Histopathology applied in mouse phenotyping for the identification of metabolic diseases

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1. Histopathology as a component of high-throughput phenotyping pipelines in the GMC/IMPC

2. Histopathology reveals correlative and unique phenotypes in a high-throughput phenotyping pipeline

3. Incidental and background lesions

4. Good laboratory practice to process tissues for histopathological analysis
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Goal: The characterization of mouse models for human diseases to understand molecular mechanisms of human disorders and for the development of new therapies.

Strategy: To offer a large scale standardized and comprehensive phenotypic analysis of mouse mutants from various sources as open platform.

Pathology Screen: Mutant mice and control littermates are examined macro- and microscopically. Up to date techniques used in human pathology (including immunophenotyping) have been adapted and established for mouse tissue. This enables the pathology screen to make a contribution to the identification of new phenotypes which aim to reproduce and understand human diseases.

www.mouseclinic.de
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Histopathology as a component of high-throughput phenotyping

IMPC pipeline

7M + 7F Mutant Adult Mice

Weight Curve - 4wk to 16wk

In life

- Open Field
- CSD
- Grip Strength
- Acoustic Startle/PPI
- Calorimetry
- ECG/Echo
- Challenge Whole Body Plethysmography
- Intraperitoneal Glucose Tolerance Test
- Body Composition (lean/fat)
- X-ray
- Auditory Brain Stem Response
- Pain Test
- Eye Morphology

Terminal

- Haematology
- Clinical Blood Chemistry
- Insulin Blood Level
- FACS (spleen)
- Heart Weight
- Gross Pathology and Tissue Collection
- Tissue embedding & block banking
- Histopathology • from blocks where required

Key:

- Mandatory tests
- Non-Mandatory tests
- Tests in development or under consideration
Histopathology as a component of high-throughput phenotyping

Animal cohort
7 Mutant males
7 Mutant females
Wildtype cohort

Necropsy
Gross pathology

Tissue collection

Weight & Measurements

Fixation in formalin

2 Mutant males
2 Mutant females
Wildtype cohort

Tissue embedding & block banking

Histopathological analysis when required

Database & report

Photomicrographs for report

Pathology Screening Platform at German Mouse Clinic
• Determination of alterations in movement or deformities
• Skin and appendages (coat color and appearance)

• Necropsy of the mouse

• Photo documentation
• Measurement of body, liver, heart and spleen weights
• Body and tibia length.

Information in the database
### Statistical records

<table>
<thead>
<tr>
<th></th>
<th>female</th>
<th>male</th>
<th>Linear model</th>
<th>Linear model</th>
<th>Linear model</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>mutant</td>
<td>genotype</td>
<td>sex</td>
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<td>n=15</td>
<td>n=11</td>
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<tr>
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<td>mean ± sd</td>
<td>mean ± sd</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Body weight [g]</strong></td>
<td>22.836 ± 0.739</td>
<td>24.012 ± 1.003</td>
<td>26.867 ± 1.876</td>
<td>26.855 ± 2.023</td>
<td>0.236</td>
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<tr>
<td><strong>Heart weight [mg]</strong></td>
<td>114.9 ± 20.5</td>
<td>113.6 ± 11.9</td>
<td>131.5 ± 26.7</td>
<td>124.6 ± 19</td>
<td>0.538</td>
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<tr>
<td><strong>Tibia length [mm]</strong></td>
<td>18.8 ± 0.62</td>
<td>18.98 ± 0.66</td>
<td>19.03 ± 0.51</td>
<td>19.2 ± 0.64</td>
<td>0.345</td>
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<tr>
<td><strong>Heart Weight / Tibia</strong></td>
<td>6.12 ± 1.11</td>
<td>6 ± 0.67</td>
<td>6.92 ± 1.4</td>
<td>6.51 ± 1.09</td>
<td>0.457</td>
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<td><strong>Length [mg/mm]</strong></td>
<td>5.03 ± 0.86</td>
<td>4.73 ± 0.42</td>
<td>4.86 ± 0.69</td>
<td>4.65 ± 0.72</td>
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**Pathology Screening Platform at German Mouse Clinic**
### Tissue embedding and block banking

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Tissue Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Cervical lymph node</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>Thymus</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Spleen</td>
</tr>
<tr>
<td>Heart</td>
<td>Thyroid</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Parathyroid</td>
</tr>
<tr>
<td>Trachea</td>
<td>Adrenal gland</td>
</tr>
<tr>
<td>Lung</td>
<td>Kidneys</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Testes</td>
</tr>
<tr>
<td>Stomach</td>
<td>Epididymis</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Funiculus spermaticus</td>
</tr>
<tr>
<td>Large intestine</td>
<td>Ovaries</td>
</tr>
<tr>
<td>Liver</td>
<td>Uterus</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Vagina</td>
</tr>
</tbody>
</table>

- **28 organs**
- **6 paraffin blocks**
- **H&E staining**

*per mouse*

Remaining organs and mice: Storage in formalin / paraffin
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Pathology is a two way process!

