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Guiding Targeted Proteomics to detect Human Missing Proteins by Protein Identification in Mouse Tissues

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Overview

- Approximately 99% of mouse genes have counterparts in the human

- Goal is to use extensive analysis of mouse tissues to guide search for human missing proteins

- Focus on tissues difficult to obtain from humans

Analysis of 41 tissues from C57BL/6 mice

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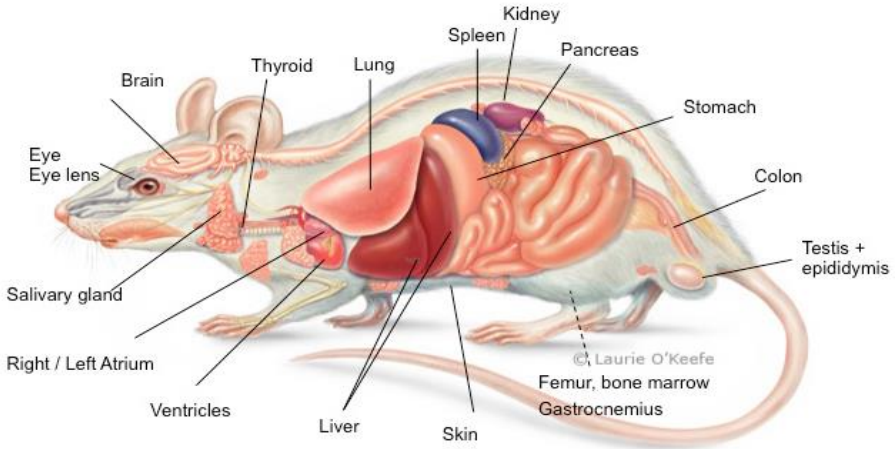
- Tissue from 3 female, 3 male mice
  - Samples homogenized and pooled together for each sex
  - Standard tryptic digestion
- Shotgun LC/MS-MS on Orbitrap Fusion
  - EASYnLC HPLC + Nanospray source
  - 2 hour gradient



Tissue Samples for Discovery Analysis	
Pancreas	Epididymis
Spleen	Seminal Vesicles
Small Intestine	Urinary Bladder
Stomach	Prostate
Cecum	Kidney
Colon	Adrenal Glands
Lymph Node	Sciatic Nerve
Liver – caudate lobe	Femur
Liver (L+R lobe)	Bone Marrow
Gallbladder	Spinal Cord
Salivary Glands	Brown Fat
Thymus	White Fat
Trachea	Skin
Heart	Plasma
Lung	White Blood Cells
Skeletal muscle	Red Blood Cells
Brain	Ovary
Eye	Uterus
Lens	Vagina
Optical Nerve	Mammary Gland
Testes	

