Ultra-sensitive Quantification of UBE3A in Human CSF Using SISCAPA-MS

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INTRODUCTION

Angelman Syndrome (AS) is a rare neurodevelopmental disorder caused by the loss of maternal ubiquitin-protein ligase E3A (UBE3A) expression in neurons, where the paternal allele is silenced by a neuron-specific antisense transcript. UBE3A encodes a ubiquitin ligase essential for synaptic development and neuronal function. Therapeutic strategies aim to restore UBE3A activity by reactivating the paternal allele or delivering functional protein to the brain. However, quantifying UBE3A in cerebrospinal fluid (CSF) is technically challenging due to its extremely low abundance and the limitations of conventional immunoassays[1]. To overcome this, we developed a highly sensitive hybrid assay using Stable Isotope Standards and Capture by Anti-Peptide Antibodies combined with LC-MS (SISCAPA-MS) to quantify UBE3A in CSF. Here, we present the development and characterization of this assay and demonstrate its application in CSF samples.

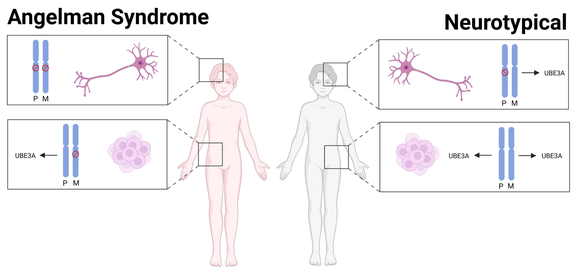

Figure 1 UBE3A expression in neurotypical individuals vs. Angelman Syndrome patients.

METHODS

DEVELOPMENT OF UBE3A SISCAPA ASSAY REAGENTS

- **Proteotypic peptides:** Selected best-performing peptide after mass spec acquisition and used as immunogens to develop high affinity anti-peptide rabbit monoclonal antibodies (mAbs).
- **B-cell sorting and screening:** Antibody-producing B-cells are isolated and mAbs screened for performance in the SISCAPA workflow to identify top candidates.
- **Recombinant mAb production:** The selected mAb is then recombinantly produced and used to enrich the target peptide from digested sample matrix.

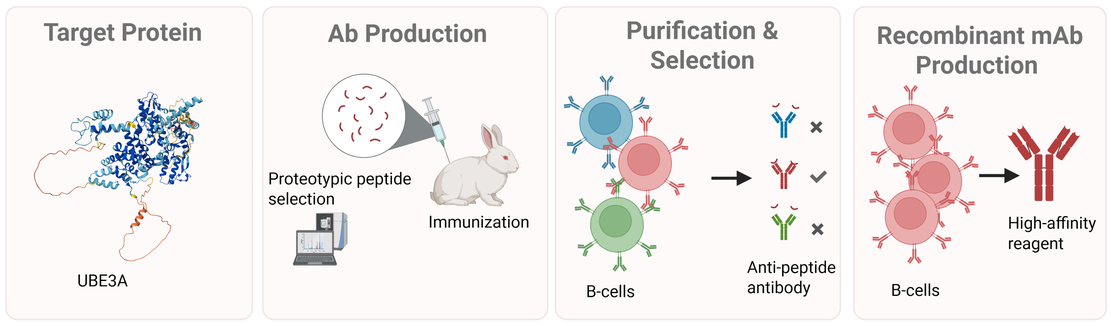

Figure 2 Workflow depicting the development of high-affinity mAbs for SISCAPA assay of UBE3A. Predicted protein structure from AlphaFold DB[2] (UniProt ID: Q05086).

SISCAPA-MS ASSAY FOR ABSOLUTE QUANTIFICATION OF UBE3A

- **Samples:** Post-mortem CSF samples from six donors (ages 54–84) with varying causes of death were analyzed.
- **Quantification:** Calibration curve for qualification ranged from 32 pg/mL to 0.25 pg/mL using recombinant UBE3A in surrogate matrix (artificial CSF + 0.1% BSA). Five QC levels were included: ULOQ (32 pg/mL), HQC (20.6 pg/mL), MQC (12.8 pg/mL), LQC (0.75 pg/mL), and LLOQ (0.25 pg/mL). All samples were spiked with stable isotope standard (SIS) peptides.
- **Automated sample preparation:** Implemented on a Hamilton STAR Plus workcell with input sample volume of 100 µL (**Figure 3**).
- **LC-MS acquisition:** Peptides were analyzed using FAIMS-assisted PRM with a 40 SPD Whisper ZOOM method on a Evosep One coupled to a Thermo Exploris 480 instrument (**Figure 3**).
- **Data analysis:** PRM data were processed using Biognosys' SpectroDive™ 12.
- **Assay characterization**: Accuracy and precision were assessed across four runs. Sentivity was assessed by extending the calibration with serial two-fold dilutions. Stability of standards and QCs were evaluated under freeze-thaw (3 cycles), benchtop (3 h), and fresh-frozen conditions. Lot-to-lot variability of mAbs was also characterized.

