Titre: Title:	Genome-wide profiling of circulating microRNAs in adolescent idiopathic scoliosis and their relation to spinal deformity severity, and disease pathophysiology. Supplément
Auteurs: Authors:	Nasrin Khatami, Iurie Caraus, Mustafizur Rahaman, Evguenia Nepotchatykh, Mohamed Elbakry, Wesam Elremaly, Anita Franco, Marie Beauséjour, Anne-Marie Laberge, Stefan Parent, Hubert Labelle, Carl-Éric Aubin, Jean Lachaine, & Alain Moreau
Date:	2025
Type:	Article de revue / Article
Référence: Citation:	Khatami, N., Caraus, I., Rahaman, M., Nepotchatykh, E., Elbakry, M., Elremaly, W., Franco, A., Beauséjour, M., Laberge, A.-M., Parent, S., Labelle, H., Aubin, C.-É., Lachaine, J., & Moreau, A. (2025). Genome-wide profiling of circulating microRNAs in adolescent idiopathic scoliosis and their relation to spinal deformity severity, and disease pathophysiology. Scientific Reports, 15, 5305 (11 pages). https://doi.org/10.1038/s41598-025-88985-3

Titre de la revue: Journal Title:	Scientific Reports (vol. 15)
Maison d'édition: Publisher:	Springer Nature
URL officiel: Official URL:	https://doi.org/10.1038/s41598-025-88985-3
Mention légale: Legal notice:	This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the

Genome-Wide Profiling of Circulating MicroRNAs in Adolescent Idiopathic Scoliosis and their Relation to Spinal Deformity Severity, and Disease Pathophysiology

Nasrin Khatami[1,2], Iurie Caraus[1,2], Mahamuda Rahaman[1], Evguenia Nepotchatykh[1,3], Mohamed Elbakry[1,4], Wesam Elremaly[1], Anita Franco[1], Marie Beauséjour[5,6,7], Anne-Marie Laberge[8], Stefan Parent[5,6,9,10], Hubert Labelle[5,6,9,10], Carl-Éric Aubin[5,10,11], Jean Lachaine[12], and Alain Moreau[1,2,3,10,13,*]

[1]Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, CHU Sainte-Justine, Azrieli Research Center, Montreal, QC, Canada;

[2]Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada;

[3]Molecular Biology PhD Program, Faculty of Graduate and Postdoctoral Studies, Université de Montréal, Montreal, QC, Canada;

[4]Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt;

[5] CHU Sainte-Justine, Azrieli Research Center, Montreal, QC, Canada;

[6]Department of Surgery, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada;

[7]Department of Community Health Sciences, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Longueuil, QC, Canada;

[8]Medical Genetics, Department of Pediatrics, CHU Sainte-Justine and Université de Montréal, Montreal, QC, Canada;

[9]Orthopedic Division, CHU Sainte-Justine, Montreal, QC, H3T 1C5, Canada;

[10]Institut TransMedTech Montreal, CHU Sainte-Justine, Montreal, QC, Canada;

[11]Polytechnique Montréal, Montreal, QC, Canada;

[12]Faculty of Pharmacy, Université de Montréal, Montreal, Quebec, Canada;

[13]Department of Stomatology, Faculty of Dentistry, Université de Montréal, Montreal, QC, Canada;

[*]Corresponding Author: Pr. Alain Moreau, PhD, Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, room 2.17.027, Sainte-Justine University Hospital Research Center, 3175 Cote-Ste-Catherine Road, Montreal, QC, H3T 1C5, Canada.
Email: alain.moreau.hsj@ssss.gouv.qc.ca

Supplementary Information

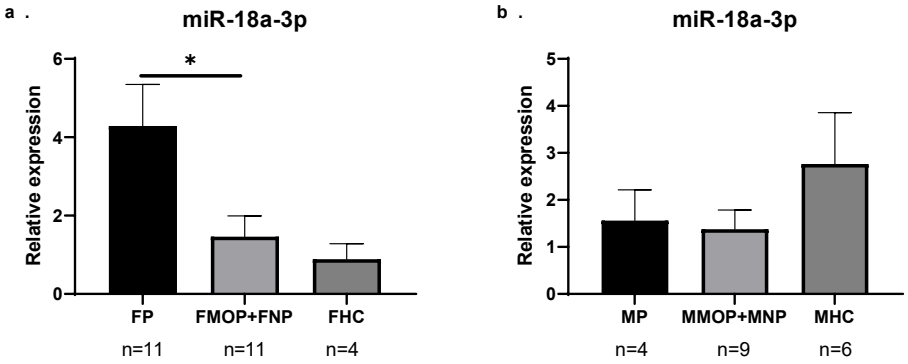
Supplementary Table S1. List of 90 circulating miRNAs differentially expressed in AIS and identified by miRNA array (discovery cohort)

