Circulating mir-28-5p is a potential biomarker of sarcopenia in patients with Cushing's Syndrome in remission

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Background

Patients with Cushing's syndrome (CS) in remission present with sustained sarcopenia. Changes in circulating levels of muscle-specific microRNAs (myomiRs) have been described in several conditions associated with muscle dysfunction, including aging-related sarcopenia.

Our study was aimed at evaluating if there are differentially expressed myomiRs in plasma of patients with CS in remission, and these are related to sarcopenia.

Patients and methods

- Thirty-six women with CS in remission [median age 51 (15); BMI 27±4 Kg/m^2] and 36 age-, BMI-matched female controls were included. Seven patients had sarcopenia acording to the definition of the European Working Group on Sarcopenia in Older People (EWGSOP).
- Small RNA sequencing to identify circulating miRNAs expressed in CS patients as compared with controls.
- Small RNA libraries were generated and indexed using a modified Illumina TruSeq small RNA protocol. Significant miRNAs were identified using bioinformatic analysis, and validation wascarried out using RT-Qpcr. For the validation, Taqman probes were performed on QuantStudio 5 equipment (Applied Biosystems).

Results

- Three miRNA were differentially expressed between the two groups. All miRNA were upregulated in patients compared to controls (Figure 1).
- miR-28-5p was significantly upregulated in patients with Cushing and sarcopenia in comparison with the remaining Cushing patients (Figure 2).
- For miR-28-5p we obtained an AUC of 0.7980 (p=0,0156) and we calculated the optimal cut-off value for the fold change as 3,80 with a sensitivity of 85,71% and a specificity of 68,97% (Figure 3).

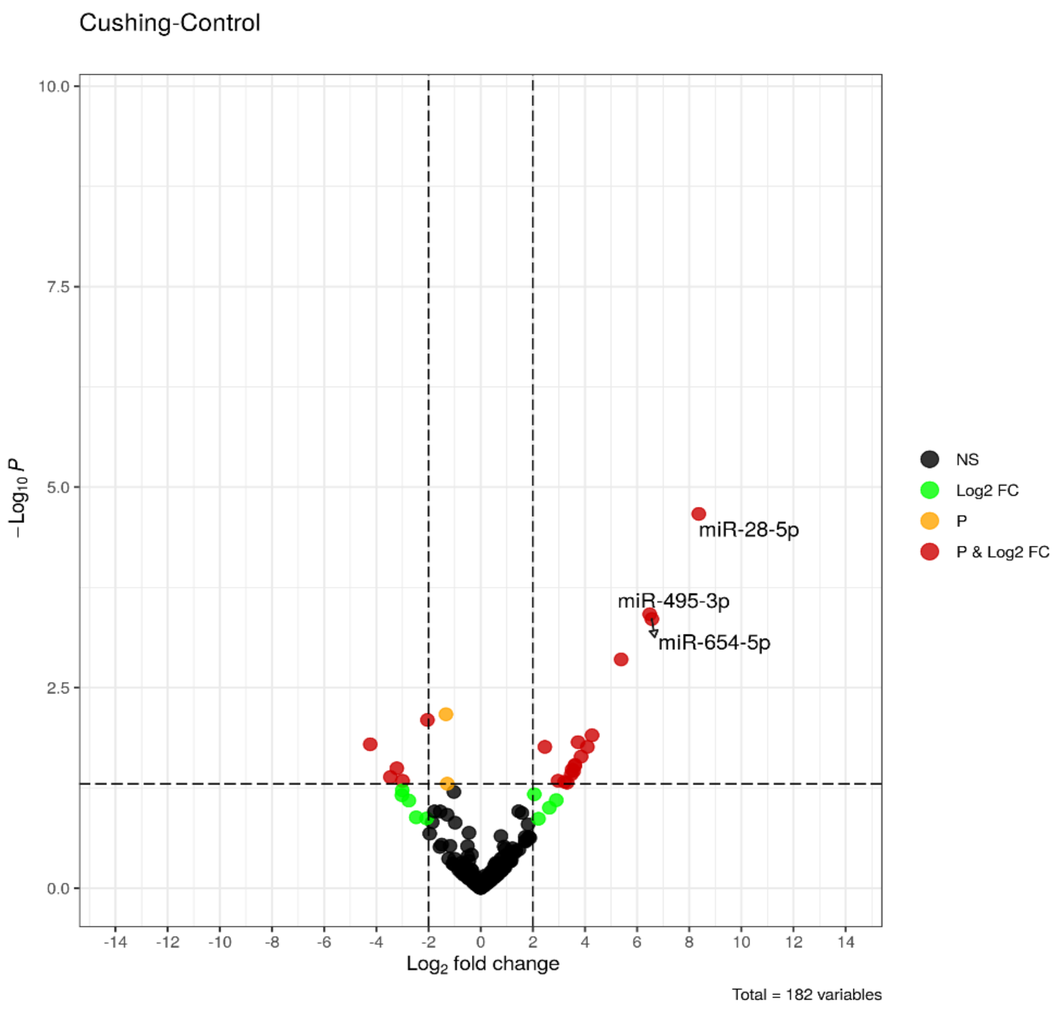

Figure 1. Volcano-plot of differential expressed miRNAs between healthy participants (Controls) (n=18) and Cushing patients (n=18). Vertical lines indicate the threshold for a relative expression fold change (FC) of 2 or -2 fold compared to controls. The horizontal line represents the threshold of a 0.05 p-value**.** NS: miRNAs not significative; Log2FC: miRNAs with Log2 fold change > 2 in absolute value; P: miRNAs with p-value < 0.05; P&Log2FC: miRNAs with p-value < 0.05 and Log2 fold change > 2 in absolute value. miRNAs with FDR less <0.05 are labelled.

