The Computational Analysis Conducted on miRNA Target Sites in Association with SNPs at 3’UTR of ADHD-implicated Genes

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Abstract: Background: Attention-deficit/hyperactivity disorder (ADHD) is a frequent chronic neuropsychiatric disorder in which different factors including environmental, genetic, and epigenetic factors play an important role in its pathogenesis. One of the effective epigenetic factors is recognized as MicroRNAs (miRNAs). On the other hand, it has been indicated that the single nucleotide polymorphism (SNPs) present within 3’UTR (3’ untranslated region) of mRNAs can influence the regulation of miRNA-mediated gene and susceptibility to a diversity of human diseases.

Methods: The purpose of this study was to analyze the SNPs within the 3’UTR of miRNA target genes associated with ADHD. 3’UTR genetic variants were identified in all genes associated with ADHD using DisGeNET, dbGaP, Ovid, DAVID, Web of knowledge, and SNPs databases. miRNA’s target prediction databases were applied in order to predict the miRNA binding sites. 124 SNPs with MAF>0.05 were identified located in the binding site of the miRNA of 35 genes amongst 51 genes associated with ADHD.

Results: Bioinformatics analysis predicted 81 MRE (miRNA recognition elements)-creating SNPs, 101 MRE-breaking SNPs, 61 MRE-enhancing SNPs, and finally predicted 41 MRE-decreasing SNPs in the 3’UTR of ADHD-implicated genes. These candidate SNPs within these genes miRNA binding sites can alter the miRNAs binding, and consequently, lead to mRNA gene regulation.

Conclusion: Therefore, these miRNA and MRE-SNPs may play important roles in ADHD, and because of that, they would be valuable for further investigation in the field of functional verification.

Keywords: ADHA, SNP, miRNA, miRNA binding sites, ADHD- related genes, miRNA target genes.

1. INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is known as a neurodevelopmental disorder [1]. It is characterized by different inattention, hyperactivity, and impulsivity levels [2]. This disorder prevalence has been estimated as 2.5-4.9% in adults and 5% in children [3]. ADHD is identified as a multifactorial disorder, and all three environmental, genetic, and epigenetic factors play significant roles in this disorder pathogenesis [4]. One of these epigenetic factors is MicroRNAs (miRNAs). Much research has indicated that alterations in the miRNAs expression or function are associated with ADHD, schizophrenia, autism, bipolar disorder, and other intellectual disorders [5]. miRNAs are considered as important non-coding RNAs class with 18-25 nucleotide length, which regulates gene expression post-transcriptionally. miRNAs are involved in different biological processes including neuronal cell growth, specification, development, differentiation, synaptic plasticity, and also memory formation [6]. The miRNAs critical region is the “seed” region (2-7 nt from 5’ end of miRNAs), which in the 3’UTR (3’ untranslated region) of the mRNA preferentially binds to a target site named as miRNA recognition elements (MREs) [7].

Therefore, any disturbance in miRNA-MRE interactions could have an influence on gene expression regulation. Single Nucleotide Polymorphisms (SNPs) in the 3’UTR of the...
target mRNAs have the ability to disturb the miRNA binding by modifying the existing MREs or by new MREs creating [8]. The pathological importance of these functional SNPs has been studied in a variety of diseases [9], including neurodegenerative diseases [10]. Additionally, these SNPs in mRNA target sites make the pathway of this disease more complicated and result in some changes in phenotype. Also, they are regularly involved in the disease's susceptibility or onset [11]. Consequently, SNPs in mRNA binding sites might have main functions that can be applied for ADHD diagnosis and treatment. This study predicted mRNA target binding sites at 3'UTR of ADHD-implicated genes in order to identify SNPs, which could modify mRNA-target mRNA interactions, and also result in target gene expression modification.

2. MATERIALS AND METHODS

2.1. In silico Analysis of ADHD-associated Genes

ADHD-related genes and their pathways were achieved from ADHD gene [12] (http://adhdd.psych.ac.cn/), DisGeNET [13], dbGaP (https://www.ncbi.nlm.nih.gov/gap/phenogli), Ovid (http://www.ovid.com), and DAVID Bioinformatics Resources 6.8 (https://david.ncicfrf.gov/).

2.2. Prediction of the SNPs at 3'UTR of Candidate Genes

The “database SNP” (http://www.ncbi.nlm.nih.gov/SNP/) was applied in order to identify the selected genes SNPs, and also the genetic variants at the 3'UTR were selected. Moreover, the allele's frequencies were investigated and the SNPs with the amount of minor allele frequency (MAF) higher than 0.05 in HapMap were chosen and documented.

2.3. The Computational Analysis of mRNA Binding Sites and the Calculation of the Binding Free Energy

miRNA target prediction databases including miRdSNP [14] (http://mirdsn.ccr.buffalo.edu/search.php/), MirSNP [15] (http://202.38.126.151/hmm/mirsnp/search/), TargetScan Human 6.2 [16] (http://www.targetscan.org), miRNASNP 2.0 [17] (http://www.bioguo.org/miRNASNP/search.php), and PolymiRTS 3.0 [18] (http://compbio.uthsc.edu/miRSNP/) were used, in order to identify putative mRNA target binding sites containing the 3'UTR SNPs of each selected gene associated with ADHD. mRNA sequences were attained from miRBase 21 (http://mirbase.org). The 3'UTR SNPs in the target gene have an effect on mRNA function. These variants could decrease, increase, break, and create a miRNA binding site [15]. The SNP sequence and the function were attained from MirSNP and investigating miRNA binding site, respectively.

The Gibbs binding free energy ($\Delta G$, kCal/mol) was assessed for the major and the minor alleles using RNAcofold (http://rna.tbi.univie.ac.at/cgi-bin/RNAcofold.cgi). After that, the free energies difference (i.e., $\Delta \Delta \Delta G$) was calculated between two alleles as “variation of $\Delta G$”. The greater the difference ($\Delta \Delta \Delta G$), the higher the stability of the mRNA: miRNA duplex. Moreover, SNPs with the energy less than 0.1 kCal/mol, consequently could perform weak activity [19].

3. RESULTS

3.1. Selection of ADHD: Associated Genes and SNPs

Different databases and electronic libraries including Ovid, PubMed, and Web of Science, were applied in order to select genes and SNPs. Also, 51 ADHA-implicated genes were found (Table 1). After that, 124 SNPs were selected in the 3'UTR of 35 genes with MAF>0.05 as shown in Table 2. About 61% of genes have more than one SNP.

3.2. The Prediction of SNPs Locating in the miRNAs Target Binding Site

This study investigated the 124 SNPs within 3'UTR of these 35 genes. The results demonstrated that 71 SNPs of 31 genes have the target binding sites for miRNA (Table 3). 284 putative miRNAs were identified using different databases as followings: 283 miRNAs by the use of MirSNP, 10 miRNAs by TargetScan Human 6.2, 44 miRNAs using PolymiRTS 3.0, 19 miRNAs by the use of miRNASNP 2.0, and 6 miRNAs using miRdSNP, which they have been overlapped with each other. These SNPs have different effects on miRNA binding site including creation, break, increase, and decrease. Totally, 81 SNPs are MRE-creating (SNPs that create new MREs for miRNAs), 101 SNPs are MRE-breaking (SNPs that disrupt the miRNA binding sites completely), 61 SNPs are MRE-enhancing (SNPs that could increase the binding affinity of the miRNA to the binding sites), and finally, 41 SNPs are MRE-decreasing (SNPs that could decrease the miRNA binding efficacy to the binding sites) (Table 3). Also, it is noteworthy to state that each SNP has a different, independent effect on each different miRNA, and that is, if one SNP is associated with four miRNAs, then it has four different effects. For example, the second SNP in Table 3 (rs3750625) has several different effects on miRNAs.

4. DISCUSSION

ADHD is identified as a disorder that has a neurobiological basis. Although the ADHD pathogenesis and etiology is still completely unidentified, family and molecular genetic studies results indicated the strong genetic influence on ADHD [20]. It has been also reported that SNPs in regulatory regions could affect the gene expression. They play a remarkable role in susceptibility to multifactorial diseases [21]. For example, two SNPs -1291C/G and rs1800544 of the Alpha-2A Adrenergic Receptor (ADRA2A) Gene was associated with the efficacy of methylphenidate for the treatment of ADHD subjects [22, 23]. The ADRA2A receptors expressed on prefrontal cortical pyramidal neurons play a significant role in the regulation of the prefrontal cortex function [24] and correlate to methylphenidate therapeutic effect [25].

