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ALBUMIN and VITAMIN D BINDING PROTEIN SYSTEMS POLYMORPHISMS of ARABIAN HORSES in TURKEY

Türkiye'deki Arap Atlarında albumin ve Vitamin D Bağlayıcı Protein Polimorfizminin Araştırılması

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SUMMARY

This study was carried out to investigate the polymorphisms of Albumin (Al) and Vitamin D Binding Protein (Gc) Systems in 1177 and 1251 blood samples collected from Arabian foals and their parents, respectively.

The results of starch gel electrophoresis (SGE) indicated that in the Al systems, the first homozygote band was visualised as FF, the last homozygote band was SS. The band between the two homozygote bands was named as heterozygote, FS. The allelic ferquencies were determined to be 0.565 for F and 0.435 for S. The phenotypic frequencies of these alleles were found as 30.41% for FF, 52.09% for FS and 17.50% for SS.

The polymorphism of the Gc system investigated in 1251 blood samples of Arabian horses demonstrated the existence of three phenotypes controlled by two autosomal, co-dominant alleles. The phenotypic frequencies were found to be 87.05% for FF, 11.75% for FS and 1.19 for SS. The allelic frequencies of F and S were determined as 0.930±0.005 and 0.07±0.005, respectively.

Key Words: Albumin, Vitamin D Binding Protein, Genetik polymorphism, Arabian Horse.

ÖZET

Bu çalışma, Albumin (Al) ve Vitamin D Bağlayıcı Proten Sistemi (Gc) polimorfizmini incelemek amacıyla yeni doğmuş Arap atı tayları ile onların ebeveynlerinden alınan sırasıyla 1177 ve 1251 kan örneğinde üzerinde yürütüldü.

Yapılan nişasta jel elektroferezinde önde ilerleyen homozigot band FF, arkada kalan homozigot band SS ve her ikisinin arasında kalan heterozigot band FS olarak isimlendirilmiştir. F alleli frekansı 0.565 ve S alleli frekansı 0.435 olarak tespit edilmiştir. Bu allelere ait fenotiplerin görülme yüzdeleri, FF % 30.41, FS: % 52.09 ve SS: % 17.50 olarak belirlenmiştir.

Gc sistemi de iki otozomal ko-dominant allel tarafından kontrol edilen 3 fenotip göstermiştir. Bu fenotiplerin görülme sıklıkları; FF: % 87.05, FS: % 11.75 ve SS: % 1.19 olarak belirlenmiştir. F ve S aleleri frekanslarının sırasıyla 0.930±0.005 ve 0.07±0.005 olarak tespit edilmiştir.

Anahtar Sözcükler: Albumin, Vitamin D Bağlayıcı Protein, Genetik polimorfizm, Arap atı.

INTRODUCTION

The albumin system is the well-known polymorphic locus used in parentage control and pedigree analysis in horses. In the albumin system (Al), the early studies showed that the extinction of AA, AB, BB phenotypes controlled by a pair of autosomal co-dominat alleles in the horse (1). Similar results reported by Braend confirmed that the phenotypes of Al system were as AA, AB and BB (2). Within the following years, Gahne (3) named the phenotypes of Al system as FF, FS and SS which seemed to be controlled by two autosomal, co-dominant alleles, Al^F and Al^S. In addition, Sandberg (4) demonstrated the third allele, AlI, in the albumin system. Al and A, B, I are accepted as the symbol of the Al locus and alleles by ISAG (5), respectively. The close linkage between Al and group specific component (Gc) in human has also been proved in the horses (6,7).

Group specific component is another polymorphic protein system in determining parentage and pedigree analysis in horses. The Gc system also known Vitamin D Binding Protein is one of the post-albumin proteins. This system, designated as Gc, has two described alleles, F and S (5). The polymorphism of Gc in horse plasma was first demonstrated with

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two autosomal, co-dominant alleles, F and S. Three different phenotypes, FF, FS and SS, were determined in Arabian and Thoroughbred horses and Shetland ponnies against goat anti-human Gc antibody using immunofixation electrophoresis (8). Later studies also showed that these phenotypes are genetically controlled by a pair of alleles of Gc, Gc^F and Gc^S (9).

