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► **To cite this version:**

Stéphane Balayssac, Thomas Cruz, Marie Gleizes, Gary Lutin, Juana Como, et al.. NMR metabolomics for understanding the role of alkaline phosphatase in neurotransmission and inflammation processes. European RFMF Metabomeeting, Jan 2020, Toulouse, France. hal-02875612

**HAL Id: hal-02875612**

**<https://hal.science/hal-02875612v1>**

Submitted on 19 Aug 2022

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

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## Introduction

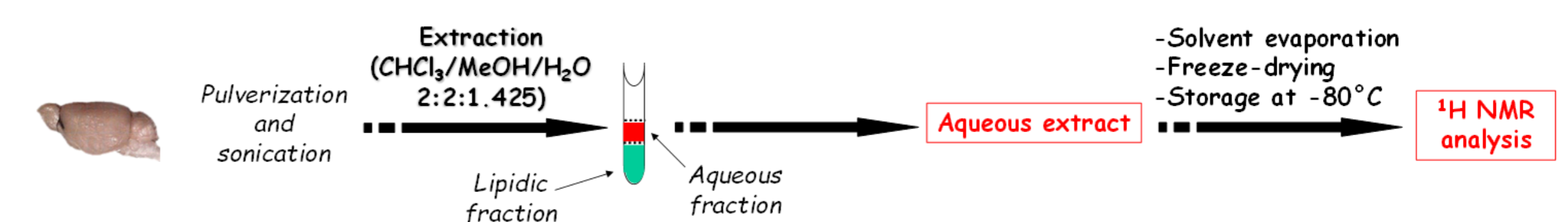
There are several genes for alkaline phosphatase (AP). One of them codes for TNAP (Tissue Non-specific Alkaline Phosphatase). It is a ubiquitous ectoenzyme located in different tissues in the body.

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 <sup>1</sup>H 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

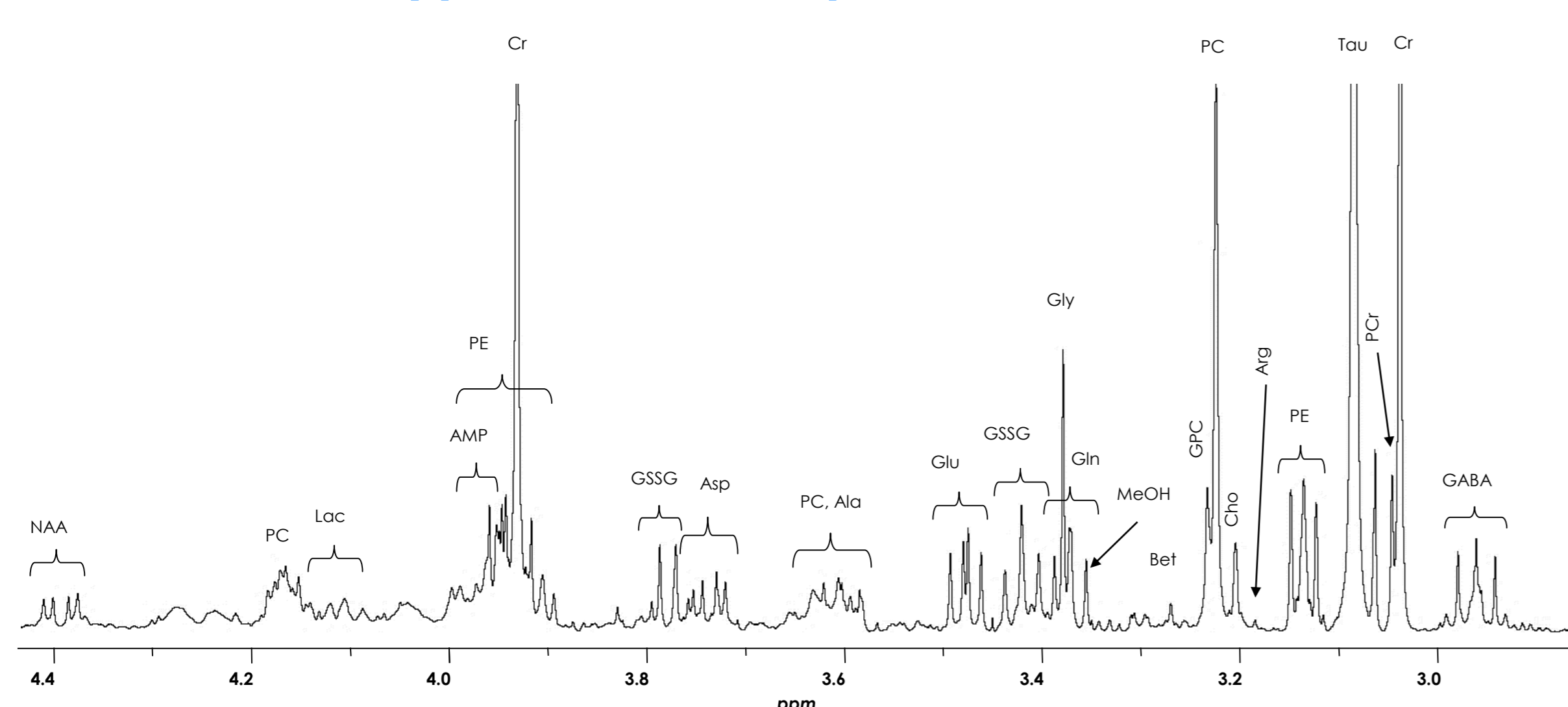
The dried aqueous extract was redissolved in deuterated borate buffer at pH 10 and a solution of sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (TSP) was added as an internal chemical shift and quantification reference.

