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NMR METABOLOMICS FOR UNDERSTANDING THE ROLE OF ALKALINE PHOSPHATASE IN NEUROTRANSMISSION AND INFLAMMATION PROCESSES

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Introduction

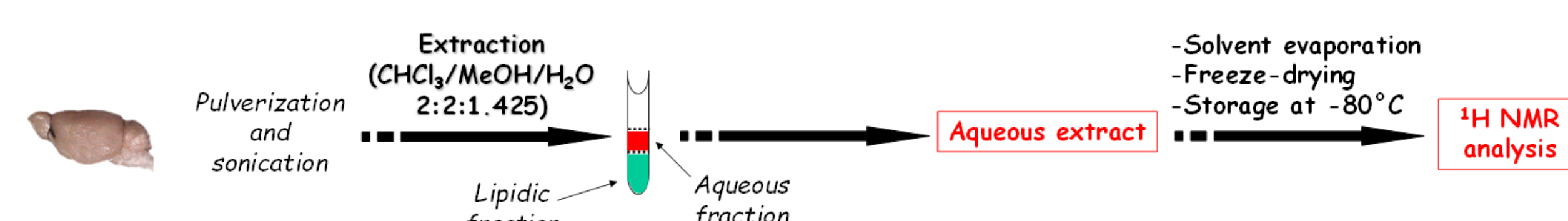
There are several genes for alkaline phosphatase (AP). One of them codes for TNAP (Tissue Non-specific Alkaline Phosphatase). It is a ubiquitous ectoenzyme located in different tissues in the body.

Mutations of the TNAP gene are responsible for hypophosphatasia, a rare disease characterized by defective bone mineralization caused by pyrophosphate accumulation. In the severe form, hypophosphatasia is associated with epileptic seizures. This neurological disorder could be explained by a decrease in GABA level due to lack of pyridoxal 5'-phosphate (PLP) dephosphorylation.

In this study, we aimed in identifying metabolic pathways associated with TNAP activity in brain and in exploring its role in the inflammatory process. We used untargeted ¹H NMR-based metabolomics on tissue extracts from two original models: (i) Akp2 mice, which is the rodent model of hypophosphatasia by invalidation of the TNAP gene, and (ii) a model of postprandial inflammation based on food intake.