In addition to the SNPs that could affect the amino acid sequence, regulatory SNPs in the genome non-coding sequences might also develop the phenotypic variation in humans. It has been indicated that the SNPs within 3'UTR may interfere with miRNAs and target genes binding, resulting in dysregulation of mRNA and protein [26], which will influence the susceptibility to ADHD. In fact, Garcia-Martínez et al. (2016)
Table 1. The list of candidate genes to analyze the genetic variants at the 3'UTR.

| Gene Name                                      | Gene Symbol | Gene Name                                      | Gene Symbol |
|-----------------------------------------------|-------------|-----------------------------------------------|-------------|
| Adrenoceptor alpha 2A                         | ADRA2A      | Brain-derived neurotrophic factor             | BDNF        |
| Astrotactin 2                                 | ASTN2       | Cholinergic Receptor, Nicotinic, Alpha 4 subunit | CHRNA4      |
| Butyrylcholinesterase                        | BCHE        | Ciliary neurotrophic factor                   | CNTF        |
| Brain-derived neurotrophic factor             | BDNF        | Catechol-O-methyltransferase                  | COMT        |
| Cholinergic Receptor, Nicotinic, Alpha 4 subunit | CHRNA4      | 5-Hydroxytryptamine receptor 3A               | HTR3A       |
| Ciliary neurotrophic factor                   | CNTF        | Monoamine oxidase A                           | MAOA        |
| Catechol-O-methyltransferase                  | COMT        | Monoamine oxidase B                           | MAOB        |
| Complexin 2                                   | CPLX2       | Nitric oxide synthase 1                       | NOS1*       |
| Dopamine beta-hydroxylase                     | DBH         | Protein kinase, cGMP-dependent, type I        | PRKG1       |
| Dopa decarboxylase                            | DDC         | Solute Carrier Family 1 Member 3              | SLC1A3      |
| DIRAS family GTPase                           | DIRAS2      | Solute Carrier Family 6 Member 2              | SLC6A2/NET1 |
| Dopamine receptor D1                          | DRD1        | Solute Carrier Family 6 Member 3              | SLC6A3/DAT1 |
| Dopamine receptor D2/Ankyrin repeat and kinase domain containing 1 | DRD2/ANNK1 | Solute Carrier Family 6 Member 4              | SLC6A4/5HTT |
| Dopamine receptor D5                          | DRD5        | Solute Carrier Family 9 Member 9              | SLC9A9/NHE9 |
| Fatty acid desaturase 2                       | FADS2       | Solute Carrier Family 18 Member 2             | SLC18A2/VMAT2 |
| Glial cell derived neurotrophic factor        | GDNF        | Synaptosome associated protein 25             | SNAP25      |
| Glutamionotropic receptor NMDA type subunit 2A | GRIN2A      | Sparc/osteonectin, cwcw and kazal-like domains proteoglycan 3 | SPOCK3 |
| Glutamate metabotropic receptor 7             | GRM7        | Syntaxin1A                                    | STX1A       |
| 5-Hydroxytryptamine receptor 1A               | HTR1A       | Synaptophysin                                  | SYP         |
| 5-Hydroxytryptamine receptor 1B               | HTR1B       | Synaptotagmin 1                               | SYT1        |
| 5-Hydroxytryptamine receptor 1E               | HTR1E       | Transcription elongation regulator 1-like     | TCERG1L     |
| 5-Hydroxytryptamine receptor 2A               | HTR2A       | Tryptophan hydroxylase 2                      | TPH2        |
| 5-Hydroxytryptamine receptor 2C               | HTR2C       | Vesicle-associated membrane protein 2         | VAMP2       |
| Adrenoceptor alpha 2A                         | ADRA2A      | 5-Hydroxytryptamine receptor 3A               | HTR3A       |
| Butyrylcholinesterase                        | BCHE        | -                                             | -           |

Table 2. The list of candidate genes associated with ADHD and SNPs with MAFs higher than 0.05.

| Gene Symbol | dbSNP ID   | Variation | MAF  |
|-------------|------------|-----------|------|
| ADRA2A      | Rs11195419 | C/A       | 0.1813 |
| -           | Rs553608   | A/G, T    | 0.3295 |
| -           | Rs3750625  | C/A       | 0.1336 |
| -           | Rs13306146 | A/G       | 0.1372 |
| ASTN2       | Rs7518     | C/T       | 0.2005 |
| BCHE        | Rs3495     | C/A, T    | 0.3522 |
| Gene Symbol | dbsNP ID       | Variation | MAF   |
|-------------|----------------|-----------|-------|
| BDNF        | Rs7124442      | C/T, G    | 0.3299|
| CHRNA4      | Rs6090378      | A/G       | 0.0677|
|             | Rs6011770      | C/T       | 0.0623|
|             | Rs2236196      | G/A, C    | 0.3858|
| CNTF        | Rs2515362      | T/C       | 0.4874|
| COMT        | Rs165599       | G/A       | 0.4908|
|             | Rs165728       | C/T, G    | 0.1593|
| CPLX2       | Rs3822674      | T/C       | 0.4984|
|             | Rs11747985     | G/A, C    | 0.4087|
|             | Rs1006101      | T/C       | 0.4639|
|             | Rs4867809      | A/G       | 0.4349|
|             | Rs1560034      | T/C       | 0.3249|
| DBH         | Rs129882       | C/T       | 0.2554|
|             | Rs13306304     | G/A, C    | 0.0857|
| DDC         | Rs11575553     | G/A       | 0.0769|
| DIRAS2      | Rs7854469      | T/A, C    | 0.1392|
|             | Rs1542478      | A/G       | 0.0527|
|             | Rs16906711     | C/G       | 0.1122|
|             | Rs726214       | G/A       | 0.1767|
| DRD1        | Rs4867798      | T/C       | 0.3329|
|             | Rs686          | G/A       | 0.3950|
| DRD2/ANNK1  | Rs6278         | C/A       | 0.2041|
|             | Rs6274         | A/T, C    | 0.0551|
|             | Rs6279         | G/C       | 0.4782|
|             | Rs6276         | C/T       | 0.4669|
| DRD5        | Rs1967551      | T/C       | 0.4255|
| GDNF        | Rs17379771     | C/T, A    | 0.2350|
|             | Rs11111        | T/C       | 0.2476|
|             | Rs3749692      | A/G       | 0.4601|
| GRIN2A      | Rs767749       | T/G       | 0.2494|
|             | Rs1420040      | A/G       | 0.4233|
|             | Rs9940680      | G/C       | 0.4163|
|             | Rs9933624      | T/A, C    | 0.4173|
|             | Rs8045712      | C/T       | 0.3676|
|             | Rs8044472      | G/A, C    | 0.2478|
|             | Rs1014531      | G/A       | 0.2682|
| HTR1A       | Rs878567       | A/C, G    | 0.3522|

Table 2. Contd…
| Gene Symbol | dbSNP ID | Variation | MAF |
|-------------|---------|-----------|-----|
| -           | Rs6449693 | G/A | 0.3512 |
| HTR1B       | Rs13212041 | C/T | 0.2847 |
| -           | Rs6297 | C/A, T | 0.0765 |
| HTR1E       | Rs11970489 | T/C | 0.1841 |
| -           | Rs11963460 | A/T, C | 0.1879 |
| -           | Rs11964260 | A/C | 0.1859 |
| HTR2A       | Rs7323441 | A/T | 0.0891 |
| -           | Rs7325168 | T/C | 0.0887 |
| -           | Rs7324017 | C/T | 0.2314 |
| -           | Rs7324218 | C/T | 0.0887 |
| -           | Rs9595552 | T/G | 0.0927 |
| -           | Rs3803189 | T/G | 0.1843 |
| -           | Rs3125 | C/G, T | 0.1260 |
| HTR2C       | Rs1801412 | T/G | 0.0628 |
| HTR3A       | Rs1150219 | G/C | 0.0940 |
| MAOA        | Rs3027407 | A/G | 0.4490 |
| MAOB        | Rs3027438 | A/G, T | 0.1015 |
| -           | Rs3027439 | A/G | 0.1340 |
| -           | Rs2072745 | A/T | 0.1338 |
| -           | Rs3027440 | A/G | 0.1009 |
| -           | Rs17462 | T/C | 0.0630 |
| NOS1        | Rs12425729 | T/C | 0.1136 |
| -           | Rs10774906 | T/C | 0.4746 |
| -           | Rs10774907 | G/A | 0.4738 |
| -           | Rs1105026 | A/G, T | 0.1446 |
| -           | Rs9658570 | G/T | 0.0911 |
| -           | Rs9658562 | A/T | 0.0673 |
| -           | Rs11068415 | G/C, T | 0.1368 |
| -           | Rs2682826 | G/A | 0.2558 |
| SLC1A3      | Rs1049522 | A/C | 0.3504 |
| -           | Rs2269272 | C/T | 0.2049 |
| SLC6A2      | Rs42879 | T/C | 0.0739 |
| -           | Rs36006 | T/C | 0.0741 |
| SLC6A3      | Rs7732456 | A/C, T | 0.0609 |
| -           | Rs3797200 | C/G, T | 0.1773 |
| -           | Rs27072 | C/T, A | 0.2051 |
| -           | Rs1042098 | A/G | 0.2951 |

