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Genetic variation within the coat color gene of Melanocortin 1 Receptor (*MC1R*) in Mong Cai pigs of Vietnam

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Abstract. *Thuy CTT, Ha CD, Long TD, Hai TV, Long ST, Chi TT, Quyen TTH, Yen VH, Khanh NTM, Trung NQ, Trung NT. 2023. Genetic variation within the coat color gene of Melanocortin 1 Receptor (MC1R) in Mong Cai pigs of Vietnam. Biodiversitas 24: 1735-1741.* Pig (*Sus scrofa*) is considered one of the major domesticated animals in Vietnam. Various indigenous pig varieties have been successfully developed as the Vietnamese's main daily meat source. However, little information has been reported about the conservation of these indigenous pig varieties. In this study, the Mong Cai, a prized domestic pig breed in Vietnam, is well-characterized by a black saddle on the hips extending to the lower abdomen. As a result of the introduction of exotic breeds and crossbreeding, Mong Cai pigs exhibited various phenotypic characteristics and coat colors. We constructed a database of Melanocortin 1 Receptor (*MC1R*) coding sequences, including the *MC1R* sequences of 26 Mong Cai and 20 other pig breeds from Europe and Asia. Subsequently, 12 haplotypes were determined using 18 mutations in 46 individuals. The phylogenetic tree result indicated that the Mong Cai population was distributed into two distinct haplotypes. Moreover, Mong Cai pigs could belong to the Asian clade; however, six Mong Cai individuals formed a distinct cluster. These six individuals carried the nt729G gene, which is prevalent in European pigs. In addition, our study could provide a solid foundation for further investigation of the evolution of indigenous pig varieties in Vietnam.

Keywords: Black saddle, coat color, haplotype diversity, Melanocortin 1 Receptor, Mong Cai pig

INTRODUCTION

The pig, also known as *Sus scrofa*, is a domesticated mammal in the family Suidae. They are commonly raised for their meat and by-products, like lard and pork rinds. Pigs are omnivorous animals and can consume a wide variety of food. The pigs' origin is in the Middle East, where wild boars have first domesticated around 9,000 years ago (Giuffra et al. 2000). Furthermore, they were brought to Europe and Asia, which further domesticated and selectively bred for specific traits, such as meat production. Today, pigs are found worldwide and are an important food source for many cultures. The Mong Cai pig is regarded as one of Vietnam's unique indigenous pig breeds. It originated in the cold Mong Cai District of Quang Ninh Province, adjacent to the Chinese border, where it was first documented. Due to their high fertility, Mong Cai sows have been bred with western boars for F1 crossbred products and meat production since the turn of the 20th century (Lapar 2014). The body shape of the Mong Cai pig breed is characteristic of the local pig breed: Short body, short neck, small ears, small and short legs, swayback backline, and a drooping-shaped belly (Ishihara et al. 2020). In addition, the black saddle coat that extends from the hips to the lower abdomen on Mong Cai pigs is distinctive. In recent decades, Mong Cai pigs were likely isolated from European breeds due to limited genetic diversity and being reared in a confined environment at the raising station (Tran et al. 2016). As a result of the introduction of exotic breeds and crossbreeding, the black saddle phenotype of Mong Cai pigs has undergone significant variation. Recently, several efforts have been recorded to reconstruct the scheme of the Vietnamese native pig genetic resources based on the single-nucleotide polymorphism array (Ba et al. 2020).

