A Thesis
For the Degree of Master of Veterinary Science

Genetic Polymorphism of the Serum Proteins of Horses in Cheju

Department of Veterinary Medicine

Graduate School, Cheju National University

Jinah Shin

2001. 2

Genetic Polymorphism of the Serum Proteins of Horses in Cheju

Jinah Shin
(Supervised by Professor Kyoungkap Lee)

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Veterinary Science.

2000. 12.

This thesis has been examined and approved.

Thesis director, Heeseok Kim, Prof. of Veterinary Medicine

Younghoon Yang, Prof. of Animal Biotechnology

Kyoungkap Lee, Prof. of Veterinary Medicine

2001. 2.

Department of Veterinary Medicine
GRADUATE SCHOOL
CHEJU NATIONAL UNIVERSITY

초 록

제주마의 혈청단백질의 유전적 다형현상

신진아

제주대학교 대학원 수의학과

제주도내 사육되고 있는 말의 혈액 유전형을 조사하기 위해, 천연기념물인 제주재래마군 45두, 제주경마장의 제주경주마군 60두, 육성마 목장의 더러브렛군 60두를 선정하여, 혈청단백질인 albumin (Alb), vitamin-D binding protein (GC), esterase (ES), A1B glycoprotein (A1B), transferrin (TF) 좌위를 polyacrylamide gel electrophoresis를 이용하여, 표현형, 빈도수와 유전적 평형상태를 구하였다.

더러브렛군에서의 TF 좌위를 제외하고, 모든 좌위에서 다형현상을 보였다. 제주재래마군에서 ES^S와 TF^{F1} 대립유전자는 관찰되지 않았다. 더러브렛군에서는 Alb^B, ES^I, TF^D와 TF^{F1}의 빈도수는 높게 나타났다. 관찰치와 기대치의 검증결과, 제주경주마군의 ES 좌위를 제외하고 세 군 모두 유전적 평형상태를 나타내었다.

Alb, ES 와 TF 좌위에서 이형접합도는 높게 나타난 반면 GS와 A1B 좌위에서는 낮게 나타났다. 평균 이형접합도는 제주재래마군, 제주경주마군, 더러브렛군 에서 각각 0.3535, 0.3555, 0.2726을 나타내었다.

중심어: 혈청단백질, 다형현상, 표현형, 빈도수, 이형접합도, 전기영동, 제주재래마

Contents

1. Introduction ---------- 1

2. Materials and Methods ---------- 3

3. Results ---------- 6

4. Discussion ---------- 19

5. References ---------- 23

1. Introduction

The Cheju native horses (CNH) are representative of the native horses in Korea, and have a particular hereditariness in process of adaptation to the climate of Cheju. In recent years, it has been assumed that some of CNH have been hybridized with foreign breeds for racing and riding in farms (Cho *et al.*, 2000).

The CNH had been identified by color, size, shape and hair characteristics (Kang *et al.*, 1986; 1988; Jung *et al.*, 1991; Yang *et al.*, 1991), but these are relatively difficult to measure (Bowling & Clark, 1985). Blood groups and protein polymorphisms can be revealed by laboratory methods which allow precise definition and discriminations of variants (Bowling & Clark, 1985; Oh *et al.*, 1992; Han *et al.*, 1995; Bowling, 1996; Cho *et al.*, 2000). Blood grouping is recognized either by clumping of erythrocytes (agglutination) or by lysis of erythrocytes (hemolysis) in the presence of complement. And several kinds of blood protein are clearly recognized by electrophoresis. Electrophoresis is a technique that uses an electrical current to separate a mixture of molecules embedded in a supporting medium (starch, agarose or acrylamide gel). When applied to blood protein, electrophoresis can reveal genetic differences between animals (Bowling, 1996). The items of blood proteins assay by electrophoresis are usually divided into albumin (Alb), tranferrin (TF), postalbumin (A1B), hemoglobin (Hb), 6-phosphogluconate dehydrogenase (6-PGD) and esterase (ES) loci, etc (Bowling & Clark, 1985;

Yokohama *et al.*, 1985; Kaminski *et al.*, 1986; Bowling & Ryder, 1987; Cothran *et al.*, 1987; Bell *et al.*, 1994; Cho *et al.*, 2000).

