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Current Medicinal Chemistry, 2020, *27*, 1-12

REVIEW ARTICLE

The Association Between Depression and Gastroesophageal Reflux Based on Phylogenetic Analysis of miRNA Biomarkers

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ARTICLE HISTORY

Received: January 05, 2020
Revised: February 10, 2020
Accepted: April 15, 2020

DOI:
10.2174/0929867327666200425214906

Abstract: A number of clinical studies have revealed that there is an association between major depression (MD) and gastroesophageal reflux disease (GERD). Both the diseases are shown to affect a large proportion of the global population. More advanced studies for understanding the comorbidity mechanism of these two diseases can shed light on developing new therapies of both diseases. To the best of our knowledge, there has not been any research work in the literature investigating the relationship between MD and GERD using their miRNA biomarkers. We adopt a phylogenetic analysis to analyze their miRNA biomarkers. From our analyzed results, the association between these two diseases can be explored through miRNA phylogeny. In addition to evidence from the phylogenetic analysis, we also demonstrate epidemiological evidence for the relationship between MD and GERD based on Taiwan biobank data.

Keywords: Major depression, gastroesophageal reflux, microRNA, phylogenetic analysis.

1. INTRODUCTION

Major depression (MD) is a common and serious mood disorder that affects approximately 4.4% of the global population. The symptoms of MD may include the feeling of hopelessness, loss of interest in normal activities, sleep disturbances and anxiety. The number of people suffering from MD continues to increase . MD can be diagnosed with psychological tests that can measure the severity of MD by asking personal questions from the participant.

MD has been shown to be associated with a number of other diseases such as obesity, diabetes, cancer, stroke, and acute coronary syndrome [1-4]. In addition, there exists evidence supporting that the gut microbiome inhabiting the gastrointestinal tract is an important risk factor for the development of MD and persistence of depressive symptoms [5]. In this study, we explore the association between MD and gastroesophageal reflux disease (GERD).

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GERD is a common digestive disorder where stomach acid moves into the esophagus. The symptoms include burning feeling in the chest, difficultly in swallowing, chronic cough and asthma. Approximately half of all adults experience reflux symptoms at some time [6]. GERD is a risk factor for esophageal cancer [7] and is the primary risk factor for Barrett's esophagus [8].

A number of clinical studies have revealed that there is an association between MD and GERD. A population-based study was conducted in the Norwegian county to investigate the association between psychiatric disorders and gastro-oesophageal reflux symptoms [9]. The Hospital Anxiety and Depression Scale (HADS) was used to evaluate the level of anxiety and depression of the subjects. This population-based study showed that there is a strong association between depression and reflux symptoms. A retrospective cross-sectional study, including 19,099 subjects who underwent upper gastrointestinal endoscopy was performed and concluded that depression levels were significantly higher in subjects with GERD than in controls [10]. In addition, GERD and depression displayed bidirectional associations in a national

0929-8673/20 $65.00+.00

sample cohort study of the Korean population [11], where the State-Trait Anxiety Inventory (STAI) scale and the Beck Depression Inventory (BDI) scale were used to assess the level of anxiety and depression in all subjects, respectively. Similarly to the study mentioned above [9], subjects in the GERD group showed higher state anxiety and depression levels than those in the control group. On the other hand, a cohort study of Australian men indicated the association between gastroesophageal reflux disease with sleep quality, depression, and anxiety [12].

The above-mentioned association is identified from clinical or population-based studies. There is seldom any research that discusses this problem based on biological methods such as genetic mechanisms or molecular biomarkers. To the best of our knowledge, there has not been any research work in the literature investigating the relationship between MD and GERD using their microRNA (miRNA) biomarkers. miRNAs are single-stranded and non-coding RNAs of about 22–24 nucleotides that were first discovered in the early 1990s while studying development in the nematode Caenorhabditis elegans [13, 14]. miRNAs contribute to the regulation of gene expression, cell proliferation, differentiation and apoptosis and physiological processes [15].

miRNA biomarkers have been discovered for many diseases. A miRNA functions as a tumor suppressor by targeting specific genes [16]. High-confident miRNA biomarkers are predicted for various cancers [17-21]. For non-small cell lung cancers (NSCLC), miR-21-5p, miR-20a-5p, miR141-3p, miR-145-5p, miR-155-5p, and miR-223-3p have been identified to be differentially expressed in different stages of the disease [22]. miR-21 was found to be upregulated in glioblastomas, breast, colon and pancreatic cancers [23]. miRNA may also contribute to neurological diseases and inflammation in the brain [24], such as amyotrophic lateral sclerosis, Parkinson's disease, and anti-NMDA receptor encephalitis [25, 26].

