the Mienturnet-tool offers the possibility to perform a functional enrichment analysis of the targets of selected miRNAs.

2.4 Expression profile of the ADHD gene set targeting miRNAs

The read counts of the small-RNA-seq data was downloaded from BrainSnap, and as RNA-seq data, we regrouped the original samples into four developmental stages (infancy (birth-12 month), childhood (1-11 year), adolescence (12-19 year) and adulthood (21-40 year), respectively). The details of small RNA-seq data, i.e., tissue acquisition, processing, and small RNA-sequencing can be found on the website. The fetal stage samples were not included in the small-RNA-seq data. The edgeR Bioconductor package (46) was used to compare these four groups and identified miRNAs significantly differentially expressed between the four developmental stages. Then the expression pattern of the identified miRNAs targeting ADHD genes (from section 2.3) was extracted from the small-RNA-seq data and their expression pattern was evaluated.

3 Results

To identify potential ADHD associated genes, a search was conducted in public databases and literature. ADHD associated genes were identified from curated databases and from supplementary data from a recent study (38). Based on such search, we constructed four gene lists and compared them. A gene was included in the potential ADHD candidate gene set if it was present in all four gene lists. From such analysis, we identified 103 shared potential ADHD candidate genes (Figure 1A and Table 1) and these genes were used in downstream analysis. It is however important to notice that the identified 103 genes did not necessarily reach genome-wide significance. To ascribe biological relevance to the selected 103 ADHD gene set, we conducted a functional enrichment analysis using the GESEASE tool (39). The result of the functional pathway enrichment analysis is presented in Figure 1B. The top ten enriched signaling pathways represented by our 103 gene set were

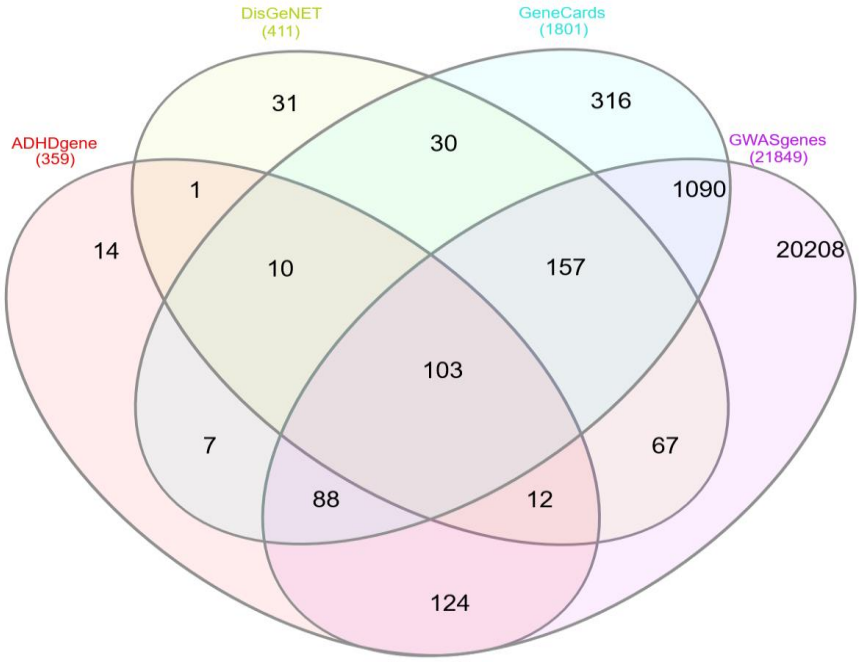
neuroactive ligand-receptor interaction, dopaminergic synapse, synaptic vesicle cycle, cocaine addiction, calcium signaling pathway, cAMP signaling pathway, serotonergic synapse, glutamatergic synapse, alcoholism and circadian entrainment, respectively (Figure 1B) and most of these pathways are implicated with ADHD.

Table 1. Potential ADHD candidate genes (n = 103 genes)

Gene symbol	Description	Gene symbol	Description
ADRA1A	Adrenoceptor Alpha 1A	GRM8	Glutamate Metabotropic Receptor 8
ADRA1B	Adrenoceptor Alpha 1B	GSK3B	Glycogen Synthase Kinase 3 Beta
ADRA2A	Adrenoceptor Alpha 2A	HK1	Hexokinase 1
ADRA2B	Adrenoceptor Alpha 2B	HTR1A	5-Hydroxytryptamine Receptor 1A
ADRA2C	Adrenoceptor Alpha 2C	HTR1B	5-Hydroxytryptamine Receptor 1B
AK8	Adenylate Kinase 8	HTR1D	5-Hydroxytryptamine Receptor 1D
ANK3	Ankyrin 3	HTR2A	5-Hydroxytryptamine Receptor 2A
ARVCF	ARVCF Delta Catenin Family Member	IL16	Interleukin 16
ASTN2	Astrotactin 2	IL1RN	Interleukin 1 Receptor Antagonist
ATP2C2	ATPase Secretory Pathway Ca2+ Transporting 2	ITGA1	Integrin Subunit Alpha 1
ATXN1	Ataxin 1	ITGA11	Integrin Subunit Alpha 11
BAIAP2	BAR/IMD Domain Containing Adaptor Protein 2	ITGAE	Integrin Subunit Alpha E
BCHE	Butyrylcholinesterase	ITIH3	Inter-Alpha-Trypsin Inhibitor Heavy Chain 3
BDNF	Brain Derived Neurotrophic Factor	MAP1B	Microtubule Associated Protein 1B
CACNA1C	Calcium Voltage-Gated Channel Subunit Alpha1 C	MC4R	Melanocortin 4 Receptor
CALY	Calcyon Neuron Specific Vesicular Protein	MOBP	Myelin Associated Oligodendrocyte Basic Protein
CDK20	Cyclin Dependent Kinase 20	MTHFR	Methylenetetrahydrofolate Reductase
CHRNA3	Cholinergic Receptor Nicotinic Alpha 3 Subunit	MTNR1A	Melatonin Receptor 1A
CHRNA4	Cholinergic Receptor Nicotinic Alpha 4 Subunit	NET1	Neuroepithelial Cell Transforming 1
CHRNA7	Cholinergic Receptor Nicotinic Alpha 7 Subunit	NGF	Nerve Growth Factor
CLOCK	Clock Circadian Regulator	NOS1	Nitric Oxide Synthase 1
CNR1	Cannabinoid Receptor 1	NPSR1	Neuropeptide S Receptor 1
CNTFR	Ciliary Neurotrophic Factor Receptor	NR3C2	Nuclear Receptor Subfamily 3 Group C Member 2
COMT	Catechol-O-Methyltransferase	NR4A2	Nuclear Receptor Subfamily 4 Group A Member 2
CPLX1	Complexin 1	NT5C2	5'-Nucleotidase, Cytosolic II
CPLX2	Complexin 2	NTF3	Neurotrophin 3
CSMD2	CUB And Sushi Multiple Domains 2	NTRK2	Neurotrophic Receptor Tyrosine Kinase 2
DBH	Dopamine Beta-Hydroxylase	OPRM1	Opioid Receptor Mu 1
DCDC2	Doublecortin Domain Containing 2	OXTR	Oxytocin Receptor
DCLK1	Doublecortin Like Kinase 1	PER2	Period Circadian Regulator 2
DDC	Dopa Decarboxylase	PPP1R1B	Protein Phosphatase 1 Regulatory Inhibitor Subunit 1B

DHCR7	7-Dehydrocholesterol Reductase	PRTG	Protogenin
DIRAS2	DIRAS Family GTPase 2	PTPRN2	Protein Tyrosine Phosphatase Receptor Type N2
DISC1	DISC1 Scaffold Protein	SH2B1	SH2B Adaptor Protein 1
DPP6	Dipeptidyl Peptidase Like 6	SLC1A3	Solute Carrier Family 1 Member 3
DRD1	Dopamine Receptor D1	SLC6A1	Solute Carrier Family 6 Member 1
DRD2	Dopamine Receptor D2	SLC6A2	Solute Carrier Family 6 Member 2
DRD3	Dopamine Receptor D3	SLC6A3	Solute Carrier Family 6 Member 3
DRD4	Dopamine Receptor D4	SLC6A4	Solute Carrier Family 6 Member 4
DRD5	Dopamine Receptor D5	SLC9A9	Solute Carrier Family 9 Member A9
EMP2	Epithelial Membrane Protein 2	SNAP25	Synaptosome Associated Protein 25
FADS1	Fatty Acid Desaturase 1	SPOCK3	SPARC (Osteonectin), Cwcv And Kazal Like Domains Proteoglycan 3
FADS2	Fatty Acid Desaturase 2	STX1A	Syntaxin 1A
FTO	FTO Alpha-Ketoglutarate Dependent Dioxygenase	SYN3	Synapsin III
GDNF	Glial Cell Derived Neurotrophic Factor	SYT2	Synaptotagmin 2
GIT1	GIT ArfGAP 1	TPH1	Tryptophan Hydroxylase 1
GPC6	Glypican 6	TPH2	Tryptophan Hydroxylase 2
GRIN2A	Glutamate Ionotropic Receptor NMDA Type Subunit 2A	TRIM32	Tripartite Motif Containing 32
GRIN2B	Glutamate Ionotropic Receptor NMDA Type Subunit 2B	TRIO	Trio Rho Guanine Nucleotide Exchange Factor
GRM1	Glutamate Metabotropic Receptor 1	VAMP2	Vesicle Associated Membrane Protein 2
GRM5	Glutamate Metabotropic Receptor 5	ZNF804A	Zinc Finger Protein 804A
GRM7	Glutamate Metabotropic Receptor 7		

A)



