BÓBEK

ISA

10-15 APRIL 2023

INTERNATIONAL SCIENTIFIC-PRACTICAL JOURNAL

XIX GLOBAL SCIENCE AND INNOVATIONS 2023: CENTRAL ASIA

Astana, Kazakhstan

ОБЪЕДИНЕНИЕ ЮРИДИЧЕСКИХ ЛИЦ
В ФОРМЕ АССОЦИАЦИИ
«ОБЩЕНАЦИОНАЛЬНОЕ ДВИЖЕНИЕ «БОБЕК»
КОНГРЕСС УЧЕНЫХ КАЗАХСТАНА

ISSN 2664-2271

«ГЛОБАЛЬНАЯ НАУКА И ИННОВАЦИЯ 2023: ЦЕНТРАЛЬНАЯ АЗИЯ»

№ 1(19). АПРЕЛЬ 2023
СЕРИЯ «БИОЛОГИЧЕСКИЕ НАУКИ»
Журнал основан в 2018 г.

III ТОМ

АСТАНА – 2023

CONSOLIDATION OF LEGAL ENTITIES IN THE FORM OF AN ASSOCIATION «NATIONAL MOVEMENT «BOBEK» CONGRESS OF SCIENTISTS OF KAZAKHSTAN

ISSN 2664-2271

BÓBEK

НАУЧНАЯ ЭЛЕКТРОННАЯ БИБЛИОТЕКА
eLIBRARY.RU
РИНЦ

«GLOBAL SCIENCE AND INNOVATIONS 2023: CENTRAL ASIA»

No. 1(19). APRIL 2023
SERIES "BIOLOGICAL SCIENCES"
The journal was founded in 2018.

III VOLUME

ASTANA – 2023

UDC 57.08

DETERMINATION OF SNP POLYMORPHISM CAUSES HEREDITARY ANOMALIES IN THE KAZAKH NATIVE HORSE POPULATION

D. Kabylbekova[1], **Y. Ussenbekov**[2], **T. Assanbayev**[3], **J. Kantanen**[4]
[1] Almaty Technological University, PhD student, Almaty, Kazakhstan;
[2]Kazakh National Agrarian Research University Almaty, Kazakhstan;
[3]S. Toraighyrov Pavlodar State University, Pavlodar, Kazakhstan;
[4]Natural Resources Institute Finland

Annotation: *Hydrocephalus in horses is a developmental disorder that often results in stillbirth of affected foals and dystocia in dams. The occurrence is probably related to a founder effect and inbreeding in the population. The aim of our study was to determine the polymorphism of the B3GALNT2 gene that causes hydrocephalus in the local Kazakh horse population. The results showed that the mutation was present in Zhabe horse breed from Pavlodar region.*

Keywords: *SNP polymorphism, kazakh horse genotyping, B3GALNT2 gene, level of homozygosity and heterozygosity of the population, DNA markers*

Introduction

Currently, according to the international information website Online Mendelian Inheritance in Animals (OMIA), there are 263 inherited anomalies found in horses, which cause significant economic damage to breeding and commercial horse industry [1]. Therefore, research on optimizing and developing methods for investigating SNP polymorphisms in local horse breeds associated with economically beneficial traits and inherited anomalies is of great importance.

B3GALNT2 is an enzyme involved in glycoylation of alpha-dystroglycan. Mutations in this gene result in various human muscular dystrophies, some of which also involve hydrocephalus. The mutation observed in Friesians introduces an early stop codon, resulting in an incomplete (and thus likely non-functional) protein. Hydrocephalus is an abnormal build up of cerebral spinal fluid around the brain. The occurrence is probably related to a founder effect and inbreeding in the population. In the Friesian, it is believed that a narrowing of a passage within the brain prevents normal fluid absorption, leading to an obvious external cranial distension. Affected foals are often stillborn and are associated with dystocia in the dams. Hydrocephalus is an autosomal recessive disorder, thus a foal must inherit two copies of the mutant allele to be affected. Horses with only one copy of the allele are known as carriers due to their ability to produce an affected foal [2]. A study by Sipma et al. (2013), found that stillborn Friesian foals with hydrocephalus had larger heads and brains compared to normal foals. The authors also found that there was a significant increase in the size of the lateral ventricles of the brain in foals with hydrocephalus. In addition, they noted that there were differences in the structure of the brain tissue between foals with hydrocephalus and normal foals [3].

