DOI: 10.21005/AAPZ2023.68.4.3

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2023, 369(68)4, 23–29

Received 11 Aug 2023
Revised 5 Sep 2023
Accepted 8 Sep 2023

Review Article

Inga KOWALEWSKA

CHARACTERISTICS OF THE *MC1R* GENE AS A LOCUS E AFFECTING COAT COLOR IN DOGS

Department of Genetics, Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology, Szczecin, Poland

Abstract. The color of dogs is the result of various genes that control the pigmentation of the coat mainly. There are many genes that influence the color, pattern and distribution of fur in dogs, one of which is the gene encoding the melanocortin 1 receptor (*MC1R*), also known as the E locus. The melanocortin 1 receptor (*MC1R*) is a protein that plays a key role in the regulation of skin, hair and eye pigmentation in animals. This receptor is responsible for the processing of peptide hormones that control the production of melanin, which is the pigment of the skin and fur. Mutations within this gene can affect the color of animals, including dogs. The main alleles identified and described for the E locus are: ***E*** (wild type), E^M (melanistic mask) and ***e*** (recessive red); they are widespread in various dog breeds, indicating that they existed at an early stage in the formation of individual breed groups. The other identified alleles are less common and are: E^G (grizzle/domino), e^2 (only among Australian Cattle Dogs and their crossbreds) and e^3 (only among Siberian Huskies and their crossbreds), e^A (ancient red) allele and the allele E^H (only among English Cocker Spaniels and American Cocker Spaniels). Of course, it's important to remember that dog color is a complex genetic phenomenon that can be controlled by many different genes working together, and that the variety of coat colors in different dog breeds results from a combination of these genes.

Key words: dogs colour, *MC1R*, locus E, melanistic mask, recessive red.

INTRODUCTION

Genes play a significant role in determining the color of dogs. The genetic makeup of a dog influences the production and distribution of pigments in their fur, which ultimately determines their coat color. There are several genes involved in this process, and different combinations and variations of these genes can result in a wide range of coat colors and patterns. The most known and described genes that affect the color of dogs are: melanocortin 1 receptor (*MC1R*), agouti signaling protein (*ASIP*), beta-defensin 103 (*CBD103*), tyrosinase-related protein 1 (*TYRP1)*. *MC1R* gene controls the production of eumelanin, which is responsible for

Corresponding author: Inga Kowalewska, Department of Genetics, Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology, Piastów Avenue 45, 71-899 Szczecin, Poland, e-mail: inga.kowalewska@zut.edu.pl.

black and brown pigments, *ASIP* gene regulates the distribution of pigments within the hair shaft; it controls the switch between eumelanin (black/brown) and phaeomelanin (red/yellow), *CBD103* gene influences the production of white or yellow coat color in certain dog breeds, *TYRP1* gene is involved in the production of eumelanin and affects the intensity of black and brown pigments. It's important to note that coat color in dogs is a complex trait influenced by the interactions of multiple genes. Different combinations and interactions of these genes can produce a wide variety of coat colors and patterns observed in different dog breeds (Schmutz et al. 2003; Schmutz and Berryere 2007; Kaelin and Barsh 2013; Saif et al. 2020; Brancalion et al. 2021).

E (EXTENSION) LOCUS

One of the most well-known genes associated with coat color in dogs is the melanocortin receptor 1 (*MC1R*) gene, also known as the E (extension) locus. This naming convention is derived from early genetic research conducted in mice, which used alphabetical letters to denote different genes and loci.

In mice, the E locus was associated with variations in coat color, and subsequent studies revealed that this locus corresponded to the *MC1R* gene. When researchers began studying the similar gene in dogs, they adopted the same nomenclature, referring to the canine *MC1R* gene as the E locus. It's important to note that the use of "E locus" as a name for the *MC1R* gene in dogs is a convention within the field of genetics, and it helps researchers identify and communicate about specific genes and genetic loci across different species (Newton et al. 2000; Brancalion et al. 2021).