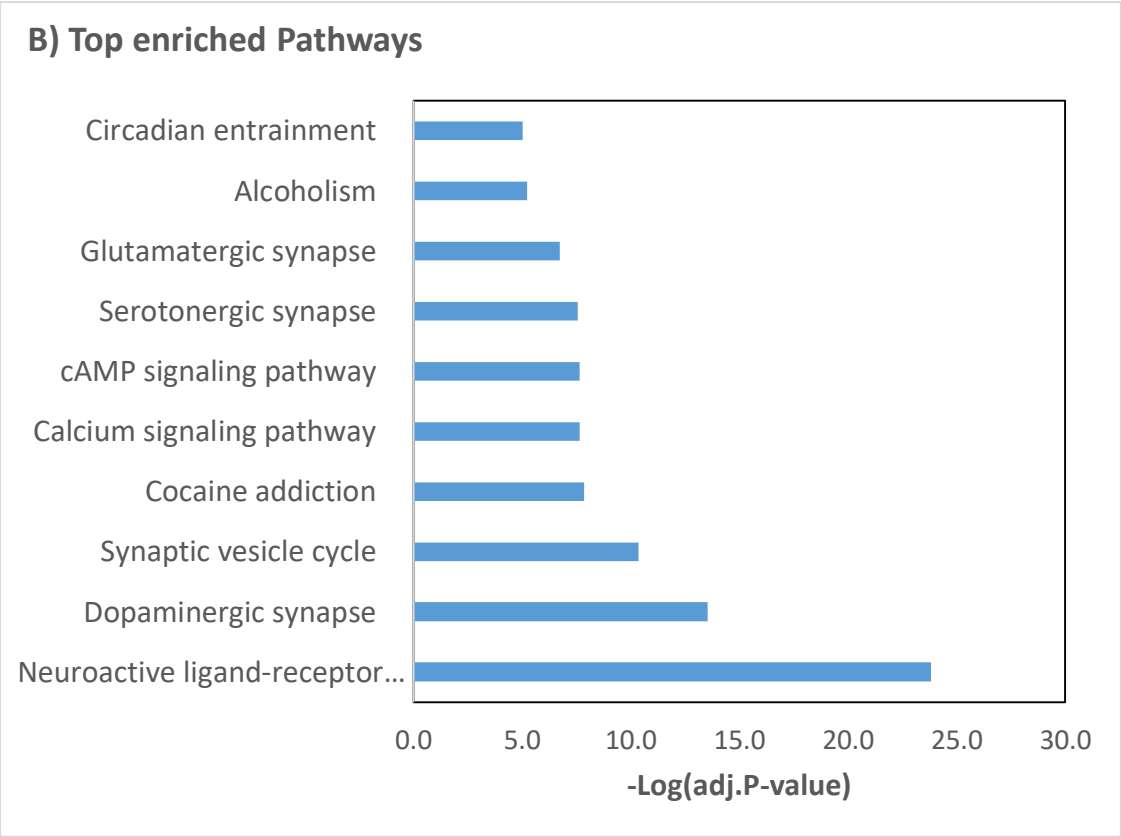


Figure 1. Identification of potential ADHD candidate gene set and functional analysis. A) Venn diagram of four ADHD gene list from curated databases and literature. The 103 shared genes are potential ADHD candidate genes and used in downstream analysis. The Venn diagram was drawn by InteractiVenn, a web-based tool (83). B) Top enriched pathways (adjusted $p < 1x\ 10^{-5}$, Benjamini-Hochberg (BH) method). Functional pathway enrichment analysis of the selected 103 ADHD gene set and the top ten most enrich pathways are presented. The enrichment analysis indicated that the identified 103 ADHD gene set were involved in a range of signaling pathways.

To get insight into the transcriptional dynamics of the identified 103 ADHD candidate gene set in developing brain, we extracted the transcription data of these genes from BrainSnap (40). We performed principal component analysis (PCA) and multi-dimensional scaling (MDS) on their transcription data. The analysis revealed that there was a clear developmental stage difference between samples, and samples from fetal stages clustered closer together than samples from other developmental stages (infancy, childhood, adolescences and adulthood stages) (Figure 2A and B). The observed differences of the gene expression pattern due to developmental stage variance contributes more than the other variables (e.g. gender or brain region) for these 103 ADHD candidate genes. We then conducted hierarchical clustering analysis in order to group samples from different developmental stages based on their transcript level similarity, and the hierarchical clustering analyses results were visualized in a dendrogram and are presented in Figure 2C. By visual inspection of the clustering dendrogram, we observed that fetal stage samples clustered close to each other;

however, there were a few samples from the fetal stages, which clustered together with the other developmental stage samples (Figure 2C). We then compared the transcript level of the five developmental stages using limma package (41). From such analysis, we observed that there were many genes which are differentially expressed between the five developmental stages (Figure 2D), and more than 60 genes were differentially expressed between fetal stage samples and samples from the other developmental stages. This further confirms the clustering results where the fetal stage samples behave differently than samples from the other stages.

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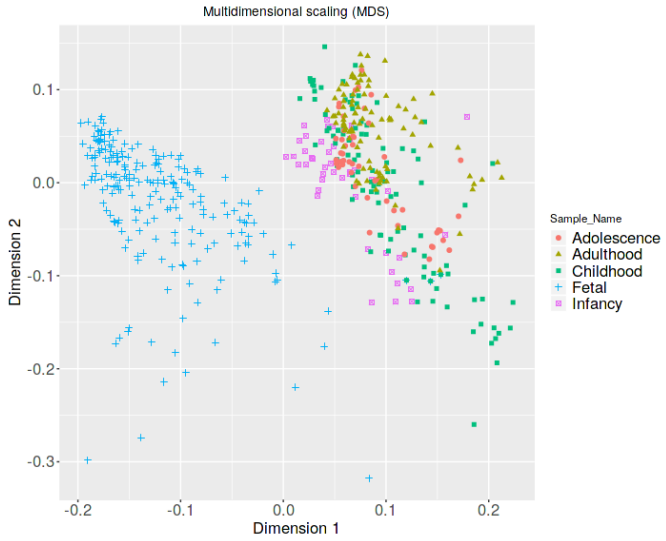

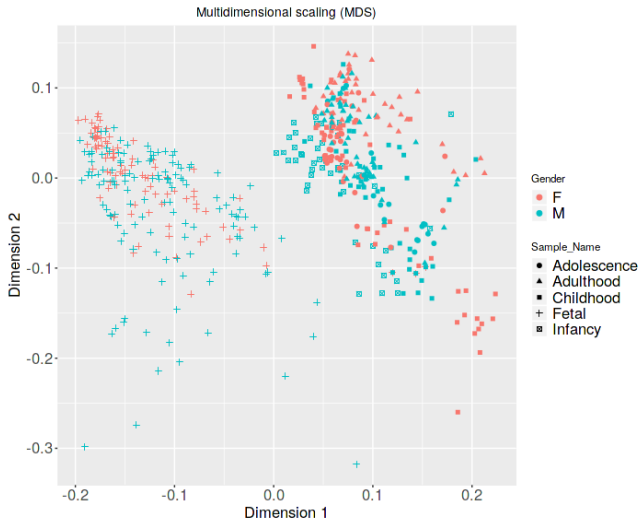

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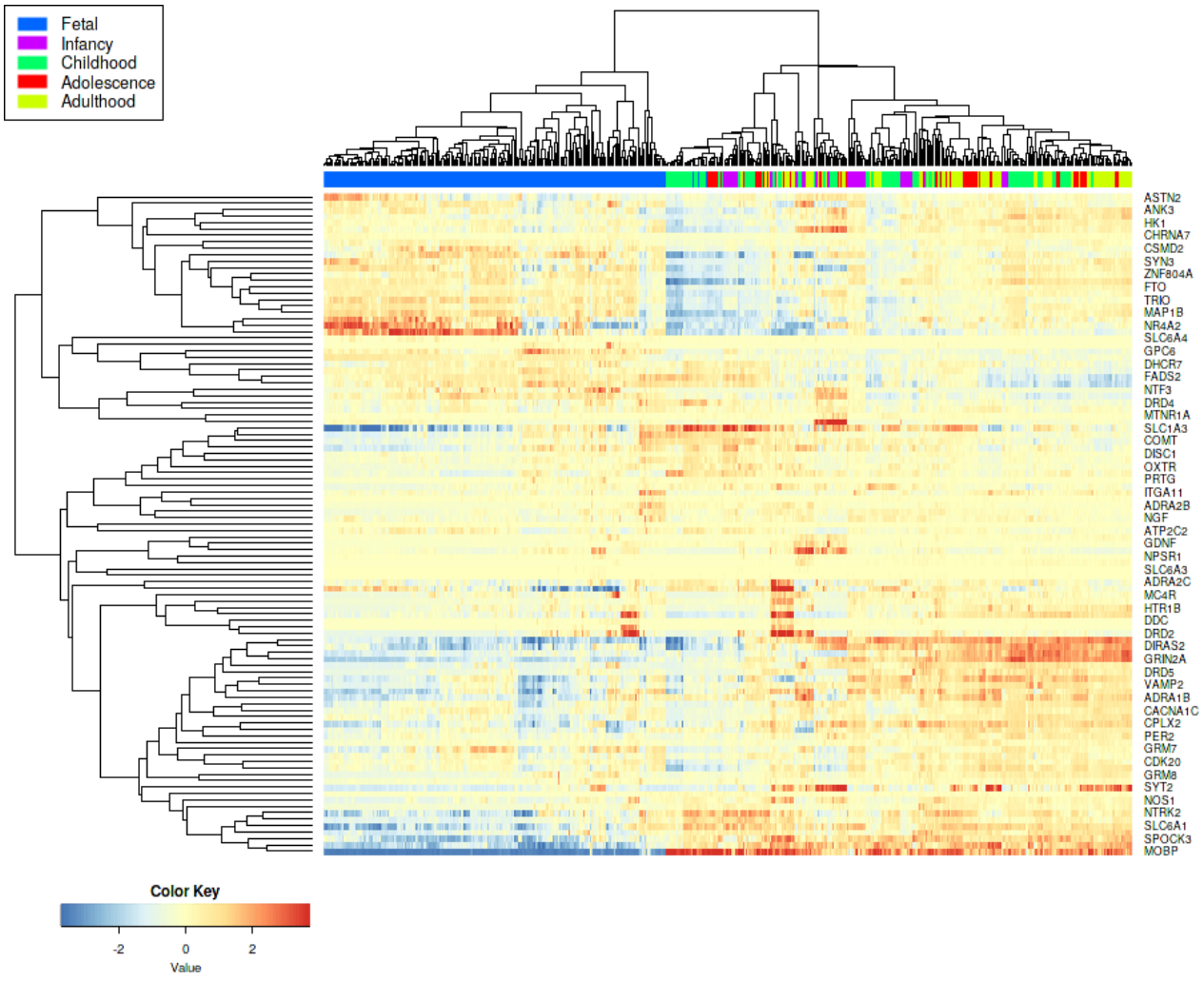

