The liver of obese patients with hepatic steatosis exhibits a severe dysregulation of key splicing machinery components as compared to obese patients without hepatic steatosis

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INTRODUCTION

Obesity, a disease that is reaching epidemic proportions worldwide, is caused by a combination of genetic and lifestyle factors. One of the most common pathologies associated with obesity is **hepatic steatosis**, an accumulation of fat within the liver that can progress to liver fibrosis, cirrhosis, and ultimately lead to hepatocellular carcinoma. There is emerging evidence suggesting that alternative mRNA splicing, the key mechanism providing transcript and protein diversity, is dysregulated in many tissues under pathological conditions, such as obesity and cancer. Moreover, the splicing variants generated by the alteration of the normal splicing process could contribute to the aggressiveness and comorbidities of these diseases.

We **hypothesized** that an alteration in the splicing machinery could occur in the liver of obese patients with hepatic steatosis, which could contribute to the dysregulation of the splicing process and might ultimately be associated with the progression to hepatic fibrosis/cirrhosis/carcinoma. Therefore, the **OBJECTIVE** of this work was to determine the pattern of dysregulation of the spliceosome components and splicing factors in the liver of obese women with steatosis compared to control women.

COHORT CHARACTERISTICS

Characteristic		Mean	SEM	t test
Age	NON STEATOSIS N=9	37.00	4.997	0.423
	STEATOSIS N=33	40.36	1.713	
Body weight	NON STEATOSIS N=9	125.6	3.066	0.296
	STEATOSIS N=33	132.9	3.470	
BMI	NON STEATOSIS N=9	48.96	0.922	0.582
	STEATOSIS N=33	50.36	1.293	
Waist circunference	NON STEATOSIS N=9	129.9	3.190	0.149
	STEATOSIS N=33	136.9	2.316	
Glucose (mg/dl)	NON STEATOSIS N=9	101.7	7.623	0.102
	STEATOSIS N=33	109.7	4.497	
Insulin (mU/l)	NON STEATOSIS N=5	9.440	1.320	0.049
	STEATOSIS=22	19.59	2.287	
Glycated hemoglobin (%)	NON STEATOSIS N=8	6.03	0.444	0.175
	STEATOSIS N=32	6.181	0.126	
HDL (mg/dl)	NON STEATOSIS N=9	43.78	4.893	0.652
	STEATOSIS N=33	41.85	1.795	
Triglycerides (mg/dl)	NON STEATOSIS N=9	103.3	18.72	0.215
	STEATOSIS N=33	136.9	19.21	
LDL (mg/dl)	NON STEATOSIS N=9	126.6	11.38	0.401
	STEATOSIS N=32	138.1	6.479	
CRP (mg/L)	NON STEATOSIS N=9	8.456	1.278	0.570
	STEATOSIS N=28	12.28	1.855	

Figure 2: **Demographic and clinical characteristics** of the patients included in this study showed no major differences in key parameters. BMI=Body Mass Index, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, CRP=C-Reactive Protein.

MATERIALS & METHODS

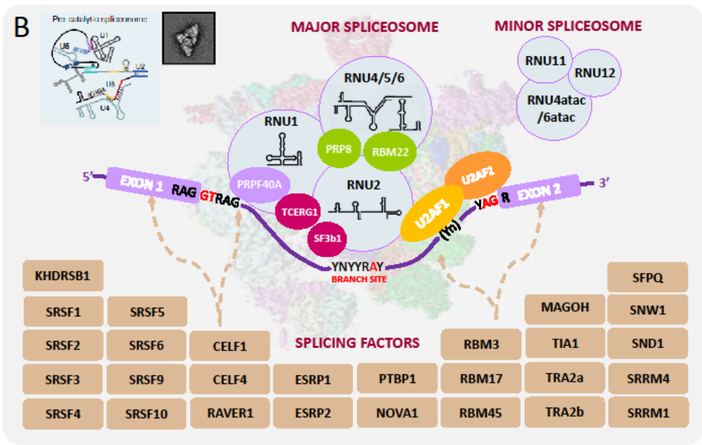
A

Liver Biopsy → RNA extraction → Retrotranscription → Dynamic Array

Obese women
- Non Steatosis (NO ST) n=9
- Steatosis I (ST I) n=8
- Steatosis II (ST II) n=12
- Steatosis III (ST III) n=13

Figure 1. A: Experimental design. RNA from liver samples from obese (BMI>30) women with and without liver steatosis classified using abdominal echography was extracted and retro-transcribed to measure the expression levels of several components of the major and minor spliceosome, as well as associated splicing factors, using the dynamic PCR array based on the microfluidic technology of Fluidigm. **B: Graphical list of the selected components** of the major (n=13) and minor spliceosome (n=4) and associated splicing factors (n=28) included in the PCR array.

B



RESULTS

The expression of a number of relevant splicing factors and spliceosome components is altered in steatosis (ST) livers compared to non-steatotic (NON ST) control livers. Interestingly, some of these alterations seem to be dependent on the degree of liver steatosis.