The melanocortin 1 receptor (MC1R) is a protein that plays a central role in regulating coat color determination in mammals, including humans. The MC1R protein belongs to the G-protein-coupled receptor (GPCR) family, which are transmembrane proteins that transmit signals from outside the cell to the inside, activating various cellular responses. The MC1R protein is primarily expressed in melanocytes, which are specialized cells responsible for producing the pigment melanin. Melanin is responsible for determining the color of our hair, skin, and eyes. There are two primary types of melanin: eumelanin, which gives color ranging from brown to black, and pheomelanin, which gives color ranging from yellow to red. The MC1R protein is involved in the production of eumelanin and pheomelanin by melanocytes. It acts as a receptor for a hormone called alpha-melanocyte-stimulating hormone (α-MSH), which is produced by the pituitary gland. When α-MSH binds to the MC1R receptor, it activates a signaling pathway that stimulates the production of eumelanin (Hubbard et al. 2010; Wolf Horrell et al. 2016).

The *MC1R* gene is a gene that codes for the melanocortin 1 receptor, which is a protein located on the surface of melanocytes (cells that produce melanin) in the skin and hair follicles. This receptor plays a crucial role in regulating the production of melanin and determining coat color in dogs (Kaelin and Barsh 2013).

The *MC1R* gene is located on a dog's chromosome 5 and is a single exon gene (Nowacka-Woszczuk et al. 2012) and encodes a protein of 317 amino acid residues and is the same length as in other mammals, with the exception of mice, which lack two amino-terminal residues in the extracellular domain (Newton et al. 2000).

The following work focuses on the characteristics of the E locus in the context of the influence on the color of dogs, and therefore a query of scientific publications of both original research and review papers was conducted. There are seven alleles at the E locus: e^{1-3}, e^{A}, E, E^{G} and E^{M}, in order of increasing dominance, of which the e^{1} (most commonly called e), E

and E^M alleles are widespread, while the other alleles are less common (Anderson et al. 2020; Brancalion et al. 2021).

MAJOR E LOCUS ALLELES

The *E* allele is associated with the expression of the full range of eumelanin production, which means it allows for the formation of both black and brown pigment. When a dog carries two copies of the *E* allele (homozygous *EE*), it has the potential to express a wide variety of coat colors, including black, liver, and their dilutions (such as blue and Isabella). This allele is considered the dominant form, as it has a stronger effect on pigmentation compared to other alleles.

The E^M allele is associated with a specific coat color phenotype known as "melanistic mask". It causes a specific pattern of coat color pigmentation, characterized by a dark mask on the face. This mask typically covers the muzzle, ears, and sometimes extends to the eyes. Importantly, the mask visible in the dog's phenotype will have the color of eumelanin that the dog can produce due to the presence of other genes responsible for coloration, so it will be black, brown, blue or lilac (Schmutz and Melekhovets 2012; Dreger et al. 2019).

The dominant E^M allele is associated with the M264V amino acid change within melanocortin receptor (Conant et al. 2011). This change is located where the transmembrane domain connects to the extracellular loop, which can affect ligand affinity, signaling, or receptor stability. Another explanation for this effect on the dog's phenotype is that it is in a linkage disequilibrium with a regulatory mutation that alters *MC1R* expression levels. In both cases, the E^M allele is assumed to affect *MC1R* signaling levels rather than its regional distribution, which in turn may indicate that different body regions have different pigment type switching thresholds, which are revealed by disturbances in *MC1R* signaling efficiency (Schmutz et al. 2003). The basis of the M264V change is the nucleotide substitution c.790A>G (rs24201590) (OMIA 2023a).