Experimental part

EXTRACTION PROCEDURE



NMR

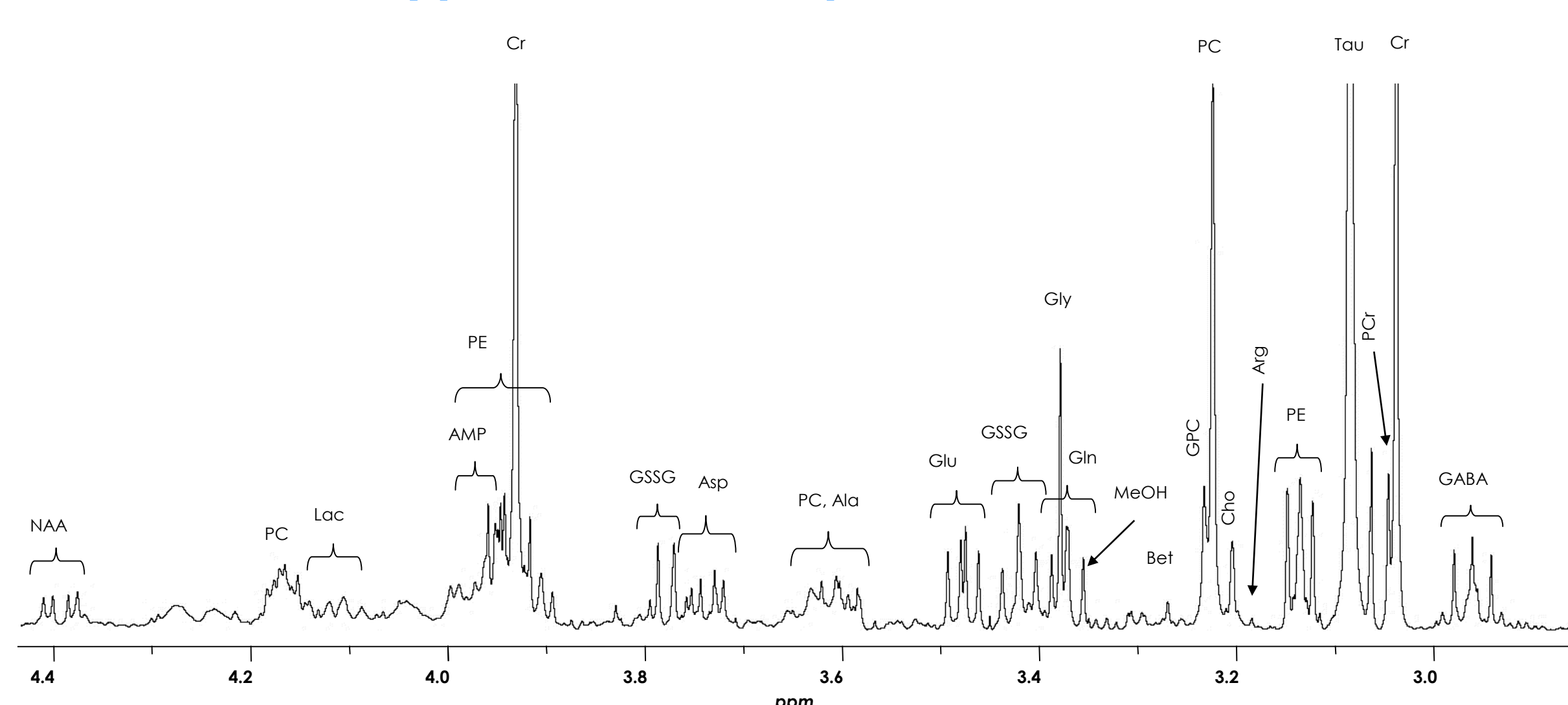
The dried aqueous extract was redissolved in deuterated borate buffer at pH 10 and a solution of sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (TSP) was added as an internal chemical shift and quantification reference.

¹H NMR experiments were recorded at 298K on a Bruker Avance 400 spectrometer equipped with a 5 mm BBI probe. A simple 1D NMR sequence (pulse-acquisition-relaxation delay) including water presaturation was used. Acquisition parameters were 3.7 s and 2.0 s for acquisition time and relaxation delay respectively, with a spectral width of 12 ppm, a flip angle of 30° and 768 scans.