miRNAs validated initially				
hsa-let-7f-5p	**hsa-miR-1-3p**	hsa-miR-342-5p	hsa-miR-486-5p	hsa-miR-6730-5p
hsa-miR-103a-3p	**hsa-miR-143-3p**	hsa-miR-3609	hsa-miR-508-5p	hsa-miR-675-3p
hsa-miR-107	hsa-miR-146b-5p	hsa-miR-376a-2-5p	hsa-miR-510-5p	hsa-miR-6757-5p
hsa-miR-10b-3p	**hsa-miR-148a-3p**	hsa-miR-3924	hsa-miR-513a-5p	hsa-miR-6774-5p
hsa-miR-1180-5p	**hsa-miR-148b-3p**	hsa-miR-3926	hsa-miR-519b-3p	hsa-miR-6778-5p
hsa-miR-1199-5p	hsa-miR-150-5p	hsa-miR-3934-5p	**hsa-miR-551b-3p**	hsa-miR-6798-5p
hsa-miR-1237-5p	**hsa-miR-152-3p**	hsa-miR-3937	hsa-miR-5587-5p	hsa-miR-6801-3p
hsa-miR-1264	hsa-miR-182-3p	hsa-miR-3972	hsa-miR-5692c	hsa-miR-6806-5p
hsa-miR-126-5p	hsa-miR-187-3p	hsa-miR-4269	**hsa-miR-576-5p**	hsa-miR-6846-5p
hsa-miR-1266-5p	hsa-miR-188-5p	hsa-miR-4483	hsa-miR-611	hsa-miR-6888-3p
hsa-miR-1271-3p	**hsa-miR-18a-3p**	hsa-miR-451b	hsa-miR-6129	hsa-miR-6892-5p
hsa-miR-1285-3p	hsa-miR-1914-3p	hsa-miR-4640	hsa-miR-6131	hsa-miR-877-5p
hsa-miR-1285-5p	hsa-miR-198	hsa-miR-4651	hsa-miR-623	hsa-miR-935
hsa-miR-1292-5p	**hsa-miR-19a-3p**	hsa-miR-4653-3p	hsa-miR-6500-5p	hsa-miR-938
hsa-miR-1306-3p	**hsa-miR-19b-3p**	**hsa-miR-4665-5p**	hsa-miR-6507-3p	
hsa-miR-133b	**hsa-miR-214-3p**	hsa-miR-4728-5p	hsa-miR-6513-5p	
hsa-miR-135a-3p	hsa-miR-2467-3p	hsa-miR-4733-3p	hsa-miR-658	
hsa-miR-138-2-3p	hsa-miR-27a-5p	hsa-miR-4746-3p	hsa-miR-6717-5p	
hsa-miR-138-5p	hsa-miR-325	hsa-miR-4793-5p	hsa-miR-6722-3p	

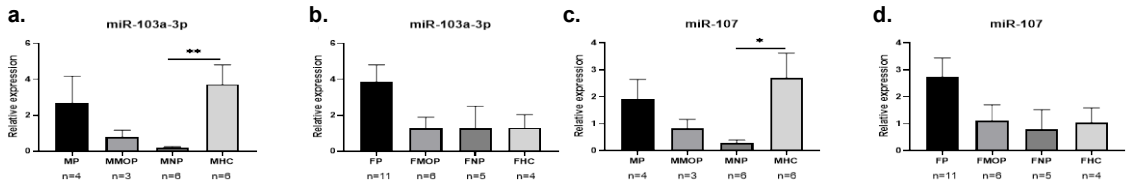
Supplementary Table S2. List of circulating miRNAs associated with AIS and validated by qPCR (validation cohort 1)

