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Polymorphisms of Coat Color Gene in Jeju Native Horse by Routine Genotyping

Department of Veterinary Medicine

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Abstracts

Phenotypes of equine coat color depend on the relative amount of eumelanin (black-brown) and phaeomelanin (red-yellow), which are controlled, by the melanocortin-1 receptor (*MC1R*) and the agouti-signaling peptide (*ASIP*) genes. Dilution of color is associated with membrane-associated transporter protein (*MATP*) gene. This study was designed to confirm polymorphisms of *ASIP*, *MC1R*, and *MATP* and to analyze genotyping of coat color in Jeju native and Thoroughbred horses.

Genomic DNA was extracted from the whole blood of 35 Jeju native and 110 Thoroughbred horses. The *ASIP* gene was analyzed by simple polymerase chain reaction(PCR) amplification and electrophoresis on 4% agarose gel. The *MC1R* and *MATP* genes were analyzed by PCR-restriction fragment length polymorphism (RFLP) and electrophoresis on 2% agarose gel.

Horses with the *ASIP* mutant type (*a/a*) genotype had a black coat regardless of *MC1R* type except for those with a gray color, while horses with the *MC1R* mutant type (*e/e*) expressed a chestnut color. Horses with *C/C* type typically had a heavier

coat color than those carrying C/C^{cr}.

Jeju native horses carrying basic coat colors can be analyzed with routine genotyping. There were no specific differences between Jeju native and Thoroughbred horses in basic coat color gene expression. Classification by genotyping is required for accurate confirmation of coat color.

Keywords : Jeju native horse, Coat color, *MC1R*, *ASIP*, *MATP*, polymorphism

Contents

Ⅰ. Introduction

Equine coat color types are very diverse and referred to by a variety of terms. The basic horse coat color phenotypes are bay, chestnut, black and gray. Coat colors such as buckskin, cremello, perlino, and palomino are dilutions of these basic colors [13]. When sorting coat color phenotypes, bay horses have body colors which range from a light reddish-brown to very dark brown with black points. Chestnut horses have a reddish body color with no black. Black horses must be completely black except for white markings [17]. Gray or grey is a coat color of horses characterized by progressive silvering of the colored hairs of the coat [6].

Buckskin features coat color faded to a yellow, cream, or gold while keeping the black points. Bay horses with one copy of the cream gene appeared to be buckskin in color. Cremello coats feature pale cream or light tan colors. Horses with a chestnut base coat and two cream genes appear cremello. Perlino is similar to cremello and consists of a bay base coat with dilute genes. Palominos feature golden, yellow, and tan shades with a flaxen or white mane and tail. Chestnut horses with one cream dilution gene have the palomino color. Equine coat colors can be classified by the aforementioned features.

Jeju native horses inhabiting on Jeju island are one of the pony breeds and are state designated natural monument No. 347 with its specific traits. Jeju native horses have a variety of coat colors and the traditional coat color classification of Jeju native horses has existed for a long time. Many phenotypes of Jeju native horses had been named by this methods. Typical and detailed names of phenotypes of Jeju native horse colors including basic and dilution types exist. However limitations also exist with regard to assigning exact horse coat colors due to subjectivity.

As a result of analysis developments at the molecular level, melanocyte may surface as a key factor to determine equine phenotypes. Pigment synthesis is known to be directly dependent on the acting, development, differentiation, proliferation, and migration of melanocytes [9]. Basic coat color is influenced by two pigments, black eumelanin and red pheomelanin. Coat phenotypes manifest differently according to the relative quantity of both pigments. Chestnut horses are characterized by eumelanin pigment in the skin and pheomelanin pigment in the hair. Black horses have uniformly distributed black pigmented skin and hair, while bay horses have pheomelanin in the body and eumelanin in the mane, tale, and lower legs [12].

