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In silico Identification of MicroRNAs Potentially Targeting the Low-density Lipoprotein Receptor-Related Protein 5 (*LRP5*) Transcript, a Crucial Factor for Osteoporosis Development and Other Genes of a Predictive Network of *LRP5*

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Abstract Low-density lipoprotein receptor-related protein 5 (*LRP5*) is the key regulator of bone metabolism. Deregulation of this gene may cause different bone metabolic diseases such as osteopororsis characterized by low BMD. Deregulation of this gene includes mutations, single nucleotide polymorphisms, altered expression and several others. Like all other genes, *LRP5* also works through a complex genetic network. This study focuses on a genetic network of LRP5 proposed by GeneMania, a bioinformatics tool. A list of microRNAs targeting *LRP5* has been retrieved from miRDB database and validated through other software. In the proposed genetic network, 20 genes are found to be interacting with *LRP5* by various means. The 22 miRNAs found to interact with *LRP5* has also found to influence the other genes from the proposed genetic network of *LRP5*. A meta-analysis shows some of these miRNAs to play important role in bone metabolism as well as osteoporosis development. As miRNAs regulate gene expression through a complex regulatory network, the rest of the miRNAs also need experimental validations of their functional role in bone. Over all this paper is a comprehensive report of miRNAs affecting *LRP5* function in bone development and diseases particularly osteoporosis.

Keywords Bioinformatics, GeneMania, *in silico*, *LRP5*, miRDB, miRNA, Osteoporosis, Venny 2.1

1. Introduction

The low-density lipoprotein receptor-related protein 5 (*LRP5*) is encoded by the *LRP5* gene in humans [1]. *LRP5* is the key component of the LRP5/LRP6/Frizzled co-receptor complex working through canonical Wnt signaling pathway regulating bone metabolism. *LRP5* thus has an important role in bone metabolism as it transduces signals by Wnt proteins [2] in osteoblasts [3]. It is evident that any alteration in the expression of *LRP5* can lead to considerable changes in bone mass following bone diseases [4].

Osteopororsis is a complex bone metabolic disease characterized by low bone mineral density. It has been raised as a major public health hazard now a days among the elderly population [5]. Through recent investigations *LRP5* gene is appearing as one of the key regulators of bone mineral density as well as of the development of osteoporosis [6].

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Lrp5 gene, located on human chromosome 11q13 [7] associated with several other genes to form a genetic network. Altered expression of *LRP5* may affect the functionality of these genes and vice versa.

MicroRNAs (miRNAs) are proved to be one of the most important regulators of eukaryotic gene expression [8]. The miRNAs are short single stranded non-coding RNAs usually 19-25 nucleotides long. They target and bind to messenger RNAs (mRNAs) and determine their fate [9]. Binding of miRNAs clog translation of target mRNAs into protein and encourage degradation of mRNA targets. In this way miRNAs regulate the expression of more than 30% of protein-coding genes at the post-transcriptional and translational level [10].

miRNAs have immense role in regulating *LRP5* expression as well as its function. Several miRNAs reported to target *LRP5* which are enlisted in various target prediction software. Comprehensive and computational analysis of miRNA targeting *LRP5* as a crucial factor in osteoporosis progression and bone metabolism has not yet been undertaken. Aim of this piece of work is to assemble all the available information about miRNAs taking part in

regulation of *LRP5* and thus affecting its role in bone metabolism.

2. Materials and Methods

2.1. Prediction of Genetic Network of LRP5

GeneMania (http://www.GeneMania.org) is an online bioinformatics tool which predicts the interaction of a number of genes with the query gene [11] using a wide set of functional association data. We put human "*LRP5*" of as our query term and retrieved a genetic interaction network of 20 genes associated with *LRP5* [12].

2.2. Mining the miRNA Pool Targeting LRP5 and Its Interacting Partners

To explore the miRNA pool targeting *LRP5,* we use miRDB (MicroRNA target prediction and functional study database http://mirdb.org/). miRDB is an online computational tool for miRNA target prediction and functional annotations [13]. We have used the search term "*LRP5*" as "Human" "Gene Symbol" and retrieved a list of 22 miRNAs targeting the query gene.

We tabulate the details of each miRNA provided by the software. To find out whether these miRNAs target other genes of the predicted network of *LRP5,* we put each miRNA name as the search item and got the list of all the targets of the query miRNA. From the list we find out the names of the genes associated with LRP5 in its predicted network.

2.3. Confirmation of the miRNA – LRP5 Interaction

The output of miRDB was confirmed with other three software *viz.*, TargetScanHuman 8.0 (https://www.targetscan.org/vert_80/); RegenDbase (https://regendbase.org/mirna-targets/search) and mirTargetLink 2.0 (https://ccb-compute.cs.uni-saarland.de/mirtargetlink2/).