The E^G allele is responsible for the grizzly phenotype. The E^G allele encodes a coat colour variant called "domino" in Afghan hounds and "grizzle" in Salukis (Dreger and Schmutz 2010). The "grizzle" phenotype in dogs refers to a specific coat color pattern that is characterized by a combination of light and dark bands on the hairs. This pattern creates a unique and attractive appearance in the dog's coat. The darker color is typically found at the base of the hair, while the rest of the hair gradually lightens towards the tips. The term "grizzle" is often used to describe the peppered or salt-and-pepper appearance of the coat. The individual hairs may have a mix of colors, giving the overall impression of a speckled or mottled coat. Grizzle dogs often have distinct facial markings, such as light-colored "eyebrows" above the eyes, which can add to their expressive appearance. Each grizzle dog's coat may vary in intensity, with some displaying a more pronounced grizzle pattern, while others may have a more subtle effect (Dreger and Schmuzt 2010; Dürig et al. 2018; Anderson et al. 2020; Brancalion et al. 2021). Interestingly, in other breeds with a phenotype similar to Alaskan Malamute or Siberian Husky, the E^G allele has not been identified. In contrast, the allele has recently been identified in a further 26 breeds, including the Akita, Dachshund, Foxhound, Greyhound and Yorkshire Terrier, indicating that it is more common than originally thought (Dreger et al. 2019).

The grizzle allele is dominant to all alleles of the E locus except the E^M allele, which is responsible for the presence of a mask, which of course means that a dog with this phenotype will never have a mask.

The E^G allele is caused by a genetic mutation at position c.233G>T in the *MC1R* gene, resulting in an amino acid change from glycine to valine at 78 position (G78V) (Dreger and Schmutz 2010; OMIA 2023b).

The *e* allele of the *MC1R* gene in dogs is another allele that affects a dog's coat color. The *e* allele is associated with the expression of a recessive red or yellow coat color in dogs. Recessive

red dogs have a non-functional variant of the gene in the E locus that prevents the pigment cells from receiving the signal they need to switch from producing pheomelanin to producing eumelanin. This loss of gene function is caused by a nonsynonymous mutation at position c.916C>T (*rs851563576*) in the promoter region causing a premature stop codon (R306ter) resulting in the elimination of the evolutionary strongly conserved 10 carboxy terminal amino acid residues (Everts et al. 2000; Newton et al. 2000). This mutation is presumed to delete 12 of the 17 residues that make up the cytoplasmic tail of the melanocortin receptor. In the case of other G protein-coupled receptors, such as MC1R, the cytoplasmic tail often contains phosphorylation and/or palmitoylation sites at amino acid residues Ser, Thr or Cys, respectively, whose post-translational modification is necessary for the proper functioning of the receptor (Everts et al. 2000; Newton et al. 2000).

A recessive red refers to a coat color that is inherited when a dog carries two copies of the recessive red gene (*ee*) and lacks dominant black or other color genes.

A recessive red dog's coat color is typically a shade of red or yellow, and it can vary in intensity, ranging from light cream to deep red. The coat color is solid, without any black or other darker pigmentation. The nose, eye rims, and paw pads of recessive red dogs are usually a liver or flesh color, which is different from the standard black found in many other dog colors. Keep in mind that the specific appearance of a recessive red dog can still vary depending on the breed and individual genetics.

MINOR E LOCUS ALLELES

It is worth noting that more and more often it is postulated to distinguish three *e* alleles – denoted as e^1, e^2 and e^3. All alleles work in the same way, and we cannot infer the presence of a given allele in a genotype from a dog's phenotype. The e^1 allele is the most common and has so far been referred to simply as the allele, i.e. the one described above. Dürig et al. (2018) reported two new e alleles namely a variant within the MITF binding site of the canine *MC1R* promoter (Chr5:63695679C>G) in Australian Cattle Dogs (e^2) and a 2-bp deletion in the coding sequence, MC1R:c.816_817delCT (p. (Ile272MetfsTer22) in Alaskan and Siberian Huskies (e^3). The first of these mutations, called the e^2 allele, is a mutation mapped 430 nucleotides upstream of the ATG start codon of the *MC1R* gene within an E-box motif. As shown in previous studies in humans and mice, this site is responsible for the binding of MITF, which is an important regulatory factor for melanocytes. In dogs, the E-box motif in the promoter sequence of the *MC1R* gene has the sequence CACGTG, and in the case of the e^2 allele, conserved guanine at the fourth position is replaced by a cytosine (CACCTG) (Dürig et al. 2018). The consequence of the second detected mutation, named by the authors the e^3 allele, is an altered expression of *MC1R*, which results in the absence of the last transmembrane domain and cytoplasmic C-terminal tail in the protein. In the examined group of Husky dogs (cohort included a complete family with seven offspring), a connection between white color and the presence of two loss-of-function alleles (e^1, e^3) was demonstrated. The authors did not detect the e^3 allele in any of the other breeds they studied (Dürig et al. 2018; Brancalion et al. 2021).