Genetic tests at the DNA level are now available which allow genotyping of individual horses for basic coat colors (chestnut, bay, black). Basic color expressions are controlled by extension (*E*) and agouti (*A*) loci [9]. The agouti locus is occupied by the agouti signalling peptide (*ASIP*) gene, which encodes same protein. and the extension locus is occupied by the melanocortin-1-receptor (*MC1R*) gene, which encods the same protein. These two loci are encoded by *MC1R* and *ASIP* [12]. *MC1R*, is encoded by the extension (*E*) locus, and its peptide antagonist *ASIP* is encoded by the agouti (*A*) locus. *ASIP* acts as an antagonist of *MC1R* by nullifying the action of α-melanocyte-stimulating hormone(α-MSH). Loss of function of *MC1R* results in a yellow pigment(pheomelanin), whereas gain of function of *MC1R* or loss of function of *ASIP* appears to result in the production of black pigment(eumelanin) [2]. Membrane-associated transporter protein (*MATP*) is associated hypopigmentation [7].

A variety of mutations of these loci were found in horses. The chestnut allele *e*, a single nucleotide mutation (*C*901*T*) within *MC1R*, was the first to be described in the horse [8]. The black allele *a*, an 11 base pair (bp) deletion in exon 2 of *ASIP* has also been detected [12] as well as the dilution allele C^{cr}, a single nucleotide mutation (G457A) [7].

Few studies concerning genotyping of Jeju native horses have been reported although genotyping analysis of other horse breeds has been done many times. The aim of the present study was to understand the relationships between genotypes and phenotypes in Jeju native horses with *MC1R*, *ASIP*, *MATP* genes and to classify and systematize Jeju native horse coat color polymorphisms.

Ⅱ. Materials and Methods

1. Animals

A total of 35 Jeju native horses and 110 Thoroughbred horses were evaluated in this study. All animals were used for horseracing at the Korea Racing Authority(KRA) Jeju and Seoul racecourses.

2. Sample preparation

Blood was collected from the jugular vein for routing genotyping. All samples were stored in EDTA-3K tubes to prevent coagulation.

3. DNA Isolation

Genomic DNA was extracted and isolated from 300 ㎕ blood samples using G-Dex™ Ⅱb DNA Extraction Kits (iNtRON Biotechnology, INC., Seoul, Korea) according to standard protocols. All concentrations of isolated DNA were adjusted to 100 ng/㎕ for analysis (NanoVue™, GE Healthcare, UK). Samples were preserved at -20℃ to insure their stability.

4. PCR

1) Primer design

Primer sets and oligonucleotide sequence used in the present study were designed by the DNASIS MAX v2.5 operating program. Three primer pairs were designed to amplify fragments of *MC1R*, *ASIP*, and *MATP* containing gene polymorphisms [7, 8, 11]. PCR primer sets used are detailed in Table 1. Primer set amplification sizes were 116 bp (*MC1R*), 120 bp (*ASIP*) and 190 bp (*MATP*).

Table 1. Oligonucleotide sequences of *MC1R*, *ASIP* and *MATP* gene primer sets, melting temperature (Tm) and amplicon size

Primer	Sequence(5'～3')	Tm (°C)	Amplicon size(bp)
MC1R-F	GGGGAATTCCCTGCACTCACCCATGTACTACTTCATCTG	58	116
MC1R-R	GGGGAATTCGCAGCAGCAAGATTGCCATCTCCAG	58	116
ASIP-F	GGGGAATTCCTTTTGTCTCTCTTTGAAGCATTG	54.4	120
ASIP-R	GGGGAATTCGAGAAGTCCAAGGCCTACCTTG	54.4	120
MATP-F	GGGGAATTCTTTGATTGCTGACCGAAGGAAGAAG	70	190
MATP-R	GGGGAATTCGAAGAGAGCGTGGTGGTGGAG	70	190

2) Simple PCR and PCR-RFLP

PCRs of three genes were conducted with a TM 600 thermal cycler(Takara Bio, Shuzo, Japan). All PCRs followed the same protocol except for melting temperature (Tm). The PCR protocol consisted of forward primer 1㎕, reverse primer 1㎕ (10 pmole/㎕), 10× PCR buffer 2㎕, 1×dNTP mixture 0.5㎕, Top-*taq*TM 0.2㎕ (2.5 unit/㎕), and DNA 1㎕ (100 ng/㎕). The total reaction mixture volume was 20㎕. The Initial denaturation stage was performed at 95℃ for 8 min and the second step was performed at 95℃ for 30 s.

