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The Genetics of Cream Coat Color in Dogs

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Abstract

Cream dogs of several breeds require a genotype of *e/e* at *MC1R* based on 27 individuals in this study. All Akita, Caucasian Mountain Dogs, German Shepherd Dogs, Miniature Schnauzer, and Puli with this genotype are cream, suggesting they are fixed at a second locus which causes the phaeomelanin pigmentation caused by this genotype to be diluted or pale. Conversely, although all Chinese Shar-Pei and Poodles that were cream had an *e/e* genotype at *MC1R*, not all dogs with this genotype are cream. Today, many Golden Retrievers and Labrador Retrievers with an *e/e* genotype are cream instead of the traditional yellow to golden color seen in the past. The second gene in these breeds must have multiple alleles, only one of which causes phaeomelanin pigment to be diluted or pale. *Tyrosinase* (*TYR*) and *solute carrier family 45, member 2* (*SLC45A2*) have been shown to cause cream coat color in other species and were therefore investigated in dogs as candidate genes for this second locus. Although polymorphisms were detected in cDNA sequence from *TYR* and *SLC45A2*, no polymorphism was consistently associated with cream dogs or cosegregated with cream coat color in any of the families used in this study. A microsatellite was detected in a published BAC sequence (GenBank no. AAEX01017083) in intron 2 and was used to map *SLC45A2* to CFA4.

Cream or white is a relatively common coat color in many breeds but the underlying genetic mechanism for this color has not been elucidated. All Samoyed, American Eskimo Dogs, and West Highland White Terriers are white. In recent years, many Labrador Retreivers, of the type that were known as "yellow labs," are often more cream than yellow (Figure 2 color plate, bottom right corner). Likewise some Golden Retreivers are more cream than golden.

Cream to white also occurs in many other breeds as one of several coat colors. In some breeds, such as German Shepherd Dogs, this coat color was excluded from the show ring but a small group of aficiandos has embraced these dogs and formed a separate club.

Little (1957) suggested that an allele c^{ch} of the C locus would pale phaeomelanin to cream and that another possible allele c^{e} might dilute phaeomelanin to white in addition to the c^{a} allele which causes albinism in homozygotes. In several species of animals the C locus is considered to be tyrosinase because albinism is caused by mutations at this locus in mice (Yokoyama et al. 1990), humans (Oetting and King 1994; Fukai et al. 1995), rabbits (Aigner et al. 2000), cattle (Schmutz et al.2004), and cats (Schmidt-Kuntzel et al. 2005; Imes et al. 2006).

Solute carrier family 45, member 2 (*SLC45A2*), previously called *MATP* or *AIM-1*, has been shown to be the gene causing the chestnut/palamino/bucksin and bay/cremello/perlino coat colors in the horse (Locke et al. 2001, Mariat et al. 2003), underwhite in the mouse (Sweet et al. 1998), a gold color in Medaka fish (Fukamachi et al. 2001), and oculocutaneous albinism type 4 in humans (Newton et al. 2001). There appears to be a codominant dilution of phaeomelanin pigmentation resulting in red, apricot, or cream in some dogs (Sponenberg and Rothschild 2001), such as poodles, with *MC1R e/e* genotypes (Newton et al. 2000) similar to the chestnut, palamino and cremello colors in horses (Locke et al. 2001; Mariat et al. 2003), and the Uw^{dbr} allele in mice (Sweet et al. 1998).

We therefore studied *TYR* and *SLC45A2* in the dog as candidate genes for cream coat color in some breeds of dogs in which dogs of *e/e* genotype at *MC1R* varied in shade of phaeomelanin pigmentation, such as Labrador Retrievers, Golden Retrievers, and Chinese Shar-Pei. We also genotyped several cream to white coat color dogs, and their family members when available, for *MC1R* alleles to determine if only phaeomelanin was expressed (*e/e* genotype) (Newton et al. 2000) or if both eumelanin and phaeomelanin were expressed (*E* or E^{M} allele present) (Schmutz et al. 2003).