Table 2. Contd…
| Gene Symbol | dbSNP ID  | Variation | MAF    |
|-------------|-----------|-----------|--------|
| SLC6A4      | Rs7224199 | G/T       | 0.4189 |
|             | Rs3813034 | A/C       | 0.4834 |
|             | Rs1042173 | A/C       | 0.4852 |
| SLC9A9      | Rs3796229 | A/G       | 0.1326 |
|             | Rs3796228 | T/C       | 0.0871 |
|             | Rs3796227 | G/C, T    | 0.0609 |
| SLC18A2     | Rs10377   | A/C       | 0.4521 |
|             | Rs14240   | T/C, A    | 0.4523 |
|             | Rs363282  | G/A       | 0.3091 |
|             | Rs363235  | T/A, C    | 0.3089 |
|             | Rs363236  | C/T, A    | 0.3091 |
|             | Rs363237  | T/A, C    | 0.4519 |
|             | Rs363238  | C/A       | 0.2218 |
| SNAP25      | Rs3746544 | G/T       | 0.2812 |
|             | Rs1051312 | T/C       | 0.1256 |
|             | Rs8636    | T/A, C    | 0.2538 |
| SPOCK3      | Rs6846930 | C/G, A    | 0.3966 |
|             | Rs3762245 | A/G       | 0.2081 |
| STX1A       | Rs867500  | G/T, A, C | 0.2314 |
|             | Rs1569061 | C/T       | 0.0931 |
| SYP         | Rs7889267 | G/A       | 0.1399 |
| SYT1        | Rs1245667 | T/C       | 0.1062 |
|             | Rs2248102 | G/A       | 0.0545 |
| TCERG1L     | Rs2944507 | A/G       | 0.2296 |
|             | Rs2280200 | A/T       | 0.3720 |
|             | Rs2280199 | C/G, T    | 0.4780 |
|             | Rs1055043 | T/C       | 0.3217 |
|             | Rs2918092 | G/A       | 0.1502 |
| TPH2        | Rs17110747| G/A       | 0.1454 |
| VAMP2       | Rs1150    | A/G       | 0.3652 |
|             | Rs1061032 | T/A, C, G | 0.2598 |
|             | Rs8636    | T/C, A    | 0.2538 |
| SPOCK3      | Rs6846930 | C/G, A    | 0.3966 |
|             | Rs3762245 | A/G       | 0.2081 |
| STX1A       | Rs867500  | G/T, A, C | 0.2314 |
|             | Rs1569061 | C/T       | 0.0931 |
| SYP         | Rs7889267 | G/A       | 0.1399 |

Table 2. Contd…
Table 3. Predicted SNPs and miRNAs analyzing using miRNA target prediction databases.

| Gene Symbol | dbSNP ID | Variation | miRNA           | [ΔΔG] (kCal/mol) | MirSNP | TagetScan | PolymiRTS | miRNASNP | miRdSNP | Effect     |
|-------------|----------|-----------|-----------------|------------------|--------|-----------|-----------|----------|---------|------------|
| SYT1        | Rs1245667| T/C       | hsa-miR-3677-5p | 6.85             | +      | +         | +         | Create   |         | Create     |
|             |          |           | hsa-miR-3926   | 2.2              | +      | +         | +         | Create   |         | Create     |
|             |          |           | hsa-miR-548s   | 0.43             | +      | +         | +         | Create   |         | Create     |
| TCERG1L     | Rs2944507| A/G       | hsa-miR-1207-5p| 0.05             | +      | +         | +         | Create   |         | Create     |
|             |          |           | hsa-miR-149-3p | 1.56             | +      |           |           |         |         | Enhance    |
|             |          |           | hsa-miR-2682-5p| 6.44             | +      |           |           | Break    |         | Break      |
|             |          |           | hsa-miR-3150a-3p| 0.02           | +      |           |           |         |         | Enhance    |
|             |          |           | hsa-miR-34a-5p | 1.38             | +      | +         | +         | Break    |         | Break      |
|             |          |           | hsa-miR-34b-5p | 1.5              | +      | +         | +         |          |         | Break      |
|             |          |           | hsa-miR-34c-5p | 1.56             | +      | +         | +         |          |         | Break      |
|             |          |           | hsa-miR-3616-3p| 0.32             | +      |           |           |         |         | Decrease   |

Table 3. Contd…
| Gene     | dbSNP ID | Variation | miRNA       | \[ΔΔG\] (kCal/mol) | MirSNP | TagScan | PolymiRTS | miRNASNP | miRdSNP | Effect |
|----------|----------|-----------|-------------|---------------------|--------|---------|-----------|----------|---------|--------|
|          |          |           | hsa-miR-4446-3p | 2.99                | +      | +       | +         | +        | +       | Create |
|          |          |           | hsa-miR-449a   | 0.0                 | +      | +       | +         | +        | +       | Break  |
|          |          |           | hsa-miR-449b-5p | 3.29                | +      |         | +         | +        | +       | Break  |
|          |          |           | hsa-miR-449c-5p | 1.11                | +      |         | +         | +        | +       | Break  |
|          |          |           | hsa-miR-4514   | 0.0                 | +      |         | +         | +        | +       | Break  |
|          |          |           | hsa-miR-4692   | 0.0                 | +      |         | +         | +        | +       | Break  |
|          |          |           | hsa-miR-4763-3p | 0.98                | +      | +       | +         | +        | +       | Break  |
|          |          |           | hsa-miR-512-3p  | 2.74                | +      | +       | +         | +        | +       | Break  |
|          |          |           | hsa-miR-940    | 0.44                | +      | +       | +         | +        | +       | Create |
| rs13306146 | A/G    |           | hsa-miR-432-5p | 0.37                | +      |         |           |          |         | Decrease |
|          |          |           | hsa-miR-646    | 0.16                | +      | +       | +         |          |         | Break  |
| ASTN2    | rs7518   | C/T       | hsa-miR-3189-3p | 0.3                 | +      | +       | +         |          |         | Break  |
|          |          |           | hsa-miR-5001-3p | 2.34                | +      |         | +         |          |         | Enhance |
|          |          |           | hsa-miR-5089   | 0.01                | +      |         | +         |          |         | Break  |
| BDNF     | rs7124442 | C/T, G    | hsa-miR-142-5p | 0.65                | +      |         |           |          |         | Decrease |
|          |          |           | hsa-miR-5590-3p | 0.1                 | +      |         |           |          |         | Enhance |
|          |          |           | hsa-miR-922    | 0.77                | +      |         |           |          |         | Break  |
| rs6090378 | A/G    |           | hsa-miR-136-5p | 0.36                | +      |         |           |          |         | Break  |
| CHRNA4   | rs6011770 | C/T       | hsa-miR-3186-3p | 0.23                | +      |         | +         |          |         | Enhance |
|          |          |           | hsa-miR-4267   | 0.04                | +      |         | +         |          |         | Break  |
|          |          |           | hsa-miR-4661-5p | 0.77                | +      |         | +         |          |         | Create |
|          |          |           | hsa-miR-629-3p | 0.14                | +      |         |            |          |         | Decrease |
| rs2515362 | T/C    |           | hsa-miR-3174   | 0.33                | +      |         | +         |          |         | Enhance |
|          |          |           | hsa-miR-548ac  | 0.94                | +      |         | +         |          |         | Break  |
|          |          |           | hsa-miR-548d-3p | 0.35                | +      |         | +         |          |         | Break  |
|          |          |           | hsa-miR-548h-3p | 0.9                 | +      |         | +         |          |         | Break  |
|          |          |           | hsa-miR-548z   | 0.84                | +      |         | +         |          |         | Break  |
| rs165728 | C/T, G  |           | hsa-miR-3138   | 0.4                 | +      |         |           |          |         | Create |
|          |          |           | hsa-miR-4520a-3p | 1.59                | +      |         | +         |          |         | Create |
|          |          |           | hsa-miR-541-3p | 1.52                | +      | +       |           |          |         | Break  |
|          |          |           | hsa-miR-654-5p | 1.76                | +      | +       | +         |          |         | Break  |
| rs3822674 | T/C    |           | hsa-miR-4287   | 0.22                | +      |         |           |          |         | Enhance |
|          |          |           | hsa-miR-4685-3p | 0.22                | +      |         | +         |          |         | Enhance |
|          |          |           | hsa-miR-498    | 0.24                | +      |         | +         |          |         | Break  |
| CPLX2    | rs1006101 | T/C       | hsa-miR-3689d  | 2.89                | +      | +       | +         |          |         | Create |
|          |          |           | hsa-miR-4802-5p | 1.85                | +      | +       | +         |          |         | Create |
|          |          |           | hsa-miR-588    | 2.9                 | +      |         |           |          |         | Decrease |
|          |          |           | hsa-miR-609    | 2.9                 | +      |         |           |          |         | Break  |

