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Sequencing Variation in Pigmentation Genes of Albino Cobra

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ABSTRACT

Animal coloration is a powerful model for studying the genetic mechanisms that determine phenotype. Various genes, McIr, ASIP, SLC45A2, MITF, and TYR, were studied in different phenotypic colors of cobra snake (Naja kaouthia). Amplification of McIr seems to contribute in different colors of cobra more than other genes. However, sequence variation of 318 bp McIr among, normal black wild type, normal black carrier with albino parents, intermediate with cream, and blanched albino, is rare. Only 5 nucleotide polymorphisms were observed among 12 cobras. It seems likely that no relationship between Mc1r polymorphism and colour variation observed in cobras. It is possible that the activity of Mc1r gene might affect only the amount of melanin produced, not genotypic sequences. Changes in the production and dispersion of melanin granules are responsible for changing in the color of cobras or relevant traits. Color variation among cobras seems to be associated with a consequence of non-genetic factors such as environmental factors, nutrition status, and maternal effects.

Key words: *McIr, albino, cobras*

INTRODUCTION

Color pattern in animals has long been implicated in the connection between genotype and phenotype. The pigmentation system is also affected by genetic, the environment, and selective forces such as nutrition status, crypsis, thermoregulation, mimicry, and warning signal. Thus, pigmentation phenotypes in natural populations present an ideal opportunity for studying the genetic basis of phenotypic diversity and evolutionary change[1,2].

Most studies on animal pigment have focused on the melanin system in which specialized cells called melanocytes produce either eumelanin (black/brown) or phaeomelanin (red/yellow) pigments. A large number of genes involved in pigmentation have been isolated, and their functions have been well characterized[3]. One gene that has been received much attention encodes the *melanocortin-I receptor* (*Mc1r*). *McIr* has been implicated in intraspecific pigmentation variation across a wide range of mammal, bird and reptile species. This protein is expressed in pigmentation cells known as melanocytes, where it plays a role in the dispersal of melanostomes through cells and initiation of the melanin-production process[4,5]. Activation of the receptor by melanin-stimulating hormone (MSH) has been shown to lead to an increase in the production of black and brown eumelanin in melanstomes.

Mutations in the receptor that lead to activation of *McIr* and increased synthesis of eumelanin are known as gain-of-function mutations, while loss-of-function mutations in *McIr* often are associated with the production of red or yellow phaeomelanin[6]. Although reptile pigment cell morphology differs from that of other animals such as mammals and birds, the function of melanin is remarkably conserved. Reptiles have three important cell layers for pigment production. The layer nearest to the epidermis consists of pigment-containing xanthophore cells that generate yellow or orange colors.

The middle layer contains iridophore cells that produce structural colors through the reflective properties of the cells. The deepest pigment cell layer produces melanin, and the overall darkness of the body is largely a sequence of the amount of melanin deposited by these melanophores. Reptile melanophores are known only to produce eumelanin which unlike mammals and birds. Thus, *McIr* activity might simply affect the amount of melanin produced rather than switching between the two types of melanin. Despite these differences, changes in the production and dispersion of melanin granules are ultimately responsible for changes in the dorsal color of reptiles[7]. However, in other cases, *McIr* does not seem to be involved in the color differences. This suggests that other candidate pigment genes could be responsible for the coloration such as *Agouti signaling protein* (*ASIP*), *Solute carrier family 45 member 2 protein* (*SLC45A2*), Microphthalmia transcription factor (*MITF*), and Tyrosinase (*TYR*)[8].

In this study, we examined the variation of pigment genes, *McIr*, *ASIP*, *SLC45A2*, *MITF*, and *TYR*, which might lead to black, cream, and blanched phenotypic colors of normal black and albino cobra snakes (*Naja kaouthia*). Additionally, we addressed whether different sequence variations are responsible for population differences in color.

MATERIAL AND METHODS

Sample collection

Snake shed skins and bloods from 12 normal black and albino cobra snakes (*Naja kaouthia,* NK) were obtained from Snake Farm, Queen Saovabha Memorial Institute (QSMI), The Thai Red Cross Society.

