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NOTE

Baboon bearing resemblance in pigmentation pattern to Siamese cat carries a missense mutation in the tyrosinase gene

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Abstract: An infant hamadryas baboon exhibiting an albino phenotype—white body hair and red eyes—was born to parents with wild-type body color. Pigmentation on some parts of its body surfaced during childhood and progressed with age. This baboon in adulthood has gray hair on parts of its body, such as the tail, distal portion of the legs, and face, with the remainder being white. This pigmentation pattern resembles that of the Siamese cat and the Himalayan variants of the mouse and the mink. The distinguishing phenotypes in these animals are known to be caused by a temperature-sensitive activity of tyrosinase, an enzyme essential for biosynthesis of melanin. We sequenced all the five exons of the tyrosinase (*TYR*) gene of this albino baboon, which were amplified by PCR, and found a base substitution leading to alteration of the 365th amino acid from Ala to Thr. Tyrosinase requires copper as a cofactor for its enzyme function. It has two copper-binding sites, the second of which contains His residues in positions 363 and 367 that are critical to its function. Thus, p.(Ala365Thr) due to a mutation in the *TYR* gene is a likely candidate for the cause of the albino phenotype in this baboon.

Key words: albinism, melanin, body color, primate, Old World monkey.

Résumé : Un jeune babouin hamadryas présentant un phénotype albinos – des poils blancs sur le corps et des yeux rouges – est né de parents ayant une pigmentation de type sauvage. Une pigmentation sur certaines parties de son corps est apparue au cours de l'enfance et s'est accentuée avec le temps. À l'âge adulte, ce babouin présente des poils gris sur certaines parties de son corps, la queue, la partie distale des jambes et le visage, tout en demeurant albinos sur le reste de son corps. La pigmentation ressemble à celle observée chez le chat siamois ainsi que chez les variants himalayens de la souris et du vison. Il est connu que les phénotypes distinctifs de ces animaux sont dus à une activité thermosensible de la tyrosinase, une enzyme essentielle à la synthèse de la mélanine. Les auteurs ont amplifié par PCR et séquencé les cinq exons du gène codant pour la tyrosinase (*TYR*) chez ce babouin albinos, et ils ont trouvé une substitution nucléotidique entraînant un changement d'acide aminé à la position 365 (Ala à Thr). La tryosinase nécessite le cuivre comme cofacteur pour sa fonction enzymatique. Elle possède deux sites de liaison du cuivre et le second contient des résidus His aux positions 363 et 367, lesquels sont critiques pour sa fonction. Ainsi, il est vraisemblable que la mutation dans le gène *TYR*, p.(Ala365Thr), soit la cause de l'albinisme observé chez ce babouin. [Traduit par la Rédaction]

Mots-clés : albinisme, mélanine, couleur du corps, primate, singe de l'Ancien Monde.

Introduction

Tyrosinase (EC 1.14.18.1) is an enzyme that catalyzes the tyrosine-to-dopa and dopa-to-dopaquinone reactions in melanin biosynthesis (Körner and Pawelek 1982) and is encoded by a single gene, *TYR*, in mice (Jiménez et al. 1989) and other mammals. The Siamese cat exhibits a distinguishing coat coloration, in which melanin pigmentation is limited to the extremities of the body, such as the tail, paws, and face. This is a specific type of albinism caused by a temperature-sensitive activity of tyrosinase (Searle 1990). Similar albino phenotypes are known, with the name of the Himalayan variant, in the mouse, rabbit, mink, and guinea pig. Many of these examples have been shown to be associated with a nonsynonymous base substitution in the *TYR* gene (Kwon et al. 1989; Lyons et al. 2005; Benkel et al. 2009).

Wanpark Kochi Animal Land (a municipal zoo located in Kochi City, Japan) houses animals including the hama-

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Fig. 1. Phenotypes of HamA (male with albino body color) and HamW (female with wild-type pigmentation). (A–C) HamA a few days after birth. (D) Whole body of HamA. (E) Whole body of HamW. (F) Face of HamA. (G) Face of HamW.

