doi 10.18699/SBB-PlantGen-2024-30

Challenges of modelling epilepsy in mice: the example of *kcna1* gene knockout

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Key words: epilepsy; animal models; genome editing, kcna1

Motivation and Aim: Epilepsy is one of the most common neurological disorders. However, its pathogenesis and disease-related physiological changes are not well understood. The main reason for this is that epilepsy is very diverse, with more than 500 different genes now associated with the development of the disease [1]. Therefore, animal models are crucial for further investigation of epilepsy pathogenesis and treatment. We aimed to create a new mouse model of epilepsy by CRISPR-Cas9-mediated knockout of the *kcna1* gene. Previously, a similar mouse strain was created by inserting a neomycin resistance cassette into the *kcna1* locus, but these mice have a very high postnatal mortality (half of the newborn animals die by the fifth week) [2].
Methods and Algorithms: The guide RNA sequence was selected using the CRISPR Design Tool (Synthego), considering the optimal location of the binding site at the end of the reading frame to reduce the likelihood of lethal mutations. *In vitro* transcription was used to synthesize the guide RNA and RNA purification was performed using the Trizol method. The guide RNA was then tested *in vitro* using Cas9 protein and DNA with a nucleotide sequence corresponding to the target site of the *kcna1* gene. The prepared construct, consisting of guide RNA and Cas9 mRNA, was microinjected into zygotes, after which the zygotes were transferred into pseudopregnant mice.
Results: We obtained the first generation of mice (5 pups) Using the ICE Analysis service (Synthego), detailed data on the presence and size of deletions were obtained. The size of the deletions ranged from 1 to 19 nucleotides, in 3 out of 5 mice multiple mutation variants were detected. We observed spontaneous epileptic seizures at an early age (1–2 weeks). Unfortunately, all obtained animals died before reaching puberty (between 2 and 3 weeks of age).
Discussion: The mortality of the animals we obtained was higher than in the previously described model. This can be explained by the fact that in previous model the whole exon was replaced by a neomycin cassette, and the target protein could not be synthesized. Similar subunits (e.g. kcna2) may be involved in the formation of tetramers replacing the missing *kcna1* protein. In our case, the synthesis of the truncated protein may result in generation of non-functional channels. Further studies will focus on increasing the viability of the strain in the presence of spontaneous epileptic seizures.
Funding: The study is supported by the Saint Petersburg State University research project ID 116959511.

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