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RESEARCH ARTICLE

Distribution of microRNA Counts Across Human Chromosomes

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Abstract: ***Introduction*:** microRNAs (miRNAs) are a class of non-coding RNAs that play important roles in gene regulation. miRNAs are transcribed from DNA sequences into primary miRNAs and then processed into precursor miRNAs and mature miRNAs. miRNA gene counts in chromosomes for different species have been studied.

***Methods*:** Certain chromosomes have higher numbers of miRNA genes in all species, such as the X chromosome, while the Y chromosome has the fewest or no miRNA genes. miRNA counts in different chromosomes might have a positive correlation with coding gene counts in many species. In this study, a regression model was used to find the relationship between the miRNA count and the coding gene count across human chromosomes, and miRNA counts for 23 human chromosomes were predicted based on this regression model. In addition, the chromosome locations for the miRNA biomarkers of major depression, diabetes, Parkinson's disease, and COVID-19 are discussed.

***Results*:** The results reveal that miRNA biomarkers of these diseases are located in various chromosomes. The dispersion of miRNA locations across different chromosomes might explain the complication of the pathology of these diseases. Moreover, diabetes and COVID-19 have the largest number of miRNA biomarkers from Chromosome X.

***Conclusion*:** As Chromosome X is a sex chromosome, this phenomenon may explain the gender difference in the prevalence or severity of diabetes and COVID-19. The significant gender difference in the prevalence or severity of diabetes and COVID-19 might be due to the regulation function of their miRNA biomarkers from Chromosome X.

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1. INTRODUCTION

microRNAs (miRNA) are small, non-coding RNAs, about 21-24 nucleotides in length that play important roles in cell differentiation, development, and apoptosis. The first miRNA lin-4 was discovered by Victor Ambros in the early 1990s while studying development in the nematode Caenorhabditis elegans, which was often studied as a model organism, regarding the gene lin-14. The lin-4 gene was found to control the timing of Caenorhabditis elegans larval development by repressing the lin-14 gene. However, in 1993, Ambros and his co-workers Rosalind Lee and Rhonda Feinbaum discovered that lin-4, unexpectedly, could not encode a regulatory protein [1]. They found that lin-4 miRNA produced a ~22-nucleotide RNA instead of producing an mRNA encoding a protein. These short non-coding RNAs contained sequences partially complementary to multiple sequences in the 3' UTR of the lin-14 mRNA. Wightman, Ha, and Ruvkun discovered that lin-4 regulated its target mRNA lin-14 by forming imperfect RNA duplexes to down-regulate translation [1]. In 2000, the second Caenorhabditis elegans miRNA, let-7, was discovered by Ruvkun lab that regulated translation of the target gene lin-41 *via* imperfect base pairing to the 3' untranslated region of that mRNA [2]. This was an indication that miRNA regulation *via* 3' UTR complementarity may be a common feature and that there were likely to be more miRNAs. The sequence and regulation of the let-7 were conserved across different animal species, including humans [2].

The biogenesis of miRNAs has multiple processes. Most miRNAs are transcribed from DNA sequences in-

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to primary miRNAs (pri-miRNAs). The pri-miRNAs are then processed into precursor miRNAs (pre-miRNAs) and mature miRNAs. The synthesis of miRNAs from the primary miRNAs has two stages by the action of two RNase III-type proteins: Drosha in the nucleus and Dicer in the cytoplasm [3, 4]. RNase III enzyme Drosha can initiate miRNA maturation in the nucleus. The Drosha-Pasha/DGCR8 complex cleaves the pri-miRNAs to release the hairpin-shaped pre-miRNAs in the nucleus. Then the pre-miRNAs are exported to the cytoplasm to be processed by Dicer into mature miRNAs. Nevertheless, Drosha activity on some pri-miRNAs was detected in the cytoplasm, which might support the existence of cytoplasmic Drosha activity [5]. Moreover, in addition to the well-characterized roles of miRNAs in cytoplasmic gene regulation, they are also involved in transcriptional gene regulation restricted to the nucleus [6].

The miRNA nucleotide sequences can be accessed in the databases miRBase (https://www.mirbase.org/) or MirGeneDB (https://mirgenedb.org/) [7-10]. miRBase is the main online resource for miRNA sequences. It provides extensive information about published miRNAs, including sequences, biogenesis precursors, genome coordinates and context, literature references, deep sequencing expression data, and community-driven annotations. For example, the stem-loop sequence, mature 3p sequence, and mature 5p sequence of human miR-27a can be accessed from miRBase (Fig. **1**). The latest version of miRBase contains miRNA sequences from 271 organisms [7]. Another miRNA database MirGeneDB provides bona fide miRNAs [10].

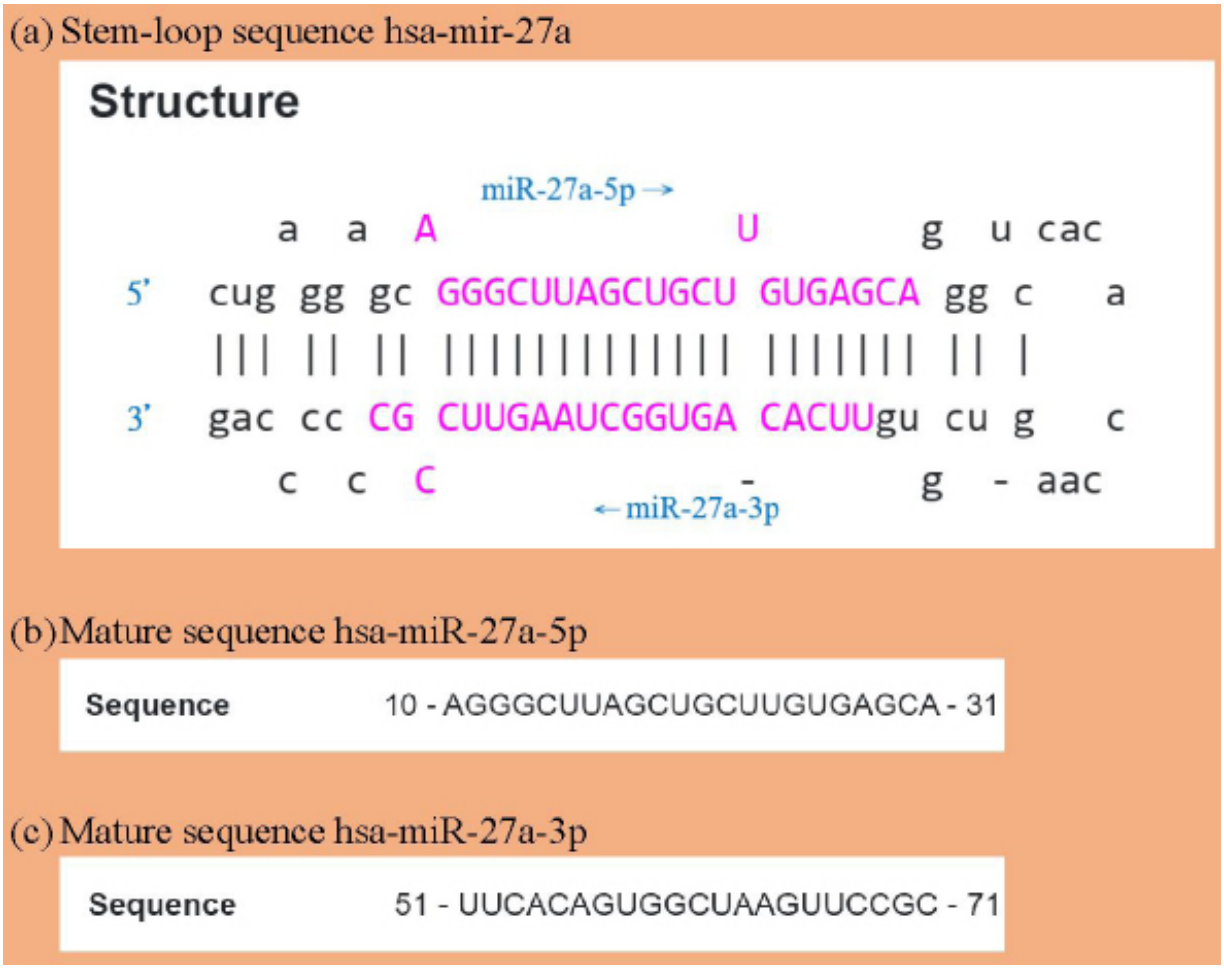


Fig. (1). The stem-loop, mature 5p, and mature 3p sequences of miR-27a obtained from miRBase (https://www.mirbase.org/cgi-bin/mirna_entry.pl?acc=MI0000085 accessed on 13 December 2021). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