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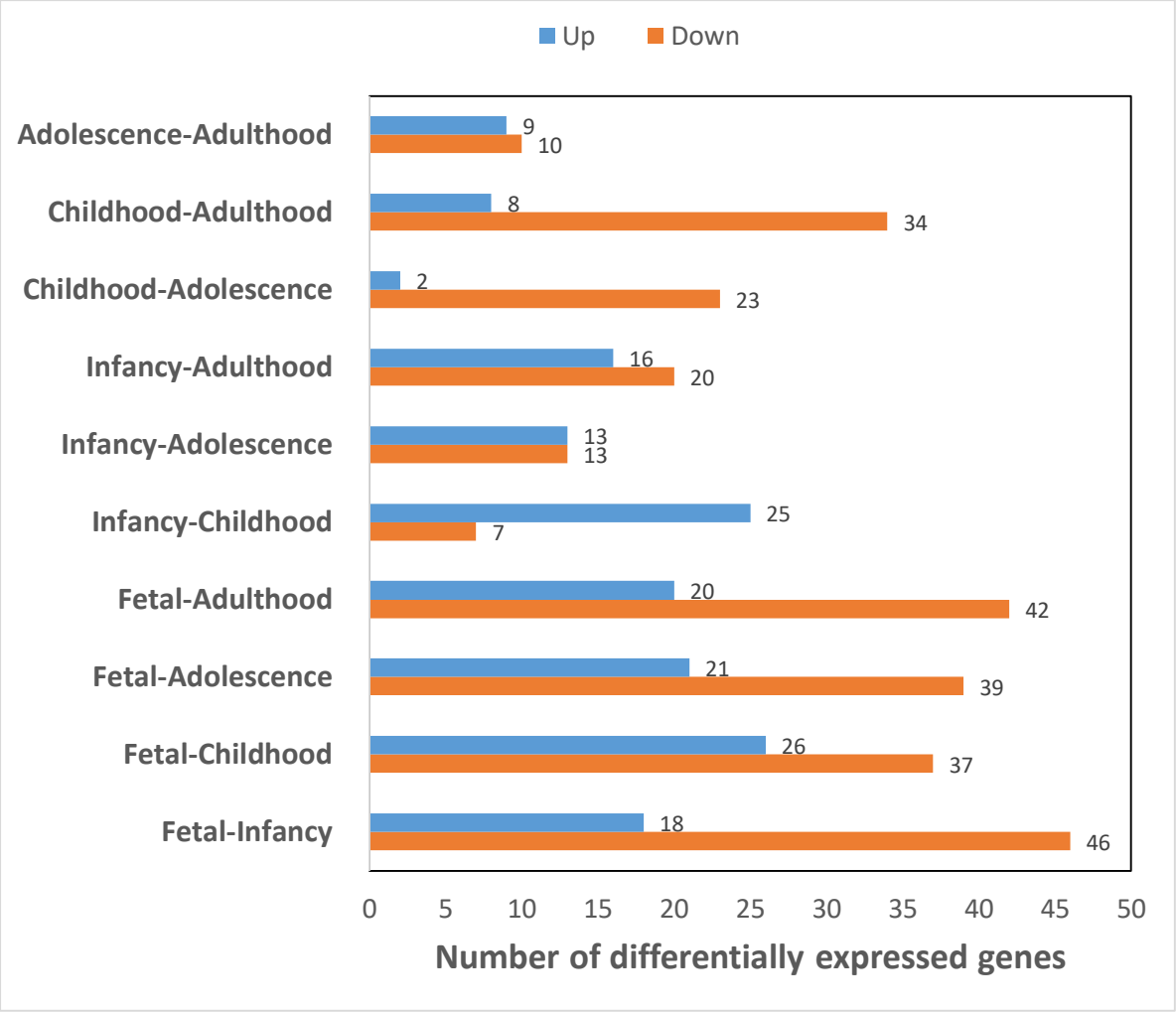


Figure 2. **The expression pattern of the 103 ADHD candidate genes in developing brain tissues**. A) Principle component analysis (PCA) and B) multi-dimensional scaling (MDS) analysis of the 103 ADHD gene set separate samples from fetal stages from the other development stages (infancy, childhood, adolescences and adulthood stages). Samples are color coded either by developmental stages, gender (F=female and M=male) or brain regions (A1C: primary auditory (A1) cortex; AMY: amygdala; CBC/CB: cerebellar cortex; CGE: caudal ganglionic eminence; DFC: dorsolateral prefrontal cortex; HIP: hippocampus; IPC: posterior inferior parietal cortex; ITC: inferior temporal cortex; M1C: primary motor (M1) cortex; MD: mediodorsal nucleus of the thalamus; MFC: medial prefrontal cortex; OFC: orbital prefrontal cortex; S1C: primary somatosensory (S1) cortex; STC: superior temporal cortex; STR: striatum; V1C: primary visual (V1) cortex and VFC: ventrolateral prefrontal cortex). C) Unsupervised hierarchical clustering analysis of the expression level of 103 genes after normalization and log2-transformation of the data. The hierarchical clustering analysis is based on similarities in gene expression. Samples are horizontally labeled based on the developmental stage they belong to. D) Comparison of the five developmental stages (fetal, infancy, childhood, adolescences and adulthood stages) to identify statistically significantly differentially expressed genes between the developmental stages (a gene is considered significantly expressed with FDR< 0.05 and fold change > 1.2).

To get a comprehensive picture of spatial-temporal expression pattern of the 103 ADHD candidate gene set in human brain regions and developmental stages, we performed *in silico* analysis of the 103 ADHD candidate genes using CSEA-tool (47). The CSEA-tool calculated the tissue-specific enrichment score (pSI: specificity index statistic) and the brain tissue expression data used to calculate the pSI are from the BrainSpan transcriptomic data. We used a pSI (specificity index statistic) threshold of 0.05 in order to examine whether the 103 ADHD genes were enriched in specific human brain regions and developmental stages. We observed that the expression levels of the 103 ADHD genes were significantly enriched in several brain regions and developmental stages (Figure 3A); i.e., significant enrichment in the cerebellum (late-fetal and mid-late childhood), hippocampus (early-infancy and mid-late childhood), striatum (early-infancy and early-late childhood, adolescence and young adulthood) and thalamus (early-infancy and mid-late childhood, adolescence and young adulthood). The genes were also enriched in the cortex of the adolescence developmental stage.