Recently, another allele from the E locus responsible for recessive red has been characterized, called ancient red, designated e^A. This is the name of the substitution of arginine for cysteine at position 301 of MC1R (R301C), which can also interfere with the expression of *MC1R* on the cell surface, because it causes changes in the cytosolic C-terminal extension. Importantly, it was first identified in dog DNA samples from 10,000 years ago and is believed to be one of the earliest mutations associated with dog domestication. The authors tested the presence of this mutation in the modern dog population and found it to be present mainly in hound breeds, like

the Drever and English Foxhound, and Spitz breeds, like the Alaskan Malamute, Alaskan Klee Kai, and Siberian Husky (Ollivier et al. 2013; Anderson et al. 2020).

The function of the e^A allele is defined as a partial recessive red allele, resulting in phenotypes of increased pheomelanin expression and decreased eumelanin expression than would be expected based on information about the dominant black (K locus) and agouti (A locus) genotypes. As a result, the phenotype of dogs carrying the ancient red allele is very variable and may look like a domino or grizzly phenotype (associated with the E^G allele). The phenotype known as "pied" in Beagles is also attributed to a variation of the e^A allele (Brancalion et al. 2021).

The last of the described alleles within the E locus is the E^H allele. It is responsible for the specific sable or dirty red color of American and English Cocker Spaniels. This type of coat colour in the phenotype is observed as a pale undercoat and areas of the body with reduced black or brown and at the same time with increased production of pheomelanin. In all dogs with the sable phenotype, the c.250G>A mutation causing a change in the transmembrane domain was detected during sequencing of the *MC1R* gene. The E^H allele is recessive to the E allele (Laukner 2015; Peist et al. 2021).

The above describes the characteristics of an important but only one gene that affects the phenotype of a dog. Of course, keep in mind that a dog's coat color is influenced by a combination of genetic factors. Genes play a key role in determining the color, pattern, and markings of a dog's fur. These genes interact in complex ways to produce the wide variety of coat colors seen in different dog breeds. It's important to note that the inheritance of coat color in dogs is quite complex due to the presence of multiple genes and their interactions. Different breeds have different combinations of these genes, leading to the incredible diversity of coat colors and patterns that we see in dogs today.

Dog breeders and geneticists often study these genetic factors to better understand how to predict and produce specific coat colors in breeding programs. However, it's important to prioritize the health and well-being of the dogs over attempting to produce specific coat colors, as some genetic combinations can also be associated with health issues.

CONCLUSIONS

The melanocortin receptor 1 gene (*MC1R*) is a gene that plays a crucial role in determining coat color in dogs, as well as in other mammals. It is often referred to as the "E locus" in dogs because it is one of the key genetic loci responsible for variations in coat color. The E locus (extension locus) is a term often used in the context of coat color genetics to describe a genetic region that influences the distribution of black and red pigments in animals, including dogs. Variations in the *MC1R* gene can lead to different coat colors and patterns in dogs. The gene produces a protein that is involved in the production of melanin, which is responsible for pigmentation in the skin, hair, and eyes. Mutations in the *MC1R* gene can result in a wide range of coat colors, including black, brown, red, and yellow, as well as variations in patterns such as brindle and sable. Different combinations of alleles at the *MC1R* gene *locus* can lead to the expression of various coat colors. Some alleles may produce more eumelanin (black and brown pigments), while others may produce more pheomelanin (red and yellow pigments). The interaction of these alleles determines the final coat color of a dog.

It's important to note that the *MC1R* gene is just one of several genes and genetic loci that influence coat color in dogs. Overall, the *MC1R* gene is a key player in the complex genetic regulation of coat color in dogs, and its variations contribute to the wide diversity of coat colors and patterns observed in different dog breeds.