miRNAs are involved in cancers through various mechanisms, including amplification or deletion of miRNA genes, abnormal transcriptional control of miRNAs, and defects in the miRNA biogenesis machinery [11, 12]. The dysregulation of miRNAs could act either as oncogenes or tumor suppressors, while miRNAs could also be regulated by oncogenes and tumor suppressor genes [13]. The earliest evidence of miRNA involvement in human cancer was the deletion or downregulation of miR-15a and miR-16-1 in clinical chronic lymphocytic leukemia cases [14]. So far, many studies focused on developing bioinformatics tools to identify the miRNA biomarkers of cancers [15, 16]. In addition to cancer, miRNAs also are involved in various diseases. miRNAs contribute to inflammation and neurological diseases [17]. Multiple sclerosis is a chronic disease of the central nervous system that affects the brain and spinal cord. A meta-analysis assessed the overall diagnostic accuracy of circulating miRNA biomarkers for multiple sclerosis and suggested that miRNAs had reference value for multiple sclerosis diagnosis [18]. Up to 50% of multiple sclerosis patients experience depressive disorders. miRNA biomarkers were used to explore the association between multiple sclerosis and major depression [19]. miRNAs were also related to other neurological diseases, including Parkinson's disease, frontotemporal dementia, Alzheimer's disease, spinal muscular atrophy, and anti-NMDA receptor encephalitis as well as eye diseases [20-28]. The association between neurological diseases and other diseases could be explored using the miRNA biomarkers [25, 29]. Moreover, miRNA also could be used to explore the association between disease and vaccination [29-31].

So far, around 2000 human miRNAs have been discovered [32], but not all of them were completely characterized and experimentally validated [33]. The chromosome localization of miRNA has also been discussed in the literature. miRNA gene counts in chromosomes vary widely in a species. Compared with other chromosomes, certain chromosomes have higher numbers of miRNA genes in a lot of species. For example, a large number of miRNAs in the X chromosome have been observed in all species, while the Y chromosome has the fewest or no miRNA genes [34]. Chromosome Y is one of two sex chromosomes. As the presence or absence of Chromosome Y determines the male or female sex, the miRNAs in Chromosome Y may play an important role in certain male-specific diseases. From this aspect, the miRNA count or coding gene count in Chromosome X may be expected to be larger than those in Chromosome Y because many diseases are not gender-specific. As a result, exploring miRNA location in chromosomes could help understand miRNA mechanisms.

miRNA counts were not correlated with the chromosome length, while chromosomal localization of coding genes is somewhat related to miRNA counts [34]. In this study, the correlation coefficient of miRNA count and coding gene count across human 23 chromosomes is explored, and the result shows a strong correlation between miRNA count and coding gene count. As there is a strong correlation, a regression model is used to predict the miRNA count for human chromosomes by coding gene count.

In addition to discussing miRNA count by chromosome, the miRNA biomarker counts of diseases across chromosomes are also investigated. In this study, the miRNA biomarker counts for major depression, diabetes, Parkinson's disease, and COVID-19 are discussed. Major depression is a mood disorder that is a comorbidity of many diseases. The abnormalities, including gut dysbiosis, mitochondrial dysfunction, neuroinflammation, chronic oxidative and nitrosative stress, can cause major depression [19, 35-37]. miRNAs were shown to play a role in these abnormalities. The manipulation of the gut microbiome modulates anxiety-like behaviors by regulating miRNA expression in brain regions [38]. Chronic inflammation is often associated with the emergence of major depression symptoms. miRNAs played an anti-inflammatory role in preventing the progression of the inflammatory response [39]. Insulin is a hormone to keep glucose levels within the normal range. Diabetes is a metabolic disease that is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to insulin. There are several major diabetes types including type I diabetes, type 2 diabetes, and gestational diabetes. Insulin is produced by beta cells in the pancreas. miRNAs play a role in regulating insulin including insulin secretion, islet development, beta cell differentiation, and indirectly control glucose and lipid metabolism [40]. miR-146a was shown to be involved in the pathogenesis of diabetic glomerulopathy [41]. Parkinson's disease is a chronic neurodegenerative disease that affects 1% of the population above 60 years. Estrogen exposure might be a risk of Parkinson's disease. Women with higher cumulative estrogen exposure had a significantly lower risk of Parkinson's disease [42, 43]. Many miRNAs were shown to have different expressions in patients with Parkinson's disease compared with healthy controls [44-46].

This study reveals that the miRNA biomarkers for major depression, diabetes, Parkinson's disease, and COVID-19 are located in various chromosomes rather

than in fewer chromosomes. The diversity of the chromosome location might explain the complications of the pathology of these diseases. Also, considering miRNA biomarker candidates in various chromosomes may increase the chance of identifying miRNA biomarkers for diseases. In addition, the prevalence of diabetes and the severity of COVID-19 infection are different in gender. This study shows that Chromosome X has the largest number of miRNA biomarkers for both diseases. This reveals that studying the count of miRNA biomarkers of diseases in Chromosomes X and Y can help understand whether gender is a risk factor for the prevalence or the severity of this disease.

2. MATERIALS AND METHODS

First, the human miRNA count in each chromosome was obtained from miRBase (Table **S1**). There are 1917 human miRNAs data in miRBase. Among these miRNAs, four of them do not have the chromosome location information. As a result, the data of 1913 miRNAs were used in this study. The miRNA counts for human chromosomes are provided in Table **1**. Chromosome 1 has the largest miRNA count 156; Chromosome Y has the smallest miRNA count 4. In addition, the other chromosome information was accessed from Ensembl, such as chromosome length and coding gene count. Ensembl is a genome browser for vertebrate genomes that supports research in comparative genomics, evolution, sequence variation, and transcriptional regulation [47]. The chromosome length and coding gene count are also provided in Table **1**. Chromosome 1 has the longest length 248956422 bps and Chromosome 21 has the smallest length 46709983 bps. Chromosome 1 has the largest coding gene count 2056 and Chromosome Y has the smallest coding gene count 64. Fig. (**2**) provides the histogram of miRNA count and coding gene count in each chromosome.

Table 1. The miRNA count, chromosome length, and gene count for each chromosome.

Chromosome	miRNA Count	Chromosome Length (bps)	Gene Count
1	156	248956422	2056
2	117	242193526	1300
3	96	198295559	1076
4	62	190214555	753
5	76	181538259	883
6	71	170805979	1050
7	82	159345973	1002
8	90	145138636	686
9	86	138394717	777
10	69	133797422	729
11	102	135086622	1320
12	80	133275309	1034
13	40	114364328	322
14	99	107043718	818
15	71	101991189	612
16	82	90338345	860
17	110	83257441	1184
18	35	80373285	269
19	143	58617616	1472
20	48	64444167	545
21	30	46709983	236
22	46	50818468	495
X	118	156040895	857
Y	4	57227415	64

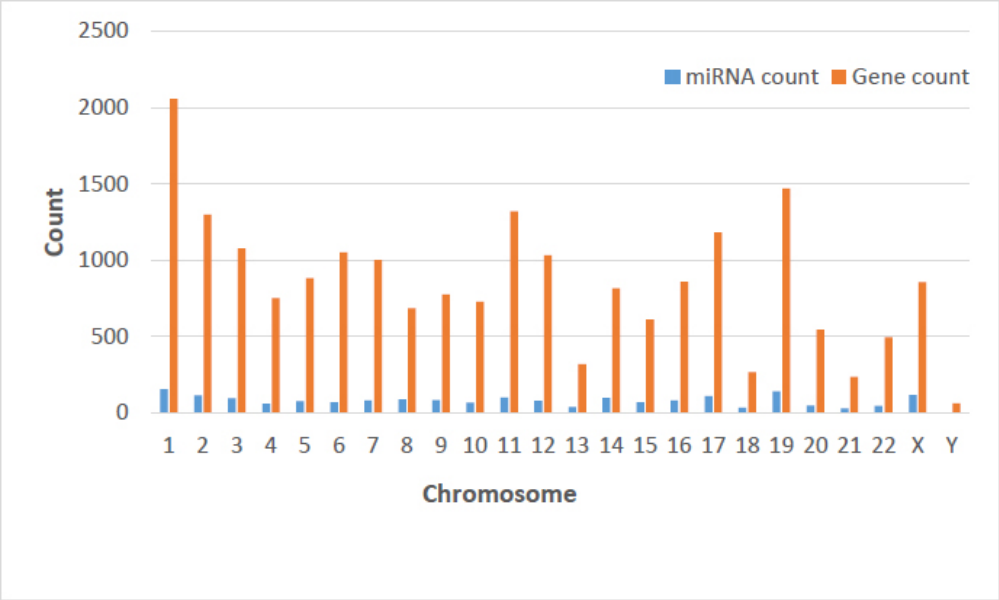


Fig. (2). The histogram of miRNA count in each human chromosome. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