To explore the association between MD and GERD based on their miRNA biomarkers, we first search miRNA biomarkers of the two diseases from the literature and then use the phylogenetic analysis tool to build the phylogenetic tree of these miRNA biomarkers. Phylogenetic tree analysis has been used in the study of miRNAs [27-30]. A combination of the phylogenetic tree analysis with a bioinformatics method can lead to more accurate identification of miRNA cancer biomarkers compared with the bioinformatics method alone [20]. The phylogenetic tree has been used as a tool to analyze the relationship between vaccination and anti-NMDAR encephalitis based on their miRNA biomarkers [31]. In addition, the phylogenetic tree analysis of miRNA biomarkers was used to analyze the association between tumor and anti-NMDAR encephalitis [32]. Following these previous studies, we employ the phylogenetic analysis in the current work.

In addition to the phylogenetic analysis, empirical data analysis from cohort studies of Taiwan Biobank (TWB) [33] was used to further validate our result. This data analysis shows the strong relationship between MD and GERD among Taiwanese people, which also coincides with the results of the previous studies for Norwegian, Korean and Australian people [9, 10, 12].

2. microRNA BIOMARKERS

A literature search was conducted for studies reporting miRNAs biomarkers of MD and GERD. The references of these studies and miRNAs are presented in Tables **1** and **2**.

Table 1. miRNA biomarkers of MD.

miRNA	References
mir-132	[34-36]
miR-16	[34, 37-41]
let-7a	[42, 43]
let-7b	[44, 45]
let-7c	[42, 44, 45]
miR-124	[24, 46-48]
miR-92a, miR-92b	[49, 50]
miR-29a	[47, 50, 51]
miR-128	[52]
miR-135a	[41, 53]
miR-1202	[41, 54, 55]
miR-218	[42, 47, 55]
miR-223	[56]
miR-130a	[57, 58]
miR-335	[57, 59]
miR-144	[50]

There are a lot of miRNA biomarkers reported to be associated with depression in the literature. We exclude those miRNAs that are related to depression indirectly through other diseases, that is, the miRNAs involved not only in depression but also in other diseases, which

are beyond our focus here, are not considered in our analysis. For searching the GERD miRNA biomarkers, since some miRNAs are related to esophageal cancer, we exclude most GERD miRNA biomarkers that are related to esophageal cancer.

Table 2. miRNA biomarkers of Gastroesophageal reflux.

miRNA	References
miR-29a-3p	[60]
miR-128-3p	[60, 61]
miR-223-3p	[60]
miR-143	[62]
miR-145	[62]
miR-205	[62]
miR-203	[63]
miR-130a	[64, 65]

In Tables **1** and **2**, there are 4 miRNAs, including miR-29a, miR-128-3p, miR-223 and miR-130a, that are common biomarkers of MD and GERD. In a rat model study, microarray analysis showed an upregulation of miR-29a-3p, miR-128-3p and miR-223-3p in reflux esophagitis [60]. Downregulation of miRNA-128-3p may protect human esophageal squamous cells Het-1A from hydrochloric acid (HCl)-induced cell injury via targeting E2F3 and inhibiting the activation of ERK and PI3K/AKT pathways [61]. miR-29a-5p showed a significant positive correlation with the Montgomery-Åsberg Depression Rating Scale score (MADRS-S) [50]. Patients with MD treated with antidepressant treatments revealed an upregulation of miR-128 in their blood [52]. In a study of patients with major depressive disorder, circulating plasma miR-223 was differentially expressed after antidepressant treatment [56]. miR-130a expression was altered in the postmortem prefrontal cortex of depressed suicide subjects compared to matched control subjects who died from natural or accidental causes [57]. Finally, miR-130a is highly expressed in the esophageal mucosa of patients with achalasia [64].

In addition to the abovementioned four common miRNA biomarkers of MD and GERD, we discussed several other miRNAs mentioned in Tables **1** and **2**. Circulating miRNAs are useful clinical biomarkers for diagnosing MD. miRNA-132 was the most replicated candidate among the 23 human studies and miRNA-16 was the most replicated candidate among the 6 animal studies [34]. Chronic mild stress is able to downregulate the expression of brain-derived neurotrophic factor (BDNF) and methyl-CpG-binding protein 2 (MeCP2), and the expression levels of MeCP2 and BDNF were negatively correlated with those of miR-132 [35]. Circulating miR-16 in major depressive disorder patients was significantly lower than that in controls [38]. The baseline expressions of let-7b and let-7c were less by ~40% and ~50% in treatment-resistant depression patients compared with controls, respectively [44].

