TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 729-733

doi:10.3906/vet-1911-91

Investigation of the SCID mutant allele in Turkish Arabian horses reared in some private farms of the Eskişehir region in Turkey

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Received: 27.11.2019 • **Accepted/Published Online:** 09.03.2020 • **Final Version:** 02.06.2020

Abstract: There are several known hereditary diseases in Arabian horses. Many studies have been conducted on molecular diagnostic methods of the mutations that cause these hereditary defects in horses. A five-base deletion in the short arm (ECA9p12) of the equine chromosome 9, starting with codon 9480, which occurs in the catalytic subunit gene of the DNA-dependent protein kinase (DNA-PKcs) results in severe combined immunodeficiency (SCID) hereditary disorder. SCID is an autosomal recessive disorder wherein the immune system of the affected foal is very weak. Purebred Turkish Arabian horses reared in the Eskişehir region have contributed greatly to Turkish Arabian horse breeding and racing. The aim of this study was to detect the SCID mutant allele by using DNA sequencing analysis in Turkish Arabian horses, raised in nine different private farms in Eskişehir. It was determined that none of the horses evaluated in this study carried the SCID mutant allele.

Key words: DNA sequencing analysis, heritable disorders, SCID, Turkish Arabian horses

1. Introduction

Hereditary defects in animal populations are important issues for researchers and breeders alike. The progress in genetics has produced valuable testing methods to determine hereditary disorders. Additionally, advanced management contributed to creating breeding protocols focusing on reducing the impact of genetic diseases on the horse sector. While the frequencies of the SCID carrier and affected foal were 25.7% and 3% before the genetic tests, with the application of the tests the frequency of the SCID carrier and affected foal decreased to 8.4% and 0.18%, respectively in the United States [1]. Horse owners and breeders desire to produce foals without genetic disorders. Another important goal is to reduce the frequency of the mutation in the gene pool while maintaining genetic diversity. The diversity is crucial in the long term for genetic improvement and animal welfare. Breeding decisions must be made wisely and carefully to prevent unnecessary suffering of foals and to preserve the future health of the Arabian horse. When a decision has to be made to use a carrier mare with superior characteristics in breeding programs, she should always be bred to a normal stud. An appropriate planning of breeding of carriers prevents the outcome of affected foals and decreases the frequency of the mutant gene in the population. Therefore, it is very important to determine the molecular basis of hereditary defects in animal husbandry. In horses, 232 genetic disorders have been determined. Among these disorders, 44 mutations and inheritance models were identified [2]. Some of these inherited defects appear only in certain breeds and their crosses. SCID, which also affects humans, dogs, and mice, is an autosomal recessive hereditary disorder that causes serious economic losses in Arabian horses and their crosses worldwide [1]. Heterozygous horses can appear normal while carrying a mutant allele of SCID. Thus, it is important to detect the carrier via molecular testing to reduce the frequency of this allele in the population [3].

The first article about SCID, published by McGuire and Poppie, studied two Arabian foals with pneumonia that did not respond to treatment. SCID-affected foals are highly susceptible to secondary infections due to insufficient antigen-specific immunity responses, which are caused by the absence of B and T lymphocytes. These foals produce no antibodies after infection or immunization and often cannot survive beyond 6 months of age even with intensive veterinary care [4]. The inheritance mode of SCID was confirmed through test mating in 1977 [5].

The mutation that causes SCID is a five nucleotide deletion in the DNA Protein Kinase catalytic subunit (DNA-PKcs) gene on the 9th equine chromosome (ECA9p12), which prevents the formation of the enzyme

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DNA-PK [6]. Once hereditary defects caused by SCID mutation are identified, SCID mutant alleles can be confirmed using the Southern Blotting method [7]. Another genetic test developed by Bernoco and Bailey used PCR primers to determine the frequency of SCID carriers. Random sampling of 250 Arabian horses revealed that the frequency of SCID was 8.4% in USA [8]. After investigating the pedigrees of carrier horses, it was shown that this mutation was introduced and distributed among the popular stallions by an imported horse.

