

GENETICS OF MELANOCORTIN 1 RECEPTOR (MC1R) IN PAPUA CUSCUSES (GENUS SPILOCUSCUS) AND ITS CORRELATION WITH COAT PIGMENTATION

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**STUDY PROGRAM OF ANIMAL BIOSCIENCES
FACULTY OF MATHEMATICS AND SCIENCES
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STATEMENT LETTER

I hereby declare that dissertation entitled “Genetics of Melanocortin 1 (*MC1R*) in Papua Cuscuses (Genus *Spilocuscus*) and Its Correlation with coat pigmentation” is original of my own research supervised by supervisory committee and has never been submitted in any form at any institution before. All information from other authors cited here are mentioned in the text and listed in the reference at the end part of the dissertation.

Bogor, October 2025

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SUMMARY

AKSAMINA MARIA YOHANITA. Genetics of Melanocortin 1 Receptor (*Mclr*) in Papua Cuscuses (Genus *Spilocuscus*) and Its Correlation with Coat Pigmentation. Supervised by TRI ATMOWIDI, BAMBANG SURYOBROTO, KANTHI ARUM WIDAYATI, and HIROO IMAI.

Coloration and color patterns are among the most variable morphological traits in animals. Animals use body coloration for camouflage, communication, and physiological regulation. Body color is mediated by melanocortin receptors in melanocyte cells of the skin. One of the most widely studied color receptors is MC1R, encoded by the *MC1R* gene. Previous studies have shown that *MC1R* variation correlates with color phenotypes among individuals. However, *MC1R* does not consistently predict coat color variation in all species.

The molecular mechanisms underlying mammalian coat color variation have been extensively studied in placental mammals (Eutheria). However, studies on the relationship between *MC1R* polymorphisms and coat color in marsupials remain limited, and agonist-induced as well as basal MC1R activity have not yet been investigated. The objectives of this study were to provide a description of the coat color phenotypes of two cuscuses species through both qualitative and quantitative approaches. For the purpose, I investigated interspecific and intraspecific nucleotide polymorphisms of the Melanocortin 1 Receptor (MC1R) gene, and conducted *in vitro* functional characterization of MC1R in relation to coat color variation, with a particular focus on Papuan cuscuses (*Spilocuscus* spp.). Phenotypic analyses of coat color variation across species show that *S. maculatus* has a wide diversity of coat color patterns, whereas *S. wilsoni* displays limited variation. Quantitatively, the two species do not differ significantly in color (L^* , a^* , b^*), but qualitative trends indicate that *S. maculatus* appears redder than *S. wilsoni*.

At the molecular level, the *MC1R* gene of *Spilocuscus maculatus* possesses three nucleotide haplotypes; however, all encode a single amino acid haplotype (Hap_A), indicating a conserved MC1R function despite the observed variation in coat coloration. In contrast, *S. wilsoni* possesses three nucleotide haplotypes that correspond to three distinct amino acid haplotypes (Hap_B, Hap_C, and Hap_D), suggesting functional diversification of MC1R. At the cellular level, functional assays further revealed species-specific differences in receptor activity: *S. maculatus* (Hap_A) exhibited high basal cAMP activity, indicating constitutive activation of MC1R in the absence of ligand binding, whereas *S. wilsoni* demonstrated lower and more variable basal and maximum activities across haplotypes. *S. maculatus* (Hap_A) also achieves maximum cAMP production as indicated by a significant response to α -MSH at concentrations from 0.1 μ M to 100 μ M ($EC_{50}=0.007 \pm 0.006$). *S. wilsoni* (Hap_D), despite having the lowest basal activity, approached maximum cAMP production at a concentration of 10 μ M to 100 μ M ($EC_{50}=0.90 \pm 0.43$). G517A mutant exhibits a concentration effect trend; however, it is not statistically significant. Conversely, concentration treatment does not affect Hap_B and Hap_C.

In general, haplotypes with higher activity are associated with dark pigment (eumelanin), whereas those with lower activity produce lighter pigment

(pheomelanin). However, in both species, the functional results do not fully align with their phenotypes (relatively light and reddish), which indicates pheomelanin production. These results showed that MC1R is not the sole determinant of coat color in cuscuses, suggesting that other pigmentation genes (e.g., ASIP, TYRP1, MITF) or environmental factors may also play a role.

Keywords: Coloration, gene, MC1R, Spilocuscus, Papua

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RINGKASAN

AKSAMINA MARIA YOHANITA. Genetika Reseptor Melanokortin 1 Receptor (*MC1R*) pada Kuskus Papua (Genus *Spilocuscus*) dan Korelasinya dengan Pigmentasi Rambut. Dibimbing oleh TRI ATMOWIDI, BAMBANG SURYOBROTO, KANTHI ARUM WIDAYATI, dan HIROO IMAI.

