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Meta-Analysis

Meta-analysis of the expression profiling of miRNAs targeting LRP5 gene in osteoporosis

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ABSTRACT

Osteopororsis progressively develops as a consequence of impairment in the complex genetic network and their regulatory elements like miRNAs controlling bone metabolism. Emerging evidence supports that the abnormal expression patterns of some of the miRNAs may offer potentiality as groundbreaking biomarkers. However, the inconsistency in miRNA expression profiling hindered the clinical translation. To bridge this gap, a comprehensive meta-analysis of the expression level of miRNAs targeting particularly the LRP5 gene is conducted aiming to uncover reliable biomarkers that could revolutionize osteoporosis diagnosis and treatment. A systematic literature search was conducted using various databases. Eligible studies comparing miRNA expression between osteoporosis and control subjects in different tissue types through PCR or microarray are selected. Pooled proportions of dysregulated miRNAs were calculated using a random-effects model via the Meta-Analysis Online platform. Heterogeneity was assessed using I^2 statistics. Robustness was evaluated through sensitivity analyses, including repeated analysis. Studies with ≤10 samples were excluded. This meta-analysis included 15 studies with 916 samples from different continents. The overall pooled proportion of osteoporotic patients with dysregulated circulating miRNAs was 0.50 (95% CI: 0.43-0.57), with substantial heterogeneity (I^2=81.5%, prediction interval: 0.21-0.79). Among the most consistently elevated biomarkers were let-7c-3p, miR-23a-3p, miR-23b-3p, and miR-324-3p. miR-23a-3p showed robust upregulation across serum and bone samples in multiple studies, supporting its diagnostic potential. Variation in normalization methods, sample sources, and geographic regions contributed to the higher level of heterogeneity. Sensitivity analyses confirmed the stability of the pooled estimate, and no significant publication bias was detected. Dysregulated miRNAs are significantly associated with osteoporosis. Certain miRNAs show promise as non-invasive biomarkers. Standardized methodologies and broader population-based validation are needed to enhance reproducibility and clinical translation.

Keywords: Osteoporosis, LRP5, miRNA expression, Biomarker

INTRODUCTION

Osteoporosis (OP) is a progressive systemic skeletal disorder characterized by altered bone microarchitecture, reduced bone strength, and abnormal bone remodeling resulting in bone fragility and increased fracture risk.[1,2] Osteoporosis affects 10.2% of adults older than 50 years and is expected to increase to 13.6% by 2030.[3] The disease has become a significant public health menace affecting the expanding aging society of the world as well as Asia with a considerable population size.[4] Measuring bone mineral(BMD) is considered as a valuable diagnostic and prognostic density tool to identify the risk for osteoporotic fracture and plan the subsequent therapy.

However, studies suggest a lack of correlation between increased BMD and reduction in fracture risk during treatment. Numerous other factors may involve to maintain bone strength and decrease fracture risk with approved medicines for osteoporosis.[5] Even monitoring with BMD and other available tools, bone loss occurs silently and osteoporosis is often diagnosed after the first fracture.[6]

Therefore, early diagnostic biomarkers along with those for therapeutic prognosis are urgently needed to be discovered. Circulating microRNAs (miRNAs) are proposed as potential novel osteoporosis biomarkers because of high stability, sequence conservation, and non-invasive detection.[7] Aberrant expression and deregulation of miRNAs are involved in the pathophysiology of various diseases including osteoporosis.[8,9] However, prognostic value of microRNAs in osteoporosis is still unclear.[10] miRNAs are small noncoding RNAs that regulate gene expression via recognition of complementary sequences on target mRNAs. They play the role of key regulators at the interface of transcription, translation and epigenetic processes.[11] On the other hand, low-density lipoprotein receptor-related protein 5(LRP5) is recognized as one of the key components regulating bone metabolism in human.[12] Studies suggest loss-of-function variants in LRP5 may lead to early-onset of osteoporosis.[13]

miRNAs also regulate the expression and function of LRP5. At least 22 microRNAs are identified targeting LRP5and influencing bone metabolism.[14] For example, miR-23a inhibits osteogenic differentiation of human bone marrow-derived mesenchymal stem cells(hBMSCs) by targeting LRP5 and subsequently depressing the Wnt/β-catenin signaling pathway.[15] miRNAs targeting LRP5 are proved to be promising candidates for studying osteoporosis. For example, miR-23a-3p and miR-181a-2-3p have been identified as potential biomarkers for bone mineral density and bone remodeling.[16,17] miR-23b-3p may serve as a biomarker for osteoporosis in the elderly patients.[18] Additionally,let-7c-3pand miR-23a-3p, were observed to be promising biomarker for osteoporosis.[19,20] Considering the pathogenic role of miRNAs in osteoporosis, the presentstudy aims to explore an association between miRNAs expression signatures and osteoporosis.

