IPD Project Details

Project ID: IPD3877

Project Title: Mass Spectrometry analysis of human matched Anterior and Posterior lobes of Pituitary

Description: This study provides a comprehensive proteomic analysis of five matched pairs of the anterior and posterior lobe of the pituitary, which was used to study the proteomic signature specific to both anterior and posterior lobes. Anterior Lobe n=5 Posterior Lobe n=5 A441 PT441 A442 PT442 A457 PT457 A459 PT459 A460 PT460

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Sample Preparation: Sample Processing Protocol- Pituitary matched anterior and posterior lobes were lysed using lysis buffer (8M Urea, 50 mM Tris pH 8.0, 75 mM NaCl, 1mM $MgCl_2$) supplemented with protease inhibitors. The homogenates were centrifuged to obtain the supernatant. Proteins in the supernatant were quantified with BCA protein quantification.

Peptide Separation: Protein enzymatic cleavage was carried out with trypsin at 37 °C for 16 h. Peptides were desalted and vacuum dried. Further, these peptides were quantified using the Scopes method. After quantification, these peptides were subjected to Lable free quantification using nLC-Orbitrap Mass Spectrometric techniques.

Protein Characterization: Data Processing Protocol The peptide mass fingerprint data obtained from MS were processed in Proteome Discoverer 2.4, using UniProt Human Proteome and contaminant database and searched with built-in three search engines Sequest HT, MSPep Search and MS Amanda 2.0. In proteome discoverer, raw files were processed with Trypsing used for digestion, maximum cleavage site of 2, default peptide length of 6 and 144 amino acid, Precursor and fragment mass tolerance 10ppm and 0.05 Dalton, Static modification containing Carbamidomethylation of Cysteine (+57.021464 Da) and dynamic modifications consisting oxidation of Methionine (+15.994915 Da), Phosphorylation at S, T, Y (+79.996 Da) and Methionine loss and Acetylation (N-terminal, +89.030Da). Target FDR for PSMs and peptides was set at 0.01 and the type of peptide identified was set to “unique.” The following data consists of anterior and posterior raw files generated from orbitrap, pd study and result files and Human and contaminant database used in the study.

Experiment Type: Shotgun proteomics

Species: Homo sapiens - 9606

Tissue: Brain (bto:0000142)

Cell Type: Pituitary gland cell (cl:2000004)

Disease: Disease free

Instrument Details: Orbitrap Fusion (MS:1002416)

Protein Modifications: monohydroxylated residue, phosphorylated residue, acetylated residue, iodoacetamide derivatized residue

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