The CNH were designated as natural monuments, and have been raised specially. Some of them were distributed to farms and have been used as racing horses at the Cheju Racing Track, a branch of Korea Racing Association. Presently, Cheju Institute is very concerned about hybrid of the CNH with foreign breeds artificially for getting excellent records when they are in a race. Therefore the preservation of pure pedigree is very important. There are some reports of morphology (Kang *et al.*, 1986; 1988; Jung *et al.*, 1991; Yang *et al.*, 1991), genetic phenotypes and frequencies of serum proteins of horses in Cheju (Yokohama *et al.*, 1989; Oh *et al.*, 1992; Kim *et al.*, 1993; Oh *et al.*, 1995; Han *et al.*, 1995; Shin *et al.*, 1999; Cho *et al.*, 2000), but there are few reports of genetic comparison of serum proteins among CNH, CRH and TB.

This study was carried out to find genetic diversity in CNH, CRH and TB by investigating the phenotypes and gene frequencies of Alb, GC, ES, A1B, and TF loci which are authorized internationally among serum proteins, to clarify the distribution and characteristics of serum proteins of CNH and to get a basic data for pedigree establishment and maintenance of purity of the CNH.

2. Materials and Methods

1) Experimental animals

This study used three different groups of horses in Cheju, and experimental individuals were gathered at random in each group; 45 Cheju native horses (CNH) which were precious natural monuments in Jeju Institute for Livestock Promotion, 60 Cheju racing horses (CRH) which were racing horses in Jeju Racing Association and 60 Thoroughbreds in (TB) in Jeju equine stud farm and training center.

2) Sampling

Blood samples were collected from 165 horses (CNH, 45; CRH, 60; TB, 60) from jugular vein. The samples were centrifuged at 2,500 rpm for 10 minutes, and then isolated serum and stored in –72 ℃.

3) Electrophoresis

The polymorphism of serum proteins was analyzed by horizontal polyacrylamide gel electrophoresis (HPAGE) (Yokohama *et al.*, 1987). The gel solutions and electrode buffer contents were as follows;

(1) Gel solution

A solution;

Acrylamide 32 g, N'-methylenebisacrylamide 0.8 g / DW 100 ㎖

B solution;

18 % Trisaminomethane 50 ㎖, N, N, N', N'-tetramethylethylenediamine (TEMED) 300 ㎖, 2-Mercaptoethanol 150 ㎕ / DW 100 ㎖, adjust pH 7.9 with 1 M citric acid.

C solution;

Ammonium persulfate 100 ㎎ / DW 50 ㎖

The compositions of solutions for making suitable gels were shown in Table 1.

Table 1. The composition of polyacrylamide gels

Components	A solution	Distilled water	B solution	C solution
Order	1	2	3	4
12%	44.8 ㎖	15.2 ㎖	30 ㎖	30 ㎖
4%	2 ㎖	8.2 ㎖	2 ㎖ + T10 ㎕	4 ㎖
8%	6 ㎖	9 ㎖	3 ㎖ + T15 ㎕	6 ㎖

(2) Electrode buffer; Trisaminomethane 7.87 g, boric acid 1.48 g. pH 9.0

The staining and destaining solutions were as follows;

(1) ES staining; 0.19 M Trisaminomethane 150ml, 0.05M Citric acid Monohydrate 200 ml, 1% α-Naphthyl acetate (dissolved in Acetone) 8 ml, Fast blue B salt

(2) Protein staining; Coomassie brilliant blue G 1 g, 60 % perchloric acid 60 ml / DW 1000 ml

(3) Destaining; Methanol 200 ml, acetic acid 70ml / DW 1000 ml

Polyacrylamide gel was cast between glass plates. A step gradient of acrylamide concentration of 12 %, 4 % and 8 % was used in turn. The gel buffer of pH 7.9 was Tris-citrate and the electrode buffer of pH 9.0 was Tris-borate. Samples were run simultaneously on a cooling plate at 5 ℃. The current was at first set at 500 V, 30 W for 8 minutes, after removing the sample loading papers, and then set at 1200 V, 50 W for 6 hours. The detection of esterase (ES) was stained in ES staining solution and the other proteins were stained in protein solution.

4) Statistical analysis

Statistical methods (Pasteur & Pasteur, 1988) used in this study were as follows;

(1) Allelic frequency: $2\{ii\} + \{ij\} / 2N = p, q$

($\{ii\}$, the number of ii homozygotes; $\{ij\}$, the number of heterozygotes having an I allele; N, number of individuals)

(2) Expected number: Ho : $p^2 \times N$, He : $2pq \times N$, Ho' : $q^2 \times N$

(3) Chi-square test: $\chi^2 = \Sigma (O - E)^2 / E$

(O, the observed number; E, the expected number)

(4) Heterozygosity: $H = 1 - \Sigma q_i^2$

(q, the frequency of the I allele of the gene at this locus)

Chi-square tests carried out to check for significant differences between observed and expected numbers for genetic equilibrium of Hardy-Weinberg law.