The authors Ducro et al. (2015), identified a single nucleotide polymorphism (SNP) in the B3GALNT2 gene that was strongly associated with hydrocephalus in Friesian horses. This SNP results in a premature stop codon in the B3GALNT2 protein, leading to a truncated and non-functional protein. The authors also confirmed the presence of this SNP in affected Friesian horses, but not in unaffected horses or other horse breeds [4]. Another study conducted by Ayala-Valdovinos et al., used two methods, PCR-RFLP and PCR-PIRA, to genotype the horses and detect the presence of the c.1423C>T mutation in the B3GALNT2 gene. The study found that the frequency of the mutation was low among the Friesian stallions in Mexico.

Researchers, including Kolb and Klein (2019), have studied the genetics of congenital hydrocephalus in Belgian draft horses and have identified the specific mutation in the B3GALNT2

gene that is responsible for this condition. They have also developed a genetic test to identify carriers of the mutation, which can help breeders to avoid breeding horses that are at risk of producing offspring with congenital hydrocephalus [6]. Thus, a literature review demonstrates that valuable information on the genetic basis of congenital hydrocephalus in horses and highlights the importance of genetic testing and responsible breeding practices in preventing the spread of this condition. This study was conducted to determine the polymorphism of the B3GALNT2 gene causes hydrocephalus in local Kazakh horse population using the PCR-RFLP method.

Experimental

For the study, 41 blood samples from horses with EDTA were used, including 8 samples of the local Zhabe breed (Peasant farm "Akimbekov" of Zhetysu region), 16 samples of the local Zhabe breed (LLP "Akshiman-Agro" of Pavlodar region), and 17 samples of the local Adai breed (Peasant farm "Kozhyr-Ata" of Mangystau region). DNA extraction from blood samples was performed in the laboratory of the Department of Obstetrics, Surgery and Biotechnology of Reproduction of Kazakh National Agrarian University.

The detection of a genetic defect associated with hydrocephalus was performed using primers F-5' CCTGTGGCTGTGTGAGAAGA-3' and R-5' TCGGGCTTTCCTCAGACTTA-3', resulting in an amplicon size of 204 bp. To identify wild-type and mutant alleles of the B3GALNT2 gene, the AciI restriction enzyme with recognition site CCGC was utilized, resulting in fragment sizes of 157 bp and 47 bp in homozygous healthy animals, and fragment sizes of 204 bp, 157 bp, and 47 bp in heterozygous carriers. The PCR conditions for the B3GALNT2 gene were as follows: initial denaturation at 95°C for 5 min, 32 cycles of denaturation at 94°C for 20 sec, annealing of primers at 58°C for 40 sec, elongation at 72°C for 30 sec, and a final extension at 72°C for 5 min.

Results

DNA samples from horses were genotyped at the Laboratory of Green Biotechnology and Cell Engineering at the Kazakh-Japanese Innovation Center of Kazakh National Agrarian Research University. Fragments of 204 bp, 157 bp, and 47 bp (figure 1) were detected on the electrophoretogram, indicating the identification of heterozygous carriers of the c.1423C > T mutation in the B3GALNT2 gene in horses of the Zhabe breed from the Pavlodar region. Out of 16 DNA tested horses from the Pavlodar region, two mares aged 4-5 years (figure 2) were found to have a heterozygous genotype, which is 12.5%. Heterozygous carriers of the mutation in the B3GALNT2 gene were not identified in horses of the Adai type (n=17) from the Mangystau region, and in horses of the Zhabe breed (n=8) from the Zhetysu region based on the results of the study.

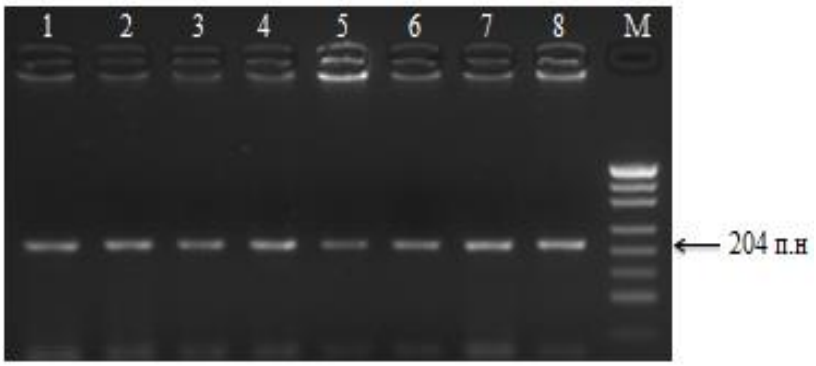

Figure 1. Electropherogram of B3GALNT2 gene amplicon, lanes 1-8 show an amplicon with a size of 204 bp, M - DNA marker pUC19/Mspl