TargetScanHuman predicts biological targets of miRNAs by searching the presence of conserved 8mer, 7mer, and 6mer sites that match the seed region of each miRNA [14]. RegenDbase, the Regeneration Database provides comprehensive set of miRNA-target predictions by integrating mRNA and microRNA expression data from diverse models of regeneration together with data from embryonic and induced pluripotent stem cells [15]. In the form of interactive network visualization, mirTargetLink 2.0 offers information on microRNA-mRNA interactions [16].

In case of TargetScanHuman, "Human" is selected as the species and "*LRP5*" as the query gene symbol. We submit the names of each of 22 miRNAs to find out the interaction of the miRNA and *LRP5* transcript. Here not only we got the confirmation of miRNA targeting LRP5 but also the region of their interaction.

For RegenDbase, *Homo sapiens* is selected as "organism" and miRBase mature miRNA accession IDs are used for the final output. It confirms whether a particular miRNA targets LRP5 or not.

Each of the mature miRNA names are used as input in mirTargetLink 2.0 to further confirm *LRP5* as the target output.

2.4. Preparing a Venn Diagram to Show the Proportion of miRNAs Confirmed by Various Software to Regulate LRP5

To represent graphically the data retrieved from the entire four target predicting software tools, Venny 2.1 is used. Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/, accessed on 12 September 2023) is a Web-Tool for Creating Venn Diagrams [17]. The list of miRNAs confirmed by each software is used as the input and the output is a Venn diagram showing the proportion of miRNAs confirmed by various software to regulate LRP5.

2.5. Meta-Analysis of MicroRNA Functions Experimentally Proved to Regulate LRP5

A meta-analysis has been done to find out the functional role of microRNAs in regulation of *LRP5*. For mining the related experimental data, miRBase (https://www.mirbase.org/) database is used primarily. National Center for Biotechnology Information's (NCBI) PubMed (https://pubmed.ncbi.nlm.nih.gov/about/) has also been utilized for these experimental resource supports.

3. Result

3.1. Genetic Network of LRP5

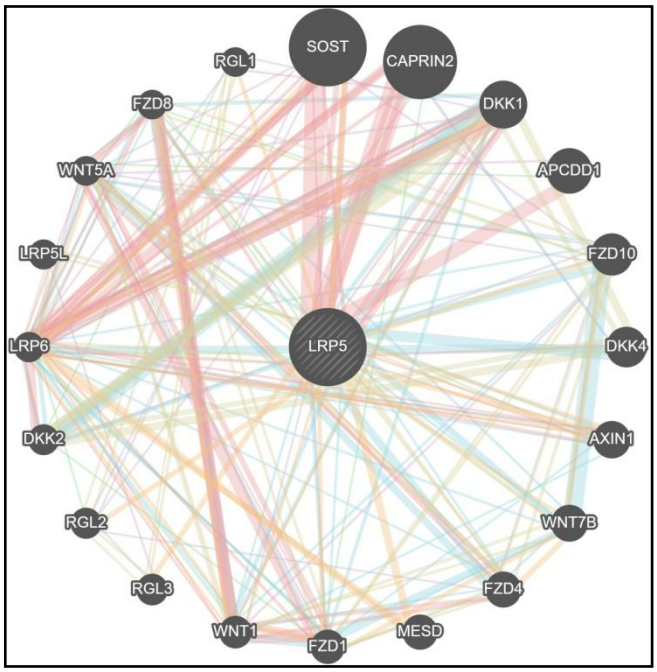

Figure 1. LRP5 genetic network showing LRP5 interactions with other related genes as predicted by online software tool GeneMANIA (Adapted from Sengupta & Das, 2023)

GeneMania identified that LRP5 interacts with 20 others genes viz. SOST, CAPRIN2, DKK1, APCDD1, FZD10,

DKK4, AXIN1, WNT7B, FZD4, MESD, FZD1, WNT1, RGL3, RGL2, DKK2, LRP6, LRP5L, WNT5A, FZD8, RGL1 according to Physical Interactions, Co-expression, Predicted, Co-localization, Genetic Interactions, Pathway, Shared protein domains etc. (Figure 1, Table 1).

Sclerostin *(SOST*) gene encodes for the protein sclerostin, a ligand for LRP5/LRP6 and an inhibitor of Wnt signaling pathway. It is a negative regulator of bone formation [18]. According to GeneMania, *SOST* holds rank 1 being the most influencing partner of *LRP5*.

Caprin family member 2 (*CAPRIN2*) encodes for protein caprin-2. It enhances the activity of the canonical Wnt signaling pathway by promoting the phosphorylation of the Wnt coreceptor *LRP6* [19]. GeneMania ranked *CAPRIN 2* as the second most interactive partner of *LRP5*.

