NMR-based metabolomic profiling provides a specific type 2 diabetes mellitus associated profile in Spanish population beyond glucose: The Di@bet.es Study

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Background

The Di@bet.es Study, the first national study in Spain to examine the prevalence of diabetes and impaired glucose regulation, showed an overall type 2 DM (DM2) prevalence of 13.8% in Spain [1].

The primary objective of our study was to characterize the NMR metabolomic profile of a sub-group of 721 individuals, – men and women from 18 to 84 years– 25% of them with DM2. We also aimed to evaluate 1H-Nuclear Magnetic Resonance (1H-NMR) molecular panels to find a specific metabolomic profile associated with DM.

Results

Lipoprotein and glycoprotein profiles, as well as a set of 13 low molecular weight metabolites (LMWM), including amino acids and catabolic metabolites, were obtained. In order to reduce the differences in age, sex, body mass index (BMI) and glucose, all diabetic individuals (n=147) were matched with non-diabetic ones by these variables (see figure 1).

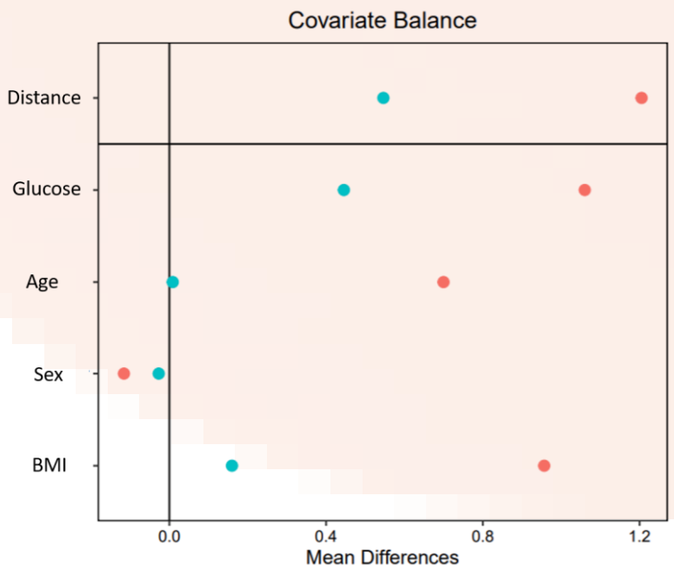


Figure 1. Mean differences between non-diabetic and diabetic individuals for each matched variable. Red points show differences before matching, while blue points show differences after matching process.

Univariate analysis showed significant differences ($p<0.05$) in: tyrosine, isoleucine, TG/cholesterol (TG/C) ratio of VLDL and HDL and in the percentage of small LDL particles (%S-LDL-P). Stronger differences ($p<0.01$) were observed in: glucose, glutamine, glycine and in the percentage of small VLDL particles (see table 1). In order to look for a DM associated metabolomic profile, four multivariate models were built: logistic regression, random forest, extreme gradient boosting and naïve Bayes. Among them, random forest was the most accurate one, so each variable importance in it was analysed (see figure 2). Although glucose remained having a great relevance in the model, additional predictors (previously masked by this metabolite) showed significant contribution. The TG/C-ratio of VLDL, glutamine, the S-LDL-P(%), glycine and creatine were the most contributing variables to DM.