DNA Extraction

Shed snake skins were washed with sterile distilled water, dried in air and cut into small pieces. DNA extraction was performed using Genomic DNA extraction mini kit (Tissue) (RBC Bioscience, Taipei, Taiwan). DNA from blood was extracted using Genomic DNA extraction kit (Blood/Bacteria/Cultured cells) (RBC Bioscience, Taipei, Taiwan).

Oligonucleotide Primers and Amplification

Oligonucleotide primers of *Mc1r*, *MITF*, *ASIP*, *SLC45A2*, and *TYR* were designed based on NCBI GenBank database as reference data (Table 1.). DNA amplification using PCR was carried out with 50 µl reaction buffer containing 10xbuffer, 100mM of each dNTP, 25 mM $MgCl_2$, 50 pmol/µl of sense and antisense primers, Taq DNA polymerase and 10 µl DNA template. The amplification was preceded on a thermocycle (MWG Biotech, MA, USA) at 94°c, 3 minutes, followed by 40 cyclers of 94°c/56°c/72°c one minute each with final extension of 72°c for 7 minutes. The final products were electrophoresed on a 1.5% agarose gel containing ethidium bromide in 1xTAE buffer along with appropriate molecular size markers. The gel fragment containing the amplified product was excised and extracted using Gel/PCR DNA fragments extraction kit (RBC Bioscience, Taipei, Taiwan).

Nucleotide Sequencing

DNA sequencing was carried out using the same primers used in the PCRs by 1st BASE sequencing (Malaysia-http://www.base-asia.com). DNA sequences from normal black and albino cobras were aligned using Clustal X program[9,10].

Table 1. Oligonucleotide primers of *Mc1r*, *ASIP*, *SLC45A2*, *MITF* and *TYR* for DNA sequencing was designed based on NCBI GenBank database: *Mc1r* (Accession number: AY586157), *ASIP* (Accession number: BR000930), *SLC45A2* (Accession number: DQ900695), *MITF* (Accession number: FJ196874), and *TYR* (Accession number: EU162134)

Primers	Nucleotides (5' ⟶ 3')	Product size (bp)
Mc1r-F	5'GACCGCTACATCACCATCTT 3'	318
Mc1r-R	5' AGCAAGATGGTGAGGGTGAT3'	
ASIP-F	5' ATGAAGGGAATACTGTTGCT 3'	142
ASIP-R	5' CCACAATAGAGATAGGAGGGAG 3'	
SLC-F	5' ATGACCCTAACGGAACAGCA 3'	438
SLC-R	5' CTGAGATCATCTCGTCCCCA 3'	
MITF-F	5' GTGCAGACTCACCTTGAGAA 3'	227
MITF-R	5'CTCTTTTTCACAGTTGGAGT 3'	
TYR-F	5' TTCTGCTGCTGTGGGAAC 3'	157
TYR-R	5' TGCTGGGCTGAGTAAATTAGGG 3'	

RESULTS AND DISCUSSION

Amplification of *Mc1r* gene could be observed in *Naja kaouthia* known as cobra snake with different phenotypic colors: normal black wild type, normal black carrier with albino parents, intermediate with cream, and blanched albino (Figure 1.). At least 2 types of color pattern in cobra were found to be albino and both were incompatible with each other. Albino is generally blanched albino by their lack of black pigment with having red eyes. Intermediate with cream is also assumed to be one type of albino with brown or black eyes. However, these are simple recessive traits. The sequence variation of 318 bp *Mc1r* gene, in color polymorphism among cobras, is rare. Only 5 nucleotide polymorphisms were observed among 12 cobras but none of them could be associated with color pattern (Figure 2.). It seems likely that *Mc1r* is highly conserved among vertebrates and has a relatively simple genetic structure, which has facilitated its identification in a diversity of taxa including lizards and snakes[11]. *Mc1r* acts as a molecular switch between eumelanin (black or brown pigment) and pheomelanin (yellow or red pigment) production based on binding with either alpha-MSH or agouti ligands, respectively. Different mutations at *Mc1r* cause either light or dark phenotypes in several laboratory and domestic animals such as mouse, dog, pig, horse, fox, and chicken. Dominant mutations are associated with a gain of *Mc1r* function and result in dark color, while recessive mutations are associated with a reduction or loss of *Mc1r* function and result in light color. On the other hand, the melanin pathway is not well characterized in reptiles such as snakes and lizards, melanophores produce mainly eumelanin and the concentration of granules within these cells determines the overall coloration[12,13] . Skins appear lighter when pigment is concentrated and darker when it is dispersed through the cells. Accordingly, changes in the production and dispersion of melanin granules are responsible for changes in the dorsal color of reptiles. It is known that temperature influences the melanocyte-stimulating hormone (MSH) that affects melanin dispersion. Thus, the activity *Mc1r* gene seems to control only the amount of melanin produced in reptiles, including cobra, not genotypic sequences. Meanwhile, body darkness in reptiles, lizards and snakes, often varies with the substrate color or temperature of the environment, and is generally presumed to be an adaptation for crypsis or thermoregulation[14,15].