3. Results

The image of horizontal polyacrylamide gel electrophoresis at 12% gel to separate horse blood serum protein was presented in Fig. 1. According to mobilities, the protein bands from fast migration to slow migration were albumin (Alb), vitamin-D binding protein (GC), esterase (ES), A1B glycoprotein (A1B) and tranferrin (TF) loci in order.

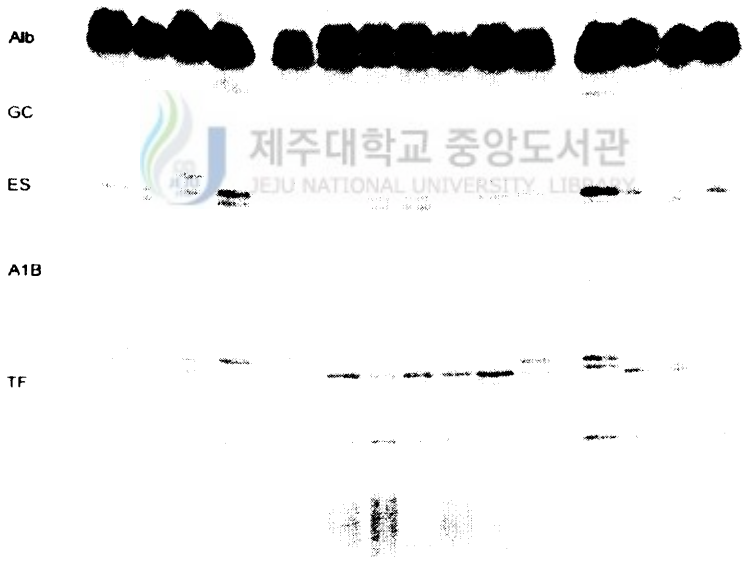

Figure 1. Serum protein loci separated on the horizontal polyacrylamide gel (HPAGE)

1) Genetic polymorphism of Albumin (Alb) locus

Albumin is the most fast migrating protein component on gel. This locus was controlled by 2 codominant autosommal allele A and B; phenotypes of albumin were the fast migrating AA, slow migrating BB and heteotype AB (Fig. 2).

Figure 2. Phenotypes of Alb locus separated on the HPAGE

The phenotype BB of TB has the highest frequency in all three groups. Over all, the frequency of Alb^B was higher than that of Alb^A. The frequencies of Alb^A and Alb^B were 0.433 and 0.567 in CNH, 0.450 and 0.550 in CRH, 0.108 and 0.892 in TB, respectively. χ^2 values from Hardy-Weinberg genetic equilibrium test were 0.0742 (p>0.05) in CNH, 0.0061 (p>0.05) in CRH and 0.1562 (p>0.05) in TB.

Table 2. Phenotypes and gene frequencies of Alb locus.

	Phenotype	No. of heads		Gene frequency	χ^2-test		
		Observed	Expected		χ^2	df	p
CNH	AA	8 (17.8*)	8.450	Alb^A= 0.433			
	AB	23 (51.1)	22.10	Alb^B= 0.567			
	BB	14 (31.1)	14.450				
	total	45			0.0742	1	0.785
CRH	AA	12 (20)	12.150	Alb^A= 0.450			
	AB	30 (50)	29.700	Alb^B= 0.550			
	BB	18 (30)	18.150				
	total	60			0.0061	1	0.938
TB	AA	1 (1.7)	0.704	Alb^A= 0.108			
	AB	11 (18.3)	11.590	Alb^B= 0.892			
	BB	48 (80)	47.70				
	total	60			0.1562	1	0.693

CNH, Cheju native horses ; CRH, Cheju racing horses ; TB, Thoroughbreds ; *, %

2) Genetic polymorphism of Vitamin-D binding protein (GC) locus

The GC variants were detected F and S; Fast migrating FF, slow migrating SS and heterotype FS (Fig. 3).

Figure 3. Phenotypes of GC locus separated on the HPAGE

The phenotype SS was not observed in all three groups. The frequencies of GC^F and GC^S were 0.967 and 0.033 in CNH, 0.992 and 0.008 in CRH and 0.950 and 0.050 in TB, respectively. χ^2 values from Hardy-Weinberg equilibrium test were 0.0535 (p>0.05) in CNH, 0.0042 (p>0.05) in CRH and 0.1662 (p>0.05) in TB.

Table 3. Phenotypes and gene frequencies of GC locus.

