

SUPPLEMENTARY MATERIALS

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“Expression analysis of microRNA and lncRNA in visceral adipose tissue of obese and non-obese individuals”

Supplementary Material 1

Table 1. Percentage of participants using different kind of medications separated by groups with abdominal obesity (AO+) and without abdominal obesity (AO–)

Medication	AO+ (%)	AO– (%)
Angiotensin converting enzyme inhibitors	42.9	16.1
Diuretics	15.7	3.2
Beta-Blockers	20	9.7
Calcium channel blockers	11.4	0
Platelet aggregation inhibitor	11.4	3.2
Others	15.7	3.2

Table 2. Primers used for lncRNA detection and quantification

LnRNA	Direct primer	Reverse primer
GAPDH	TGCACCACCAACTGCTTAGC ^[53]	GGCATGGACTGTGGTCATGAG ^[53]
GAS5 ENST00000702964.1	GACAGTTGTGTCCCAAGGA	TGTGTGCCAATGGCTTGAGT
P5549 ENST00000560705.1	GGCCTTGAGGAGGCAAGTAA	TCAGATGTGTCGCTTCTCGG
P5549 ENST00000559600.1	CCCACACAGCACGATGAGAT	GAAGCCAGTATTTCCAGCTAAT
p19461 ENST00000558120.5	ATGCAAGGGAGATGCATTGGT	GGATTTCACCCAGTGTTTCC
p19461 ENST00000663878.1	ATGCAAGGGAGATGCATTGGT	AGGTGAGCTGGTTCCTCTCTGA
p19461 ENST00000664388.1	TTCCTGAGAGCCTAGGGACC	ACGACAAGGCCCAAGGAAAA
p19461 ENST00000664536.1	ATGCAAGGGAGATGCATTGGT	AGGTGAGCTGGTTCCTCTCTGA
p19461 ENST00000666463.1	ATGCAAGGGAGATGCATTGGT	AGGGTACTCCTGCTGCTCAA
SNGH9 ENST00000531523.3	GGGAATCCACCCGAAGAGTG	TGGGAGGACCAGTGTCTCTAA
SNGH9 ENST00000564014.1	GGGAATCCACCCGAAGAGTG	ATTTTCAACCAACGCCGGTC
ASMER1 ENST00000625278.2	AAGTAGCACCATGTGGCAGAA	GAAAGGGTGTGTTCTCGTGG
ASMER1 ENST00000630211.2	ACCAGCCTCTCCTACTGACT	GAAAGGGTGTGTTCTCGTGG
ASMER1 ENST00000418954.2	CCCAGACTCCAGAACTACTCC	TTGGGGAAACTCAGCTCTCTT
ASMER1 ENST00000412426.5	CCCAGACTCCAGAACTACTCC	TTGGGGAAACTCAGCTCTCTT

Supplementary Material 1 (continued)

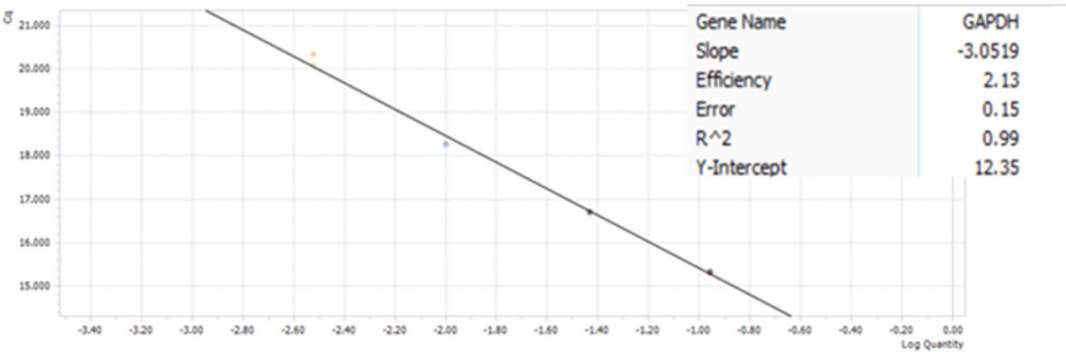


Fig. 1. Standard curve indicating efficiency of primer set used for quantification of reference gene GAPDH expression levels.