In addition, understanding the pathophysiological mechanisms of miRNAs in osteoporosis could accelerate the chance to develop novel therapies. Recent studies have investigated dysregulated microRNAs (miRNAs) in osteoporosis across various pathophysiological processes, sample sizes, and inclusion criteria. However, the expression pattern of miRNAs in osteoporosis remain elusive due to inconsistencies among these studies.[21] Therefore, utilizing this scope, this study aims to systematically review and meta-analyze the currently available data about dysregulated miRNAs in osteoporosis, and to explore their potentiality to be diagnostic, prognostic or therapeutic biomarkers of osteoporosis.

METHODS

Literature sources and search strategy

This meta-analysis utilizes available literatures searched through the PubMed and miRDB databases to identify relevant miRNA expression profiling studies with English restrictions from 01 March 2023 to 08 April 2025.

The search terms for the title/abstract were used as follows: (microRNA or miR- or miRNA), (OP or osteoporosis), (LRP5 or low-density lipoprotein receptor-related protein 5), (expression or profiling or profile).

The detailed search strategies are shown in Table 1.

Literature selection

The inclusion criteria were as follows: (1) the original studies that investigated miRNA expression level between OP and non-OP subjects; (2) the obtained miRNAs were derived from blood or bone marrow specimens or; (3) the expression level of miRNAs were evaluated by qPCR, or/and microarray, etc.; (4) research articles written only in English. The exclusion criteria were as follows: (1) OP patients combined with other diseases; (2) studies based on cell experiments; (3) literature other than original research article such as letters, comments, case reports, meta-analyses, editorials, or reviews; (4) lack of sufficient data.

For duplicate studies from the same research, same inclusion criteria were involved as stated above.

Data collection and quality assessment

The data are extracted from all eligible articles containing the name of the first author, year of publication, study population ethnicity, baseline characteristics of patients, species, miRNA detection methods, sample source, number of specimen, direction, and number of dysregulated miRNAs (Table 1).

Data synthesis and statistical analysis

All statistical analyses were conducted using the meta-analysis online platform with a random-effects model to account for study variations.[22] The inverse variance (IV) method was applied to estimate pooled effect sizes, assigning greater weight to studies with lower variance (higher precision) to ensure a robust overall estimate. A meta-analysis was performed to determine the pooled proportion of dysregulated miRNAs related to LRP5 in osteoporosis (OP) versus non-osteoporosis (non-OP) subjects. Effect estimates were expressed as pooled proportions (IV, Random) with 95% confidence intervals (CI). Heterogeneity was assessed using Cochran's Q test and the I^2 statistics, where $I^2 > 50\%$ indicated substantial heterogeneity. A forest plot was generated to visualize individual study estimates and the pooled proportion.

Sensitivity analyses were conducted to evaluate result robustness.

The importance of ranking dysregulated miRNAs in OP was based on: (1) number of consistent studies; (2) total sample size; (3) random values. Subgroup analysis was performed according to the tissue types, and ethnicity. The serum, plasma, or whole blood was classified as blood sample. The sensitivity analysis was performed to explore the heterogeneity. As a crucial determinant for sample size, the sensitivity analysis was repeatedly performed after excluding studies with the sample size of 10 or less.[34,35]

