MicroRNAs Correlate with Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorder in a Chinese Population

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Background: Recent studies identified a set of differentially expressed miRNAs in whole blood that may discriminate neuromyelitis optica spectrum disorders (NMOSD) from relapsing-remitting multiple sclerosis (RRMS). This study invalidated 9 known miRNAs in Chinese patients.

Material/Methods: The levels of miRNAs in whole blood were assayed in healthy controls (n=20) and patients with NMOSD (n=45), RRMS (n=17) by quantitative real-time polymerase chain reaction (qRT-PCR), and pairwise-compared between groups. They were further analyzed for association with clinical features and MRI findings of the diseases.

Results: Compared with healthy controls, miR-22b-5p, miR-30b-5p and miR-126-5p were down-regulated in NMOSD, in contrast, both miR-101-5p and miR-126-5p were up-regulated in RRMS. Moreover, the levels of miR-101-5p, miR-126-5p and miR-660-5p were significantly higher in RRMS than in NMOSD (P=0.04, 0.01 and 0.02, respectively). The level of miR-576-5p was significantly higher in patients underwent relapse for ≤3 times than those for >4 times. In addition, its level was significantly higher in patients suffered from a severe visual impairment (visual sight ≤0.1). Moreover, the levels of each of the 9 miRNAs were lower in NMOSD patients with intracranial lesions (NMOSD-IC) than those without (NMOSD-non-IC). Despite correlations of miRNAs with these disease subtypes, all AUCs of ROC generated to discriminate patients and controls, as well as intracranial lesions, were <0.8.

Conclusions: Certain miRNAs are associated with RRMS and NMOSD. They are also related to the clinical features, especially intracranial lesions of NMOSD. However, none of the miRNAs alone or in combination was powerful to ensure the diagnosis and differentiation of the 2 disease subtypes.

MeSH Keywords: MicroRNAs • Multiple Sclerosis • Neuromyelitis Optica

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Background

Multiple sclerosis (MS) and neuromyelitis optica spectrum disorders (NMOSD) are autoimmune demyelinating disorder of the central nervous system. Only a few biomarkers are available in the clinical practice, such as cerebrospinal fluid oligoclonal bands and serum anti-aquaporin 4 antibodies. Thus, there is a significant unmet need for biomarkers to assess diagnosis and prognosis. MicroRNAs, a kind of small non-coding RNA present in stable form in the human blood, have attracted much attention as novel diagnostic biomarkers for many diseases, such as tumors and autoimmune diseases [1]. Functionally, these miRNAs regulate gene expression involving cell division, metabolism, stress response, and angiogenesis [2–5]. Others play roles in proliferation, invasion and migration of cancer [6–10].

Previous studies demonstrated that miRNA expression profiles in whole blood or purified blood cell subtypes are correlated with MS and that circulating miRNAs are differentially expressed in different stages of MS [11–14], making them easily accessible for monitoring MS [15]. Moreover, recent study identified a set of differentially expressed miRNAs in whole blood that may discriminate neuromyelitis optica spectrum disorders (NMOSD) from relapsing-remitting multiple sclerosis (RRMS) in Europeans [16]. However, there are less reports on the correlation between miRNAs and clinical features and pathology of NMOSD. For instance, it is unclear how certain miRNAs contribute specifically to brain pathology in NMOSD.

So far there is no accurate epidemiological data on NMOSD worldwide, but it is well known that NMOSD accounts for a much higher proportion of idiopathic inflammatory demyelinating diseases (IIDDS) (40%) in Asians than in white populations (1%) [17]. Regarding a predominance of NMOSD in Chinese and remarkable differences of clinical features and genetic backgrounds between Eastern and Western populations [18], we sought to re-evaluate the correlation of these miRNAs with NMOS and RRMS Chinese. We also analyzed the association of these miRNAs with the clinical features of these diseases.

Material and Methods

Patients

A total of 62 patients were diagnosed and treated in The First Affiliated Hospital of Fujian Medical University from November 2013 to July 2016. Twenty healthy adults (18 females, 2 males, aged 44.7±9.8 years) were recruited as control normals. Among all the cases, 45 were diagnosed as NMOSD according to 2015 International Consensus Diagnostic Criteria for Neuromyelitis Optica Spectrum Disorders [18], and 17 were diagnosed with RRMS according to the McDonald 2010 criteria [19] and 2016 MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines [20]. We defined patients within 8 weeks after an acute attack with NMOSD or RRMS as active phase, more than 8 weeks as a stable phase according to the diagnostic criteria for MS [14].

All clinical information including MRI and laboratory tests were collected and evaluated by senior neurologists with expertise in neuroimmunology. The clinical features of the 3 categories of patients were listed in Table 1. The 2 patient groups were significantly different in age and female preponderance. Among 40 NMOSD patients who underwent anti-AQP4 antibody detection by cell-based transfection immunofluorescence assay (CBA, EUROIMMUM Medical Diagnostic, China Co. Ltd.), 34 (85.0%) were positive, the other 5 patients who did not make detection were diagnosed by AQP4 negative diagnostic criteria. Among 10 RRMS patients underwent anti-AQP4 antibody detection, none was positive. The proportion of B lymphocyte in peripheral blood mononuclear cells (PBMCs) was detected in 23 NMOSD patients, among which 8 were decreased and 12 were increased. 15 of 36 NMOSD patients underwent other autoantibodies detection, including ANA, ANA spectrum, dsDNA, ACA, AnCA, and 15(41.7%) were positive. Among 15 RRMS patients underwent autoantibodies detection, 1 (6.7%) was positive. Parenchymal lesions were found in 19(42.2%) NMOSD cases among which 14(31.1%) met the neuroimaging criteria of the 2015 International Consensus Diagnostic Criteria for Neuromyelitis Optica Spectrum Disorders and 2016 MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. These lesions located extensively in the brain regions, including medulla oblongata and area postrema (6/14), midbrain (2/14), thalamus (1/14), periaqueductal, lateral ventricle and the third ventricle (3/14), corpus callosum (3/14) and cerebral hemisphere (4/14). All NMOSD cases received 500–1000 mg of methyl prednisolone treatment in acute stage, which were gradually reduced to 10mg as maintenance dosage for 3 to 36 months. Thirteen patients were treated with gamma globulin 400 mg/kg intravenous injection for 5 days together with prednisolone in the acute phase. Twelve cases used azathioprine 100–150 mg/day for 3 to 60 months, and 3 of them also used cyclosporine 100–150 mg/day for 12 to 36 months. This study was approved by the Ethics Committee of The First Affiliated Hospital of Fujian Medical University (ID: clinical research 2014y0022) and written informed consent was obtained from all study participants.

Selection of miRNAs for measurement

A total of 9 miRNAs were selected for verification in our study, including miR-15b-3p (chr3: 16040458-160404685), miR-22b-5p (chr5: 13813148-13813229), miR-30b-5p (chr8: 134800520-134800607), miR-101-5p (chr1: 65058434-65058508),...
miR-126-5p (chr9: 136670602-136670686), miR-223-5p (chrX: 66018870-66018979), miR-335-3p (chr7: 130496111-130496204), miR-576-5p (chr4: 109488698-109488795) and miR-660-5p (chrX: 50013241-50013337). All of them showed significantly different expression levels in both NMOSD vs. CIS/RRMS and NMOSD vs. healthy controls in whole blood according to the Keller’s study [23].

Peripheral blood RNA isolation and qRT-PCR

A 5-ml blood sample was collected in EDTA tubes from each of the participants and stored at –80°C. MiRNAs was extracted from peripheral whole blood using Tri-Reagent (Life Technologies) according to the manufacturer’s instructions. The purity and concentration of RNA were determined using NanoDrop One (Thermo Scientific). For quantitative detection of miRNA by RT-PCR, purified whole blood miRNA was converted to cDNA by reverse transcription reactions using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) and miRNA-specific stem-loop primers were supplied by the TaqMan MicroRNA Assays (Applied Biosystems).