Warna dan pola warna adalah salah satu karakter morfologi hewan yang paling bervariasi. Hewan menggunakan pewarnaan tubuh untuk kamuflase, komunikasi, dan proses regulasi fisiologi. Warna tubuh dimediasi oleh reseptor warna pada sel melanosit di kulit. Salah satu reseptor warna yang paling banyak dipelajari adalah *MC1R*, yang disandikan oleh gen *MC1R*. Studi sebelumnya menunjukkan variasi *MC1R* berkorelasi dengan fenotipe warna antar individu. Namun, *MC1R* tidak selalu dapat memprediksi variasi warna bulu pada semua spesies.

Mekanisme molekuler yang mendasari variasi warna rambut pada mamalia umumnya telah banyak diteliti pada mamalia plasenta (*Eutheria*). Namun, studi mengenai hubungan antara polimorfisme *MC1R* dan warna rambut pada marsupial masih terbatas, termasuk aktivitas *MC1R* yang diinduksi agonis maupun aktivitas basal belum pernah dipelajari. Tujuan penelitian ini adalah pertama, mendeskripsikan fenotipe warna bulu kuskus secara kualitatif dan kuantitatif. Kedua, menyelidiki polimorfisme nukleotida *MC1R* secara interspesifik dan intraspesifik yang berasosiasi dengan warna bulu. Ketiga, menyelidiki karakterisasi fungsional gen reseptor Melanocortin 1 (*MC1R*) secara *in vitro*, yang berkaitan dengan fenotipe warna bulu kuskus Papua (genus *Spilocuscus*).

Analisis fenotipe variasi warna bulu antarspesies menunjukkan bahwa *S. maculatus* memiliki keragaman pola warna bulu yang luas, sedangkan *S. wilsoni* menampilkan variasi yang terbatas. Fenotip *S. wilsoni* menunjukkan dikromatism jantan dan betina dengan pola warna rambut yang berbeda. Secara kuantitatif, kedua spesies tidak berbeda signifikan dalam warna (L^* , a^* , b^*), tetapi tren kualitatif menunjukkan bahwa *S. maculatus* tampak lebih merah dibandingkan *S. wilsoni*.

Di tingkat molekuler, gen *MC1R* *S. maculatus* memiliki tiga haplotipe nukleotida tetapi semuanya mengkode satu haplotipe asam amino (Hap_A), menunjukkan fungsi *MC1R* yang terjaga (conserved) meskipun warna rambut bervariasi. Sebaliknya *S. wilsoni* memiliki tiga haplotipe nukleotida yang menghasilkan tiga haplotipe asam amino berbeda (Hap_B, Hap_C, Hap_D), menandakan adanya diversifikasi fungsi *MC1R*. Di tingkat seluler, uji fungsional mengindikasikan adanya perbedaan aktivitas reseptor antar spesies. *S. maculatus* (Hap_A) menunjukkan aktivitas basal tinggi, menandakan *MC1R* konstitutif aktif tanpa ligan. *S. wilsoni* menunjukkan aktivitas basal yang lebih rendah dan bervariasi antar haplotipe. *S. maculatus* (Hap_A) juga mencapai produksi maksimum cAMP yang ditunjukkan oleh respons signifikan terhadap α -MSH pada konsentrasi 0.1 μ M sampai 100 μ M ($EC_{50}=0.007 \pm 0.006$). *S. wilsoni* (Hap_D) walaupun basal aktivitas paling rendah namun mendekati produksi cAMP maksimum pada konsentrasi 10 μ M sampai 100 μ M ($EC_{50}=0.90 \pm 0.43$). G517A mutant



menunjukkan tren adanya pengaruh konsentrasi, tetapi belum kuat secara statistika. Berbeda dengan Hap_B dan Hap_C yang tidak merespon perlakuan konsentrasi.

Secara umum, haplotipe dengan aktivitas lebih tinggi diasosiasikan dengan pigmen gelap (eumelanin), sedangkan yang rendah menghasilkan pigmen terang (pheomelanin). Namun, pada kedua spesies, hasil fungsional ini tidak sepenuhnya selaras dengan fenotipe (relatif terang dan kemerahan) yang mengarah kepada produksi feomelanin. Hasil ini menunjukkan bahwa MC1R bukan satu-satunya gen penentu warna bulu pada kuskus, sehingga kemungkinan ada gen pigmen lain (misalnya ASIP, TYRP1, MITF) atau faktor lingkungan yang berperan.

Kata kunci: Pewarnaan, gen, MC1R, Spilocuscus, Papua

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AKSAMINA MARIA YOHANITA

Dissertation
Submitted in partial fulfillment of the requirements for
Doctoral Degree
In the Animal Biosciences Study Program

**STUDY PROGRAM OF ANIMAL BIOSCIENCES
FACULTY OF MATHEMATICS AND SCIENCES
IPB UNIVERSITY
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PREFACE

This dissertation represents the culmination of my academic journey and years of research dedicated to understanding the evolutionary and ecological aspects of my study. It has been a journey of continuous learning, exploration, and collaboration, made possible through the guidance, support, and encouragement of many individuals and institutions.

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It is my sincere hope that this scientific work will not only serve as a contribution to the advancement of knowledge but also inspire future research in related fields. May it be of benefit to those who seek to understand and protect our natural world.

Bogor, September 2025

Aksamina Maria Yohanita



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