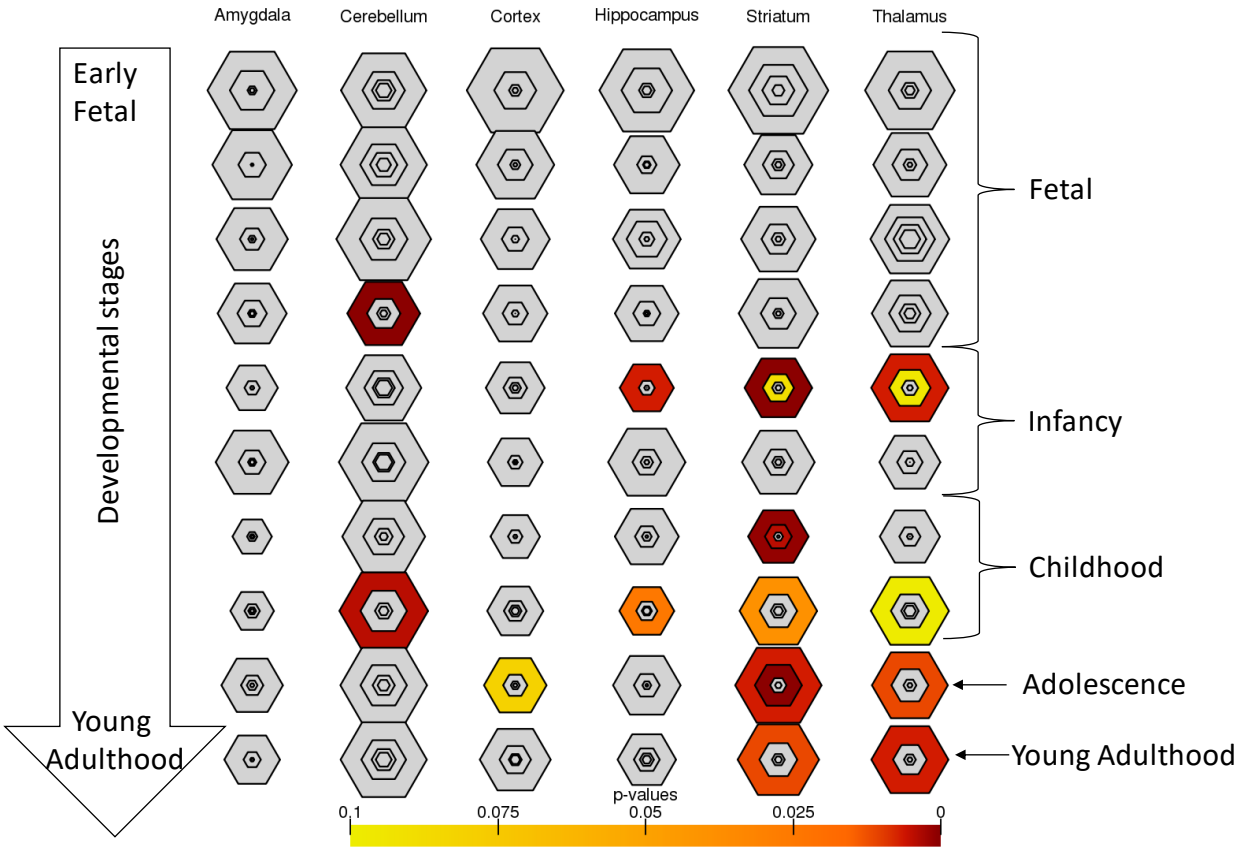
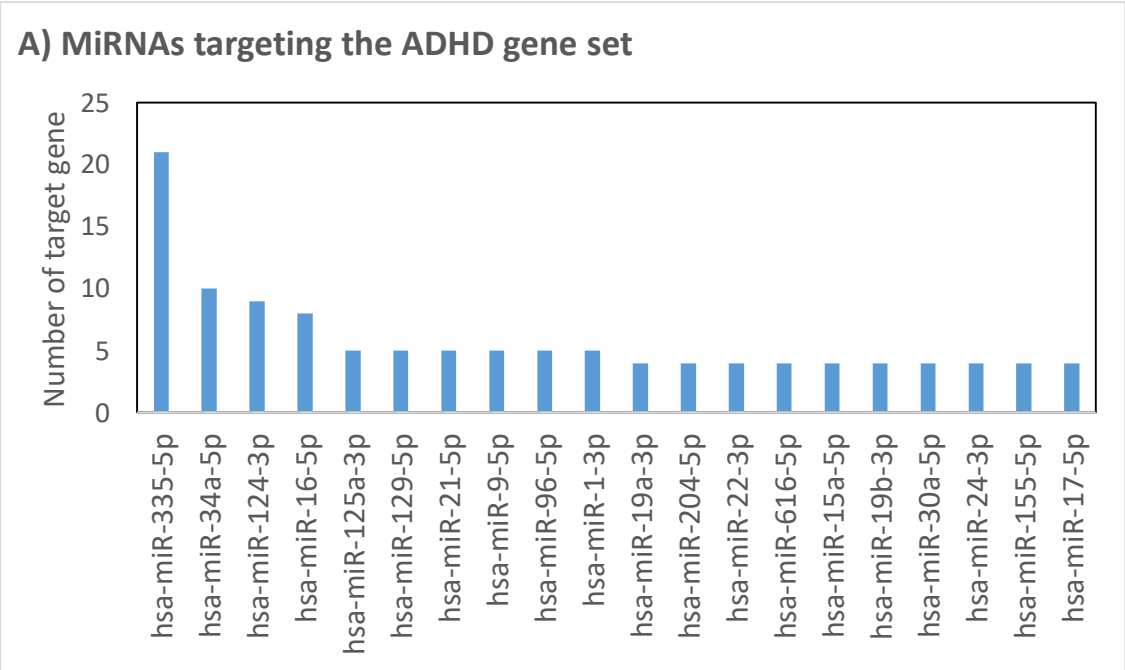


Figure 3. Brain region and developmental enrichment analysis of the 103 ADHD gene set. The CSEA-tool was used to analyze the 103 ADHD gene set enrichment in specific human brain regions and developmental stages. The color codes represent the Benjamini-Hochberg (BH) corrected one-tailed Fisher's Exact test p-values, and shaded regions closer to the center of each hexagon indicate increasing tissue specificity.

MiRNAs are expressed in mammalian brains specifically and influence multiple circuits in the brain, suggesting their unique roles in neurodevelopment and brain function (48), and their involvement in anxiety, exploration, learning and memory has been reported (49). Further, a number of developmental and adult brain disorders are associated with abnormal changes in synaptic connectivity and plasticity, including fragile X syndrome and autism disorder (50). To identify miRNAs regulating the selected 103 ADHD gene set and explore how these miRNAs expressed in normal developing human brain tissues, we first searched for miRNAs targeting the 103 ADHD genes using the Mienturnet-tool (42). Since each miRNA can regulate numerous target genes and therefore has the potential to alter multiple biochemical pathways, we selected only experimentally validated miRNA-target interactions with strong evidence from the miRTarBase (43). We were interested on miRNAs that target several genes, because miRNAs targeting the same genes may infer a broader range of target level alteration and they usually have similar target genes (51). Further, miRNAs that co-regulated similar target genes may probably have a greater influence in determining phenotypic outcomes and are more important in a global biological context than miRNAs that modulate just a few genes (45, 52). MiRNA target gene sharing principle is based on that if two miRNAs share a common set of target genes, they may probably influence or co-regulate similar biological pathway(s) (51). Therefore, we selected only validated miRNA-target interactions with strong evidence and at the same time regulate several target genes. Based on these criteria, we identified 20 miRNAs which target at least four or more genes (Figure 4A). We then used the miRmapper package to identify miRNAs that work cooperatively among the identified 20 miRNAs, and the results are presented in Figure 4B. Four miRNAs (miR-17-5p, miR-129-5p, miR-19a-3p and miR-19b-3p) clustered closer together and share similar target genes (Figure 4B and C). Further, miR-17, miR-19a and miR-19b belongs to the miR-17~92 family of miRNA clusters composed of three related, highly conserved, poly-cistronic miRNA genes that collectively encode for a total of fifteen miRNAs (53), and members of this family cooperate together to fine-tune signaling and developmental pathways. It has been reported that mutations or dysregulation of this miRNA-family contributes to the pathogenesis of a variety of human diseases, including cancer and congenital developmental defects (53). The fourth miRNA, miR-129-5p does not belong to the miR-17~92 family, however, miR-129-5p cluster with this family based on target gene similarity. Another two miRNAs (miR-15a-5p and miR-16-5p) were also clustered together by their target similarity (Figure 4B and C), and these two miRNAs belong to a highly conservative miR-15/107 family (54). The members of the miR-15/107 family consist of ten miRNAs and are strongly implicated in multiple human disorders. Furthermore, four miRNAs (miR-335-5p, miR-34a-5p, miR-124-3p and miR-16-5p) had more than five targets (Figure 4A), and these miRNAs may have significant impact in the regulation of our ADHD genes.



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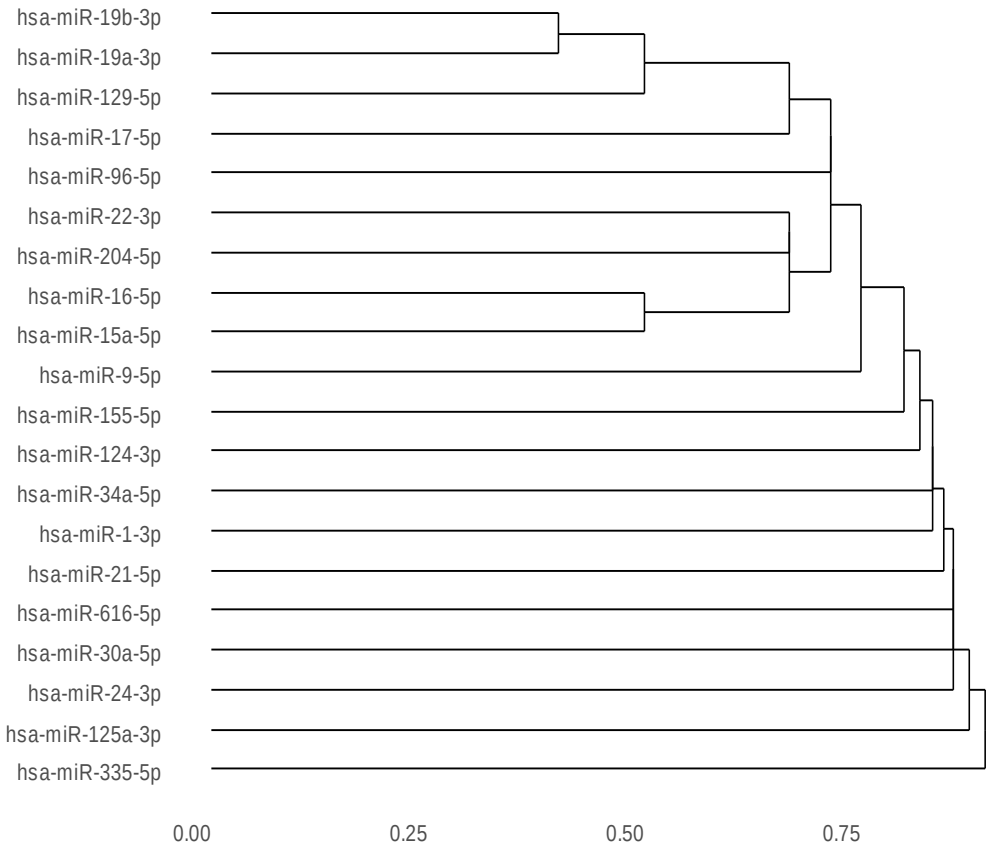