Dickkopf WNT signaling pathway inhibitor (*DKK*) family genes codes for Dickkopf-related protein 1, 2 and 4 (*DKK1, DKK2 and DKK4* respectively). They are reported to prevent canonical Wnt signaling by inhibiting LRP5/6 interaction with Wnt by internalization of LRP5/6 [20] by forming a ternary complex with the transmembrane protein KREMEN [21]. Dkks are implicated in bone formation and bone disease [22,23,24]. GeneMania specified the rank of interaction for *DKK1, DKK4* and *DKK2* is rank 3, 6 and 15 respectively.

APC down-regulated 1 (*APCDD1*) encodes for the protein *APCDD1*. It negatively regulates the Wnt signaling pathway by interacting with Wnt and LRP proteins [25]. It is the 4th gene to interact with *LRP5* as ranked by GeneMania.

Frizzled (*FZD*) family genes encode for a number of seven transmembrane receptor proteins. *FZD* 1, 4, 8 and 10 proteins act as receptors for Wnt proteins [26,27,28,29]. They influence the WNT signaling by various activities. According to GeneMania, *FZD10* is ranked as 5th, *FZD 4* is ranked as 9th, *FZD1* is ranked as 11th and *FZD8* is ranked as 19th with reference to the interaction with LRP5 itself.

Axin 1 (*AXIN1*) gene encodes Axin-1 protein. The stability of β-catenin is crucial for canonical Wnt signaling. *Axin/APC/GSK3β* complex phosphorylates β-catenin to be degraded by ubiquitination [30]. According to GeneMania, Axin1 is the 7th to interact with *LRP5*.

Wnt family member (*WNT*) 1, 5A, 7B encodes Protein Wnt-1, Wnt-5a and Wnt-7b respectively. These act as ligands for frizzled family receptors that functions in the canonical Wnt/beta-catenin signaling pathway [31]. Mutations in *WNT1* cause different forms of bone fragility [32]. It has a role in osteoblast function, bone development and bone homeostasis [33]. *WNT5A* can promote or suppress osteoclast differentiation and development [34]. Wnt7b is important in osteogenesis as it participates in the bone formation [35]. GeneMania ranked *WNT1*, *WNT5A* and *WNT7B* as the 12th, 18th and 8th interactive partners of *LRP5* respectively.

Mesoderm development LRP chaperone (*MESD*) gene encodes *MESD* protein which acts as a molecular chaperon of *LRP5 and LRP6* [36]. It is ranked as the 10th interacting partner of *LRP5* as mentioned by GeneMania.

Ral guanine nucleotide dissociation stimulator like (*RGL*) genes (*RGL1, RGL 2* and *RGL 3*) encodes related proteins Rgl-1, Rgl-2 and Rgl-3 respectively. These proteins probably act as Guanine nucleotide exchange factor (*GEF*) [37]. Over expression of *RGL2* inhibits the protein degradation of β-catenin [38]. *RGL1, RGL2* and *RGL3* are ranked as 20th, 14th and 13th respectively.

Low-density lipoprotein receptor-related (LDL) 6 gene encoded protein *LRP6* is a component of the Wnt-Fzd-LRP5-LRP6 complex that triggers beta-catenin signaling through inducing aggregation of receptor-ligand complexes into ribosome-sized signalosomes. This coreceptor of Wnt plays a pivotal role in bone formation [39]. *LRP6* is at the 16th position specified by GeneMania in respect to interact with *LRP5*.

LDL receptor related protein 5 like (*LRP5L*) encodes Low-density lipoprotein receptor-related protein 5-like protein [40]. Rank 17 is the interaction rank as specified by GeneMania for *LRP5L* interaction with *LRP5*.

3.2. miRNAs Targeting LRP5 and Its Interacting Partners

Data of 22 specific miRNAs targeting *LRP5* were retrieved using miRDB database (Table 2). All the 22 miRNAs bind to the 214 bp sequence of 3' UTR region of *LRP5*. Interestingly several of these miRNAs also target some of the major genes interacting with *LRP5* (Table 3).