3. RESULTS

The correlation coefficient between miRNA count and chromosome length is 0.528, which is not high. Although miRNA count and chromosome length have a positive correlation, they are not very closely related. The correlation coefficient between miRNA count and coding gene count is 0.9163, which reveals a strong relationship between miRNA count and coding gene count. To explore their relationship, a regression model was used to fit the data of miRNA count, chromosome length, and coding gene count. Let Y denote the miRNA count, X denote the coding gene count, and L denote the chromosome length. The regression model is:

$$Y = a + bX + cL$$

The estimators of the coefficients, a, b and c are $\hat{a}$ = 18.407, $\hat{b}$ = 0.0768 and $\hat{c}$ = -3.1 * 10^{-8}. As a result, the fitted model is:

$$Y = 18.407 + 0.0768\mathrm{X} - 3.1 * 10^{-8}L \quad (1)$$

In this model, the estimator of the coefficient c is very small. This means that compared with the coding gene count, the chromosome length is not an important factor contributing to miRNA count. To assess the effect of the chromosome length on the miRNA count, I also consider another regression model:

$$\mathrm{Y} = a^* + c^*L \quad (2)$$

The least squared estimator of the coefficients of the linear regression (2) is $\hat{a}^*$ = 37.6595 and $\hat{c}^*$ = 3.27 * 10^{-7}. This result shows that the effect of the chromosome length on the miRNA count can be negligible.

This regression model (1) has a high R^2 value 0.8412. This indicates that this fitted model is acceptable. Based on Model (1), we can calculate the estimated value of Y (predicted value of miRNA count) for each chromosome and compare them with the miRNA count (Fig. **3**).

The miRNA count, the predicted value of miRNA count, and their difference for each chromosome are provided in Table **2**, along with the ratios of the absolute value of the difference to miRNA count and to the predicted miRNA count. Coding genes have been studied for a longer time than miRNA. As coding gene count and miRNA count have a high correlation, using coding gene count to predict miRNA count may be a feasible way. If more coding genes are discovered on a chromosome, we can expect more miRNAs to be discovered from that chromosome. However, some chromosomes may be exceptions such as Chromosome Y. As mentioned in Section 1, Chromosome Y has the fewest or no miRNA genes [34]. From Table **2**, there

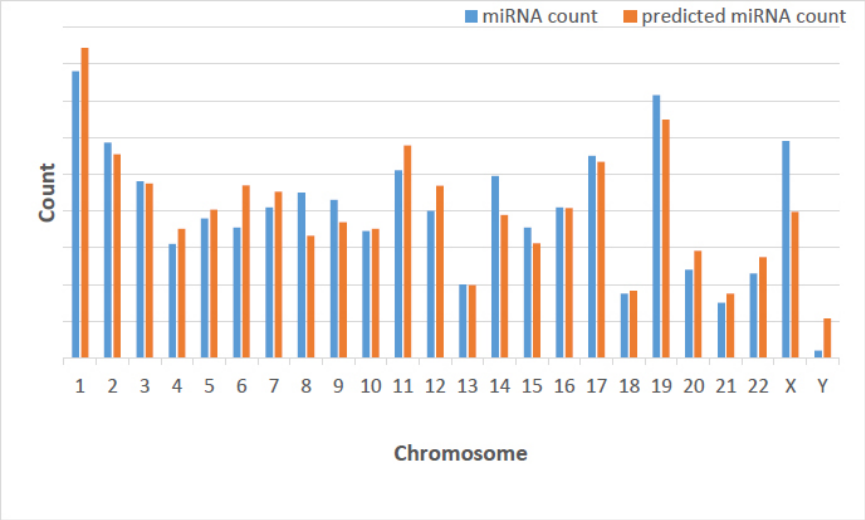


Fig. (3). miRNA count and predicted miRNA count in each chromosome. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 2. miRNA count, predicted miRNA count, and their differences for each chromosome, as well as the ratios of the absolute value of the difference to miRNA count and to the predicted miRNA count.

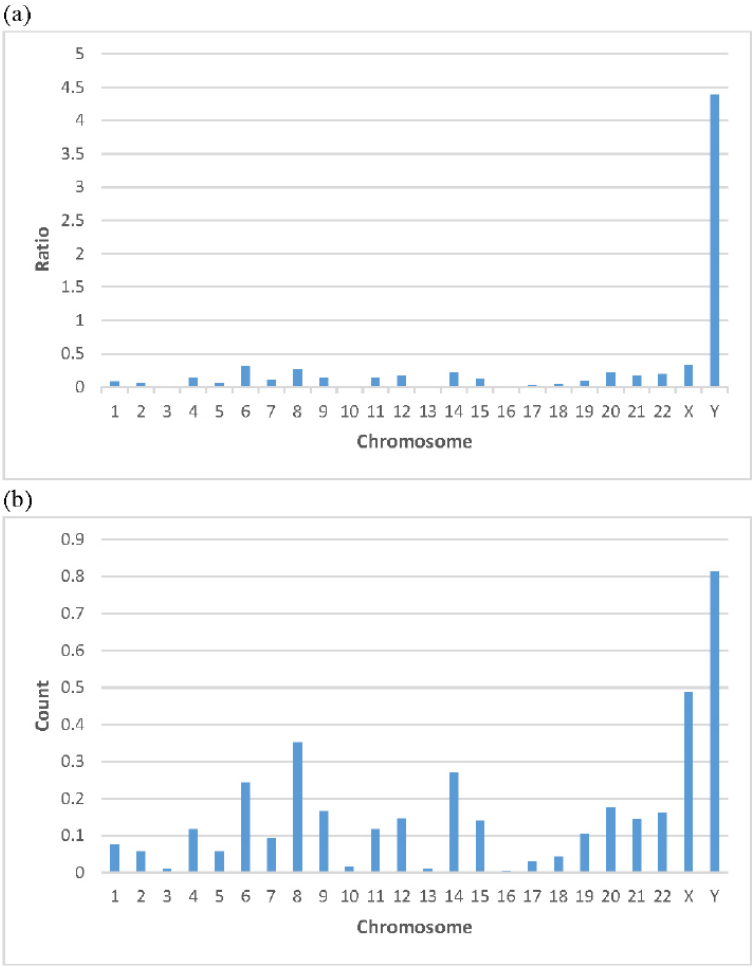
Chromosome	miRNA count (*A*)	Predicted miRNA Count (*B*)	A - B	|A - B|/A	|A - B|/B
1	156	168.61	-12.61	0.080833333	0.074787972
2	117	110.76	6.24	0.053333333	0.056338028
3	96	94.91	1.09	0.011354167	0.011484564
4	62	70.36	-8.36	0.13483871	0.11881751
5	76	80.61	-4.61	0.060657895	0.057188934
6	71	93.77	-22.77	0.320704225	0.242828197
7	82	90.43	-8.43	0.102804878	0.093221276
8	90	66.61	23.39	0.259888889	0.351148476
9	86	73.80	12.2	0.141860465	0.165311653
10	69	70.26	-1.26	0.01826087	0.01793339
11	102	115.60	-13.6	0.133333333	0.117647059
12	80	93.70	-13.7	0.17125	0.146211313
13	40	39.60	0.4	0.01	0.01010101
14	99	77.92	21.08	0.212929293	0.270533881
15	71	62.26	8.74	0.123098592	0.140379056
16	82	81.66	0.34	0.004146341	0.004163605
17	110	106.76	3.24	0.029454545	0.030348445
18	35	36.58	-1.58	0.045142857	0.043193002
19	143	129.64	13.36	0.093426573	0.103054613
20	48	58.27	-10.27	0.213958333	0.176248498
21	30	35.09	-5.09	0.169666667	0.145055571
22	46	54.85	-8.85	0.192391304	0.161349134
X	118	79.40	38.6	0.327118644	0.486146096
Y	4	21.55	-17.55	4.3875	0.814385151

are 13 chromosomes whose miRNA count is less than the predicted miRNA count, including Chromosome 1, 4, 5, 6, 7, 10, 11, 12, 18, 20, 21, 22, and Y, and the other 11 chromosomes are the opposite. In the last two columns of Table **2**, the largest two values of the ratios for each column occur on Chromosomes X and Y (Fig. **4**). This may indicate that the numbers of miRNAs located on both sex chromosomes are quite different from the expected number compared with other chromosomes.