	Phenotype	No. of heads		Gene frequency	χ^2-test		
		Observed	Expected		χ^2	df	p
CNH	FF	42 (93.3*)	42.050	GC^F = 0.967			
	FS	3 (6.7)	2.900	GC^S = 0.033			
	SS	-	0.050				
	total	45			0.0535	1	0.817
CRH	FF	59 (98.3)	59.004	GC^F = 0.992			
	FS	1 (1.7)	0.992	GC^S = 0.008			
	SS	-	0.004				
	total	60			0.0042	1	0.948
TB	FF	54 (90)	54.150	GC^F = 0.950			
	FS	6 (10)	5.700	GC^S = 0.050			
	SS	-	0.150				
	total	60			0.1662	1	0.684

3) Genetic polymorphism of Esterase (ES) locus

Three ES variants, F, I and S, showed to be controlled by codominant alleles; Fast migrating FF, moderate migrating II, slow migrating SS and heterotype FI, IS and FS (Fig. 4).

Figure 4. Phenotypes of ES locus separated on the HPAGE

The frequency of ES^I was high in all three groups, and this was the highest in TB. S allele was not observed in CNH. The frequencies of ES^F, ES^I and ES^S, were 0.389, 0.611and 0 in CNH, 0.308, 0.575 and 0.117 in CRH and 0.108, 0.808 and 0.083 in TB, respectively. χ^2 values from Hardy-Weinberg equilibrium test were 0.5613 (p>0.05) in CNH, 10.3885 (p<0.05) in CRH and 4.5567 (p>0.05) in TB.

Table 4. Phenotypes and gene frequencies of ES locus.

	Phenotype	No. of heads		Gene frequency	χ^2-test		
		Observed	Expected		χ^2	df	p
CNH	FF	8 (17.8*)	6.806				
	II	18 (40)	16.806	ES^F= 0.389			
	SS	-	-	ES^I = 0.611			
	FI	19 (42.2)	21.389				
	IS	-	-	ES^S = 0			
	FS	-	-				
	total	45			0.5613	1	0.454
CRH	FF	11 (18.3)	5.704				
	II	24 (40)	19.838	ES^F= 0.308			
	SS	1 (1.7)	0.817	ES^I = 0.575			
	FI	12 (20)	21.275				
	IS	9 (15)	8.050	ES^S = 0.117			
	FS	3 (5)	4.317				
	total	60			10.3885	3	0.016
TB	FF	2 (3.3)	0.704				
	II	39 (65)	39.204	ES^F= 0.108			
	SS	-	0.417	ES^I = 0.808			
	FI	9 (15)	10.508				
	IS	10 (16.7)	8.083	ES^S = 0.083			
	FS	-	1.083				
	total	60			4.5567	3	0.207

4) Genetic polymorphism of A1B glycoprotein (A1B) locus

Generally, three allelic variants F, K and S were detected according to mobilities, but this locus was detected K and S variants in this study (Fig. 5).

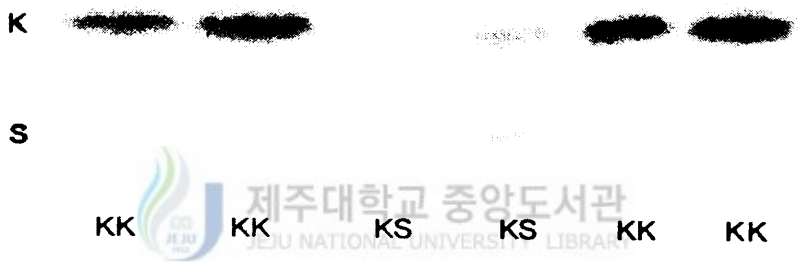

Figure 5. Phenotypes of A1B locus separated on the HPAGE

In TB only phenotype KK was detected. The frequencies of $A1B^K$ and $A1B^S$ in CNH, CRH and TB were 0.967 and 0.033, 0.983 and 0.017, 1 and 0, respectively. χ^2 values from Hardy-Weinberg equilibrium test were estimated to be 0.0535 (p>0.05) in CNH, 0.0172 (p>0.05) in CRH.

Table 5. Phenotypes and gene frequencies of A1B locus.

	Phenotype	No. of heads		Gene frequency	χ^2-test		
		Observed	Expected		χ^2	df	p
CNH	FF	-	-				
	KK	42 (93.3*)	42.050	$A1B^F = 0$			
	SS	-	0.050				
	FK	-	-	$A1B^K = 0.967$			
	KS	3 (6.7)	2.900	$A1B^S = 0.033$			
	FS	-	-				
	total	45			0.0535	1	0.817
CRH	FF	-	-				
	KK	58 (96.7)	58.017	$A1B^F = 0$			
	SS	-	0.017				
	FK	-	-	$A1B^K = 0.983$			
	KS	2 (3.3)	1.967	$A1B^S = 0.017$			
	SS	-	-				
	total	60			0.0172	1	0.896
TB	FF	-	-				
	KK	60 (100)	60	$A1B^F = 0$			
	SS	-	-				
	FK	-	-	$A1B^K = 1$			
	KS	-	-	$A1B^S = 0$			
	SS	-	-				
	total	60					1

5) Genetic polymorphism of Transferrin (TF) locus

TF locus was detected D, F1, F2, H2, O and R in order of decreasing mobility to the anode (Fig. 6).

