METABOLIC CHANGES IN THE CSF OF MIGRAINE PATIENTS – MEASURED WITH NMR.

Molecular BioSystems

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Supplementary material:

Table s-1: Overview of 2D JRES peak annotations expressed in parts per million

	Name	HMDB	Peak							
			1	2	3	4	5	6	7	8
	TSP (internal standard)		0							
1	2-hydroxyisovalerate	HMDB00407	0.83	0.97						
2	2-hydroxybutyrate	HMDB00008	0.90							
3	Valine	HMDB00883	0.99	1.04						
4	2-oxoisovalerate	HMDB00019	1.13							
5	Ethanol	HMDB00108	1.19	3.66						
6	3-hydroxyisovalerate	HMDB00754	1.27							
7	Lactate	HMDB00190	1.33	4.12						
8	Alanine	HMDB00161	1.48							
9	Acetate	HMDB00042	1.92							
10	Acetone	HMDB01659	2.24							
11	Pyruvate	HMDB00243	2.38							
12	Glutamine	HMDB00641	2.43	2.45	2.47					
13	Citrate	HMDB00094	2.54	2.69						
14	Creatinine	HMDB00562	4.06							
15	Choline	HMDB00097	3.20							
16	Glucose	HMDB00122	3.24	3.40	3.49	3.54	3.72	3.73	3.775	5.24
17	Myo-inositol	HMDB00211	3.63							
18	Creatine	HMDB00064	3.94							
19	Fructose	HMDB00660	4.03							
20	Formate	HMDB00142	8.46							

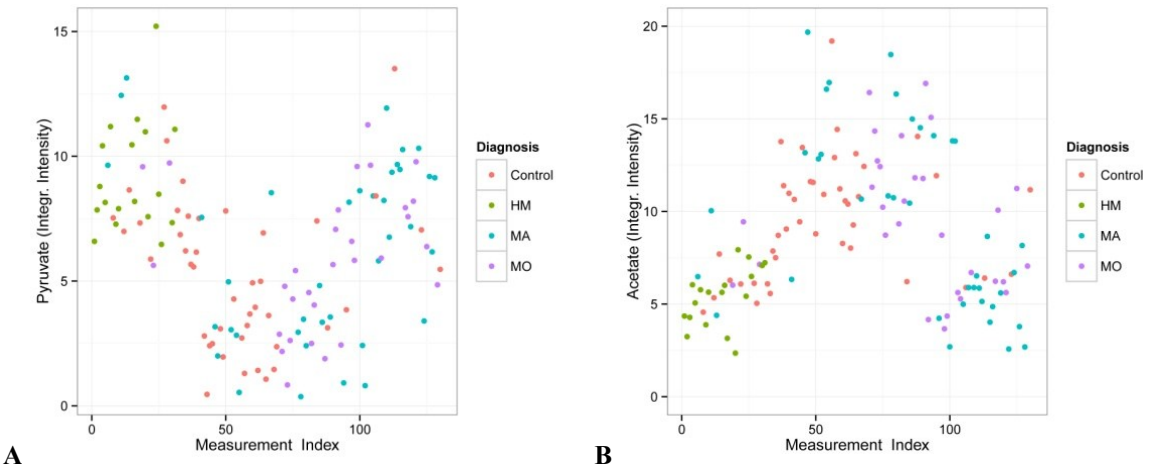


Figure s-1: Apparent batch effect of pyruvate an acetate. Pyruvate (A) and acetate (B) show an apparent batch effect in relation to their measurement index, i.e. the order in which the sample was collected, stored, prepared and measured. Reversed correlation between acetate and pyruvate levels can be explained by pyruvate conversion into acetate due to unknown cause.

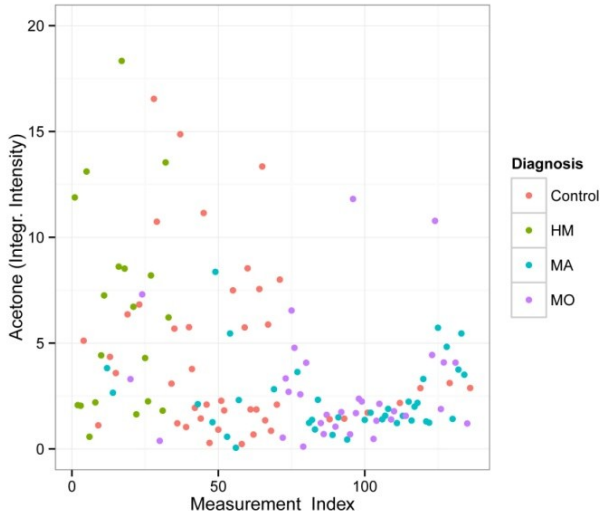


Figure s-2: Spread in acetone signal intensity correlates with the order in which the samples were collected, prepared and measured, i.e. the measurement index. As acetone concentration varies greatly in the first 80 samples and last 15 samples, but not within the samples in-between, it's likely an external source was present during their collection or immediate handling or that the acetone is a result of ketosis. Therefore, acetone was not taken into account during the analysis.