4. DISCUSSION

The abnormal expression of a miRNA may cause diseases, and the mechanism of diseases also are related to miRNAs. In this section, the dispersion of disease biomarker location across chromosomes is discussed. The chromosome location of the miRNA biomarkers of the four diseases, including major depression, diabetes, Parkinson's disease, and COVID-19, are discussed in this study. Their miRNA biomarkers are located in various chromosomes rather than in a few chromosomes.

First, for major depression, 17 miRNA biomarkers were listed as potential biomarkers that were directly involved in the mechanism of major depression [48]. The chromosome location of these 17 miRNAs is listed in Table **3**. These 17 miRNAs are located in 14 chromosomes. Only Chromosomes 7, 13, and 17 are the locations of two miRNA biomarkers of these 17 miRNAs. The other chromosomes are the locations of only one miRNA biomarker. This reveals that the chromosome location of the major depression is dispersed. Although the mechanism of miRNA biomarker location in different chromosomes is not very clear now, the dispersion of the chromosome location may indicate more disease causes of the major depression.



(Fig. 4). (**a**) The ratio of the absolute value of the difference between miRNA count and the predicted miRNA count in each chromosome to the miRNA count; (**b**) The ratio of the absolute value of the difference between miRNA count and the predicted miRNA count in each chromosome to the predicted miRNA count. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 3. The chromosome location for the 17 miRNA biomarkers of major depression.

Chromosome	miRNA
1	miR-92b
2	miR-128
3	miR-135a
4	miR-218
6	miR-1202
7	miR-29a, miR-335
8	miR-124
9	let-7a
11	miR-130a
13	miR-16, miR-92a
17	mir-132, miR-144
21	let-7c
22	let-7b
X	miR-223

Certain major depression biomarkers in Table **3** are reviewed. Circulating miR-16 expression level was significantly lower in major depression patients than in controls [49]. The baseline expressions of let-7b and let-7c were lower in treatment-resistant major depression patients compared with controls [50]. The blood expression level of miR-128 was higher in major depression patients treated with antidepressant treatments [51]. miR-223 was downregulated in the peripheral blood of major depression patients with antidepressant therapy studies [52].

Major depression is known as a complex disease that affects approximately 4.4% of the global population [48]. A serious medical illness, life changes, trauma, genetic factors, and other reasons may cause depression. Chronic inflammation might increase the risk of development and persistence of inflammation-associated depression [53]. The endocrine system plays a significant role in the pathology of major depression. A positive association was found between depressive disorder and insulin resistance [54]. Family and twin studies also demonstrated that genetic factors played a role to increase the risk of depressive disorders [55]. This reveals that the mechanism of major depression is complex and many possible causes can trigger major depression. The diversity of the chromosome location of the miRNA biomarkers for major depression might partially explain the complication of the pathology of major depression.

In addition to major depression, the Human microRNA Disease Database (HMDD) (http://www.cuilab.cn/hmdd) was used to find the miRNAs that were identified to be related to diabetes and Parkinson's disease, respectively [56, 57]. Since the HMDD database does not provide many depression-related miRNA biomarkers, the biomarkers from this database for the major depression case were not searched. For the diabetes case, the keyword used to search for the miRNA biomarkers was "diabetes" in HMDD. The related miRNAs as well as their reference could be found in the HMDD. The chromosome location of these miRNAs is listed in Table **4**. Almost all human chromosomes are the location of these miRNA biomarkers except Chromosome Y. Chromosomes X and 1 are the two chromosomes with the first and second top numbers of these miRNAs. This also reveals that the chromosome location of diabetes is very dispersed.

Certain miRNAs in Table **4** are reviewed. The expression levels of miR-1-3p and miR-34a-5p were lower in type 2 diabetes patients compared with controls [58]. miR-146a-5p was downregulated in type 1 diabetes patients [59]. Plasma miR-210 was significantly upregulated in type 2 diabetes compared to controls [60]. The serum samples from patients with gestational diabetes had a higher miR-195-5p expression level compared with healthy pregnancies [61]. Serum miR-7 was significantly increased in patients with type 2 diabetes and patients with type 2 diabetes-related microvascular complications compared with controls [62].

Obesity, sedentary behavior, Western diet, metabolic syndrome, and family history are risk factors for diabetes [63]. High-calorie diet might lead to an abnormal generation of inflammatory molecules. Inflammation might induce oxidative stress that could exacerbate the development of diabetes and its complications [64]. Sedentary behaviors and reduced physical activity were associated with obesity and chronic inflammation that increased the risk of diabetes [65]. In addition, gut microbiota is a relatively new field that was studied to be related to type 2 diabetes [65]. Intestinal dysbiosis might promote gut barrier integrity, pancreatic β-cell proliferation, and insulin biosynthesis and disrupt glucose homeostasis that could trigger diabetes development.

Next, Parkinson's disease is considered. The keyword used to search for the miRNA biomarkers for Parkinson's disease is "Parkinson" in HMDD. Table **5** provides the chromosome location of these miRNAs. Almost all human chromosomes are the location of these miRNA biomarkers except Chromosomes 16, 21, and Y. Chromosome 14 has the largest number of the miRNA count. Similar to the major depression and diabetes cases, the chromosome location of Parkinson's disease is very dispersed.

Table 4. The chromosome location for the miRNA biomarkers of diabetes.

Chromosome	miRNA	miRNA Count
1	mir-101-1, mir-137, mir-181a-1, mir-181b-1, mir-186,mir-194-1, mir-197, mir-199a-2, mir-200a, mir-200b, mir-205, mir-214, mir-29b-2,mir-29c, mir-30c-1, mir-30e, mir-34a, mir-429, mir-9-1	19
2	mir-128-1, mir-149, mir-217, mir-26b, mir-375	5
3	let-7g, mir-128-2, mir-135a-1, mir-138-1,mir-15b, mir-191, mir-26a-1, mir-28	8
4	mir-572, mir-95	2
5	mir-103a-1, mir-1271, mir-143, mir-145, mir-146a, mir-9-2,	6
6	mir-1275, mir-133b, mir-206, mir-30a, mir-30c-2, mir-4463	6
7	mir-148a, mir-182, mir-29a,mir-29b-1, mir-550a-1, mir-550a-2,mir-550a-3, mir-590, mir-93,mir-96	10
8	mir-124-1, mir-124-2, mir-30b, mir-30d, mir-320a, mir-383, mir-486-1, mir-486-2	8
9	let-7a-1, let-7f-1, mir-101-2, mir-126, mir-147a, mir-181a-2, mir-181b-2, mir-204, mir-23b,mir-24-1, mir-27b, mir-31, mir-3960, mir-455, mir-6853, mir-7-1	16
10	mir-107, mir-146b, mir-202, mir-3158-1, mir-3158-2, mir-5699	6
11	let-7a-2, let-7d,mir-100, mir-125b-1, mir-130a, mir-192, mir-194-2, mir-210, mir-326, mir-34c, mir-4687, mir-483	12
12	mir-135a-2, mir-196a-2, mir-200c, mir-26a-2	4
13	mir-15a, mir-17, mir-18a,mir-19a,mir-19b-1, mir-20a, mir-320d-1, mir-92a-1,	8
14	mir-208a, mir-299, mir-342, mir-370, mir-376a-1, mir-376a-2, mir-409, mir-410, mir-487a, mir-494,mir-495, mir-665	12
15	mir-7-2, mir-9-3	2
16	mir-138-2, mir-193b	2
17	mir-132, mir-142, mir-144, mir-195, mir-21, mir-212, mir-22, mir-301a, mir-33b, mir-423, mir-451a, mir-454, mir-497, mir-657	14
18	mir-1-2, mir-122, mir-133a-1,mir-187	4
19	mir-125a, mir-150, mir-181c, mir-199a-1, mir-23a,mir-24-2, mir-27a, mir-518d, mir-7-3	9
20	mir-1-1, mir-103a-2, mir-124-3, mir-133a-2	4
21	mir-125b-2, mir-155, mir-802, mir-99a	4
22	let-7a-3, mir-130b, mir-185, mir-33a, mir-4534	5
X	let-7f-2, mir-19b-2, mir-20b, mir-221, mir-222, mir-223,mir-320d-2,mir-362, mir-374a, mir-384,mir-424, mir-452, mir-502, mir-5, mir-508, mir-532, mir-652, mir-766, mir-92a-2, mir-98	20
Y	--	0

Table 5. The chromosome location for the miRNA biomarkers of Parkinson's disease.