Selected miRNAs were measured by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) using the qPCR Master Mix (Promega) and QuantStudio® 5 Real-Time PCR System (Applied Biosystems) according to the manufacturer’s instructions. The reactions were incubated in a 96-well optical plate at 95°C for 5 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 40 s. Reactions were performed in triplicate. The cycle threshold (CT) was recorded, which was defined as the number of PCR cycles required for the fluorescent signal to be higher than a threshold indicating baseline variability. Cel-miR-39-3p was chosen as the exogenous reference control. Amplification and melting working curves of all miRNAs are shown in Supplementary Figures 1 and 2. Relative changes of miRNA expression were represented by 2-ΔΔCT.

Bioinformatics analysis

We used the miEAA (http://www.ccb.uni-saarland.de/mieaa_tool/) as a tool to characterize the association of the miRNAs with molecular pathways. MiEAA is based on GeneTrail [24] and used for standard enrichment analyses, such as over-representation analysis or gene set enrichment analysis in the context of miRNAs. Adjusted p values <0.05 were considered significant enrichment.

Statistical analysis

Numeric data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using the professional statistical computer software, GraphPad Prism 5. Differences between groups were tested using the one-way ANOVA rank test or two-tailed student t-test, P<0.05 for two-tailed test was set as the level of statistical significance. Post hoc testing was carried out between the samples. The P values were corrected by the Tukey-Kramer standard.

Results

Clinical features of the NMOSD and RRMS patients

Clinical features of the patients with NMOSD, RRMS are listed in Table 1 and compared with healthy controls (HCs). Compared to RRMS, NMOSD patients had older onset (P=0.04), more

Table 1. Clinical features of the studied subjects.

| Clinical feature                  | NMOSD (n=45) | RRMS (n=17) | CIS (n=14) | HC (n=20) | P value               |
|----------------------------------|--------------|-------------|----------|----------|----------------------|
| Female/male ratio                | 6.5:1        | 1.4:1       | 2.5:1    | 9.1      | 0.02 0.53 0.08       |
| Age at study (year)              | 40.9±12.8    | 31.2±9.3    | 46.4±17.3| 44.7±9.8 | 0.01 0.25 0.06       |
| Age at onset (year)              | 36.1±13.3    | 28.9±8.1    | 44.4±17.3|          | 0.04                 |
| Disease duration (year)          | 4.9±6.6      | 3.8±4.6     | 1.8±5.3  |          | 0.49                 |
| Relapse (time)                   | 3.4±2.1      | 2.2±0.9     |          |          | 0.03                 |
| EDSS score at the last visit     | 3.4±2.0      | 2.4±1.1     | 2.4±1.2  |          | 0.06                 |
| Ratio of visual impairment       | 9:36         | 1:16        | 6:8      |          | 0.17                 |
| Ratio of anti-AQP4-Ab positivity | 34/6         | 0/10        | 0/5      |          | <0.0001             |
| Ratio of autoantibody positivity | 15/21        | 1/14        | 0/10     |          | 0.002               |

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significant female preponderance (P=0.02), higher frequency of recurrence (P=0.03), as well as higher positive rate of anti-AQP4 antibody (P<0.0001) and autoantibody (P=0.002).

Alterations of the miRNA expression level in NMOSD and RRMS

The levels of all measured miRNAs are shown in Figure 1. As compared with healthy controls (HCs), miR-22-5p, miR-30b-5p and miR-126-5p were down-regulated in NMOSD (P=0.02, P<0.001 and P=0.04, respectively). In contrast, miR-101-5p and miR-126-5p were expressed at higher levels in RRMS (P=0.03 and P=0.04) than in controls. Moreover, the levels of miR-101-5p, miR-126-5p as well as miR-660-5p, were significantly higher in RRMS than in NMOSD (P=0.04, 0.01 and 0.02, respectively).

Correlation between miRNA levels and the clinical features of NMOSD

Based on a significant correlation between miRNAs with the development of NMOSD, we next analyzed the correlation between miRNA expression level and clinical features of NMOSD, including age, gender, disease duration, recurrence times, severity of visual impairment, EDSS score, AQP4 antibody titers, proportion of B lymphocyte subsets, and MRI findings.

Figure 1. The expression level of the 9 miRNAs in HCs, NMOSD and RRMS separately, as well as the statistical significance among all groups. NMOSD – neuromyelitis optica spectrum disorders; RRMS – relapsing-remitting multiple sclerosis; HCs – healthy controls. NMOSD (n=45), MS (n=17), HCs (n=20). The bar diagram shows the mean 2-ΔCT values and standard deviations. * P<0.05, ** P<0.01, *** P<0.001.
By comparing the miRNA levels in patients displaying each of the two-categorized clinical features, we found that the level of miR-576-5p was significantly higher in patients underwent relapse for ≤3 times than those for ≥4 times (P=0.01). In addition, its level was significantly higher in patients suffered from a severe visual impairment (visual sight ≤0.1) (P=0.003). Similar changes were revealed in the level of miR-223-5p in patients with more relapses and visual impairment, but with lower statistical significance (P=0.05 and 0.04, respectively). There was no significant correlation between the expression level of the remaining 7 miRNAs and the NMOSD features (Table 2).

**Correlation between miRNAs with intracranial lesions in NMOSD patients**

The demyelinating lesions in CNS of NMOSD are mainly confined within the optic nerve and spinal cord. However, it has been demonstrated that intracranial (IC) lesions are also common, and that different molecular mechanisms may account for cases with and without intracranial. Thus, we asked whether this difference may be related to miRNAs. To address this, we further divided the NMOSD patients into 2 subgroups, showing typical intracranial (IC) and without (non-IC) lesions according to MRI findings, and compared the miRNA levels of patients in RRMS patients. Among 45 NMOSD cases, 14 (31.1%) had typical intracranial lesions distributed widely in the white matters, including paraventricular, subcortical regions and corpus callosum. As shown in Figure 2, the levels of each of 9 miRNAs were lower in NMOSD patients with intracranial lesions (NMOSD-IC) than those without (NMOSD-non-IC). In addition, although the level of miR-15b-3p, miR-22b-5p, miR-30b-5p and miR-126b-5p were reduced in NMOSD as a whole, they were only significantly down-regulated in the NMOSD-IC subgroup, as compared with HCs. Similarly, only the NMOSD-IC patients showed lower miR-15b-3p, miR-30b-5p, miR-223-5p and miR-576b-5p levels than the NMOSD-non-IC patients. Interestingly, the level of miR-15b-5p was significantly lower in the NMOSD-IC patients than in RRMS and HCs, although its level in all NMOSD patients was not significantly different from patients with these groups (Figure 1). In contrast, there

### Table 2. Correlation between miRNA expression and the clinical features of NMOSD.

| Clinical features | Categorized comparisons | miR-15b-3p | miR-22-5p | miR-30b-5p | miR-335-3p | miR-101-5p | miR-126-5p | miR-223-5p | miR-576-5p | miR-660-5p |
|-------------------|-------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Gender            | Female (n=39) vs. Male (n=6) | 0.93       | 0.89       | 0.84       | 0.09       | 0.51       | 0.71       | 0.48       | 0.67       | 0.78       |
| Phase of clinical course | Active (n=30) vs. stable (n=15) | 0.27       | 0.09       | 0.26       | 0.1        | 0.83       | 0.71       | 0.92       | 0.65       | 0.36       |
| Times of relapse  | ≤3 (n=25) vs. ≥4 (n=20) | 0.67       | 0.34       | 0.09       | 0.87       | 0.44       | 0.3        | 0.05       | 0.01       | 0.08       |
| EDSS score        | ≤3 (n=23) vs. >3 (n=22) | 0.5        | 0.81       | 0.52       | 0.39       | 1          | 0.62       | 0.55       | 0.48       | 0.21       |
| Visual impairment | Yes (n=18) vs. No (n=27) | 0.85       | 0.74       | 0.63       | 0.55       | 0.28       | 0.99       | 0.49       | 0.16       | 0.37       |
| AQP4-Ab(titre)    | Negative (n=6) vs. Positive (n=34) | 0.71       | 0.47       | 0.65       | 0.44       | 0.32       | 0.48       | 0.4        | 0.48       | 0.7        |
| Autoantibody      | ≤1: 32 (n=20) vs. ≥1: 100 (n=20) | 0.23       | 0.39       | 0.11       | 0.49       | 0.51       | 0.25       | 0.95       | 0.72       | 0.86       |
| MRI enhancement   | Positive (n=14) vs. Negative (n=20) | 0.52       | 0.31       | 0.94       | 0.77       | 0.43       | 0.17       | 0.8        | 0.44       | 0.44       |
| Spinal cord involved (segment) | <6 (n=17) vs. >6 (n=19) | 0.36       | 0.38       | 0.23       | 0.62       | 0.59       | 0.91       | 0.68       | 0.64       | 0.66       |
| B lymphocyte proption (%) | <9.0 (n=8) vs. >14.1 (n=12) | 0.29       | 0.28       | 0.66       | 0.36       | 0.35       | 0.6        | 0.56       | 0.57       | 0.63       |
was no significant difference in the level of any of the 9 miRNAs between NMOSD-non-IC subgroup with RRMS, CIS and HCs. These results collectively suggested that it was the intracranial lesions in the NMOSD that correlate with the peripheral down-regulated miRNAs.

