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# In search for novel biomarkers of frontotemporal dementia – developing a hypothalamic peptide panel

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## Introduction

### What is frontotemporal dementia (FTD)?

*Figure 1. Lobes of the human brain. Frontal lobe in red and temporal lobe in green are affected in FTD.*

FTD is a progressive, neurodegenerative disorder that affects the frontal and temporal lobes. It can cause problems with behaviour, language and/or movement, and is therefore clinically separated into behavioural variant FTD (bvFTD) or primary progressive apahasia (PPA).

## Aim

### Developing a targeted MRM peptide assay

Reliable biomarkers will help to diagnose, prognose and stratify FTD subtypes. They are also paramount for monitoring treatment response in future clinical trials.

Changes in eating behaviour is one of the clinical features of bvFTD. Piguet *et al* found that patients with high feeding disturbance had reduced posterior hypothalamic volume, suggesting hypothalamic involvement in abnormal feeding behaviour in FTD.

A targeted proteomics panel evaluating appetite-regulating hypothalamic peptides is being developed using Multiple Reaction Monitoring assays (MRM). It includes central and peripheral peptides, involved in the orexigenic and anorexigenic pathways.

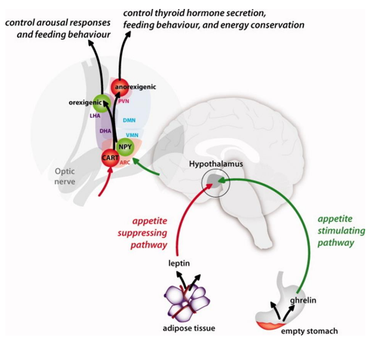



*Figure 2. Appetite-controlling central and peripheral pathways. Main neurons involved in anorexigenic pathway include CART, whereas NPY acts as the main modulator of the orexigenic pathway. From Piguet et al.*

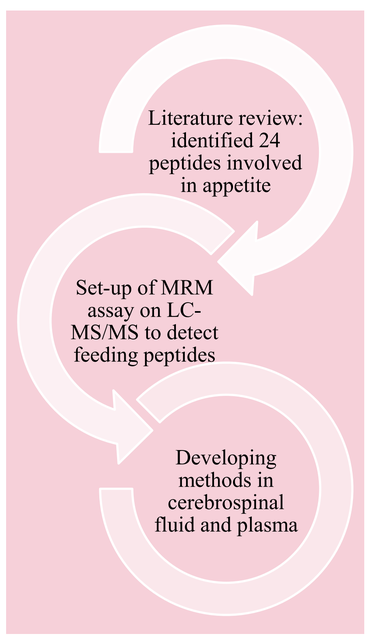



*Figure 3. Overview of study design.*

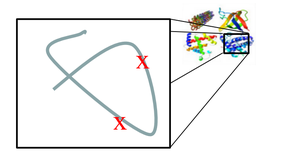
## Methods

The processing methods prior to LC-MS/MS analysis differ from CSF to plasma, due to the complexity of sample matrix. For CSF, proteins are precipitated first by freeze-drying. In comparison, plasma samples are pre-treated with 1:1 Acetonitrile:MeOH containing 1% acetic acid. Various other pre-treatment protocols will be tested to maximize peptide enrichment. Subsequent processing steps are depicted in the figure 5 below.