Expressed				Undetermined *
hsa-let-7f-5p	**hsa-miR-148b-3p**	hsa-miR-4653-3p	hsa-miR-6730-5p	hsa-miR-1180-5p
hsa-miR-103a-3p	hsa-miR-150-5p	**hsa-miR-4665-5p**	hsa-miR-675-3p	hsa-miR-1199-5p
hsa-miR-107	**hsa-miR-152-3p**	hsa-miR-4728-5p	hsa-miR-6757-5p	hsa-miR-1237-5p
hsa-miR-10b-3p	hsa-miR-182-3p	hsa-miR-4746-3p	hsa-miR-6778-5p	hsa-miR-1914-3p
hsa-miR-1264	hsa-miR-187-3p	hsa-miR-4793-5p	hsa-miR-6801-3p	hsa-miR-2467-3p
hsa-miR-126-5p	hsa-miR-188-5p	hsa-miR-486-5p	hsa-miR-6888-3p	hsa-miR-3609
hsa-miR-1266-5p	**hsa-miR-18a-3p**	hsa-miR-508-5p	hsa-miR-6892-5p	hsa-miR-3926
hsa-miR-1271-3p	hsa-miR-198	hsa-miR-510-5p	hsa-miR-877-5p	hsa-miR-3937
hsa-miR-1285-3p	**hsa-miR-19a-3p**	hsa-miR-513a-5p	hsa-miR-935	hsa-miR-4269
hsa-miR-1285-5p	**hsa-miR-19b-3p**	hsa-miR-519b-3p	hsa-miR-938	hsa-miR-4640
hsa-miR-1292-5p	**hsa-miR-214-3p**	**hsa-miR-551b-3p**		hsa-miR-4651
hsa-miR-1306-3p	hsa-miR-27a-5p	hsa-miR-5692c		hsa-miR-4733-3p
hsa-miR-133b	hsa-miR-325	**hsa-miR-576-5p**		hsa-miR-5587-5p
hsa-miR-135a-3p	hsa-miR-342-5p	hsa-miR-6129		hsa-miR-611
hsa-miR-138-2-3p	hsa-miR-376a-2-5p	hsa-miR-6131		hsa-miR-6507-3p
hsa-miR-138-5p	hsa-miR-3924	hsa-miR-623		hsa-miR-658
hsa-miR-1-3p	hsa-miR-3934-5p	hsa-miR-6500-5p		hsa-miR-6774-5p
hsa-miR-143-3p	hsa-miR-3972	hsa-miR-6513-5p		hsa-miR-6798-5p
hsa-miR-146b-5p	hsa-miR-4483	hsa-miR-6717-5p		hsa-miR-6806-5p
hsa-miR-148a-3p	hsa-miR-451b	hsa-miR-6722-3p		hsa-miR-6846-5p

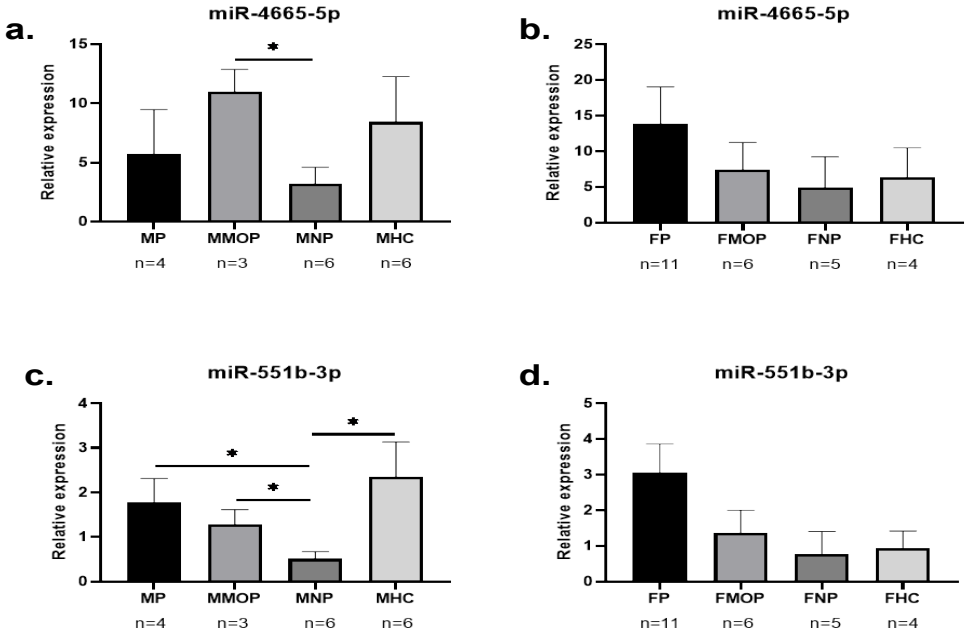
* "Undetermined" or not detectable means that miRNAs' amplification did not reach the detection threshold.