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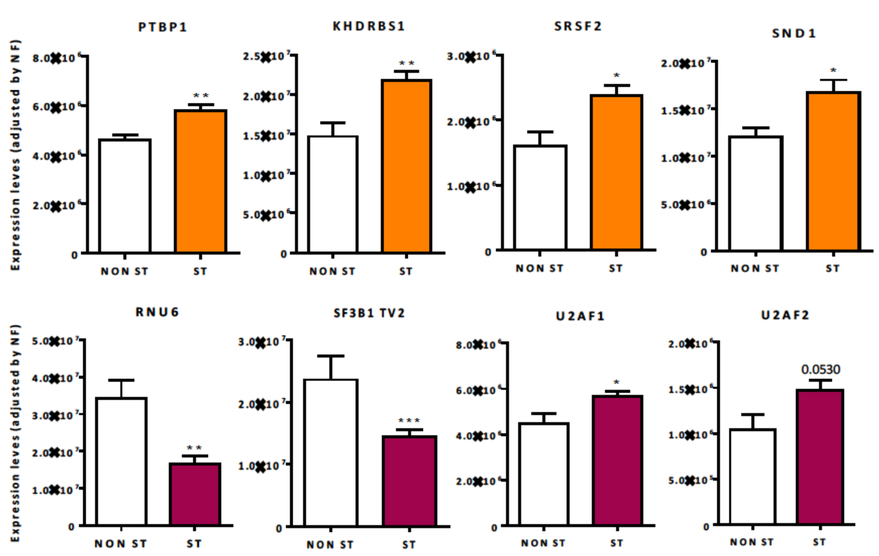

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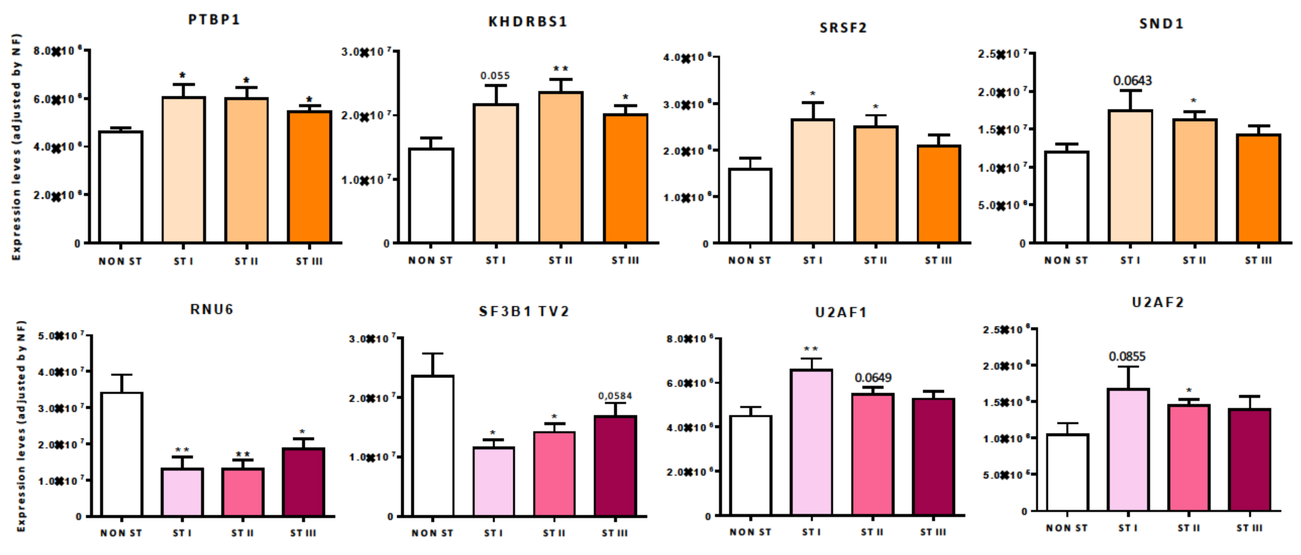
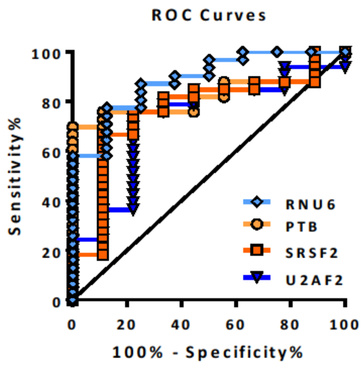


Figure 3. A: Effect of hepatic steatosis on the expression of spliceosome components and splicing factors. mRNA expression levels [adjusted by a normalization factor (NF) calculated from the expression level of HPRT and beta-actin] of the different spliceosome components and splicing factors in the liver of obese women with and without steatosis. B: Effect of hepatic steatosis on the expression of spliceosome components and splicing factors in the liver of obese women with different degrees of steatosis. Values represent the mean ± SEM, *Asterisks* indicate values that significantly differ from Non-Steatosis values (t-test *p<0.05, **p<0.01, ***p<0.001).