Of our interest, the coloring of the skin and hair in mammals is a complex process influenced by various factors, like genetics, hormones, and environmental conditions. The pigment responsible for mammal skin and hair coloration is called melanin, produced by specialized cells called melanocytes. The amount and distribution of melanin in the skin and hair are responsible for the wide range of skin and hair colors seen in different mammal species. Thus, understanding the mechanisms that control pigmentation can provide insight into the evolution and adaptation of different mammal species, perhaps pig species. Local pigs have diverse

coat color phenotypes, including black, red, white, spotted, brown, belted, and two-end black, resulting from an artificial selection over an extended period (Wu et al. 2019). Several important genes affecting pig coat color phenotypes have been identified to date, including mast/ stem-cell growth factor receptor (*KIT)*, Melanocyte Inducing Transcription Factor (*MITF)*, antagonist Agouti Signaling Protein (*ASIP)*, *DCT*, Tyrosinase-related Protein 1 (*TYRP1*) (Du et al. 2022). Melanocortin 1 Receptor (*MC1R*) gene determines dominant black, black-spotted, and red coat colors in Western and Chinese pigs (Zhong et al. 2022). The skin and hair pigmentation in mammals results from cellular activities and cooperation between two cell types, melanocytes producing melanin and keratinocytes distributing melanin throughout the skin (Le et al. 2021). In addition, the *MC1R* is a G-protein coupled receptor predominantly expressed in melanocytes. Therefore, it plays a key role in melanogenesis by regulating the production of red/yellow pheomelanin and dark eumelanin (Horrell et al. 2016). *MC1R* variants associated with the domestication of Asian and European pig breeds have been identified through characterization (Lu et al. 2017). However, the variation of the *MC1R* gene in Vietnam native pig varieties, perhaps in the Mong Cai pig breed, is still lacking.

The aim of this study was to analyze the variation in *MC1R* sequence and locus in the Mong Cai pig population. Firstly, we performed sequencing of the *MC1R* gene isolated from Mong Cai pig individuals. We then analyzed the variation between these *MC1R* genes. Finally, a phylogenetic tree has been generated to gain insight into the evolution and classification of the Mong Cai pigs.

MATERIALS AND METHODS

Sampling and DNA extraction

These pigs were raised in Mong Cai swine farms in Quang Ninh Province, Vietnam: 12 from the farm of Thien Thuan Tuong Mining JSC, two from the farm of Quang Ninh Nong Lam Ngu Development MTV Co. Ltd, seven from the farm of An Loc Organic Agriculture Cooperative, and five from the farm of Van Thanh Phat Cooperative. A total of 26 ear tissues were collected from individuals from four months to three and a half years old, including 21 female and five male pigs (more details see Table 2). All tissue samples were collected and preserved in 75% ethanol at 4°C. The phenotypes of these individuals were also recorded according to the farmers' knowledge of pedigree information. The seamless black coat and knotted black coat phenotypes were identified by the spotted area characteristics that extended from the hips to the lower abdomen (Figure 1).

Genomic DNA was extracted from ear tissues according to the method by Pearson and Stirling (2003). The study protocols complied with the Institutional Guidelines for the care and use of laboratory animals, which were approved by the Ministry of Agriculture and Rural Development of Vietnam (TCVN 8402:2010).

Polymerase chain reaction (PCR) amplification and sequencing

An *MC1R* fragment, including the entire coding sequence plus introns at 5'-Untranslated Regions (UTR) and 3'-UTR directions, was amplified using two primer pairs, according to Wu et al. (2017) (Table 1). Each PCR is conducted in a 40 µL volume containing 4 µL template DNA (approximately 25- 50 ng/µL), 20 µL of 2X Mytaq Mix PCR Mastermix (Meridian Bioscience), 4 µL of primer (10 pmol each primer), and nuclease-free water. The following steps comprised PCR amplifications: 95°C for 3 min, followed by 35 cycles of amplification, including 15 s at 95°C, annealing at the Tm (Table 1) for 15 s and 20 s at 72°C, and finally 72°C for 2 min. The products were detected by electrophoresis on a gel-red stained 2% agarose gel and then purified using the TopPURE® PCR/GEL DNA PURIFICATION KIT (ABT Co. Ltd). Competent PCR products were sent for sequencing (1st BASE).