Table 3. Contd…
| Gene       | dbSNP ID  | Variation | miRNA                | $\Delta\Delta G$ (kCal/mol) | TagScan | PolymiRTS | miRNASNP | miRdSNP | Effect |
|------------|-----------|-----------|----------------------|----------------------------|---------|-----------|----------|---------|--------|
|            | rs4867809 | A/G       | hsa-miR-4471         | 0.09                       | +       | +         | +        |        | Create |
|            |           |           | hsa-miR-892b         | 0.1                        | +       | +         | +        | +      | Create |
|            | rs1560034 | T/C       | hsa-miR-4435         | 0.09                       | +       |           |          |        | Create |
|            |           |           | hsa-miR-548s         | 0.39                       | +       |           |          |        | Break  |
| DBH        | rs129882  | C/T       | hsa-miR-1268a        | 1.79                       | +       | +         |          |        | Break  |
|            |           |           | hsa-miR-1268b        | 1.68                       | +       | +         |          |        | Break  |
|            |           |           | hsa-miR-1285-3p      | 0.98                       | +       |           | +        |        | Create |
|            |           |           | hsa-miR-3187-5p      | 1.69                       | +       |           |          |        | Create |
|            |           |           | hsa-miR-4253         | 2.13                       | +       |           |          |        | Create |
|            |           |           | hsa-miR-4486         | 0.35                       | +       | +         |          |        | Break  |
|            |           |           | hsa-miR-5189         | 0.03                       | +       |           |          |        | Create |
|            |           |           | hsa-miR-612          | 0.36                       | +       |           | +        |        | Create |
|            | rs1330630 | G/A, C    | hsa-miR-1908         | 0.13                       | +       |           |          |        | Decrease |
|            |           |           | hsa-miR-3180         | 0.02                       | +       |           |          |        | Enhance |
|            |           |           | hsa-miR-3180-3p      | 0.07                       | +       |           |          |        | Enhance |
|            |           |           | hsa-miR-3196         | 2.77                       | +       |           |          |        | Enhance |
|            |           |           | hsa-miR-4697-5p      | 0.01                       | +       | +         | +        |        | Break  |
|            |           |           | hsa-miR-4787-5p      | 0.34                       | +       | +         |          |        | Break  |
|            |           |           | hsa-miR-609          | 0.01                       | +       |           | +        |        | Break  |
| DIRAS2     | rs7854469 | T/A,C     | hsa-miR-3163         | 0.07                       | +       |           | +        |        | Decrease |
|            |           |           | hsa-miR-374a-5p      | 0.09                       | +       |           | +        |        | Create |
|            |           |           | hsa-miR-374b-5p      | 0.18                       | +       |           | +        |        | Create |
|            | rs16906711| C/G       | hsa-miR-139-5p       | 0.28                       | +       | +         | +        |        | Break  |
|            |           |           | hsa-miR-633          | 0.4                        | +       |           |          |        | Create |
|            | rs726214  | G/A       | hsa-miR-3117-3p      | 2.43                       | +       |           |          |        | Create |
|            |           |           | hsa-miR-3169         | 0.1                        | +       |           |          |        | Create |
|            |           |           | hsa-miR-3199         | 0.18                       | +       | +         |          |        | Break  |
|            |           |           | hsa-miR-4648         | 2.29                       | +       | +         |          |        | Break  |
| DRD1       | rs686     | G/A       | hsa-miR-4323         | 0.45                       | +       |           |          |        | Decrease |
| DRD2/ANK1  | rs6278    | C/A       | hsa-miR-214-3p       | 1.58                       | +       | +         | +        |        | Break  |
|            |           |           | hsa-miR-298          | 1.3                        | +       |           | +        |        | Create |
|            |           |           | hsa-miR-3154         | 2.16                       | +       |           | +        |        | Create |
|            |           |           | hsa-miR-3619-5p      | 1.82                       | +       | +         | +        |        | Break  |
|            |           |           | hsa-miR-3714         | 4.48                       | +       |           |          |        | Decrease |
|            |           |           | hsa-miR-3918         | 0.92                       | +       |           |          |        | Enhance |
|            |           |           | hsa-miR-761          | 1.59                       | +       | +         | +        |        | Break  |

Table 3. Contd...
| Gene     | dbSNP ID | Variation | miRNA          | \[\text{ΔΔG} (\text{kCal/mol})\] | MirSNP | TagetScan | PolymiRTS | miRNASNP | miRdSNP | Effect  |
|----------|----------|-----------|----------------|---------------------------------|--------|-----------|-----------|----------|---------|---------|
|          | rs6279   | G/C       | hsa-miR-4311   | 0.31                            | +      | +         |           |          |         | Break   |
|          |          |           | hsa-miR-4323   | 1.31                            | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-4758-3p| 1.85                            | +      |           |           |          |         | Enhance |
|          | rs6276   | C/T       | hsa-miR-1234   | 0.69                            | +      | +         |           |          |         | Create  |
|          |          |           | hsa-miR-3064-5p| 0.42                            | +      |           |           |          |         | Break   |
|          |          |           | hsa-miR-3176   | 0.03                            | +      |           |           |          |         | Break   |
|          |          |           | hsa-miR-3922-3p| 0.8                             | +      |           |           |          |         | Break   |
|          |          |           | hsa-miR-4316   | 0.68                            | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-4710   | 0.08                            | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-485-5p | 5.2                             | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-5580-5p| 0.02                            | +      |           |           |          |         | Break   |
|          | rs1967551 | T/C       | hsa-miR-210    | 0.58                            | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-4697-3p| 0.41                            | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-636    | 0.3                             | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-654-3p | 0.15                            | +      |           |           |          |         | Break   |
|          | rs11111  | T/C       | hsa-let-7f-2-3p| 2.29                            | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-1185-1-3p| 3.66                          | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-1185-2-3p| 3.69                          | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-3934   | 0.75                            | +      |           |           |          |         | Enhance |
|          | rs3749692 | A/G       | hsa-let-7u-2-3p| 0.69                            | +      |           |           |          |         | Break   |
|          |          |           | hsa-let-7g-3p  | 0.65                            | +      |           |           |          |         | Break   |
|          |          |           | hsa-miR-1915-3p| 0.45                            | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-3649   | 2.81                            | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-4700-3p| 2.22                            | +      |           |           |          |         | Break   |
|          |          |           | hsa-miR-5685   | 2.81                            | +      |           |           |          |         | Enhance |
|          | rs767749  | T/G       | hsa-miR-3618   | 1.84                            | +      |           |           |          |         | Break   |
|          | rs1420040 | A/G       | hsa-miR-4645-5p| 0.37                            | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-580    | 0.15                            | +      |           |           |          |         | Create  |
|          | rs9940680 | G/C       | hsa-miR-181a-5p| 2.91                            | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-181b-5p| 1.31                            | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-181d   | 3.91                            | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-3663-5p| 4.64                            | +      |           |           |          |         | Break   |
|          | rs9933624 | T/A, C    | hsa-miR-22-5p  | 0.45                            | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-607    | 0.71                            | +      |           |           |          |         | Create  |