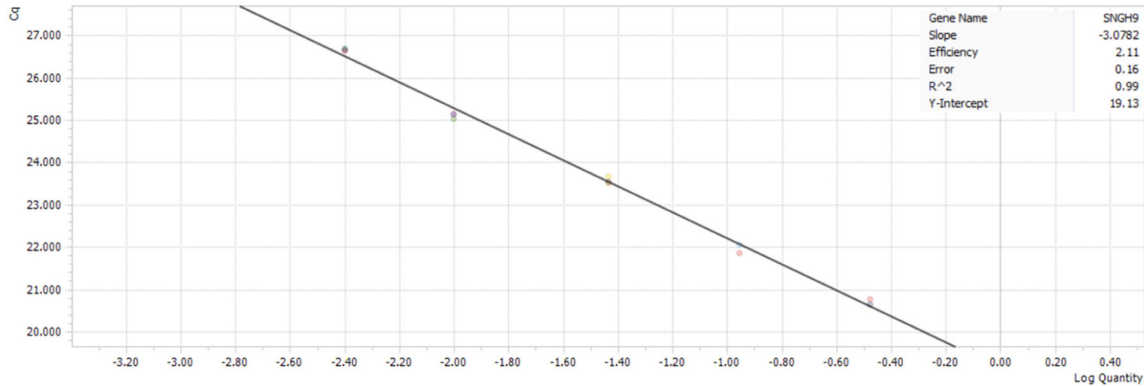


Fig. 2. Standard curve indicating efficiency of primer set used for quantification of SNGH9 lncRNA expression levels.

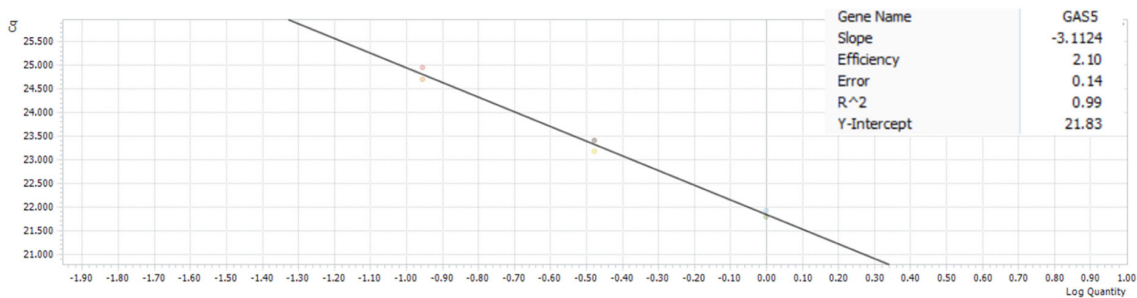


Fig. 3. Standard curve indicating efficiency of primer set used for quantification of GAS5 lncRNA expression levels.

Supplementary Material 1 (end)

Table 3. Primers used for miRNA reverse transcription and quantification

Stem loop primer	hsa-miR-221	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGACTGGATACGACGAAACC
Stem loop primer	hsa-miR-222	GTGCAGGGTCCGAGGTCTAGAGCCACCTGGGCAATTTTTCCTTACCCAG
Stem loop primer	hsa-miR-26a	GTGCAGGGTCCGAGGTCTAGAGCCACCTGGGCAATTTTTCCTTACCCCTA
Stem loop primer	hsa-miR-155	GTGCAGGGTCCGAGGTCTAGAGCCACCTGGGCAATTTTTCCTTAAACCC
Stem loop primer	hsa-miR-103a	GTGCAGGGTCCGAGGTCTAGAGCCACCTGGGCAATTTTTCCTTCATAGC ^[55]
Specific primers	Direct	Reverse
hsa-miR-221	AACACGCAGCTACATTGTCTG	GTCGTATCCAGTGCAGGGT
hsa-miR-222	TTCGGAGCTACATCTGGCTA	CAGTGCAGGGTCCGAGGT ^[55]
hsa-miR-26a	TTCGGTTCAAGTAATCCAGGA	CAGTGCAGGGTCCGAGGT ^[55]
hsa-miR-155	AAGCGACCTTAATGCTAATCGTGA	CAGTGCAGGGTCCGAGGT ^[55]
hsa-miR-103a	GGAGCAGCATTGTACAGGG ^[55]	CAGTGCAGGGTCCGAGGT ^[55]

