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Tyrosinase and *Tyrosinase Related Protein 1* Alleles Specify Domestic Cat Coat Color Phenotypes of the *albino* and *brown* Loci

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Abstract

The genes encoding enzymes of the *tyrosinase* family are strong candidates for coat color variation in mammals. To investigate their influence in domestic cat coat color, we determined the complete nucleotide coding sequence of the domestic cat genes *tyrosinase* (*TYR*)—a plausible candidate gene for the *albino* (*C*) locus, and *tyrosinase related protein 1* (*TYRP1*)—a candidate gene for the *brown* (*B*) locus. Sequence variants between individuals exhibiting variation in pigmentation were submitted to association studies. In *TYR*, two nonsynonymous substitutions encoding TYR-G301R and TYR-G227W were associated with the siamese and burmese phenotypes of the *albino* locus, respectively. *TYRP1* was mapped on chromosome D4 within 5 cM of a highly polymorphic microsatellite, previously found to be fixed in a cat breed selected for the *chocolate* (b) allele of the *B* locus, which reinforced *TYRP1* as a candidate gene for the *B* locus in the domestic cat. Two DNA polymorphisms, one leading to a TYRP1-A3G substitution in the signal peptide and another to an in-frame insertion TYRP1-421ins17/18 caused by a donor splice site mutation in intron 6, were associated with the *chocolate* (b) allele. A premature UAG stop codon at position 100 of TYRP1 was associated with a second allele of the *B* locus, *cinnamon* (b^l). The results provide very strong evidence that the specific nucleotide variants of feline *TYR* (chromosome D1) are causative of the *siamese* (c^s) and *burmese* (c^b) alleles of the *albino* locus, as well as nucleotide variants of *TYRP1* (chromosome D4) as specifying the *chocolate* (b) and *cinnamon* (b^l) alleles of the *B* locus.

Introduction

Coat color is a clearly identifiable and selectable phenotype that is seldom associated with lethality, making it a convenient tool for the study of genetic variation in underlying genes. The genes responsible for coat color variation were therefore candidates for the first mapping exercises and have served as landmarks for the earliest genetic linkage maps in the rat and mouse (reviewed in Baker et al. 1979; Silvers 1979). Today over 50 loci associated with mammalian hair pigmentation have been cloned in the mouse (reviewed in Bennett and Lamoreux 2003), and numerous loci have been characterized (reviewed in Silvers 1979). The coat color loci influence the development, maturation, and migration of melanocytes as well as the synthesis of melanin and the formation, transport, and transfer of melanosomes (Barsh 1996; Bennett and Lamoreux 2003; Hearing and Tsukamoto 1991; Jackson 1994). A change in number of mature melanocytes located in the hair follicles or a change in the composition of their pigment-loaded melanosomes will affect the color of the growing hair shaft. Genes involved in the determination of coat color often have pleiotropic effects, which impact other important biochemical pathways. Several human homologs of loci involved in mouse coat color variants are associated with heritable pathologies; examples include Griscelli disease I and II, associated with the *dilute* and *ashen* loci, respectively (Bahadoran et al. 2003), and Chediak-Higashi Syndrome, associated with the *beige* locus (Holcombe et al. 1987).

The melanogenic pathway (reviewed in del Marmol and Beermann 1996; Hearing and Tsukamoto 1991; Searle 1968)

mediates the synthesis of coat color pigments by catalyzing the metabolism of tyrosine to eumelanic (dark; brown to black) and/or pheomelanic (light; reddish to yellow) pigments. The key enzymes of the melanogenic pathway, encoded by the *tyrosinase* gene family, are tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and tyrosinase-related protein 2 (TYRP2) or dopachrome tautomerase (DCT). TYR is the rate-limiting enzyme, involved in multiple steps of the pathway, including catalysis in the initial steps in which tyrosine is transformed into an intermediate metabolite that is common to the pheomelanogenic and eumelanogenic pathways. TYRP1 and TYRP2 are active in the eumelanogenic pathway. In the mouse, *TYR, TYRP1*, and *TYRP2* correspond to the *albino* (*C*), *brown* (*B*), and *slaty* (*Slt*) loci, respectively.