<sup>1</sup>H 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

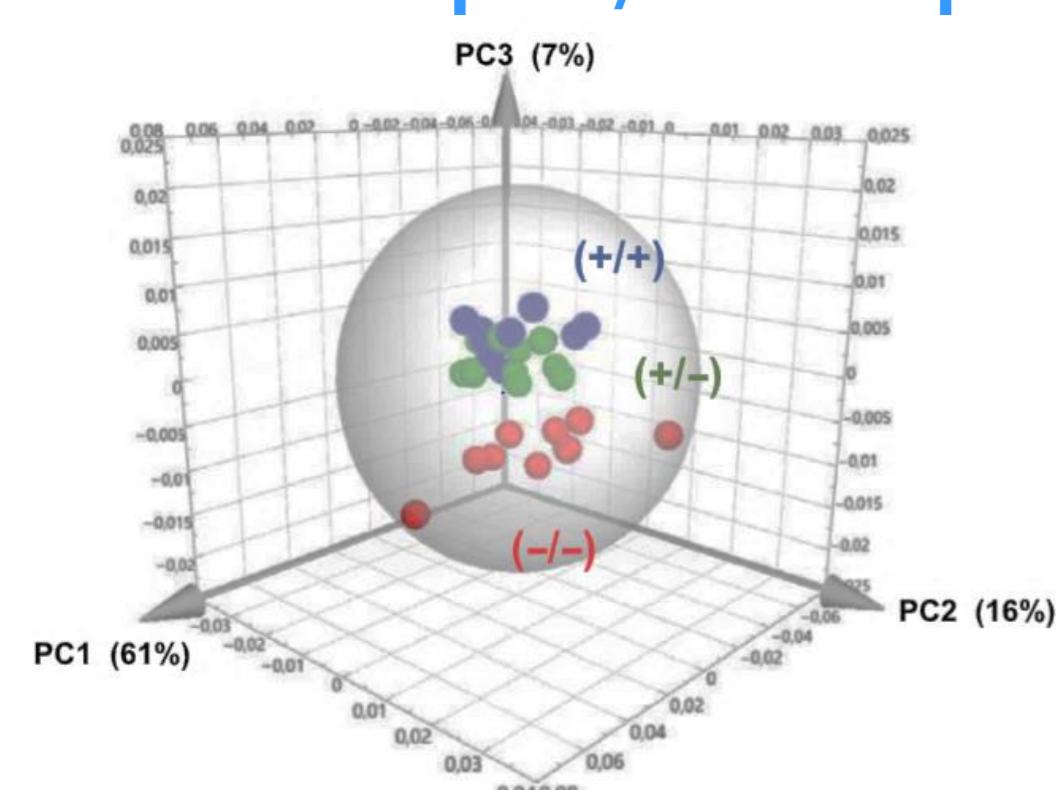
The bin area method based on intelligent bucketing (KnowItAll®, BIO-RAD) was used for segmenting NMR spectra. After normalization, univariate (boxplot, p-value) and multivariate analyses (PLS-DA (Partial Least Squares-Discriminant Analysis)) were applied to NMR data using the free R and SIMCA P+ (Umetrics®) softwares. Each statistical model was validated thanks to the Q<sup>2</sup> (predictive value of the model) and CV-ANOVA (ANalysis Of Variance of Cross-Validated predictive residuals) values.