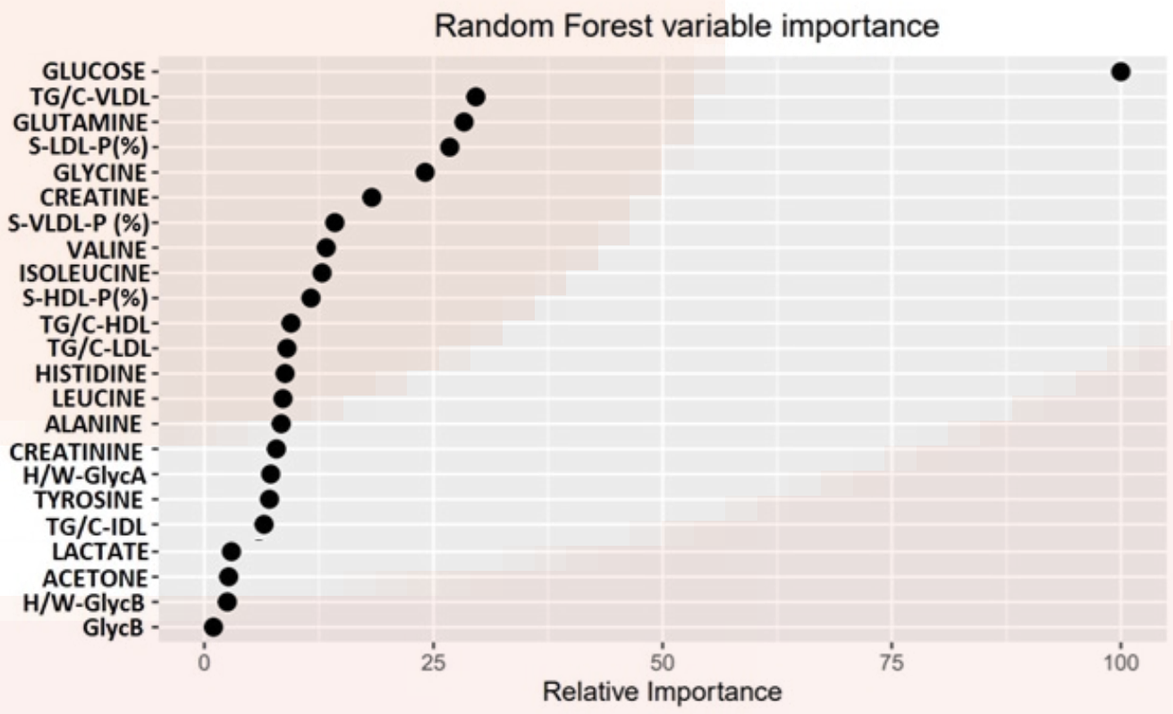


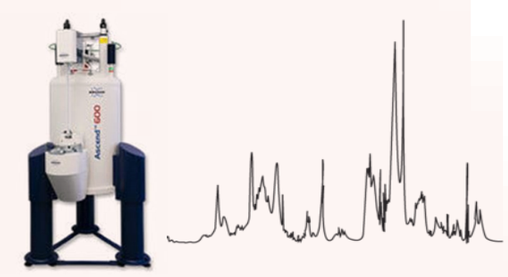
Figure 2. Relative importance of each variable in the random forest model.

Materials & Methods

Study Population

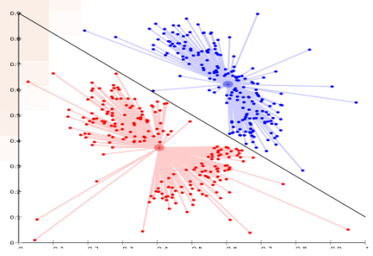
721 individuals of the Di@bet.es. men (44%) and women (56%) in a wide age range were included, 25% of them being diabetic.

H-NMR Metabolomics



Statistical Analysis

Univariate and multivariate statistical analyses were made between non-diabetic and diabetic individuals.



Advanced Metabolomic Profile

Advanced lipoprotein, glycoprotein and low molecular weight metabolites (LMWM) and glycoprotein profiles were obtained

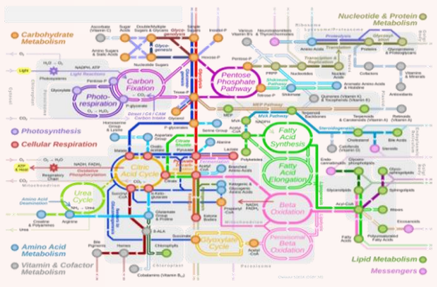


Table 1. Univariate analysis. Median and interquartile range are included. Negative values are a consequence of logarithmic transformation.

Metabolite	Type 2 DM		p overall
	No (N = 147)	Yes (N= 147)	
GlycB (µmol/L)	0.34 [-0.26;1.09]	0.27 [-0.35;0.96]	0.445
GlycA (µmol/L)	0.29 [-0.23;0.91]	0.26 [-0.16;1.03]	0.665
H/W GlycB	0.34 [-0.23;1.06]	0.25 [-0.26;0.97]	0.419
H/W GlycA	0.41 [-0.19;0.95]	0.34 [-0.15;0.93]	0.857
TG/Chol-VLDL	-0.35 [-0.76;0.18]	-0.14 [-0.62;0.32]	0.031
TG/Chol-IDL	0.15 [-0.29;0.95]	0.31 [-0.24;0.98]	0.459
TG/Chol-LDL	-0.23 [-0.71;0.26]	-0.47 [-0.74;0.12]	0.132
TG/Chol-HDL	0.09 [-0.42;0.74]	0.37 [-0.09;1.26]	0.002
Small VLDL particles (%)	-0.12 [-0.62;0.54]	0.32 [-0.20;0.98]	<0.001
Small LDL particles (%)	-0.01 [-0.78;0.57]	0.22 [-0.25;1.06]	0.002
Small HDL particles (%)	0.13 [-0.54;0.85]	0.09 [-0.57;0.67]	0.375
Acetone (mmol/L)	0.03 [-0.36;0.58]	0.13 [-0.27;0.73]	0.292
Alanine (mmol/L)	0.39 [-0.14;0.97]	0.63 [-0.02;1.17]	0.175
Creatinine (mmol/L)	0.22 [-0.30;0.83]	0.12 [-0.62;0.65]	0.068
Creatine (mmol/L)	0.06 [-0.55;0.73]	-0.20 [-0.67;0.49]	0.055
Glucose (mmol/L)	0.49 [0.18;0.77]	0.87 [0.16;1.41]	<0.001
Glutamine (mmol/L)	0.30 [-0.45;0.93]	-0.18 [-0.90;0.56]	<0.001
Lactate (mmol/L)	0.31 [-0.32;0.90]	0.43 [-0.27;0.91]	0.495
Valine (mmol/L)	0.08 [-0.31;0.70]	0.39 [-0.23;1.09]	0.061
Tyrosine (mmol/L)	0.34 [-0.18;0.80]	0.56 [-0.11;1.21]	0.037
Glycine (mmol/L)	0.08 [-0.52;0.76]	-0.33 [-0.74;0.34]	0.001
Histidine (mmol/L)	0.12 [-0.59;0.76]	0.22 [-0.64;0.85]	0.893
Isoleucine (mmol/L)	0.19 [-0.41;0.63]	0.45 [-0.13;1.05]	0.008
Leucine (mmol/L)	0.32 [-0.31;0.87]	0.48 [-0.24;1.03]	0.223

Conclusion

[1]H-NMR is a powerful tool to characterize DM-associated metabolomic profile, beyond traditional biomarkers, helping to a better understanding of DM etiopathology.

REFERENCES

[1] S. Chatterjee et. al., Type 2 diabetes. Lancet. 2027 Jun; 389(10085): 2239-2251.