During the annealing stage, the Tm settings for each gene were different (58℃, 30s/ *MC1R*; 54.4℃, 30s/ *ASIP*; 70℃, 30s/ *MATP*). Annealing was repeated for 40 cycles. The elongation stage setting was 72℃ for 7min. All PCR products were stored at -4℃. The annealing temperature selected for each gene was determined by the most efficient range found in preliminary tests and published results.

3) Polymorphism detection

PCR and PCR-RFLP were used to differentiate wild and mutant genetic types. For the *ASIP* gene, simple PCR amplification was performed with 5㎕ of PCR product on 4% agarose gel electrophoresis with EtBr (ethidium bromide). For *MC1R* and *MATP* genes, PCR-RFLP was conducted after amplification because the mutation of *MC1R* and *MATP* genes is only a single nucleotide and general PCR methods cannot separate bands by simple amplification. The RFLP preparation was made up PCR product DNA (5㎕), restriction enzyme (0.5㎕, 1unit/㎕), and 10×buffer (1㎕) for a total reaction mixture volume of 10 ㎕. This mixture was incubated at 65℃ for 1hr and RFLP product (5㎕) was confirmed by 2% agarose gel electrophoresis with EtBr.

Restriction enzymes used for RFLP were *Taq* Ⅰ (Takara Bio) for *MC1R* and *Tsp 509 I* (New England Biolabs Inc.) for *MATP*. Mutant type *MC1R* was cut by *Taq* Ⅰ (*T!CGA*), The total 116 bp PCR product was divided into 54 bp and 62 bp. Mutant type *MATP* was cut by *Tsp509 I* (T!TAA) and the total PCR products was divided into 79bp and 111bp.

4) Comparison between coat color's phenotype and genotype

After obtaining all genotypes, animals participating in the experiment were compared for phenotype and genotype by referring to photographs. Phenotype classification was determined by the KRA registry methods.

Ⅲ. Results

The experiment evaluated a sample of 35 Jeju native horses and 110 Thoroughbreds (Table 2) for coat color phenotypes recorded by KRA horse registry.

The bay color type was found in 7 Jeju native and 44 Thoroughbred horses. Of these bays, 5 Jeju native and 44 Thoroughbred horses had common traits *E/-* and *A/-*. There were two Jeju native horses with *e/e*, *A/a* and *C/C* genotypes.

The chestnut color type was found in four Jeju native horses and 25 Thoroughbred horses. All chestnut Jeju native horses had *e/e* genotypes, and 23 of 25 Thoroughbreds had the same *e/e* genotypes.

A total of 30 Thoroughbred horses were dark bay in color. Various genotypes, i.e. *E/-* + *a/a*, *E/-* + *A/A*, and *E/-* + *A/a* were found.

Many Jeju native horses in this study were gray in color; however, no specific genotype pattern was found for 18 gray Jeju native horses and 11 gray Thoroughbreds. In the array of genotypes detected three genes were found. A total of 11 different genotypes with *MATP*, *ASIP,* and *MC1R* were found in 18 gray Jeju native horses and nine different genotypes were found in the 11 gray colored Thoroughbreds.

A total of six different genotypes were expressed in six pinto colored(spotted) Jeju native horses. No pinto Thoroughbred horses were found in this study.

The results depicted in Table 2 were acquired thorough simple PCR amplification and PCR-RFLP. Patterns of electrophoresis are shown in figures 1, 2, and 3. Figure 1 depicts *ASIP* gene polymorphisms after simple PCR amplification. Wild, homozygous type (*A/A*) PCR fragment size was 120 bp, homozygous recessive type (*a/a*) size was 109 bp with a single band. Wild, heterozygous type (*A/a*) amplicon sizes were 109 and 111 bps with double bands.

In relation to the *ASIP* gene, the homozygous recessive type (*a/a*) was found in gray Jeju native horses which actually appeared to have black coats and in black Thoroughbred horses. The *a/a* genotype horses showed black phenotypes in both Jeju native horse and Thoroughbreds regardless of *MC1R* genotype.

Figure 2 shows *MC1R* gene polymorphisms after PCR-RFLP. Wild homozygous type (*E/E*) fragment size was only 116 bp, homozygous recessive type (*e/e*) amplicon sizes were 54 and 62bp, and the wild heterozygous type (*E/e*) had bands at 54, 62 and 116 bp.