Figure 1. The phenotypes of Mong Cai pigs. A. Knotted black saddle, B. Seamless black saddle. The white arrow addresses the knotted position in the black saddle area, only seen on the Mong Cai knotted black saddle coat group

Table 1. PCR primers and conditions used for amplification of Melanocortin 1 Receptor (*MC1R*)

Primer name	Primer sequence	Primer binding region	Size (bp)	Tm (°C)
*MC1R*1 - F	5'-GCTGAGCACAGGCGAGGTTG-3'	5'UTR	900	60
*MC1R*1 - R	5'-AGGAAGCAGAGGCTGGACAC-3'	Exon 1		
*MC1R*2 - F	5'-CGCCAAGAACCGCAACCTG-3'	Exon 1	950	59.5
*MC1R*2 - R	5'-GTCCAGCGTCCATACCTTCAG-3'	3'UTR		

Table 2. Information of 26 Mong Cai pigs which were studied in analyzing of *MC1R* gene

Individuals	Age (month)	Sex	Location
010	24	F	Thien Thuan Tuong Mining JSC
020	24	F	Thien Thuan Tuong Mining JSC
080	24	M	Thien Thuan Tuong Mining JSC
12006	12	F	Thien Thuan Tuong Mining JSC
12007	12	F	Thien Thuan Tuong Mining JSC
12008	12	F	Thien Thuan Tuong Mining JSC
12009	12	F	Thien Thuan Tuong Mining JSC
13002	24	F	Thien Thuan Tuong Mining JSC
13013	42	F	Thien Thuan Tuong Mining JSC
13014	36	F	Thien Thuan Tuong Mining JSC
13015	36	F	Thien Thuan Tuong Mining JSC
13153	24	F	Thien Thuan Tuong Mining JSC
3628	06	F	Quang Ninh Nong Lam Ngu Development MTV Co. Ltd
21026	36	M	Quang Ninh Nong Lam Ngu Development MTV Co. Ltd
32007	07	F	An Loc Organic Agriculture Coop.
32016	05	F	An Loc Organic Agriculture Coop.
32029	06	F	An Loc Organic Agriculture Coop.
32033	04	F	An Loc Organic Agriculture Coop.
32046	36	F	An Loc Organic Agriculture Coop.
31089	12	M	An Loc Organic Agriculture Coop.
32095	12	M	An Loc Organic Agriculture Coop.
41021	24	M	Van Thanh Phat Cooperative
42022	24	F	Van Thanh Phat Cooperative
42024	24	F	Van Thanh Phat Cooperative
42027	24	F	Van Thanh Phat Cooperative
42030	24	F	Van Thanh Phat Cooperative

Data analysis

MC1R full-length sequences were assembled using the CAP3 sequence assembly program (Pôle Rhône-Alpes de Bioinformatique). Subsequently, these sequences were aligned with the published sequences of *S. scrofa MC1R* (AF326520) in GenBank to identify the coding region. We constructed an *MC1R* CDs dataset consisting of the *MC1R* sequences of 26 Mong Cai pigs and 20 other pig breeds from Europe and Asia. GenBank was used to retrieve the *MC1R* sequences of the other breeds. The position and number of polymorphic sites and their corresponding haplotypes were analyzed by DnaSP version 6.12.03 (Rozas et al. 2017). The MEGAv11 software (Kumar et al. 2018) was then used for phylogenetic analysis. A Maximum-Likelihood phylogenetic tree was constructed employing the Hasegawa-Kishino-Yano model and bootstrap 1000.