The 22 bp long miRNA hsa-miR-340-5p is synthesized from a region of chromosome 5 and targets a 7 bp region at the 178th nucleotide of the 3' UTR of *LRP5*. This miRNA targets *CAPRIN2* and *LRP6* among *LRP5* interacting network. hsa-miR-605-5p is a 23 bp miRNA synthesized from a region of chromosome 10 and targets a 7 bp region at the 101th nucleotide of the 3' UTR of *LRP5*. hsa-miR-524-5p which is 22 bp, synthesized from a region of chromosome 19 and binds with two 7 bp regions at the 158th and 192nd nucleotide of the 3' UTR of *LRP5* respectively. This miRNA also targets *DKK2* and *LRP6* along with *LRP5*. hsa-miR-520d-5p being 20 bp, synthesized from chromosome 19 and pairs with two 7 bp region at the 158th and 192nd nucleotide of the 3' UTR of *LRP5* respectively. Along with *LRP5*, this miRNA also targets *FZD10, DKK2* and *LRP6*. hsa-miR-4668-3p is synthesized from a region of chromosome 9, this 23 bp miRNA targets a 8 bp region at the 102nd nucleotide of the 3' UTR of *LRP5*. It also targets LRP6. The 24 bp miRNA hsa-miR-1277-5p is synthesized from chromosome X and targets a 7 bp region at the 68th nucleotide of the 3' UTR of *LRP5*. It also targets *DKK2* and *LRP6* of the *LRP5* interacting network. hsa-miR-let-7c-3p has a length of 22 bp and is synthesized from a region of chromosome 21 and targets a 8 bp region at the 160th nucleotide of the 3' UTR of *LRP5*. This miRNA not only targets *LRP5* but also 5 other major genes among *LRP5* network viz. *DKK1, FZD4, FZD1, LRP6* and *WNT5A*. The 22 bp hsa-miR-23c is synthesized from chromosome X and targets an 8 bp region at the 121st nucleotide of the 3' UTR of *LRP5*. The 21 bp hsa-miR-23a-3p is synthesized from a region of chromosome 19 and targets an 8 bp region

at the 121st nucleotide of the 3' UTR of *LRP5*. The 23 bp hsa-miR-23b-3p is synthesized from a region of chromosome 9 and targets an 8 bp region at the 121st nucleotide of the 3' UTR of *LRP5*. The 21 bp hsa-miR-1206 is synthesized from a region of chromosome 8 and targets a 7 bp region at the 52nd nucleotide of the 3' UTR of *LRP5*. *APCDD1*, a predicted partner of *LRP5* is also targeted by this miRNA. The 22 bp hsa-miR-6853-3p is synthesized from a region of chromosome 9 and targets a 7 bp region at the 52nd nucleotide of the 3′ UTR of *LRP5*. Besides *LRP5*, *FZD8* is also a candidate of this miRNA. The 22 bp hsa-miR-551b-5p is synthesized from a region of chromosome 3 and targets a 7 bp region at the 78th nucleotide of the 3' UTR of *LRP5*. It also targets *LRP6*. The 17 bp hsa-miR-1279 is synthesized from a region of chromosome 12 and targets a 7 bp region at the 49th nucleotide of the 3' UTR of *LRP5*. The 21 bp hsa-miR-6844 is synthesized from a region of chromosome 8 and targets an 8 bp region at the 56th nucleotide of the 3' UTR of *LRP5*. This miRNA also targets *FZD4*. The 22 bp hsa-miR-5007-3p is synthesized from a region of chromosome 13 and targets a 7 bp region at the 50th nucleotide of the 3' UTR of *LRP5*. It can also target *SOST*. The 22 bp hsa-miR-4733-5p is synthesized from a region of chromosome 17 and targets a 7 bp region at the 99th nucleotide of the 3' UTR of *LRP5*. *DKK1* is also a target of this miRNA. The 21 bp hsa-miR-7151-3p is synthesized from a region of chromosome 10 and targets a 7 bp region at the 33th nucleotide of the 3' UTR of *LRP5*. The 22 bp hsa-miR-3163 is synthesized from a region of chromosome 11 and targets a 7 bp region at the 72th nucleotide of the 3' UTR of *LRP5*. *DKK2*, *WNT5A*, *FZD4*, *FZD10* and *LRP6* are also the target of this miRNA along with *LRP5*. The 22 bp hsa-miR-629-3p is synthesized from a region of chromosome 15 and targets two 7 bp regions at the 151th and 169th nucleotide of the 3' UTR of *LRP5*. Two more candidate of this particular miRNA is *DKK2* and *MESD*. The 22 bp hsa-miR-181a-2-3p is synthesized from a region of chromosome 9 and targets a 7 bp region at the 165th nucleotide of the 3' UTR of *LRP5*. The 23 bp hsa-miR-324-3p is synthesized from a region of chromosome 17 and targets a 7 bp region at the 165th nucleotide of the 3' UTR of *LRP5*. This also targets *FZD4* which is one among the major genes interacting with *LRP5*. The miRNAs targeting each of the predictive partners of *LRP5* are enlisted comprehensively in Table 3.