The utility of miRNAs in diagnosis and differentiation of NMOSD and RRMS

The correlation between miRNA levels with the development and clinical features of NMOSD and RRMS suggested that they could help in diagnosing and differentiating them. To test how well these miRNAs discriminate individuals with demyelinating disease and controls and patients with different subtypes, we generated receiver operating characteristic (ROC) curves by plotting the sensitivity of the levels of these miRNAs against 1-specificity and calculating the area under the ROC curves (C statistic) for each population. As shown in Figure 3, the AUCs of miR-101-5p and miR-126-5p for discriminating NMOSD and control were 0.74 and 0.72(A), the AUCs of miR-101-5p, miR-126-5p and miR-660-5p for discriminating NMOSD from RRMS were 0.71, 0.72 and 0.69 respectively(B). When we combined these 3 miRNAs, the AUC was 0.72, 0.69, 0.71 and 0.72 in discriminating these 2 subtypes (C). We also calculated the
AUC of ROC for miR-15-5p, miR-30-5p, miR-223-5p and miR-576-5p, alone and in combination, in discriminating NMOSD-IC and NMOSD-non-IC. It turned out that all the AUCs were <0.8 (D). Combined, the results showed that none of the miRNA has enough power in the diagnosis and differential diagnosis of RRMS or NMOSD.

Enrichment of miRNAs in molecular pathways

For the 5 miRNAs differentially expressed in NMOSD patients as compared to controls or RRMS, we found the most enrichment of miRNAs in pathways in cancer (4 of 5 ranked on position 1). Moreover, the neurotrophin signaling pathway, though not ranked before many pathways, was shared by all the 5 miRNAs (Supplementary Table 1).

Discussion

NMOSD miRNA profiling was studied by next-generation sequencing (NGS), and the whole blood is thought to be an appropriate biospecimen for identification with neuroinflammatory diseases [16]. Previous research showed that a part of the miRNAs we selected are associated with inflammatory disease (miR-15b-5p and miR-30b-5p), others are associated with autoimmune disease (miR-22-5p, miR-101-5p, miR-223-5p and miR-660-5p). MiRWalk database showed that all the 9 miRNAs were specifically enriched in neurotrophin signaling pathway. Signaling activated by neurotrophins leads to a series of neuronal functions, such as axonal growth, cell survival, differentiation, dendritic arborization, synapse formation, plasticity and axonal guidance [21, 22].
We found that some miRNAs (miR-22-5p, miR-30b-5p and miR-126-5p) were down-regulated in NMOSD, while others (miR-101-5p and miR-126-5p) were up-regulated in RRMS. Moreover, miR-223-5p and miR-576b-5p are associated with the certain clinical features in NMOSD, including the relapse and extent of visual impairment. MiR-30b-5p participates in restoration of injured optic nerve by regulating sema3A [23]. However, we did not observe any different expression between the patients with relapse or visual impairment. Instead, we found that the miR-576b-5p and miR-223-5p levels were associated with severe visual damage. These results confirm that miRNAs are correlated with CNS inflammatory demyelinating diseases, yet different subtypes may have different miRNA profiles. Nonetheless, the numbers of the patients RRMS was too small and the results look preliminary.

A major strength of the study is the finding of a strong reverse correlation between the peripheral miRNA expression levels with the intracranial (IC) lesions in NMOSD. In fact, the down-regulation of miRNAs (such as miR-22b-5p, miR-30b-5p and miR-126b-5p) revealed in NMOSD were confined to patients with intracranial lesions. In contrast, there was no significant difference in any of the 9 miRNAs between NMOSD patients without intracranial lesions (NMOSD-non-IC), RRMS and HCs, suggesting that these miRNAs were only associated with the NMOSD-IC subgroup, but not all the NMOSD patients. These observations are contrasted with Keller’s study, in which miR-30b-5p and miR-15b-5p were demonstrated as differentiation biomarkers for NMOSD and MS/CIS [20]. The explanations for such differences could be multifold, the most important of which could be the considerable variation of incidence of intracranial lesions in NMOSD across different ethnicities, ranging from 12.5 to 89% [24–29]. The low incidence of intracranial lesions in our study might be a second explanation, with 5 non-specific small lesions locating in subcortical white matter and less than 3mm excluded from counting according to the MRI definition in the guidelines [20]. However, we do not really understand the causes of NMOSD-IC. In MS patients, Th17/Th1 ≥1 relates to more lesions in brain than in spinal cord. Since NMOSD has more prominent imbalance of Th17/Th1 ratio than RRMS in the peripheral blood [30], the intracranial lesion-specific miRNAs could be also involved in the regulation of Th17 polarization, which, in turn, may increase the permeability and destruction of BBB through ICAM, VCAM, MMP-9 [31–33]. Studies have demonstrated the important roles of miR-30b-5p in regulation of humoral immune response as an inflammatory related factor [4], and bioinformatics analysis has also shown its acting on the IL-17 pathway. So, we consider that the cause of NMOSD-IC is the same as that of MS.

The functional significance of these NMOSD-associated miRNAs is not clear. It is interesting to find that these miRNAs were dominantly enriched in the cancer pathways and neurotrophin signaling pathway. Although there is no functional study confirming the involvement of cancer signaling pathway in inflammatory demyelinating diseases, there have been several studies confirming the role of neurotrophin factors, e.g. ciliary neurotrophic factor (CNTF) and p75NTR neurotrophin receptor, in multiple sclerosis [21,22]. Thus, it is intriguing to further investigate what neurotrophin factor genes are targeted by these miRNAs and what mechanisms by which are involved in NMOSD. The difference of miRNA levels in whole blood between patients and controls suggest that they may be candidate diagnostic and differential biomarkers for these disease entities. However, the discriminating power of any of the miRNAs alone or in combination were not strong enough (all AUCs of ROC were less than 0.8) to ensure diagnosis and differentiation of NMOSD or RRMS. Nor was the discrimination ensured by any miRNA alone or in combination between NMOSD patients with intracranial lesions from those without at the diagnostic level.

**Conclusions**

In summary, in a verification study, we confirmed that certain miRNAs in the whole blood are associated with NMOSD and RRMS with distinct profiles. We also demonstrated that miRNAs are only reversely correlated to the intracranial lesions in NMOSD. However, contrasting to Keller’s study, none of the miRNA alone or in combination was powerful to ensure the diagnosis and differentiation of these disease entities. Future studies with expanded sample size (especially that of RRMS and CIS patients) and functional studies are needed to verify our findings.

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**Conflict of interest**

The authors declare that they have no conflict of interest.
Supplementary Figure 1. Amplification plot of the miRNAs. A is the amplification curves of miR-15b-3p, miR-22b-5p, miR-30b-5p and cel39, B is the amplification curves of miR-101-5p, miR-126-5p, miR-223-5p and cel39, C is the amplification curves of miR-335-3p, miR-576-5p, miR-660-5p and cel39.

