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Quantification of cholesterol metabolites in the brain by on-tissue derivatization mass spectrometry imaging in a mouse model of Huntington's disease

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Huntington's disease (HD) is an autosomal neurodegenerative disorder caused by a mutation of the IT15 gene on chromosome 4 [1], translating for the mutant huntingtin protein (mHTT). mHTT reduces the sterol-regulatory element-binding protein (SREBP) transcription factor translocation, essential for cholesterol (Chol) synthesis, leading to a significant reduction of Chol metabolism in the brain. Notably, different Chol metabolites have been shown to be down-regulated in the brain of R6/2 mice [2].

Here, we proposed a high-resolution imaging-mass spectrometry (IMS) approach, for the study of the spatial distribution of Chol brain metabolites and their simultaneous quantitation using in-house developed software.

In the present study, the whole brain of both WT and R6/2 mice at 12 weeks was employed and three to five sagittal sections were cut and mounted on the target at–20°C as replicates.

The IMS method was developed on an AP-MALDI source (Mass-Tech), installed on an LTQ Orbitrap XLmass spectrometer (Thermo Scientific), and allowed the simultaneous quantitation and spatial distribution study of the free form of 24OHC, Chol, desmosterol, and 7-dehydrocholesterol in the brain.

We set up the IMS method to evaluate the distribution of the selected Chol metabolites in the brain from R6/2 mice, focusing on the striatum, the most affected area by neurodegeneration in HD. Our IMS approach included two-derivatization steps directly on tissue slices that use Chol oxidase and Girard's T reagent and MS/MS experiments. The evidences confirmed the data previously obtained with LC-MS analysis *(unpublished data),* highlighting a significant reduction of desmosterol, 24OHC and chol levels. As desmosterol is a Chol metabolite representative of the Bloch pathway, our results suggested that this is the most affected pathway by HD.

References

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