Materials and Methods

Dogs and Families

Cheek brush DNA samples (Epicentre, Madison, WI) from 11 dog families that segregated for cream coat color were

Table 1. Primers and the fragment of the dog *SLC45A2* gene amplified

Fragment detected	Direction	Sequence
Exon 1 SNP	Forward	ATGACCACTTTGATCC TGTGGAG
Exon 1 SNP	Reverse	CTAGGAGAGACAATC CGTTC
Intron 2 microsatellite	Forward	CCACCCTTGCTTCTGT GCTC
Intron 2 microsatellite	Reverse	GTTAAATGAGGTCAT GAGGG
cDNA (5′ UTR–Exon 2)	Forward	GACCATCTCTGTTGG CTGCTCAG
cDNA (5′ UTR–Exon 2)	Reverse	GGTAGTGGAGGCCCC TCTCC
cDNA (exons 2–5)	Forward	CATTAAAGCCTACTTA TTTG
cDNA (exons 2–5)	Reverse	GATGCACAAGCCCCA ACAT
cDNA (exon 5–3′ UTR)	Forward	GTGCACACAACTCCA CAGAG
cDNA (Exon 5–3′ UTR)	Reverse	GTAGGGACAGTGTCT CTTTATTG

obtained from dog breeders and owners and used for genotyping. These included Akita (1), Caucasian Mountain Dog (1), Chinese Shar-Pei (2), Golden Retriever (1), Labrador Retriever (1), Poodle (3), and Puli (2) families. Photographs were also supplied to document coat color. Additional dog families were used for linkage mapping. In addition, 27 cream- to white-colored individual dogs of several breeds were also available through the course of our collection of various coat color studies.

We used cDNA prepared from skin biopsies, tail dockings, and surgeries collected from previous coat color studies (Schmutz et al. 2002, 2003) and placed in liquid nitrogen or RNAlater (Ambion Inc., Austin, TX) within 20 min of collection to obtain RNA sequence of *TYR* and *SLC45a2*.

Polymorphism Detection

MC1R genotypes were obtained from all dogs in the study using the PCR–RFLP tests to detect the E^M, E, and e alleles reported previously (Schmutz et al. 2003).

A *TYR* polymorphism was detected as previously reported (Schmidtz and Schmutz 2002). Microsatellite alleles of *FH2312* were detected after polyacrylamide electrophoresis (Mellersh et al. 2000).

In order to study the cosegregation of these dilute phenotypes in these dog families, we tried to identify polymorphisms to use as markers in cosegregation analysis. *SLC45A2* dog sequence was obtained using primers (Table 1) that were initially designed from a dog BAC sequence (GenBank no. AAEX01017083) identified by using a blast search of human *SLC45A2*sequence (GenBank no. NM_01618). The PCR for exons 1 and 2 of *SLC45A2* consisted of 15 μl which contained 50–100 ng of DNA template, 1.5 μl of 10× PCR buffer (Invitrogen Co., Carlsbad, CA), 1–3 mM $MgCl_2$ (Invitrogen), 0.2 mM dNTP (Invitrogen), 0.5 U *Taq* DNA polymerase (Invitrogen), 0.66 pmol of each primer, and 9.6 μl of ddH_20. The reaction began with an initial 4-min denaturation step at 94 °C; followed by 35 cycles of 50 s at 94 °C, 50 s at 57 °C, and 50 s at 72 °C; and finished with a final 4-min extension step at 72 °C.