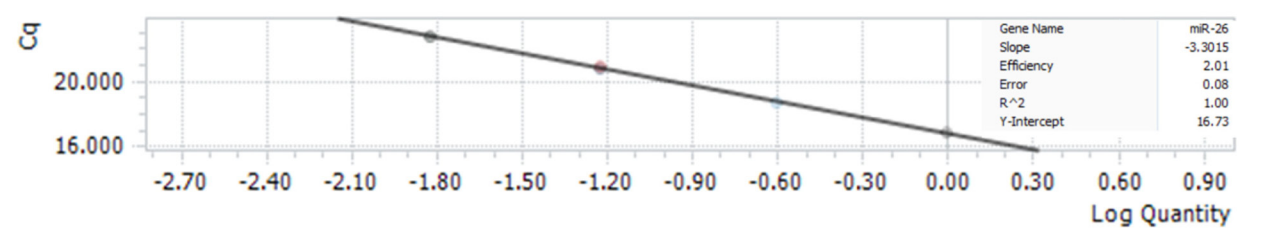


Fig. 4. Standard curve indicating efficiency of primer set used for quantification of miRNA-26A expression levels.

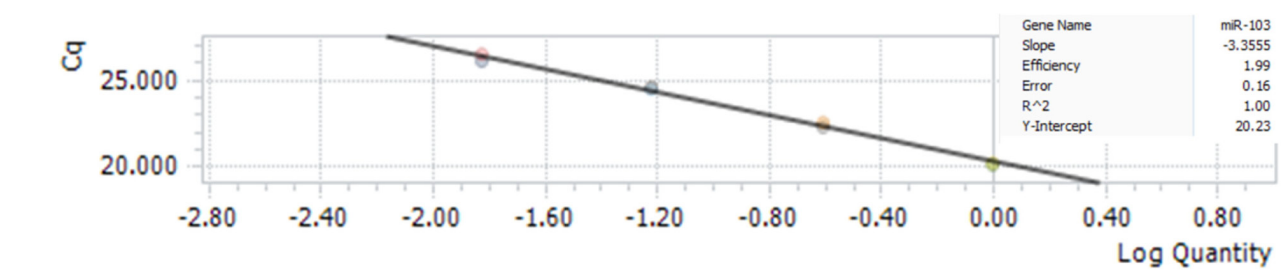


Fig. 5. Standard curve indicating efficiency of primer set used for quantification of reference miRNA-103 expression levels.

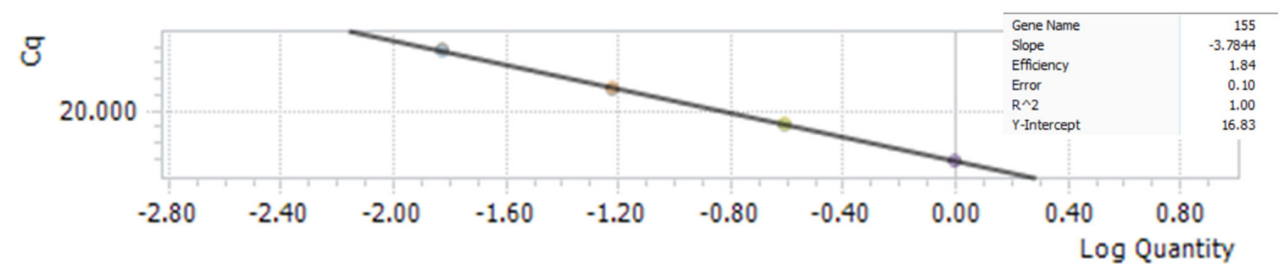


Fig. 6. Standard curve indicating efficiency of primer set used for quantification of miRNA-155 expression levels.

