ApoCIII glycoforms determination and proteomic analysis in plasma of coronary patients with different ApoCIII levels

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BACKGROUND: The aims of this study were:i) to analyze and quantify the apolipoproteinCIII glycoforms characterized by none, one or two sialic acids,(having different lipoprotein lipase-LPL-inhibitory activity), ii) to assess their relationship with LPL activity and ApoA-V in CAD patients, iii) to analyze some previously identified plasma proteins in relation to lipids status.

METHODS: ApoCIII glycoforms in four groups of patients (from "Verona Heart Study" biobank,) classified according to the total plasma concentration of ApoCIII and different triglyceride (TG) levels, were analyzed by a classical (isoelectric focusing/western blotting) and by a shotgun MS approach. LPL activity (Fluorescent assay) and ApoA-V concentration (ELISA assay) were determined, and their correlations with lipid metabolism parameters were analyzed.

RESULTS & DISCUSSION:

Tabel 1. Characteristics of the subjects

groups	Number of samples	ApoCIII levels	Fatty acids profile
1	7	low (7.25 ± 1.45 mg/dL)	A (poly- unsaturated > 40%)
2	5	low (7.25 ± 1.45 mg/dL)	B (poly- unsaturated < 40%)
3	7	high (17.31 ± 3.98 mg/dL)	A (poly- unsaturated > 40%)
4	7	high (17.31 ± 3.98 mg/dL)	B (poly- unsaturated < 40%)

Note: The level of apolipoprotein C-III was determined using an automated turbidimetric immunoassay; are considered low values <9.2 mg / dL and higher than ≥ 12.6 mg / dL. The polyunsaturated (PUFA) profile has been defined by gas chromatography.

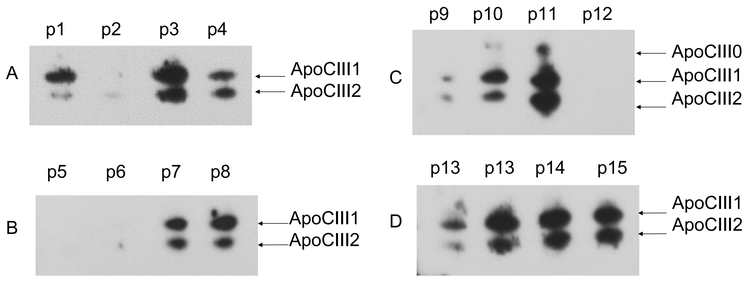

Figure1. ApoCIII detection after IEF and diffusion blotting. (A) GROUP 1 low ApoCIII ;(B) GROUP 2 low ApoCIII; (C) GROUP 3 high ApoCIII;(D) GROUP 4 high ApoCIII

The distribution of the three ApoCIII glycoforms in the selected groups of patients are related to the TG levels, particularly the mono-sialylated isoform (ApoCIII-1) prevails in patients with the highest TG levels.

Table2. ApoAV and LPL correlations with apolipoprotein CIII and triglycerides

Variable	Concentration	Triglycerides	ApoCIII
LPL (µmol/ml)*	3,10 (2,72 -3,53)	r= 0,034 p= 0,805	r= 0,188 p= 0,165	r= 0,153 p= 0,259
Apo A-V ng/ml*	496,90 (369,70-667,87)	r= -0,245 p= 0,143	-	-

r = correlation coefficient, p = p-value

Table 3. Mean values, with standard deviation, range and normal values, relative to the concentration of apolipoprotein and LPL activity

Variable		Range (min-max)	Normality value
Apo C-III (mg/dl)	11,81 ± 3,84	3,17 - 20,44	< 10.5
LPL (µmol/ml)*	3,10 (2,72 -3,53)	0,78 - 7,21	-
Apo A-V ng/ml*	496,90 (369,70 -667,87)	66 - 2395,95	-

The mean concentration of ApoAV measured in our study group (496.90 ng / ml, CI 369.70 to 667.87), falls within the normal range, however upper than prevoiusly reported. LPL activity, measured as Vmax, does not show significant correlations with ApoCIII.