Pigmentation also impacts thermoregulation process because darker reptiles are able to reach a higher body temperature than lighter reptiles therefore have benefits in cool areas. Furthermore, climate change leads to a rise in temperature and UV radiation and dark coloration plays a role in UV protection, dark animals may be less affected from global warming. In contrast, as desertification increases, pale/cream colouration may expand in those regions, whereas dark colouration may expand in regions where humidity is predicted to increase. Geographical variation in coloration is frequent in reptiles and selective pressures may be involved[16,17,18,19].

Other genes have been looked for the interaction between genes which might affect phenotype (Table 2.). However, it remains difficult to determine precisely how many genes underlie color change. Agouti signaling peptide (*ASIP*) is a product of the agouti gene. It acts as an inverse agonist at melanocortin receptors, to be specific *Mc1r*. Agouti gene is responsible for determining whether a mammal's coat is banded (agouti) or of a solid color (non-agouti). *ASIP* has been reported that it triggers pheomelanin production. However, reptiles and fish do not produce pheomelanin. Hubbard et al (2010) mentioned that agouti-like sequences have not been reported in reptiles. This knowledge led to the faint band of 142 bp *ASIP* which was detected on agarose gel after amplification but unable to be sequenced.

Solute carrier family 45 member 5 (*SLC45A2*) was also detected using PCR with 438 bp from cobra. However, nucleotide sequencing was failed to analyze and only sequences of primer pairs were matched. It could be associated with inefficient primers and unachieved sequencing. *SLC45A2* is a transporter protein that mediates melanin synthesis. *SLC45A2* gene codes for a transporter protein involved in pigment production, and the mutation results in the substitution of an amino acid, which may partially block the transporter channel cavity. Mutation of this gene inhibits the production of pheomelanin (red to yellow pigments), but does not seem to affect black pigment. It has been found to play a role in pigmentation in several species such as tigers, horses, mice, fish, chickens and also in light skinned people. Nevertheless, this gene never been reported in reptile including snakes and lizards[20].

Microphthalmia-associated transcription factor (*MITF*) and Tyrosinase (*TYR*) genes have been proposed to play a vital role in coat color genesis in vertebrates. *MITF* is also known as a master regulator of melanin production. Melanin pigment is synthesized from tyrosine via an enzymatic process. This process is catalyzed by tyrosinase family proteins, tyrosinase (*TYR*), and tyrosinase-related protein 1 (TYRP1). After melanin production, melanin pigment is stored in melanosomes, organelles containing melanin, and is transported to the skin for UV protection. *MITF* gene controls not only expression of pigmentation-related genes but also genes involved in diverse biological processes in melanoma cells such as proliferation, invasion, resistance to apoptosis and stress mediated by reactive oxygen species, and possibly metastasis[21,22]. The product size 227 bp of *MITF* could be amplified from cobra but sequencing of this gene was homologous to various animals such as Burmese python (*Python bivittatus*), chicken (*Gallus gallus*), and Painted turtle (*Chrysemys picta*). Meanwhile, *TYR* gene with 157 bp with aberrant band was detected in all cobras. It is probably that *TYR* gene codes for a melanogenic enzyme involved in the production of eumelanin which has been associated with color variation in several domestic animals including dogs, cats, and cattles but less affected in reptiles. It could be possible that this gene was non-specific product from primer design.