Table 3. Contd...
| Gene     | dbSNP ID | Variation | miRNA               | $|\Delta\Delta G| (kCal/mol) | MirSNP | TagetScan | PolymiRTS | miRNASNP | miRdSNP | Effect  |
|----------|----------|-----------|---------------------|----------------|--------|-----------|-----------|----------|---------|---------|
|          | rs8045712| C/T       | hsa-miR-1343        | 0.29           | +      |           |           |          |         | Decrease|
|          | rs8044472| G/A, C    | hsa-miR-4637        | 0.09           | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-4653-3p     | 0.04           | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-520a-5p     | 0.14           | +      |           |           |          | +       | Break   |
|          |          |           | hsa-miR-525-5p      | 0.13           | +      |           |           |          | +       | Break   |
|          | rs1014531| G/A       | hsa-miR-1185-5p     | 1.44           | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-1266        | 1              | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-197-5p      | 1              | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-3132        | 0.99           | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-3664-3p     | 1.04           | +      |           |           |          |         | Break   |
|          |          |           | hsa-miR-3679-5p     | 1.02           | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-4518        | 1.87           | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-510         | 1              | +      |           |           |          |         | Decrease|
| HTR2A    | rs3125   | C/G, T    | hsa-miR-3662        | 0.08           | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-3976        | 2.92           | +      |           |           |          | +       | Break   |
|          |          |           | hsa-miR-5689        | 0.2            | +      | +         | +         |          |         | Create  |
| HTR2C    | rs1801412| T/G       | hsa-miR-10a-5p      | 0.4            | +      | +         |           |          |         | Break   |
|          |          |           | hsa-miR-10b-5p      | 2.66           | +      | +         |           |          |         | Break   |
|          |          |           | hsa-miR-141-3p      | 4.61           | +      |           | +         |          |         | Create  |
|          |          |           | hsa-miR-200a-3p     | 4.6            | +      |           | +         |          |         | Create  |
|          |          |           | hsa-miR-2054        | 0.86           | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-2115-3p     | 1.62           | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-339-5p      | 5.82           | +      |           | +         |          |         | Break   |
| MAOA     | rs3027407| A/G       | hsa-miR-3120-5p     | 2.49           | +      | +         | +         |          |         | Create  |
|          |          |           | hsa-miR-4652-3p     | 0.22           | +      |             | +         |          |         | Break   |
|          | rs3027439| A/G       | hsa-miR-3173-3p     | 0.1            | +      | +         |           |          |         | Create  |
|          |          |           | hsa-miR-3689d       | 0.2            | +      |           |           |          |         | Create  |
|          |          |           | hsa-miR-4668-5p     | 0.18           | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-4668-5p     | 0.18           | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-4668-5p     | 0.18           | +      |           |           |          |         | Enhance |
|          |          |           | hsa-miR-583         | 0.03           | +      |           |           |          |         | Break   |
| MAOB     | rs2072745| A/T       | hsa-miR-4511        | 0.01           | +      |           |           |          |         | Enhance |
|          | rs3027440| A/G       | hsa-miR-1226-5p     | 0.0            | +      |           |           |          |         | Decrease|
|          |          |           | hsa-miR-3616-3p     | 0.02           | +      |           |           |          |         | Break   |
|          |          |           | hsa-miR-4744        | 0.04           | +      |           |           |          |         | Enhance |
|          | rs17462  | T/C       | hsa-miR-4299        | 0.47           | +      | +         |           |          |         | Break   |
|          |          |           | hsa-miR-4738-3p     | 0.84           | +      | +         | +         |          |         | Break   |
|          |          |           | hsa-miR-582-3p      | 0.7            | +      | +         |           |          |         | Break   |

Table 3. Contd…
| Gene     | dbSNP ID | Variation | miRNA         | $| \Delta \Delta G |$(kCal/mol) | MirSNP | TargetScan | PolymiRTS | miRNASNP | miRdSNP | Effect      |
|----------|----------|-----------|---------------|----------------|-----------|---------|------------|-----------|----------|---------|-------------|
| NOS1     | rs12425729 | T/C       | hsa-miR-548v  | 0.97           | +         |         |            |           |          |         | Decrease    |
|          | rs10774906 | T/C       | hsa-miR-4752  | 1.47           | +         |         |            |           |          |         | Create      |
|          | rs10774907 | G/A       | hsa-miR-3145-3p | 0.81          | +         | +       |            |           |          |         | Break       |
|          | rs9658570  | G/T       | hsa-miR-3120-5p | 0.04          | +         |         |            |           |          |         | Enhance     |
| SLC1A3   | rs9658562  | A/T       | hsa-miR-302b-5p | 1.56          | +         | +       |            |           |          |         | Create      |
|          | rs2682826  | G/A       | hsa-miR-302c-5p | 0.35          | +         | +       |            |           |          |         | Create      |
|          | rs9658562  | A/T       | hsa-miR-302d-5p | 0.29          | +         | +       |            |           |          |         | Create      |
|          | rs9658562  | A/T       | hsa-miR-3143   | 0.17          | +         | +       |            |           |          |         | Create      |
|          | rs9658562  | A/T       | hsa-miR-593-5p  | 0.06          | +         |         |            |           |          |         | Enhance     |
| SLC6A2   | rs1049522  | A/C       | hsa-miR-3171   | 0.0           | +         | +       | +           |           |          |         | Break       |
|          | rs42879    | T/C       | hsa-miR-30a-3p  | 2.73          | +         |         |            |           |          |         | Enhance     |
|          | rs42879    | T/C       | hsa-miR-30d-3p  | 4.22          | +         |         |            |           |          |         | Enhance     |
|          | rs42879    | T/C       | hsa-miR-4263   | 1.53          | +         |         |            |           |          |         | Break       |
|          | rs36006    | T/C       | hsa-miR-4329   | 3.34          | +         |         |            |           |          |         | Create      |
|          | rs36006    | T/C       | hsa-miR-4786-5p | 0.49          | +         |         |            |           |          |         | Create      |
|          | rs36006    | T/C       | hsa-miR-5693   | 2.68          | +         |         |            |           |          |         | Enhance     |
|          | rs36006    | T/C       | hsa-miR-3692-3p | 0.13          | +         |         |            |           |          |         | Break       |
| SLC6A3   | rs7732456  | A/C, T    | hsa-miR-3976   | 1.86          | +         |         |            |           |          |         | Break       |
|          | rs1042098  | A/G       | hsa-miR-187-3p  | 3.05          | +         |         |            |           |          |         | Enhance     |
|          | rs1042098  | A/G       | hsa-miR-2116-3p | 0.44          | +         |         |            |           |          |         | Enhance     |
|          | rs1042098  | A/G       | hsa-miR-4713-5p | 2.45          | +         |         |            |           |          |         | Enhance     |
|          | rs1042098  | A/G       | hsa-miR-5187-5p | 2.28          | +         |         |            |           |          |         | Break       |

Table 3. Contd…
| Gene     | dbsNP ID | Variation | miRNA          | $|ΔΔG|$ (kCal/mol) | MirSNP | TargetScan | PolymiRTS | miRNAsNP | miRdSNP | Effect     |
|----------|----------|-----------|----------------|----------------|--------|------------|-----------|----------|---------|------------|
| SLC6A4   | rs7224199| G/T       | hsa-miR-1252   | 0.03           | +      |            |           |          |         | Break      |
|          |          |           | hsa-miR-3185   | 0.09           | +      |            |           |          |         | Break      |
|          | rs3813034| A/C       | hsa-miR-2053   | 0.37           | +      |            |           |          |         | Break      |
|          |          |           | hsa-miR-569    | 0.42           | +      |            |           |          |         | Break      |
|          |          |           | hsa-miR-571    | 0.31           | +      |            |           |          |         | Decrease   |
|          | rs1042173| A/C       | hsa-miR-3163   | 0.71           | +      |            |           |          |         | Enhance    |
|          |          |           | hsa-miR-3942-5p| 0.1            | +      |            |           |          |         | Enhance    |
| SLC9A9   | rs3796229| A/G       | hsa-miR-15a-3p | 0.62           | +      |            |           |          |         | Decrease   |
|          |          |           | hsa-miR-1972   | 0.34           | +      |            |           |          |         | Enhance    |
|          | rs3796228| T/C       | hsa-miR-1260a  | 5.37           | +      | +          |           |          |         | Create     |
|          |          |           | hsa-miR-1260b  | 0.38           | +      | +          |           |          |         | Create     |
|          | rs10377  | A/C       | hsa-miR-3145-3p| 0.78           | +      |            |           |          |         | Enhance    |
|          |          |           | hsa-miR-3163   | 3.11           | +      |            |           |          |         | Enhance    |
|          |          |           | hsa-miR-3163   | 3.11           | +      |            |           |          |         | Enhance    |
|          |          |           | hsa-miR-3646   | 3.12           | +      |            |           |          | +       | Create     |
|          |          |           | hsa-miR-3662   | 3.28           | +      | +          |           |          | +       | Create     |
|          | rs14240  | T/C, A    | hsa-miR-1297   | 1.98           | +      | +          |           |          |         | Break      |
|          |          |           | hsa-miR-26a-5p | 2.26           | +      | +          | +         |          |         | Break      |
|          |          |           | hsa-miR-26b-5p | 2.27           | +      | +          |           |          |         | Break      |
|          |          |           | hsa-miR-3671   | 1.87           | +      | +          | +         |          |         | Create     |
|          |          |           | hsa-miR-4465   | 1.48           | +      | +          |           |          |         | Break      |
|          |          |           | hsa-miR-5002-5p| 2.32           | +      | +          |           |          |         | Decrease   |
|          |          |           | hsa-miR-607    | 1.8            | +      | +          | +         |          |         | Create     |
| SLC18A2  | rs363282 | G/A       | hsa-miR-2278   | 2.64           | +      |            |           |          |         | Decrease   |
|          | rs363235 | T/A, C    | hsa-miR-1205   | 0.97           | +      |            |           |          |         | Enhance    |
|          |          |           | hsa-miR-125b-2-3p| 3.2        | +      | +          | +         |          |         | Create     |
|          |          |           | hsa-miR-1297   | 0.83           | +      | +          |           |          |         | Break      |
|          |          |           | hsa-miR-26a-5p | 1             | +      | +          | +         |          |         | Break      |
|          |          |           | hsa-miR-26b-5p | 0.82           | +      | +          | +         |          |         | Break      |
|          |          |           | hsa-miR-4320   | 2.51           | +      |            |           |          |         | Break      |
|          |          |           | hsa-miR-4418   | 1.35           | +      |            |           |          |         | Enhance    |
|          |          |           | hsa-miR-4465   | 0.7            | +      |            |           |          |         | Break      |
|          |          |           | hsa-miR-509-3-5p| 0.41         | +      |            |           |          |         | Enhance    |
|          |          |           | hsa-miR-509-5p | 0.78           | +      |            |           |          |         | Enhance    |
|          |          |           | hsa-miR-513b   | 1.4            | +      |            |           |          |         | Create     |
|          |          |           | hsa-miR-513c-5p| 3.87           | +      | +          | +         |          |         | Break      |
|          |          |           | hsa-miR-514b-5p| 4.36           | +      | +          | +         |          |         | Break      |