3.3. Validation of miRNA-LRP5 Interaction by Other Software

In this study, we collect responsible miRNAs from miRDB database individually for *LRP5*. After that, common responsible miRNAs were collected by using Venny online tools. The miRNAs specified by miRDB regulating *LRP5* were reconfirmed by other software. Among 22 derivatives of miRDB, TargetScan validated 21, RegenDbase confirmed 3, MirTargetLink 2.0 confirmed only one miRNA targeting *LRP5* (Table 4). These proportions of miRNAs confirmed by different software are graphically represented by a Venn diagram (Figure 2). It shows that 22 miRNAs predicted by miRDB to be 100%. Accordingly, targetScan confirms 95.4%, RegenDbase confirms 13.6%, MirTargetLink 2.0 confirms 4.5% of total miRNAs predicted by miRDB targeting *LRP5*. All the four software confirm only one (4.5%) miRNA *viz*. hsa-miR-23a-3p. Three software i.e., miRDB, TargetScan and RegenDbase commonly confirm two miRNAs (9.1%) *viz* hsa-miR-524-5p and hsa-miR-3163. Two software i.e., miRDB and TargetScan confirm eighteen miRNAs (81.8%) *viz*. hsa-miR-340-5p, hsa-miR-605-5p, hsa-miR-520d-5p, hsa-miR-4668-3p, hsa-miR-1277-5p, hsa-let-7c-3p, hsa-miR-23c, hsa-miR-23b-3p, hsa-miR-1206, hsa-miR-6853-3p, hsa-miR-551b-5p, hsa-miR-1279, hsa-miR-6844, hsa-miR-5007-3p, hsa-miR-4733-5p, hsa-miR-7151-3p, hsa-miR-629-3p, hsa-miR-181a-2-3p. One miRNA *viz*.hsa-miR-324-3p is confirmed only by miRDB.

Table 1. Types of interactions of *LRP5* with other related genes of its network predicted by GeneMANIA

Category	Genes
Physical Interactions	*SOST, CAPRIN2, DKK1, APCDD1, DKK4, AXIN1, , FZD4, FZD1, WNT1, DKK2, LRP6, WNT5A, FZD8*
Co-expression	*SOST, CAPRIN2, DKK1, APCDD1, FZD10, FZD4, FZD1, WNT1, RGL3, RGL2, DKK2, LRP6, LRP5L, WNT5A, RGL1*
Predicted	*SOST, DKK1, FZD10, AXIN1, WNT7B, FZD4, MESD, FZD1, WNT1, RGL3, RGL2, LRP6, WNT5A, FZD8, RGL1*
Co-localization	*CAPRIN2, DKK4*
Genetic Interactions	*CAPRIN2, APCDD1, AXIN1, FZD10, FZD4, FZD1, RGL2, DKK2, LRP6, WNT5A, FZD8, RGL1*
Pathway	*DKK1, FZD10, DKK4, AXIN1, WNT7B, FZD4, FZD1, WNT1, DKK2, LRP6, WNT5A, FZD8*
Shared protein domains	*DKK1, FZD10, DKK4, WNT7B, FZD4, FZD1, WNT1, RGL3, RGL2, DKK2, LRP6, LRP5L, WNT5A, FZD8, RGL1*

Table 2. List of miRNAs prdicted by online tool miRDB to target *LRP5* transcript