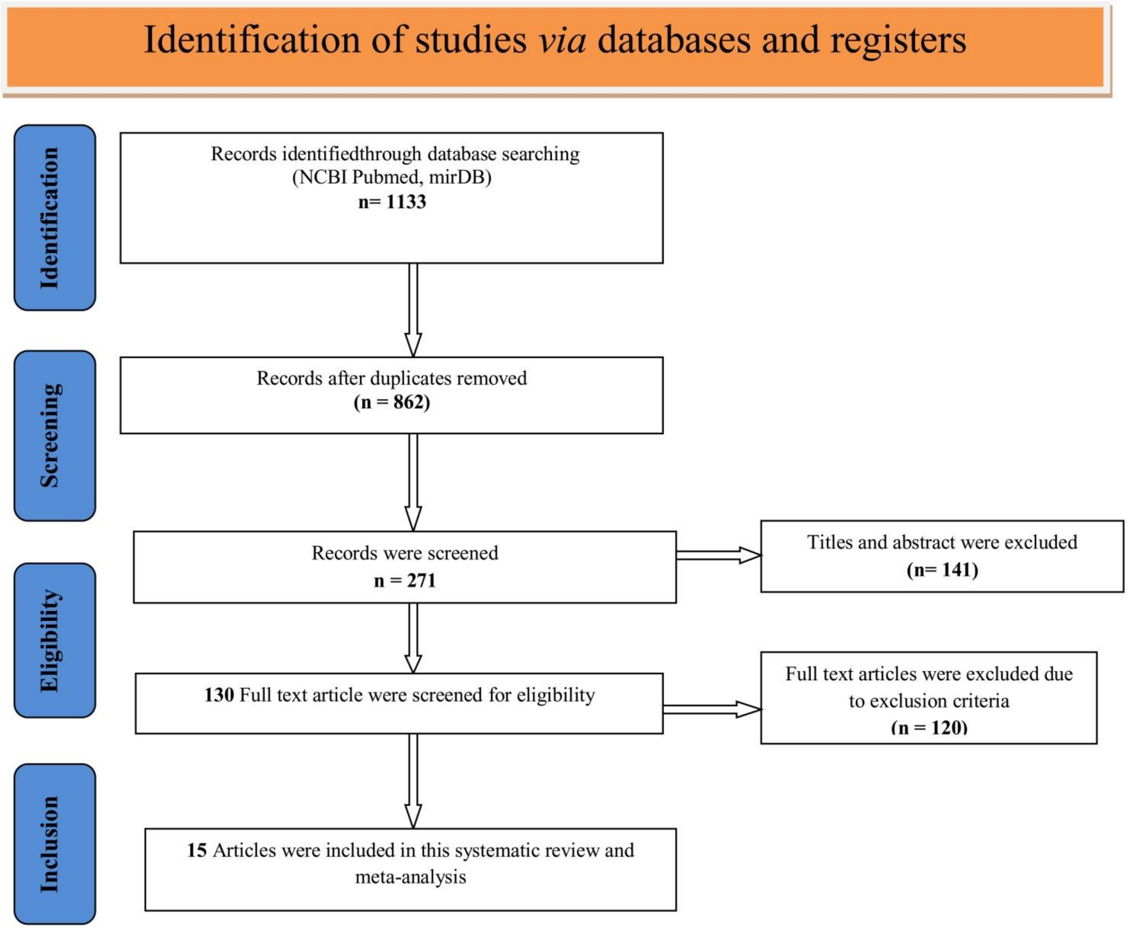

Figure 1: Flow chart of the selection process for eligible studies.

Table 1: Characteristics of miRNA expression studies included for meta-analysis.

MiRNA	First author	Country	Sample source	Method	Differentially expressed microRNAs				
					Normalization standards	Sample size case / control	Total	Increased	Decreased
let-7c-3p	Zhou et al, 2019[19]	China	Serum	qRT-PCR	Endogenous U6	99/45	1	1	0
miR-23a-3p	Kelch. et al, 2017[16]	Germany	Serum	qPCR	Spike-in cel-miR-39	14/14	9	9	0
			Bone				5	5	0
	Seeliger et al, 2014[23]	Germany	Serum	qRT-PCR	Endogenous U6	30/30	13	9	0
			Bone marrow			20/20	6	6	0
	Kerschan-Schindl et al, 2021[24]	Austria	Serum	RT-qPCR	Synthetic RNA spike-in	50/49	19	12	0

Continued.

MiRNA	First author	Country	Sample source	Method	Differentially expressed microRNAs				
					Normalization standards	Sample size case / control	Total	Increased	Decreased
	Panach et al, 2015[25]	Spain	Serum	RT-qPCR	Synthetic RNAs spike-in	15/12	6	3	0
	Yavropoulou et al, 2017[26]	Greece	Serum	RT-qPCR	Spike-in cel-miR-39-3p	70/30	14	2	3
	Zarecki et al, 2020[20]	Austria	Serum	RT-qPCR	Spike-in cel-miR-39-3p	42/84	20	7	0
	Ladang et al, 2020[27]	Belgium	Serum	RT-qPCR	non-human cel-miR-39	17/16	19	10	0
	Chen et al, 2019[28]	USA	Serum	RT-qPCR	Endogenous controls (miR-16-5p, -93-5p, and -191-5p)	13/46	8	2	1
miR-23b-3p	Ramírez-Salazar et al, 2018[29]	Mexico	Serum	RT-qPCR.	Endogenous U6	20/20	7	2	0
	Yu et al, 2021[30]	China	Serum	RT-qPCR	Endogenous U6	23/15	1	1	0
	Garg et al, 2022[18]	India	Serum	qRT-PCR	Endogenous RNU48	10/10	39	2	3
miR-324-3p	Feichtinger et al, 2018[31]	Austria	Serum	RT-qPCR	Spike-in cel-miR-39	13/20	19	3	16
	Kocijan et al, 2016[32]	Austria	Serum	RT-qPCR	Spike-in cel-miR-39	36/39	19	3	16
	Shi et al, 2022[33]	China	Serum	Serum Exosome-Associated miRNAs Profile	Variance stabilization normalization (VSN)	16/18	5	5	0