RESULTS AND DISCUSSION

Sequencing and polymorphism detection results

A sequence of ~1600 bp comprising the coding region and 5' and 3'UTR of the *MC1R* gene was amplified successfully in 26 Mong Cai individuals. The complete coding sequence of 26 Mong Cai pigs had a length of 963 bp and encoded 321 amino acids. The sequence alignment revealed 18 polymorphic sites, including two insertion-deletion sites (indel), four synonymous single nucleotide polymorphisms (SNPs), and 12 non-synonymous SNPs. Within the Mong Cai population, there were five detected substitutions. In addition, 11 variants (c.6T>C, c.51A>G, c.61G>A, c.68_69insCC, c.283A>G, c.305C>T, c.363C>T, c.364G>A, c.370G>A, c.491C>T, and c.729A>G) have also been reported (Li et al. 2010; Liu et al. 2016; Lu et al. 2017; Wu et al. 2017). Five nucleotide substitutions (c.28>G, c.152T>G, c.674C>A, c.712T>A, and c.727G>A) were identified in the Mong Cai population. The 2-bp indel at codon 23 (c.68_69insCC) in the *MC1R* CDs is a frameshift mutation, which has been reported in recent coat color research (Wu et al. 2017). However, the Mong Cai population under study does not exhibit this trait. Next, using the chi-square test (df=3, *P*-value <0.05), all polymorphic sites were significantly associated with the coat color trait. As a result, all novel sequences were submitted to GenBank with references numbered OP142697-OP142700.

Haplotype diversity

Twelve haplotypes from 18 mutations among 46 pigs have been identified (Tables 4 and 5). Specifically, Hap2 to Hap7 are European original pig breeds with white dominant and white with two-end black coat colors. Hap1 comprises seven Chinese pig breeds with various coat color phenotypes and 18 Mong Cai pigs. However, the remaining six Mong Cai members constitute the Hap12 group. Notably, nt729 (c.729A>G) is different between Hap1 and Hap12, and it is a synonymous mutation. Intriguingly, all European pig breeds and six Mong Cai pigs are homozygous for the 729GG allele. ntA was recorded at site 729 for Hap1 and from Hap8 to Hap11.

Phylogenic analysis

The constructed phylogenetic tree revealed that European and Asian pig breeds were separated into two distinct clusters, with the Mong Cai population belonging to the Asian clade (Figure 2). Interestingly, the six Mong Cai individuals with the Hap12 genotype formed a distinct cluster within the Asian population.

This result provided evidence to support the earlier conclusion that Mong Cai pigs are of Asian origin (Bui et al. 2018; Ishihara et al. 2020), and the *MC1R* variation distinguishes between European and Asian pig breeds. However, the remaining six members of Hap12 may have a different ancestry than the entire population.

Table 3. Variations of the Melanocortin 1 Receptor (*MC1R)* gene and allelic frequency in the European pigs, Asian pigs, and Mong Cai pigs based on coat color phenotype