Among Arabian horse populations tested using molecular methods, the SCID mutant gene was detected in the USA [8], the UK [9], Brazil [10], Morocco [11], Egypt [1], and South Africa [12], but could not be detected in Ukraine [13], Syria [14], Iran [15], Romania [16], Slovenia [17], Poland [18], or Turkey [19]. There was only one study on SCID in Turkish Arabian horses. This study was carried out in the Turkish State farm and showed that no animals examined were the carrier of SCID.

Eskişehir is one of the most important breeding grounds in Turkish horseracing. When the pedigrees of horses that are successful in the race fields are examined, the enterprises in Eskişehir come to the forefront. Purebred horse breeding is carried out in 73 private farms in Eskişehir, mainly in the Mahmudiye district. As of 2014, there were approximately 1600 purebred mares and stallions at various ages in Eskişehir [20].

The aim of this study was to investigate the presence of the mutant allele of the SCID gene in Turkish Arabian horses reared in Eskişehir using DNA sequencing analysis.

2. Materials and methods

All experimental techniques, including animal handling and sample collection, were approved by the Medical and Surgical Experimental Research Center Committee (TICAM) of Eskişehir Osmangazi University, Decision 2015/466-1.

2.1. Animals

In this study, a total of 115 Turkish Arabian horses (10 of 115 were stud and rest were mare) from nine private horse enterprises in the Mahmudiye district of Eskişehir in Turkey were genotyped. The age of the horses included in the study ranged from 5 to 15 years.

2.2. DNA extraction and genotyping

Genomic DNA was purified from whole blood samples using the salting out method [21] and stored at –20 °C until analysis. To detect the SCID mutant allele, polymerase chain reaction (PCR) was performed to amplify with a pair of primers (F: 5'-AAGTTGGTCTTGTCATTGAGC-3'; R: 5'-TTTGTGATGATGTCATCCCAG-3') [22] covering the five nucleotide deletion sequence in the DNA-PKcs gene. The amplicon length of the primers used in this study was 174 bp. A 50-µL PCR reaction mixture contained a 50–100 ng DNA template, 10X *Taq* polymerase buffer, 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 0.5 U *Taq* DNA polymerase, and 5 pmol of each primer. The 174 bp PCR products were checked with RedSafe (iNtRON Biotechnology, Korea) stained 2% agarose gel electrophoresis. PCR products were cleaned with a PCR clean-up kit (Macherey-Nagel, Germany) and DNA sequencing analysis was performed on an ABI 3500 instrument (Applied Biosystems, CA, USA) using a BigDye ™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). DNA sequencing analysis was performed using MEGA X [23] and CodonCode [24] computer programs.

3. Results

In this study, 115 Turkish Arabian horses from nine different private farms in Eskişehir, Turkey were screened for the SCID mutant allele using DNA sequencing. Before sequence analysis, 174 bp PCR products were controlled on 2% agarose gel electrophoresis (Figure 1). DNA sequence analysis revealed that there was no deletion in any of the samples tested in the study (Figure 2).

4. Discussion

DNA sequencing was used to investigate the five-nucleotide deletion in the DNA-PKcs gene in Arabian horses reared on Eskişehir private farms. Cinar Kul et al. [19] showed that there were no SCID genetic defects in Turkish Arabian horse populations in state breeding farms. Supporting the previous study, our research indicates that all samples obtained from the private horse farms of Eskisehir were normal in terms of SCID hereditary defects. These results suggest that the Turkish Arabian horse population in Eskişehir has no SCID mutant allele, and thus has a higher genetic value. However, Turkish Arabian horse populations should be investigated for genetic defects throughout Turkey. In particular, for the protection of the Turkish Arabian horse population, which seems to be free of SCID, it is crucial that imported horses and semen should be tested for known genetic defects, such as SCID, prior to use in breeding.