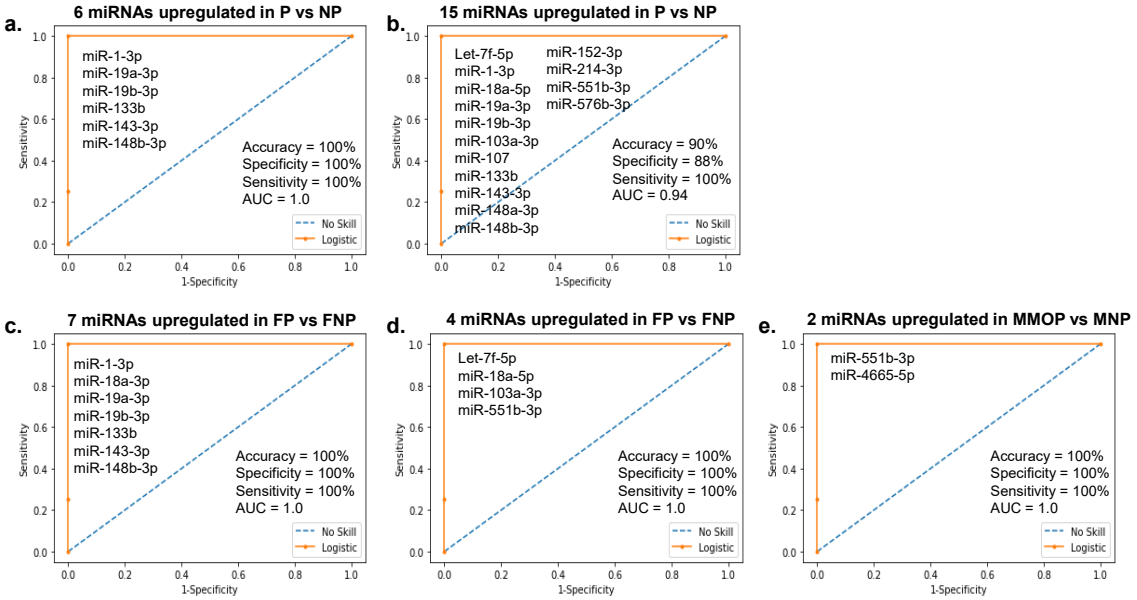
Supplementary Figure S1. Elevation of circulating miR18a-3p is sex dependent in AIS progressors. Expression of MiR-18a-3p is upregulated explicitly in females classified in the severe (P) group. **Panel a** show the comparison of miR-18a-3p expression between females exhibiting a severe scoliosis (FP group) with the female with mild-to-moderate scoliosis (FMOP+FNP group). **Panel b** shows the comparison of miR-18a-3p expression in males exhibiting a severe scoliosis (MP group) compared to males classified in the non-severe group (FMOP+FNP). GraphPad Prism 9 was used to generate graphs and statistical analysis. Statistical significance between each group was determined using the T-test for two groups and ANOVA for more than two groups (* p-value<0.05).



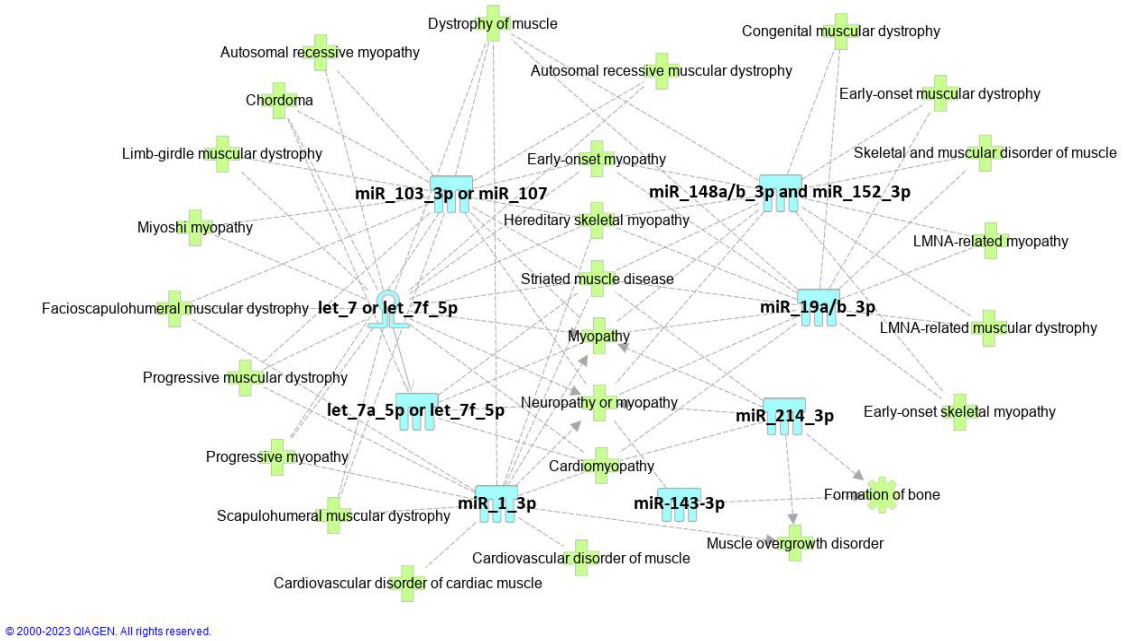
Supplementary Figure S2. Circulating miRNAs downregulated in AIS non-progressor males. Panel a and b illustrate miR-103a-3p expression in male and female patients respectively, with a severe downregulation only in males non-progressor (MNP). **Panel c and d** represent a similar comparison showing a significant downregulation of miR-107 in male non-progressor group (MNP). Of note, female patients and controls exhibited to opposite behavior in term of expression for both miRNAs. GraphPad Prism 9 was used to generate the graphs and perform the statistical analysis. To compare the statistical significance between each group, we used the T-test for two populations and the ANOVA test for more than two populations. (* p-value<0.05, ** p-value<0.01).