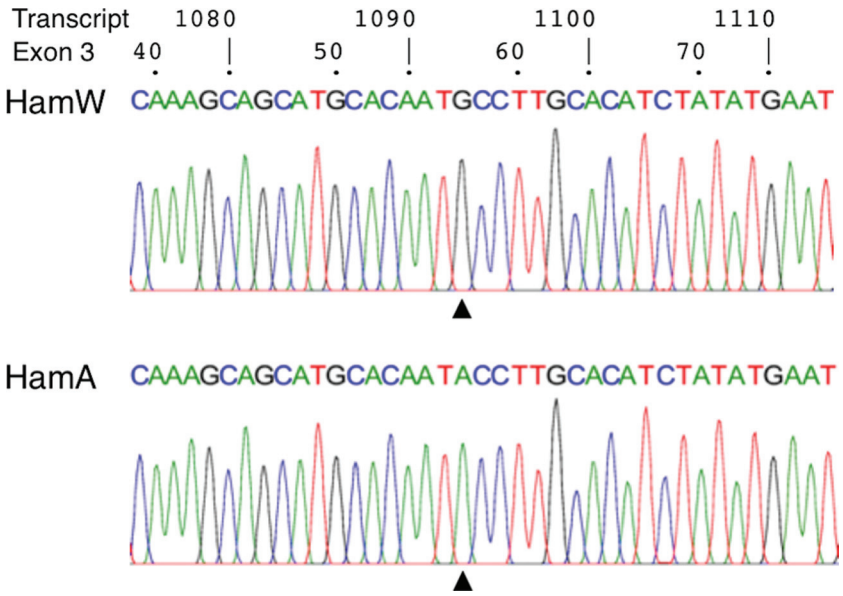
dryas baboon (*Papio hamadryas*). On 3 November 1994 an albino male infant of hamadryas baboon (Cima) was born to parents with wild-type body color (Patra and Caesar). At birth Cima exhibited a complete oculocutaneous albinism, with white hair on the whole body and red eyes (Fig. 1). When Cima was 2 or 3 years old, pigmentation started surfacing on some parts of his body that progressed with age. On gaining sexual maturity, he exhibited a pigmentation pattern similar to that of the Siamese cat—gray hair on the tail, distal portion of legs, and face. This coloration has been maintained until now (Fig. 1). The eye color also underwent changes. Currently, the pupil is dark red, and the iris shows gradation from blue to brown from the center outwards. Considering the possibility that a *TYR* mutation is responsible for Cima's albino phenotype, we amplified all five exons of this gene by PCR using feces samples from both Cima and a wild-type hamadryas baboon and sequenced the fragments. This analysis revealed a mutation leading to an amino acid substitution, which is located in a functionally important region of the tyrosinase enzyme.

Materials and methods

This study did not include any animal experiments—sample collection from zoo animals was conducted by a noninvasive method, and all experiments performed in this study were in vitro experiments using these samples. The sample collection was registered in advance at the Animal Welfare and Animal Care Committee of the Primate Research Institute, Kyoto University (registration number 2919A-001). This study involved a recombinant DNA experiment and it was approved in advance by the Recombinant DNA Experiment Safety Committee of Kyoto University (approval number 190058).

In addition to Cima, as a target for comparison, we also collected and used a sample from a female hamadryas baboon of the wild-type body color (Pong). She was born in Tobe Zoological Park of Ehime Prefecture (Tobe, Japan)

Fig. 2. Wave patterns obtained from the sequencing analysis. The 57th site on *TYR* exon 3 (black triangle), which corresponds to the 1093th site on the *TYR* transcript, was found to carry different nucleotides: G in HamW and A in HamA. The wave patterns around this site are shown. The numbers in the top and second lines show nucleotide positions on the transcript and exon 3, respectively.



in 1997. As far as we could trace their pedigrees, Cima and Pong do not share an ancestor. Hereafter, for ease of explanation, Cima and Pong will be denoted by HamA and HamW, respectively (Ham, hamadryas baboon; A, albino; W, wild-type color), as well as samples and data originating from the respective animals.

Feces naturally egested and left in their sleeping chamber were picked up, and DNA was extracted using the NucleoSpin DNA Stool kit (product of Macherey-Nagel). The concentration of these DNA samples was roughly estimated by comparing the intensity of the band on an agarose electrophoresis gel photograph and then adjusted to approximately 25 ng/μL. These DNA samples were expected to contain baboon genomic DNA originating from their intestinal epithelium, with the remainder coming from other sources, such as bacteria or food residuum.

We obtained nucleotide sequences of the *TYR* gene region from the genomic DNA assembly of the olive baboon (*Papio anubis*) (Panu_3.0, released in April 2017). Comparing this sequence data with that of a *TYR* transcript (file ID, ENSPANT00000015629.2), we selected five pairs of 30-nucleotide regions that encompassed the five *TYR* exons (Fig. S1[1]). The selection was conducted so that the distance from either the start or end points of the exon would be 200–600 nucleotides and the four nucleotides would be contained at nearly equal frequencies. We then synthesized oligomers that represented these selected regions.

We conducted PCR amplification of the exon regions from the DNA samples as template, using PrimeSTAR GXL DNA Polymerase (product of Takara Bio Inc.). The PCR conditions were as follows: 2 min at 98 °C, 4 cycles of 10 s at 98 °C and 2*n* s at 68 °C, 36 cycles of 10 s at 98 °C and *n* s at 68 °C, and then 2 min at 68 °C, in which *n* was determined based on the expected product length (30 s for 1000 nucleotides).