Chromosome	miRNA	miRNA Count
1	mir-135b, mir-205, mir-214, mir-29b-2, mir-29c, mir-30c-1, mir-30e, mir-34a, mir-9-1	9
2	mir-153-1	1
3	let-7g, mir-135a-1, mir-16-2, mir-26a-1, mir-28, mir-4271	6
4	mir-218-1	1
5	mir-218-2, mir-449a, mir-449b, mir-9-2	4
6	mir-133b,mir-30a, mir-30c-2	3
7	mir-129-1, mir-153-2, mir-182, mir-183, mir-29a, mir-29b-1, mir-335	7
8	mir-151a, mir-30b, mir-30d	3

(Table 5) contd....

Chromosome	miRNA	miRNA Count
9	let-7a, let-7d, mir-126, mir-147a, mir-199b, mir-7-1	6
10	mir-146b	1
11	let-7a, mir-129-2, mir-34b,mir-34c, mir-4697	5
12	mir-135a-2, mir-141, mir-26a-2	3
13	mir-16-1, mir-19b-1	2
14	mir-134, mir-203a, mir-299, mir-342, mir-376a, mir-376b,mir-382, mir-409, mir-433, mir-495, mir-543	11
15	mir-7-2, mir-9-3	2
16	--	0
17	mir-10a, mir-132, mir-195, mir-21, mir-22, mir-301a	6
18	mir-1-2	1
19	mir-1283, mir-199a-1, mir-199a-2, mir-518e, mir-519a, mir-7-3	6
20	mir-1-1,	1
21	--	0
22	let-7a	1
X	mir-106a, mir-19b-2, mir-221, mir-374a, mir-374b	5
Y	--	0

Finally, Coronavirus disease 2019 (COVID-19) was considered. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which has had a huge impact all over the world, affecting people's lifestyle, economy, and livelihood [66]. It was first identified in Wuhan, China, in December 2019, and then was transmitted to Europe, and finally arrived in the USA [67]. COVID-19 infection or COVID-19 vaccines may induce serious comorbidities such as anti-N-methyl-d-aspartate (anti-NMDA) receptor encephalitis. However, the risk of triggering anti-NMDA receptor encephalitis is not high [68]. Collagen treatments or supplements can be used to treat comorbid diseases and prevent complications of COVID-19 [69]. Table **6** provides the chromosome location of the miRNA biomarkers discussed in [68]. Almost all human chromosomes are the location of these miRNA biomarkers except Chromosomes 18, 20, and Y. Chromosome X has the largest miRNA count. Similar to the above-mentioned three diseases, the chromosome location of COVID-19 is very dispersed.

In the four diseases, diabetes and COVID-19 have the largest number of miRNA biomarkers from Chromosome X. The phenomenon does not occur for major depression and Parkinson's disease. As Chromosome X is a sex chromosome, this phenomenon may explain the gender difference in the prevalence or severity of diabetes and COVID-19. Diabetes was more prevalent in men than in women in most of the world [70]. This might be due to the difference in energy balance and glucose metabolism between males and females. The severity and mortality between male and female patients with COVID-19 were different. While men and women had the same prevalence of COVID-19, men were at a higher risk for severity and death [71]. These two diseases have a significant gender difference in prevalence or severity that might be due to the regulation function of miRNAs from Chromosome X.

Moreover, numerous miRNAs located on the X chromosome play critical roles in immunity and cancer [72]. The distinct inheritance pattern of Chromosome X is a key factor in the immune disadvantage observed in males and the improved survival rates of females [72]. In addition, epigenetic mechanisms also encompass Chromosome X inactivation in females. During embryonic development, one of the two X chromosomes is inactivated in females to ensure balanced X chromosome expression, similar to males who possess only one X chromosome [73]. Incomplete inactivation of the X chromosome results in the biallelic expression of miRNA [74]. Rheumatoid arthritis (RA) is an autoimmune disorder that primarily impacts women. The roles of biased Chromosome X inactivation in the higher prevalence of RA among females has been explored [75]. The expression levels of specific X chromosome-linked miRNAs vary between RA patients and healthy donors, as well as between males and females, and these have been examined [76]. The findings emphasized sex differences in the expression of several X chromosome-linked miRNAs.

Table 6. The chromosome location for the miRNA biomarkers of COVID-19.

Chromosome	miRNA	miRNA Count
1	mir-12136, mir-29b-2, mir-181a-1, mir-194-1, mir-135b, mir-429	6
2	mir-26b	1
3	mir-16-2, mir-15b,mir-26a-1	3
4	mir-4454	1
5	mir-146a	1
6	mir-6891, mir-4463,mir-1275	3
7	mir-183,mir-4284, mir-29b-1	3
8	mir-548d-1,mir-30b	2
9	mir-181a-2, let-7a-1,mir-23b, let-7f-1,mir-7-1,mir-126	6
10	mir-107,mir-1296	2
11	mir-4485, mir-483, mir-125b-1, let-7a-2, mir-194-2	5
12	mir-618, mir-548c,mir-26a-2	3
13	mir-15a	1
14	mir-409, mir-299	2
15	mir-627, mir-5701	2
16	mir-365a,mir-940	2
17	mir-144,mir-548d-2, mir-365b, mir-21, mir-142	5
18	--	0
19	mir-4745	1
20	--	0
21	mir-6501, mir-125b-2, mir-155	3
22	let-7a-3	1
X	mir-505, mir-501, mir-374a, mir-450b,mir-23c,mir-374c, mir-374b,mir-362	8
Y	--	0

CONCLUSION

miRNAs are useful biomarkers for many diseases. New miRNAs have been discovered in recent years. The chromosome location for miRNAs and coding genes are discussed in this study. A regression model was used to explore the relationship between the human miRNA count and coding gene count across chromosomes. In addition, chromosome location for miRNA biomarkers of major depression, diabetes, Parkinson's disease, and COVID-19 are discussed. The miRNA biomarkers for any one of these diseases are from various chromosomes rather than in fewer chromosomes. The dispersion of miRNA locations across different chromosomes may partially explain the complex pathology of these diseases. Moreover, diabetes and COVID-19 have the largest number of miRNA biomarkers in Chromosome X. For diabetes and COVID-19, the regulation function of the miRNA biomarkers in Chromosome X may be a factor causing the gender difference in prevalence or severity of both diseases.

AUTHORS' CONTRIBUTIONS

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

LIST OF ABBREVIATIONS

miRNAs	=	microRNAs
COVID-19	=	Coronavirus disease 2019
SARS-CoV-2	=	Severe Acute Respiratory Syndrome Coronavirus 2
anti-NMDA	=	anti-N-methyl-d-aspartate

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Lee, R.C.; Feinbaum, R.L.; Ambros, V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell,* **1993**, *75*(5), 843-854. http://dx.doi.org/10.1016/0092-8674(93)90529-Y PMID: 8252621

[2] Pasquinelli, A.E.; Reinhart, B.J.; Slack, F.; Martindale, M.Q.; Kuroda, M.I.; Maller, B.; Hayward, D.C.; Ball, E.E.; Degnan, B.; Müller, P.; Spring, J.; Srinivasan, A.; Fishman, M.; Finnerty, J.; Corbo, J.; Levine, M.; Leahy, P.; Davidson, E.; Ruvkun, G. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature,* **2000**, *408*(6808), 86-89. http://dx.doi.org/10.1038/35040556 PMID: 11081512

[3] O'Brien, J.; Hayder, H.; Zayed, Y.; Peng, C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front. Endocrinol.,* **2018**, *9*, 402. http://dx.doi.org/10.3389/fendo.2018.00402 PMID: 30123182

[4] Kim, V.N.; Han, J.; Siomi, M.C. Biogenesis of small RNAs in animals. *Nat. Rev. Mol. Cell Biol.,* **2009**, *10*(2), 126-139. http://dx.doi.org/10.1038/nrm2632 PMID: 19165215

[5] Dai, L.; Chen, K.; Youngren, B.; Kulina, J.; Yang, A.; Guo, Z.; Li, J.; Yu, P.; Gu, S. Cytoplasmic Drosha activity generated by alternative splicing. *Nucleic Acids Res.,* **2016**, *44*(21), gkw668. http://dx.doi.org/10.1093/nar/gkw668 PMID: 27471035

[6] Turunen, T.A.; Roberts, T.C.; Laitinen, P.; Väänänen, M.A.; Korhonen, P.; Malm, T.; Ylä-Herttuala, S.; Turunen, M.P. Changes in nuclear and cytoplasmic microRNA distribution in response to hypoxic stress. *Sci. Rep.,* **2019**, *9*(1), 10332. http://dx.doi.org/10.1038/s41598-019-46841-1 PMID: 31316122

