SNP regulation of miRNA Expression and its association with Osteoporosis

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Introducction

miRNAs have been extensively studied in bone research, particularly their relationship to osteoporosis (De-Ugarte et al, BMC Med Genomics, 2015; Seeliger et al, J Bone Min Research, 2014) . However, the miRNA expression signatures described in patients with osteoporosis do not provide evidence of causality because the altered pattern could be a consequence of the disease or even unrelated to the pathogenesis. Another approach in miRNAs studies is the association analysis between one SNP within a candidate miRNA (miR-SNP) or in a miRNA target site, and one disease related-outcome. In this case, the associated variant is likely involved in the pathophysiology or confers susceptibility to develop the disease (Estrada et al, Nat Genet, 2012).

Objectives

The aims of this study were (1) to identify SNPs within candidate miRNAs in order to perform an association study with bone mineral density (BMD), the main outcome used to define osteoporosis and (2) to validate in bone cells this miR-SNP association with the osteoporotic phenotype.

Methods

SNPs located in pre-miRNA sequences that (1) bind to the mRNA 3'UTR of genes related with bone metabolism or (2) found to be highly expressed in human osteoblasts were selected. Validated SNPs with a MAF>0.01 (n=5) were genotyped in the OSTEOMED2 cohort (Table 1) to assess their association with LS BMD and FN BMD (Table 3). Multivariate linear regression models were fitted to assess the association between genotyped SNPs and BMD. Potential confounders considered for adjustment were densitometer devices, body mass index (BMI) and age.
MiRNAs which harbored BMD-associated SNPs were quantified by qPCR in order to compare the expression levels between OP and non-OP bone samples (Table 2). Mann-Whitney U test was performed for OP and non-OP group comparisons.
Human primary osteoblasts were cultured for DNA and RNA extraction and sorted by genotype for both rs6430498 and rs12512664. The correlation between expression levels of mature miRNA miR-3679-3p and miR-4274 with its corresponding genotypes was analyzed using linear regression. All analyses were two-tailed, and p-values<0.05 were considered significant.

Table 1. Baseline characteristics of the OSTEOMED2 cohort.

Patient characteristic	Mean ± SD	
	LS BMD n=2183	FN BMD n=2015
Age (years)	57.61 ± 9.26	58.80 ± 8.99
Age of menopause (years)	48.7 ± 3.94	48.7 ± 3.92
BMI (kg/m^2)	26.56 ± 4.18	26.48 ± 4.13
BMD (g/cm^2)	0.870 ± 0.16	0.707 ± 0.14

Abbreviations: BMI=body mass index; BMD=bone mineral density; LS=lumbar spine; FN=femoral neck

Table 2. Patient characteristics for osteoporotic fracture and non-osteoporotic groups.

	n	Age (Mean ± SD)	BMI (kg/m^2) (Mean ± SD)	BMD (g/cm^2) (Mean ± SD)
Biological groups				
Osteoporotic	10	75.6 ± 6.38	27.11 ± 2.94	Fragility fracture
Non-osteoporotic	10	71.7 ± 7.36	27.42 ± 3.15	0.882 ± 0.137

Abbreviations: SD: Standard Deviation; BMI: Body Mass Index; BMD: Bone Mineral Density

Results

SNP rs6430498 in the miR-3679 and rs12512664 in the miR-4274 were significantly associated with FN BMD (Table 4). The A alleles for rs6430498 (minority allele) and rs12512664 (majority allele) were found to be associated with lower BMD values.

Table 4. SNPs associated with FN BMD in linear-regression analysis

SNP ID	miRNA	Genotyping efficiency (%)	HWE	Beta coefficient[a] [95% CI]	*p* value
rs6430498	miR-3679	97.96	1	-0.017 [-0.032 to -0.003]	0.021^R
rs12512664	miR-4274	98.72	0.47	0.015 [0.004 to 0.027]	0.01^R

[a]Adjusted for DXA device, age and BMI
Abbreviations: FN BMD=Femoral neck bone mineral density; HWE=Hardy-Weinberg equilibrium; R=recessive model

Both miRNAs miR-3679-3p and miR-4274 were significantly overexpressed in the OP samples (Table 5).

Table 5. miRNA expression levels, comparison between osteoporotic and non-osteoporotic bone samples

miRNA	Biological Group	RQ (Median)	IQR	*P* value
miR-3679	Osteoporotic	89.601	220.636	0.001
	Control	1.423	0.964	
miR-4274	Osteoporotic	144.268	318.409	0.001
	Control	1.197	2.154	

Abbreviations: RQ=Relative quantification; IQR=Interquartile Range

A significant correlation was observed between miRNA levels and the genetic variant (Figure 1). The A allele for both SNPs was associated with higher expression of each corresponding miRNA (miR-3674; log-additive model; p-value=0.015, and miR-4274; dominant model; p-value=0.013). Additionally, in order to corroborate that the differences among expression levels are due to genotypes "per se" and not for other cellular circumstances, another bone-related miRNAs were checked in these cells and no differences in expression were found irrespective of genotypes.