| Peptide | Cleaved peptide (Tryptic) | Mass (Da) | Modifications |
|---|---|---|---|
| MCH | DFDM LR\|CMLGR\|VYR PCWQV | 2388 | |
| α-MSH | SYS MEHFR\|WGKPV | 1665 | N-terminal acetylation |
| NPY | YP SKPDNPGEDA PAEDMAR\|YYS ALR\|HYINLIT R\|QR\|Y | 4271 | C-terminal midation |
| AGRP | SSR\|R\|CVR\|L HESCLGQQVP CCDPCATCYC R\|FFNAFCYCR\| K\|LGTAMNPCS R\|T | 12472 | |
| OXT | CYIQNCPLG | 1010 | C-terminal midation |
| LEP | VPIQK\|VQDDTK\|TLIK\|TIVTR\|INDI SHTQSVSSK\|QK\|VTGLDFIPGLHPI LTLSK\|MDQTLAVYQQILTSMPSR\| NVIQISNDLENLR\|DLLHVLAFSK\|S CHLPWASGLETLDSLGGVLEASG YSTEVVALSR\|LQGSLQDMLWQL DLSPGC | 16026 | |
| CART | VPIYEK\|K\|YGQVPMCDAGEQCAV R\|K\|GAR\|IGK\|LCDCPR\|GTSCNSFL LK\|CL | 5251 | |
| NPW | WYK\|HVASPR\|YHTVGR\|AAGLLM GLR\|R\|SPYLW | 3543 | |
| GALA | GWTLNSAG YLLGPHAVGN HR\|SFSDK\|NGL TS | 3157 | |
| GHRL | GSSFLSPEHQR\|VQQR\|K\|ESK\|KPPA K\|LQPR | 3381 | Octanoylated serine |
| GALP | APAHR\|G R\|GGWTLNSAG YLLGPVLHLP QMGDQDGK\|R\|E TALEILDLWK\| AIDGLPYSHP PQPS | 6500 | |
| CBLN | SGSAK\|VAFSAIR\|STNH | 1633 | |
| CRH | SEEPPISLDLTFHLLR\|EVLEMAR\|A EQLAQQAHSNR\|K\|LMEIL | 4759 | C-terminal amidation |
| NSFT | VPIDIDK\|TK\|VQNIHPVESAK\|IEPP DTGLYYDEYLK\|QVIDVLETDK\|HF R\|EK\|LQK\|ADIEEIK SGR\|LSK\|ELDLVSHHVR\|TK\|LDEL | 9551 | |
| CCK | VSQR\|TDGESR\|AHLGALLAR\|YIQ QAR\|K\|APSGR\|MSIVK\|NLQNLDPS HR\|ISDR\|DYMGWMDF | 6644 | Sulfotyrosine; C-terminal amidation |
| OBE | FNAPFDVGIK\|LSGVQYQQHSQAL | 2546 | C-terminal amidation |
| GRP | VPLPAGGGTVLTK\|MYPR\|GNHWA VGHLM | 2859 | C-terminal amidation |
| OXM | HSQGTFTSDYSK\|YLDSR\|R\|AQDF VQWLMNTK\|R\|NR\|NNIA | 4448 | Phosphoserine |
| INS-B | FVNQHLCGSHLVEALYLVCGER\|G FFYTPK\|T | 3429 | |
| PPP | APLEPVYPGDNATPEQMAQYAAD LR\|R\|YINMLTRPR\|Y | 4181 | C-terminal amidation |
| IGF2 | AYRPSETLCGGELVDTLQFVCGD R\|GFYFSRPASR\|VSR\|R\|SR\|GIVEEC CFR\|SCDLALLETYC ATPAKSE | 7469 | |
| Orexin | AGAEPAPRPCLGR | 1294 | |
| GLUC | HSQGTFTSDYSK\|YLDSR\|R\|AQDF VQWLMNT | 3482 | |
| C-PEP | EAEDLQVGQVELGGGPGAGSLQP LALEGSLQ | 3020 | |