Gahne and Juneja (10) reported a human homologue of Gc protein other than cattle and horse in plasma. The electrophoretic pattern of post-albumin protein in the autoradiographic study was distinct in cattle and horse. In cattle, each of the homozygous showed two bands while the heterozygous had four bands. In horse, single band was visualised in homozygous while two bands were seen in heterozygous.

The breeding studies for the Arabian horses in Turkey started during the Ottoman time and this horses were first used for military purpose for a long time. In the early years of Turkish Republic (1925-1936), the cross breeding studies were carried out between in the Arabian horses originated from Anatolian, Iraq and Saudia Arabian horses. Many years of breeding studies resulted in a new horse bred to be named as Turkish-Arabian horse (11). In this study, we aimed to determine the allelic frequencies of these two systems in Arabian horse population in Turkey and compare to other horse populations in the world.

MATERIALS and METHODS

The horses used in the present study belonged to the Turkish-Arabian breed. Blood samples was obtained from the newborn foals and their parents between 1995-1998. The blood samples were collected for the routine evaluation of parentage control and pedigree record. In this study, the polymorphism of Al and Gc systems were investigated in 1177 and 1251 blood samples, respectively. All the analysis were performed in the Institute of Etlik Veterinary Control and Research Center, Ankara.

The serum of each blood sample was separated and kept at -20 °C till the analyse stage. Both of polymorphic systems were analysed using polyacrylamide gel electrophoresis (PAGE) at pH 8.5. The PAGE electrophoresis lasted for about 3.5-4 hours (12). In addition, acidic starch gel electrophoresis (SGE) was used for the determination of the Al system in order to recognise the phenotypes which were not identified clearly by PAGE.

Allelic frequencies for protein polymorphism was computed by direct counting from phenotypes (13). The chi-square goodness of fit test was performed to find out the differences between expected and observed values for the Al and Gc system. The population was evaluated in Hardy-Weinberg equilibrium (14).

RESULTS

The polymorphism of Al was investigated in 1177 blood samples from Arabian horses. Three phenotypes, FF, FS and SS, determined by two autosomal co-dominant alleles were observed in 1177 blood samples for the Al system. The allelic frequencies of F and S were estimated to be 0.565 and 0.435, in order. The phenotype frequencies for FF, FS and SS were 30.41%, 42.09%, 17.50%, respectively (Table 1).

The blood samples from 1251 Arabian horses were analysed to determine the polymorphismin in the Gc system. Three phenotypes and two autosomals, co-dominant alleles were also visualised by PAGE as observed in Gc. The phenotypic percentages were determined for FF (87.05%), FS (11.75%) and SS (1.19%) within the population. The allelic frequency of F was 0.930 and F allele was significantly higher than S allele (Table 1). It was observed that the population was in Hardy-Weinberg equilibrium for both polymorphic systems.

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Table 1. The polymorphic values for the Al and Gc systems in Arabian horses
Tablo 1. Arap atlarında Al ve Gc sistemleri için polimorfik değerler

Polymorphic system	Phenotype	Observation (%)	Observed (n)	Expected (n)	Allele	Genotype frequency
Al	FF	30.41	358	375	F	0.565± 0.01
	FS	52.09	613	579		
	SS	17.50	206	223	S	0.435± 0.01
	Total	100	1177	1177		
Gc	FF	87.05	1089	1082	F	0.930± 0.005
	FS	11.75	147	163		
	SS	1.19	15	6	S	0.070± 0.005
	Total	100	1251	1251		

DISCUSSION and CONCLUSION

The albumin system which has been used for the analysis of parentage testing, the best known polymorphic loci in horse populations. For the analysis of Arabian horse population in Turkey, PAGE and SGE demonstrated that the FF, FS and SS phenotypes were determined by two autosomal, co-dominant alleles. Though some researchers reported the Al system had three co-dominant alleles, most studies confirmed the idea of 2 alleles (1,4,15-17). The analytical works performed by Kaminski in Arabian and Anglo-Arabian horses could not determine the third allele, I. In the same study, the allelic frequency of I was also investigated in different Ponny breds and found around 0.01 in only two ponnies. These results were found insignificant (16).