WORKFLOW AND INSTRUMENTATION FOR SISCAPA-MS ASSAY OF UBE3A

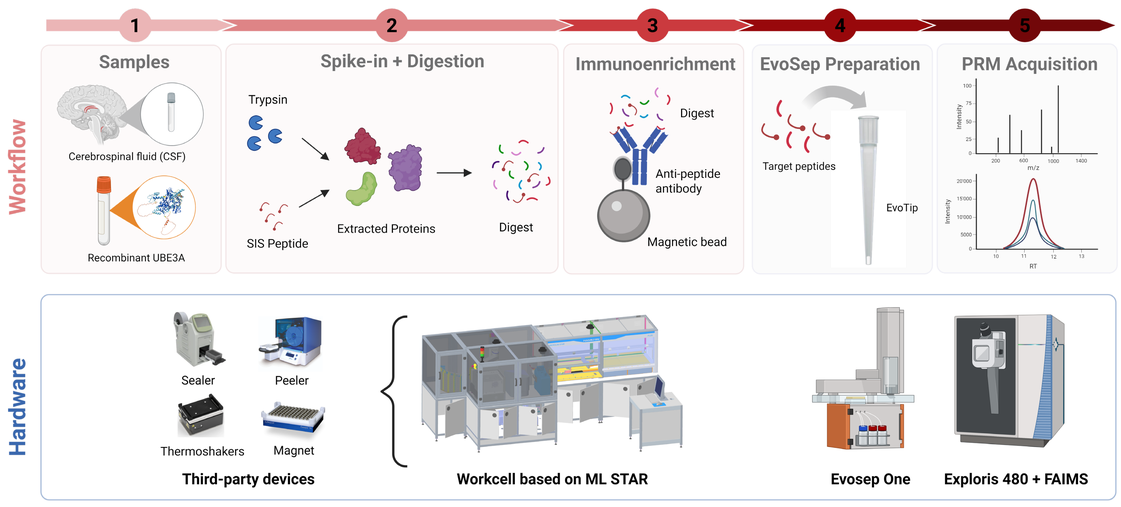

Figure 3 Graphic depicting the processing of CSF or recombinant UBE3A samples with SISCAPA-MS assay: Samples were spiked with SIS peptides and digested with trypsin, followed by immunoenrichment using monoclonal anti-peptide antibodies. Enriched peptides were loaded onto EvoTips and analyzed using LC-PRM acquisition.