Variation description	Variation	Allele				Amino acid	*P*-value	X^2
		Solid black	Spotted	Solid white	Mong Cai			
Synonymous	c.6T>C	T/1.00	T/1.00	T/0.67	T/1.00	Pro	<0.05	13.43
		C/0.00	C/0.00	C/0.33	C/0.00			
Non-synonymous	c.28C>G	C/1.00	C/1.00	C/1.00	C/0.96	p.Leu10Val	<0.05	8.81
		G/0.00	G/0.00	G/0.00	G/0.04			
Synonymous	c.51A>G	A/0.67	A/0.63	A/0.00	A/1.00	Ala	<0.05	26.89
		G/0.33	G/0.37	G/1.00	G/0.00			
Non-synonymous	c.61G>A	G/1.00	G/1.00	G/0.83	G/1.00	p.Ala21Thr	<0.05	9.07
		A/0.00	A/0.00	A/0.17	A/0.00			
Indel	c.68_69insCC	-/0.83	-/0.75	-/0.33	-/1.00	-	<0.05	15.98
		CC/0.17	CC/0.25	CC/0.67	CC/0.00			
Indel	c.70insC	-/1.00	-/1.00	-/0.67	-/1.00	-	<0.05	13.43
		C/0.00	C/0.00	C/0.33	C/0.00			
Non-synonymous	c.85A>G	A/1.00	A/1.00	A/0.83	A/1.00	p.Asn28Asp	<0.05	9.07
		G/0.00	G/0.00	G/0.17	G/0.00			
Non-synonymous	c.152T>G	T/1.00	T/1.00	T/1.00	T/0.96	p.Leu50Arg	<0.05	8.81
		G/0.00	G/0.00	G/0.00	G/0.04			
Non-synonymous	c.283A>G	A/0.67	A/0.50	A/0.00	A/1.00	p.Meth94Val	<0.05	27.40
		G/0.33	G/0.50	G/1.00	G/0.00			
Non-synonymous	c.305C>T	C/0.67	C/0.50	C/0.00	C/1.00	p.Pro101Leu	<0.05	27.40
		T/0.33	T/0.50	T/1.00	T/0.00			
Synonymous	c.363C>T	C/0.67	C/0.63	C/0.00	C/1.00	Asn	<0.05	26.89
		T/0.33	T/0.37	T/1.00	T/0.00			
Non-synonymous	c.364G>A	G/0.83	G/1.00	G/1.00	G/1.00	p.Val122Ile	<0.05	9.07
		A/0.17	A/0.00	A/0.00	A/1.00			
Non-synonymous	c.370G>A	G/0.67	G/0.63	G/0.33	G/1.00	pAsp124Asn	<0.05	15.64
		G/0.33	A/0.37	A/0.67	A/0.00			
Non-synonymous	c.491C>T	C/1.00	C/1.00	C/0.67	C/1.00	p.Ala164Val	<0.05	13.42
		T/0.00	T/0.00	T/0.33	T/0.00			
Non-synonymous	c.674C>A	C/1.00	C/1.00	C/1.00	C/0.96	p.Ala225Asp	<0.05	8.81
		A/0.00	A/0.00	A/0.00	C/0.04			
Non-synonymous	c.712T>A	T/1.00	T/1.00	T/1.00	T/0.96	p.Cys274Ser	<0.05	8.81
		A/0.00	A/0.00	A/0.00	A/0.04			
Non-synonymous	c.727G>A	G/1.00	G/1.00	G/0.67	G/1.00	p.Ala243Thr	<0.05	13.43
		A/0.00	A/0.00	A/0.33	A/0.00			
Synonymous	c.729A>G	A/0.67	A/0.63	A/0.00	A/0.77	Ala	<0.05	12.44
		G/0.33	G/0.37	G/1.00	G/0.23			

Table 4. Haplotypes and polymorphism in the Melanocortin 1 Receptor (*MC1R*) gene among Mong Cai pigs, European pigs, and Asian pigs

Haplotypes	Alleles																	
	6	28	51	61	68-69	70/ 72	85/88	152/ 155	283/ 286	305/ 308	363/ 366	364/367	370/373	491/494	674/677	712/715	727/730	729/ 732
1	T	C	A	G	-	-	A	T	A	C	C	G	G	C	C	T	G	A
2	.	.	G	.	CC	-	.	.	G	T	T	.	A	.	.	.	.	G
3	.	.	G	.	-	-	.	.	G	T	T	.	A	.	.	.	.	G
4	C	.	G	.	CC	C	.	.	G	T	T	.	A	.	.	.	.	G
5	.	.	G	.	-	-	.	.	G	T	T	.	.	.	.	.	A	G
6	C	.	G	.	CC	C	G	.	G	T	T	.	A	T	.	.	.	G
7	.	.	G	A	CC	-	.	.	G	T	T	.	A	.	.	.	.	G
8	.	.	.	.	-	-	.	.	.	.	.	A	.	.	.	.	.	.
9	.	.	.	.	-	-	.	.	G	T	.	.	.	.	.	.	.	.
10	.	.	.	.	-	-	.	G	.	.	.	.	.	.	.	.	.	.
11	.	G	.	.	-	-	.	.	.	.	.	.	.	.	A	A	.	.
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G

Note: "." denotes that the nucleotide at that point is the same as it at Haplotype 1; "-" denotes that there was no nucleotide found in this haplotype

Table 5. Haplotypes distribution in a data set of Mong Cai, European, and Asian pigs