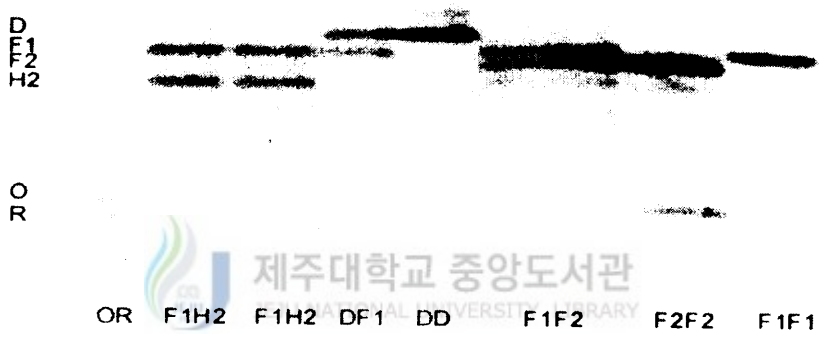

Figure 6. Phenotypes of TF locus separated on the HPAGE

There were 21 different phenotypes and 6 alleles at TF locus. F1 allele was not observed in CNH, but was observed in CRH. F2 and R alleles were high in CNH, D, F2 and R alleles were high in CRH, D, F1 and F2 alleles were quantitative in TB. χ^2 from Hardy-Weinberg equilibrium test were 9.8776 (p>0.05) in CNH, 11.5255 (p>0.05) in CRH and 12.1406 (p>0.05) in TB.

Table 6-1. Phenotypes and gene frequencies of TF locus.

	Phenotype	No. of heads		Gene frequency	χ^2 –test		
		Observed	Expected		χ^2	df	p
CNH	DD	1 (2.2*)	0.356				
	DF1	-	-				
	DF2	5 (11.1)	3.822				
	DH2		0.089				
	DO	1 (2.2)	1.956	$TF^D = 0.089$			
	DR		1.422				
	F1F1	-	-	$TF^{F1} = 0$			
	F1F2	-	-				
	F1H2	-	-				
	F1O	-	-	$TF^{F2} = 0.478$			
	F1R	-	-				
	F2F2	10 (22.2)	10.272	$TF^{H2} = 0.011$			
	F2H2		0.478				
	F2O	10 (22.2)	10.511				
	F2R	8 (17.8)	7.644	$TF^O = 0.244$			
	H2H2	-	0.006				
	H2O	1 (2.2)	0.244	$TF^R = 0.178$			
	H2R		0.178				
	OO	4 (8.9)	2.689				
	OR	2 (4.4)	3.911				
	RR	3 (6.7)	1.422				
	total	45			9.8776	10	0.451

to be continued

Table 6-2. Phenotypes and gene frequencies TF locus.

	phenotype	No. of heads		Gene frequency	χ^2 –test		
		Observed	Expected		χ^2	df	p
CRH	DD	-	0.817				
	DF1	-	0.583				
	DF2	9 (15)	7.117				
	DH2	2 (3.3)	0.817				
	DO	-	0.817	$TF^D = 0.117$			
	DR	3 (5)	3.033				
	F1F1	-	0.104	$TF^{F1} = 0.042$			
	F1F2	2 (3.3)	2.542				
	F1H2	-	0.292				
	F1O	-	0.292	$TF^{F2} = 0.508$			
	F1R	3 (5)	1.083				
	F2F2	15 (25)	15.504	$TF^{H2} = 0.058$			
	F2H2	3 (5)	3.558				
	F2O	6 (10)	3.558				
	F2R	11 (18.3)	13.217	$TF^O = 0.058$			
	H2H2	-	0.204				
	H2O	-	0.408	$TF^R = 0.217$			
	H2R	2 (3.3)	1.517				
	OO	-	0.204				
	OR	1 (1.7)	1.517				
	RR	3 (5)	2.817				
	total	60			11.5255	15	0.715
TB	DD	8 (13.3)	6.338				
	DF1	9 (15)	12.350				
	DF2	8 (13.3)	7.475				
	DH2	2 (3.3)	0.975				
	DO	2 (3.3)	2.925	$TF^D = 0.325$			
	DR	2 (3.3)	2.601				
	F1F1	7 (11.7)	6.017	$TF^{F1} = 0.317$			
	F1F2	7 (11.7)	7.283				
	F1H2	-	0.950				
	F1O	4 (6.7)	2.850	$TF^{F2} = 0.192$			
	F1R	4 (6.7)	2.535				
	F2F2	3 (5)	2.204	$TF^{H2} = 0.025$			
	F2H2	1 (1.7)	0.575				
	F2O	1 (1.7)	1.725				
	F2R	-	1.534	$TF^O = 0.075$			
	H2H2	-	0.038				
	H2O	-	0.225	$TF^R = 0.067$			
	H2R	-	0.200				
	OO	1 (1.7)	0.338				
	OR	-	0.600				
	RR	1 (1.7)	0.267				
	total	60			12.1406	15	0.668