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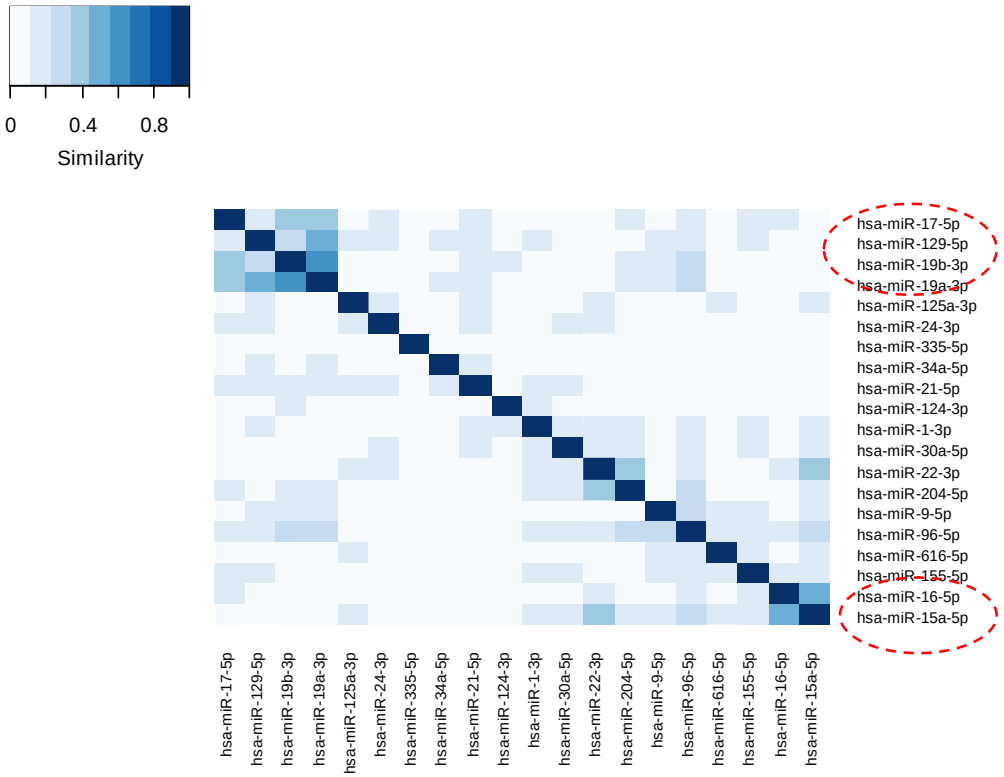
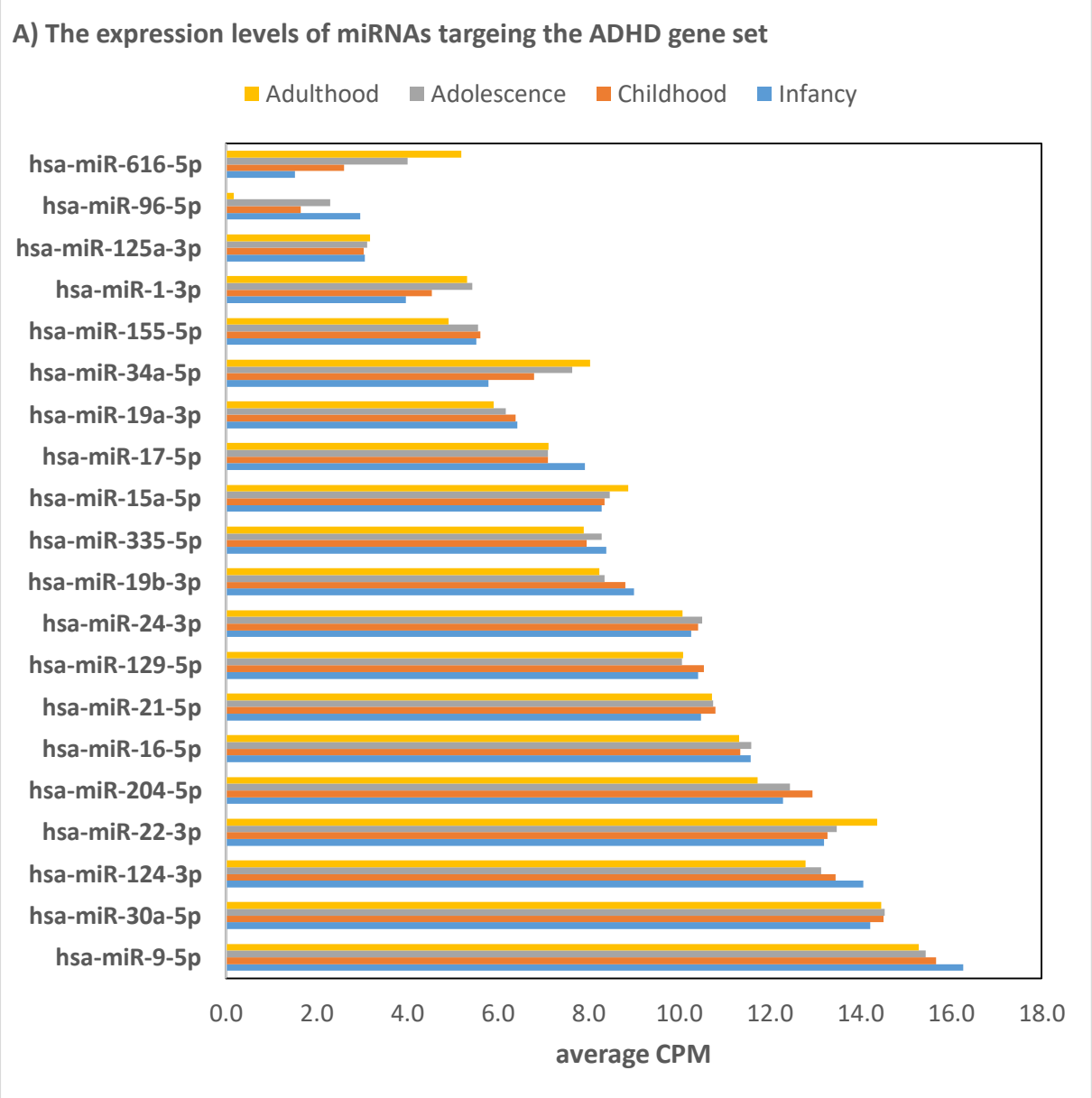
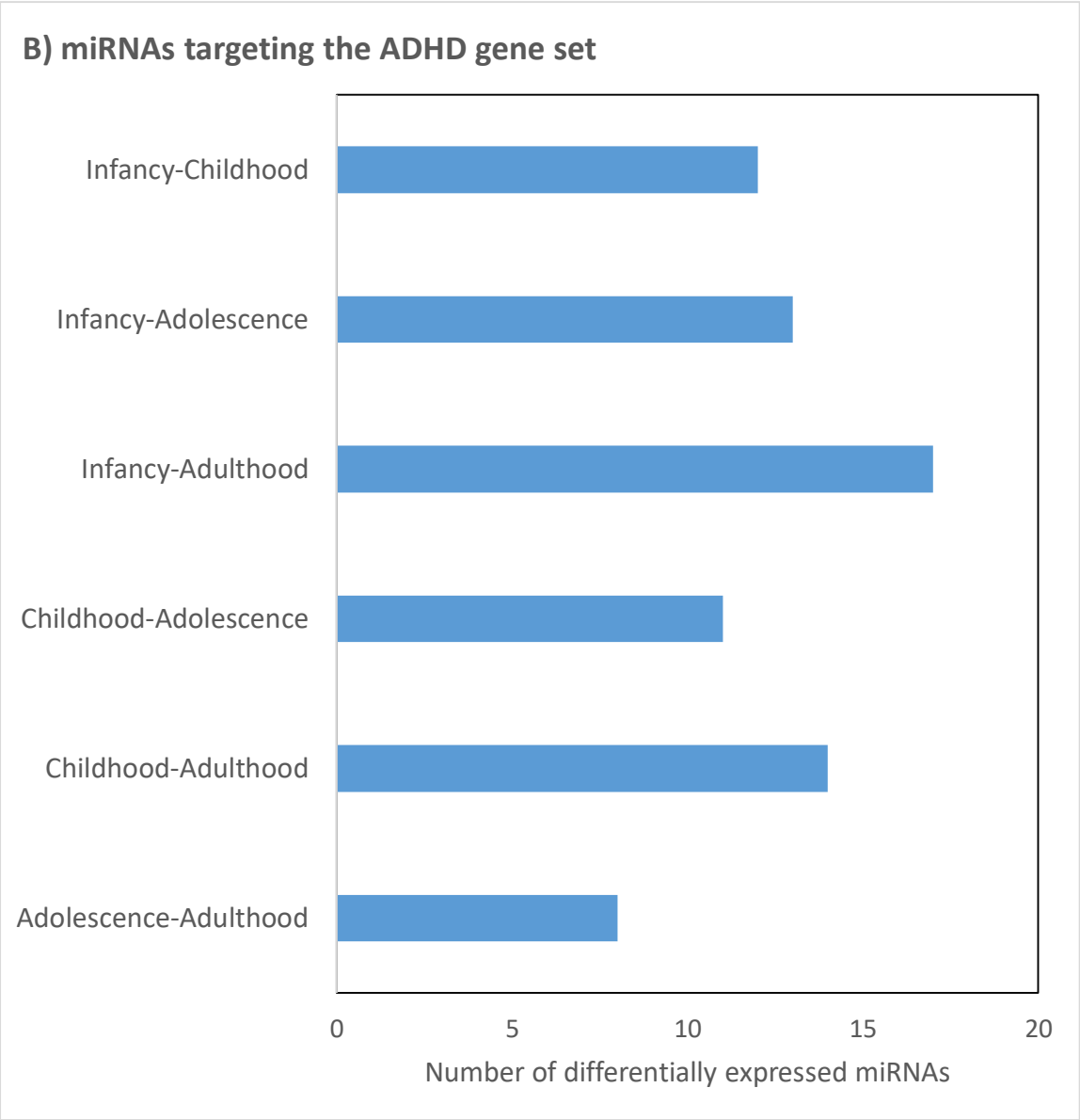