STATISTICAL ANALYSES

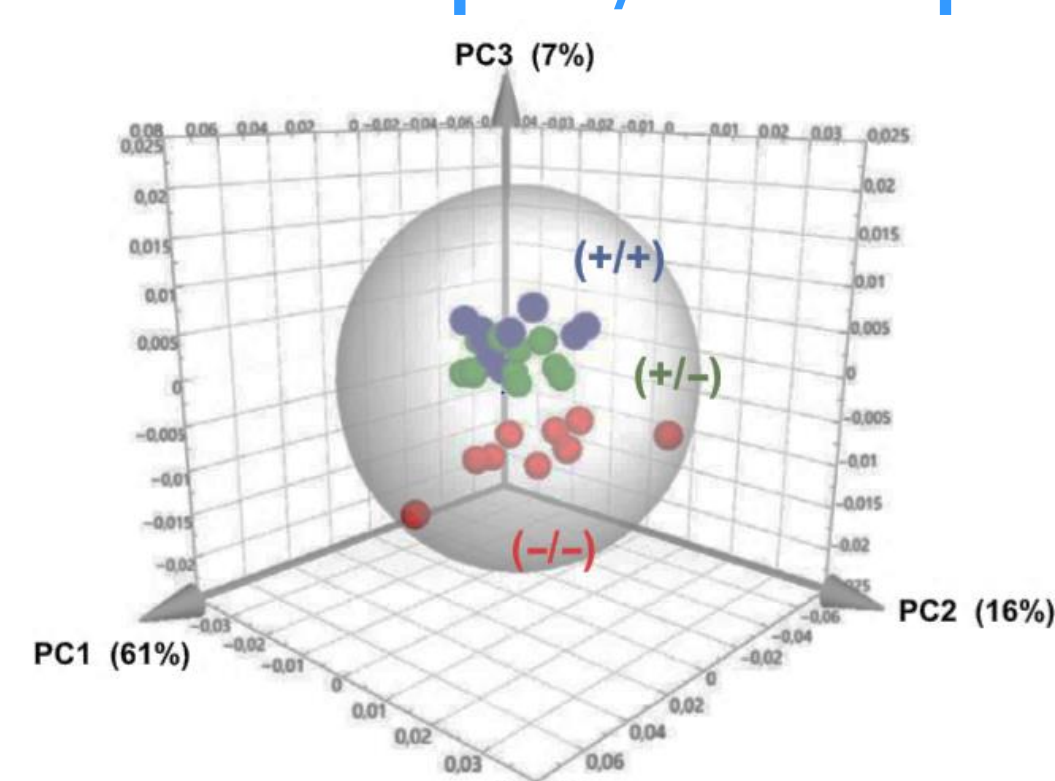
The bin area method based on intelligent bucketing (KnowItAll®, BIO-RAD) was used for segmenting NMR spectra. After normalization, univariate (boxplot, p-value) and multivariate analyses (PLS-DA (Partial Least Squares-Discriminant Analysis)) were applied to NMR data using the free R and SIMCA P+ (Umetrics®) softwares. Each statistical model was validated thanks to the Q² (predictive value of the model) and CV-ANOVA (ANalysis Of Variance of Cross-Validated predictive residuals) values.