For each exon region, after confirming amplification of each DNA fragment by gel electrophoresis, the fragment was purified by polyethylene glycol precipitation. The fragment was then sequenced by the Sanger method using a 3730xl DNA analyzer (Applied Biosystems). The same primers used for PCR amplification were used separately for sequencing.

Results

PCR reactions yielded single fragments of the expected lengths for all five exon regions in both cases of HamW and HamA (Fig. S2[1]). For each exon region, we prepared two fragments by setting up two PCR reaction mixtures, and collected sequence data from both. The purpose was to exclude PCR or sequencing errors. Discrepancy between the two fragments was not found in any of the pairs. As shown in sequence data alignments (Fig. S3[1]), insertion or deletion of nucleotides was not found in the exons. As for nucleotide substitutions between olive baboon (Anu) and hamadryas baboon, or between HamW and HamA, three sites were identified in exon 1 and one

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2020-0003.

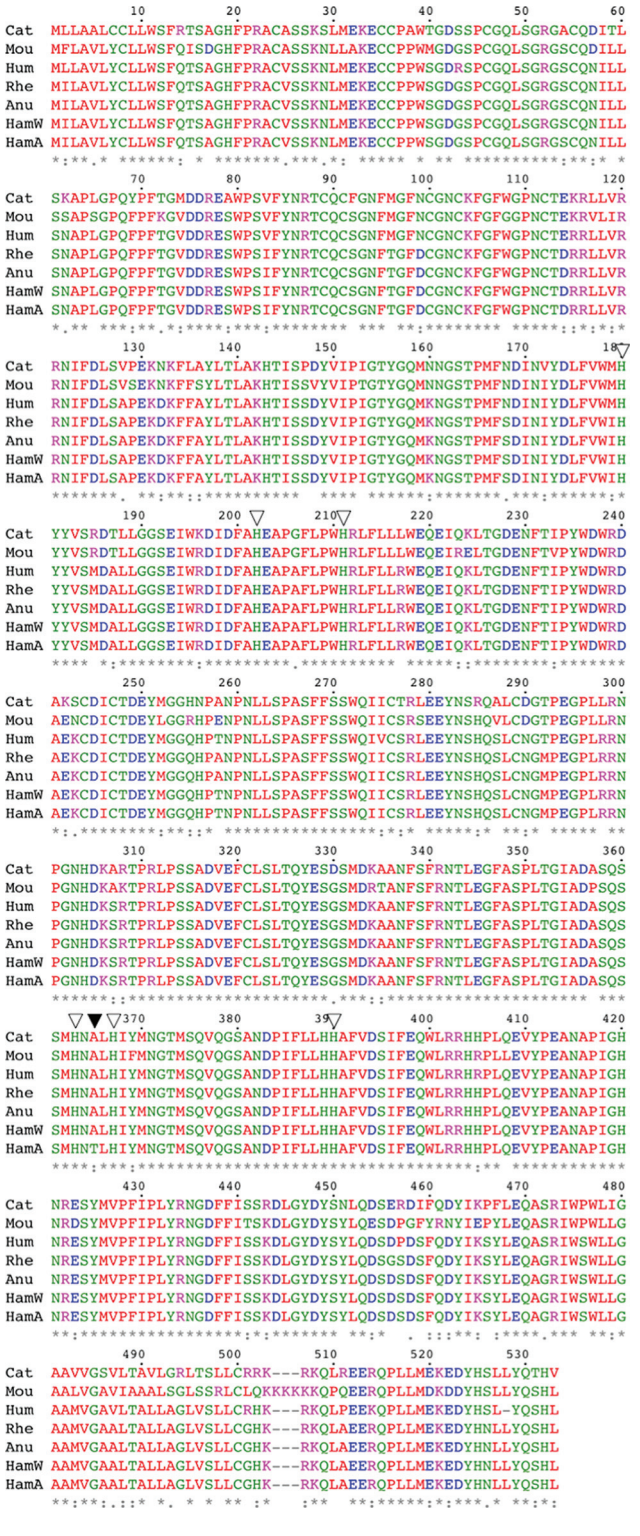


Fig. 3. Alignment of amino acid sequences. Amino acid sequences deduced from nucleotide sequences of the exons were aligned. Mou, mouse; Hum, human; Rhe, rhesus macaque; Anu, olive baboon. The numbers indicate site positions in the tyrosinase amino acid sequence. However, the portions after the 503rd site do not necessarily imply accurate positions because of insertions or deletions in some species. The open triangles indicate His residues that play key roles in tyrosinase function. The closed triangle shows the p.(Ala365Thr) substitution found in HamA. The color coding for amino acids follows the definition by the Clustal Omega program.

was found in exon 3. All introns were found to start with GT (splicing donor site) and end with AG (splicing acceptor site).