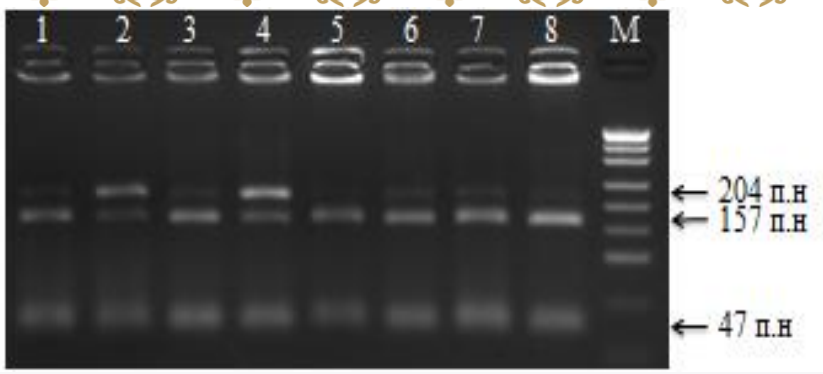

Figure 2. Electropherogram of B3GALNT2 gene amplicon after AciI endonuclease restriction, lanes 1, 3, 5-8 show homozygous healthy individuals with a size of 157 bp and 47 bp, lanes 2 and 4 show heterozygous carriers of the gene mutation with sizes of 204 bp, 157 bp, and 47 bp, M - DNA marker pUC19/Mspl

Conclusion

The identification of heterozygous individuals in horses from the Pavlodar region indicates that heterozygous carriers of the harmful c.1423C>T mutation in the B3GALNT2 gene are present in domestic horse breeds. According to foreign scientists, the c.1423C>T mutation determines the phenotypic trait of hydrocephalus, which in horses is accompanied by abortions and stillbirths. Due to the increase in the horse population in our country, the development of molecular genetic diagnostic methods is becoming an urgent issue in horse breeding. Therefore, we consider it necessary to conduct genetic screening of horses in Kazakhstan. In the future, it is planned to carry out experimental work on a large sample from different regions of the country.

Funding

This work was carried out within the framework of grant funding for scientific and (or) scientific and technical projects for 2022–2024 of the Ministry Education and Science of the Republic of Kazakhstan AP14869181 “The study of microbiome ecogenomics of Kazakh horse breed by NGS sequencing”.

REFERENCES:

1 Online Mendelian Inheritance in Animals, OMIA. Sydney School of Veterinary Science Retrieved from https://omia.org/OMIA000487/9796/

2 Etalondx.com Retrieved from https://www.etalondx.com/hydrocephalus

3 Sipma KD, Cornillie P, Saulez MN, Stout TAE, Voorhout G, Back W. (2013). Phenotypic Characteristics of Hydrocephalus in Stillborn Friesian Foals. Veterinary Pathology.;50(6):1037-1042. doi:10.1177/0300985813488955

4 Ducro, B. J., Schurink, A., Bastiaansen, J. W., Boegheim, I. J., Steenbeek, F. G., Vos-Loohuis, M., Nijman, I.J., Monroe, G.R., Hellinga, I., Dibbits, B.W., Back, W., & Leegwater, P. A. (2015). A nonsense mutation in B3GALNT2 is concordant with hydrocephalus in Friesian horses. *BMC Genomics*, *16*(1). doi: 10.1186/s12864-015-1936-z

5 Ayala-Valdovinos, M. A., Galindo-García, J., Sánchez-Chiprés, D., & Duifhuis-Rivera, T. (2016). Genotyping of Friesian horses to detect a hydrocephalus-associated c.1423c>T mutation in B3GALNT2 using PCR-RFLP and PCR-PIRA methods: Frequency in stallion horses in México. *Molecular and Cellular Probes*, *32*, 69–71. https://doi.org/10.1016/j.mcp.2016.12.005

6 Kolb DS, Klein C. (2019) Congenital hydrocephalus in a Belgian draft horse associated with a nonsense mutation in B3GALNT2. Can Vet J. 60(2):197-198. PMID: 30705458; PMCID: PMC6340252.