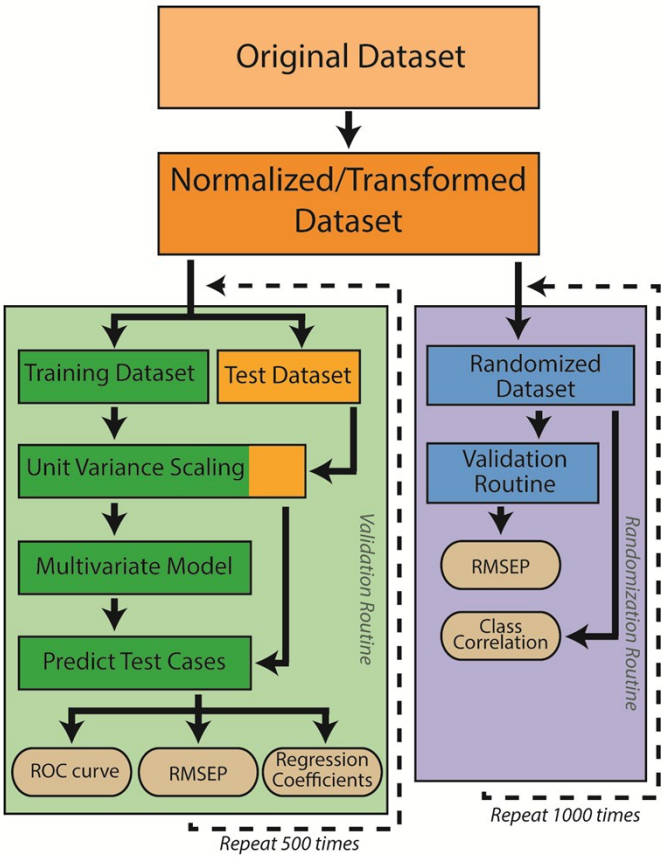


Figure s-3: Multivariate model building. Graphical representation of model building exercise by penalized logistic regression using elastic net. The complete dataset is normalized and transformed, prior to the manual selection of age- and gender-matched groups. Models are built and validated by a 10-fold cross-validation routine, which is repeated 500 times. During this routine, the receiver operator characteristic/root mean square error of prediction (ROC/RMSEP) statistics, and regression coefficients are extracted. To estimate the amount of overfitting, a randomization routine was used. By randomizing the dataset 1,000 times, and comparing the RMSEP statistic on each run to the RMSEP of the original dataset, we estimated the percentage of models which performed better at random. In this case, a low number means a low amount of over fitting.