In relation to the *MC1R* gene, the *e/e* genotype appeared in only chestnut and some gray colored horses. All chestnut phenotype Jeju native horses had the *e/e* genotype and five gray Jeju horses and three chestnut and white pinto horses had the *e/e* genotype as well.

Fiqure 3 shows *MATP* gene polymorphisms following PCR-RFLP. Wild, homozygous type (*C/C*) PCR fragment size was only in 190 bp with one band. Wild, heterozygous type (C/C^{cr}) amplicon size included 190, 111 and 79 bps with three bands. Homozygous recessive type (C^{cr}/C^{cr}) had two bands of 111 and 79 bps. There were no homozygous recessive (C^{cr}/C^{cr}) types in this study.

In relation to the *MATP* gene, *C/C* wild types were found in bay and chestnut Jeju native horses. There were two genotypes, *C/C* and C/C^{cr}, in gray and pinto horses. Thoroughbred horses also had the C/C^{cr} genotype.

Table 2. Genotype expression with *ASIP*, *MATP*, *MC1R* genes in Jeju native and Thoroughbred horses

Phenotype	Genotype							
	Jeju native horse				Thoroughbred			
	n	*MC1R*	*ASIP*	*MATP*	n	*MC1R*	*ASIP*	*MATP*
Bay	2	*E/E*	*A/A*	*C/C*	4	*E/E*	*A/A*	*C/C*
	1	*E/e*	*A/A*	*C/C*	22	*E/e*	*A/A*	*C/C*
	2	*E/e*	*A/a*	*C/C*	2	*E/e*	*A/A*	*C/C*^{cr}
	2	*e/e*	*A/a*	*C/C*	12	*E/e*	*A/a*	*C/C*
					2	*E/e*	*A/a*	*C/C*^{cr}
					1	*E/e*	*A/A*	*C/C*
					1	*E/E*	*A/a*	*C/C*
Chestnut	2	*e/e*	*A/A*	*C/C*	11	*e/e*	*A/A*	*C/C*
	2	*e/e*	*A/a*	*C/C*	8	*e/e*	*A/a*	*C/C*
					1	*E/E*	*A/a*	*C/C*
					1	*E/e*	*A/A*	*C/C*
					2	*e/e*	*A/a*	*C/C*^{cr}
					2	*e/e*	*A/A*	*C/C*^{cr}
Dark Bay					3	*E/E*	*A/a*	*C/C*
					2	*E/E*	*a/a*	*C/C*
					3	*E/e*	*A/A*	*C/C*
					10	*E/e*	*A/a*	*C/C*
					7	*E/e*	*a/a*	*C/C*
					1	*E/E*	*A/A*	*C/C*
					1	*e/e*	*A/a*	*C/C*
					1	*E/e*	*A/a*	*C/C*^{cr}
					1	*E/E*	*A/A*	*C/C*^{cr}
					1	*e/e*	*A/a*	*C/C*^{cr}
Gray	1	*E/E*	*A/A*	*C/C*	1	*E/E*	*A/A*	*C/C*
	1	*E/E*	*A/A*	*C/C*^{cr}	3	*E/e*	*A/A*	*C/C*
	1	*E/E*	*A/a*	*C/C*	1	*E/e*	*A/a*	*C/C*^{cr}
	1	*E/E*	*A/a*	*C/C*^{cr}	1	*E/e*	*a/a*	*C/C*
	4	*E/e*	*A/A*	*C/C*	1	*E/e*	*a/a*	*C/C*^{cr}
	2	*E/e*	*A/A*	*C/C*^{cr}	1	*e/e*	*A/A*	*C/C*^{cr}
	2	*E/e*	*A/a*	*C/C*	1	*e/e*	*A/a*	*C/C*
	1	*E/e*	*a/a*	*C/C*	1	*e/e*	*A/A*	*C/C*
	1	*E/e*	*a/a*	*C/C*^{cr}	1	*E/e*	*A/a*	*C/C*
	2	*e/e*	*A/A*	*C/C*				
	2	*e/e*	*A/a*	*C/C*				
Pinto	1	*E/e*	*A/A*	*C/C*				
	1	*E/e*	*A/A*	*C/C*^{cr}				
	1	*E/e*	*A/a*	*C/C*^{cr}				
	1	*e/e*	*A/A*	*C/C*^{cr}				
	1	*e/e*	*A/a*	*C/C*				
	1	*e/e*	*A/a*	*C/C*^{cr}				
Total	35				110			