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to compare miRNA expression levels in oesophageal mucosa from individuals without pathological gastro-oesophageal reflux to individuals with ulcerative oesophagitis, and miR-143, miR-145, and miR-205 levels were significantly higher in gastro-oesophageal reflux group [62]. miR-203 was significantly downregulated in GERD patients as compared to controls [63].

3. METHODS AND MATERIALS

3.1. Phylogenetic Tree Analysis

A phylogenetic tree analysis was adopted to cluster miRNAs in order to investigate the relationship between MD and GERD. The similarity of two nucleotide sequences can be measured using substitution models. Using the MATLAB software [66], we can plot phylogenetic trees based on different substitution models.

To begin with, we need to find miRNA biomarkers for both diseases. A number of miRNA biomarkers for MD and GERD, respectively, have already been listed in Table **1**. To plot the phylogenetic tree of these miRNAs, the stem-loop sequences of these biomarkers were used because they may provide more information than the mature -5p sequence and mature -3p sequence. The stem-loop sequences can be accessed from miRBase (http://www.mirbase.org/) [67]. In addition to miRBase, recently, an alternative miRNA database MirGeneDB [68] was published [69, 70]. We separately analyzed the miRNA sequences of these two databases.

To classify these miRNA sequences, the distances between any two miRNA sequences were calculated. Then the sequences were classified such that sequences with smaller distances can be clustered into a group. The study applied the phylogenetic tree method to classify these sequences. In the Matlab software [57], the two steps are implemented as the selection of a distance (evolutionary) model to calculate the distance between two nucleotide sequences, as well as the selec-

tion of a clustering method to build a tree. The distance models considered in the Matlab software [57] include the p-distance, Jukes-Cantor distance, alignment-score distance models, etc. The clustering methods (linkage functions) in the Matlab software include the median, single, and average methods, and so on. In this paper, we used the Jukes-Cantor distance (or the alignment-score distance) to calculate the distances and the median method (or the average method) as the linkage function to build a tree.

3.2. Taiwan Biobank Data

The TWB community–based cohort plans to recruit 200,000 volunteers between 30 and 70 years of age with no history of cancer. The recruitment is still ongoing, and up to now, the cohort has accumulated more than 120,000 participants. Demographical characteristics and disease/symptom/health information for the TWB community–based cohort are collected through carefully collected and examined questionnaire data. Details about the TWB cohort and the data access can be found in webpages [71] and [72], respectively.

For the purpose of our analysis of the empirical association between MD and GERD, the released TWB community-based cohort sample of size 5000 was used. The mean age of the TWB community sample is 48.8 years with standard deviation 10.8 years. The male:female ratio in the sample is 2445:2555.

4. RESULTS

4.1. Phylogenetic Tree Analysis

By applying the phylogenetic trees method, the trees were plotted using the miRNA biomarkers listed in Tables **1** and **2**. First, the miRNA sequences obtained from miRBase were used. Fig. (**1a**) presents the phylogenetic trees based on the Jukes-Cantor model distance and average method; Fig. (**1b**) presents the phylogenetic trees based on the Jukes–Cantor model distance and median method. Fig. (**2a**) presents the phylogenetic trees based on the alignment-score distance and average method; Fig. (**2b**) presents the phylogenetic trees based on the alignment-score distance and median method. Fig. (**3a**) presents the phylogenetic trees based on the p distance and average method; Fig. (**3b**) presents the phylogenetic trees based on the p distance and median method.

From (Figs. **1-3**), most of the MD and GERD biomarkers cannot be classified into two groups. It shows that from the miRNA biomarker viewpoint, these two diseases may have an association. In particular, the two miRNAs miR-29a and miR-335 are different from the other miRNAs. In (Fig. **1a**), (Fig. **2ab**) and (Fig. **3a,b**), miR-29a or miR-335 is in a separate branch of the tree. These figures are based on different evolutionary models and clustering methods. Most of them show similar results for these two miRNAs. Since miR-29a is a common miRNA biomarker of MD and GERD and