Typical ¹H NMR spectrum



¹H NMR spectrum 4.4- 2.9 ppm region of an aqueous extract of the brain from an Akp2 (+/+) mouse

Cluster analysis revealed separation of Akp2 -/- vs Akp2 +/- and +/+ mice

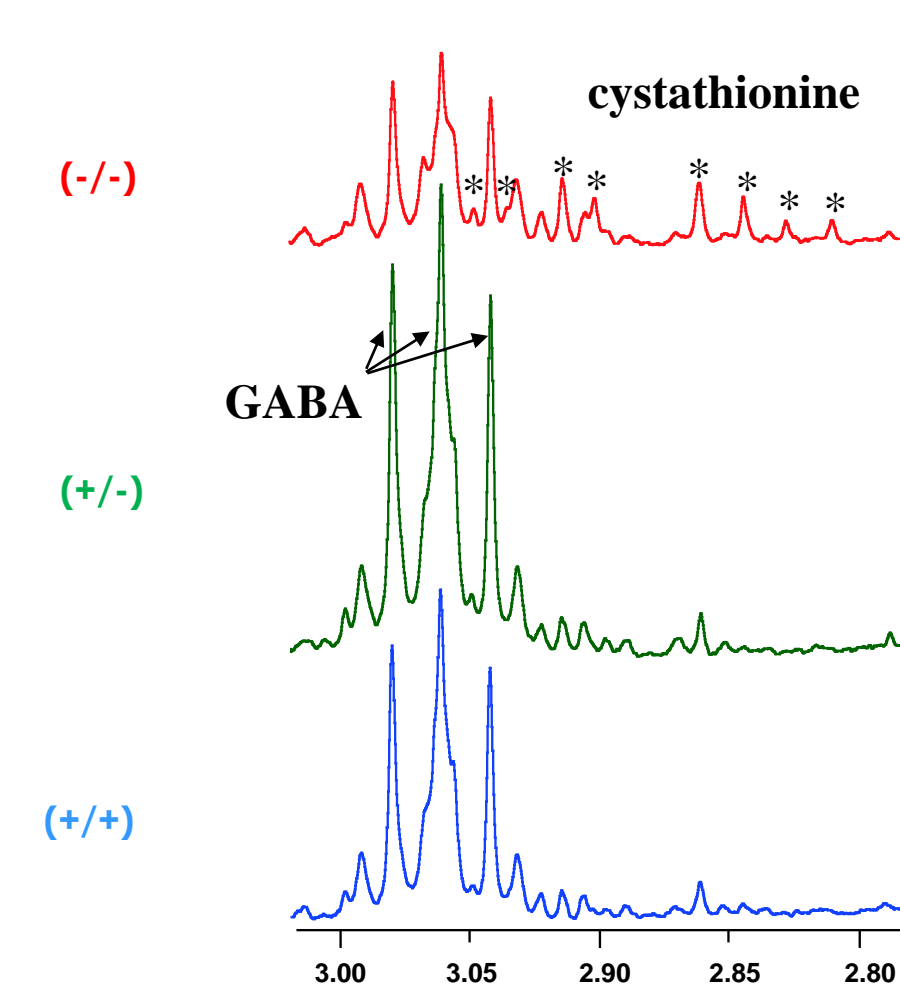


- Unsupervised statistical analysis (PCA) indicated that the metabolome of Akp2^{-/-} mice was distinct from that of Akp2^{+/-} and Akp2^{+/+} mice.

Akp2 mice model

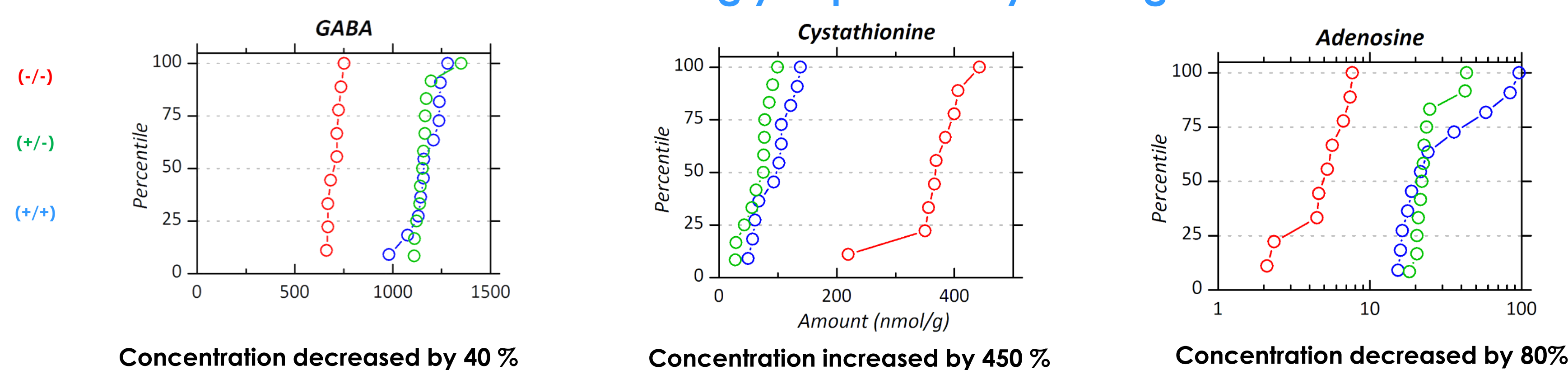
- 39 metabolites identified in whole brain extract.
- 8 metabolites** significantly impacted by TNAP gene invalidation (cystathionine, adenosine, GABA, methionine, histidine, 3-methylhistidine, N-acetylaspartate and N-acetyl-aspartylglutamate).
- Akp2 (+/-) / (+/+) mice have very **similar metabolomes**.
- Very large changes in Knockout mice for **GABA, cystathionine and adenosine** concentration compared to Akp2 (+/+) and Akp2 (+/-).
- GABA and adenosine levels were decreased** in Akp2 (-/-) mice compared to others.
- Cystathionine levels were increased** in Akp2 (-/-) mice compared to others.