Thermosensitive alleles of the *albino* locus were attributed to nonsynonymous substitutions in the *TYR* exon 4 region encoding a copper-binding domain, H420R and R422G, in the Himalayan mouse (Kwon et al. 1989) and in the human temperature-sensitive type I oculocutaneous albinism (Giebel et al. 1991), respectively. A *TYR* variant E294G was also found in the Himalayan rabbit (Aigner et al. 2000). At the *B* locus, sequence variants in *TYRP1* were attributed to recessive brown coat color in mice (C86Y; Zdarsky et al. 1990), dogs (C41S, G331X, and/or P345del; Schmutz et al. 2002), cows (H434T; Berryere et al. 2003), and to rufous oculocutaneous albinism in humans (S166X, or A368del; Manga et al. 1997).

The genes of the *tyrosinase* family have been implicated in cases of human genetic pigmentation disorders, for example, oculocutaneous albinism (Spritz and Hearing 1994). The enzymes of the *tyrosinase* family have also gained importance as markers for melanocytes-derived tumors (Sarantou et al. 1997). *TYR* expression has been used to detect melanoma cells in peripheral blood (Smith et al. 1991). *TYRP1* expression is negatively associated with the metastatic potential of non-melanoma tumor cells and has been suggested as a candidate for prognostic marker and therapeutic tool (Agarwal et al. 2003).

In the domestic cat, at least eight loci involved in the determination of coat color are well-defined monogenic traits, and several others are likely involved as modifiers of these known variants (Robinson 1991). The combination of genotypes at the different loci leads to extensive variation in phenotypes. Artificial selection by cat fanciers and owners influences coat color frequencies in domestic cat populations, and natural selection on coat color following an urban–rural cline has been suggested (Dreux 1978). Coat color is clearly defined in cat breed standards throughout the world, and several breeds are fixed for specific color variants (e.g., chocolate in the Havana Brown, black dilute [i.e., gray] in the Chartreux; The Cat Fanciers' Association [CFA], www.cfa.org; Fédération Internationale Féline [FIFE], www.fifeweb.org, and The International Cat Association [TICA], www.tica.org).

The first coat color gene in the domestic cat was characterized only recently. A 2-bp deletion in the *agouti* (*ASIP*) gene was identified as the likely causative mutation for melanism in the domestic cat, whereas deletions in the receptor gene *MC1R* are associated with melanistic jaguars and darkly pigmented jaguarundis (Eizirik et al. 2003). One of the first mapping associations in the domestic cat (O'Brien et al. 1986) demonstrated that a phenotypic variant of the *C* locus, siamese, was closely linked to *Beta Hemoglobin* (*HBB*), which mapped to cat chromosome D1 using a cat × rodent somatic cell hybrid panel. Recently *TYR* was mapped to ~ 13 cM from *HBB* (Menotti-Raymond et al. 2003b) reaffirming *TYR* as a candidate gene for the *albino* locus in the domestic cat.

