Cloning and identification of the ASIP gene in Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*)

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**ABSTRACT.** The quantity, quality, and distribution of eumelanin and pheomelanin determine a wide variety of coat colors in animals. Three coat color variants exist in farmed wild-type Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*), which is an important fur-bearing animal species. The ASIP gene is an important candidate gene for coat color variation in some species. In this study, the complete cDNA sequences of ASIP were amplified from a wild-type Chinese raccoon dog. Sequence analysis revealed the coding region of ASIP in Chinese raccoon dog to be 396-bp in length and two transcripts (accession Nos. KT224450 and KT224451) were identified due to the alternative use of exon 1 (1A and 1C). However, the alternative splicing pattern and the coding sequence of ASIP in three types of coat color variants were the same as those identified in the wild-type individual. Based on the results obtained in this study, we can exclude a role for alternative splicing of exon 1 and the coding sequence of ASIP in coat color variation in Chinese raccoon dog.

**Key words:** Chinese raccoon dog; Coat color; ASIP gene; Alternative splicing
INTRODUCTION

Diversity of mammalian coat color arises through the differential expression and regional distribution of eumelanin (brown/black) and pheomelanin (red/yellow). The switch between eumelanin and pheomelanin synthesis is mainly controlled by the products encoded by the \textit{MC1R} and \textit{ASIP} genes. The \textit{ASIP} gene encodes the agouti signaling protein, which is involved in the switch from eumelanin to pheomelanin synthesis in melanocytes. Mutations or polymorphisms in the \textit{ASIP} gene have been implicated in coat color phenotype in a variety of animals including mice (Vrieling et al., 1994; Hustad et al., 1995), pigs (Drögemüller et al., 2006), sheep (Norris and Whan, 2008; Li et al., 2014), goats (Badaoui et al., 2011), horses (Rieder et al., 2001), rabbits (Fontanesi et al., 2010), red foxes (Våge et al., 1997), and dogs (Dreger and Schmutz, 2011; Ciampolini et al., 2013).

The Chinese raccoon dog (\textit{Nyctereutes procyonoides procyonoides}), which has a mixed coat color of black-to-brown with grey hairs (Han et al., 2012), is an important fur-bearing animal species worldwide (Nowacka-Woszuk et al., 2013; Yan et al., 2013). Nowadays, three coat color variants exist in farmed wild-type Chinese raccoon dog (Figure 1), which are brown, dominant-white, and black. Because the mutation responsible for these variants in Chinese raccoon dog remains unknown, the aim of this study was to clone and analyze cDNA sequences of the \textit{ASIP} gene from animals presenting four coat-color phenotypes, and to further determine whether these phenotypes are associated with polymorphisms in this species.

![Figure 1. Coat-color phenotypes of the adult Chinese raccoon dogs in winter. A. wild-type; B. black; C. brown; D. dominant-white.](image)

MATERIAL AND METHODS

Skin tissues were taken from four farmed Chinese raccoon dogs presenting four types of coat color from three fur-bearing farms in Shandong, Hebei, and Jilin, China. Animal experiments were performed in accordance with the guidelines for animal care established by the Jilin University Animal Care and Use Committee. Total RNA was extracted using the RNaPrep pure Tissue Kit (TIANGEN, Beijing, China) according to the manufacturer protocol and immediately frozen at...
-80°C. The RNA concentration was quantified using an ultraviolet spectrophotometer, and RNA quality was assessed on 1.0% agarose gels.

In order to obtain the full-length cDNA sequence of ASIP from Chinese raccoon dog, 5’ and 3’ rapid-amplification of cDNA ends (RACE) was carried out according to the protocol reported by Cosentino et al. (2010). Gene-specific primers were designed based on the canine ASIP mRNA sequence (GenBank accession No. NM_001007263). In 5’ RACE, first-strand cDNA was synthesized from total RNA using a PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China) following the manufacturer instructions. A poly (dC) tail was then added to the 3’ end of the cDNA using TdT terminal transferase (TaKaRa). The first round of PCR was performed with 5-AP (5’-GGCCACGCGTCGACTAGTACGGGGGGGGGGGGGGG-3’) and HAP-R1 (5’- TTCTTTTCCG CCTCTTTTCTGCT-3’) as primers. PCR was performed in a 25-μL reaction under the following conditions: 95°C for 2 min; 30 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 5 min. Nested PCR was carried out using 5-AP1 (5’-GGCCACCGCTCGACTAGTAC-3’) and HAP-R2 (5’-AGACAGAAGGAAATCCAAAAAGG-3’) as primers and 0.5 μL of the product from the first-round of PCR as the template. The PCR components and reaction conditions were the same as those described above. In 3’ RACE, first-strand cDNA was synthesized using 3-AP1 (5’-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
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Figure 2. Agarose gel electrophoresis of PCR products. Lane 1, DL2000 marker. Lane 2, 5'-RACE amplification using primer set 5-AP1 and HAP-R2. Lane 3, 3'-RACE amplification using primer set HAP-F1 and 3-AP1.

Figure 3. CDNA and deduced amino acid sequences of *ASIP* from Chinese raccoon dogs. The vertical lines mark the junctions of exons with the exon numbers. The stop codon is represented by the asterisk. Underlined text indicates the probable polyadenylation signal.

By assembling the cloned 3'- and 5'-end sequences obtained by RACE, two full-length cDNAs of *ASIP* from Chinese raccoon dog were generated. The transcripts of *ASIP* are 726- and 718-bp long excluding the poly(A) tail, and contain a 396-bp open reading frame, a 95-bp (87-bp for 1C) 5'-untranslated region (UTR), and a 235-bp 3'-UTR. These sequences have been deposited in NCBI GenBank under accession No. KT224450 and KT224451, respectively. The *ASIP* of Chinese raccoon dog encodes a putative protein containing 131 amino acids, which is 99.2, 97.7, 82.2, 81.4, and 81.4% identical to that of red fox (accession No. P79407), dog (NP_001007264), cattle (NP_996674), mouse (NP_056585), and human (NP_001663), respectively. According to the genomic sequence of canine *ASIP* (NC_006606) and mRNA data obtained in this study, we can deduce that the *ASIP* gene in Chinese raccoon dog is composed of four exons and three introns.
RT-PCR amplification using primer sets HAP-1A-F/HAP-R3 and HAP-1C-F/HAP-R3 produced 502- and 527-bp fragments from three other individuals representing three types of coat-color variations. Sequencing revealed that two transcripts resulting from the alternative splicing of exon 1 (1A and 1C) of the ASIP gene were present in three types of coat-color variants. Therefore, we excluded a possible association between alternative splicing and coat-color variation in Chinese raccoon dog. In addition, no polymorphism was found in the coding region of the ASIP gene among any individuals examined in this study.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES