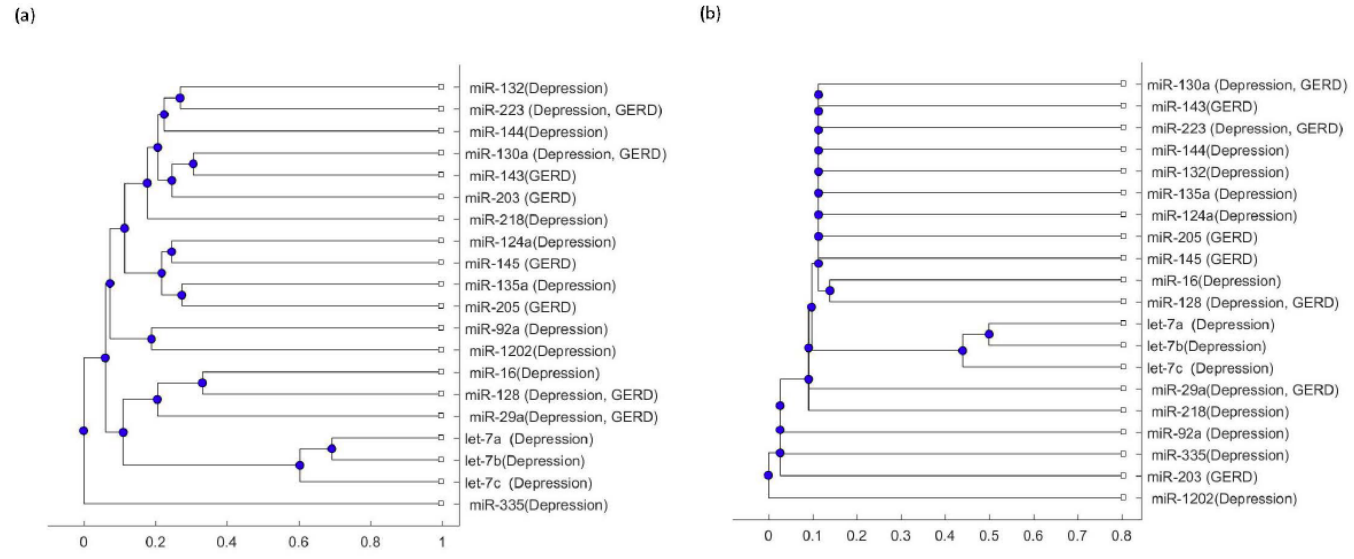

Fig. (1). (**a**) The phylogenetic tree of miRNA sequences obtained from miRBase based on the Jukes-Cantor model distance and the average method. (**b**) The phylogenetic tree of miRNA sequences obtained from miRBase based on the Jukes-Cantor model distance and the median method. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

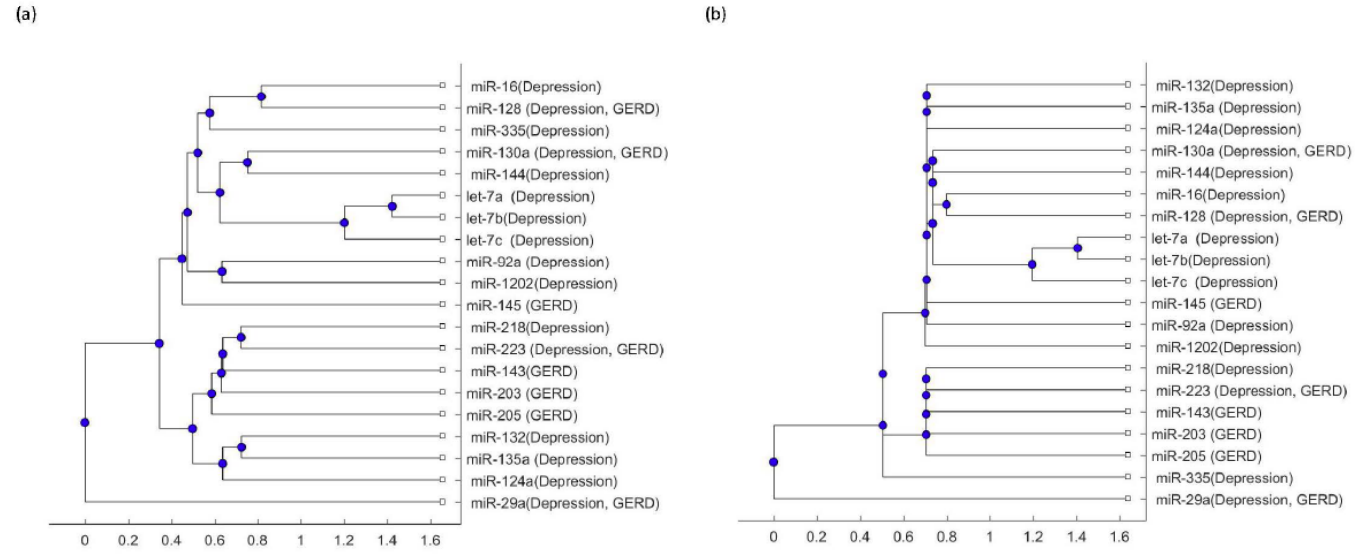

Fig. (2). (**a**) The phylogenetic tree of miRNA sequences obtained from miRBase based on the alignment-score distance and the average method. (**b**) The phylogenetic tree of miRNA sequences obtained from miRBase based on the alignment-score distance and the median method. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