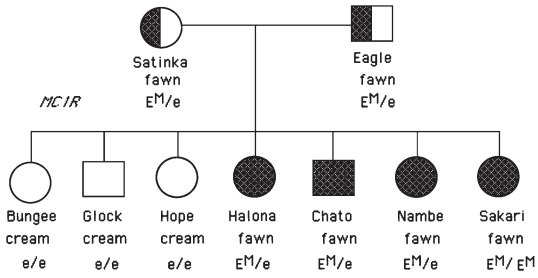

Figure 1. Pedigree of an Akita Family that segregated for cream coat color (open symbols) illustrating that all the cream pups inherited an *e/e MC1R* genotype.

Two polymorphisms were identified in *SLC45A2*. A single-nucleotide polymorphism (SNP) (150G>A) (GenBank no. DQ118774) was identified in exon 1 of *SLC45A2* that did not alter the amino acid. A natural *Aci*I cut site occurs at this SNP. The A allele cuts into 226- and 248-bp fragments. The G allele cuts into 93, 115, and 226-bp fragments that can be resolved on 2% agarose gel.

A microsatellite in intron 2 of *SLC45A2* was identified in the BAC sequence (GenBank no. AAEX01017083). In order to detect this microsatellite, the forward primer was end-labeled in a 10-μl reaction consisting of 2.0 μl of forward primer, 5.0 μl dH_2O, 1.0 μl of 10× polynucleotide kinase (PNK) buffer, 1 μl ATP^{32}, and 1 μl of PNK (New England BioLabs Inc., Beverly, MA). This reaction was then incubated at 37 °C for 30 min. The PCR protocol was the same as for the exon 1 SNP with the exception of the annealing temperature being 55 °C. The alleles were differentiated on a 6% polyacrylamide gel and visualized on autoradiograph film. Seven alleles were identified among the 35 dogs genotyped for the microsatellite in intron 2. The alleles ranged in size from 220 to 296 bp.

Results and Discussion

In the Akita (Figure 1), Caucasian Mountain Dog, both Chinese Shar-Pei, all three Poodle, and both Puli families, the cream-colored pups had an *e/e* genotype at *MC1R*, as shown in Figure 2 (Supplemental Table 1). In addition, we also found that 17 individual dogs that were cream to white from breeds, in which cream is one of several coat colors, all had an *e/e* genotype at *MC1R*. These included 3 Cardigan Welsh Corgi, 3 Chinese Shar-Pei, 5 German Shepherd Dogs, 2 Great Pyrenees, and 3 Miniature Schnauzer. Furthermore white dogs of other breeds where this is the only color, such as American Eskimo Dog (6), Samoyed (3), and West Highland White Terrier (1), also genotyped as *e/e* at *MC1R*. In

Figure 2. Photographs of representative cream dogs that had a genotype of *e/e* at *MC1R*. Top to bottom: Akita pup, Miniature Schnauzer, German Shepherd Dog, Puli, and Caucasian Mountain Dog. The Labrador Retriever littermates in the final photo illustrate the difference between cream or pale yellow and dark yellow seen in some breeds.

both the Golden Retriever and yellow Labrador Retriever families, although all individuals had an *e/e* genotype at *MC1R*, some pups were golden/yellow and others were cream (Figure 2, bottom right).

In all the families studied, cream coat color fit an autosomal recessive inheritance pattern among the dogs with *e/e MC1R* genotype. This *e/e* genotype alone does not cause cream in all dogs of all breeds, and so we assumed that some other gene, which varied in some breeds, must interact to cause cream instead of yellow or red.

TYR

We obtained the complete coding sequence of *TYR* (GenBank no. AY336053) from a cream Poodle, an albino Lhasa Apso, and 2 albino Doberman Pinschers as well as a blue Doberman Pinscher and black-and-white Large Munsterlanders as controls. Although several SNPs were detected (780A>T, 882A>G, 1223G>A which caused an arginine to be replaced by a glutamine, 1312C>T which caused a lysine to be replaced by a phenylalanine), none were consistent