REFERENCES

Anderson H., Honkanen L., Ruotanen P., Mathlin J., Donner J. 2020. Comprehensive genetic testing combined with citizen science reveals a recently characterized ancient *MC1R* mutation associated with partial recessive red phenotypes in dog. Canine Med. Genet. 7(1), 16. DOI: 10.1186/s40575-020-00095-7.

Brancalion L., Haase B., Wade C.M. 2021. Canine coat pigmentation genetics: A review. Anim. Genet. 53, 3–34. DOI: 10.1111/age.13154.

Conant E.K., Juras R., Cothran E.G. 2011. Incidence of the mask phenotype *M264V* mutation in Labrador Retrievers. Res. Vet. Sci. 91(3), e98–e99. DOI: 10.1016/j.rvsc.2011.02.002.

Dreger D.L., Hooser B.N., Hughes A.M., Ganesan B., Donner J., Anderson H., Holtvoigt L., Ekenstedt K.J. 2019. True Colors: Commercially-acquired morphological genotypes reveal hidden allele variation among dog breeds, informing both trait ancestry and breed potential. PLoS ONE 14(10), e0223995. DOI: 10.1371/journal.pone.0223995.

Dreger D.L., Schmutz S.M. 2010. A new mutation in *MC1R* explains a coat color phenotype in 2 ‘‘old’’ breeds: Saluki and Afghan Hound. J. Hered. 101(5), 644–649. DOI: 10.1093/jhered/esq061.

Dürig N., Letko A., Lepori V., Hadji Rasouliha S., Loechel R., Kehl A., Hytönen M.K., Lohi H., Mauri N., Dietrich J., Wiedmer M., Drögemüller M., Jagannathan V., Schmutz S.M., Leeb T. 2018. Two *MC1R* loss-of-function alleles in cream-coloured Australian Cattle Dogs and white Huskies. Anim. Genet. 49(4), 284–290. DOI: 10.1111/age.12660.

Everts R.E., Rothuizen J., van Oost B.A. 2000. Identification of a premature stop codon in the melanocyte-stimulating hormone receptor gene (*MC1R*) in Labrador and Golden retrievers with yellow coat colour. Anim. Genet. 31(3), 194–199. DOI: 10.1046/j.1365-2052.2000.00639.x.

Hubbard J.K., Uy J.A., Hauber M.E., Hoekstra H.E., Safran R.J. 2010. Vertebrate pigmentation: From underlying genes to adaptive function. Trends Genet. 26(5), 231–239. DOI: 10.1016/j.tig.2010.02.002.

Kaelin C.B., Barsh G.S. 2013. Genetics of pigmentation in dogs and cats. Annu. Rev. Anim. Biosci. 1, 125–156. DOI: 10.1146/annurev-animal-031412-103659.

Laukner A. 2015. ZOBEL – was gibt es Neues zur GENETIK? Das Jagdspaniel, 6, 36–38.

Newton J.M., Wilkie A.L., He L., Jordan S.A., Metallinos D.L., Holmes N.G., Jackson I.J., Barsh G.S. 2000. Melanocortin 1 receptor variation in the domestic dog. Mamm. Genome 11(1), 24–30. DOI: 10.1007/s003350010005.

Nowacka-Woszuk J., Salamon S., Gorna A., Switonski M. 2012. Missense polymorphisms in the *MC1R* gene of the dog, red fox, arctic fox and Chinese raccoon dog. J. Anim. Breed. Genet. 130 (2), 136–141. DOI: 10.1111/jbg.12005.

Ollivier M., Tresset A., Hitte C., Petit C., Hughes S., Gillet B., Duffraisse M., Pionnier-Capitan M., Lagoutte L., Arbogast R.M., Balasescu A., Boroneant A., Mashkour M., Vigne J.D., Hänni C. 2013. Evidence of coat color variation sheds new light on ancient canids. PLoS One 8(10), e75110. DOI: 10.1371/journal.pone.0075110.