Precursor miRNA		Mature miRNA				Target Gene specifications		
miRBase ID	**Precursor name**	**miRBase ID**	**miRNA name**	**Sequence**	**Length**	**Genomic Location**	**Seed sequence**	**Seed Location**
MI0000802	hsa-mir-340	MIMAT0004692	hsa-miR-340-5p	5' - uuauaaagcaaugagacugauu - 3'	22	chr5:180015303-180015397 (-)	TTTATAA	178
MI0003618	hsa-mir-605	MIMAT0003273	hsa-miR-605-5p	5' - uaaaucccauggugccuucuccu - 3'	23	chr10:51299573-51299655 (+)	GGGATTT	101
MI0003160	hsa-mir-524	MIMAT0002849	hsa-miR-524-5p	5' - cuacaaagggaagcacuuucuc - 3'	22	chr19:53711002-53711088 (+)	CTT TGTA, TTTGTAA	158, 192
MI0003164	hsa-mir-520d	MIMAT0002855	hsa-miR-520d-5p	5' - cuacaaagggaagcccuuuc - 3'	20	chr19:53720096-53720182 (+)	CTT TGTA, TTTGTAA	158, 192
MI0017298	hsa-mir-4668	MIMAT0019746	hsa-miR-4668-3p	5' - gaaaauccuuuuuguuuuuccag - 3'	23	chr9:111932100-111932169 (+)	GGATTTTA	102
MI0006419	hsa-mir-1277	MIMAT0022724	hsa-miR-1277-5p	5' - aaauauauauauauauguacguau - 3'	24	chrX:118386394-118386471 (+)	ATA TATT	68
MI0000064	hsa-let-7c	MIMAT0026472	hsa-let-7c-3p	5' - cuguacaaccuucuagcuuucc - 3'	22	chr21:16539828-16539911 (+)	TTGTACA	160
MI0016010	hsa-mir-23c	MIMAT0018000	hsa-miR-23c	5' - aucacauugccagugauuaccc - 3'	22	chrX:20017088-20017187 (-)	AATGTGAA	121
MI0000079	hsa-mir-23a	MIMAT0000078	hsa-miR-23a-3p	5' - aucacauugccagggauuucc - 3'	21	chr19:13836587-13836659 (-)	AATGTGAA	121
MI0000439	hsa-mir-23b	MIMAT0000418	hsa-miR-23b-3p	5' - aucacauugccagggauuaccac - 3'	23	chr9:95085208-95085304 (+)	AATGTGAA	121
MI0006339	hsa-mir-1206	MIMAT0005870	hsa-miR-1206	5' - uguucauguagauguuuaagc - 3'	21	chr8:128008898-128008956 (+)	ATGAACA	52
MI0022699	hsa-mir-6853	MIMAT0027607	hsa-miR-6853-3p	5' - uguucauuggaacccugcgcag - 3'	22	chr9:35732922-35732995 (+)	ATGAACA	52
MI0003575	hsa-mir-551b	MIMAT0004794	hsa-miR-551b-5p	5' - gaaaucaagcgugggugagacc - 3'	22	chr3:168551854-168551949 (+)	TGA TTTA	78
MI0006426	hsa-mir-1279	MIMAT0005937	hsa-miR-1279	5' - ucauauugcuucuuucu – 3	17	chr12:69273157-69273218 (-)	AA TATGA	49
MI0022690	hsa-mir-6844	MIMAT0027589	hsa-miR-6844	5' - uucuuuguuuuuaauucacag - 3'	21	chr8:124508515-124508576 (-)	ACAAAGAA	56
MI0017874	hsa-mir-5007	MIMAT0021036	hsa-miR-5007-3p	5' - aucauaugaaccaaacucuaau - 3'	22	chr13:55174454-55174548 (+)	ATATGAA	50
MI0017370	hsa-mir-4733	MIMAT0019857	hsa-miR-4733-5p	5' - aaucccaaugcuagacccggug - 3'	22	chr17:31094350-31094425 (-)	TTGGGAT	99
MI0023611	hsa-mir-7151	MIMAT0028213	hsa-miR-7151-3p	5' - cuacaggcuggaaugggcuca - 3'	21	chr10:67403351-67403410 (-)	CCTGTAA	33
MI0014193	hsa-mir-3163	MIMAT0015037	hsa-miR-3163	5' - uauaaaaugagggcaguaagac - 3'	22	chr11:66934434-66934506 (-)	ATTTTAT	72
MI0003643	hsa-mir-629	MIMAT0003298	hsa-miR-629-3p	5' - guucucccaacguaagcccagc - 3'	22	chr15:70079372-70079468 (-)	GGGAGAA, GGAGAAA	151, 169
MI0000269	hsa-mir-181a-2	MIMAT0004558	hsa-miR-181a-2-3p	5' - accacugaccguugacuguacc - 3'	22	chr9:124692442-124692551 (+)	CAGTGGA	165
MI0000813	hsa-mir-324	MIMAT0000762	hsa-miR-324-3p	5' - cccacugccccaggugcugcugg - 3'	23	chr17:7223297-7223379 (-)	CAGTGGA	165

Table 3. miRNAs targeting other genes of *LRP5* network

miRNA targeting LRP5	Genes associated with and along with LRP5
hsa-miR-340-5p	*CAPRIN2, LRP6*
hsa-miR-605-5p	Nil
hsa-miR-524-5p	*DKK2, LRP6*
hsa-miR-520d-5p	*FZD10, DKK2, LRP6*
hsa-miR-4668-3p	*LRP6*
hsa-miR-1277-5p	*DKK2, LRP6*
hsa-let-7c-3p	*DKK1, FZD4, FZD1, LRP6, WNT5A*
hsa-miR-23c	Nil
hsa-miR-23a-3p	Nil
hsa-miR-23b-3p	Nil
hsa-miR-1206	*APCDD1*
hsa-miR-6853-3p	*FZD8*
hsa-miR-551b-5p	*LRP6*
hsa-miR-1279	Nil
hsa-miR-6844	*FZD4*
hsa-miR-5007-3p	*SOST*
hsa-miR-4733-5p	*DKK1*
hsa-miR-7151-3p	Nil
hsa-miR-3163	*DKK2, WNT5A, FZD4, FZD10, LRP6*
hsa-miR-629-3p	*DKK2, MESD*
hsa-miR-181a-2-3p	Nil
hsa-miR-324-3p	*FZD4*

3.4. Functional Relevance of the miRNAs Targeting LRP5

A total of 20 studies were identified in the systematic review indicating important roles of the miRNAs enlisted in this work in osteoporosis, low BMD conditions and other bone related diseases. These studies depicted an altered expression level of 10 miRNAs from our list (Table 5) in bone related abnormalities. One miRNA hsa-miR-3163 has been reported [41] to regulate oncogenic Wnt/β-catenin pathway which is also highly associated with bone development.