## Typical <sup>1</sup>H NMR spectrum



<sup>1</sup>H 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

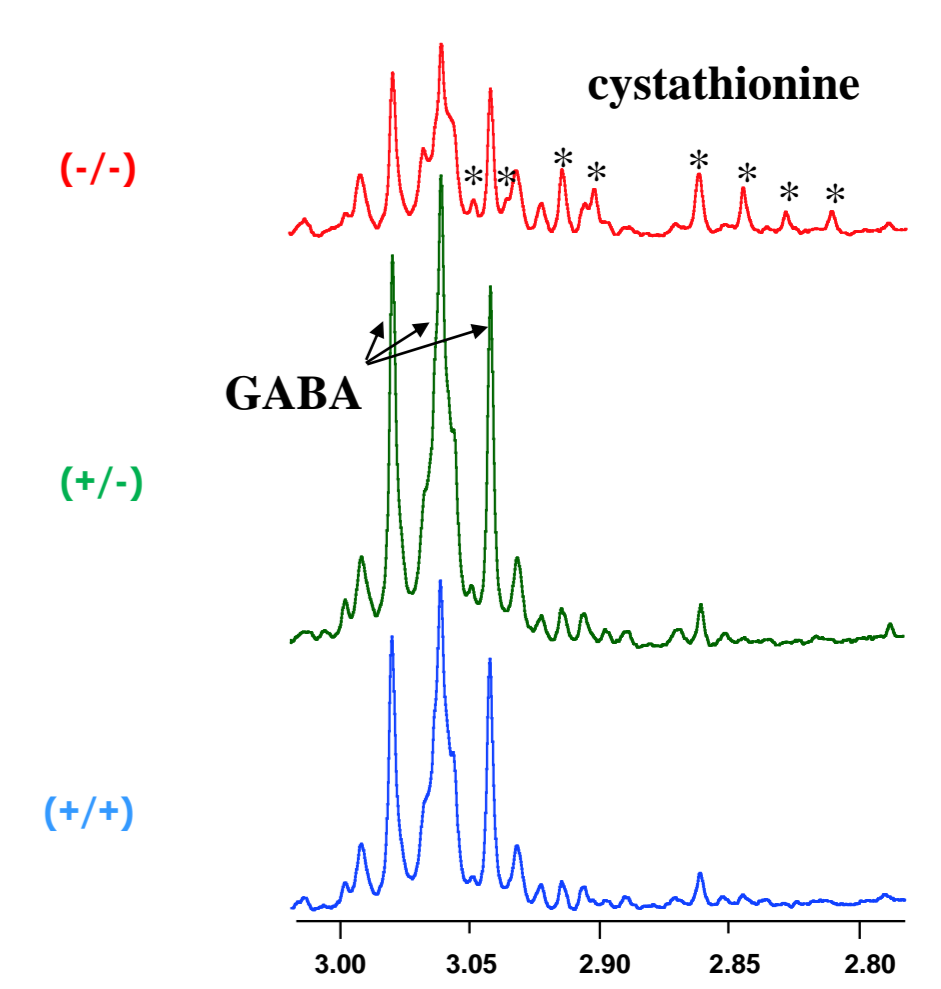


- Unsupervised statistical analysis (PCA) indicated that the metabolome of Akp2<sup>-/-</sup> mice was distinct from that of Akp2<sup>+/-</sup> and Akp2<sup>+/+</sup> mice.