[7] Kozomara, A.; Birgaoanu, M.; Griffiths-Jones, S. miRBase: From microRNA sequences to function. *Nucleic Acids Res.,* **2019**, *47*(D1), D155-D162. http://dx.doi.org/10.1093/nar/gky1141 PMID: 30423142

[8] Kozomara, A.; Griffiths-Jones, S. miRBase: Annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res.,* **2014**, *42*(D1), D68-D73. http://dx.doi.org/10.1093/nar/gkt1181 PMID: 24275495

[9] Fromm, B.; Billipp, T.; Peck, L.E.; Johansen, M.; Tarver, J.E.; King, B.L.; Newcomb, J.M.; Sempere, L.F.; Flatmark, K.; Hovig, E.; Peterson, K.J. A uniform system for the annotation of vertebrate microRNA genes and the evolution of the human microRNAome. *Annu. Rev. Genet.,* **2015**, *49*(1), 213-242. http://dx.doi.org/10.1146/annurev-genet-120213-092023 PMID: 26473382

[10] Fromm, B.; Domanska, D.; Høye, E.; Ovchinnikov, V.; Kang, W.; Aparicio-Puerta, E.; Johansen, M.; Flatmark, K.; Mathelier, A.; Hovig, E.; Hackenberg, M.; Friedländer, M.R.; Peterson, K.J. MirGeneDB 2.0: The metazoan microRNA complement. *Nucleic Acids Res.,* **2020**, *48*(D1), D132-D141. http://dx.doi.org/10.1093/nar/gkz885 PMID: 31598695

[11] Peng, Y.; Croce, C.M. The role of microRNAs in human cancer. *Signal Transduct. Target. Ther.,* **2016**, *1*(1), 15004. http://dx.doi.org/10.1038/sigtrans.2015.4 PMID: 29263891

[12] Jansson, M.D.; Lund, A.H. microRNA and cancer. *Mol. Oncol.,* **2012**, *6*(6), 590-610. http://dx.doi.org/10.1016/j.molonc.2012.09.006 PMID: 23102669

[13] Zhou, K.; Liu, M.; Cao, Y. New insight into microRNA functions in cancer: Oncogene–microRNA–tumor suppressor gene network. *Front. Mol. Biosci.,* **2017**, *4*, 46. http://dx.doi.org/10.3389/fmolb.2017.00046 PMID: 28736730

[14] Calin, G.A.; Dumitru, C.D.; Shimizu, M.; Bichi, R.; Zupo, S.; Noch, E.; Aldler, H.; Rattan, S.; Keating, M.; Rai, K.; Rassenti, L.; Kipps, T.; Negrini, M.; Bullrich, F.; Croce, C.M. Frequent deletions and down-regulation of micro-RNA genes *miR15* and *miR16* at 13q14 in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*(24), 15524-15529. http://dx.doi.org/10.1073/pnas.242606799 PMID: 12434020

[15] Wang, H. Predicting microRNA biomarkers for cancer using phylogenetic tree and microarray analysis. *Int. J. Mol. Sci.,* **2016**, *17*(5), 773. http://dx.doi.org/10.3390/ijms17050773 PMID: 27213352

[16] Hsieh, W.J.; Lin, F.M.; Huang, H.D.; Wang, H. Investigating microRNA-target interaction-supported tissues in human cancer tissues based on miRNA and target gene expression profiling. *PLoS One,* **2014**, *9*(4), e95697. http://dx.doi.org/10.1371/journal.pone.0095697 PMID:

24756070
[17] Wang, H.; Taguchi, Y.H.; Liu, X. Editorial: MiRNAs and neurological diseases. *Front. Neurol.,* **2021**, *12*, 662373. http://dx.doi.org/10.3389/fneur.2021.662373 PMID: 33959091
[18] Zhou, Z.; Xiong, H.; Xie, F.; Wu, Z.; Feng, Y. A meta-analytic review of the value of miRNA for multiple sclerosis diagnosis. *Front. Neurol.,* **2020**, *11*, 132. http://dx.doi.org/10.3389/fneur.2020.00132 PMID: 32158427
[19] Wang, H. microRNAs, multiple sclerosis, and depression. *Int. J. Mol. Sci.,* **2021**, *22*(15), 7802. http://dx.doi.org/10.3390/ijms22157802 PMID: 34360568
[20] Taguchi, Y.; Wang, H. Exploring microRNA biomarker for amyotrophic lateral sclerosis. *Int. J. Mol. Sci.,* **2018**, *19*(5), 1318. http://dx.doi.org/10.3390/ijms19051318 PMID: 29710810
[21] Goh, S.Y.; Chao, Y.X.; Dheen, S.T.; Tan, E.K.; Tay, S.S.W. Role of microRNAs in parkinson's disease. *Int. J. Mol. Sci.,* **2019**, *20*(22), 5649. http://dx.doi.org/10.3390/ijms20225649 PMID: 31718095
[22] Grasso, M. Plasma microRNA profiling distinguishes patients with frontotemporal dementia from healthy subjects. *Neurobiol Aging,* **2019**, *240*, 240-e1. http://dx.doi.org/10.1016/j.neurobiolaging.2019.01.024
[23] Wang, W.X.; Rajeev, B.W.; Stromberg, A.J.; Ren, N.; Tang, G.; Huang, Q.; Rigoutsos, I.; Nelson, P.T. The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of β-site amyloid precursor protein-cleaving enzyme 1. *J. Neurosci.,* **2008**, *28*(5), 1213-1223. http://dx.doi.org/10.1523/JNEUROSCI.5065-07.2008 PMID: 18234899
[24] Magri, F.; Vanoli, F.; Corti, S. mi RNA in spinal muscular atrophy pathogenesis and therapy. *J. Cell. Mol. Med.,* **2018**, *22*(2), 755-767. http://dx.doi.org/10.1111/jcmm.13450 PMID: 29160009
[25] Wang, H. microRNAs, parkinson's disease, and diabetes mellitus. *Int. J. Mol. Sci.,* **2021**, *22*(6), 2953. http://dx.doi.org/10.3390/ijms22062953 PMID: 33799467
[26] Taguchi, Y.; Wang, H. Exploring microRNA biomarkers for parkinson's disease from mRNA expression profiles. *Cells,* **2018**, *7*(12), 245. http://dx.doi.org/10.3390/cells7120245 PMID: 30563060
[27] Zhang, J.; Xu, X.; Zhao, S.; Gong, Z.; Liu, P.; Guan, W.; He, X.; Wang, T.; Peng, T.; Teng, J.; Jia, Y. The expression and significance of the plasma let-7 family in anti-n-methyl-d-aspartate receptor encephalitis. *J. Mol. Neurosci.,* **2015**, *56*(3), 531-539. http://dx.doi.org/10.1007/s12031-015-0489-6 PMID: 25603816
[28] Wang, H. microRNA pathological mechanisms between Parkinson's disease, Alzheimer's disease, glaucoma and macular degeneration. *Expert Rev. Mol. Med.,* **2023**, *25*, e24. http://dx.doi.org/10.1017/erm.2023.19 PMID: 37469309
[29] Wang, H. Phylogenetic analysis to explore the association between anti-NMDA receptor encephalitis and tumors based on microRNA biomarkers. *Biomolecules,* **2019**, *9*(10), 572. http://dx.doi.org/10.3390/biom9100572 PMID: 31590348
[30] Wang, H. Anti-NMDA receptor encephalitis and vaccination. *Int. J. Mol. Sci.,* **2017**, *18*(1), 193. http://dx.doi.org/10.3390/ijms18010193 PMID: 28106787
[31] Wang, H. Anti-NMDA receptor encephalitis, human papillomavirus, and microRNA. *Curr. Med. Chem.,* **2024**. PMID: 38549528
[32] Hammond, S.M. An overview of microRNAs. *Adv. Drug Deliv. Rev.,* **2015**, *87*, 3-14. http://dx.doi.org/10.1016/j.addr.2015.05.001 PMID: 25979468
[33] Alles, J.; Fehlmann, T.; Fischer, U.; Backes, C.; Galata, V.; Minet, M.; Hart, M.; Abu-Halima, M.; Grässer, F.A.; Lenhof, H.P.; Keller, A.; Meese, E. An estimate of the total number of true human miRNAs. *Nucleic Acids Res.,* **2019**, *47*(7), 3353-3364. http://dx.doi.org/10.1093/nar/gkz097 PMID: 30820533
[34] Ghorai, A.; Ghosh, U. miRNA gene counts in chromosomes vary widely in a species and biogenesis of miRNA largely depends on transcription or post-transcriptional processing of coding genes. *Front. Genet.,* **2014**, *5*, 100. http://dx.doi.org/10.3389/fgene.2014.00100 PMID: 24808907
[35] Sherwin, E.; Rea, K.; Dinan, T.G.; Cryan, J.F. A gut (microbiome) feeling about the brain. *Curr. Opin. Gastroenterol.,* **2016**, *32*(2), 96-102. http://dx.doi.org/10.1097/MOG.0000000000000244 PMID: 26760398
[36] Bansal, Y.; Kuhad, A. Mitochondrial dysfunction in depression. *Curr. Neuropharmacol.,* **2016**, *14*(6), 610-618. http://dx.doi.org/10.2174/1570159X14666160229114755 PMID: 26923778
[37] Franscina Pinto, E.; Andrade, C. Interferon-related depression: A primer on mechanisms, treatment, and prevention of a common clinical problem. *Curr. Neuropharmacol.,* **2016**, *14*(7), 743-748. http://dx.doi.org/10.2174/1570159X14666160106155129 PMID: 26733280
[38] Hoban, A.E.; Stilling, R.M.; M Moloney, G.; Moloney, R.D.; Shanahan, F.; Dinan, T.G.; Cryan, J.F.; Clarke, G. Microbial regulation of microRNA expression in the amygdala and prefrontal cortex. *Microbiome,* **2017**, *5*(1), 102. http://dx.doi.org/10.1186/s40168-017-0321-3 PMID: 28838324
[39] Tahamtan, A.; Teymoori-Rad, M.; Nakstad, B.; Salimi, V. Anti-inflammatory microRNAs and their potential for inflammatory diseases treatment. *Front. Immunol.,* **2018**, *9*, 1377. http://dx.doi.org/10.3389/fimmu.2018.01377 PMID: 29988529
[40] Tang, X.; Tang, G.; Ozcan, S. Role of microRNAs in diabetes. *Biochim. Biophys. Acta. Gene Regul. Mech.,* **2008**, *1779*(11), 697-701. http://dx.doi.org/10.1016/j.bbagrm.2008.06.010 PMID: 18655850
[41] Lee, H.W.; Khan, S.Q.; Khaliqdina, S.; Altintas, M.M.; Grahammer, F.; Zhao, J.L.; Koh, K.H.; Tardi, N.J.; Faridi, M.H.; Geraghty, T.; Cimbaluk, D.J.; Susztak, K.; Moita, L.F.; Baltimore, D.; Tharaux, P.L.; Huber, T.B.; Kretzler, M.; Bitzer, M.; Reiser, J.; Gupta, V. Absence of miR-146a in podocytes increases risk of diabetic glomerulopathy *via* up-regulation of ErbB4 and notch-1. *J. Biol. Chem.,* **2017**, *292*(2), 732-747. http://dx.doi.org/10.1074/jbc.M116.753822 PMID: 27913625
[42] Haaxma, C.A.; Bloem, B.R.; Borm, G.F.; Oyen, W.J.G.; Leenders, K.L.; Eshuis, S.; Booij, J.; Dluzen, D.E.; Horstink, M.W.I.M. Gender differences in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry,* **2007**, *78*(8),