6) Average heterozygosity

The heterozygosity reflects the variety of sources from which this breed is being created. Calculated heterozygosity were estimated to be 0.4911, 0.4950 and 0.1932 at Alb locus, 0.0644, 0.0165 and 0.0950 at GC locus, 0.4753, 0.5607 and 0.3279 at ES locus, 0.0646, 0.0328 and 0 at A1B locus 0.6723, 0.6725 and 0.7467 at TF locus in CNH, CRH and TB, respectively. The TF locus showed the highest value at 5 protein loci. Heterozygosity values of TB were low at all loci, especially A1B locus, but value of TF locus was high. Average heterozygosity values ranged from 0.2726 (TB) to 0.3555 (CRH). TB had the lowest value compared with the other groups. Heterozygosity values of Alb, ES and TF loci were high, but GC and A1B loci were low.

Table 7. Heterozygosity of serum proteins in three groups.

Locus	CNH	CRH	TB
Alb	0.4911	0.4950	0.1932
GC	0.0644	0.0165	0.0950
ES	0.4753	0.5607	0.3279
A1B	0.0644	0.0328	0
TF	0.6723	0.6725	0.7466
Average	0.3535	0.3555	0.2726

4. Discussion

Horizontal polyacrylamide gel electrophoresis was resulted in a separation of proteins, according to mobilities; albumin (Alb), vitamin-D binding protein (GC), esterase (ES), A1B glycoprotein (A1B) and tranferrin (TF) loci were given for CNH, CRH and TB. Mogi *et al.* (1970) reported that Alb locus is controlled by A and B alleles, and there are genetic differences in frequency between Asia and European's horses. It was reported that GC locus is comprised of F and S alleles (Bowling & Clark, 1985; Bell, 1994) and ES locus is comprised of F, G, H, I, S, O and R alleles (Bowling & Clark, 1985). Andersson (1983) and Cho *et al.* (2000) reported that A1B locus is controlled by F, K and S alleles and the frequencies were different between breeds. Yokohama *et al.* (1989) and Schmid & Braend (1990) reported that TF is identified 14 alleles, C, D1, D2, D, F1, F2, F3, G, H1, H2, J, M, O, R and silent, and phenotypes are different between breeds. In this study, restricted alleles were accomplished by HPAGE.

Studies for CNH have been reported of Alb locus (Oh *et al.*, 1992; Oh *et al.*, 1995; Cho *et al.*, 2000), GC locus (Kim *et al.*, 1993; Cho *et al.*, 2000), ES locus (Yokohama *et al.* 1989; Oh *et al.*, 1992; Oh *et al.*, 1995; Cho *et al.*, 2000), A1B locus (Kim *et al.*, 1993; Oh *et al.*, 1995; Cho *et al.*, 2000), TF locus (Yokohama *et al.*, 1989; Cho *et al.*, 2000), almost all of their results appeared to be similar to these results. But at GC locus, results (GC^F, 0.411; GC^S, 0.589) of Kim *et al.* (1993) showed differences in frequencies, it is

probably due to a difference of population examined. And at ES locus, results (ES^F, 0.274; ES^I, 0.479; ES^S, 0) of Cho *et al.* (2000) showed somewhat different frequencies. It is considered that the differences were due to the electrophoresis method. And S allele of ES locus and F1 allele of TF locus in this study were not observed, this could be also identified by Yokohama *et al.*(1989) and Cho *et al.*(2000).

Cho *et al.* (2000) reported of CRH at Alb, GC, ES, A1B and TF loci. The phenotypes and frequencies in this study were similar to previous study. But at Alb locus, his results (Alb^A, 0.280; Alb^B, 0.720) showed differences in frequency. At ES locus, his results (ES^F, 0.203; ES^I, 0.661; ES^S, 0.076) showed slight differences in frequency, it is considered that the differences were due to the electrophoresis method.