Supplementary Figure 2. Melting curves of the miRNAs. A is the melt curves of miR-15b-3p, miR-22b-5p, miR-30b-5p and cel39, B is the melt curves of miR-101-5p, miR-126-5p, miR-223-5p and cel39, C is the melt curves of miR-335-3p, miR-576-5p, miR-660-5p and cel39.
Supplementary Table 1. Enrichment of NMOSD-associated miRNAs in the molecular pathways.

| MiRNA         | PathName                  | PathFg | PathBg | GenomeFG | GenomeBG | P value       | BH      |
|---------------|---------------------------|--------|--------|----------|----------|---------------|---------|
| hsa-miR-101-5p | Insulin signaling pathway | 87     | 139    | 8485     | 19747    | 2.34E-06      | 0.00043571 |
| hsa-miR-101-5p | Endocytosis                | 109    | 187    | 8485     | 19747    | 1.65E-05      | 0.003114265 |
| hsa-miR-101-5p | Long term potentiation     | 48     | 71     | 8485     | 19747    | 2.36E-05      | 0.004468536 |
| hsa-miR-101-5p | Colorectal cancer          | 56     | 86     | 8485     | 19747    | 2.78E-05      | 0.005217761 |
| hsa-miR-101-5p | Neurotrophin signaling pathway | 78   | 129    | 8485     | 19747    | 4.52E-05      | 0.009146105 |
| hsa-miR-101-5p | Glioma                     | 44     | 65     | 8485     | 19747    | 4.89E-05      | 0.00905575 |
| hsa-miR-101-5p | Adherens junction           | 57     | 94     | 8485     | 19747    | 4.92E-05      | 0.009146105 |
| hsa-miR-101-5p | Pathways in cancer         | 176    | 330    | 8485     | 19747    | 8.55E-05      | 0.015646108 |
| hsa-miR-101-5p | Endometrial cancer         | 36     | 52     | 8485     | 19747    | 0.000115328   | 0.020759015 |
| hsa-miR-101-5p | Wnt signaling pathway      | 88     | 152    | 8485     | 19747    | 0.000143178   | 0.025628775 |
| hsa-miR-101-5p | Axon guidance              | 76     | 129    | 8485     | 19747    | 0.000185502   | 0.032833892 |
| hsa-miR-101-5p | T cell receptor signaling pathway | 66   | 110    | 8485     | 19747    | 0.000231552   | 0.040753187 |
| hsa-miR-101-5p | ErbB signaling pathway     | 55     | 89     | 8485     | 19747    | 0.000260331   | 0.045579725 |
| hsa-miR-101-5p | Chronic myeloid leukemia   | 47     | 75     | 8485     | 19747    | 0.000452969   | 0.077910691 |
| hsa-miR-101-5p | Phosphatidylinositol signaling system | 47  | 76     | 8485     | 19747    | 0.000695386   | 0.117520317 |
| hsa-miR-101-5p | Calcium signaling pathway  | 98     | 178    | 8485     | 19747    | 0.000741421   | 0.125300102 |
| hsa-miR-101-5p | Chemokine signaling pathway | 103    | 189    | 8485     | 19747    | 0.000887525   | 0.148216608 |
| hsa-miR-101-5p | Ubiquitin mediated proteolysis | 76  | 134    | 8485     | 19747    | 0.000905223   | 0.151172313 |
| hsa-miR-101-5p | Melanoma                   | 44     | 71     | 8485     | 19747    | 0.000956101   | 0.158712713 |
| hsa-miR-101-5p | Renal cell carcinoma       | 44     | 71     | 8485     | 19747    | 0.000956101   | 0.158712713 |
| hsa-miR-101-5p | Non small cell lung cancer | 35     | 54     | 8485     | 19747    | 0.000981599   | 0.16294541 |
| hsa-miR-101-5p | B cell receptor signaling pathway | 46   | 75     | 8485     | 19747    | 0.001018064   | 0.167980536 |
| hsa-miR-101-5p | Type II diabetes mellitus  | 32     | 49     | 8485     | 19747    | 0.001327476   | 0.215051074 |
| hsa-miR-101-5p | mTOR signaling pathway     | 34     | 53     | 8485     | 19747    | 0.001507004   | 0.241366957 |
| hsa-miR-101-5p | Gap junction               | 53     | 90     | 8485     | 19747    | 0.002335429   | 0.361944148 |
| hsa-miR-101-5p | Fc gamma R mediated phagocytosis | 56  | 97     | 8485     | 19747    | 0.002362227   | 0.36615908 |
| hsa-miR-101-5p | Adipocytokine signaling pathway | 42   | 70     | 8485     | 19747    | 0.003005223   | 0.453791189 |
| hsa-miR-101-5p | MAPK signaling pathway     | 139    | 272    | 8485     | 19747    | 0.003964632   | 0.582800901 |
| hsa-miR-101-5p | Pancreatic cancer          | 44     | 75     | 8485     | 19747    | 0.004398819   | 0.637828798 |
| hsa-miR-101-5p | Focal adhesion             | 106    | 203    | 8485     | 19747    | 0.004776226   | 0.78776614 |
| hsa-miR-101-5p | Purine metabolism          | 84     | 158    | 8485     | 19747    | 0.006105552   | 0.84734415 |
| hsa-miR-101-5p | GnRH signaling pathway     | 58     | 105    | 8485     | 19747    | 0.00745423    | 0.94695317 |
| hsa-miR-101-5p | Long term depression       | 40     | 73     | 8485     | 19747    | 0.027582947   | 1.000000001 |
| MiRNA     | PathName                                      | PathFg | PathBg | GenomeFg | GenomeBG | P value          | BH   |
|-----------|-----------------------------------------------|--------|--------|----------|----------|------------------|------|
| hsa-miR-101-5p | Jak STAT signaling pathway                    | 82     | 156    | 8485     | 19747    | 0.009686644     | 1    |
| hsa-miR-101-5p | Small cell lung cancer                        | 46     | 84     | 8485     | 19747    | 0.019339805     | 1    |
| hsa-miR-101-5p | SNARE interactions in vesicular transport     | 23     | 39     | 8485     | 19747    | 0.032118483     | 1    |
| hsa-miR-101-5p | Acute myeloid leukemia                        | 34     | 58     | 8485     | 19747    | 0.011708417     | 1    |
| hsa-miR-101-5p | Vascular smooth muscle contraction            | 57     | 116    | 8485     | 19747    | 0.052274252     | 1    |
| hsa-miR-101-5p | Thyroid cancer                                | 19     | 29     | 8485     | 19747    | 0.011984069     | 1    |
| hsa-miR-101-5p | Lysine degradation                            | 27     | 45     | 8485     | 19747    | 0.015826415     | 1    |
| hsa-miR-101-5p | Epithelial cell signaling in Helicobacter pylori infection | 38     | 71     | 8485     | 19747    | 0.047178139     | 1    |
| hsa-miR-101-5p | Metabolic pathways                            | 499    | 1091   | 8485     | 19747    | 0.030975475     | 1    |
| hsa-miR-101-5p | VEGF signaling pathway                        | 44     | 78     | 8485     | 19747    | 0.011418856     | 1    |
| hsa-miR-101-5p | Fc epsilon RI signaling pathway               | 43     | 82     | 8485     | 19747    | 0.052777799     | 1    |
| hsa-miR-101-5p | Amyotrophic lateral sclerosis ALS             | 30     | 55     | 8485     | 19747    | 0.055429268     | 1    |
| hsa-miR-101-5p | Primary bile acid biosynthesis                | 11     | 16     | 8485     | 19747    | 0.039489998     | 1    |
| hsa-miR-101-5p | Nicotinate and nicotinamide metabolism        | 16     | 24     | 8485     | 19747    | 0.01649091      | 1    |
| hsa-miR-101-5p | Inositol phosphate metabolism                 | 32     | 54     | 8485     | 19747    | 0.01154892      | 1    |
| hsa-miR-101-5p | Peroxisome                                    | 44     | 79     | 8485     | 19747    | 0.015200198     | 1    |
| hsa-miR-101-5p | PPAR signaling pathway                        | 39     | 70     | 8485     | 19747    | 0.021327693     | 1    |
| hsa-miR-101-5p | Cell adhesion molecules CAMs                  | 67     | 133    | 8485     | 19747    | 0.050655518     | 1    |
| hsa-miR-101-5p | Nitrogen metabolism                           | 16     | 23     | 8485     | 19747    | 0.009100855     | 1    |
| hsa-miR-101-5p | Aldosterone regulated sodium reabsorption    | 24     | 42     | 8485     | 19747    | 0.045107589     | 1    |
| hsa-miR-101-5p | O Glycan biosynthesis                         | 20     | 30     | 8485     | 19747    | 0.007542929     | 1    |
| hsa-miR-101-5p | Melanogenesis                                 | 54     | 102    | 8485     | 19747    | 0.026728523     | 1    |
| hsa-miR-101-5p | Lysosome                                      | 62     | 121    | 8485     | 19747    | 0.040474391     | 1    |
| hsa-miR-101-5p | Tight junction                                | 68     | 132    | 8485     | 19747    | 0.029075318     | 1    |
| hsa-miR-101-5p | Regulation of actin cytoskeleton              | 103    | 212    | 8485     | 19747    | 0.056232548     | 1    |
| hsa-miR-101-5p | TGF beta signaling pathway                    | 46     | 86     | 8485     | 19747    | 0.031594456     | 1    |
| hsa-miR-101-5p | Pathways in cancer                            | 176    | 330    | 8124     | 19747    | 4.43E-06        | 1    |
| hsa-miR-101-5p | Small cell lung cancer                        | 55     | 84     | 8124     | 19747    | 5.61E-06        | 0.00085946 |
| hsa-miR-101-5p | Neurotrophin signaling pathway                | 76     | 129    | 8124     | 19747    | 3.35E-05        | 1    |
| hsa-miR-101-5p | Colorectal cancer                             | 54     | 86     | 8124     | 19747    | 4.05E-05        | 1    |
| hsa-miR-101-5p | Chronic myeloid leukemia                      | 48     | 75     | 8124     | 19747    | 5.29E-05        | 0.01004258 |
| hsa-miR-101-5p | Apoptosis                                     | 53     | 87     | 8124     | 19747    | 0.000151403     | 0.02770625 |
| hsa-miR-101-5p | Ubiquitin mediated proteolysis                | 76     | 134    | 8124     | 19747    | 0.000189394     | 0.034280231 |
| MiRNA     | PathName                                      | PathFg | PathBg | GenomFg | GenomBG | P value         | BH       |
|-----------|-----------------------------------------------|--------|--------|---------|---------|-----------------|----------|
| hsa-miR-126-5p | Insulin signaling pathway                      | 78     | 139    | 8124    | 19747   | 0.000249692    | 0.044445215 |