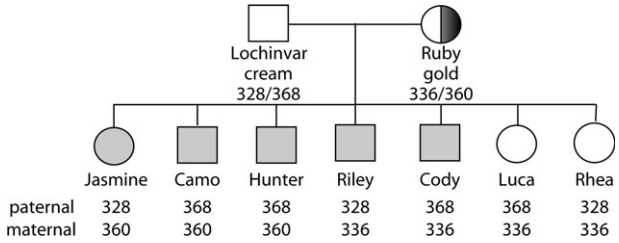

Figure 3. Pedigree of a Golden Retriever Family that segregated for cream coat color (open symbols) illustrating the paternally and maternally inherited alleles of*FH2312* alleles, a microsatellite near *TYR*. All Golden Retrievers have an *e/e MC1R* genotype.

with either cream or albinsim when compared with the sequence obtained from additional dogs of the same phenotype.

We had previously identified a polymorphism in *TYR*, 175G>A (GenBank no. AF473807), which changed a valine to an isoleucine (Schmidtz and Schmutz 2002) that was used to map this gene to CFA21 with no recombinants to *FH2312*. Some cream dogs were heterozygous, suggesting that this variant was not responsible for cream coat color which is inherited as a recessive. Neither the informative Golden Retriever family of 7 pups (Figure 3) nor the Labrador Retriever family of 4 pups showed cosegregation with cream coat color and *TYR* using the *FH2312* microsatellite (LOD = −5.02). This also excludes mutations in the promoter and introns as being associated with variation in phaeomelanin pigmentation.

SLC45A2

No mutations affecting amino acids were found in the coding sequence of *SLC45A2* (GenBank no. DQ302162) in a cream poodle compared with a Brittany Spaniel of darker red pigmentation and *e/e* genotype at *MC1R* or a black-and-white Large Munsterlander of *E/E* genotype. We did identify an SNP, 150G>A, in exon 1 (GenBank no. DQ118774S1), but it was not informative in the families segregrating for shades of phaeomelanin. We also identified a microsatellite in intron 2. This did not cosegregate with pale and dark yellow in the Labrador Retriever family (Figure 4) (LOD = −2.8).

This microsatellite was polymorphic in several additional dog families. Linkage mapping was accomplished using one French Brittany, one Newfoundland, one Cardigan Welsh Corgi, and one Tervuren family. *SLC45A2* was mapped to canine chromosome 4.9 cm from *FH2097* (LOD = 5.158).

Because cream dogs always have an *e/e* genotype at *MC1R*, DNA testing for an *e* allele should be predictive that the dog is heterozygous for cream coat color in breeds such as Akita, Caucasian Mountain Dogs, German Shepherd Dogs, Miniature Schnauzers, and Puli. Neither *TYR* nor *SLC45A2* appeared to co-segregate with cream coat color in dogs from breeds with an *e/e* genotype where the variation

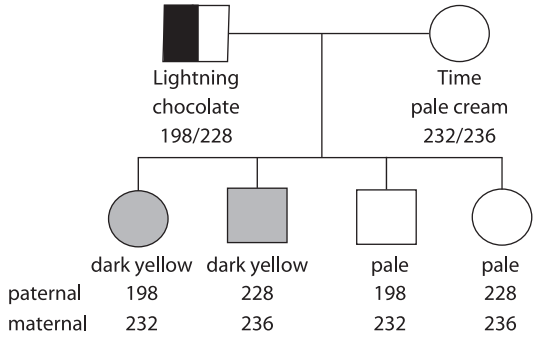

Figure 4. Pedigree of a Labrador Retriever Family that segregated for pale (open symbols) and dark yellow among the yellow pups which all have an *e/e MC1R* genotype. The paternally and maternally inherited alleles of the microsatelllite detected in intron 2 of *SLC45A2*.

in phaeomelanin can be cream through yellow and even red, such as Chinese Shar-Pei, Golden Retrievers, Labrador Retrievers, and Poodles.

Supplementary Material

Supplementary Table 1 can be found at http://www.jhered.oxfordjournals.org/.

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