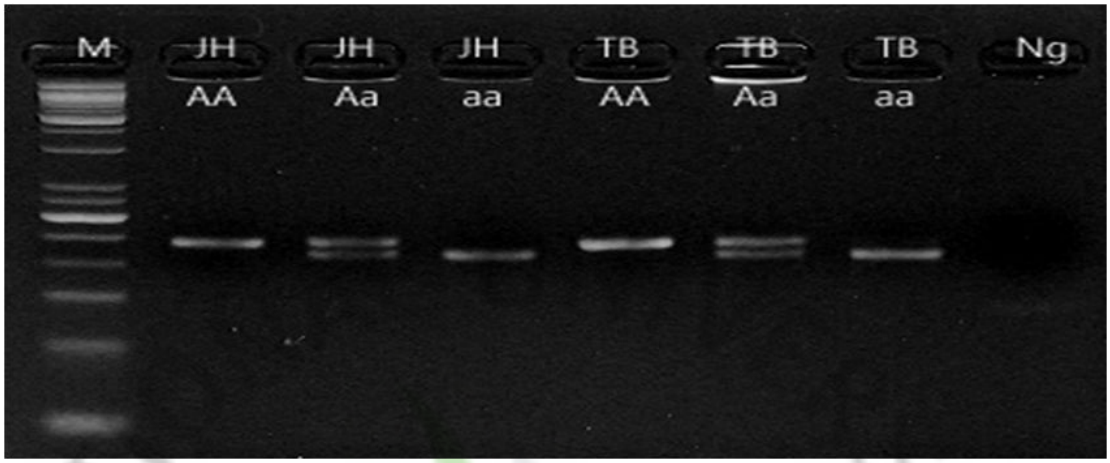

Fig 1. *ASIP* PCR products after simple amplification
M: 25/100 bp ladder, Genotype: *A/A, A/a, a/a*, JH: Jeju horse, TB: Thoroughbred, JH(*a/a*), TB(*a/a*): Black

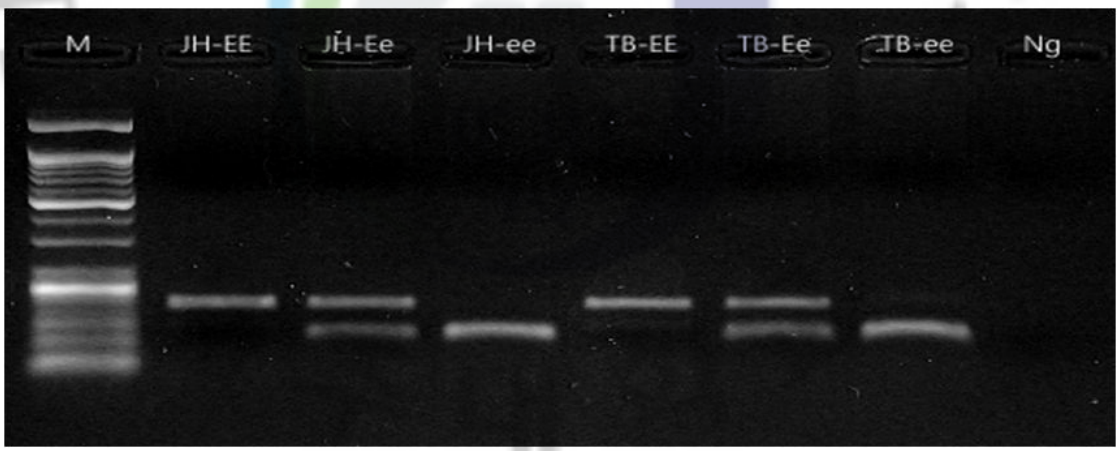

Fig 2. *MC1R* PCR-RFLP products after digestion with *Taq* I
M: 25/100 bp ladder, Genotype : *E/E, E/a, e/e*, JH: Jeju horse, TB: Thoroughbred, JH(*e/e*), TB(*e/e*): Chestnut