OMIA. 2023a. OMIA:001590-9615: Coat colour, melanistic mask in Canis lupus familiaris, https://omia.org/OMIA001590/9615/, access: 11.08.2023.

OMIA. 2023b. OMIA:001495-9615: Coat colour, grizzle in Canis lupus familiaris, https://omia.org/OMIA001495/9615/, access: 11.08.2023.

Peist I., Pałka S., Migdał Ł. 2021. Czynniki genetyczne i pozagenetyczne wpływające na umaszczenie psów domowych (*Canis lupus familiaris*). Rocz. Nauk. Zoot. 48(2), 155–178.

Saif R., Iftekhar A., Asif F., Alghanem M.S. 2020. Dog coat colour genetics: A review. Adv. Life Sci. 7(4), 215–224.

Schmutz S.M., Berryere T.G. 2007. Genes affecting coat colour and pattern in domestic dogs: A review. Anim. Genet. 38(6), 539–549. DOI: 10.1111/j.1365-2052.2007.01664.x.

Schmutz S.M., Berryere T.G., Ellinwood N.M., Kerns J.A., Barsh G.S. 2003. *MC1R* studies in dogs with melanistic mask or brindle patterns. J. Hered. 94(1), 69–73. DOI: 10.1093/jhered/esg014.

Schmutz S.M., Berryere T.G., Goldfinch A.D. 2002. *TYRP1* and *MC1R* genotypes and their effects on coat color in dogs. Mamm. Genome. 13(7), 380–387. DOI: 10.1007/s00335-001-2147-2.

Schmutz S.M., Melekhovets Y. 2012. Coat color DNA testing in dogs: Theory meets practice. Mol. Cell. Probes. 26(6), 238–242. DOI: 10.1016/j.mcp.2012.03.009.

Wolf Horrell E.M., Boulanger M.C., D'Orazio J.A. 2016. Melanocortin 1 receptor: Structure, function, and regulation. Front Genet. 7, 95. DOI: 10.3389/fgene.2016.00095.

CHARAKTERYSTYKA GENU *MC1R* JAKO LOCUS E WPŁYWAJĄCEGO NA UMASZCZENIE PSÓW

Streszczenie. Umaszczenie psów jest wynikiem działania różnych genów, które kontrolują pigmentację głównie sierści. Istnieje wiele genów, które wpływają na kolor, wzór i rozkład barw sierści u psów, a jednym z nich jest gen kodujący receptor melanokortyny 1 (*MC1R*), znany także jako locus E. Receptor melanokortyny 1 (MC1R) jest białkiem, które odgrywa kluczową rolę w regulacji pigmentacji skóry, sierści i oczu u zwierząt. Receptor ten jest odpowiedzialny za przetwarzanie hormonów peptydowych, które kontrolują produkcję melaniny, czyli barwnika skóry i sierści. Mutacje w obrębie tego genu mogą wpływać na umaszczenie zwierząt, w tym również psów. Główne allele zidentyfikowane i opisane dla locus E to allele: *E* (typ dziki), E^M (melanistyczna maska) oraz *e* (recesywna czerwień); są one szeroko rozpowszechnione u różnych ras psów, co wskazuje, że istniały już na wczesnym etapie formowania się poszczególnych grup ras. Pozostałe zidentyfikowane allele są mniej powszechne i są to: E^G (grizzle/domino), e^2 (tylko w rasie australijski pies pasterski i u mieszańców) i e^3 (tylko w rasie husky syberyjski i u mieszańców), allel e^A (antyczny czerwony) oraz allel E^H (tylko w rasach cocker spaniel angielski i cocker spaniel amerykański). Należy oczywiście pamiętać, że umaszczenie psów jest złożonym zjawiskiem genetycznym, które może być kontrolowane przez wiele różnych genów działających wspólnie, a różnorodność umaszczeń dla poszczególnych ras psów wynika z kombinacji tych genów.

Słowa kluczowe: umaszczenie psów, *MC1R*, locus E, melanistyczna maska, recesywna czerwień.