Several previous genetic studies have investigated the five nucleotide deletion in the DNA-PKcs gene using primers that yield a PCR product of 521 bp [25], 259 bp [15], 235 bp [16], 179 bp [10], 174 bp [22], 169 bp [8,26], 163 bp [1,9,19,27], 162 bp [11], and 135 bp [13,17] in different Arabian horse populations.

To detect wild and mutant SCID alleles, the amplified PCR products were electrophoresed on 10% [10], 8% [13,15], and 6% Polyacrylamide gel [8,27], 4% agarose gel [17], or capillary electrophoresis [9,11,16,19]. In addition to electrophoresis, sequencing analysis could be performed to identify the genotypes of a population in terms of the presence/absence of SCID mutant alleles, as in

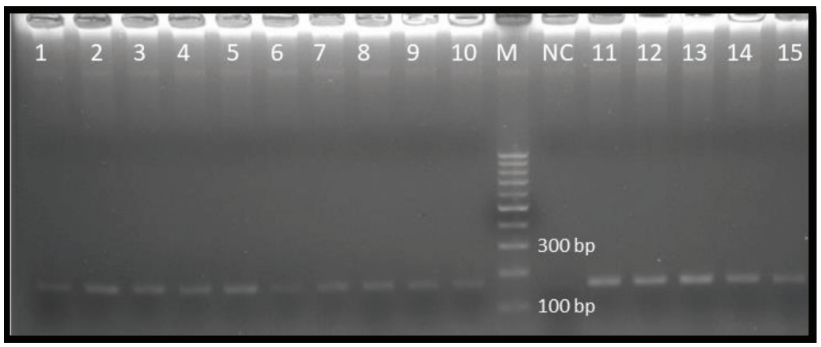

Figure 1. Electrophoretic band pattern of SCID genotypes (M: 100 bp GeneRuler DNA marker, NC: Negative control).