**In vivo**
- Clinical history
  - mice strain
  - gene function
  - known phenotype(s)
  - results - internal flow
  - possible disease models

**Post mortem**
- To detect and record abnormal findings in internal and external organs

### Screens

<table>
<thead>
<tr>
<th>Screens</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behaviour</td>
<td>Open field</td>
</tr>
<tr>
<td></td>
<td>Acoustic startle response &amp; PPI</td>
</tr>
<tr>
<td>Neurology</td>
<td>Modified SHIRPA, grip strength</td>
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<tr>
<td></td>
<td>Rotarod</td>
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<tr>
<td>Clinical Chemistry</td>
<td>Clinical Chemistry after fasting</td>
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<tr>
<td>Nociception</td>
<td>Hot plate</td>
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<tr>
<td>Dysmorphology</td>
<td>Anatomical observation</td>
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<tr>
<td>Allergy</td>
<td>Transepidermal water loss (TEWL)</td>
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<td>Energy Metabolism</td>
<td>Indirect calorimetry, NMR</td>
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<tr>
<td>Clinical Chemistry</td>
<td>IpGTT</td>
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<tr>
<td>Cardiovascular</td>
<td>Awake ECG / Echocardiography</td>
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<tr>
<td>Eye</td>
<td>Scheimpflug imaging, OCT, LIR, drum</td>
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<tr>
<td>Clinical Chemistry</td>
<td>Clinical Chemical analysis, hematology</td>
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### Methods

<table>
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<tr>
<td>Immunology</td>
<td>FACS analysis of PBCs</td>
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<tr>
<td>Allergy</td>
<td>BIOPLEX ELISA (Ig concentration)</td>
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<td>Steroid Metabolism</td>
<td>Corticost., Androst., Testosterone</td>
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<td>Lung Function</td>
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<td>Molecular Phenotyping</td>
<td>Expression profiling</td>
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<tr>
<td>Pathology</td>
<td>Macro &amp; microscopic analysis</td>
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The Carboxypeptidase E (Cpe) mouse line

Characterised by the metabolism, clinical chemistry and pathology screens

Carboxypeptidase E is an enzyme that catalyzes the release of C-terminal arginine or lysine residues from polypeptides. Thereby, it cleaves active neuropeptides and peptide hormones like insulin from their intermediate precursor. It is involved in insulin and glucose homeostasis (Chu KY et al. Islets. 2011 Jul-Aug;3(4):155-165).

A significant increase in body mass of mutant mice was detected during the pipeline.

The Cpe mutant mice showed an altered glucose clearance and impaired insulin action.
Gross pathology

<table>
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<td>20.5 ± 1.3</td>
<td>35.4 ± 4.9</td>
<td>25.6 ± 2.7</td>
<td>35 ± 4.9</td>
<td>0.026</td>
<td>&lt; 0.001</td>
</tr>
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</table>

- Increased body weight in mutant mice.
- Increased liver weight (6 mutants).
- Liver was enlarged with a pale yellow brown greasy cut surface.

*High Fat Diet-Induced Changes in Hepatic Protein Abundance in Mice. Luo et al., J Proteomics Bioinform 2012, 5:3*
Histopathology Liver

In the histological analysis a diffuse vacuolation of hepatocytes was observed. **Severe hepatic periportal micro- to macrovesicular steatosis in all mutant mice.**
The islet of Langerhans are small aggregates generally in contact with ducts (arrows). The islets range in diameter from less than 50mm to 300mm in control mice.

In the Cpe mutant mouse line, we observed enlarged islets (hypertrophic islets) with increased vascularization.
The Carboxypeptidase E (Cpe) mouse line

Testicular histopathology in a mutant mouse

Testicular atrophy was detected. It was manifested by a bilateral, decreased testicular size, seminiferous tubules degeneration and the presence (also in the epididymis) of multinucleated giant cells and round spermatids (arrows).
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Histological differences among mouse strains

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Fixing tissue: **Why ?** Prevents tissue:

- **Autolysis** - degeneration by lysosomal enzymes
- **Putrefaction** – destruction by microorganisms
- Retains **tissue morphology**

Standard immersion fixation
What is 10% neutral buffered formalin?

Formaldehyde gas is soluble at 40% in water therefore most commercial solutions of formaldehyde are 40%.

The standard fixative is made up of 1 part formaldehyde solution to 10 parts water = 10% formalin solution

The 10% formalin solution therefore contains 4% formaldehyde

Many labs buffer the 10% formalin solution with a phosphate buffer to pH 7.2-7.4 = neutral buffered formalin

*taked from Chery L. Scudamore talk infrafrontier gross pathology workshop München*
Common causes of poor fixation

Inadequate time in fixative, inadequate volume

- Ideal fixative ratio: **10 parts formalin : 1 part tissue**
- Leave in fixative for **24 hrs at room temp.**

Tissue can be stored in formalin for prolonged periods
Make most efficient use of resources: pro’s & con’s

(+) Gaining a lot of information in one analysis
(-) But: Cost intensive, personnel and time-intensive

Young academics for old pathologists!!!
- Attempts for high throughput
- Automatisation and digitalisation
- Statistician support for analysis
Many Thanks for your Attention