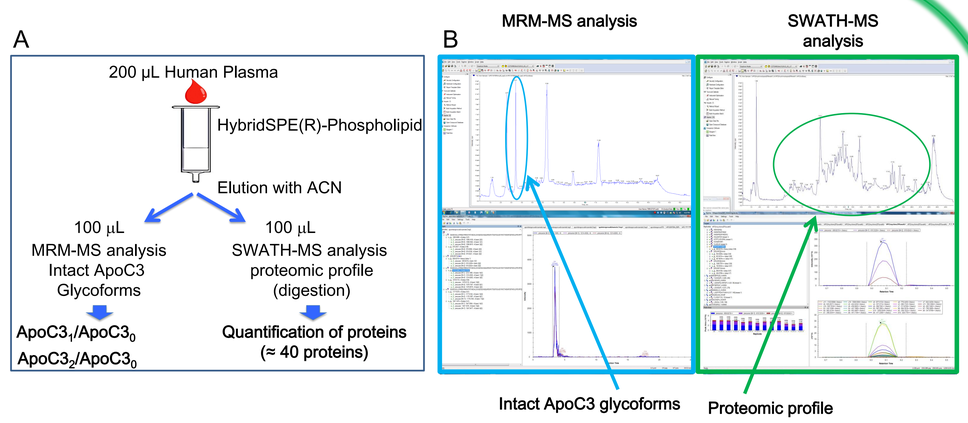

Figure2. (A)workflow MS analysis;(B) Chromatogram of the LC-MS analysis of intact ApoC3 glycoforms (left-up) and skyline interface for glycoforms quantification (left-down); chromatogram of the LC-SWATH-MS analysis of proteomic profile of plasma sample (right-up) and skyline interface protein quantification (right-down);

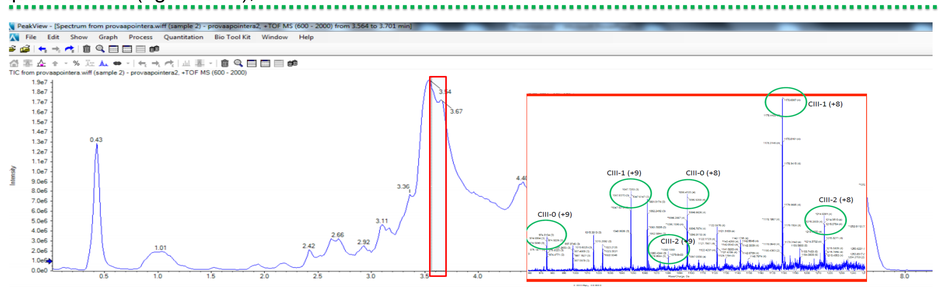

Figure3. Chromatogram and TOF MS with the ions of the glycoforms Sum of the three most abundant ions for +8 and +9 charge state of each glycoforms for relative quantification

Good agreement between IEF analysis and MS approach in terms of abundance % of isoforms. The MS analysis on a new set (n=60) of CAD patients is actually ongoing!

CONCLUSIONS: As compared with the other ones, mono – sialylated isoform of apo CIII is preferentially associated with TG levels. Samples with elevated levels of apoCIII are characterized by specific proteomic patterns.

REFERENCES:

Olivieri O. et al. 2010 Apolipoprotein C-III predicts cardiovascular mortality in severe coronary artery disease and is associated with an enhanced plasma thrombin generation. Journal of thrombosis and haemostasis 2010; 8(3): 463–47.

Yoshinao Wada et al.2012 Mass spectrometry of apolipoprotein C-III, a simple analytical method for mucin-type O-glycosylation and its application to an autosomal recessive cutis laxa type-2 (ARCL2) patient

Wenying Jian et al., 2013 Relative Quantitation of Glycoisoforms of Intact Apolipoprotein C3 in Human Plasma by Liquid Chromatography–High-Resolution Mass Spectrometry

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