Table 3. Contd...
| Gene     | dbSNP ID | Variation | miRNA         | $\Delta\Delta G$ (kCal/mol) | MirSNP | TargetScan | PolymiRTS | miRNASNP | miRdSNP | Effect  |
|----------|----------|-----------|---------------|-----------------------------|--------|------------|-----------|----------|---------|---------|
| SNAP25   | rs363238 | C/A       | hsa-miR-297   | 0.77                        | +      |            |           |          |         | Decrease|
|          |          |           | hsa-miR-3149 | 0.98                        | +      | +          |           |          |         | Break   |
|          |          |           | hsa-miR-4677-5p | 0.53                      | +      | +          |           |          |         | Decrease|
|          |          |           | hsa-miR-4774-5p | 6.41                      | +      | +          |           |          |         | Create  |
|          |          |           | hsa-miR-578   | 0.98                        | +      | +          | +         |          |         | Break   |
|          |          |           | hsa-miR-643   | 0.98                        | +      |             | +         |          |         | Break   |
| SNAP25   | rs3746544| G/T       | hsa-miR-3617  | 0.56                        | +      |            |           |          |         | Break   |
|          |          |           | hsa-miR-3913-3p | 0.14                      | +      |             |           |          |         | Break   |
|          |          |           | hsa-miR-641   | 0.38                        | +      |             |           |          |         | Break   |
|          | rs1051312| T/C       | hsa-miR-3646  | 0.31                        | +      |             |           |          |         | Decrease|
|          |          |           | hsa-miR-3664-3p | 0.24                      | +      |             | +         |          |         | Break   |
|          | rs8636   | T/A, C    | hsa-miR-510   | 1.86                        | +      |             | +         |          |         | Break   |
| SPOCK3   | rs6846930| C/G, A    | hsa-miR-3617  | 0.56                        | +      |             |           |          |         | Break   |
|          |          |           | hsa-miR-3913-3p | 0.14                      | +      |             |           |          |         | Break   |
|          | rs3762245| A/G       | hsa-miR-4260  | 0.05                        | +      |             |           |          |         | Decrease|
| STX1A    | rs1569061| C/T       | hsa-miR-4945  | 0.32                        | +      |             |           |          |         | Break   |
|          |          |           | hsa-miR-499b-5p | 2.59                      | +      |             | +         |          |         | Break   |
|          | rs7889267| G/A       | hsa-miR-3137-5p | 4.34                      | +      |             |           |          |         | Decrease|
|          |          |           | hsa-miR-661   | 0.0                         | +      |             |           |          |         | Enhance |
|          |          |           | hsa-miR-3173-3p | 0.18                      | +      |             | +         |          |         | Enhance |
| SYP      |          |           | hsa-miR-1266  | 0.11                        | +      |             |           |          |         | Decrease|
|          |          |           | hsa-miR-1321  | 0.14                        | +      |             |           |          |         | Create  |
|          |          |           | hsa-miR-149-3p | 0.0                        | +      |             | +         |          |         | Create  |
|          |          |           | hsa-miR-3173-3p | 0.18                      | +      |             | +         |          |         | Break   |
|          |          |           | hsa-miR-4270  | 0.16                        | +      |             |           |          |         | Create  |
|          |          |           | hsa-miR-4441  | 0.06                        | +      |             |           |          |         | Create  |
|          |          |           | hsa-miR-4518  | 0.1                         | +      |             |           |          |         | Decrease|
|          |          |           | hsa-miR-3173-3p | 0.18                      | +      |             | +         |          |         | Create  |
|          |          |           | hsa-miR-4728-5p | 0.02                      | +      |             |           |          |         | Create  |
|          |          |           | hsa-miR-4739  | 2.12                        | +      |             |           |          |         | Create  |
|          |          |           | hsa-miR-4756-5p | 0.18                      | +      |             |           |          |         | Create  |
|          |          |           | hsa-miR-4779  | 0.0                         | +      |             |           |          |         | Break   |

Table 3. Contd…
| Gene | dbSNP ID | Variation | miRNA | ΔΔG (kCal/mol) | MirSNP | TargetScan | PolymiRTS | miRNASNP | miRdSNP | Effect |
|------|----------|-----------|-------|---------------|--------|------------|-----------|----------|---------|--------|
| SYT1 | rs1245667 | T/C       | hsa-miR-143-5p | 1.25 | + | + | + | + | Create |
|      |          |           | hsa-miR-148a-3p | 1.17 | + | + | + | + | Create |
|      |          |           | hsa-miR-148b-3p | 0.83 | + | + | + | + | Create |
|      |          |           | hsa-miR-152 | 1.35 | + | + | | + | Create |
|      |          |           | hsa-miR-3189-3p | 1.85 | + | | | | Decrease |
|      |          |           | hsa-miR-34c-5p | 1.46 | + | | | | Enhance |
|      |          |           | hsa-miR-449a | 1.47 | + | | | | Enhance |
|      |          |           | hsa-miR-449b-5p | 1.47 | + | | | | Enhance |
|      |          |           | hsa-miR-4650-3p | 1.78 | + | | | | Break |
|      |          |           | hsa-miR-635 | 0.72 | + | | | | |
|      |          |           | hsa-miR-936 | 1.3 | + | + | | | Create |
|      | rs2248102 | G/A      | hsa-miR-4327 | 3.67 | + | | | | Break |
| VAMP2 | rs1150 | A/G | hsa-miR-5583-3p | 0.0 | + | | | | Enhance |
|      |          |           | hsa-miR-601 | 0.0 | + | | | | Create |
|      | rs1061032 | T/A, C, G | hsa-miR-127-3p | 0.02 | + | | | | |
|      |          |           | hsa-miR-149-3p | 2.7 | | | | | Decrease |
|      |          |           | hsa-miR-4447 | 2.56 | + | + | | | Break |
|      |          |           | hsa-miR-4472 | 2.12 | + | + | | | Break |
|      |          |           | hsa-miR-4481 | 1.76 | + | + | | | Break |
|      |          |           | hsa-miR-4728-5p | 2.47 | + | | | | Create |
|      |          |           | hsa-miR-4745-5p | 3.53 | + | + | | | Break |

Demonstrated that an SNP (rs4938723) located in the promoter region of the pri-miR-34b/c could affect the binding of transcription factor GATA, and consequently, result in a reduction of the miR-34b and miR-34c expression levels in PBMCs of ADHD patients [27].