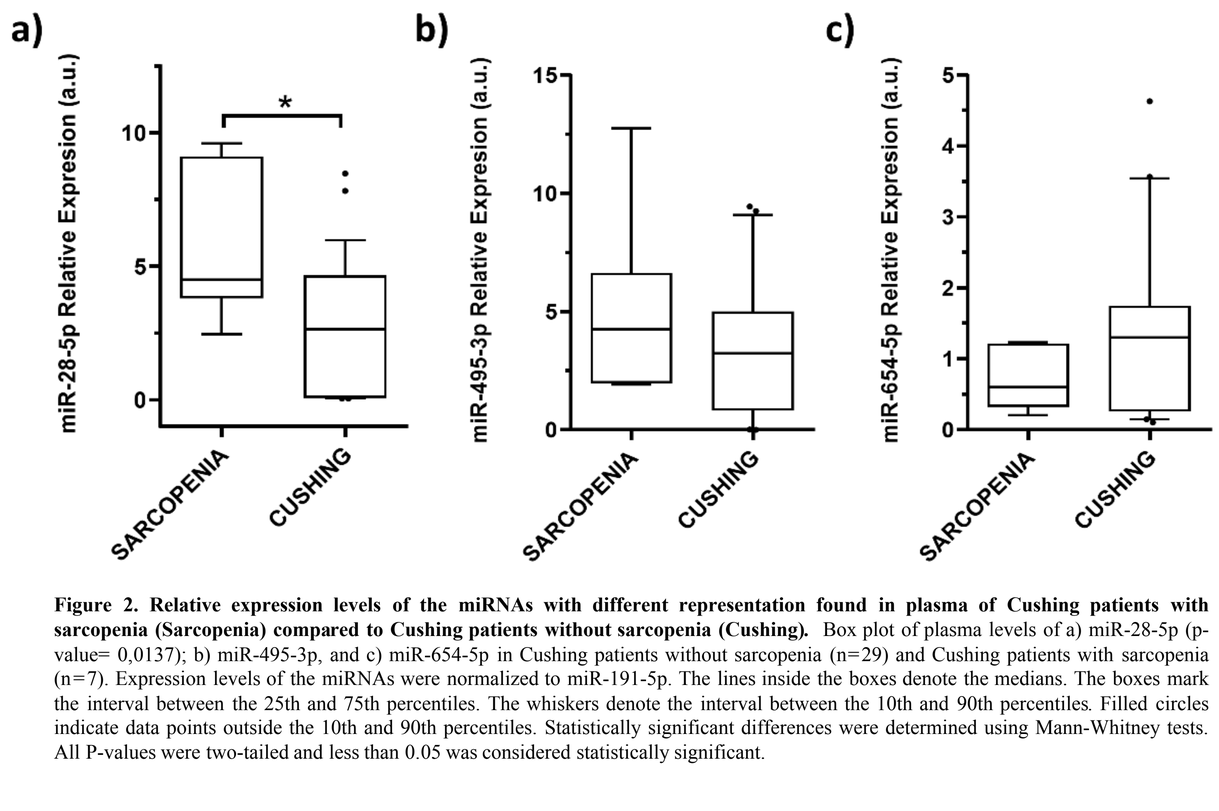

Figure 2. Relative expression levels of the miRNAs with different representation found in plasma of Cushing patients with sarcopenia (Sarcopenia) compared to Cushing patients without sarcopenia (Cushing). Box plot of plasma levels of a) miR-28-5p (p-value= 0,0137); b) miR-495-3p, and c) miR-654-5p in Cushing patients without sarcopenia (n=29) and Cushing patients with sarcopenia (n=7). Expression levels of the miRNAs were normalized to miR-191-5p. The lines inside the boxes denote the medians. The boxes mark the interval between the 25th and 75th percentiles. The whiskers denote the interval between the 10th and 90th percentiles. Filled circles indicate data points outside the 10th and 90th percentiles. Statistically significant differences were determined using Mann-Whitney tests. All P-values were two-tailed and less than 0.05 was considered statistically significant.

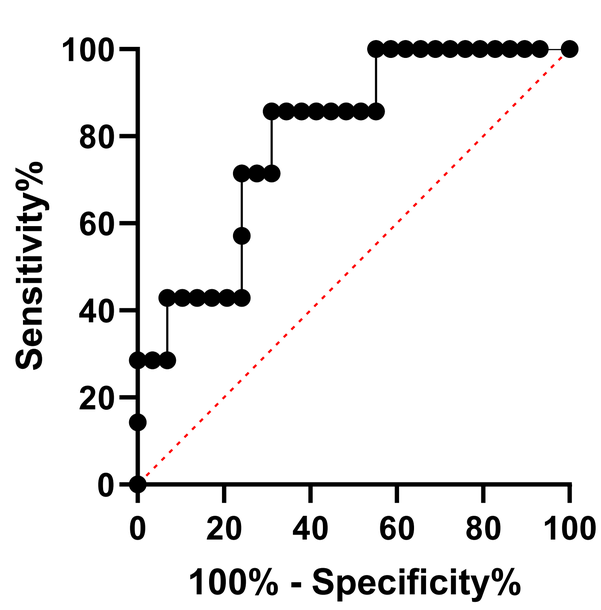

Figure 3. Area under the curve of receiver operating characteristic (ROC) for miR-28-5p**.**

Conclusion

MiR-28-5p, a myomiR involved in myotube proliferation and differentiation in vivo, may serve as an independent biomarker for identifying CS patients at high-risk of sarcopenia after biochemical remission.