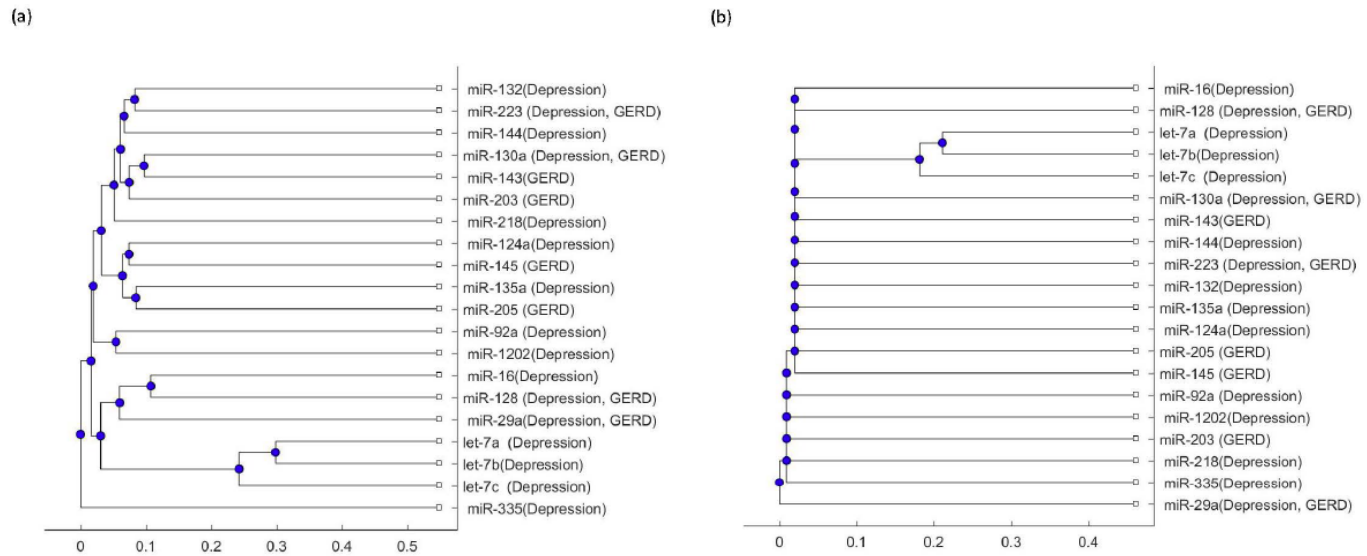

Fig. (3). (**a**) The phylogenetic tree of miRNA sequences obtained from miRBase based on the p distance and the average method. (**b**) The phylogenetic tree of miRNA sequences obtained from miRBase based on the p distance and the median method. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

miR-335 is only a miRNA biomarker of MD, miR-335 may be a useful miRNA that can be used to investigate the different disease mechanism between MD and GERD.

Next, the miRNA sequences obtained from MirGeneDB were analyzed. The phylogenetic trees of miRNA sequences obtained from MirGeneDB are plotted in (Figs. **4-6**). Similar to (Figs. **1-3**), from (Figs. **4-6**), we can see that most of the MD and GERD biomarkers cannot be classified into two groups. It shows that from the miRNA biomarker viewpoint, these two diseases may have an association. In particular, the miRNA miR-335 is different from the other miRNAs. In (Fig. **4a,b**), (Fig. **5a,b**) and (Fig. **6a,b**), miR-335 is in a separate branch of the tree. These figures are based on different evolutionary models and clustering methods. Most of them show similar results for these two miRNAs. As a result, miR-335 may be a useful miRNA that can be used to investigate the different disease mechanisms between MD and GERD.

From (Figs. **4-6**), most of the MD and GERD biomarkers cannot be classified into two groups. It shows