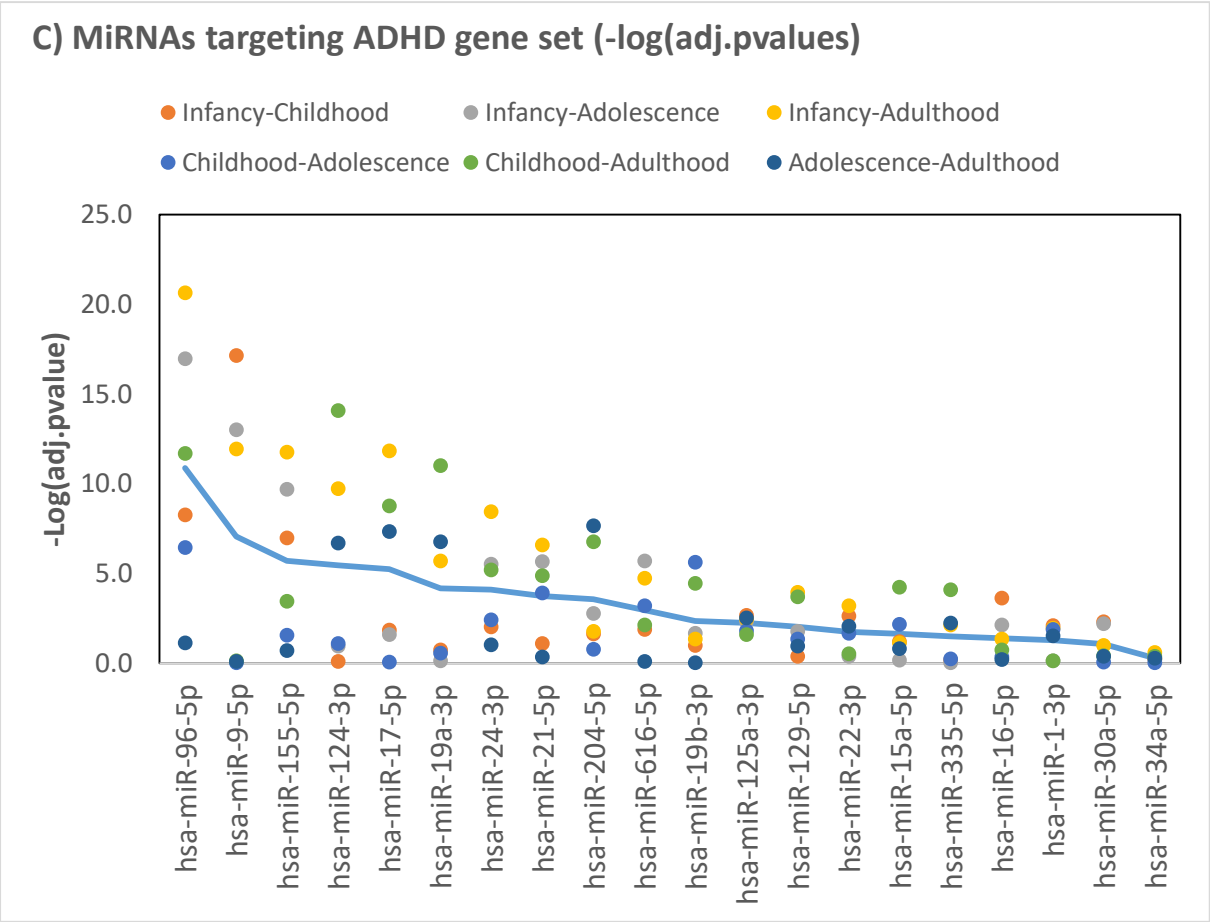
Figure 4. MiRNAs targeting the 103 ADHD gene set. A) The identified miRNAs (N = 20 miRNAs) targeting the ADHD gene set. Each miRNA targets at least four or more genes. B) A clustering dendrogram based on the similarity of the miRNAs' Jaccard index values to each other analyzed by miRmapper package. C) Correlation plot with the miRNAs clustered by target similarity. The distances were based on the similarity of the miRNAs' Jaccard index values to each other analyzed by miRmapper package.

To get insight into the transcriptional response of the identified 20 miRNAs regulating our 103 ADHD candidate genes in developing brain, we extracted the miRNA transcriptional data of these miRNAs from BrainSnap (40), and as RNA-seq data, we regrouped the original samples into four developmental stages (infancy (birth-12 month), childhood (1-11 year), adolescence (12-19 year) and adulthood (21-40 year)). The expression profiles of the 20 miRNAs are presented in Figure 5A, and we observed a developmental stage dependent expression level fluctuation for several miRNAs. We then compared the transcript level of the four developmental stages using edgeR package (46). From such analysis, we observed that there were many miRNAs which are differentially expressed between the four developmental stages (Figure 5B and C), and more than eleven miRNAs were differentially expressed between developmental stages. We then performed miRNA-target interaction network using topological properties computed for each node, and the resulting miRNA-target interaction network is presented in Figure 5D. Only miRNAs with five or more connections were included in the network, and miR-335-5p was the most connected miRNA.

Finally, a functional enrichment analysis of the targets of identified 20 miRNAs reveal that these miRNAs were involved in functional pathways relevant to neurodevelopment (alcoholism, circadian rhythm, cocaine addiction, dopaminergic synapse, glutamatergic synapse, long-term depression, synaptic vesicle cycle and neuroactive ligand-receptor interaction), and the most enriched disease ontologies includes attention deficit hyperactivity disorder, autism spectrum disorder, bipolar disorder, borderline personality disorder, mental depression, mood disorder and obsessive-compulsive disorder.







D)

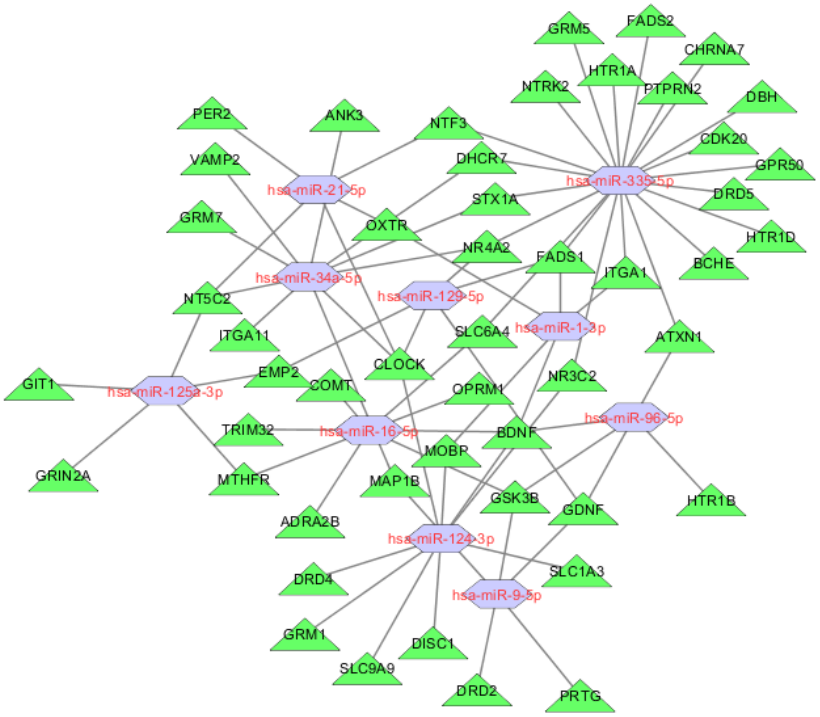


Figure 5. The expression profile of miRNAs (N=20 miRNAs) targeting the ADHD gene set. A) The expression level of the 20 miRNAs. The expression levels are presented as average CPM (counts pre million) of brain tissue samples from each developmental stages. B) Comparison of the four developmental stages (infancy, childhood, adolescences and adulthood stages) to identify statistically significantly differentially expressed miRNAs between the developmental stages (miRNAs are considered significantly expressed with FDR< 0.05 and fold change > 1.2). C) The –log adjusted p-values of the 20 miRNA expression level comparison. D) miRNA-mRNA network. Ten miRNAs connected to five or more target genes are shown, and miR-335-5p is the most connected miRNA. Genes are colored with green color and miRNA with blue color. The miRNA-mRNA network was constructed by CytoScape v3.7.1.

4 Discussion

Attention-deficit/hyperactivity disorder is a complex neuropsychiatric disorder, and is associated with increased healthcare costs for patients and their family members. The disorder is thus of great societal concern, and increased knowledge of the etiology may lead to earlier diagnosis and improved treatment. In this study, we evaluated the expression pattern of potential ADHD candidate genes and miRNAs that regulate these genes in normally developing human brain tissues from BrainSnap (40). We identified 103 ADHD candidate genes and these genes were expressed and significantly enriched in several brain regions and developmental stages such as cerebellum, hippocampus, striatum, thalamus and cortex which are implicated with ADHD. Brain studies of patients with ADHD have shown structural abnormalities in frontal-striatal and cerebellar circuitry, lower grey matter density, reduced total brain

volume and volume of some brain structures (55);(56);(57);(58);(59).

Human brain development is a dynamic and complex process. It requires precise coordination of cellular and molecular events in order to achieve proper brain regions interconnectivity and specialization (60). In order to understand pathogenesis mediated by gene expression modulations, it is important to get a complete picture of the normal gene expression regulation during human brain development, and how dysregulation of these processes contribute to the molecular pathogenesis of neurodevelopmental disorders, including ADHD. Understanding of the transcriptional fluctuation of the identified ADHD candidate genes during neurodevelopment and their transcriptional regulation mediated by miRNAs targeting them during normal neurodevelopment is essential for understanding the abnormal changes that may occur in the onset and course of common neurological conditions, including ADHD.