| hsa-miR-126-5p | Pentose and glucuronate interconversions       | 21     | 28     | 8124    | 19747   | 0.000289367    | 0.051279554 |
| hsa-miR-126-5p | ErbB signaling pathway                         | 53     | 89     | 8124    | 19747   | 0.00034132     | 0.060413711 |
| hsa-miR-126-5p | Non small cell lung cancer                     | 35     | 54     | 8124    | 19747   | 0.000373669    | 0.065765685 |
| hsa-miR-126-5p | MAPK signaling pathway                         | 139    | 272    | 8124    | 19747   | 0.000528068    | 0.092411913 |
| hsa-miR-126-5p | Glioma                                         | 40     | 65     | 8124    | 19747   | 0.000709157    | 0.123393247 |
| hsa-miR-126-5p | Ascorbate and aldarate metabolism              | 19     | 26     | 8124    | 19747   | 0.000976366    | 0.168911356 |
| hsa-miR-126-5p | p53 signaling pathway                          | 41     | 68     | 8124    | 19747   | 0.001096874    | 0.189759278 |
| hsa-miR-126-5p | Endocytosis                                     | 98     | 187    | 8124    | 19747   | 0.001158087    | 0.200349415 |
| hsa-miR-126-5p | Type II diabetes mellitus                      | 311    | 109    | 8124    | 19747   | 0.001400798    | 0.222783392 |
| hsa-miR-126-5p | Prostate cancer                                | 51     | 89     | 8124    | 19747   | 0.001486867    | 0.254558697 |
| hsa-miR-126-5p | Pancreatic cancer                              | 44     | 75     | 8124    | 19747   | 0.001614467    | 0.274459422 |
| hsa-miR-126-5p | Renal cell carcinoma                            | 42     | 71     | 8124    | 19747   | 0.001632088    | 0.277455025 |
| hsa-miR-126-5p | T cell receptor signaling pathway              | 61     | 110    | 8124    | 19747   | 0.001662737    | 0.282665317 |
| hsa-miR-126-5p | Axon guidance                                   | 70     | 129    | 8124    | 19747   | 0.001728524    | 0.293849129 |
| hsa-miR-126-5p | Wnt signaling pathway                           | 79     | 152    | 8124    | 19747   | 0.004369155    | 0.642265816 |
| hsa-miR-126-5p | Melanoma                                        | 40     | 71     | 8124    | 19747   | 0.00685062     | 0.897431176 |
| hsa-miR-126-5p | Focal adhesion                                  | 101    | 203    | 8124    | 19747   | 0.007764884    | 0.978375349 |
| hsa-miR-126-5p | VEGF signaling pathway                          | 41     | 78     | 8124    | 19747   | 0.027028017    | 1          |
| hsa-miR-126-5p | ECM receptor interaction                        | 43     | 84     | 8124    | 19747   | 0.039640345    | 1          |
| hsa-miR-126-5p | GnRH signaling pathway                          | 52     | 105    | 8124    | 19747   | 0.05015675     | 1          |
| hsa-miR-126-5p | Progesterone mediated oocyte maturation         | 44     | 88     | 8124    | 19747   | 0.057399498    | 1          |
| hsa-miR-126-5p | ABC transporters                                | 26     | 44     | 8124    | 19747   | 0.012230769    | 1          |
| hsa-miR-126-5p | Endometrial cancer                              | 28     | 52     | 8124    | 19747   | 0.043389343    | 1          |
| hsa-miR-126-5p | Non homologous end joining                     | 9      | 13     | 8124    | 19747   | 0.038757914    | 1          |
| hsa-miR-126-5p | Adipocytokine signaling pathway                 | 39     | 70     | 8124    | 19747   | 0.009610695    | 1          |
| hsa-miR-126-5p | B cell receptor signaling pathway               | 39     | 75     | 8124    | 19747   | 0.037002266    | 1          |
| hsa-miR-126-5p | Primary immunodeficiency                       | 20     | 35     | 8124    | 19747   | 0.040787619    | 1          |
| hsa-miR-126-5p | Cell adhesion molecules CAMs                   | 68     | 133    | 8124    | 19747   | 0.012382036    | 1          |
| hsa-miR-126-5p | Cell cycle                                     | 64     | 124    | 8124    | 19747   | 0.01604645     | 1          |
| hsa-miR-126-5p | Acute myeloid leukemia                          | 31     | 58     | 8124    | 19747   | 0.038959432    | 1          |
| hsa-miR-126-5p | Drug metabolism other enzymes                  | 29     | 51     | 8124    | 19747   | 0.016791375    | 1          |
| hsa-miR-126-5p | PPAR signaling pathway                         | 36     | 70     | 8124    | 19747   | 0.052375031    | 1          |
| hsa-miR-126-5p | Starch and sucrose metabolism                  | 29     | 52     | 8124    | 19747   | 0.023721708    | 1          |
| hsa-miR-126-5p | mTOR signaling pathway                         | 29     | 53     | 8124    | 19747   | 0.03154144     | 1          |
| MiRNA          | PathName                                  | PathFg | PathBg | GenomeFg | GenomeBG | P value            | BH   |
|----------------|-------------------------------------------|--------|--------|----------|----------|--------------------|------|
| has-miR-126-5p | Aldosterone regulated sodium reabsorption | 25     | 42     | 8124     | 19747    | 0.012305735       | 1    |
| has-miR-126-5p | Tight junction                            | 64     | 132    | 8124     | 19747    | 0.052078934       | 1    |
| has-miR-126-5p | Porphyrin and chlorophyll metabolism      | 25     | 41     | 8124     | 19747    | 0.008117058       | 1    |
| has-miR-126-5p | Regulation of actin cytoskeleton          | 99     | 212    | 8124     | 19747    | 0.057272856       | 1    |
| has-miR-126-5p | Protein export                            | 14     | 23     | 8124     | 19747    | 0.044570896       | 1    |
| has-miR-22-5p  | Pathways in cancer                        | 257    | 330    | 11812    | 19747    | 1.83E-12           | 3.56E-10 |
| has-miR-22-5p  | Axon guidance                             | 110    | 129    | 11812    | 19747    | 2.59E-10           | 5.00E-08 |
| has-miR-22-5p  | Endocytosis                               | 149    | 187    | 11812    | 19747    | 4.95E-09           | 9.40E-07 |
| has-miR-22-5p  | Wnt signaling pathway                     | 124    | 152    | 11812    | 19747    | 6.55E-09           | 1.24E-06 |
| has-miR-22-5p  | MAPK signaling pathway                    | 207    | 272    | 11812    | 19747    | 8.91E-09           | 1.69E-06 |
| has-miR-22-5p  | Colorectal cancer                         | 75     | 96     | 11812    | 19747    | 2.32E-08           | 4.45E-06 |
| has-miR-22-5p  | Cell adhesion molecules CAMs              | 108    | 133    | 11812    | 19747    | 9.92E-08           | 1.85E-05 |
| has-miR-22-5p  | ErbB signaling pathway                    | 76     | 89     | 11812    | 19747    | 1.37E-07           | 2.56E-05 |
| has-miR-22-5p  | Neurotrophin signaling pathway            | 103    | 129    | 11812    | 19747    | 9.50E-07           | 0.000173927 |
| has-miR-22-5p  | Focal adhesion                            | 154    | 203    | 11812    | 19747    | 9.68E-07           | 0.000177219 |
| has-miR-22-5p  | Chronic myeloid leukemia                   | 64     | 75     | 11812    | 19747    | 1.43E-06           | 0.000260548 |
| has-miR-22-5p  | Small cell lung cancer                    | 70     | 84     | 11812    | 19747    | 2.90E-06           | 0.000522275 |
| has-miR-22-5p  | Glioma                                    | 56     | 65     | 11812    | 19747    | 3.48E-06           | 0.000622806 |
| has-miR-22-5p  | Type II diabetes mellitus                 | 44     | 49     | 11812    | 19747    | 3.52E-06           | 0.000629532 |
| has-miR-22-5p  | B cell receptor signaling pathway         | 63     | 75     | 11812    | 19747    | 5.33E-06           | 0.000948843 |
| has-miR-22-5p  | Prostate cancer                           | 73     | 89     | 11812    | 19747    | 5.80E-06           | 0.00102714 |
| has-miR-22-5p  | T cell receptor signaling pathway         | 87     | 110    | 11812    | 19747    | 1.34E-05           | 0.002351804 |
| has-miR-22-5p  | Leukocyte transendothelial migration      | 91     | 116    | 11812    | 19747    | 1.57E-05           | 0.00274659 |
| has-miR-22-5p  | Regulation of actin cytoskeleton          | 156    | 212    | 11812    | 19747    | 1.73E-05           | 0.003024113 |
| has-miR-22-5p  | Apoptosis                                 | 70     | 87     | 11812    | 19747    | 3.23E-05           | 0.005550083 |
| has-miR-22-5p  | Adherens junction                         | 62     | 76     | 11812    | 19747    | 4.08E-05           | 0.006975744 |
| has-miR-22-5p  | Pancreatic cancer                         | 63     | 75     | 11812    | 19747    | 5.61E-05           | 0.009538741 |
| has-miR-22-5p  | Fc gamma R mediated phagocytosis          | 76     | 97     | 11812    | 19747    | 8.38E-05           | 0.014085998 |
| has-miR-22-5p  | Endometrial cancer                        | 44     | 52     | 11812    | 19747    | 0.000101849        | 0.017008724 |
| has-miR-22-5p  | Ubiquitin mediated proteolysis            | 101    | 134    | 11812    | 19747    | 0.000107433        | 0.01794134 |
| has-miR-22-5p  | Insulin signaling pathway                 | 104    | 139    | 11812    | 19747    | 0.000141148        | 0.023485705 |
| has-miR-22-5p  | Non small cell lung cancer                | 48     | 54     | 11812    | 19747    | 0.000179093        | 0.029550271 |
| has-miR-22-5p  | Melanogenesis                             | 78     | 102    | 11812    | 19747    | 0.000288309        | 0.047282657 |
| has-miR-22-5p  | VEGF signaling pathway                    | 61     | 78     | 11812    | 19747    | 0.000454736        | 0.073212496 |