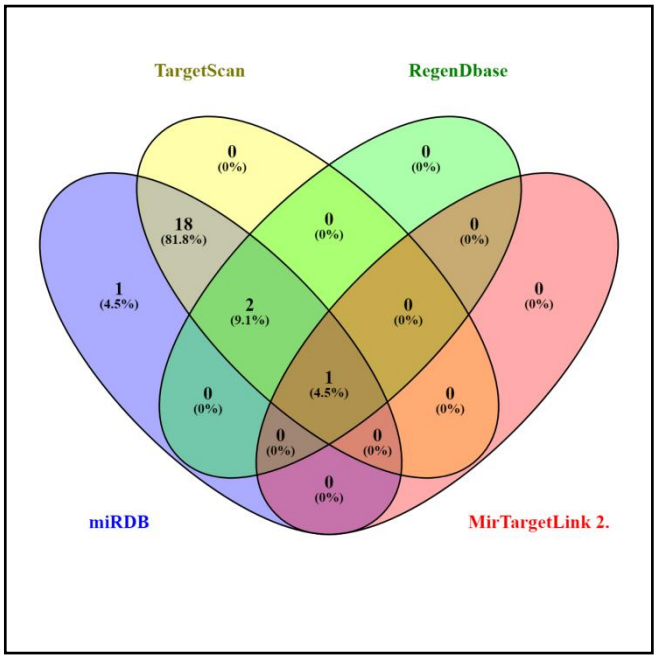

Figure 2. Venn diagrams created by using Venny 2.1 graphically representing the data retrieved from the entire four target predicting software tools

Table 4. A comprehensive data from four different software

miRDB	TargetScan	RegenDbase	miRTargetlink2
hsa-miR-340-5p	hsa-miR-340-5p	Nil	Nil
hsa-miR-605-5p	hsa-miR-605-5p	Nil	Nil
hsa-miR-524-5p	hsa-miR-524-5p	hsa-miR-524-5p	Nil
hsa-miR-520d-5p	hsa-miR-520d-5p	Nil	Nil
hsa-miR-4668-3p	hsa-miR-4668-3p	Nil	Nil
hsa-miR-1277-5p	hsa-miR-1277-5p	Nil	Nil
hsa-let-7c-3p	hsa-let-7c-3p	Nil	Nil
hsa-miR-23c	hsa-miR-23c	Nil	Nil
hsa-miR-23a-3p	hsa-miR-23a-3p	hsa-miR-23a-3p	hsa-miR-23a-3p
hsa-miR-23b-3p	hsa-miR-23b-3p	Nil	Nil
hsa-miR-1206	hsa-miR-1206	Nil	Nil
hsa-miR-6853-3p	hsa-miR-6853-3p	Nil	Nil
hsa-miR-551b-5p	hsa-miR-551b-5p	Nil	Nil
hsa-miR-1279	hsa-miR-1279	Nil	Nil
hsa-miR-6844	hsa-miR-6844	Nil	Nil
hsa-miR-5007-3p	hsa-miR-5007-3p	Nil	Nil
hsa-miR-4733-5p	hsa-miR-4733-5p	Nil	Nil
hsa-miR-7151-3p	hsa-miR-7151-3p	Nil	Nil
hsa-miR-3163	hsa-miR-3163	hsa-miR-3163	Nil
hsa-miR-629-3p	hsa-miR-629-3p	Nil	Nil
hsa-miR-181a-2-3p	hsa-miR-181a-2-3p	Nil	Nil
hsa-miR-324-3p	Nil	Nil	Nil