MiRNAs are expressed in mammalian brains specifically, suggesting the unique regulatory roles of miRNAs in neuronal development and are likely an important mediator of neuronal plasticity (48). Around 70% of known miRNAs are expressed in the human brain, and there is a growing list of brain-specific miRNAs (61);(62);(63). It has also been reported that specific miRNAs are involved in learning and memory, exploration, and anxiety behavior (49). From a comprehensive analysis, we identified 20 miRNAs regulating the 103 ADHD candidate genes (Figure 4). Some of the identified 20 miRNAs have been implicated to regulate neurodevelopment; for instance, miR-9 and miR-124 are reported to be highly expressed in the brain (64);(65);(66);(67), and overexpression of miR-9 negatively regulates proliferation, promotes neuronal differentiation and migration, and controls neural stem cell differentiation (64);(68);(69);(70);(71);(72). Increased expression of miR-124 promotes neural differentiation and specification (73). Further, miR-125 and miR-129 also have important roles in synapse formation and plasticity (74);(75);(76). Three other miRNAs, miR-17, miR-19a and miR-19b, which belongs to the miR-17~92 family of miRNA clusters (53), cooperate together to fine-tune signaling and developmental pathways. Overexpression of the miR-17-92 cluster modulates PTEN protein levels and increases axonal growth (77);(53). Moreover, overexpression of miR-34a alters hippocampal spinal morphology by modulating the expression of synaptic genes (synaptotagmin-1 and syntaxin-1A) (78). These illustrate that the identified 20 miRNAs targeting the 103 ADHD candidate genes have important roles in brain function and development. It will be interesting to elucidate the role of these miRNAs as potential biomarkers of ADHD. Among the identified 20 miRNAs, two miRNAs (hsa-miR-22-3p and hsa-miR-24-3p) have been linked to ADHD (79), where aberrant expression levels of these miRNAs were observed in ADHD patients (79). A neuroprotective activity of miR-22-3p has been reported (80) and as well as its implication as a potential biomarker of schizophrenia (81). Further, miR-24-3p has been shown to regulate neuronal differentiation by controlling hippocalcin (HPCA), a neuron-specific calcium-binding protein predominantly expressed in the nervous system (82). There are several reports on the role of miRNAs in ADHD (27);(28, 29);(30);(31);(32);(33);(34); however, these studies are preliminary evidence and there is so far little overlap between the identified ADHD linked miRNAs.

In a clinical setting, a minimally invasive diagnostic assay for early detection of ADHD is required to select optimal patient groups in clinical trials, monitor disease progression and response to treatment, and to

better plan patient clinical care. An advantage of using blood-based markers is the ease and possible frequency of collection of sample from patients. Circulating profiles of miRNAs have been shown to discriminate different tumor types, indicate staging and progression of the disease and to be useful as prognostic markers. Recently their role in neurodevelopmental diseases, both as diagnostic biomarkers as well as explaining basic disease etiology has come into focus. Most importantly, in an ongoing project, we are investigating these miRNAs in plasma samples from cord blood of ADHD cases and matched controls to assist in the prediction and early diagnosis of ADHD. Identification of dysregulated miRNAs in cord blood plasma samples may uncover associations between perinatal (early life) environmental stressors and ADHD.

In conclusion, this study identified 103 potential ADHD candidate genes and their regulating miRNAs. These genes and the regulating miRNAs were enriched in functional pathways and disease ontologies implicated with neurodevelopmental disorders, including ADHD. The knowledge of the expression pattern of potential ADHD candidate genes and miRNAs, which regulate these genes across different stages of brain development, is essential for understanding normal brain development and their implication to brain disease development. Identification of miRNA-regulated ADHD candidate genes can be used to develop blood-based molecular markers to be investigated in future studies of ADHD patients.

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References

1. Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. The American journal of psychiatry. 2007;164(6):942-8.
2. Polanczyk GV, Salum GA, Sugaya LS, Caye A, Rohde LA. Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. Journal of child psychology and psychiatry, and allied disciplines. 2015;56(3):345-65.
3. Thomas R, Sanders S, Doust J, Beller E, Glasziou P. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Pediatrics. 2015;135(4):e994-1001.
4. American Psychiatric Association. (2013). Diagnostic and statistical manual of

mental disorders (5th ed.). Washington, DC: Author. http://dx.doi.org/10.1176/appi.books.9780890425596. American Psychiatric Association Publishing2013.
5. Biedermann F, Fleischhacker WW. Psychotic disorders in DSM-5 and ICD-11. CNS spectrums. 2016;21(4):349-54.
6. Williams NM, Zaharieva I, Martin A, Langley K, Mantripragada K, Fossdal R, et al. Rare chromosomal deletions and duplications in attention-deficit hyperactivity disorder: a genome-wide analysis. Lancet (London, England). 2010;376(9750):1401-8.
7. Faraone SV, Biederman J. Is attention deficit hyperactivity disorder familial? Harvard review of psychiatry. 1994;1(5):271-87.
8. Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, et al. Molecular genetics of attention-deficit/hyperactivity disorder. Biological psychiatry. 2005;57(11):1313-23.
9. Faraone SV, Mick E. Molecular genetics of attention deficit hyperactivity disorder. The Psychiatric clinics of North America. 2010;33(1):159-80.
10. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747-53.
11. Faraone SV, Larsson H. Genetics of attention deficit hyperactivity disorder. Mol Psychiatry. 2019;24(4):562-75.
12. Faraone SV, Biederman J, Mick E. The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychological medicine. 2006;36(2):159-65.
13. Lara C, Fayyad J, de Graaf R, Kessler RC, Aguilar-Gaxiola S, Angermeyer M, et al. Childhood predictors of adult attention-deficit/hyperactivity disorder: results from the World Health Organization World Mental Health Survey Initiative. Biological psychiatry. 2009;65(1):46-54.
14. Rutter M, Kim-Cohen J, Maughan B. Continuities and discontinuities in psychopathology between childhood and adult life. Journal of child psychology and psychiatry, and allied disciplines. 2006;47(3-4):276-95.
15. Gizer IR, Ficks C, Waldman ID. Candidate gene studies of ADHD: a meta-analytic review. Human genetics. 2009;126(1):51-90.
16. Demontis D, Walters RK, Martin J, Mattheisen M, Als TD, Agerbo E, et al. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. Nature genetics. 2019;51(1):63-75.
17. McClellan J, King MC. Genetic heterogeneity in human disease. Cell. 2010;141(2):210-7.
18. Anway MD, Skinner MK. Epigenetic programming of the germ line: effects of endocrine disruptors on the development of transgenerational disease. Reproductive biomedicine online. 2008;16(1):23-5.
19. Ashe A, Whitelaw E. Another role for RNA: a messenger across generations. Trends in genetics : TIG. 2007;23(1):8-10.
20. Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, Cuzin F. RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. Nature. 2006;441(7092):469-74.
21. Jin P, Alisch RS, Warren ST. RNA and microRNAs in fragile X mental retardation. Nature cell biology. 2004;6(11):1048-53.
22. Camkurt MA, Karababa F, Erdal ME, Bayazıt H, Kandemir SB, Ay ME, et al. Investigation of Dysregulation of Several MicroRNAs in Peripheral Blood of Schizophrenia Patients. Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology. 2016;14(3):256-60.
23. Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K, Newman MA, et al. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome biology. 2007;8(2):R27.

24. Sarachana T, Zhou R, Chen G, Manji HK, Hu VW. Investigation of post-transcriptional gene regulatory networks associated with autism spectrum disorders by microRNA expression profiling of lymphoblastoid cell lines. Genome medicine. 2010;2(4):23.
25. Lukiw WJ, Zhao Y, Cui JG. An NF-kappaB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. The Journal of biological chemistry. 2008;283(46):31315-22.
26. Maes OC, Chertkow HM, Wang E, Schipper HM. MicroRNA: Implications for Alzheimer Disease and other Human CNS Disorders. Current genomics. 2009;10(3):154-68.
27. Wu LH, Peng M, Yu M, Zhao QL, Li C, Jin YT, et al. Circulating MicroRNA Let-7d in Attention-Deficit/Hyperactivity Disorder. Neuromolecular medicine. 2015;17(2):137-46.
28. Kovács-Nagy R, Hu J, Rónai Z, Sasvári-Székely M. SNAP-25: a novel candidate gene in psychiatric genetics. Neuropsychopharmacologia Hungarica : a Magyar Pszichofarmakologiai Egyesulet lapja = official journal of the Hungarian Association of Psychopharmacology. 2009;11(2):89-94.
29. Kovacs-Nagy R, Sarkozy P, Hu J, Guttman A, Sasvari-Szekely M, Ronai Z. Haplotyping of putative microRNA-binding sites in the SNAP-25 gene. Electrophoresis. 2011;32(15):2013-20.
30. Garcia-Martínez I, Sánchez-Mora C, Pagerols M, Richarte V, Corrales M, Fadeuilhe C, et al. Preliminary evidence for association of genetic variants in pri-miR-34b/c and abnormal miR-34c expression with attention deficit and hyperactivity disorder. Translational psychiatry. 2016;6(8):e879.
31. Wang LJ, Li SC, Kuo HC, Chou WJ, Lee MJ, Chou MC, et al. Gray matter volume and microRNA levels in patients with attention-deficit/hyperactivity disorder. European archives of psychiatry and clinical neuroscience. 2019.
32. Aydin SU, Kabukcu Basay B, Cetin GO, Gungor Aydin A, Tepeli E. Altered microRNA 5692b and microRNA let-7d expression levels in children and adolescents with attention deficit hyperactivity disorder. Journal of psychiatric research. 2019;115:158-64.
33. Sánchez-Mora C, Soler Artigas M, Garcia-Martínez I, Pagerols M, Rovira P, Richarte V, et al. Epigenetic signature for attention-deficit/hyperactivity disorder: identification of miR-26b-5p, miR-185-5p, and miR-191-5p as potential biomarkers in peripheral blood mononuclear cells. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2019;44(5):890-7.
34. Cao P, Wang L, Cheng Q, Sun X, Kang Q, Dai L, et al. Changes in serum miRNA-let-7 level in children with attention deficit hyperactivity disorder treated by repetitive transcranial magnetic stimulation or atomoxetine: An exploratory trial. Psychiatry research. 2019;274:189-94.
35. Zhang L, Chang S, Li Z, Zhang K, Du Y, Ott J, et al. ADHDgene: a genetic database for attention deficit hyperactivity disorder. Nucleic Acids Res. 2012;40(Database issue):D1003-9.
36. Piñero J, Bravo À, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, Centeno E, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res. 2017;45(D1):D833-d9.
37. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Current protocols in bioinformatics. 2016;54:1.30.1-1..3.
38. Alonso-Gonzalez A, Calaza M, Rodriguez-Fontenla C, Carracedo A. Gene-based analysis of ADHD using PASCAL: a biological insight into the novel associated