| has-miR-22-5p  | Acute myeloid leukemia                    | 47     | 58     | 11812    | 19747    | 0.000474205        | 0.076346974 |
| MiRNA       | PathName                             | PathFg | PathBg | GenomeFG | GenomeBG | P value       | BH     |
|------------|--------------------------------------|--------|--------|----------|----------|---------------|--------|
| hsa-miR-22-5p | p53 signaling pathway                | 54     | 68     | 11812    | 19747    | 0.000481292  | 0.077197402 |
| hsa-miR-22-5p | Dorso ventral axis formation          | 22     | 24     | 11812    | 19747    | 0.000620089  | 0.098594229 |
| hsa-miR-22-5p | Melanoma                             | 55     | 71     | 11812    | 19747    | 0.001297563  | 0.197229622 |
| hsa-miR-22-5p | Calcium signaling pathway             | 125    | 178    | 11812    | 19747    | 0.002461358  | 0.356896943 |
| hsa-miR-22-5p | Long term potentiation                | 54     | 71     | 11812    | 19747    | 0.002984255  | 0.426748527 |
| hsa-miR-22-5p | Renal cell carcinoma                  | 54     | 71     | 11812    | 19747    | 0.002984255  | 0.426748527 |
| hsa-miR-22-5p | Aldosterone regulated sodium reabsorption | 34   | 42     | 11812    | 19747    | 0.003015903  | 0.431274084 |
| hsa-miR-22-5p | Basal cell carcinoma                  | 43     | 55     | 11812    | 19747    | 0.003152907  | 0.447712777 |
| hsa-miR-22-5p | Amyotrophic lateral sclerosis ALS     | 43     | 55     | 11812    | 19747    | 0.003152907  | 0.447712777 |
| hsa-miR-22-5p | Adipocytokine signaling pathway       | 36     | 45     | 11812    | 19747    | 0.003356409  | 0.476610046 |
| hsa-miR-22-5p | mTOR signaling pathway                | 41     | 53     | 11812    | 19747    | 0.003848062  | 0.542576696 |
| hsa-miR-22-5p | Epithelial cell signaling in Helicobacter pylori infection | 53   | 71     | 11812    | 19747    | 0.006391491  | 0.824502359 |
| hsa-miR-22-5p | Hypertrophic cardiomyopathy HCM       | 63     | 86     | 11812    | 19747    | 0.006423679  | 0.828654588 |
| hsa-miR-22-5p | Arrhythmogenic right ventricular cardiomyopathy ARVC | 55   | 74     | 11812    | 19747    | 0.006436624  | 0.830010217 |
| hsa-miR-22-5p | Phosphatidylinositol signaling system | 56     | 76     | 11812    | 19747    | 0.0081223    | 0.96653692 |
| hsa-miR-22-5p | Prion diseases                        | 28     | 35     | 11812    | 19747    | 0.009551427  | 1       |
| hsa-miR-22-5p | Heparan sulfate biosynthesis          | 21     | 26     | 11812    | 19747    | 0.020411929  | 1       |
| hsa-miR-22-5p | Fc epsilon RI signaling pathway       | 58     | 82     | 11812    | 19747    | 0.026603879  | 1       |
| hsa-miR-22-5p | GnRH signaling pathway                | 73     | 105    | 11812    | 19747    | 0.025144507  | 1       |
| hsa-miR-22-5p | Chemokine signaling pathway           | 128    | 189    | 11812    | 19747    | 0.014802878  | 1       |
| hsa-miR-22-5p | N Glycan biosynthesis                 | 35     | 46     | 11812    | 19747    | 0.01561363   | 1       |
| hsa-miR-22-5p | TGF beta signaling pathway            | 62     | 86     | 11812    | 19747    | 0.0120408    | 1       |
| hsa-miR-22-5p | Hedgehog signaling pathway            | 40     | 56     | 11812    | 19747    | 0.048585074  | 1       |
| hsa-miR-22-5p | ABC transporters                     | 33     | 44     | 11812    | 19747    | 0.026093417  | 1       |
| hsa-miR-22-5p | Type I diabetes mellitus             | 32     | 44     | 11812    | 19747    | 0.05300307   | 1       |
| hsa-miR-22-5p | Jak STAT signaling pathway            | 108    | 156    | 11812    | 19747    | 0.069973444  | 1       |
| hsa-miR-22-5p | Valine leucine and isoleucine degradation | 33   | 45     | 11812    | 19747    | 0.04218212   | 1       |
| hsa-miR-22-5p | Long term depression                 | 51     | 73     | 11812    | 19747    | 0.049326477  | 1       |
| hsa-miR-22-5p | SNARE interactions in vesicular transport | 30   | 39     | 11812    | 19747    | 0.019292832  | 1       |
| hsa-miR-22-5p | Caffeine metabolism                  | 7      | 7      | 11812    | 19747    | 0.027380781  | 1       |
| hsa-miR-22-5p | Dilated cardiomyopathy               | 67     | 94     | 11812    | 19747    | 0.013884057  | 1       |
| MiRNA          | PathName                                      | PathFg | PathBg | GenomeFg       | GenomeBG       | P value          | BH          |
|----------------|-----------------------------------------------|--------|--------|----------------|----------------|------------------|-------------|
| hsa-miR-22-5p  | Progesterone mediated oocyte maturation       | 61     | 88     | 11812          | 19747          | 0.04174596      | 1           |
| hsa-miR-30b-5p | Pathways in cancer                            | 156    | 330    | 6411           | 19747          | 1.23E-08         | 2.38E-06    |
| hsa-miR-30b-5p | Adherens junction                             | 46     | 76     | 6411           | 19747          | 4.52E-07         | 8.67E-05    |
| hsa-miR-30b-5p | Colorectal cancer                             | 49     | 86     | 6411           | 19747          | 2.38E-06         | 0.000450104 |
| hsa-miR-30b-5p | ErbB signaling pathway                        | 50     | 89     | 6411           | 19747          | 3.30E-06         | 0.000623737 |
| hsa-miR-30b-5p | Glioma                                        | 37     | 65     | 6411           | 19747          | 4.53E-06         | 0.000851658 |
| hsa-miR-30b-5p | Colorectal cancer                             | 45     | 87     | 6411           | 19747          | 5.81E-05         | 0.010463047 |
| hsa-miR-30b-5p | Wnt signaling pathway                         | 73     | 152    | 6411           | 19747          | 4.52E-05         | 0.008184389 |
| hsa-miR-30b-5p | Non small cell lung cancer                    | 33     | 54     | 6411           | 19747          | 1.41E-05         | 0.002637103 |
| hsa-miR-30b-5p | Apoptosis                                     | 45     | 87     | 6411           | 19747          | 8.38E-06         | 0.01567818  |
| hsa-miR-30b-5p | Long term potentiation                        | 38     | 71     | 6411           | 19747          | 0.000193569      | 0.034068087 |
| hsa-miR-30b-5p | Endocytosis                                   | 38     | 71     | 6411           | 19747          | 0.000193569      | 0.034068087 |
| hsa-miR-30b-5p | Endocytosis                                   | 84     | 187    | 6411           | 19747          | 0.000238243      | 0.041930686 |
| hsa-miR-30b-5p | Prostate cancer                               | 45     | 89     | 6411           | 19747          | 0.000295855      | 0.052070558 |
| hsa-miR-30b-5p | Neurotrophin signaling pathway                | 60     | 129    | 6411           | 19747          | 0.000594457      | 0.099868714 |
| hsa-miR-30b-5p | Long term depression                          | 37     | 73     | 6411           | 19747          | 0.000929249      | 0.150577329 |
| hsa-miR-30b-5p | Amyotrophic lateral sclerosis ALS             | 29     | 55     | 6411           | 19747          | 0.001463924      | 0.229836089 |
| hsa-miR-30b-5p | Regulation of actin cytoskeleton              | 89     | 212    | 6411           | 19747          | 0.002188848      | 0.334837477 |
| hsa-miR-30b-5p | Renal cell carcinoma                          | 38     | 71     | 6411           | 19747          | 0.005227257      | 0.788434095 |
| hsa-miR-30b-5p | Neurotrophin signaling pathway                | 45     | 87     | 6411           | 19747          | 0.000193569      | 0.034068087 |
| hsa-miR-30b-5p | Long term potentiation                        | 38     | 71     | 6411           | 19747          | 0.001463924      | 0.229836089 |
| hsa-miR-30b-5p | Regulation of actin cytoskeleton              | 84     | 187    | 6411           | 19747          | 0.000238243      | 0.041930686 |
| hsa-miR-30b-5p | Neurotrophin signaling pathway                | 45     | 87     | 6411           | 19747          | 0.000295855      | 0.052070558 |
| hsa-miR-30b-5p | Neurotrophin signaling pathway                | 60     | 129    | 6411           | 19747          | 0.000594457      | 0.099868714 |
| hsa-miR-30b-5p | Long term potentiation                        | 85     | 203    | 6411           | 19747          | 0.002953579      | 0.44599037  |
| hsa-miR-30b-5p | Focal adhesion                                | 42     | 90     | 6411           | 19747          | 0.003411486      | 0.511728266 |
| hsa-miR-30b-5p | Focal adhesion                                | 42     | 90     | 6411           | 19747          | 0.003411486      | 0.511728266 |
| hsa-miR-30b-5p | Progesterone mediated oocyte maturation       | 41     | 88     | 6411           | 19747          | 0.003926436      | 0.585346476 |
| hsa-miR-30b-5p | Acute myeloid leukemia                        | 29     | 58     | 6411           | 19747          | 0.004116963      | 0.613427489 |
| hsa-miR-30b-5p | Protein export                                | 14     | 23     | 6411           | 19747          | 0.004720087      | 0.703292942 |
| hsa-miR-30b-5p | O Glycan biosynthesis                         | 17     | 30     | 6411           | 19747          | 0.005227257      | 0.788434095 |
| hsa-miR-30b-5p | Arrhythmogenic right ventricular cardiomyopathy ARVC | 35     | 74     | 6411           | 19747          | 0.005487362      | 0.806642248 |
| hsa-miR-30b-5p | Inositol phosphate metabolism                 | 27     | 54     | 6411           | 19747          | 0.005531749      | 0.813167132 |