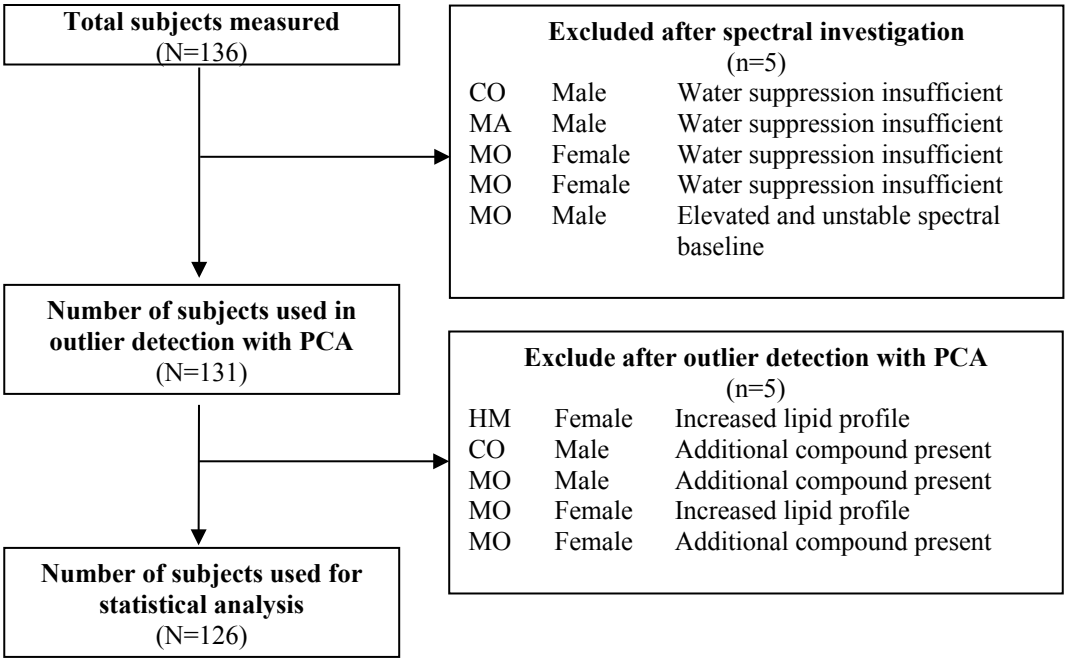


Figure s-4: Flow chart of case selection based on spectral quality and outlier detection. HM: hemiplegic migraine, MO: migraine without aura, MA: migraine with aura, CO: healthy controls.