Supplementary Material 2

Table 1. Correlation of lncRNA expression with various clinical and biochemical parameters

RNA		BMI	Waist circumference, cm	Glucose in blood	Cholesterol in blood	Triglycerides	LDL-C	HDL-C
		mmol/L						
AO+								
SNHG9	PC	0.255*	0.010	0.035	0.086	−0.028	0.096	0.071
	P (2-tailed)	0.042	0.935	0.783	0.498	0.826	0.452	0.576
GAS5	PC	0.218	−0.059	0.233	0.154	−0.061	0.136	0.135
	P (2-tailed)	0.074	0.632	0.056	0.210	0.623	0.269	0.272
AO−								
SNHG9	PC	−0.030	0.087	−0.070	−0.176	0.064	−0.005	−0.201
	P (2-tailed)	0.889	0.686	0.755	0.411	0.767	0.982	0.346
GAS5	PC	−0.177	−0.155	−0.030	−0.131	0.113	−0.024	−0.150
	P (2-tailed)	0.358	0.423	0.877	0.497	0.561	0.900	0.437

Table 2. Correlation of miRNA with various clinical and biochemical parameters

RNA		BMI	Waist circumference, cm	Glucose in blood	Cholesterol in blood	Triglycerides	LDL-C	HDL-C
		mmol/L						
AO+								
miR-26A	PC	0.008	0.030	−0.309*	−0.042	−0.081	−0.044	0.006
	P (2-tailed)	0.951	0.816	0.014	0.748	0.533	0.736	0.961
miR-155	PC	0.015	0.224	0.109	−0.239	−0.024	−0.325**	−0.122
	P (2-tailed)	0.905	0.073	0.389	0.056	0.848	0.008	0.334
AO−								
miR-26A	PC	−0.326	−0.224	0.145	−0.121	−0.260	0.025	−0.115
	P (2-tailed)	0.112	0.283	0.490	0.563	0.210	0.905	0.585
miR-155	PC	−0.179	−0.208	0.342	0.390	0.076	−0.016	0.430*
	P (2-tailed)	0.391	0.318	0.094	0.054	0.720	0.939	0.032

Table 3. Expression levels of miRNAs and lncRNAs in studied groups described as (Mean Standard ± error of the mean) *p*-value for comparison between AO+/AO− and T2D+/T2D− is presented

Transcript symbol	AO+	AO−	<i>p</i>	T2D+	T2D−	<i>p</i>
miRNA-155	0.55 ± 0.08	0.49 ± 0.1	0.83	0.69 ± 0.26	0.55 ± 0.08	0.55
miRNA-26A	7.76 ± 0.48	11.23 ± 1.02	~0.001**	7.40 ± 0.95	7.80 ± 0.54	0.78
lncRNA SNHG9	0.52 ± 0.09	0.41 ± 0.07	0.48	0.66 ± 0.24	0.50 ± 0.09	0.57
lncRNA GAS5	1.78 ± 0.29	1.79 ± 0.41	0.45	1.55 ± 1.55	4.49 ± 0.22	~0.001**

Supplementary Material 2 (continued)

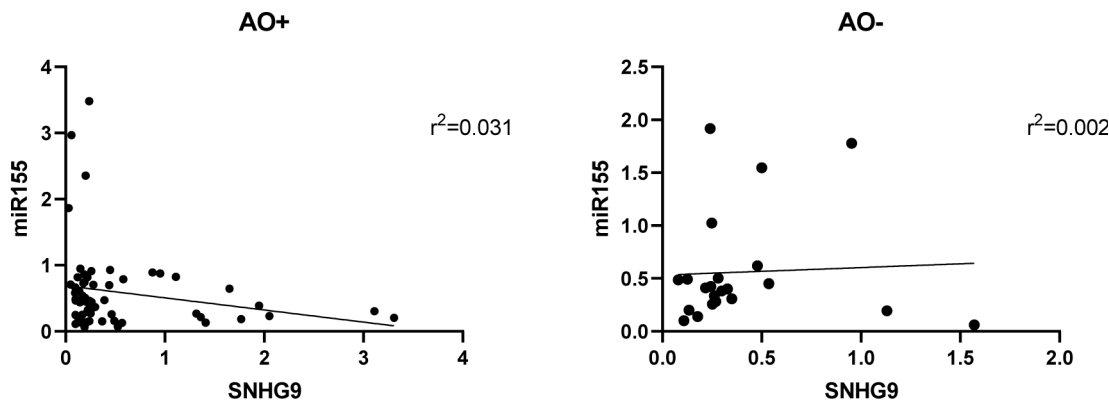


Fig. 1. Scatter plot Graph showing the correlation between expression levels of SNHG9 and miRNA-155 in obese individuals (AO+) and individuals without abdominal obesity (AO-).