Hap	n	Individuals
Hap1	25	Bamei Xiang Jinhua Tong-Cheng Shengxian-spotted Rong-Chang Qingyu 010 080 12006 12007 12008 13014 13015 32016 32029 41021 42022 42024 42027 42030 32095 32033 13153 32007
Hap2	4	Pietrain Berkshire Large-White Portugal-wild-boar
Hap3	2	Large-Black Hampshire
Hap4	1	Landrace
Hap5	2	Leicoma Duroc
Hap6	1	Yorkshire
Hap7	1	British-Lop
Hap8	1	Chinese-wild-boar
Hap9	1	Chinese-native-pig
Hap10	1	12009
Hap11	1	13013
Hap12	6	020 13002 21026 31089 32046 3628

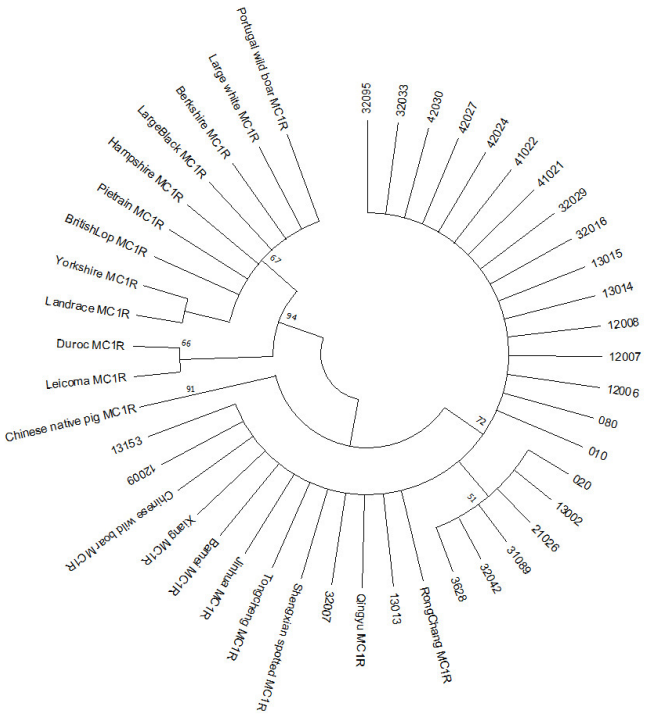

Figure 2. ML phylogenic tree based on the coding sequence of the *MC1R* gene in the dataset (26 Mong Cai pigs, 20 Asian and European pigs). Only values higher than 50% are shown

Discussion

Diversity in the Mong Cai pig population

Our research led to five substitutions in the *MC1R* gene of the Mong Cai pigs. It appears to be lower than the numbers previously recorded for Min, Qingyu, and Tibetan pigs, which were 9, 7, and 6, respectively (Mao et al. 2010; Lu et al. 2017; Wu et al. 2017). We discovered that the substitution c.729A>G frequently occurred in both our analysis and the results of these studies. In addition to the previously identified mutations in other pig breeds, our study identified five novel substitutions in Mong Cai pigs, including four non-synonymous and one synonymous SNP (c.28>G, c.152T>G, c.674C>A, c.712T>A, and c.729A>G).

The mutation c.152T>G, an SNP that causes p.Leu50Arg substitution, was only present in the Hap10 population. Hap10 consists of individual code 12009, a pig with a knotted black saddle but unequal between its sides. Crossing with imported male breeds has resulted in numerous variations in the saddle-shaped coat areas of Mong Cai pigs, as illustrated by the coat phenotype of Mong Cai pig progeny.

The remaining three SNPs identified in the Mong Cai population belong to Hap11, which consists of a single individual with the code 13013. In the *MC1R* gene of pigs, these SNPs represent novel substitutions. For example, individual 13013 was a sow with a curved, unbroken head and an extremely short black saddle compared with other Mong Cai individuals.