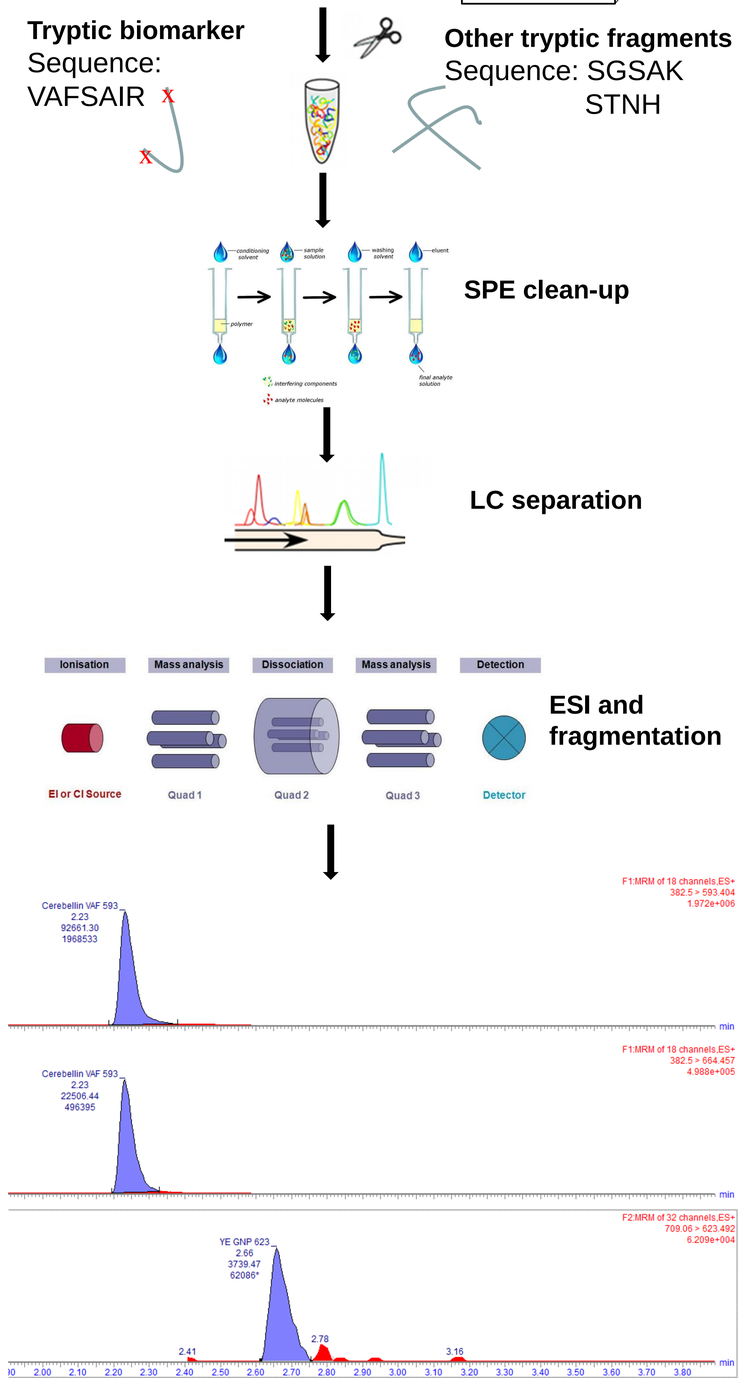
*Figure 4. List of hypothalamic peptides included in MRM assay.*

**Proteotypic peptide selection**
Sequence:
SGSAK|VAFSAIR|STNH



**Tryptic biomarker**
Sequence:
VAFSAIR

**Other tryptic fragments**
Sequence: SGSAK
STNH



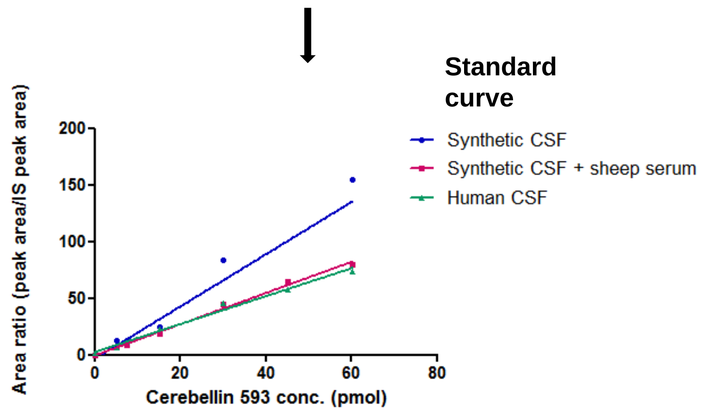




*Figure 5. Methodology. After protein enrichment, sample subject to enzymatic digestion using trypsin (specifically cleaves at arginine and lysine). Subsequent SPE clean-up is used to eliminate interfering substances, such as salts. Finally, samples are separated during a liquid chromatography run using a C18+ cortects column. Following electron spray ionization and fragmentation, parent and daughter ions are selected in the triple quad. Data is analysed using targetlynx and normalized to yeast enolase added to the initial sample. Standard curves have been set-up in various sample matrices for each peptide, including synthetic CSF and human CSF.*

## Conclusion

In summary, a targeted proteomics panel investigating hypothalamic peptides is being developed for testing biofluids including CSF and plasma/serum. Further exploration on a large clinically defined cohort will enable understanding of the differences in hypothalamic peptides in FTD and investigate whether such a panel could be used as a biomarker in FTD disease diagnosis, prognosis or stratification.

## References

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