Research Update for The PAF- July 2008

Establishing a PCCA knockout mouse colony.

We have obtained heterozygous breeding mice from Dr. Barry, and have viable litters. We have developed an improved genotyping scheme, which allows easy discrimination between PCCA knockout (PCCA +/- or PCCA -/-) and normal mice (PCCA +/+). Since PCCA -/- mice are not viable, this distinction is good enough at this point.

Construction of a transgenic mouse expressing muscle-specific PCCA.

We have constructed a plasmid containing the PCCA gene driven by the MCK promoter. The plasmid contains several potentially harmful mutations. These mutations will be replaced with synthesized DNA of the proper sequence via digestion and religation. Once the plasmid is corrected, a transgenic mouse will be created. This mouse will be bred to the PCCA -/- mouse to ultimately create a PCCA -/- mouse which contains a transgenic PCCA gene driven by the MCK promoter, which will express PCCA only in skeletal muscle. This will verify that PCCA expression within muscle alone is sufficient to allow survival, and support a gene therapy for muscle-specific treatment of propionic acidemia.

Technical Pitfalls.

Our two overall aims for this project have both been hampered by technical difficulties. Upon acquiring breeding mice from Dr. Barry, it was learned that the published protocol for genotyping was unreliable. Nevertheless, we tried it several times with several conditions, and found that it failed. We have solved this problem, and can maintain the breeding colony.

Our other aim is to construct a PCCA -/- mouse expressing the PCCA gene from the MCK promoter. The first step was construction of the MCK cloning vector, followed by insertion of the PCCA gene. After the initial construction, sequencing revealed several mutations which could have perhaps affected expression of the gene. Thus, using an improved vector (in which the gene is oriented in the opposite direction to the direction of the kanamycin gene), we have obtained a second construct. This one also contains mutations, and we have found a way to eliminate these mutations.