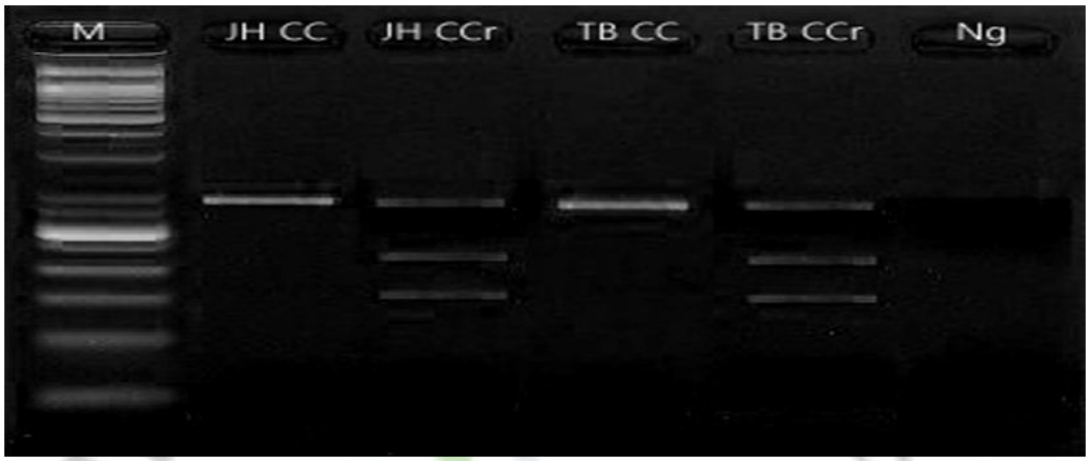

Fig 3. *MATP* PCR-RFLP products after digestion with *Tsp509 I*
M: 25/100 bp ladder, Genotype : *C/C*, *C/C*^{cr}, *C*^{cr}/*C*^{cr}, JH: Jeju horse, TB: Thoroughbred, JH(*C/C*^{cr}), TB(*C/C*^{cr}): Gray

In Jeju native horses, various coat color types could be explained by different combinations of the genotype allelic variants of the *MC1R*, *ASIP* and *MATP* genes, except for cases of gray and pinto colors. There were no differences between Jeju native and Thoroughbred horses in basic coat color gene expression.

Ⅳ. Discussion

The Genetic makeup of basic coat colors of Thoroughbred horses can be summarized as follows:

The extension (*E*) and agouti (*A*) locus are involved in bay color production. Genotype *E/-* and *A/-* leads to bay color and the mode of inheritance is autosomal dominant. The extension (*E*) locus is also involved in chestnut color expression. The agouti (*A*) locus is irrelevant, as the horse cannot produce eumelanin. As a result of a *C*901*T* missense mutation, a homozygous recessive type (*e/e*) in which *MC1R* cannot act in melanocytes will produce a chestnut color. Genotype A/- and *e/e* produces a chestnut color. *MC1R* helps to the production of the brown or black pigment eumelanin. The mode of inheritance is autosomal recessive.

The agouti (*A*) locus is involved in black color expression. The *a/a* genotype is formed by an 11 bp deletion at 2174-2184. Homozygous recessive type (*a/a*) having a character with *ASIP* cannot work in melanocytes. *ASIP* helps to synthesize pheomelanin, a yellow pigment, instead of eumelanin. Genotype *E/-* and *a/a* produces a black color. The mode of inheritance is also autosomal recessive.

The Cream (Cr) locus is involved in dilution of coat color. The mutant allele (C^{cr}) evolves from a *G*457*A* missense mutation. The Cream gene wild type (*C*) is recessive. This allele cannot dilute hair pigmentation.

The mutant allele (C^{cr}) exhibits incomplete dominance. In the case of heterozygotes (C/C^{cr}), the mutant allele dilutes red to yellow. Homozygote (C^{cr}/C^{cr}), double diluted type, is the strongest color dilution type. The red and the black hair pigment are diluted to cream color. Homozygote (C^{cr}/C^{cr}) types were no found in this study. The mode of inheritance is autosomal codominant.