The present study for the polymorphism of the Al system within the Arabian horse population demonstrated a higher gene frequency for F allele (0.565) compared to S allele (0.435). In France, similar statistical results were previously provided for the Arabian horses originated from Polland. The same study reported the gene frequencies of F and S alleles as 0.570 and 0.430, in order (15). The research in Arabian horses in France appeared to produce the same results as in this study. On the other hand, the same study found that the gene frequencies of F and S alleles were as 0.490-0.510 and 0.400-0.600 in Arabian horses originated from Iberia and France, respectively (15). The latter studies showed a higher frequency for S allele than F allele and this is distinct from our results. In United States between 1977-1987, blood samples were collected from 5392 Thoroughbred horses for the routine parentage test and reproduction control. The analysis determined two alleles known as A and B in the Al system. The blood samples from Arabian horse population were examined for the allelic frequency of F and S and estimated to be S allele (0.565) higher than F allele (0.435). In addition, the gene frequency for S allele was higher compared to F allele in four different pony breds. Only in Thoroughbred horses, F allele (0.615) showed a higher frequency than S allele (0.385) (17).

The examination of the Gc system revealed that two alleles, F and S, and three phenotypes. These results agreed with the earlier studies. These three phenotypes, FF, FS and SS, were observed at 87.05%, 11.75% and 1.19%, respectively. In consideration of the observation percentages of these phenotypes, the gene frequency of F and S alleles were determined

as 0.930 and 0.070. In conclusion, the F allele is observed significantly higher than S allele in the Gc system of Arabian horse population.

Similar polymorphic results in the Gc system are alşo seen in feral horse populations. The gene frequency of F allele was determined lower than 1.000 in 6 out of 7 feral horse populations, such as Beaty Bute (0.951), Clan Alpine 1 (0.938), Clan Alpine 2 (0.944), Flanigan (0.951), Stone Cabin 1 (0.954) and Wassuk (0.985), (18) Only Stone Cabin 2, the F allele was observed 1.000. The gene frequency of F and S was determined as 0.968 and 0.032 in Fresian horses, respectively (19). The data given above for the gene frequencies of the Gc appear to be similar to the frequence of 0.930 in the F allele in the present study.

Additionally, Bowling and Clark, (17) performed a study in Thorougbred and Arabian horse populations to determine the gene frequencies of F and S alleles. In Thoroughbred horses, the F allele frequency was 0.939 and the S allele was 0.061. Similar results for F allele and S allele in Arabian horses were also obtained in this study. A recent study by Ouragh and his colleagues (20) also obtained the same results in the Gc system for Arabian horses in Morocco (the allelic frequency for F: 0.940, s: 0.060).

Fifty-nine Standardbred horses in USA were examined for the Gc system and 32 FF, 25 FS and 2 SS phenotypes were determined. The allelic frequencies of F and S were reported to be 0.770 and 0.230, respectively. In the same study, different horse populations were examined for the existence of FF and FS phenotypes and detected 15 FF, 2 FS in 17 Thoroughbred, 14 FF and 4 FS in 18 Shetland ponnies, 3 FF and 1 FS in 4 Arabian horses, 4 FF in 4 Qarter. The allelic frequencies for F and S were estimated to be 0.940 and 0.060 in Thoroughbred and 0.890 and 0.110 in Shetland ponnies, respectively (9). The lack of significant number of horse populations makes difficult to compare with the present study for the analysis of FF and FS phenotypes. On the other hand, the allelic frequencies of F and S alleles in Thoroughbred and Shetland ponnies were found to have close values.

In conclusion, three phenotypes determined by two autosomal, co-dominant alleles are found in the Al system for the Arabian horse population in Turkey. The gene frequency of F allele is observed high compared to S allele. The heterozygous phenotype of FS is detected high in relation to homozygous phenotype. Similar results were also obtained for the Gc system. The gene frequency of F allele is found to be very high of 1251 Arabian horse population. The lomozygote phenotype of SS is determined in only 6 horses. The population analysis of both Al and Gc polymorphic systems agreed with the Hardy-Weinberg equilibrium.

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