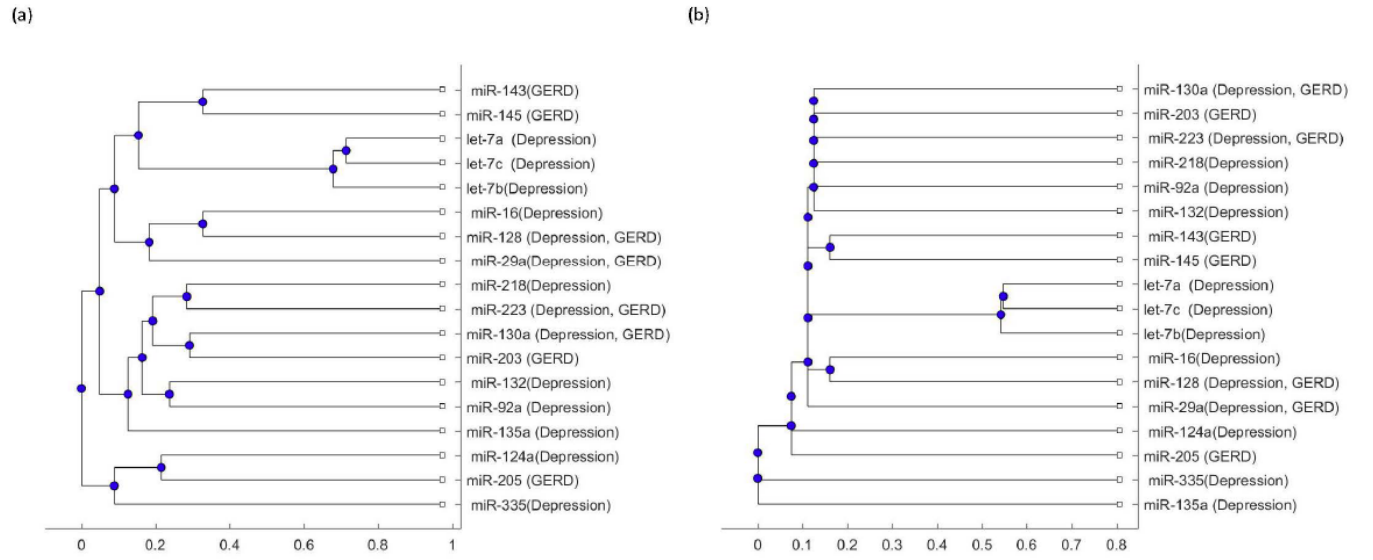

Fig. (4). (**a**) The phylogenetic tree of miRNA sequences obtained from MirGeneDB based on the Jukes-Cantor model distance and the average method. (**b**) The phylogenetic tree of miRNA sequences obtained from MirGeneDB based on the Jukes-Cantor model distance and the median method. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

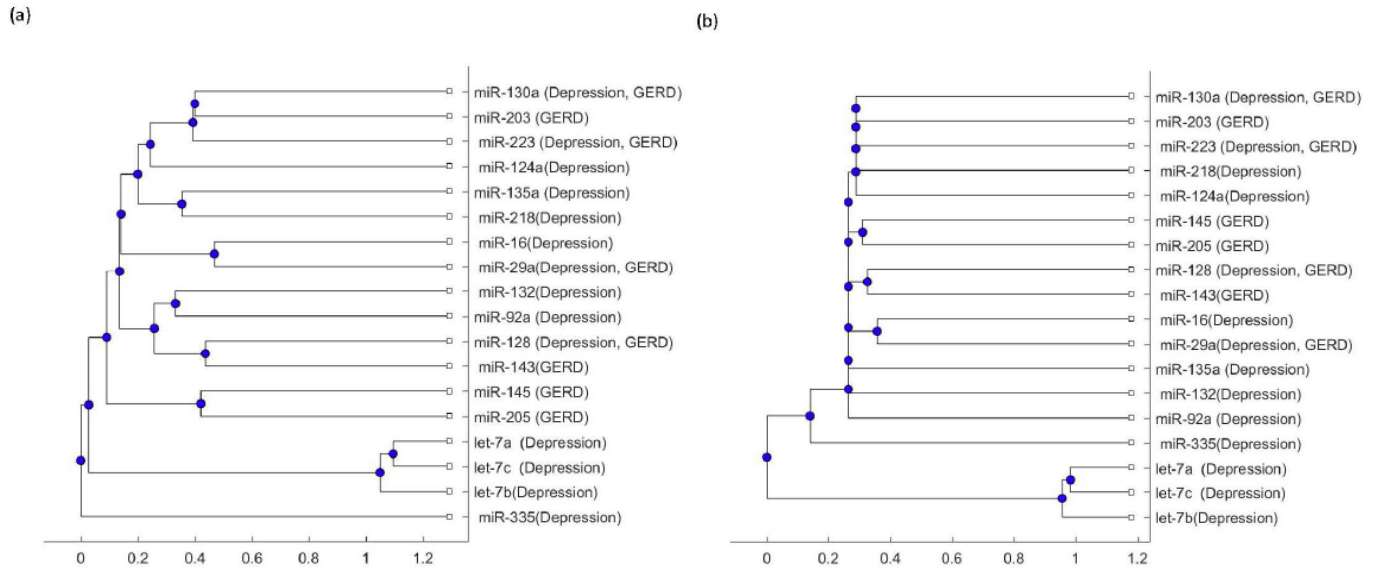

Fig. (5). (**a**) The phylogenetic tree of miRNA sequences obtained from MirGeneDB based on the alignment-score distance and the average method. (**b**) The phylogenetic tree of miRNA sequences obtained from MirGeneDB based on the alignment-score distance and the median method. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