Studies for TB have been reported of Alb locus (Mogi *et al.*, 1970; Bowling & Clark, 1985; Kaminski *et al.*, 1986), GC locus (Bowling & Clark, 1985), Es locus (Bowling & Clark, 1985; Kaminski *et al.*, 1986; Yokohama *et al.*, 1989), A1B locus (Bowling & Clark, 1985; Kaminski *et al.*, 1986) and TF locus (Bowling & Clark, 1985; Kaminski *et al.*, 1986; Bell *et al.*, 1988; Yokohama *et al.*, 1989), these present results appeared to be similar to previously described results. TB were characterized by a very large preponderance of ES^I and TB which had only the phenotype KK showed monomorphism at A1B locus in this study.

Over all, the frequency of Alb^B was higher than that of Alb^A and especially TB had higher proportions of Alb^B than other groups. In this study

F allele of GC locus was observed predominantly. Phenotype II was high at ES locus. And phenotype KK was the highest and F allele was not observed at A1B locus. The frequency of TF^{F1} was about two times higher than that of TF^{F2} in TB, while F1 allele lacked in CNH and was rare in CRH. In CNH, lacking of F1 allele could be also identified by Yokohama et al. (1989) and Cho et al. (2000). The frequencies of D and F1 alleles in TB were the highest in all three groups, these results were similar to those of Kaminski *et al.* (1986) and Yokohama *et al.* (1989). The occurrence of ES^S and TF^{F1} in CRH, even though at low frequencies, is one of difference between CRH and CNH, lacking of these variants and the relatively frequencies of ES^S and TF^{F1} in TB were high.

A Chi-square test to determine whether the fit is sufficiently close to expected Hardy-Weinberg proportion revealed that almost of all the polymorphic loci, except ES locus in CRH, showed to be in genetic equilibrium in all three groups. Result of ES in CRH suggested that CRH have been selectively bred as racing horses in farms.

Heterozygosity estimates at Alb, GC, ES, A1B and TF loci were reported previously for CNH and CRH by Cho *et al.* (2000). His results appeared to be similar to these results. But these results were different from previous results at GC locus in CNH, and A1B locus in CNH and CRH. TB showed the lowest value all of the loci, except TF locus. It might be from the relationship between individuals within small pedigreed data. Heterozygosity of CNH and CRH showed higher than TB, suggested that these groups are different from

TB.

In conclusion, these results of genetic polymorphisms and equilibrium in blood serum proteins loci and the other reports of morphological characteristics (Kang et al., 1986; Yang et al., 1991) indicated that CRH might be a hybrid or mixed population between CNH and TB or other imported breed.

5. References

Andersson, L., Juneja RK., Sandberg K.,1983. *Animal Blood Groups Biochemical Genetics.* 14, 45-50.

Bell, K., 1994. Blood protein polymorphisms in the donkey [Equus asinus]. *Animal Genetics.* 25 suppl 1, 109-113.

Bell, K., Pollitt C.C., Patterson S.D., 1988. Subdivision of equine Tf into H1 and H2. *Animal Genetics.* 19, 177-183.

Bowling, A.T., 1996. Horse Genetics. CAB INTERNATIONAL.pp.82-154.

Bowling, A.T. & Clark, R.S., 1985. Blood group and protein polymorphism gene frequencies for seven breeds of horses in the United States. *Animal Blood Groups and Biochemical Genetics.* 16, 93-108.

Bowling, A.T. & Ryder, O.A., 1987. Genetic studies of blood markers in Przewalski's horses. *The journal of Heredity.* 78, 75-80.

Cho, G.J., Kim, B.H., Lee, D.S., Lee, K.K., 2000. Genetic studies of blood markers in Cheju horses II. Blood protein types. *Korean j Vet Res.* 40(2), 283-290.

Cothran, E.G., MacCluer, J.W., Weitkamp, L.R., Bailey, E., 1987. Genetic differentiation associated with gait within American Standardbred horses. *Animal Genetic*. 18, 285-296.

Han, S.K., Chung, E.Y., Shin, Y.C., Byun, H.D., 1995. Studies on Serum Proteins and Enzyme Polymorphisms for Conservation of Cheju native Horses. *Korean J. Anim. Sci.* 37(1), 52-58.

Jung, C.J., Yang, Y.H., Kim, J.K., Kang, M.S., 1991. Studies on the Classification for the Registration of the Cheju Native Horse I. Body measurements by location sex and age. *Korean J. Anim. Sci.* 33(6), 418-422.