Metabolic changes

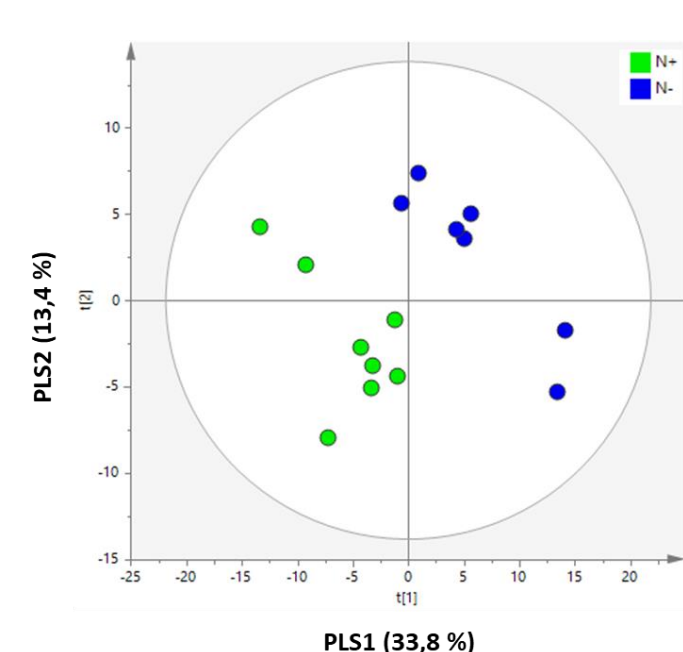


Comparison of ¹H NMR spectra (zoom around 2.9 ppm) of aqueous brain extracts from Akp2^{+/-} (blue), Akp2^{+/-} (green), and Akp2^{-/-} (red) mice.

3 metabolites strongly impacted by TNAP gene invalidation



Brain extract analysis



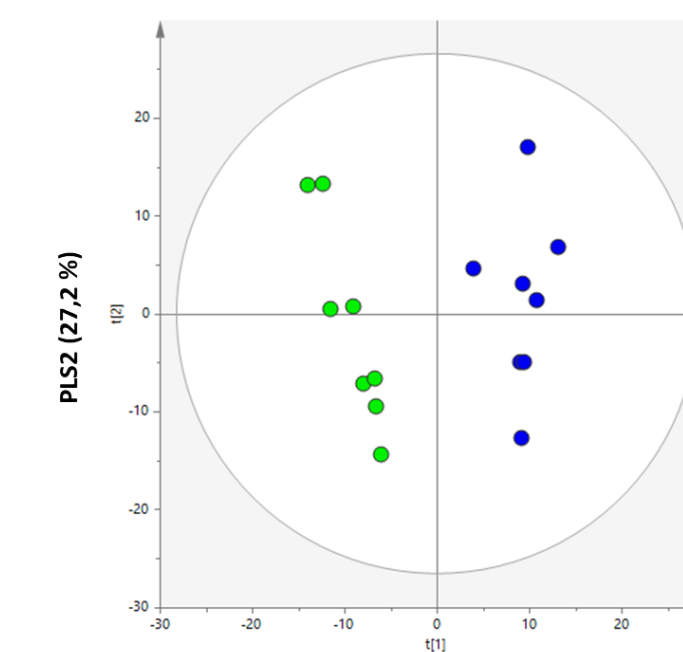
- 3 main discriminating metabolites were found.
- Levels of **methionine**, lactate and succinate increased in the postprandial model.

Postprandial model (Preliminary results)

PLSDA models were validated for brain (R²Y = 0.89 ; Q² = 0.65) and liver extract (R²Y = 0.49 ; Q² = 0.89).

Metabolites were selected according to coefficient plots (confidence intervals derived from jack-knifing ($\alpha = 0.95$) < coefficient values), VIP values (>1.5), and *p*-values of Wilcoxon test.

Liver extract analysis



- 10** main discriminating metabolites were identified : glucose, lactate, GSH, alanine, betaine, GPC, PC, choline, hypotaurine and succinate.
- Levels of identified discriminating metabolites except choline increase in the postprandial model.

Conclusion

- Pyridoxal phosphate (PLP) dephosphorylation by TNAP is required for cystathionine and GABA production; moreover TNAP ectonucleotidase function produces adenosine.

→ The metabolomic approach showed that TNAP deficiency **reduces the level of GABA and adenosine** and **increases cystathionine level** in the brain.

- In parallel with its regulatory role in neuronal synaptic transmission, **TNAP could be involved in inflammation**.

→ The metabolic changes observed in this study suggest that TNAP is directly involved in the production of anti-inflammatory agents (**adenosine**) or indirectly from precursor agents (**methionine** and **cystathionine**, precursor of H₂S).