that from the miRNA biomarker viewpoint, these two diseases may have an association. In particular, the miRNA miR-335 is different from the other miRNAs. In (Fig. **4a,b**), (Fig. **5a,b**) and (Fig. **6a,b**), miR-335 is in a separate branch of the tree. These figures are based on different evolutionary models and clustering methods. Most of them show similar results for these two miRNAs. As a result, miR-335 may be a useful miRNA that can be used to investigate the different disease mechanisms between MD and GERD.

4.2. Taiwan Biobank Data Analysis

As an empirical and validation study for the relationship between MD and GERD, we analyzed the community-based sample of size 5000 from Taiwan Biobank (TWB, [33]) as mentioned in Section 3.2.

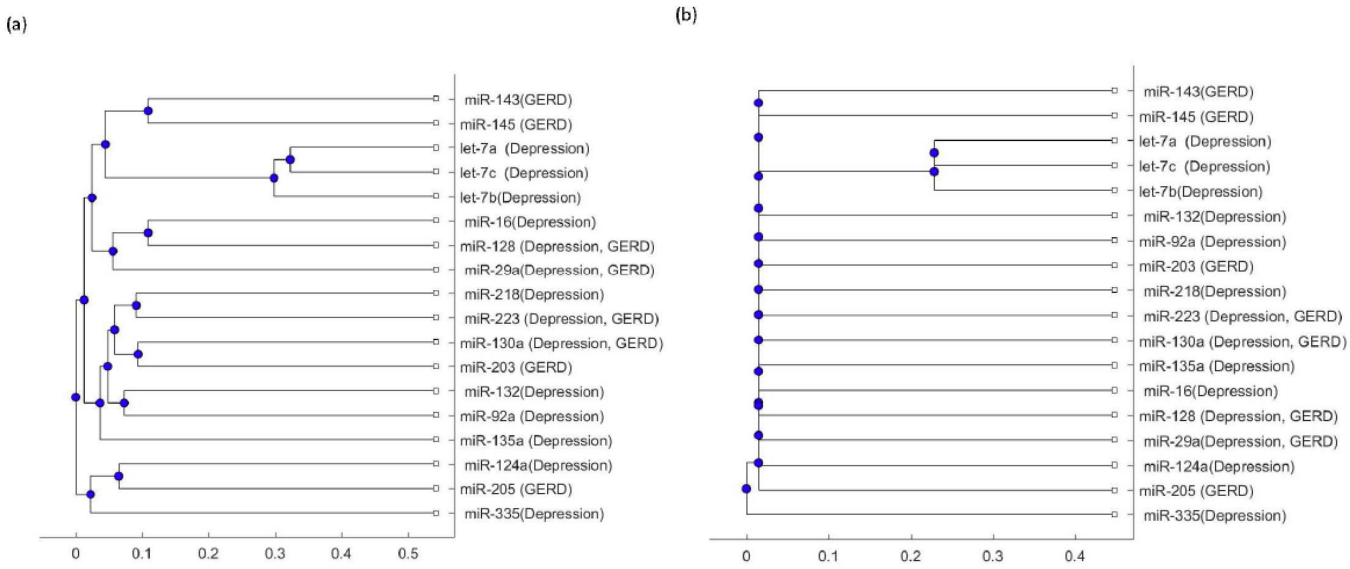

Fig. (6). (**a**) The phylogenetic tree of miRNA sequences obtained from MirGeneDB based on the p distance and the average method. (**b**) The phylogenetic tree of miRNA sequences obtained from MirGeneDB based on the p distance and the median method. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Based on the TWB data sample, the prevalence of MD was estimated to be 2.98%, while that of GERD, it was estimated to be 12.52%(please see Table **3** for details). The results are comparable to the recent literature [73, 74], although the GERD prevalence in the TWB sample is relatively lower than that obtained in another study [74]. The prevalence rates of the two diseases are similar between the female and male subsamples (Table **3b,c**).