RESULTS

BIOANALYTICAL CHARACTERIZATION[3] OF THE UBE3A SISCAPA-MS ASSAY

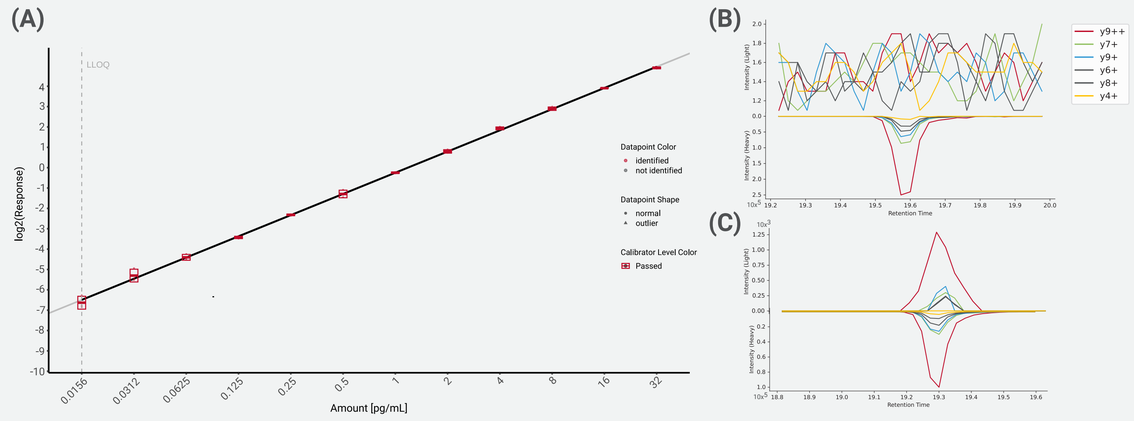

Figure 4 (**A**) Sensitivity assessment of the assay using a 12-point, 2-fold dilution curve. LLOQ was established after applying a q-value filter (0.01), zero calibrator filter (≥3× blank intensity) and accuracy and precision filters (≤20%). Mirror plot of MS2 XICs showing endogenous (top) and SIS (bottom) peptides for monitored fragment ions in (**B**) blank and (**C**) 0.016 pg/mL rUBE3A samples.

QC Sample (rUBE3A [pg/mL])	Mean Accuracy (%)	Precision (CV%)
ULOQ (32)	101.4	3.3
HQC (20.6)	102.9	9.2
MQC (12.8)	97.9	8.5
LQC (0.75)	99.4	7.0
LLOQ (0.25)	105.8	6.7

Table 1 Mean accuracy and precision across 4 runs for all QC levels.

- Sensitivity of the assay was determined at 0.016 pg/mL using a 12-point, 2-fold dilution curve (**Figure 4**). QC samples showed accuracy 97.9–105.8% and precision (%CV) 3.3–9.2% across 4 runs (**Table 1**).
- QC stability was confirmed under freeze–thaw, benchtop and fresh-frozen conditions with accuracy 88–100% and precision (%CV) <10%. Testing of different mAb lots showed high reproducibility with accuracy >95% and precision (%CV) <2.2% (data not shown).

MEASUREMENT OF UBE3A LEVELS IN CSF SAMPLES

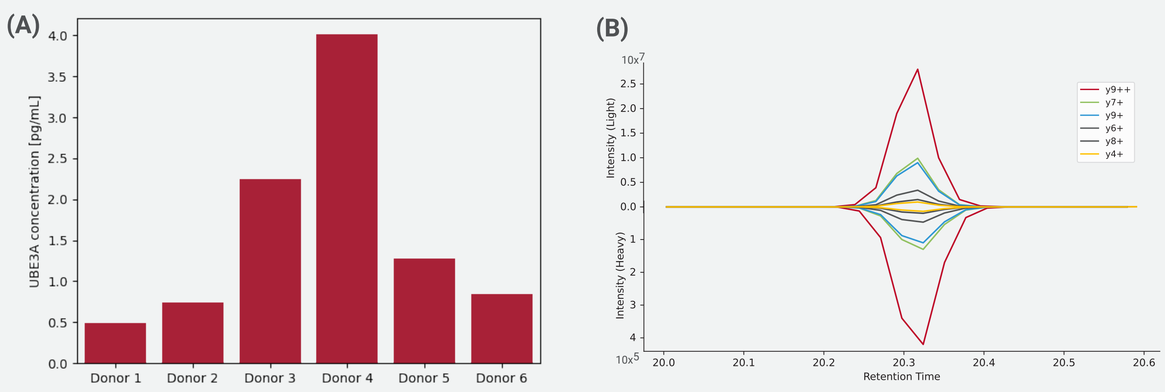

Figure 5 (**A**) UBE3A concentrations in CSF from six post-mortem donors. (**B**) Mirror plot of MS2 XIC showing endogenous (top) aligned with SIS (bottom) peptides for monitored fragment ions of Donor 1.

REFERENCES

[1] Mabrouk et. al. Novel method for detection of UBE3A protein in CSF from individuals with Angelman Syndrome, Molecular Genetics and Metabolism, 2025

[2] Abramson et. al. Accurate structure predictionof biomolecular interactions with Alphafold 3, Nature, 2024

[3] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). M10 Bioanalytical Method Validation and Study Sample Analysis: Guidance for Industry, 2022

[4] Graphical illustrations created with biorender.com

CONCLUSIONS

- **A highly sensitive SISCAPA-MS assay was developed and successfullly qualified for absolute quantification of UBE3A in human CSF.**
- **The assay was qualified over a quantification range of 0.25 to 32 pg/mL, demonstrating a true sensitivity of 0.016 pg/mL with accuracies of 98-106% and precision of 3-9%.**
- **The qualified assay was successfully applied to post-mortem CSF samples, with UBE3A concentrations measured between 0.4-4 pg/mL**
- **Next step: Assay application quantify UBE3A in Angelman Syndrome cohort samples to support drug development and to longitudinally monitor treatment prognosis.**

CONTACT

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