Up to date, there have been a few investigations conducted on miRNAs in ADHD. Wu et al. (2015) demonstrated that the miRNA let-7d expression was increased in the patient's group serum [28]. In addition, Srivastav et al. (2018) reported that miRNAs could regulate the expression of DAT1, SNAP-25, HTR2C, BDNF, HTR1B, and those genes associated with ADHD etiology. miRNAs dysregulation influences the genes regulation mechanisms, which could affect neurodevelopmental processes, and also investigating the role of miRNAs in ADHD appears to be a promising step in understanding its etiology [29]. In addition, Kandemir et al. (2014) indicated that the miRNA 155a-5p levels were increased in ADHD subjects, and the levels of miRNA 18a-5p, 22-3p, 24-3p, 106b-5p, and 107 were significantly decreased in patients [30].

In this study, 284 miRNAs were predicted, and amongst them were several miRNAs that were investigated in earlier studies, but they were not the studies in the field of ADHD.
over, hsa-miR-34a-5p dysregulation was reported in schizophrenia prefrontal cortex samples; while its expression was enhanced upon lithium treatment. Therefore, hsa-miR-34a-5p was recommended for schizophrenia predicting [34].

In one study, hsa-miR-432-5p appears to predict Schizophrenia and its clinical symptoms [35].

The miR-646 expression levels decreased in tumor tissues, and along with that in metastatic renal cell carcinoma. Actually, miR-646 controlled the NOB1 negatively and repressed the renal cancer cell migration and proliferation throughout MAPK pathway [36]. Other miRNAs including miR-3189-3p [37], hsa-miR-142-5p [38, 39], has-miR-3-617 [40], and miR-143-5p were also examined in different diseases [41].

Therefore, understanding the ADHD associated miRNAs-target genes interactions in neurodevelopment and neural function are not only important for the ADHD etiology but also can affect the diagnosis, prognosis, and treatment of ADHD.

Most of the previous investigations have evaluated the association between genes and their SNPs with ADHD; however, nowadays it is very significant for identifying the different genetic polymorphisms within miRNA binding sites of the ADHD-related genes. This study identified the ADHD-associated genes and their 3'UTR polymorphisms. Although, a large number of SNPs were investigated in the 3'UTR of 51 genes, which were predicted to have association with ADHD, but at the end most SNPs with MAF ≤ 0.05 or without HapMap data were excluded from this study, and only 124 SNPs in the 3'UTR of 35 genes were selected in order to be investigated.

miRNAs involved in the post-transcriptional regulation could recognize the target miRNAs by binding to MRE-sequences within the target genes 3'UTR [42]. Genetic variants within the binding site can influence the miRNAs function by target sites disrupting or creating [15]. These MRE-SNPs can modify the miRNA:MRE interaction, therefore, may be a regulatory mechanism underlying the gene expression, and could be involved in predisposition, individual variation in ADHD gene expression, pathogenesis, and heterogeneity.

CONCLUSION

In conclusion, this study results recommended that SNPs within MRE of the genes might confer susceptibility risk to ADHD and contribute to ADHD heterogeneity, and phenotypic variability. As a result, SNPs within miRNAs binding sites result in better ADHD mechanism perception, and consequently better diagnostic, prognostic, or therapeutic tools. However, predicted SNPs into 3'UTR and miRNAs were just theoretical and further studies in regard are required in order to validate the function of these candidate genetic polymorphisms.
Zhang, L.; Chang, S.; Li, Z.; Zhang, K.; Du, Y.; Ott, J.; Wang, J. [http://dx.doi.org/10.1186/1471-2164-13-661] [PMID: 23173617]

Kumar, A.; Wong, A.K.-L.; Tizard, M.L.; Moore, R.J.; Lefèvre, C. miRNA Targets: A database for miRNA target predictions in coding and non-coding regions of mRNAs. Genomics, 2012, 100(6), 352-356.

[http://dx.doi.org/10.1016/j.ygeno.2012.08.006] [PMID: 22940442]

Lipchitz, I.; Elkabetz, Y.; Hafner, M.; Sheridan, R.; Mihailovic, A.; Tuschl, T.; Sander, C.; Studer, L.; Betel, D. Genome-wide identification of microRNA targets in human ES cells reveals a role for miR-302 in modulating BMP response. Genes Dev., 2011, 25(20), 2173-2186.

[http://dx.doi.org/10.1010/j.gaden.2011.2012111] [PMID: 22012620]

Ziebarth, J.D.; Bhattacharya, A.; Chen, A.; Cui, Y. PolymiRTS Database 2.0: linking polymorphisms in microRNA target sites with human diseases and complex traits. Nucleic Acids Res., 2012, 40(Database issue), D216-D221.

[http://dx.doi.org/10.1038/nar.gkl2012] [PMID: 22080514]

Landi, D.; Barale, R.; Gemignani, F.; Landi, S. Prediction of the biological effect of polymorphisms within microRNA binding sites. In: MicroRNA and Cancer; Humana Press: Totowa, NJ, 2011, pp. 197-210.

[http://dx.doi.org/10.1007/978-1-60761-863-8_14] [PMID: 21758923]

Faraone, S.V.; Perlis, R.H.; Doyle, A.E.; Smoller, J.W.; Goralnik, J.J.; Holmgren, M.A.; Sklar, P. Molecular genetics of attention-deficit/hyperactivity disorder. Biol. Psychiatry, 2005, 57(11), 1313-1323.

[http://dx.doi.org/10.1016/j.biopsych.2004.11.024] [PMID: 15950004]

ShastrY, B.S. SNPs: Impact on gene function and phenotype. Methods Mol. Biol., 2009, 578, 3-22.

[http://dx.doi.org/10.1038/10321707.01.021] [PMID: 29486545]

Huang, H.-C.; Wu, L.-S.-H.; Yu, S.-C.; Wu, B.-J.; Lua, A.C.; Lee, S.-M.; Liu, C.-Z. The alpha2A adrenergic receptor gene-1291C single nucleotide polymorphism is associated with the efficacy of methylphenidate in treating taiwanese children and adolescents with attention-deficit hyperactivity disorder. Psychiatry Investig., 2015, 12(3), 306-312.

[http://dx.doi.org/10.3773/pi.2017.07.24] [PMID: 29486545]

Lario, S.; Callis, J.; Cases, A.; Oriola, J.; Torras, A.; Rivera, F. Mspl identifies a biallelic polymorphism in the promoter region of the alpha 2A-adrenergic receptor gene. Clin. Genet., 1997, 51(2), 129-130.

[http://dx.doi.org/10.1111/j.1399-0004.1997.tb02436.x] [PMID: 9112004]

Alamo, C.; López-Muñoz, F.; Sánchez-García, J. Mechanism of action of guanfacine: A postsynaptic differential approach to the treatment of attention deficit hyperactivity disorder (adhd). Actas Esp. Psiquiatr., 2016, 44(3), 107-112.

[https://www.ncbi.nlm.nih.gov/pubmed/27254403] [PMID: 27254403]

Stahl, S.M. Mechanism of action of alpha 2A-adrenergic agonists in attention-deficit/hyperactivity disorder and with no oppositional symptoms. J. Clin. Psychiatry, 2010, 71(3), 223-224.

[http://dx.doi.org/10.4088/JCP.09m0589pur] [PMID: 20331927]

Kertesz, M.; Iovino, N.; Unnertall, U.; Gaul, U.; Züchner, S.; Scott, W.K.; Martin, E.R.; Vance, J.M. Variation in the miR-NA-433 binding site of FGFR2 confers risk for Parkinson disease by overexpression of alpha-synuclein. Am. J. Hum. Genet., 2008, 82(2), 283-289.

[http://dx.doi.org/10.1016/j.ajhg.2007.09.021] [PMID: 18252210]

Ghanbari, M.; Ikarov, M.A.; de Looper, H.W.J.; Hofman, A.; Erkildsen, S.J.; Franco, O.H.; Delghan, A. Genome-wide identification of microRNA-related variants associated with risk of Alzheimer’s disease. Sci. Rep., 2016, 6, 28387.

[http://dx.doi.org/10.1038/srep28387] [PMID: 27328823]

Georges, M.; Coppet, W.; Charlier, C. Polymorphic miRNA-mediated gene regulation: Contribution to phenotype variation and disease. Curr. Opin. Genet. Dev., 2007, 17(3), 166-176.

[http://dx.doi.org/10.1016/j.coged.2007.04.005] [PMID: 17467975]

Zhong, L.; Chang, S.; Li, Z.; Zhang, K.; Du, Y.; Ott, J.; Wang, J. ADHGDgene: A genetic database for attention deficit hyperactivity disorder. Nucleic Acids Res., 2012, 40(Database issue), D1003-D1009.