Table 5. Scientific reports investigating expression alterations of miRNAs associated with bone diseases and other related diseases

miRNA	Expression (up/ down regulated)	Related Disease	Author
hsa-miR-340-5p	Up-regulation	Osteoporosis	Lu et al., 2023 Fang et al., 2013
hsa-let-7c-3p	Up-regulation	Osteoporosis	Zhou et al., 2019
hsa-miR-23a-3p	Up-regulation	Osteoporosis	Kelch et al., 2017 Li et al. 2016 Seeliger et al., 2014
hsa-miR-23b-3p	Up-regulation	Osteoporosis	Garg et al. 2022; Wu et al., 2021; Ramírez-Salazar et al., 2018
hsa-miR-324-3p	Down-regulation	Osteoporosis Osteosarcoma	Feichtinger et al., 2018 Weng et al., 2023
hsa-miR-524-5p	Up-regulation	Osteosarcoma	Zhuang et al., 2018 Zhou et al., 2021
hsa-miR-520d-5p	Down-regulation	Chondrogenesis	Lu et al., 2020
hsa-miR-1277-5p	Down-regulation	Osteoarthritis	Guo et al., 2021 Wang et al., 2019
hsa-miR-3163	Down-regulation	Wnt/β-catenin pathway regulation	Liu et. al., 2021
hsa-miR-629-3p	Up-regulation	Osteosarcoma	Wang et al., 2022
hsa-miR-181a-2-3p	Down-regulation	Osteoarthiritis Ossification	Tu et al., 2016 Liu et al., 2020

4. Discussion

The role of LRP5 in bone metabolism is well established through scientific studies. Mutations, low expression, and many other variations in this gene are correlated with the development of bone related diseases [12]. Eukaryotic genes are regulated post transcriptionally by miRNAs [42]. The regulation of any gene by miRNAs is resulted through a complex regulatory network involving many other genes and factors [43].

Like other genes, LRP5 also functions through a network of associated genes. We have identified twenty genes interacting with LRP5 by various means. Interestingly, many of the miRNAs targeting LRP5 also target its (LRP5) interacting partners. This supports the idea of the existence of a miRNA-LRP5 regulatory network yet to be explored by scientific community.

The list of miRNAs provided by miRDB is validated with other software also. The very particular hsa-miR-23a-3p confirmed by all the software has been reported to be up-regulated in both male and female osteoporotic patients [44]. Over expression of microRNA-23a targeting LRP5 prevents osteogenic differentiation in human bone marrow-derived mesenchymal stem cells (hBMSCs) and down regulation of the same miRNA enhanced the process of osteogenic differentiation of hBMSCs [45]. This miRNA has also been reported to be a diagnostic and prognostic marker of osteopororsis [46]. Elevated serum level of miR-340-5p is observed osteoporotic postmenopausal women. Circulating miR-340-5p has been proposed as an osteo-miRNAs in postmenopausal women and potential biomarker of osteoporosis in postmenopausal women [47]. It has also been found to prevent Wnt/β-Catenin signaling pathway by decreasing β-catenin protein levels by 2- to 5-fold [48]. In 2019, Zhou et al. [49] reported that upregulated miRNA let-7c inhibits Wnt/β-catenin signaling and in postmenopausal osteoporotic patients. Up-regulated miR-23b-3p is associated with osteoporotic hip fractures and suggested as the risk factor for osteoporosis by [50]. It has also been proposed as the diagnostic marker for human osteoporosis and fragility fracture [51]. High serum level of miR-23b-3p thus may be a key regulator of bone mineral density [52]. Interestingly down-regulation of miR-324 is correlated with osteoporortic fractures. The serum level of miR-324 is positively correlated to bone mineral density [53]. MicroRNA-324-3p is found to inhibit osteosarcoma (OS) progression. Reduced expression is identified in in OS cell lines and tissues [54]. A role of circITCH/miR-524/RASSF6 axis is reported to suppress OS progression [55]. Another report of significant up-regulation of miR-524 in OS denotes its role in promoting cell proliferation in the disease [56]. The up-regulation and down-regulation of miR-520d-5p have been reported to promote and inhibit chondrogenesis respectively, and regulate chondrocyte metabolism [57]. Down regulation of miR-1277-5p is associated with osteoarthritis development [58,59]. A role of miR-3163 is proved in activating Wnt/β-catenin pathway in pancreatic cancer. But the functional role has not yet been investigated in Wnt/β-catenin pathway related to bone metabolism. Anti-calcification effects of hsa-miR-629-3p are reported in osteogenic differentiation-induced human aortic valve

interstitial cells (hVICs) [60]. A role of miR181 family members has been depicted in chondrocyte differentiation and formation [61]. It is reported that MicroRNA-181 regulates the development of Ossification of Posterior longitudinal ligament [62].

5. Conclusions

It can be concluded that there is functional significance of predicted miRNA in regulation of LRP5. These regulatory miRNAs may influence the expression level of LRP5, a key regulator of bone mineral density and its interacting partners. This altered expression in LRP5 network may hinder the down signaling cascade of WNT-β catenin pathway. This ultimately may affect the bone metabolism and develop into osteopororsis. There are reports of regulatory functions of about 11 miRNAs from our list. The rest 11 need more concern of the scientific world for finding out their plausible role(s) in bone metabolism and osteoporosis consequently.

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