819-824.
http://dx.doi.org/10.1136/jnnp.2006.103788 PMID: 17098842

[43] Cerri, S.; Mus, L.; Blandini, F. Parkinson's disease in women and men: What's the difference? *J. Parkinsons Dis.,* **2019**, *9*(3), 501-515.
http://dx.doi.org/10.3233/JPD-191683 PMID: 31282427

[44] Roser, A.E.; Caldi Gomes, L.; Schünemann, J.; Maass, F.; Lingor, P. Circulating miRNAs as diagnostic biomarkers for parkinson's disease. *Front. Neurosci.,* **2018**, *12*, 625.
http://dx.doi.org/10.3389/fnins.2018.00625 PMID: 30233304

[45] Serafin, A.; Foco, L.; Zanigni, S.; Blankenburg, H.; Picard, A.; Zanon, A.; Giannini, G.; Pichler, I.; Facheris, M.F.; Cortelli, P.; Pramstaller, P.P.; Hicks, A.A.; Domingues, F.S.; Schwienbacher, C. Overexpression of blood microRNAs 103a, 30b, and 29a in L -dopa–treated patients with PD. *Neurology,* **2015**, *84*(7), 645-653.
http://dx.doi.org/10.1212/WNL.0000000000001258 PMID: 25596505

[46] Xing, R.X.; Li, L.G.; Liu, X.W.; Tian, B.X.; Cheng, Y. Down regulation of MIR -218, MIR -124, and MIR -144 relates to Parkinson's disease *via* activating NF-κB signaling. *Kaohsiung J. Med. Sci.,* **2020**, *36*(10), 786-792.
http://dx.doi.org/10.1002/kjm2.12241 PMID: 32492291

[47] Howe, K.L.; Achuthan, P.; Allen, J.; Allen, J.; Alvarez-Jarreta, J.; Amode, M.R.; Armean, I.M.; Azov, A.G.; Bennett, R.; Bhai, J.; Billis, K.; Boddu, S.; Charkhchi, M.; Cummins, C.; Da Rin Fioretto, L.; Davidson, C.; Dodiya, K.; El Houdaigui, B.; Fatima, R.; Gall, A.; Garcia Giron, C.; Grego, T.; Guijarro-Clarke, C.; Haggerty, L.; Hemrom, A.; Hourlier, T.; Izuogu, O.G.; Juettemann, T.; Kaikala, V.; Kay, M.; Lavidas, I.; Le, T.; Lemos, D.; Gonzalez Martinez, J.; Marugán, J.C.; Maurel, T.; McMahon, A.C.; Mohanan, S.; Moore, B.; Muffato, M.; Oheh, D.N.; Paraschas, D.; Parker, A.; Parton, A.; Prosovetskaia, I.; Sakthivel, M.P.; Salam, A.I.A.; Schmitt, B.M.; Schuilenburg, H.; Sheppard, D.; Steed, E.; Szpak, M.; Szuba, M.; Taylor, K.; Thormann, A.; Threadgold, G.; Walts, B.; Winterbottom, A.; Chakiachvili, M.; Chaubal, A.; De Silva, N.; Flint, B.; Frankish, A.; Hunt, S.E.; IIsley, G.R.; Langridge, N.; Loveland, J.E.; Martin, F.J.; Mudge, J.M.; Morales, J.; Perry, E.; Ruffier, M.; Tate, J.; Thybert, D.; Trevanion, S.J.; Cunningham, F.; Yates, A.D.; Zerbino, D.R.; Flicek, P. Ensembl 2021. *Nucleic Acids Res.,* **2021**, *49*(D1), D884-D891.
http://dx.doi.org/10.1093/nar/gkaa942 PMID: 33137190

[48] Chen, Y.H.; Wang, H. The association between depression and gastroesophageal reflux based on phylogenetic analysis of miRNA biomarkers. *Curr. Med. Chem.,* **2020**, *27*(38), 6536-6547.
http://dx.doi.org/10.2174/0929867327666200425214906 PMID: 32334497

[49] Song, M.F.; Dong, J.Z.; Wang, Y.W.; He, J.; Ju, X.; Zhang, L.; Zhang, Y.H.; Shi, J.F.; Lv, Y.Y. CSF miR-16 is decreased in major depression patients and its neutralization in rats induces depression-like behaviors *via* a serotonin transmitter system. *J. Affect. Disord.,* **2015**, *178*, 25-31.
http://dx.doi.org/10.1016/j.jad.2015.02.022 PMID: 25779937

[50] Gururajan, A.; Naughton, M.E.; Scott, K.A.; O'Connor, R.M.; Moloney, G.; Clarke, G.; Dowling, J.; Walsh, A.; Ismail, F.; Shorten, G.; Scott, L.; McLoughlin, D.M.; Cryan, J.F.; Dinan, T.G. microRNAs as biomarkers for major depression: A role for let-7b and let-7c. *Transl. Psychiatry,* **2016**, *6*(8), e862.
http://dx.doi.org/10.1038/tp.2016.131 PMID: 27483380

[51] Bocchio-Chiavetto, L.; Maffioletti, E.; Bettinsoli, P.; Giovannini, C.; Bignotti, S.; Tardito, D.; Corrada, D.; Milanesi, L.; Gennarelli, M. Blood microRNA changes in depressed patients during antidepressant treatment. *Eur. Neuropsychopharmacol.,* **2013**, *23*(7), 602-611.
http://dx.doi.org/10.1016/j.euroneuro.2012.06.013 PMID: 22925464