Table 2. Phenotypic variations of cobras are presented. In each gene column, the presence of band is indicated with Y and absence with N. Some genes show aberrant or faint bands are noted in the column as AR and FB. Sex: F is Female, M is male. Parents of cobra: P is Paternal and M is Maternal. Offspring consists of normal black (B), intermediate with cream (C), and blanched albino (A).

Sample Code	Phenotypic color	Sex	Parents		Offspring			*Mc1r* gene	*ASIP* gene	*SLC45A2* gene	*MITF* gene	*TYR* gene
			P	M	B	C	A					
NK01	Normal black wild type	F	-	-	-	-	-	Y	Y,FB	Y	Y	Y,AR
NK02	Normal carrier with albino parents	M	A	A	-	-	-	Y	Y,FB	Y	Y	Y,AR
NK03	Normal black wild type	F	-	-	-	-	-	Y	Y,FB	Y	Y	Y,AR
NK04	Blanched albino	M	C	C	3	1	7	Y	Y,FB	Y	Y	Y,AR
NK05	Normal carrier with albino parents	M	A	A	10	-	10	Y	Y,FB	Y	Y	Y,AR
NK06	Blanched albino	F	B	B	10	-	10	Y	Y,FB	Y	Y	Y,AR
NK07	Intermediate with cream	F	-	-	3	1	7	Y	N	N	N	Y,AR
NK08	Normal black wild type	F	-	-	-	-	-	Y	N	Y,FB	Y	Y,AR
NK09	Blanched albino	-	B	C	-	-	-	Y	N	Y,AR	N	Y,AR
NK10	Intermediate with cream	M	-	-	-	-	-	Y	Y,FB	Y	Y	Y,AR
NK11	Blanched albino	M	C	C	-	-	-	Y	Y,FB	Y	Y	Y,AR
NK12	Blanched albino	F	C	C	-	-	-	Y	Y,FB	Y	Y	Y,AR

Fig. 1: Illustration shows color variation in natural population of *Naja kaouthia* (Cobra) in Thailand. There are at least 4 different phenotypic color patterns of cobra; A: Normal black wild type, B: Normal black carrier with albino parents, C: Blanched albino with red eyes, and D: Intermediate with cream with brown or black eyes (Photos by Dr. Lawan Chanhome).

Fig. 2: The partial nucleotide sequences of 318 bp *Mc1r* gene among 12 cobras. * Denotes nucleotide identical and only 5 nucleotide polymorphisms were observed. Normal black wild type: NK01, NK03, NK08; Normal black carrier with albino parents: NK02, NK05; Blanched albino with red eyes: NK04, NK06, NK09, NK11, NK12; Intermediate with cream with brown or black eyes: NK07, NK10.