Kaminski, M. & Damian, F., 1986. Electrophorestic markers of Andalusian Horses: Comparison of Spanish and lusitanian lineages. *Comp. Biochem. Physiol.* 83B(3), 575-588.

Kang, M.S., Choung, C.C., Kang, T.S., Choung, J.J., 1988. Coat Color Frequencies in the Cheju Island Pony. Cheju National University. 3, 129-132

Kang, M.S., Choung, C.C., Kang, T.S., Park, H.S., Kim, D.H., Choung, J.J., 1986. Study on the Body Type of Cheju Native Horses. Cheju National University. 1, 119-125.

Kim, S.Y., Kang, M.S., Jung, C.J., 1993. Study of blood protein polymorphism of Cheju native horses. Cheju National University. 313-317.

Mogi, K., Abe, T., Hosoda, T., 1970. Studies on serum protein types in horses. I. Classification and inheritance of transferrin and serum albumin types and efficacy of their typing in determining of doubtful parentage. *Jap. J. Zootech. Sci.* 41(8), 400-406.

Oh, M.Y., Ko, M.H., Kim, K.O., Kim, S.J., Chung, C.C., Kim, K.I., 1992. Genetic variations of the blood proteins in Cheju Native Horses. *Korean J. Genetics*. 14(1), 39-50.

Oh, Y.S., Oh, M.Y., Kim, S.J., Kim, K.O., Ko, M.H., Yang, Y.H., 1995. Polymorphisms of Blood Proteins in Cheju Native Horses and Tsushima Native Horses. *Korean J. Zool.* 38, 324-329.

Pasteur, N. & Pasteur, G., 1988. Practical isozyme genetics: Ellis horwood limited. pp. 165-189.

Schmid, D.O., EK, N., Braend, M., 1990. Further evidence for a silent allele in the transferrin locus of the horse. *Animal Genetics*. 21, 423-426.

Shin, J.A., Kim, S.H., Kim, Y.H., Chun, C.I., Lee, K.K., 1999. Genetic

Polymorphism of Serum Protein in Horses on Cheju. *J. Res. Ins. Anim.Sci.* 14, 149-157.

Yang, Y.H., Jung, C.J., Lee, H.J., Kang, T.S., 1991. Studies on the Classification for the Registration of the Cheju Native Horse. *Korean J. Anim. Sci.* 33(6), 438-443.

Yokohama, M., Watanabe, Y., Kobayashi, E., 1989. Classification for Transferrin and Esterase types in Horses. *Jpn. J. Zootech. Sci.* 60(2), 115-120.

Yokohama, M., Watanabe, Yand., Mogi, K., 1985. A New Equine Transferrin phenotype Detected by Isoelectric Focusing. *Jpn J. Zootech, Sci.* 56(2), 116-121.

Yokohama, M., Watanabe, Y., Gawahara, H., Kobayashi, E., 1987. Horizontal Polyacrylamide Gel Electrophoresis for Equine Serum Protein Types. *ABRI.* 15, 22-27.

Abstract

Genetic Polymorphism of the Serum Proteins of Horses in Cheju

Jinah Shin

Department of Veterinary Medicine
Graduate School, Cheju National University, Cheju, Korea

The study was carried out to investigate the genetic polymorphism of the serum proteins of horses in Cheju, they were assigned to three groups; 45 Cheju native horses (CNH), 60 Cheju racing horses (CRH) and 60 Thoroughbreds (TB). We analyzed the phenotypes and gene frequencies of serum proteins at albumin (Alb), vitamin-D binding protein (GC), esterase (ES), A1B glycoprotein (A1B) and transferrin (TF) loci in three groups by using horizontal polyacrylamide gel electrophoresis (HPAGE).

All of the loci, except A1B in TB, showed polymorphisms and different allelic and phenotypic frequencies in all three groups. ES^S and TF^{F1} were not observed in CNH. Allelic frequencies of Alb^B, ES^I, TF^D and TF^{F1} were high in TB. All of the loci, except ES locus in CRH, appeared to be in a state of Hardy-Weinberg equilibrium from *goodness-of-fit* test in all three groups

Heterozygosity estimates at Alb, ES and TF loci were high, but GC and A1B loci were low in all three groups. Average heterozygosities in CNH, CRH and TB were 0.3535, 0.3555 and 0.2726, respectively.

Results showed differences in the frequencies of alleles and phenotypes of

several serum protein loci between CNH and CRH, suggested that CRH might be the horses crossed with other breeds in some degree.

Key words: serum protein, polymorphism, phenotype, frequency, heterozygosity, HPAGE, Cheju native horse.