pHENOMMenal: Genetic manipulation of probiotic bacteria for the treatment of PKU

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With assistance from
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Disclosures: Provisional Patent U.S. PTO 61/676,461
Outline of Talk

- Origin of idea
- Probiotic and DNA vector information
  - pHENOMMenal’s *in vitro* phe metabolism
- pHENOMMenal mouse dosage tests
- Assess pHENOMMenal for prolonged function *in vivo* (mouse)
What can bacteria eat?
Phenylalanine Eating Bacteria

L-phenylalanine $\xrightarrow{\text{PAL}}$ trans-cinnamic acid

PAL = Phenylalanine Ammonia Lyase
Probiotics – the good bacteria
Our Probiotic- *Lactobacillus reuteri* 100-23 for the mouse study

Electron micrograph of *L. reuteri* 100-23 in mouse gut

Getting PAL into the probiotic
Back-Up Plasmid creation
Electroporation

Select for bacteria with plasmid
Functional PAL in our probiotic

Trans-cinnamic acid production by plasmid carried/100-23 cell line

- pSLERGT
- pHENOMM
PKU mouse colony

### JAX® Mice Pup Appearance by Age

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The approximate age of mouse pups can be determined by their physical attributes during the first two weeks of life. Examples of the developmental stages of albino, agouti, and black pups are shown.
PKU mouse genetics and bone forming IGF-1 protein

- Insulin-like growth factor 1 (IGF-1) aids in building bone
- In mice the gene for IGF-1 is right next to the gene for PAH
- Genes on other chromosomes in mice also impact IGF-1
PKU Mouse Chromosome 10

PAH = Blue vertical line
IGF-1 = Green vertical line
Jax SNPs = Red vertical lines
Grey vertical line every 10,000,000 bp
PKU mouse genetics and bone forming IGF-1 protein cont.

**Plasma IGF-1 in PAHenu2 colony**

- **PKU AA**
- **PKU LL2**
- **Het LL2**

**Plasma IGF-1**

- **5LL2 Het**
- **5LL2 B6**
The PKU mouse model

- The PKU mouse mutation is called the PAHenu2
- Similar to human disease in terms of phe and other health effects
- We use the PAHenu2 mutation in the C57BL6 (black 6) strain of mice
- Our litters are always born as a mix of mice that have PKU or are carriers for PKU
Comparing mouse/human PKU: phe intake

- **Mice eat 120mg phe/kg body weight**
  A 20 gram mouse eats at about 2 grams of chow per day, with 16% protein for 800mg phe per kg chow. This chow is considered a medium protein quantity for mouse chow. On other diets for growth or simply higher phe content, this value can be as high as 210mg/kg!!!

- **People eat 30.5mg phe/kg body weight**
  The average human weighs in at roughly 81.72kg (180lbs) and eats the recommended 50 grams of protein per day for roughly 2500mg phe daily
Comparing mouse/human PKU: Digestive transit
Dosage Determination/Does it work?

Dosage Test

Bacteria + Chow → Mice
Dosage test: Probiotic works in-vivo

P value less than 0.01
Longer term study:
*In vivo* experimental design

Day 0
Before starting treatment, collect blood from each animal for baseline phe test.

Week 1
Place animals on plain probiotic (no PAL) for 1 week*, collect blood, test phe.

Week 2 and 3
Place animals on pHENOMMMenal probiotic (with PAL) for 10-14 days, collect blood once per week on treatment to test phe.
We have created a bacteria that can reduce blood phenylalanine (phe) when administered orally in the mouse model. We hope the human version will provide an effective and inexpensive treatment for PKU while allowing a better quality of life.
Special thanks

- **The NPKUA**  Christine Brown, Dr. Tom Franklin
- **NSPKU and ESPKU**  Eric Lange, Dave Stening, and Paula Hallam
- **Dr. Ione Hunt von Herbing’s laboratory**  University of North Texas
- **Dr. Michael Allen’s laboratory**  University of North Texas/ UNT Health Science Center
- **Dr. Chris Elkins**  Food and Drug Administration (FDA), USA
- **Dr. Gerald Tannock & Dr. Nick Heng**  University of Otago (New Zealand)
Questions?