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MCIR_NK11       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK12       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK09       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK06       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK04       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK07       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK10       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK01       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK03       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK08       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK02       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
MCIR_NK05       TTGACCGCTACATCACCATCTTCTATGCCTTGCGCTATCACAGCATCATGACCATCCAGC 60
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MCIR_NK11       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK12       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK09       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK06       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK04       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK07       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK10       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK01       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK03       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK08       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK02       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
MCIR_NK05       GGGCCGCCATCCTTATGGTGGCTGTCTGGCTGGTCAGCATCGTCTCCAGCATCCTCTTCA 120
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MCIR_NK11       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK12       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK09       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK06       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK04       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK07       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK10       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK01       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK03       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK08       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK02       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
MCIR_NK05       TCGCCTACGACAGCACGGCCGTCCTCATGTGCCTGGTGGCCTTCTTCCTCTCGGTGCTGA 180
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MCIR_NK11       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAGA 240
MCIR_NK12       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAGA 240
MCIR_NK09       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAGA 240
MCIR_NK06       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAGA 240
MCIR_NK04       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGACGCACCGGCACGCCAGGCAGA 240
MCIR_NK07       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGACGCACCGGCACGCCAGGCAAA 240
MCIR_NK10       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAAA 240
MCIR_NK01       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAAA 240
MCIR_NK03       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAAA 240
MCIR_NK08       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAAA 240
MCIR_NK02       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAAA 240
MCIR_NK05       CCCTCATTGCAGGGCTCTATATCCACATGTTCATGCTGGCGCACCGGCACGCCAGGCAAA 240
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MCIR_NK11       TTTCAACCATGTATGGCAAGCAGCATGCGCCCCAATTTACCAGCATGAAGGGGGCCATCA 300
MCIR_NK12       TTTCAACCATGTATGGCAAGCAGCATGCGCCCCAATTTACCAGCATGAAGGGGGCCATCA 300
MCIR_NK09       TTTCAACCATGTATGGCAAGCAGCATGCGCCCCAATTTACCAGCATGAAGGGGGCCATCA 300
MCIR_NK06       TTTCAACCATGTATGGCAAGCAGCATGCGCCCCAATTTACCAGCATGAAGGGGGCCATCA 300
MCIR_NK04       TTTCAACCATGTATGGCAAGCAGCATGCGCCCCAATTTACCAGCATGAAGGGGGCCATCA 300
MCIR_NK07       TTTCAACCATGTATGGCAAGCAGCATGCGCCCCAATTTACCAGCATGAAGGGGGCCATCA 300
MCIR_NK10       TTTCAACCATGTATGGCAAGCAGCATGCGCCCCAATTTACCAGCATGAAGGGGGCCATCA 300
MCIR_NK01       TTTCAACCATGTATGGCAAGCAACATGCGCCCCAATTTACCAACATGAAAGGGGCCATCA 300
MCIR_NK03       TTTCAACCATGTATGGCAAGCAACATGCGCCCCAATTTACCAACATGAAAGGGGCCATCA 300
MCIR_NK08       TTTCAACCATGTATGGCAAGCAACATGCGCCCCAATTTACCAACATGAAAGGGGCCATCA 300
MCIR_NK02       TTTCAACCATGTATGGCAAGCAACATGCGCCCCAATTTACCAACATGAAGGGGGCCATCA 300
MCIR_NK05       TTTCAACCATGTATGGCAAGCAACATGCGCCCCAATTTACCAACATGAAGGGGGCCATCA 300
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MCIR_NK11       CCCTCACCATCTTGCTAA 318
MCIR_NK12       CCCTCACCATCTTGCTAA 318
MCIR_NK09       CCCTCACCATCTTGCTAA 318
MCIR_NK06       CCCTCACCATCTTGCTAA 318
MCIR_NK04       CCCTCACCATCTTGCTAA 318
MCIR_NK07       CCCTCACCATCTTGCTAA 318
MCIR_NK10       CCCTCACCATCTTGCTAA 318
MCIR_NK01       CCCTCACCATCTTGCTAA 318
MCIR_NK03       CCCTCACCATCTTGCTAA 318
MCIR_NK08       CCCTCACCATCTTGCTAA 318
MCIR_NK02       CCCTCACCATCTTGCTAA 318
MCIR_NK05       CCCTCACCATCTTGCTAA 318
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CONCLUSION

Mc1r gene could be detected in all different phenotypic color of cobras: normal black wild type, normal black carrier with albino parents, intermediate with cream, and blanched albino. However, the sequence variation of *Mc1r* gene in color pattern polymorphism of cobras was rare. It seems likely that the sequence variation in *Mc1r* gene was not associated with the color pattern feature of cobras. The activity of this gene seems to control only the amount of melanin produced in reptiles including cobras. Other genes such as *ASIP*, *SLC45A2*, *MITF*, and *TYR* were studied but could be less affected to coloration in cobras. Non-genetic factors such as environmental factors, nutrition status, maternal effects and is generally presumed to be an adaptation for crypsis or thermoregulation.

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The authors have no conflict of interest to declare in this study.

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