Analysis of 41 tissues from C57BL/6 mice

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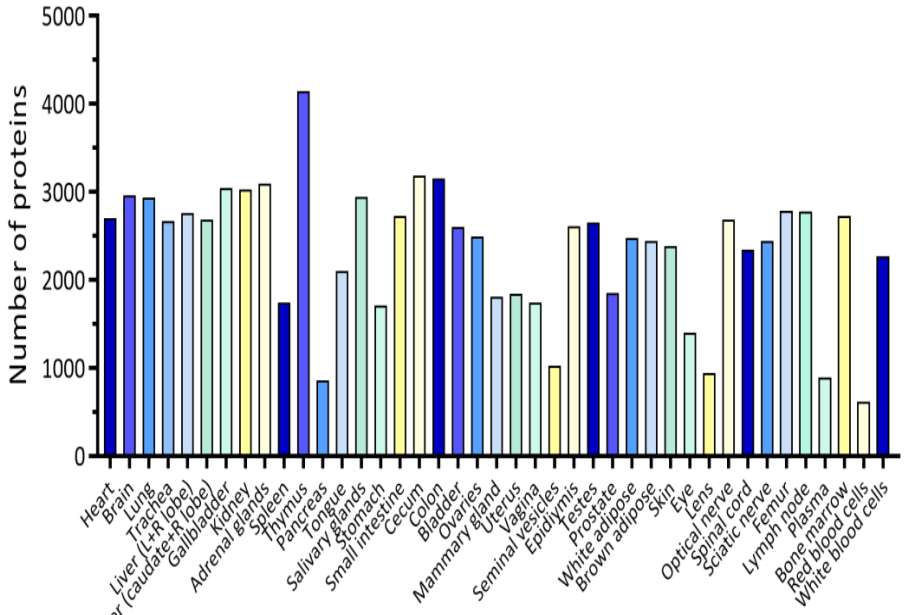
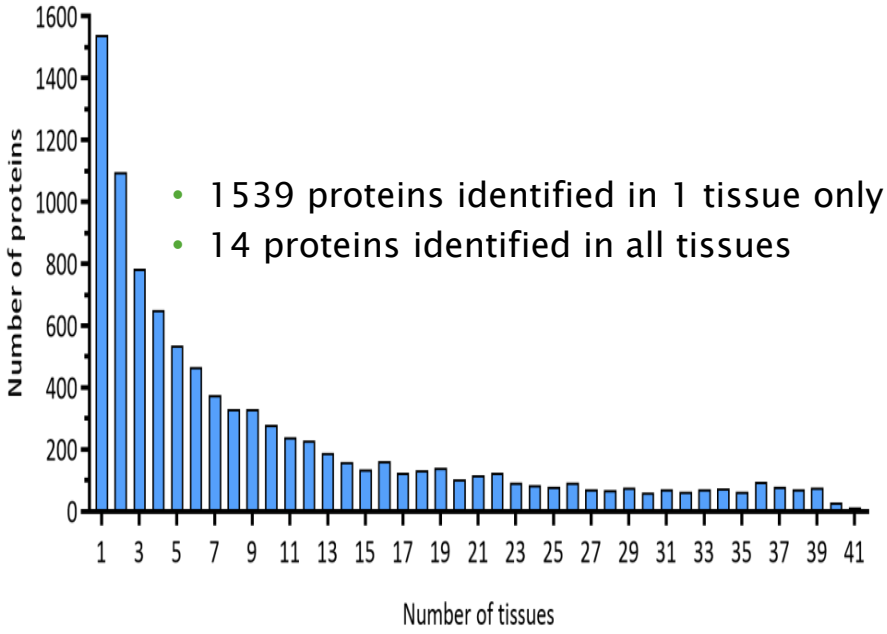
- Search using Comet/TPP
- PeptideProphet, ProteinProphet at 1% FDR
- Minimum of two peptides per protein



- 9,601 proteins were identified
- Many proteins unique to 1 tissue
- A few proteins identified in all tissues



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Homologous of missing proteins

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Human Chromosome	Number of corresponding mouse protein homologs detected	Tissues with largest number of expressed gene products (number of expressed proteins is mentioned after the colon next to each tissue)
1	19	testes:4, bone marrow:3, colon:3, skin:3, thymus:3
2	18	adrenals glands:1, gall bladder:1, liver:1, liver:1, skin:1
3	10	thymus:3, femur:2, kidneys:2, ovaries:2, trachea:2
4	8	cecum:2, salivary glands:2, adrenals glands:1, brain:1, brown fat:1
5	8	tongue:2, uterus:2, brain:1, heart:1, kidneys:1
6	9	prostate:3, spleen:2, bone marrow:1, cecum:1, colon:1
7	10	brownfat:2, thymus:2, adrenals glands:1, epididymis:1, femur:1
8	6	kidneys:6, salivary glands:6, seminal vesicles:5, epididymis:2, adrenals glands:1
9	10	colon:1, epididymis:1, lung:1, tongue:1, uterus:1
10	6	mln:4, femur:3, lens right:2, skin:2, small intestine:2
11	7	liver:7, liver c-r:6, salivary glands:5, adrenals glands:4, bone marrow:4
12	14	eye left:3, bone marrow:2, kidneys:2, lens right:2, liver:2
13	2	epididymis:3, salivary glands:2, thymus:2, adrenals glands:1, brain:1
14	9	bone marrow:2, eye left:1, lung:1, ovaries:1, plasma:1
15	5	eye left:2, plasma:2, brain:1, cecum:1, liver c-r:1
16	5	bone marrow:2, cecum:2, liver l rlateral:2, testes:2, thymus:2
17	9	kidneys:2, bone marrow:1, brain:1, brownfat:1, cecum:1
18	4	salivary glands:4, colon:3, mln:3, ovaries:3, vagina:3
19	14	femur:2, gall bladder:1, heart:1, kidneys:1, liver l rlateral:1
20	3	trachea:2, urinary bladder:2, brownfat:1, colon:1, femur:1
21	2	cecum:2, colon:2, testes:2, thymus:2, bone marrow:1
22	5	kidneys:1, liver c-r:1, liver l rlateral:1
X	12	pancreas:3, bone marrow:2, cecum:1, epididymis:1, gallbladder:1