As explained below, the single nucleotide difference observed in exon 3 (G in HamW, A in HamA) (Fig. 2) was a nonsynonymous base substitution. To further confirm that this difference was not an artifact due to a PCR or sequencing error, we prepared additional three PCR fragments for exon 3 of HamW and exon 3 of HamA, and sequenced them. All five fragments from HamW carried G at this nucleotide site, and all fragments from HamA carried A.

We cut out and combined nucleotide sequences of the five exons and aligned the deduced amino acid sequences between HamW and HamA (Fig. 3). Corresponding sequences from the cat, mouse, and some other Catarrhini primates (Old World monkeys and hominoids) were also included in this alignment. Several amino acid substitutions were observed, and one of these was unique to HamA—Thr in HamA, but Ala in HamW and other species. From this distribution pattern, the change in this site could be from Ala to Thr. This change was observed at the 365th amino acid of the olive baboon tyrosinase, leading to the denotation of A0A096MRE4: p.(Ala365Thr). This amino acid change is due to the base substitution (G in HamW, A in HamA) observed in exon 3 (Fig. S3[1]). This base substitution is located at the 1093th site in the nucleotide sequence of the olive baboon *TYR* transcript, which can be denoted by ENSPANT00000015629.2:c.1093G>A.

Discussion

The parents of HamA produced 10 offspring, of which eight were wild-type and two were albino. These figures suggest that (*i*) there is a single locus that controls the wild-type/albino body color, (*ii*) the albino allele is recessive to the wild-type allele, (*iii*) the parents were both heterozygous, and (*iv*) HamA is homozygous for the albino allele. Cima grew into adulthood, but the other albino infant died soon after birth.

As we report here, *TYR* gene of HamA carries a base substitution that leads to the amino acid alteration of p.(Ala365Thr). There were no other nucleotide changes that fell into missense, nonsense, or frame shifting mutations over the five exons. These results lead to the hypothesis that the albino phenotype of HamA is caused by p.(Ala365Thr). This is, however, no more than a hypothesis as long as genetic information remains absent about the rest of the *TYR* gene, and other genes that may cause albinism, including *P*, tyrosinase-related protein-1, and membrane-associated transporter protein (Kamaraj and Purohit 2014). On the other hand, considering the loca-

tion of the amino acid change, this mutation can be regarded as a likely candidate for the cause of the HamA phenotype. Tyrosinase requires copper as a cofactor for its enzyme function (Olivares et al. 2002). It carries two copper-binding sites that are called CuA and CuB. Each site contains three His residues by which a copper ion is coordinated. These His residues are well conserved among type-3 copper proteins, including tyrosinase and hemocyanin (Schweikardt et al. 2007). The importance of the CuA and CuB regions is also supported by plenty of reports of human mutations that cause complete oculocutaneous albinism, including, in the case of the CuB region, missense mutations for p.Ser361Arg, p.Asn364His, p.His367Tyr, and p.Met370Thr (the P14679 file of the UniProtKB database). The albinism observed in HamA is not a complete oculocutaneous albinism but retains partial pigmentation. The effect of p.(Ala365Thr) on the tyrosinase function may be milder than that of the human mutations cited above.

If the hypothesis is correct, the mutation for p.(Ala365Thr) may be more useful for studying tyrosinase mechanisms than the human mutations aforementioned. While those human mutations totally abolish the enzyme function, p.(Ala365Thr) leaves the enzyme partially or intermediately functional. This may be helpful in revealing an important aspect of the enzyme function. Another point to note is that this mutation was found in a primate species genetically close to humans. Hamadryas baboon belongs to parvorder Catarrhini that includes superfamily Hominoidea (humans and apes) and superfamily Cercopithecoidea (baboons and macaques). Humans do not have a tail, and are only slightly hairy, particularly on the hands, feet, and face. Even if an equivalent mutation occurs, it may be unnoticeable in humans.

The temperature-sensitive tyrosinase activity of the Siamese cat has been shown to be associated with a missense mutation for p.(Gly302Arg) (Lyons et al. 2005). Similar albinisms in Himalayan mouse and Himalayan mink are known to be associated with mutations for p.His420Arg (Kwon et al. 1989) and p.His420Gln (Benkel et al. 2009), respectively. The phenotype of HamA resembles the phenotypes of these mutant animals. However, the position of the mutation on the *TYR* gene differs. If the hypothesis about the cause of the HamA phenotype is correct, HamA may also contribute to the clarification of mechanisms of temperature dependence of the tyrosinase activity.

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