[http://dx.doi.org/10.1093/nar/gkr992] [PMID: 22080511]

Piñero, J.; Bravo, Á.; Queralt-Rosinaich, N.; Gutiérrez-Sacristán, A.; Deu-Pons, J.; Centeno, E.; García-García, J.; Sanz, F.; Furlong, L.I. DisGeNET: A comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res., 2017, 45(DI), D833-D839.

[http://dx.doi.org/10.1093/nar/gkw943] [PMID: 27924018]

Bruno, A.E.; Li, L.; Kalabus, J.J.; Pan, Y.; Yu, A.; Hu, Z. miRDiSNP: A database of disease-associated SNPs and microRNA target sites on 3’UTRs of human genes. BMC Genomics, 2012, 13(1), 44.

[http://dx.doi.org/10.1186/1471-2164-13-44] [PMID: 22276777]

Liu, C.; Zhang, F.; Li, T.; Lu, M.; Wang, L.; Yue, W.; Zhang, D. MiRSNP, a database of polymorphisms altering miRNA target sites, identifies miRNA-related SNPs in GWAS SNPs and eQTLs. BMC Genomics, 2012, 13(1), 661.

[http://dx.doi.org/10.1186/1471-2164-13-661] [PMID: 22173617]

Kumar, A.; Wong, A.K.-L.; Tizard, M.L.; Moore, R.J.; Lefèvre, C. miRNA Targets: A database for miRNA target predictions in coding and non-coding regions of mRNAs. Genomics, 2012, 100(6), 352-356.

[http://dx.doi.org/10.1010/j.ygeno.2012.08.006] [PMID: 22940442]

Lipchitz, I.; Elkabetz, Y.; Hafner, M.; Sheridan, R.; Mihailovic, A.; Tuschl, T.; Sander, C.; Studer, L.; Betel, D. Genome-wide identification of microRNA targets in human ES cells reveals a role for miR-302 in modulating BMP response. Genes Dev., 2011, 25(20), 2173-2186.

[http://dx.doi.org/10.1010/j.gaden.2011.2012111] [PMID: 22012620]
[28] Wu, L.H.; Peng, M.; Yu, M.; Zhao, Q.L.; Li, C.; Jin, Y.T.; Jiang, Y.; Chen, Z.Y.; Deng, N.H.; Sun, H.; Wu, X.Z. Circulating microRNA Let-7d in attention-deficit/hyperactivity disorder. *Neuromolecular Med.*, 2015, **17**(2), 137-146. [http://dx.doi.org/10.1007/s12017-015-8345-y] [PMID: 25274585]

[29] Srivastav, S.; Wallitza, S.; Grünblatt, E. Emerging role of miRNA in attention deficit hyperactivity disorder: A systematic review. *Attention Deficit Hyperact. Disord.*, 2018, **10**(1), 49-63. [http://dx.doi.org/10.1007/s12017-012-0232-y] [PMID: 28493018]

[30] Kandemir, H.; Erdal, M.E.; Selck, S.; Ay, O.I.; Karababa, I.F.; Kandemir, S.B.; Ay, M.E.; Yılmaz, S.G.; Bayazıt, H.; Taşdelen, B. Evaluation of several micro RNA (miRNA) levels in children and adolescents with attention deficit hyperactivity disorder. *Neurosci. Lett.*, 2014, **580**, 158-162. [http://dx.doi.org/10.1016/j.neulet.2014.07.060] [PMID: 25123444]

[31] Papagregoriou, G.; Erguler, K.; Dweep, H.; Voskarides, K.; Koupepidou, P.; Athanasiou, Y.; Pierides, A.; Gretz, N.; Felekis, K.N.; Deltas, C. A miR-1207-5p binding site polymorphism abolishes regulation of HBEGF and is associated with disease severity in CFHR5 nephropathy. *PLoS One*, 2012, **7**(2), e31021. [PMID: 22319602]

[32] He, Y.; Yu, D.; Zhu, L.; Zhong, S.; Zhao, J.; Tang, J. miR-149 in human cancer: A systemic review. *J. Cancer*, 2018, **9**(2), 375-388. [http://dx.doi.org/10.7150/jca.21044] [PMID: 29344284]

[33] Gallelli, L.; Cione, E.; Peltrone, F.; Siviglia, S.; Verano, A.; Chirichella, D.; Zampogna, S.; Guidetti, V.; Sammartino, L.; Montana, A.; Caroleo, M.C.; De Sarro, G.; Di Mizio, G. Hsa-miR-34a-5p and hsa-miR-375 as biomarkers for monitoring the effects of drug treatment for migraine pain in children and adolescents: A pilot study. *J. Clin. Med.*, 2019, **8**(7), E928. [http://dx.doi.org/10.3937/jem8070928] [PMID: 31256988]

[34] Kim, A.H.; Reimers, M.; Maher, B.; Williamson, V.; McMichael, O.; McClay, J.L.; van den Oord, E.J.C.G.; Riley, B.P.; Kendler, K.S.; Vladimirov, V.I. MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. *Schizophr. Res.*, 2018, **124**(1-3), 183-191. [http://dx.doi.org/10.1016/j.schres.2010.07.002] [PMID: 20675101]

[35] Hommers, L.G.; Domschke, K.; Deckert, J. Heterogeneity and individuality: microRNAs in mental disorders. *J. Neural Transm.*, 2015, **122**(1), 79-97.

[36] Li, W.; Liu, M.; Feng, Y.; Xu, Y.F.; Huang, Y.F.; Che, J.P.; Wang, G.C.; Yao, X.D.; Zheng, J.H. Downregulated miR-646 in clear cell renal carcinoma correlated with tumour metastasis by targeting the ninf one binding protein (NOB1). *Br. J. Cancer*, 2014, **111**(6), 1188-1200. [http://dx.doi.org/10.1038/bjc.2014.382] [PMID: 25010867]

[37] Bian, Y.; Guo, J.; Qiao, L.; Sun, X. miR-3189-3p mimics enhance the effects of S100A4 siRNA on the inhibition of proliferation and migration of gastric cancer cells by targeting CFL2. *Int. J. Mol. Sci.*, 2018, **19**(1), 236. [http://dx.doi.org/10.3390/ijms19010236] [PMID: 29342841]

[38] Collares, C.V.A.; Evangelista, A.F.; Xavier, D.J.; Rassi, D.M.; Arns, T.; Foss-Freitas, M.C.; Foss, M.C.; Puthier, D.; Sakamoto-Hojo, E.T.; Passos, G.A.; Donadi, E.A. Identifying common and specific microRNAs expressed in peripheral blood mononuclear cell of type 1, type 2, and gestational diabetes mellitus patients. *BMC Res. Notes*, 2013, **6**, 491. [http://dx.doi.org/10.1186/1756-0500-6-491] [PMID: 24279768]

[39] Cheng, D.; Li, J.; Zhang, L.; Hu, L. miR-142-5p suppresses proliferation and promotes apoptosis of human osteosarcoma cell line, HOS, by targeting PLA2G16 through the ERK1/2 signaling pathway. *Onco. Lett.*, 2019, **17**(1), 1363-1371. [PMID: 30655907]

[40] Wang, Q.; Wang, Y.; Ji, W.; Zhou, G.; He, K.; Li, Z.; Chen, J.; Li, W.; Wen, Z.; Shen, J.; Qiang, Y.; Ji, J.; Wang, Y.; Shi, Y.; Yi, Q.; Wang, Y. SNAP25 is associated with schizophrenia and major depressive disorder in the Han Chinese population. *J. Clin. Psychiatry*, 2015, **76**(1), e67-e82. [http://dx.doi.org/10.4088/JCP.13m08962] [PMID: 25650683]

[41] He, Z.; Yi, J.; Liu, X.; Chen, J.; Han, S.; Jin, L.; Chen, L.; Song, H. MiR-143-3p functions as a tumor suppressor by regulating cell proliferation, invasion and epithelial-mesenchymal transition by targeting QKI-5 in esophageal squamous cell carcinoma. *Mol. Cancer*, 2016, **15**(1), 51. [http://dx.doi.org/10.1186/s12943-016-0533-3] [PMID: 27358073]

[42] Satoh, J. Molecular network analysis of human microRNA targetome: from cancers to Alzheimer’s disease. *BioData Min.*, 2012, **5**(1), 17. [http://dx.doi.org/10.1186/1756-0381-5-17] [PMID: 23034144]