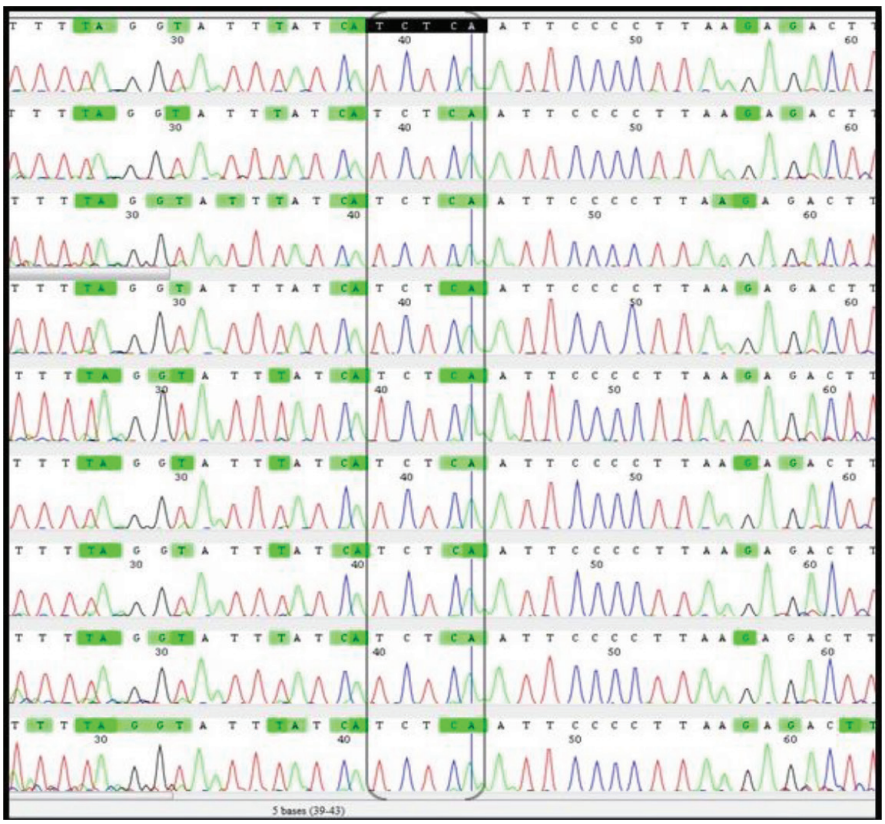

Figure 2. Alignment of the mutation site (TCTCA) of several sample sequences with the CodonCode aligner program (selected nucleotides show the deletion site).

these studies [1,25]. The studies that investigated SCID in Arabian horses in the literature were summarized in Table.

The SCID mutant allele was not observed in Arabian horse populations in Poland [18], Romania [16], Slovenia [17], Latvia [27], Iran [15], Syria [14], Ukraine [13], Egypt [1,28], or Turkey [19]. Conversely, the SCID mutant allele was identified in Arabian horse populations in the following prevalence: 8.4% in the USA [8], 2.8% in England [9], 1.5% in Brazil [10], 6.4% (in the year 2004) and 3.4% (in the year 2009) in South Africa [12], 5.6% in Morocco [11], 1.6% in Iran [26], and 0.4% in Poland [25].

After using diagnostic genetic testing, the frequency of the SCID mutant allele in South Africa was reduced from 6.4% in 2004 to around 3.4% in 2009 [12]. On the other hand, while the SCID mutant allele was not found in Poland in 1999 [18] or in Iran in 2011 [15], studies performed later showed that the frequencies of SCID carrier horses were determined as 0.19% [25] and 0.8% [26] in Poland in 2015,

Table. Genetic test results of SCID in some countries.

Country	Methods	Number of horses	Observed Heterozygotes	Reference
USA	PCR	250	21	8
UK	PCR	106	3	9
Brazil	PCR	205	3	10
Morocco	PCR	377	21	11
South Africa	PCR	800	51 (2004)	12
		699	24 (2009)	
Poland	PCR	271	-	18
	Sequencing	808	3	25
Iran	PCR	120	-	15
	PCR	244	4	26
Slovenia	PCR	128	-	17
Romania	PCR	60	-	16
Latvia	PCR	57	-	27
Egypt	Commercial kits	10	-	28
	Sequencing	103	-	1
Syria	SNP Genotyping Assay	250	-	14
Ukraine	PCR	16	-	13
Turkey	PCR	239	-	19

and Iran in 2017, respectively. According to Arabian Horse Association, from the start of testing in 1997 through December 31, 2011, a commercial testing company has tested >10,600 horses with a frequency of 15.7% SCID carriers and 0.3% SCID affected [29]. In order to avoid the spread of carriers and to keep the Turkish Arabian horse population SCID-free, it is necessary to test all Arabian horses in Turkey through genetic testing.

Knowing the molecular mechanism behind a hereditary disease is of great importance, and genetic testing to reduce the frequency of genetic disorders is necessary to maintain herds that are free from hereditary disease. Genetic testing for hereditary disorders like SCID is easy and highly accurate. With the help of genetic analysis, horses with mutant alleles can be removed from the breeding program, and these genetic defects can be prevented from being carried over to new generations [17]. With the spread of artificial insemination in animal husbandry, the frequency of some hereditary defects has increased [30]. Therefore, male and female breeding candidates should be screened for breed-specific hereditary diseases and carriers should be removed from breeding programs.

Acknowledgments

This study was supported by a grant from Scientific Research Projects of Eskişehir Osmangazi University (Project No: 2018-2033; Project coordinator: Dr M. Kaya). This work was performed by Mustafa Doğan in partial fulfillment of his master's degree in Agricultural Biotechnology at Eskisehir Osmangazi University, Turkey.

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