[52] Shi, Y. Non-coding RNAs in depression: Promising diagnostic and therapeutic biomarkers. *EBioMedicine.,* **2021**, *71*, 103569.
http://dx.doi.org/10.1016/j.ebiom.2021.103569

[53] Dantzer, R.; O'Connor, J.C.; Freund, G.G.; Johnson, R.W.; Kelley, K.W. From inflammation to sickness and depression: When the immune system subjugates the brain. *Nat. Rev. Neurosci.,* **2008**, *9*(1), 46-56.
http://dx.doi.org/10.1038/nrn2297 PMID: 18073775

[54] Pearson, S.; Schmidt, M.; Patton, G.; Dwyer, T.; Blizzard, L.; Otahal, P.; Venn, A. Depression and insulin resistance: Cross-sectional associations in young adults. *Diabetes Care,* **2010**, *33*(5), 1128-1133.
http://dx.doi.org/10.2337/dc09-1940 PMID: 20185745

[55] Shadrina, M.; Bondarenko, E.A.; Slominsky, P.A. Genetics factors in major depression disease. *Front. Psychiatry,* **2018**, *9*, 334.
http://dx.doi.org/10.3389/fpsyt.2018.00334 PMID: 30083112

[56] Cui, C.; Zhong, B.; Fan, R.; Cui, Q. HMDD v4.0: A database for experimentally supported human microRNA-disease associations. *Nucleic Acids Res.,* **2024**, *52*(D1), D1327-D1332.
http://dx.doi.org/10.1093/nar/gkad717 PMID: 37650649

[57] Huang, Z.; Shi, J.; Gao, Y.; Cui, C.; Zhang, S.; Li, J.; Zhou, Y.; Cui, Q. HMDD v3.0: A database for experimentally supported human microRNA–disease associations. *Nucleic Acids Res.,* **2019**, *47*(D1), D1013-D1017.
http://dx.doi.org/10.1093/nar/gky1010 PMID: 30364956

[58] Kokkinopoulou, I.; Maratou, E.; Mitrou, P.; Boutati, E.; Sideris, D.C.; Fragoulis, E.G.; Christodoulou, M.I. Decreased expression of microRNAs targeting type-2 diabetes susceptibility genes in peripheral blood of patients and predisposed individuals. *Endocrine,* **2019**, *66*(2), 226-239.
http://dx.doi.org/10.1007/s12020-019-02062-0 PMID: 31559537

[59] Assmann, T.S.; Recamonde-Mendoza, M.; Puñales, M.; Tschiedel, B.; Canani, L.H.; Crispim, D. microRNA expression profile in plasma from type 1 diabetic patients: Case-control study and bioinformatic analysis. *Diabetes Res. Clin. Pract.,* **2018**, *141*, 35-46.
http://dx.doi.org/10.1016/j.diabres.2018.03.044 PMID: 29679626

[60] Pordzik, J.; Jakubik, D.; Jarosz-Popek, J.; Wicik, Z.; Eyileten, C.; De Rosa, S.; Indolfi, C.; Siller-Matula, J.M.; Czajka, P.; Postula, M. Significance of circulating microRNAs in diabetes mellitus type 2 and platelet reactivity: Bioinformatic analysis and review. *Cardiovasc. Diabetol.,* **2019**, *18*(1), 113.
http://dx.doi.org/10.1186/s12933-019-0918-x PMID: 31470851

[61] Wang, J.; Pan, Y.; Dai, F.; Wang, F.; Qiu, H.; Huang, X. Serum miR-195-5p is upregulated in gestational diabetes mellitus. *J. Clin. Lab. Anal.,* **2020**, *34*(8), e23325.

http://dx.doi.org/10.1002/jcla.23325 PMID: 32301163

[62] Wan, S.; Wang, J.; Wang, J.; Wu, J.; Song, J.; Zhang, C.Y.; Zhang, C.; Wang, C.; Wang, J.J. Increased serum miR-7 is a promising biomarker for type 2 diabetes mellitus and its microvascular complications. *Diabetes Res. Clin. Pract.,* **2017**, *130*, 171-179. http://dx.doi.org/10.1016/j.diabres.2017.06.005 PMID: 28646700

[63] Wang, H. microRNA, diabetes mellitus and colorectal cancer. *Biomedicines,* **2020**, *8*(12), 530. http://dx.doi.org/10.3390/biomedicines8120530 PMID: 33255227

[64] Johansen, J.S.; Harris, A.K.; Rychly, D.J.; Ergul, A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovasc. Diabetol.,* **2005**, *4*(1), 5. http://dx.doi.org/10.1186/1475-2840-4-5 PMID: 15862133

[65] Galicia-Garcia, U.; Benito-Vicente, A.; Jebari, S.; Larrea-Sebal, A.; Siddiqi, H.; Uribe, K.B.; Ostolaza, H.; Martín, C. Pathophysiology of type 2 diabetes mellitus. *Int. J. Mol. Sci.,* **2020**, *21*(17), 6275. http://dx.doi.org/10.3390/ijms21176275 PMID: 32872570

[66] Wang, H. Tolerance limits for mixture-of-normal distributions with application to COVID-19 data. *Wiley Interdiscip. Rev. Comput. Stat.,* **2023**, *15*(6), e1611. http://dx.doi.org/10.1002/wics.1611

[67] Chen, Y.H.; Wang, H. Exploring diversity of COVID-19 based on substitution distance. *Infect. Drug Resist.,* **2020**, *13*, 3887-3894. http://dx.doi.org/10.2147/IDR.S277620 PMID: 33149633

[68] Wang, H. COVID−19, anti-NMDA receptor encephalitis and microRNA. *Front. Immunol.,* **2022**, *13*, 825103. http://dx.doi.org/10.3389/fimmu.2022.825103 PMID: 35392089

[69] Wang, H. A review of the effects of collagen treatment in clinical studies. *Polymers,* **2021**, *13*(22), 3868. http://dx.doi.org/10.3390/polym13223868 PMID: 34833168

[70] Tramunt, B.; Smati, S.; Grandgeorge, N.; Lenfant, F.; Arnal, J.F.; Montagner, A.; Gourdy, P. Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia,* **2020**, *63*(3), 453-461. http://dx.doi.org/10.1007/s00125-019-05040-3 PMID: 31754750

[71] Jin, J.M.; Bai, P.; He, W.; Wu, F.; Liu, X.F.; Han, D.M.; Liu, S.; Yang, J.K. Gender differences in patients with COVID-19: Focus on severity and mortality. *Front. Public Health,* **2020**, *8*, 152. http://dx.doi.org/10.3389/fpubh.2020.00152 PMID: 32411652

[72] Pinheiro, I.; Dejager, L.; Libert, C. X-chromosome-located microRNAs in immunity: Might they explain male/female differences? *BioEssays,* **2011**, *33*(11), 791-802. http://dx.doi.org/10.1002/bies.201100047 PMID: 21953569

[73] Caserta, S.; Gangemi, S.; Murdaca, G.; Allegra, A. Gender differences and miRNAs expression in cancer: Implications on prognosis and susceptibility. *Int. J. Mol. Sci.,* **2023**, *24*(14), 11544. http://dx.doi.org/10.3390/ijms241411544 PMID: 37511303

[74] Bellenghi, M.; Puglisi, R.; Pontecorvi, G.; De Feo, A.; Carè, A.; Mattia, G. Sex and gender disparities in melanoma. *Cancers,* **2020**, *12*(7), 1819. http://dx.doi.org/10.3390/cancers12071819 PMID: 32645881

[75] Chabchoub, G.; Uz, E.; Maalej, A.; Mustafa, C.A.; Rebai, A.; Mnif, M.; Bahloul, Z.; Farid, N.R.; Ozcelik, T.; Ayadi, H. Analysis of skewed X-chromosome inactivation in females with rheumatoid arthritis and autoimmune thyroid diseases. *Arthritis Res. Ther.,* **2009**, *11*(4), R106. http://dx.doi.org/10.1186/ar2759 PMID: 19589151

[76] Khalifa, O.; Pers, Y.M.; Ferreira, R.; Sénéchal, A.; Jorgensen, C.; Apparailly, F.; Duroux-Richard, I. X-linked miRNAs associated with gender differences in rheumatoid arthritis. *Int. J. Mol. Sci.,* **2016**, *17*(11), 1852. http://dx.doi.org/10.3390/ijms17111852 PMID: 27834806