The association between MD and GERD, measured by odds ratio (OR), is estimated to be OR=2.48 [calculated by (the number of MD/the number of non-MD) in the subsample of subjects with GERD, divided by (the number of MD/the number of non-MD) in the subsample of subjects without GERD=(111/4263)/(38/588)=2.48] with 95% confidence interval 1.70-3.62 and p-value < 10^{-5}, showing a strong relationship in the TWB sample. The result further strengthens the findings obtained from the miRNA analysis mentioned above. A further examination by gender reveals that the association between MD and GERD is stronger in females (OR =(64/2165)/(26/300)=2.93 with 95% confidence interval 1.83-4.70 and p-value < 10^{-5}) than in males (OR=(47/2098)/(12/288)=1.86 with 95% confidence interval 0.98-3.55 and p-value=0.09).

5. DISCUSSION

This study provides evidence of the association between MD and GERD using miRNA biomarkers of the two diseases and data from TWB. In particular, the TWB data analysis shows a strong relationship between these two diseases with an OR=2.48. In addition to GERD, the TWB also contains questionnaire data on the presence of two other gastrointestinal symptoms: peptic ulcer (PU) and irritable bowel syndrome (IBS). The empirical association of MD with the additional gastrointestinal symptoms revealed by TWB data may serve as further evidence for the underlying association between MD and GERD and hence will also be of interest. Based on the TWB data, the OR between MD and PU is obtained as 2.12 (95% confidence interval: 1.46-3.08; p-value<10-4), and the OR between MD and IBS is 3.09 (95% confidence interval: 1.63-5.86; p-value<10-3). All these results consistently indicate strong associations between MD with gastrointestinal symptoms.

The analysis of miRNA biomarkers also supports the connection between these two diseases. Compared with MD, there are much fewer miRNA biomarkers discovered or reported in the literature for GERD. Although there are many miRNA biomarkers that were reported to be related to depression, many of them are also related to other diseases. For example, the expression levels of miR-499, miR-708 and miR-1908 during a depression episode of bipolar disorder patients were lower than the remission state [75]. To avoid confounding from other diseases, we restricted attention to the miRNA biomarkers that only depend on MD, and excluded the miRNAs that are related not only to MD but also other diseases which is beyond the focus of this study. There are a lot of miRNA biomarkers discovered for depression. We think our concern about confound-

Table 3. Cross-table of numbers of MD and GERD cases in the TWB sample. (a) overall sample; (b) female sample; (c) male sample.

(a) Overall	**MD**		
GERD	no	yes	row %
no	4263	111	87.48%
yes	588	38	12.52%
column %	97.02%	2.98%	
(b) Female	**MD**		
GERD	no	yes	row %
no	2165	64	87.24%
yes	300	26	12.76%
column %	96.48%	3.52%	
(c) Male	**MD**		
GERD	no	yes	row %
no	2098	47	87.73%
yes	288	12	12.27%
column %	97.59%	2.41%	

ing from other diseases is necessary because MD is a comorbidity of many other diseases or symptoms. For example, depression and painful symptoms are closely related. The prevalence of pain in depressed cohorts and depression in pain cohorts are higher than when these conditions are individually examined [76]. Comorbidity of depression and anxiety was observed in the elderly [77]. Therefore, a more prudential selection of miRNA biomarkers in our analysis can help delineate the pure relationship between MD and GERD.

CONCLUSION

MD is one of the most common mental disorders in the world. It affects a large proportion of people nowadays. Similarly, GERD is a chronic disease affecting millions of people worldwide. Both of them are treatable diseases. A number of cohort studies have shown significant evidence for the strong association between these two diseases. In this study, their relationship was explored through miRNA biomarker intervention. This study first identified miRNA biomarkers for depression and GERD, respectively, and then constructed phylogenetic trees of these miRNA biomarkers and identified the common miRNAs for the two diseases. Among the miRNA biomarkers considered in this study, there are four common miRNA biomarkers of MD and GERD, and the phylogenetic trees show that most of miRNA biomarkers are not separate from the others except miR-29a.

As further empirical evidence for the relationship between MD and GERD, Taiwan biobank data were analyzed and revealed similar results to those of the cohort studies in the Norwegian, Korean and Australian people that there does exist a strong relationship between MD and GERD in Taiwanese people.

Our study is the first one to explore the MD-GERD relationship using their miRNA biomarkers. Given the evidence we provide for the relationship between MD and GERD based on both molecular and epidemiological data, this work may suggest potential directions to explore the underlying mechanism of these two highly-related diseases from molecular and clinical viewpoints.

AUTHOR CONTRIBUTIONS

Y.-H. C. and H.W. conceived the study, analyzed the data and wrote the paper.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This work was supported by the Ministry of Science and Technology 107-2118-M-009 -002 -MY2, Taiwan and 106-2118-M-001-016-MY3

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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