## Akp2 mice model

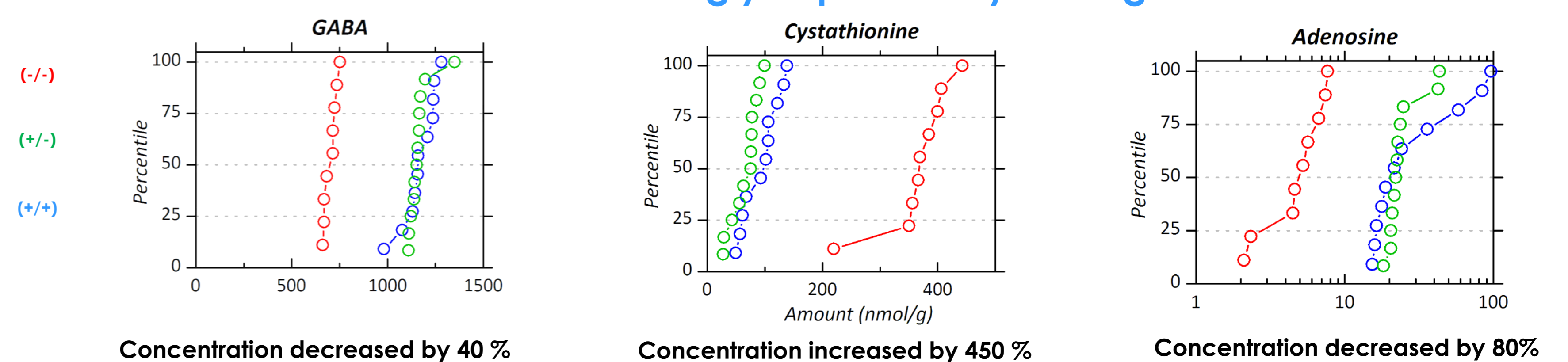
## Metabolic changes

- 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.

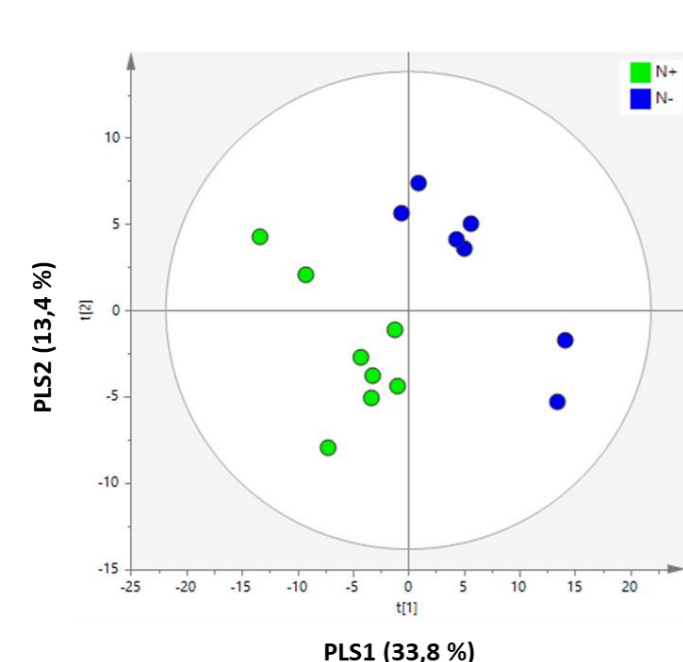


Comparison of <sup>1</sup>H NMR spectra (zoom around 2.9 ppm) of aqueous brain extracts from Akp2<sup>+/+</sup> (blue), Akp2<sup>+/-</sup> (green), and Akp2<sup>-/-</sup> (red) mice.

## 3 metabolites strongly impacted by TNAP gene invalidation



## Brain extract analysis



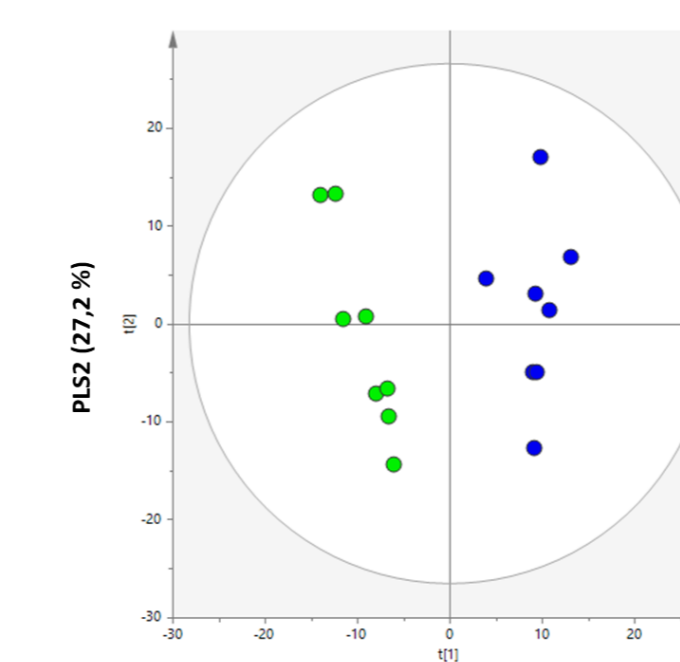
- 3 main discriminating metabolites were found.
- Levels of methionine, lactate and succinate increased in the postprandial model.

## Postprandial model (Preliminary results)

PLSDA models were validated for brain ( $R^2Y = 0.89$ ;  $Q^2 = 0.65$ ) and liver extract ( $R^2Y = 0.49$ ;  $Q^2 = 0.89$ ).

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 H<sub>2</sub>S).