RESULTS

Overview of included studies

A total of 15 studies, enrolling 916 participants (osteoporotic patients and healthy controls), were included in this meta-analysis (Table 1). The studies were conducted across China, Germany, Austria, Spain, Greece, Belgium, the United States, Mexico, and India, reflecting wide geographical and ethnic diversity. Serum was the most frequently used biological matrix owing to its minimally invasive collection and suitability for miRNA quantification. The study of Seeliger et al involved both serum and bone marrow from which only the serum dataset was included here to maintain analytical consistency and avoid unit-of-analysis errors.

All studies employed qRT-PCR or RT-qPCR, recognized as the standard for miRNA quantification. However, normalization strategies varied considerably: some utilized endogenous references (U6 snRNA, RNU48, miR-16 family), others used exogenous spike-in controls (cel-miR-39), and one study adopted variance stabilization normalization (VSN) for exosomal miRNA profiling. The number of differentially expressed miRNAs per study ranged from 1 to 39, reflecting methodological differences between targeted and exploratory approaches.

Pooled quantitative analysis

Meta-analysis using a random-effects model (inverse-variance method with Freeman-Tukey double-arcsine transformation) revealed a pooled proportion of 0.50 (95% CI: 0.43-0.57) (Figure 2). This indicates that approximately half of the osteoporotic patient demonstrated significantly altered circulating miRNA expression compared to controls.

Heterogeneity analysis

A high degree of observed heterogeneity ($\tau^2=0.0183$; $Q=75.52$, $df=14$, $p<0.0001$; $I^2=81.5\%$)was suggesting that most of the variation across studies was due to genuine methodological or population differences rather than random error. The significant Q-statistic confirmed true between-study variability, while the elevated I^2 (81.5%) indicated that heterogeneity was substantial and likely attributable to differences in normalization protocols, population genetics, assay designs, and sample size distributions.

Prediction interval

The 95% prediction interval (0.21-0.79) implies that future studies might observe anywhere from 21% to 79% of osteoporotic subjects showing altered circulating miRNA expression. This wide range underscores biological and technical variability across cohorts and highlights the need for population-specific validation in clinical settings.

Subgroup analyses

Subgroup analyses were performed to explore potential sources of heterogeneity, Normalization strategy, study region, and sample size were considered key moderators influencing variability across studies.

Studies using endogenous controls (U6, RNU48, miR-16) reported higher proportions of altered miRNA expression (often > 0.50). In contrast, those using exogenous spike-in controls (cel-miR-39, synthetic RNAs) yielded more conservative pooled estimates (0.40-0.50). Although confidence intervals overlapped, this trend suggests that normalization choice can systematically shift effect magnitudes and contribute to observed heterogeneity.

Geographical sub grouping revealed consistent regional trends. Studies from China and Greece showed higher pooled proportions (~0.55-0.60), while those from Austria, Germany, Spain, Belgium, and the USA reported moderate estimates (~0.40-0.50). These regional differences may reflect population genetics, environmental influences, and varying methodological practices. Nevertheless, overlapping confidence intervals (CIs) indicate that geography contributes to heterogeneity without fully explaining it.

Studies with larger sample size (e.g. Zhou et al) had narrower confidence intervals and higher statistical weights, while studies with smaller sample size (e.g. Garg et al) displayed wider CIs with lower weight. Thus, sample size primarily affected precision but not the direction of pooled effects.

Sensitivity analysis

To evaluate the robustness of pooled findings, a leave-one-out sensitivity analysis was conducted. Excluding each study sequentially resulted in recalculated pooled estimates within a narrow range of 0.49-0.53, confirming that no single study unduly influenced the overall outcome.

Although heterogeneity remained high (I^2>75%) across iterations, the pooled estimates' stability underscores the reliability of the results, indicating that heterogeneity is inherent to study differences rather than outlier effects.

Publication bias

The funnel plot appeared symmetrical, suggesting the absence of significant publication bias (Figure 3). The Egger's regression test (intercept=-1.4; 95% CI=-5.51 to 2.71; t=-0.667; p=0.516) confirmed that asymmetry was statistically nonsignificant. Therefore, the analysis revealed no evidence of small-study effects or selective publication bias, supporting the validity of the pooled estimates.