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| MiRNA       | PathName                                               | PathFg | PathBg | GenomeFG | GenomeBG | P value       | BH         |
|------------|--------------------------------------------------------|--------|--------|----------|----------|---------------|------------|
| hsa-miR-30b-5p | Aldosterone regulated sodium reabsorption             | 22     | 42     | 6411     | 19747    | 0.005864446  | 0.862073569 |
| hsa-miR-30b-5p | mTOR signaling pathway                                 | 26     | 53     | 6411     | 19747    | 0.008761618  | 1          |
| hsa-miR-30b-5p | Ascorbate and aldarate metabolism                      | 14     | 26     | 6411     | 19747    | 0.019511549  | 1          |
| hsa-miR-30b-5p | Type II diabetes mellitus                              | 24     | 49     | 6411     | 19747    | 0.011815574  | 1          |
| hsa-miR-30b-5p | p53 signaling pathway                                  | 30     | 68     | 6411     | 19747    | 0.029133167  | 1          |
| hsa-miR-30b-5p | Small cell lung cancer                                 | 38     | 84     | 6411     | 19747    | 0.009658053  | 1          |
| hsa-miR-30b-5p | T cell receptor signaling pathway                      | 47     | 110    | 6411     | 19747    | 0.015157895  | 1          |
| hsa-miR-30b-5p | Leukocyte transendothelial migration                   | 49     | 116    | 6411     | 19747    | 0.016941064  | 1          |
| hsa-miR-30b-5p | Cell adhesion molecules CAMs                          | 53     | 133    | 6411     | 19747    | 0.008582357  | 1          |
| hsa-miR-30b-5p | Vascular smooth muscle contraction                     | 50     | 116    | 6411     | 19747    | 0.010372332  | 1          |
| hsa-miR-30b-5p | Thyroid cancer                                         | 16     | 29     | 6411     | 19747    | 0.008582357  | 1          |
| hsa-miR-30b-5p | Insulin signaling pathway                              | 39     | 86     | 6411     | 19747    | 0.008411963  | 1          |
| hsa-miR-30b-5p | Tight junction                                         | 55     | 132    | 6411     | 19747    | 0.01623801   | 1          |
| hsa-miR-30b-5p | Hypertrophic cardiomyopathy HCM                       | 36     | 86     | 6411     | 19747    | 0.042112097  | 1          |
| hsa-miR-30b-5p | Bladder cancer                                         | 20     | 43     | 6411     | 19747    | 0.03804409   | 1          |
| hsa-miR-30b-5p | Fc gamma R mediated phagocytosis                       | 42     | 97     | 6411     | 19747    | 0.01622241   | 1          |
| hsa-miR-660-5p  | Pathways in cancer                                     | 145    | 330    | 5834     | 19747    | 1.63E-08     | 3.12E-06   |
| hsa-miR-660-5p  | Glioma                                                 | 41     | 65     | 5834     | 19747    | 2.14E-08     | 4.08E-06   |
| hsa-miR-660-5p  | Insulin signaling pathway                              | 71     | 139    | 5834     | 19747    | 7.71E-08     | 1.46E-05   |
| hsa-miR-660-5p  | Chronic myeloid leukemia                               | 47     | 75     | 5834     | 19747    | 2.75E-09     | 5.30E-07   |
| hsa-miR-660-5p  | Pathways in cancer                                     | 145    | 330    | 5834     | 19747    | 1.63E-08     | 3.12E-06   |
| hsa-miR-660-5p  | Glioma                                                 | 41     | 65     | 5834     | 19747    | 2.14E-08     | 4.08E-06   |
| hsa-miR-660-5p  | Insulin signaling pathway                              | 71     | 139    | 5834     | 19747    | 7.71E-08     | 1.46E-05   |
| hsa-miR-660-5p  | Chronic myeloid leukemia                               | 47     | 75     | 5834     | 19747    | 2.75E-09     | 5.30E-07   |
| hsa-miR-660-5p  | Pathways in cancer                                     | 145    | 330    | 5834     | 19747    | 1.63E-08     | 3.12E-06   |
| hsa-miR-660-5p  | Glioma                                                 | 41     | 65     | 5834     | 19747    | 2.14E-08     | 4.08E-06   |