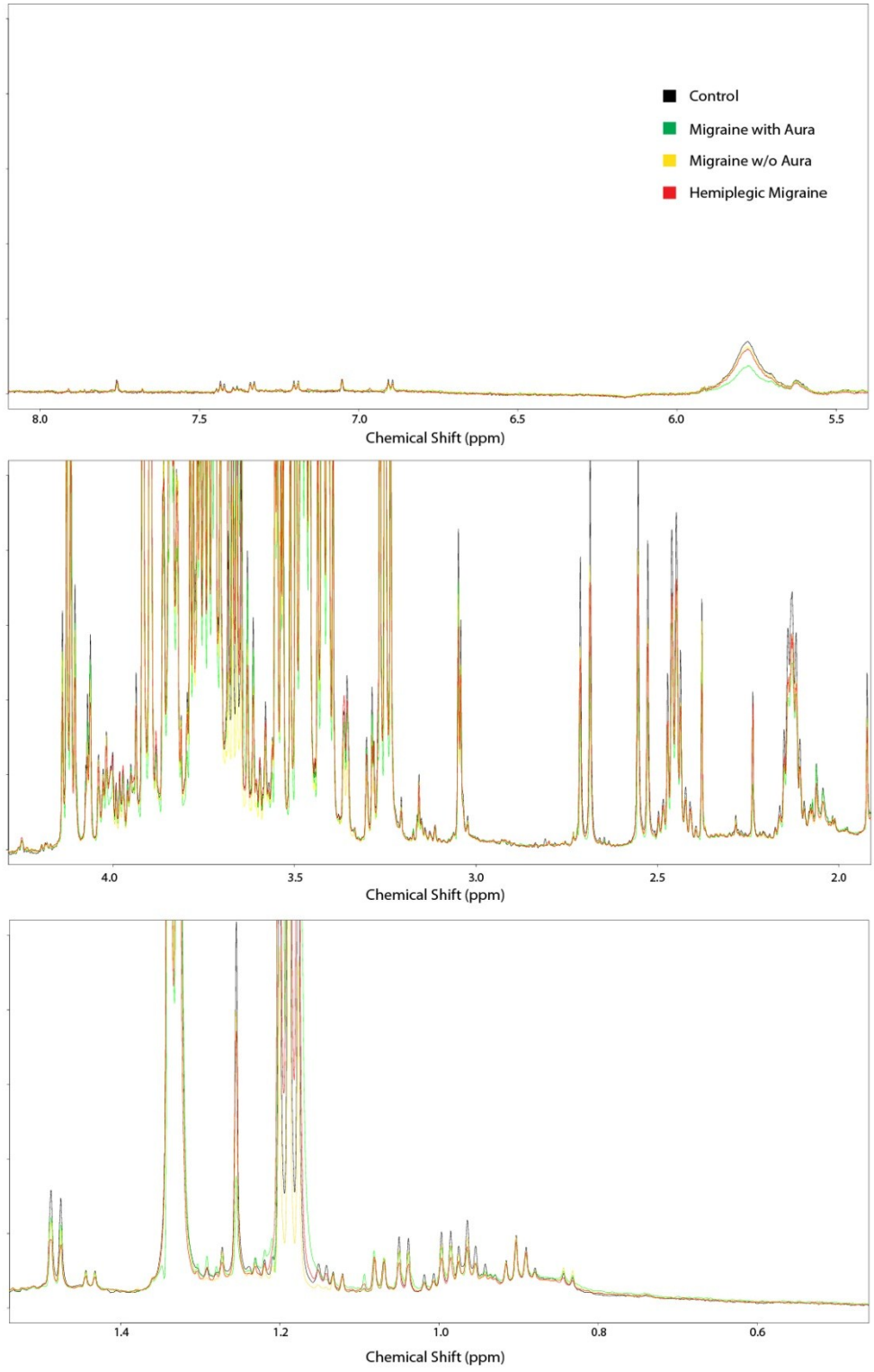


Figure s-5: Overlay of representative 1D-NOESY spectra, one for each diagnostic group. Spread in signal intensity is representative for the spread of metabolite concentration observed in the entire dataset. Separate parts of the spectrum are scaled differently, to fit peak height and spectral crowding.

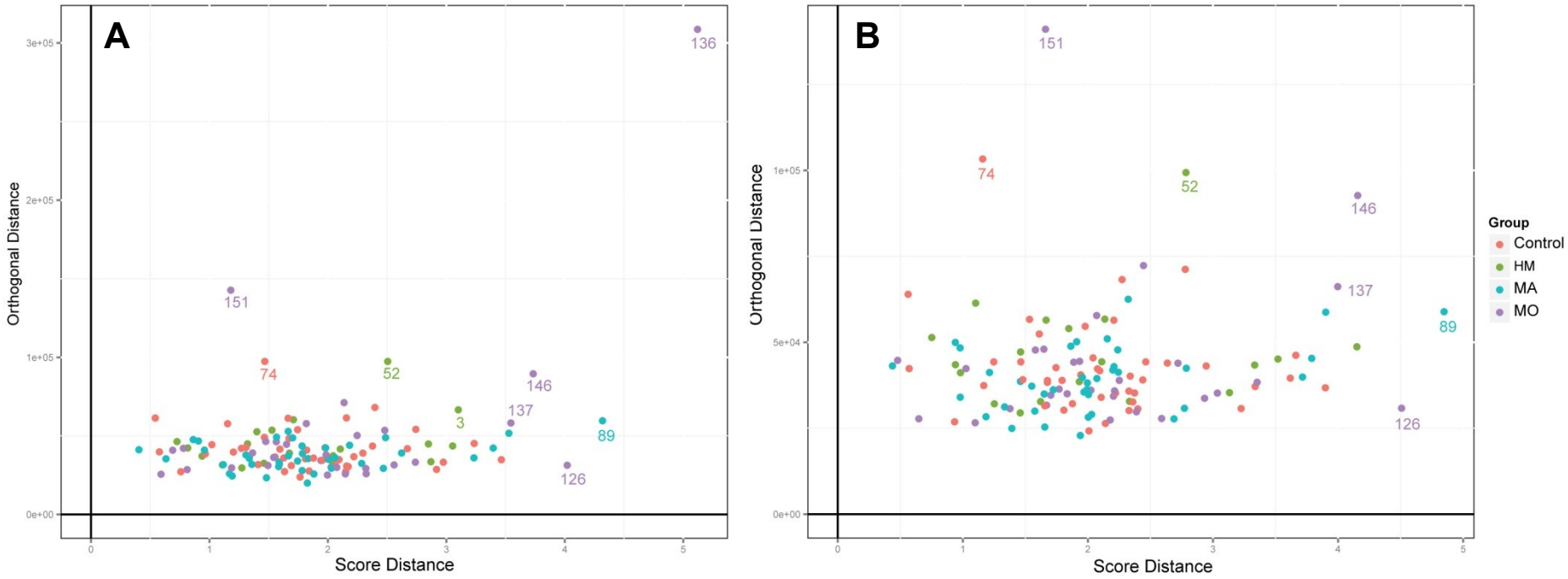
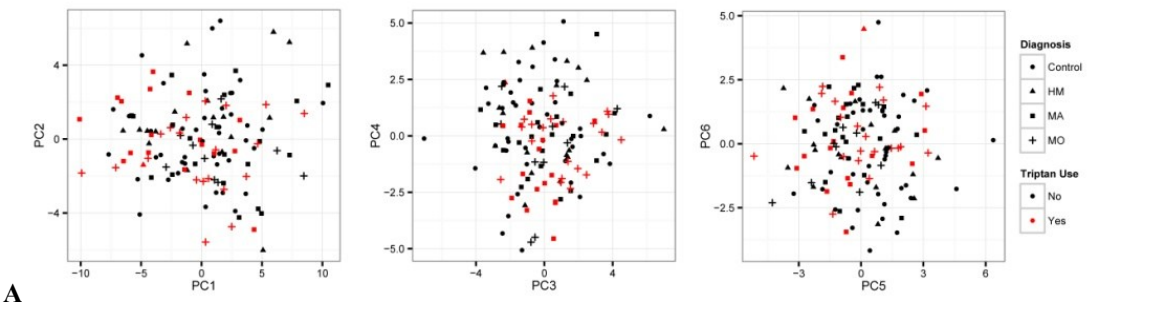
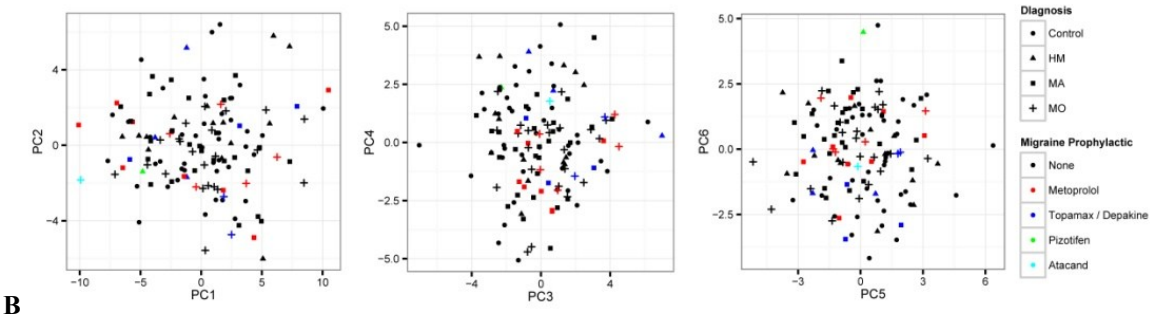