Missing Protein Analysis in Mouse

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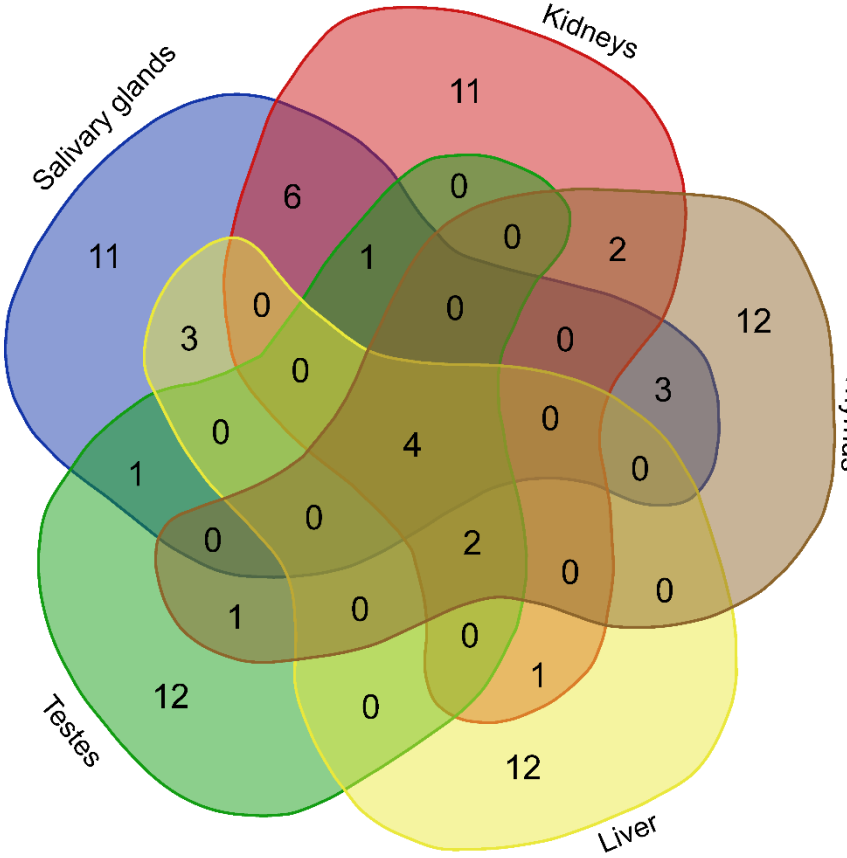
- 195 mouse proteins in various tissues that correspond to human missing proteins from several chromosomes
  - 9 from chromosome 6
- The majority of were identified in 5 or fewer tissues
- More than half were found in a single tissue
  - **Salivary Glands**
  - Half are membrane proteins



Human Sample Follow Up

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Tissue samples obtained from 3 individuals

- Only cadaver samples available
  - Varying donor health
- Samples processed with same protocol as mouse tissue, in addition
  - Besides PBS, a second protocol (urea) was used
  - 3 analytical replicates of each biological sample
- Each individual analyzed separately on orbitrap fusion
  - Data processing pipeline same as used for mouse

Discovery Results

- However 5 olfactory receptor (missing) proteins were identified
- Unable to reproduce the proteins that were found in mouse salivary glands
- Examining the individual mass spectra concluded additional in-depth bioinformatics analysis is needed
- Validation using MRM with internal standards is necessary

Progress & Future Directions

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Ongoing questions:

- Quality / freshness of human salivary tissue obtained from cadavers in comparison to mouse tissues
- Further validating discovery results in mouse and human datasets
  - Importance of selection criteria
  - Balance stringency with FDR when translating results in mouse to human

Future directions:

- Focus on tissue relevant protein groups (olfactory proteins)
- Follow up by targeted analysis using QqQ
  - Synthesize stable isotope labelled internal standards
- Alternative sample preparation for difficult-to-analyze proteins
  - Different digestion enzyme for non-tryptic peptides
  - Alternative sample preparation (eg., membrane fraction)