It is not yet known whether these novel mutations result in functional or phenotypic changes; therefore, their regulation should be the subject of further research. We also considered that Hap1 and Hap12 are the two main haplotypes in Mong Cai pigs. Our research suggests that the Mong Cai pig may have slightly elevated haplotypic diversity.

The potential association between the MC1R gene and the coat color of Mong Cai pig

The extension/*MC1R* locus is one of pigs' most important coat color loci (Lin and Fisher 2007). Therefore, several studies have investigated the association between genetic variations in the coding sequence (CDs) region of the *MC1R* gene and the phenotype of the porcine coat color. Interestingly, nt68insCC at codon 23 of the *MC1R* sequence is associated with the white-and-black-spotted coat color (Lu et al. 2017). However, it has not been observed in Mong Cai pigs.

MC1R and other identified genes play a significant role in regulating coat color variation. However, many factors influence the distribution and expression of melanin, which is responsible for the black saddle characteristics of the Mong Cai. Therefore, the true-melanogenesis operating mechanism should be initiated by melanosome formation and adding to genes associated with coat color (Moreiras et al. 2021). Furthermore, during melanosome biogenesis, numerous molecules are involved in intracellular trafficking (Du et al. 2022).

Notably, we discovered the potential phenotype segregation between haplotypes 1 and 12 regarding saddle coat color. In Hap1, 14 of 18 Mong Cai individuals exhibited the knotted black saddle coat phenotype, while the remaining individuals had the seamless black saddle coat phenotype. In contrast, five of six Hap12 individuals have a seamless black saddle-shaped coat. It would be noted that the climate of the Mong Cai region is generally humid subtropical, with four distinct seasons. The cool temperature in most seasons is not considered to be associated with the brighter coat color in Mong Cai pigs. Therefore, we inferred that the black saddle coat phenotype of Mong Cai pigs might link to the *MC1R* gene. Hap1 presented a black saddle coat with a knotted inheritance

mode, whereas Hap12 displayed a black saddle coat with a seamless inheritance mode. Our data indicated that the SNP c.729A>G was significantly related to the phenotypic difference among coat color types in Mong Cai pigs (Table 3). This substitution marked the divergence between Hap1 and Hap12, the knotted black saddle and the seamless black type. Although it was a synonymous SNP, much evidence indicates that synonymous SNPs can perturb cellular functions and elicit distinct clinical phenotypes (McCarthy et al. 2017). Synonymous SNPs can affect messenger RNA splicing, stability, structure, and protein folding by altering the ability of RNA-binding proteins to recognize the transcript (Hunt et al. 2014). The other mechanism is that synonymous SNPs affect transcription factors' and miRNAs' regulatory binding sites (Stergachis et al. 2013). Then, mutations in *MC1R* can be used as a candidate marker for Mong Cai breeding to distinguish between knotted and seamless black saddles.

Origin of Mong Cai breed

The previous mitochondrial whole-genome analysis demonstrated that Mong Cai pigs are closely related to the Bama miniature pigs found in China's Guangxi Province, which borders Quang Ninh Province. In addition, according to the Domestic Animal Diversity Information System (FAO, http://fao.org/dad-is), the Mong Cai breed shares characteristics with Luchuan pigs, native to China's Guangxi Province (Pham et al. 2014; Bui et al. 2018). Other evidence from mtDNA and microsatellite studies also revealed that the Mong Cai pig and Vietnamese indigenous pig breeds have a close genetic relationship with Asian pig breeds regarding maternal lineage and are grouped into a separate branch from European breeds (Hartatik et al. 2016; Bui et al. 2018).

Accordingly, our research proved that the origin of Mong Cai pigs is in Asia. Concurrently, we discovered that these pigs have divergence in two coat colors: A knotted black saddle and a seamless black saddle. Moreover, the nt729G gene found only in European pigs is present in the seamless black saddle pigs. Most Mong Cai pigs have a black saddle with knots, while individuals with a seamless black saddle phenotype comprise a small portion of the population.

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