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| MiRNA          | PathName                                       | PathFg | PathBg | GenomeFG | GenomeBG | P value     | BH        |
|---------------|-----------------------------------------------|--------|--------|-----------|-----------|-------------|-----------|
| hsa-miR-660-5p| Aldosterone regulated sodium reabsorption     | 25     | 42     | 5834      | 19747     | 5.11E-05   | 0.008849739|
| hsa-miR-660-5p| Prostate cancer                                | 44     | 89     | 5834      | 19747     | 6.07E-05   | 0.010434751|
| hsa-miR-660-5p| Calcium signaling pathway                      | 77     | 178    | 5834      | 19747     | 6.61E-05   | 0.011367391|
| hsa-miR-660-5p| Axon guidance                                  | 59     | 129    | 5834      | 19747     | 7.01E-05   | 0.012062999|
| hsa-miR-660-5p| Vascular smooth muscle contraction             | 54     | 116    | 5834      | 19747     | 7.81E-05   | 0.01334723 |
| hsa-miR-660-5p| VEGF signaling pathway                         | 39     | 78     | 5834      | 19747     | 0.000114223| 0.019189454|
| hsa-miR-660-5p| Dilated cardiomyopathy                         | 40     | 86     | 5834      | 19747     | 0.000133217| 0.022247276|
| hsa-miR-660-5p| Hypertrophic cardiomyopathy HCM               | 40     | 86     | 5834      | 19747     | 0.000290082| 0.046703244|
| hsa-miR-660-5p| Ether lipid metabolism                         | 21     | 36     | 5834      | 19747     | 0.000300946| 0.048452314|
| hsa-miR-660-5p| Adherens junction                              | 37     | 76     | 5834      | 19747     | 0.000335452| 0.056963838|
| hsa-miR-660-5p| Long term potentiation                         | 35     | 71     | 5834      | 19747     | 0.000356024| 0.056963838|
| hsa-miR-660-5p| Melanoma                                       | 35     | 71     | 5834      | 19747     | 0.000356024| 0.056963838|
| hsa-miR-660-5p| Type II diabetes mellitus                      | 26     | 49     | 5834      | 19747     | 0.000474592| 0.075460141|
| hsa-miR-660-5p| T cell receptor signaling pathway              | 49     | 110    | 5834      | 19747     | 0.000588087| 0.092917806|
| hsa-miR-660-5p| Colorectal cancer                              | 40     | 86     | 5834      | 19747     | 0.000640805| 0.10124723 |
| hsa-miR-660-5p| Heparan sulfate biosynthesis                   | 16     | 26     | 5834      | 19747     | 0.000700414| 0.110665477|
| hsa-miR-660-5p| Phosphatidylinositol signaling system          | 36     | 76     | 5834      | 19747     | 0.00077014  | 0.120911911|
| hsa-miR-660-5p| Fc epsilon RI signaling pathway                | 38     | 82     | 5834      | 19747     | 0.000942721| 0.148007141|
| hsa-miR-660-5p| Chondroitin sulfate biosynthesis               | 14     | 22     | 5834      | 19747     | 0.000946761| 0.148614111|
| hsa-miR-660-5p| B cell receptor signaling pathway              | 35     | 75     | 5834      | 19747     | 0.001262727| 0.195722739|
| hsa-miR-660-5p| GnRH signaling pathway                         | 46     | 105    | 5834      | 19747     | 0.001305999| 0.202429883|
| hsa-miR-660-5p| Glycerophospholipid metabolism                | 33     | 70     | 5834      | 19747     | 0.001386882| 0.214966783|
| hsa-miR-660-5p| Endometrial cancer                             | 26     | 52     | 5834      | 19747     | 0.001513571| 0.23460344 |
| hsa-miR-660-5p| alpha Linolenic acid metabolism                | 12     | 19     | 5834      | 19747     | 0.002432594| 0.36245658 |
| hsa-miR-660-5p| Acute myeloid leukemia                         | 27     | 58     | 5834      | 19747     | 0.004528781| 0.638558156|
| hsa-miR-660-5p| Ubiquitin mediated proteolysis                 | 54     | 134    | 5834      | 19747     | 0.004925757| 0.689605994|
| hsa-miR-660-5p| Tight junction                                 | 53     | 132    | 5834      | 19747     | 0.005783875| 0.798174774|
| hsa-miR-660-5p| Regulation of actin cytoskeleton               | 80     | 212    | 5834      | 19747     | 0.006113676| 0.84368735 |
| hsa-miR-660-5p| mTOR signaling pathway                         | 24     | 53     | 5834      | 19747     | 0.010883105| 1          |
| hsa-miR-660-5p| Oocyte meiosis                                 | 43     | 112    | 5834      | 19747     | 0.027327133| 1          |
| hsa-miR-660-5p| Cell adhesion molecules CAMs                   | 52     | 133    | 5834      | 19747     | 0.011277543| 1          |
| hsa-miR-660-5p| Glycerolipid metabolism                       | 19     | 46     | 5834      | 19747     | 0.059110806| 1          |
| hsa-miR-660-5p| Keratan sulfate biosynthesis                   | 8      | 15     | 5834      | 19747     | 0.045846187| 1          |
| hsa-miR-660-5p| Gap junction                                  | 35     | 90     | 5834      | 19747     | 0.035823644| 1          |
| hsa-miR-660-5p| Chemokine signaling pathway                   | 67     | 189    | 5834      | 19747     | 0.045513457| 1          |
MicroRNAs correlate with multiple sclerosis and neuromyelitis optica spectrum disorder

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