This study showed various genotype patterns in Jeju native horses and Thoroughbreds. There were many common coat color genotype patterns in both breeds. Horses which had *E/-* and *A/-* alleles showed bay colors (except for those with gray or pinto phenotypes). The *a/a* alleles played an important role in revealing black coat color. Black color in this study was classified as dark bay. Dark bay and some gray horses with *a/a* alleles had black coats. The chestnut color phenotype corresponded closely with horses having *e/e* alleles.

This study was also classified by each coat color gene, It is as in the following. Upon comparing *E* with *e* allele of *MC1R*, both Jeju native and Thoroughbred horses with *e/e* had chestnut coat colors, except for those with a gray phenotype. The *e/e* + *A/-* + *C/C* types corresponded to chestnut color horses. This result coincided with earlier studies [8, 11]. In relation to the *MC1R* gene, two chestnut horses had *E/E, E/e* genotypes were initially identified erroneously as chestnut by phenotype and two dark bay horses with *e/e* mutant homozygotes actually had a chestnut color confirmed by checking both phenotype and genotype.

All bay colored horses had *E/-* + *A/a* + *C/-* genotypes except for these two horses with *e/e* type. These horses had *e/e* were mistaken for bay. They had same traits with chestnut. Their coat colour was also initially wrongly identified.

A total of nine genotypes were revealed in 30 dark bay Thoroughbreds. Upon comparing *A* with *a* allele of *MC1R*, all dark bay horses possessed *E/-* + *A/a* + *C/-* genotype patterns similar to bays. Black coat color dark bay horses had *E/-* + *a/a* + *C/-* types. These horses were correctly identified as black. The same pattern applied to gray-black Jeju native *a/a* type horses.

This study had assumed that the *MATP* gene might affect gray color; however, no distinct evidence to support this association was found. Some light colored bay, dark

bay, and chestnut horses had C/C^{cr} alleles. In particular, bay horses with C/C^{cr} (*E/e* + *A/A* + C/C^{cr}) had more diluted coat colors than bays with *C/C* thus this may also be referred to as buckskin, a dilution of bay. Similar results were reported in a previous study [7]. Bays, carrying the C^{cr} allele were determined to be buckskin after *MATP* genotyping.

A variety of genotypes were found in gray colored horses. Some diluted gray horses had C/C^{cr} type while other diluted gray horses had *C/C* type. No standard genotypic patterns with *MATP* were found in this study.

Thus, further study of the gray color with *Gray, PMEL17,* and *SILV* gene is needed [10]. In the case of Jeju native Pintos (spotting), there were no correlations found. The six pinto horses had six different genotypes. However, *e/e* mutant types were only found in pintos mixed with chestnut and white colors. No regular patterns were found involving the *MATP* gene. As with gray, pinto color was controlled by genes other than *MC1R, ASIP* and *MATP* [17]. Additional studies are also needed with regard to this color.

Bay, chestnut, and black Jeju native horses in this study could be differentiated by routine genotyping of *ASIP, MC1R* and *MATP* genes. Jeju native horses have same traits on expressing basic coat color comparing with Thoroughbred horses. Uncertain phenotypes in basic colors (bay, chestnut and dark bay) could be separated. Additional studies covering more genes involved in gray; and pinto colours will be required for flawless classification of Jeju native horses.

This sorting by routing genotyping will help to classify horses on the basis of coat colors and enable genetic counseling for breeding of preferred coat color types. Routine genotyping of Jeju native horses is less time consuming then genotyping methods such as RT-PCR and simultaneously genotyping methods for multiple coat color genes like SNaPshot [5]. Errors associated with color classification determined

by phenotype alone will be reduced by genotyping. Coat color genotyping could be added as criteria on the Jeju native horse registry.

Ⅴ. Conclusion

Basic color expression and cream dilution in Jeju native and Thoroughbred horses was confirmed in this study using *MC1R*, *ASIP* and *MATP*. There were no differences in coat color genes activity found between Jeju native and Thoroughbred horses.

With regard to *ASIP*, black color was shown in Jeju native and Thoroughbred horses carrying the *a*/*a* genotype. It is also possible that any horses carrying *E*/-, *a*/*a* and *C*/*C* will also express a black coat color.