Supplementary Figure S3. **Circulating miRNAs showing an upregulation in moderate scoliosis progressor group. AIS**. **Panel a** show an upregulation of miR-4665-5p only in male patients with AIS classified in the moderate progressor group (MMOP) compared to the other two group, while **panel b** shows no difference among the female groups. Panel c and d represent a similar analysis with miR-551b-5p in males and female patients respectively indicating a significant upregulation of this microRNA only in male progressor group (MP) and male moderate progressor group (MMOP). GraphPad Prism 9 was used to make graphs and statistical analysis. To compare the statistical significance between each group, we used the T-test for two populations and the ANOVA test for more than two populations. (* p-value<0.05, ** p-value<0.01).



Supplementary Figure S4. Receiver-operator curve (ROC) analysis for predicting scoliosis severity in symptomatic AIS patients. Panel a represents the ROC curve generated by combining the relative expression ($2^{(-\Delta\Delta CT)}$) values from six miRNAs (miR-1-3p, miR-19a-3p, miR-19b-3p, miR-133b, miR-143-3p, miR-148b-3p) upregulated in AIS progressors (P) group in both sexes, which shows an accuracy, specificity and sensitivity of 100%, with a ROC curve AUC = 1.0**. Panel b** represents the same approach with the inclusion of the 15 miRNAs upregulated in the AIS progressors (P) group and shows a lesser predictive performance with an accuracy of 90%, a specificity of 88%, a sensitivity of 100% and a ROC curve AUC = 0.94. **Panel c** illustrates a ROC curve for predicting female progressor (FP) vs female non-progressor (FNP) with seven circulating miRNAs (miR-1-3p, miR-18a-3p, miR-19a-3p, miR-19b-3p, miR-133b, miR-143-3p, miR-148b-3p) shows an accuracy, specificity and sensitivity of 100%, with a ROC curve AUC = 1.0. **Panel d** corresponds to ROC curve for predicting female progressor (FP) vs female on-progressor (FNP) with four circulating miRNAs (let-7f-5p, miR-18a-3p, miR-103a-3p, miR-551b-3p) and shows an accuracy, specificity and sensitivity of 100%, with a ROC curve AUC = 1.0. **Panel e** shows a ROC curve for predicting male moderate progressor (MMOP) vs male non-progressor (MNP), with an accuracy, specificity and sensitivity of 100%, with a ROC curve AUC = 1.0.



Supplementary Figure S5. Muscle, bone, cartilage disease related to our candidate miRNAs. Using the Ingenuity Pathway Analysis (IPA) software (QIAGEN Inc. software version 51,963,813), we found out 11 miRNAs (let-7f-5p, miR-1-3p, miR-19a-3p, miR-19b-3p, miR-103a-3p, miR-107, miR-143-3p, miR-148a-3p, miR-148b-3p, miR-152-3p, miR-214-3p) over the 15 candidate miRNAs has been reported to be related to muscle, bone, cartilage related disease. These miRNAs have been reported in bone and mostly in muscle-related diseases.