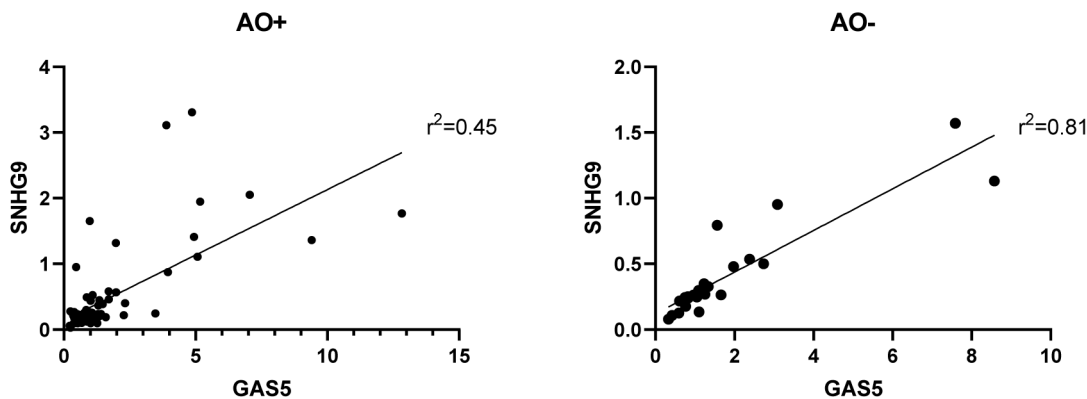


Fig. 2. Scatter plot Graph showing the correlation between expression levels of GAS5 and SNHG9 in obese individuals (AO+) and individuals without abdominal obesity (AO-).

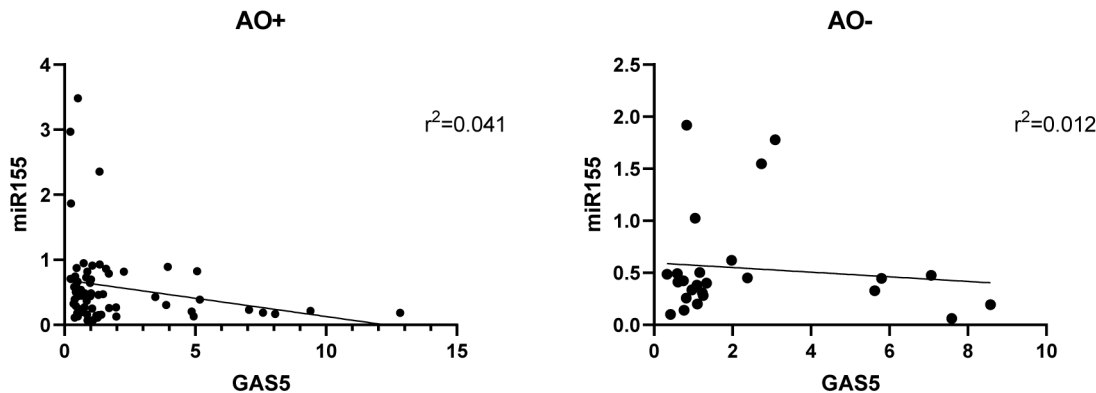


Fig. 3. Scatter plot Graph showing the correlation between expression levels of GAS5 and miRNA-155 obese individuals (AO+) and individuals without abdominal obesity (AO-).

Supplementary Material 2 (end)

