The Development of a PKU Swine Model

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A PKU swine model

• Rationale for a pig model

• Strategy & optimization of gene editing in cell culture

• Generation of a PAH KO Yucatan mini-pig model for PKU

Jamie Wyeth
Portrait of a Pig
Brandywine River Museum
Why use animal models?

- biomedical understanding of complexity
- testing of new therapeutic approaches
Why a pig model:

Mouse models often fail to model human clinical features
- physiology body size & anatomy is too different

Improved animal models for many disorders
- pig fulfills key criteria: physiology, anatomy, body size, genome, genetic technologies, success stories (eg., Cftr; metabolic disorder)
  - neurobehavioral studies are feasible in pig

An accurate animal model is essential
- to understand complex human syndromes
- to develop therapeutic approaches that can translate to human
Why a minipig model for PKU?

- have a similar physiology, biochemistry, & pathology
- similar body & organ size, & anatomy
- sequenced genome
Which species brain is the best model for human? - which are rat, pig, macaque

Brain size and gyral complexity


David W Howells¹, Michelle J Porritt¹, Sarah SJ Rewell¹, Victoria O'Collins¹, Emily S Sena², H Bart van der Worp³, Richard J Travstman⁴ and Malcolm R Macleod⁵
The pig is the best model for human disease studies.

Brain size and gyral complexity

*Journal of Cerebral Blood Flow & Metabolism (2010) 30, 1412–1431*

David W Howells¹, Michelle J Porritt¹, Sarah SJ Rewell¹, Victoria O'Collins¹, Emily S Sena², H Bart van der Worp³, Richard J Travstman⁴ and Malcolm R Macleod²
Comparison of human, pig & mouse brain

Table 1  Brain weights and sizes of adult humans, pigs, dogs, cats, rats, and mice

<table>
<thead>
<tr>
<th>Species</th>
<th>Human</th>
<th>Pig</th>
<th>Dog</th>
<th>Cat</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain size</td>
<td>![Brain size diagram]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Approximate brain weight (grams)</th>
<th>1,300–1,400</th>
<th>80–180 (large variation between breeds)</th>
<th>70–130 (large variation between breeds; e.g., ≈72 in Beagles)</th>
<th>≈30</th>
<th>≈2</th>
<th>≈0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gyri and sulci</td>
<td>Gyrencephalic</td>
<td>Gyrencephalic</td>
<td>Gyrencephalic</td>
<td>Gyrencephalic</td>
<td>Lissencephalic</td>
<td>Lissencephalic</td>
</tr>
</tbody>
</table>

- Pig brain anatomy, growth, development more like human
Pig as a model for neurodevelopmental studies using MRI

3D-brain reconstruction showing the anatomical position of:
- left hippocampus
- right hippocampus
- cortex
- diencephalon
- brainstem
- cerebellum

Major brain growth from late prenatal to early postnatal: pig (to 12 weeks) & human (1-2 years)

Can pig models be used for learning & memory studies?

- 2-week old piglets can perform T-maze tasks to assess hippocampal-dependent learning & memory

“The piglets, which were being fed on infant formula, had no interest in solid food, nor were they motivated to perform the tasks if the reward was the same as their regular food. They were, however, very willing to work for chocolate milk, specifically Nesquik™.”

Rodney W. Johnson,
University of Illinois at Urbana-Champaign
Pig models for behavioral studies

- Pigs rapidly learn to use a mirror to find hidden food
- shows assessment awareness (significance of a situation in relation to themselves)

D. Broom, Univ. of Cambridge
Biochemical data on Yucatan minipig

10 one-month old Yucatan
(5 male, 5 female)

Compared pig and children < 1
year, for normal range for plasma
amino acids

Actual slide to be added once data published
Organ systems for which genetically engineered pigs have been created.

How do you make a pig model?
How scientists perform gene editing
For the scientists: CRISPR/Cas9 ribonucleoprotein complex with Watson-Crick base pairing of the gRNA to the target

PAM = protospacer adjacent motif

RNA-guided genome editing of mammalian cells.
Pyzocha NK, Ran FA, Hsu PD, Zhang F. Methods Mol Biol. 2014;1114:269-77
How do scientists identify a broken gene (mutation)?

How do scientists identify a broken gene (mutation)?
PAH gene editing in cell culture: strategy to optimize tools

A  pSpCas9(BB) vector

U6 pr  CBh pr  STN  hSpCas9  bGHpA

CRISPR gRNA

https://www.addgene.org/42230/

B

Actual slide to be added once data published
Next 4 slides to be added once data published
Zygote RNA injection

Next 6 slides to be added once data published
## Dietary needs

<table>
<thead>
<tr>
<th>Estimated Needs</th>
<th>Formula RX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td><strong>Kcal Requirement</strong></td>
</tr>
<tr>
<td></td>
<td>3265/kg body weight</td>
</tr>
<tr>
<td>2</td>
<td>6,530</td>
</tr>
<tr>
<td>3</td>
<td>9,795</td>
</tr>
<tr>
<td>4</td>
<td>13,060</td>
</tr>
<tr>
<td>5</td>
<td>16,325</td>
</tr>
<tr>
<td>6</td>
<td>19,590</td>
</tr>
<tr>
<td>7</td>
<td>22,855</td>
</tr>
<tr>
<td>8</td>
<td>26,120</td>
</tr>
<tr>
<td>9</td>
<td>29,385</td>
</tr>
<tr>
<td>10</td>
<td>32,650</td>
</tr>
</tbody>
</table>
A lot of formula!

150 cases of Phenex-1 in first month of life
Hepatocyte transplantation for therapeutics: Kristen Skvorak, Jerry Vockley

courtesy I. J. Fox
Conclusions:

A pig model of PKU
- need more animals
- biomedical basis of any aspect of PKU
- dietary, small molecule, GI, gene therapy, & hepatocyte transplantation

Photo to be added once data published
Pittsburgh Team

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Stephanie Murphy

Pig care:
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Sabrina Hammond
Elizabeth Queathem
Thank you