genes. BMC medical genomics. 2019;12(1):143.
39. Ghandikota S, Hershey GKK, Mersha TB. GENEASE: real time bioinformatics tool for multi-omics and disease ontology exploration, analysis and visualization. Bioinformatics (Oxford, England). 2018;34(18):3160-8.
40. Miller JA, Ding S-L, Sunkin SM, Smith KA, Ng L, Szafer A, et al. Transcriptional landscape of the prenatal human brain. Nature. 2014;508(7495):199-206.
41. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research. 2015;43(7):e47-e.
42. Licursi V, Conte F, Fiscon G, Paci P. MIENTURNET: an interactive web tool for microRNA-target enrichment and network-based analysis. BMC Bioinformatics. 2019;20(1):545.
43. Huang H-Y, Lin Y-C-D, Li J, Huang K-Y, Shrestha S, Hong H-C, et al. miRTarBase 2020: updates to the experimentally validated microRNA–target interaction database. Nucleic Acids Research. 2019;48(D1):D148-D54.
44. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research. 2003;13(11):2498-504.
45. da Silveira WA, Renaud L, Simpson J, Glen WB, Jr., Hazard ES, Chung D, et al. miRmapper: A Tool for Interpretation of miRNA¯mRNA Interaction Networks. Genes. 2018;9(9).
46. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics (Oxford, England). 2010;26(1):139-40.
47. Dougherty JD, Schmidt EF, Nakajima M, Heintz N. Analytical approaches to RNA profiling data for the identification of genes enriched in specific cells. Nucleic Acids Res. 2010;38(13):4218-30.
48. Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, et al. Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(1):360-5.
49. Parsons MJ, Grimm CH, Paya-Cano JL, Sugden K, Nietfeld W, Lehrach H, et al. Using hippocampal microRNA expression differences between mouse inbred strains to characterise miRNA function. Mammalian genome : official journal of the International Mammalian Genome Society. 2008;19(7-8):552-60.
50. Belmonte MK, Allen G, Beckel-Mitchener A, Boulanger LM, Carper RA, Webb SJ. Autism and abnormal development of brain connectivity. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(42):9228-31.
51. Duale N, Eide DM, Amberger ML, Graupner A, Brede DA, Olsen AK. Using prediction models to identify miRNA-based markers of low dose rate chronic stress. The Science of the total environment. 2020;717:137068.
52. Peter ME. Targeting of mRNAs by multiple miRNAs: the next step. Oncogene. 2010;29(15):2161-4.
53. Concepcion CP, Bonetti C, Ventura A. The microRNA-17-92 family of microRNA clusters in development and disease. Cancer journal (Sudbury, Mass). 2012;18(3):262-7.
54. Finnerty JR, Wang WX, Hébert SS, Wilfred BR, Mao G, Nelson PT. The miR-15/107 group of microRNA genes: evolutionary biology, cellular functions, and roles in human diseases. Journal of molecular biology. 2010;402(3):491-509.
55. Proal E, Reiss PT, Klein RG, Mannuzza S, Gotimer K, Ramos-Olazagasti MA, et al. Brain gray matter deficits at 33-year follow-up in adults with attention-deficit/hyperactivity

disorder established in childhood. Archives of general psychiatry. 2011;68(11):1122-34.
56. Ellison-Wright I, Ellison-Wright Z, Bullmore E. Structural brain change in Attention Deficit Hyperactivity Disorder identified by meta-analysis. BMC psychiatry. 2008;8:51.
57. Nakao T, Radua J, Rubia K, Mataix-Cols D. Gray matter volume abnormalities in ADHD: voxel-based meta-analysis exploring the effects of age and stimulant medication. The American journal of psychiatry. 2011;168(11):1154-63.
58. Valera EM, Faraone SV, Murray KE, Seidman LJ. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. Biological psychiatry. 2007;61(12):1361-9.
59. Hoogman M, Rijpkema M, Janss L, Brunner H, Fernandez G, Buitelaar J, et al. Current self-reported symptoms of attention deficit/hyperactivity disorder are associated with total brain volume in healthy adults. PloS one. 2012;7(2):e31273.
60. Silbereis JC, Pochareddy S, Zhu Y, Li M, Sestan N. The Cellular and Molecular Landscapes of the Developing Human Central Nervous System. Neuron. 2016;89(2):248-68.
61. Nowak JS, Michlewski G. miRNAs in development and pathogenesis of the nervous system. Biochemical Society transactions. 2013;41(4):815-20.
62. Adlakha YK, Saini N. Brain microRNAs and insights into biological functions and therapeutic potential of brain enriched miRNA-128. Molecular cancer. 2014;13:33.
63. Shao NY, Hu HY, Yan Z, Xu Y, Hu H, Menzel C, et al. Comprehensive survey of human brain microRNA by deep sequencing. BMC genomics. 2010;11:409.
64. Delaloy C, Liu L, Lee JA, Su H, Shen F, Yang GY, et al. MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. Cell stem cell. 2010;6(4):323-35.
65. Coolen M, Katz S, Bally-Cuif L. miR-9: a versatile regulator of neurogenesis. Frontiers in cellular neuroscience. 2013;7:220.
66. Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Molecular cell. 2007;27(3):435-48.
67. Cheng LC, Pastrana E, Tavazoie M, Doetsch F. miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. Nature neuroscience. 2009;12(4):399-408.
68. Zhao C, Sun G, Li S, Shi Y. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. Nature structural & molecular biology. 2009;16(4):365-71.
69. Clovis YM, Enard W, Marinaro F, Huttner WB, De Pietri Tonelli D. Convergent repression of Foxp2 3'UTR by miR-9 and miR-132 in embryonic mouse neocortex: implications for radial migration of neurons. Development (Cambridge, England). 2012;139(18):3332-42.
70. Conaco C, Otto S, Han JJ, Mandel G. Reciprocal actions of REST and a microRNA promote neuronal identity. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(7):2422-7.
71. Tan X, Wang S, Yang B, Zhu L, Yin B, Chao T, et al. The CREB-miR-9 negative feedback minicircuitry coordinates the migration and proliferation of glioma cells. PloS one. 2012;7(11):e49570.
72. Tan SL, Ohtsuka T, González A, Kageyama R. MicroRNA9 regulates neural stem cell differentiation by controlling Hes1 expression dynamics in the developing brain. Genes to cells : devoted to molecular & cellular mechanisms. 2012;17(12):952-61.
73. Åkerblom M, Jakobsson J. MicroRNAs as Neuronal Fate Determinants. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry. 2014;20(3):235-42.

74. Muddashetty RS, Nalavadi VC, Gross C, Yao X, Xing L, Laur O, et al. Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. Molecular cell. 2011;42(5):673-88.
75. Sosanya NM, Brager DH, Wolfe S, Niere F, Raab-Graham KF. Rapamycin reveals an mTOR-independent repression of Kv1.1 expression during epileptogenesis. Neurobiology of disease. 2015;73:96-105.
76. Kocerha J, Kauppinen S, Wahlestedt C. microRNAs in CNS disorders. Neuromolecular medicine. 2009;11(3):162-72.
77. Zhang Y, Ueno Y, Liu XS, Buller B, Wang X, Chopp M, et al. The MicroRNA-17-92 cluster enhances axonal outgrowth in embryonic cortical neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(16):6885-94.
78. Agostini M, Tucci P, Steinert JR, Shalom-Feuerstein R, Rouleau M, Aberdam D, et al. microRNA-34a regulates neurite outgrowth, spinal morphology, and function. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(52):21099-104.
79. Kandemir H, Erdal ME, Selek S, Ay Ö, Karababa IF, Kandemir SB, et al. Evaluation of several micro RNA (miRNA) levels in children and adolescents with attention deficit hyperactivity disorder. Neuroscience letters. 2014;580:158-62.
80. Jovicic A, Zaldivar Jolissaint JF, Moser R, Silva Santos Mde F, Luthi-Carter R. MicroRNA-22 (miR-22) overexpression is neuroprotective via general anti-apoptotic effects and may also target specific Huntington's disease-related mechanisms. PloS one. 2013;8(1):e54222.
81. Ma J, Shang S, Wang J, Zhang T, Nie F, Song X, et al. Identification of miR-22-3p, miR-92a-3p, and miR-137 in peripheral blood as biomarker for schizophrenia. Psychiatry research. 2018;265:70-6.
82. Kang M-J, Park S-Y, Han J-S. MicroRNA-24-3p regulates neuronal differentiation by controlling hippocalcin expression. Cellular and Molecular Life Sciences. 2019;76(22):4569-80.
83. Heberle H, Meirelles GV, da Silva FR, Telles GP, Minghim R. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. BMC Bioinformatics. 2015;16(1):169.