For *MC1R*, both Jeju native and Thoroughbred horses carrying the *e*/*e* genotype had a chestnut color except for those with a gray phenotype. In general, *e*/*e*, *A*/-, and *C*/*C* types show a chestnut phenotype.

For *MATP*, *C*/*C* types were more common than C/C^{cr}, and thicker coat colour was expressed in *C*/*C* types compared to C/C^{cr} except for in cases of gray and pinto colored horses.

This study has limits in its ability to account for all coat colors in both types of horses. In particular, the different genes involved in gray and pinto coat colors require further research. This study will aid in the accumulation Jeju native horse' genotypes data and provide genetic backgrounds information for accurate classification, as opposed to rough determination by phenotype. This information can also improve the efficiency of genetic counseling for better breed management.

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국문초록

유전자형 분석을 통한 제주마 모색 다형성(多形性) 연구

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본 연구는 제주마에서 *ASIP*, *MC1R*와 *MATP* 위치다형성 확인 및 기본 모색에 대한 유전형 분포가 더러브렛 모색 발현과 어떤 차이가 있는 지를 비교 분석해 보는 것이었다.

ASIP, *MC1R*와 *MATP* 각 유전자형의 변이를 살펴보기 위해 각 유전자 특이 primer sets을 합성하여 중합효소연쇄반응(PCR) 후 전기영동으로 확인하였다. 전기영동 시 *ASIP*분석은 4% agarose gel에서 유전자형을 확인했고 *MC1R*과 *MATP*는 중합효소연쇄반응-제한효소단편길이 다형성(PCR-RFLP) 방법을 실시한 후 2% agarose gel에서 유전자형을 확인했다.

ASIP mutant type(*a*/*a*)을 보유한 말은 *MC1R*의 유전형과는 관계없이 검정 모색을 나타내었으나 회색 말에서는 일정한 패턴이 확인되지 않았다. *MC1R* 변이형(*e*/*e*)을 보인 말은 제주마와 더러브렛 모두에서 밤색을 나타내었다. *MATP* 유전형에서는 얼룩모색과 회색 모색을 제외하고 *C*/*C* type에서 C/C^{cr} type를 가진 말보다 더 진한 모색이 나타나는 것을 확인할 수 있었다. 이상의 결과에서 기본 모색을 가진 제주마에서 일반적인 유전형 분석이 가능하였고 더러브렛과 비교하였을 때에도 차이를 보이지 않았으나 회색이나 얼룩 등 기본 모색 외 관련 유전자에 대

해서는 추가 연구가 필요하다. 표현형만으로는 제주마 및 더러브렛 모두 정확한 모색 분류를 할 수 없고 오류가 존재하므로 말 등록 시 정확한 모색 확인을 위해서 표현형과 유전형 분석이 병행해야 할 것으로 사료된다.

주요어 : 제주마, 모색, *MCRI*, *ASIP*, *MATP*, 다형성

감사의 글

먼저 결혼하고 제주에 같이 와서 학위를 잘 마치게 도와주며 쌍둥이까지 선물해 준 아내 원아에게 감사의 말을 전하고 싶습니다.
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회사를 다니면서 대학원 과정을 무사히 마칠 수 있게 도와주시고 격려해주신 송대영 팀장님, 장종덕 차장님, 문규환 과장님 및 우리 제주마사보건팀원들에게 정말 감사의 말씀 전하고 싶습니다. 그리고 서울경마공원의 보건원 원장님을 비롯한 말보건원 식구들, 부산의 양재혁 과장님에게도 이 글을 통해 감사하다는 말씀을 올립니다.

그리고 제게 정말 많은 도움을 주셨던 김소연, 송정환 선생님을 비롯한 제주대학교 수의내과학 교실 여러분들께도 다시 한 번 감사의 말씀을 올립니다. 특히 실험 때 많이 도와주었던 태균 · 윤기 · 영신 정말 고마웠습니다. 여러분이 아니었으면 정말 이 글을 못 썼을 것이라고 생각합니다.

이 외에도 제가 미처 헤아리지 못하였지만 저를 항상 도와주신 많은 분들께도 감사의 말씀을 전하며 모든 분들의 가정에 평화가 함께 하시길 바랍니다.