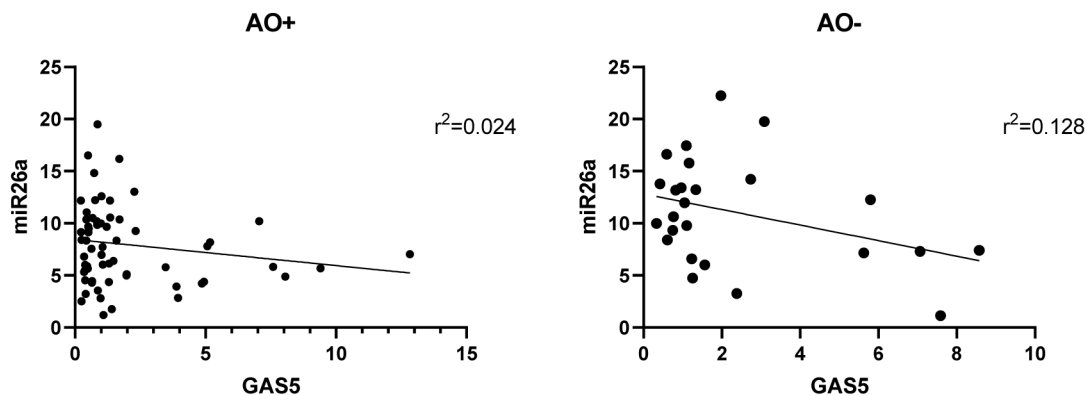


Fig. 4. Scatter plot Graph showing the correlation between expression levels of GAS5 and miRNA-26A in obese individuals (AO+) and individuals without abdominal obesity (AO-).

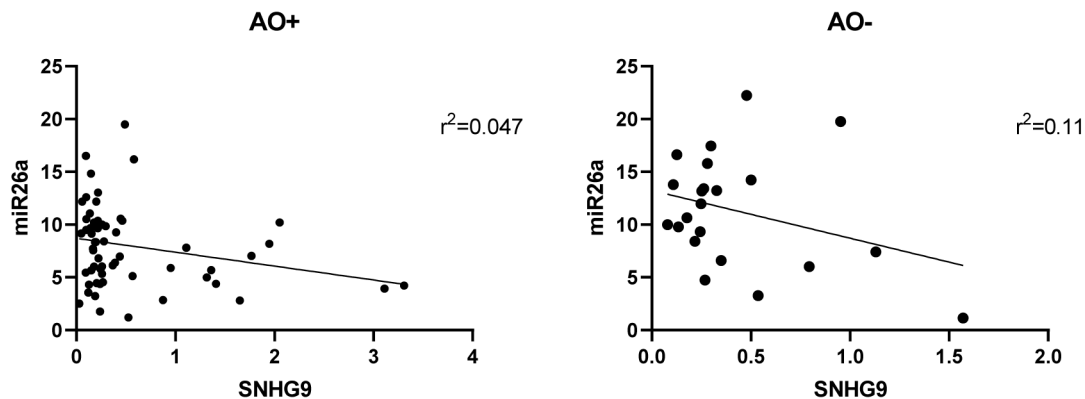


Fig. 5. Scatter plot Graph showing the correlation between expression levels of SNHG9 and miRNA-26A in obese individuals (AO+) and individuals without abdominal obesity (AO-).

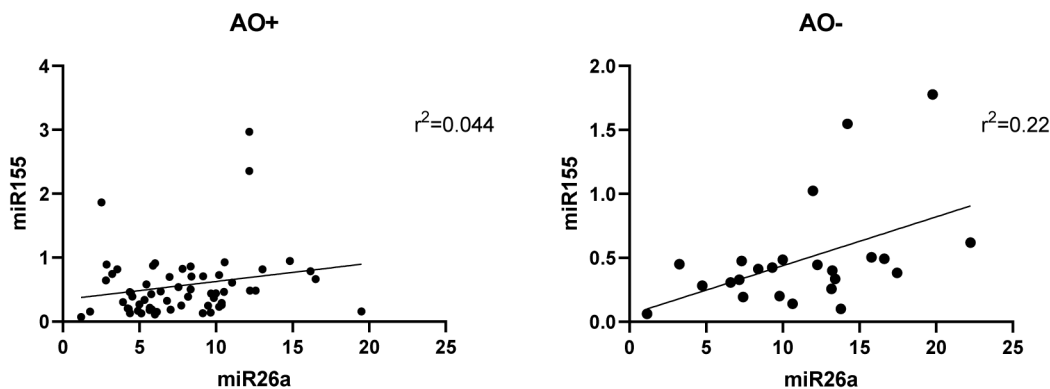


Fig. 6. Scatter plot Graph showing the correlation between expression levels of miRNA-26A and miRNA-155 in obese individuals (AO+) and individuals without abdominal obesity (AO-).