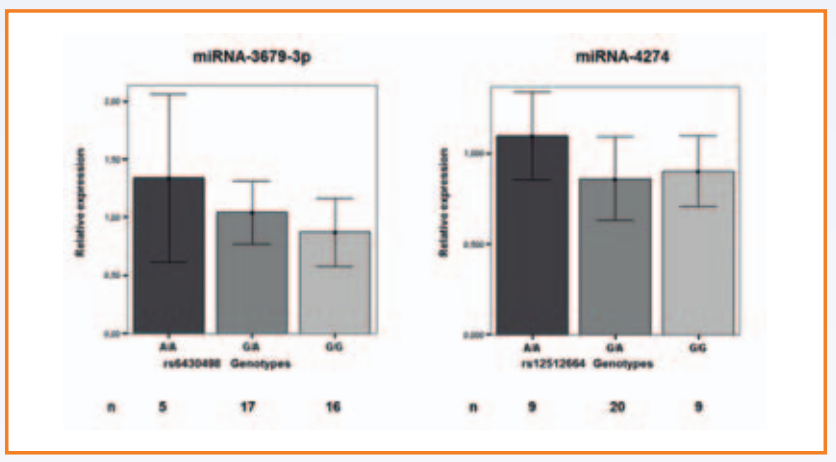

Figure 1. Correlation between miRNA expression levels and genotypes for miR-3679 and miR-4274. MiRNA expression levels are represented as a mean±SD of the relative expression in Real-Time PCR. U6 was used for normalization. Samples of 38 human primary osteoblasts were used for experiments. (n) is the number of samples for each genotype group.

Table 3. Validation of miR-SNPs for the BMD association analysis.

TARGET GENE	miRNA	SNP	MAFB
ESR1	miR-106b	rs72631827	not polym.
	miR-130b	rs72631822	not polym.
	miR-148b	rs74878365	not polym.
	miR-18a	rs41275866	not polym.
	miR-222	rs72631825	not polym.
	miR-373	rs80338016	not polym.
	miR-520c	rs7255628	not polym.
	miR-93	rs72631824	not polym.
	miR-96	rs41274239	0.0033
		rs73159662	0.0058
TGFB2	miR-141	rs34385807	not polym.
	miR-149	rs71428439	not polym.
	miR-182	rs77586312	not polym.
		rs75953509	not polym.
		rs80041074	0.0033
	miR-199b	rs72631835	not polym.
	miR-193a	rs60406007	not polym.
	miR-200b	rs72563729	not polym.
	miR-33a	rs77809319	not polym.
	miR-431	rs76090066	0.00083
		rs128840'05	not polym.
	miR-590	rs6971711	not polym.
	miR-7-1	rs76662330	not polym.
	miR-7-2	rs41276930	0.005
		rs75737367	not polym.
PTH1R	miR-339	rs13232101	not polym.
		rs72631820	not polym.
		rs72631831	not polym.
RUNX2	miR-122	rs41292412	0.0033
	miR-154	rs41286570	0.0004
IL6R	miR-124-2	rs72631829	not polym.
	miR-124-3	rs34059726	not polym.
	miR-125a	rs12975333	not polym.
	miR-140	rs7205289	not polym.
	miR-320d-1	rs74826059	not polym.
	miR-499	**rs3746444**	**0.21**
		rs7267163	0.0025
LRP5	**miR-27a**	**rs11671784**	**0.0162**
IL6	**miR-146a**	**rs2910164**	**0.26**
	miR-146b	rs76149940	not polym.
	miR-202	rs12355840	not polym.
	miR-365-2	rs35143473	not polym.
VDR	miR-10a	rs72631828	not polym.
	miR-223	rs34952329	not polym.
CYP24A1	miR-30b	rs111424617	not polym.
	miR-30e	rs112439044	not polym.
	miR-183	rs72631833	not polym.
		rs41281222	not polym.
	miR-101-2	rs78851134	0.0004
Highly expressed in HObs	miR-1282	rs11269	not polym.
	miR-3679	**rs6430498**	**0.35**
		rs10175383	not polym.
	miR-4274	**rs12512664**	**0.47**

MAFB; Minor allele frequency in BARCOS cohort
In bold; Validated SNPs for genotyping in total OSTEOMED2 cohort

Conclusions

- Genetic variants in miR-3679 and miR-4274 are associated with femoral neck bone mineral density. In both cases, alleles associated with lower BMD correlated with higher expression levels of respective mature miRNAs in human osteoblastic cells which were found overexpressed in fractured bone samples.
- Our results open new exploratory avenues for future studies in the bone field and the treatment of osteoporosis.

References

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Disclosures

The authors state that they have no conflict of interest.

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