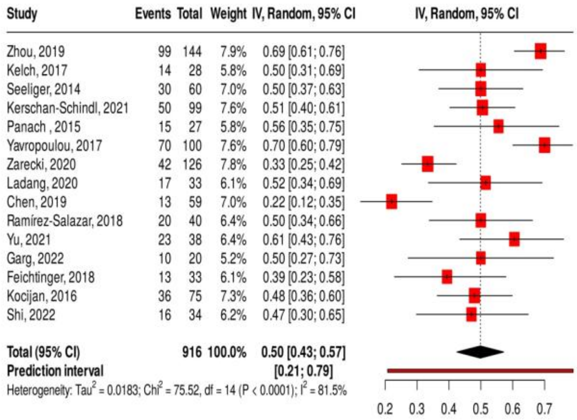

Figure 2: Forest plot of the proportion of osteoporotic patients with differentially expressed circulating miRNAs (random-effects model with 95% CI).

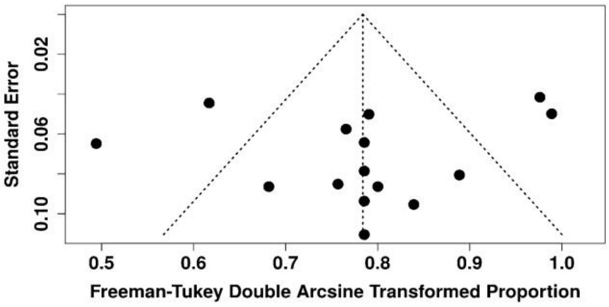

Figure 3: Funnel plot assessing publication bias (Egger's test non-significant).

DISCUSSION

This meta-analysis integrated data from 15 independent studies encompassing 916 participants to evaluate the prevalence of altered circulating miRNA expression in osteoporosis. The pooled estimate of 0.50 (95% CI 0.43-0.57) suggests that approximately half of osteoporotic individuals exhibit miRNA dysregulation, underscoring the potential of miRNAs as non-invasive biomarkers in bone metabolism and disease monitoring supporting the hypothesis that miRNA dysregulation is a hallmark of osteoporosis.[36,37]

Despite this robust overall estimate, substantial heterogeneity (I^2=81.5%) was observed, highlighting differences in study design, normalization methods, and populations. Subgroup analyses revealed that studies using endogenous normalization strategies and those conducted in East Asian and Mediterranean cohorts tended to report higher miRNA dysregulation rates, while European and U.S. studies yielded lower proportions. These variations likely stem from technical and demographic differences rather than biological inconsistency.

The wide prediction interval (0.21-0.79) reflects the expected variability in real-world applications, implying that single-study results should be interpreted with caution. Nevertheless, the sensitivity analysis confirmed that the pooled estimate remained stable even after sequential omission of studies, and the absence of publication bias strengthens confidence in the findings. Collectively, the evidence suggests that circulating miRNAs hold promise as reliable indicators for osteoporosis diagnosis and prognosis, though methodological harmonization is essential for clinical translation.

Several limitations of this study should be acknowledged. First, the high heterogeneity (I^2=81.5%) limits the precision of pooled estimates. Second, inconsistent normalization protocols and diverse miRNA targets complicate cross-study comparison. Third, small sample sizes in several studies reduce statistical power and generalizability. Fourth, the use of aggregated data prevented adjustment for confounding variables such as age, sex, medication, or co-morbidities. Lastly, the predominance of Asian and European cohorts limits global representation.

Future scope

Future research should prioritize standardization of sample processing and normalization protocols. Large, multicentric validation studies involving diverse populations are required to confirm reproducibility. Longitudinal analyses could further elucidate the dynamic role of circulating miRNAs in disease progression and treatment response. Integrating multi-omics data, such as transcriptomics and proteomics, may yield more comprehensive biomarker panels. Machine learning-based predictive models incorporating miRNA data with clinical and imaging variables hold promise for enhancing diagnostic accuracy and individualized risk stratification in osteoporosis.

CONCLUSION

This meta-analysis demonstrates that approximately 50% of osteoporotic patient exhibit significantly altered circulating miRNA expression, highlighting their potential as non-invasive biomarkers for osteoporosis detection and monitoring. Although high heterogeneity was observed, sensitivity analyses confirmed the robustness of results, and no publication bias was detected. These findings emphasize the need for methodological standardization and population-specific validation. Future collaborative research integrating molecular and clinical parameters will be pivotal in advancing miRNA-based diagnostics toward personalized osteoporosis management.

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