Interestingly, the expression levels of some of these splicing machinery components were associated with relevant clinical parameters in patients with steatotic liver, including glucose levels (i.e. SRSF2, U2AF1, U2AF2 and RNU6), gamma-glutamyltransferase (i.e. SRSF2 and U2AF1) or HDL (i.e. SND1, U2AF2).

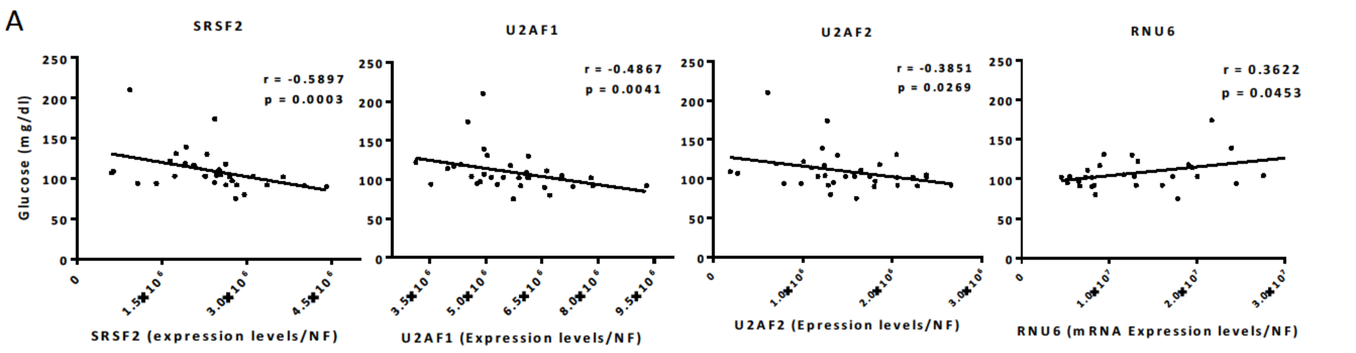
ROC analysis revealed that the expression of specific splicing factors, especially SRSF2 and PTBP1, and spliceosome components (i.e. RNU6 and U2AF2), can clearly discriminate between patients with or without hepatic steatosis.



	RNU6	PTB	SRSF2	U2AF2
AUC	0.85	0.82	0.76	0.73
p value	0.001	0.003	0.01	0.03
Sensitivity (%)	84.38	75.79	75.76	75.76
Specificity (%)	75	88.89	77.78	77.78

Figure 5: Receiver operating characteristic (ROC) curve analysis to determine the accuracy of the components of the splicing machinery as test to discriminate between obese patients with or without liver steatosis [the closer the ROC curve is to the upper left corner of the graphic (i.e., higher sensitivity and specificity), the higher the overall accuracy of the marker used].

A



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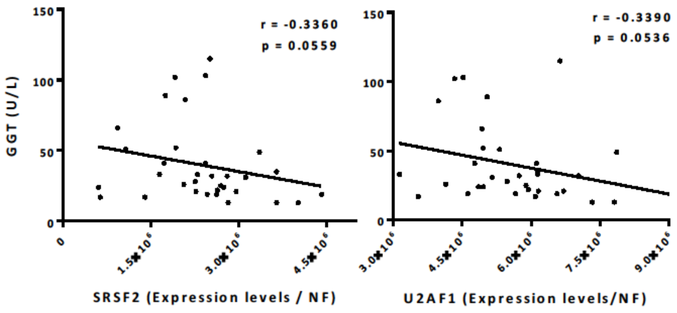

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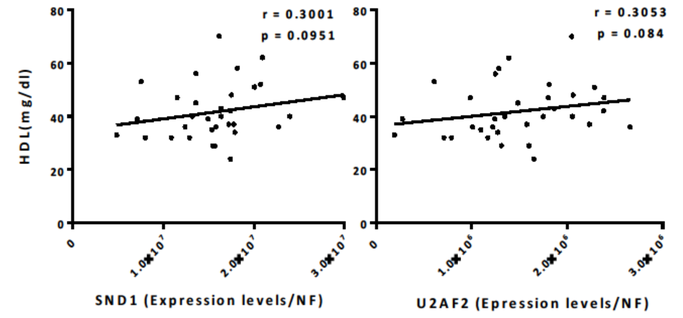

Figure 4: **Correlations** between the hepatic mRNA expression levels of **splicing factors and spliceosome components** and circulating levels of **Glucose (A), GGT (B) and HDL (C)** in patients with steatosis. Correlation coefficients were calculated by Spearman´s test.

CONCLUSION

In conclusion, the expression of specific splicing machinery components is significantly altered in the liver of obese patientswith hepatic steatosis, wherein correlates with relevant clinical parameters.
Ongoing studies would clarify the potential pathological implications of these findings, which could help to predict a worsening in steatosis, and may provide novel diagnostic biomarkers and therapeutic tools for this disease.

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