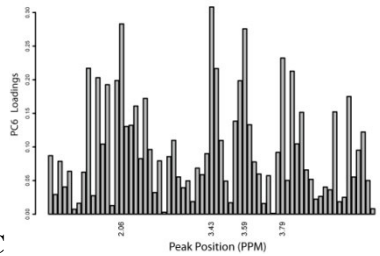
Figure s-6: PCA analysis on complete NMR spectral profiles to identify outliers. PCA analysis on complete NMR spectral profiles where the orthogonal distance to model and distance to score center are evaluated to identify outliers. Cases with a large distance indicates a spectral profile which differs greatly from the other cases. **A:** PCA plot including case 136 which shows both a large orthogonal and score distance. **B:** PCA plot without case 136 showing several other cases with a large orthogonal distance.



A



B



C

Figure s-7: PCA analysis shows no major effects of triptan (A) and migraine prophylactic (B) use. No clear separation can be seen for medicated subjects. Some clustering is observed in the 6th PC for subjects using Topamax or Depakine (B). However, the important loadings constituting the 6th PC do not involve any identified metabolites.

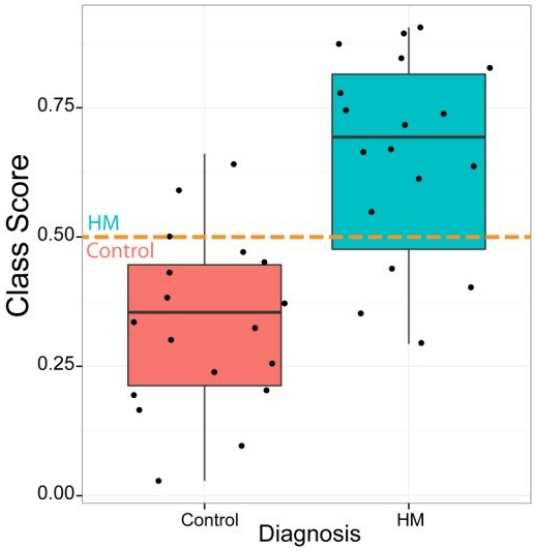


Figure s-8: Separation of controls and hemiplegic migraine patients in model training-data. Separation of the training-data according to migraine status by the penalized logistic regression (elastic net) model.

Table s-2: Result from CV-Anova of averaged cross-validation results.

	Df	SS	MS	F	p
Regression	**1**	**0.815**	**0.81**	**3.39**	**0.08**
Residual	**32**	**7.68**	**0.24**		