A NEW CRISPR/CAS9 EDITED PKU MOUSE MODEL CARRYING THE FREQUENT SPlicing VARIANT c.1066-11G>A

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INTRODUCTION
Phenylketonuria (PKU), one of the most common inherited diseases of amino acid metabolism, is caused by mutations in the PAH gene coding for the hepatic enzyme phenylalanine hydroxylase, leading to high blood Phe levels that cause alterations in brain development and function. Current treatment relies on dietary restriction of Phe and/or treatment with sapropterin dihydrochloride (Kuvan), a synthetic form of the cofactor. Given the drawbacks of these approaches that limit the quality of life for patients, alternative treatments are needed. In this study, we report the generation by CRISPR/Cas9 technology of a novel PKU mouse model carrying the highly prevalent splice intronic variant c.1066-11G>A (B6.86CBA-Pahem5Cебem).

BACKGROUND

As intronic sequences are not conserved between mice and human, we first validated the gene editing strategy by designing a humanized mouse model substituting 90 bp from the 3’ end of mouse Phe intron 10 with the human sequence containing the variant. This hybrid minigene reproduced the aberrant splicing pattern.

RESULTS

Mouse minigene

The PKU mouse model was developed following the same strategy using CRISPR/Cas9 genome editing technology through RNP microinjection and proven to have the correct genotype. The colony was backcrossed to F5 with wild-type mice (C57BL/6J) and the breeding animals from F5 x F5 backcrossing have been used for the experiments of this work.

Table 1. Levels of Phenylalanine and Tyrosine in mouse serum and brain. Values are presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Phenylalanine (μM)</th>
<th>Tyrosine (μM)</th>
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<tbody>
<tr>
<td>WT</td>
<td>59.6 ± 1.5</td>
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<tr>
<td>Het</td>
<td>73.5 ± 1.7</td>
</tr>
<tr>
<td>PKU</td>
<td>1718.6 ± 170.5</td>
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We tested for behavioral abnormalities. In the open field PKU mice showed stereotypical running at the cage periphery: this was probably an anxiety response, as in the social approach, PKU mice did not show a preference to interact with the cage containing a mouse over the empty one. Motor coordination deficiency was detected with the rotarod test.

CONCLUSIONS

1. The mice present behavioral deficits, mainly hypactivity and less social interaction, as well as locomotor deficiencies.

2. This novel mouse model represents a reliable translational tool for PKU preclinical research and drug development.