Siamese (c^s) is an allele of the *C* locus in the domestic cat, recessive to the wild-type allele (Tjebbes 1924; Weir 1889; Wright 1917). (For clarity's sake, siamese and burmese phenotypes and alleles will be referred to in lowercase to distinguish them from the cat breeds in uppercase.) The corresponding phenotype is caused by temperature sensitivity of pigment production (Iljin and Iljin 1930) and was experimentally attributed to thermosensitive activity of TYR in mouse and human (Kidson and Fabian 1981; King et al. 1991). Dark coloration of the fur is observed at the cooler extremities of the body, and the warmer parts become progressively lighter due to the impairment of the enzymatic activity by temperature. Homologous phenotypes are exhibited in the mouse, rat, guinea pig, rabbit, chinchilla, mink, Mongolian gerbil, and Syrian hamster (Castle 1905; Green 1961; Robinson 1959, 1973; Searle 1968; Wright 1915), as well as in the human temperature-sensitive oculocutaneous albinism (King et al. 1991). *Burmese* (c^b), a second thermosensitive domestic cat color variant exhibiting a moderate gradient ranging from black to dark brown, was attributed to an additional allele of the *albino* locus, recessive to the wild type (uniform coloration) and semi-dominant with the *siamese* (c^s) allele (Thompson et al. 1934; Weir 1889). Heterozygote individuals bearing a *siamese* (c^s) and a *burmese* (c^b) allele display an intermediate phenotype.

An early scientific paper describing a chocolate coat color variant in the domestic cat was published by Bamber and Herdman in 1931, and its autosomal recessive mode of inheritance was demonstrated through controlled breeding (von Ullmann and Hargreaves 1958). A second allele of the *B* locus corresponding to a lighter coat color was named *cinnamon* (b^l; red in the Abyssinian) and is inherited in an autosomal recessive mode (Dyte 1962).

In this article, we map *TYRP1* and identify and characterize domestic cat sequence variants associated with the *siamese* (c^s) and *burmese* (c^b) alleles of the *C* locus, and with the *chocolate* (b) and *cinnamon* (b^l) alleles of the *B* locus. Two independent nonsynonymous substitutions in *TYR* were found to be associated with the *siamese* (c^s) and with the *burmese* (c^b) alleles, respectively. A nonsynonymous substitution and a missplicing event in *TYRP1* were found to be associated with the *chocolate* (b) allele, and a nonsense mutation in *TYRP1* with the *cinnamon* (b^l) allele.

Materials and Methods

Animals

DNA was extracted from blood or buccal swab samples with the QIAamp DNA Blood MiniKit (Qiagen) following the manufacturer's protocol. RNA was extracted from skin

Table 1. Primer pairs for amplification of coding regions of *TYR* exons from genomic DNA. Cat-specific primers are in boldface

Product	Forward primer (5′–3′)	Reverse primer (5′–3′)
Exon 1	GGGGTTCCTGGAAAGAGAAG	AGGAACCTGGCAGAGGATATTA
Exon 1 cat-specific; *burmese* allele: 679G>T	**GATCCGTGAAGACGAGGGTA**	**TCTCTGATATTGTATGTCCAGGATG**
Exon 2; *siamese* allele: 901G>A	AGGTGGCCACAACTCCTTTA	CAGGAGTTCAGAAAAACAGAAGAA
Exon 3	GCAAACCAAATGAGATAATCACTG	TGGAAAAGTGTCAACCAACC
Exon 4	GACCCAGTTGTCACTCAACC	AAAGGCTTTGGGGATAACATT
Exon 4 cat-specific forward primer	**CAGGGGCTGACAGAATGAAT**	
Exon 5	TAGCAGAGCTGGCATTCAAA	AAAATGAGGTCAACCTTGTTGG

biopsies using the RNAqueous-4PCR kit (Ambion). The skin biopsies were stored in RNA-later buffer (Ambion) prior to extraction. Individual phenotypes were determined from pedigree information and/or direct visual examination of cats (or photographs thereof) sampled at CFA- or TICA-sponsored cat shows.

PCR, RT-PCR, and DNA Sequencing

Characterization of the two candidate genes was performed using a genomic approach for *TYR* and a cDNA approach for *TYRP1*.

Standard PCR reactions were performed with *Taq* Gold DNA polymerase (Perkin Elmer) in a 25-μl reaction with ~ 50 ng of DNA and 1.5 mM $MgCl_2$. Amplification was performed on a GeneAmp PCR system 9700 thermocycler (Applied Biosystems) under the following conditions; 10 min at 94°C, followed by 25–40 cycles of 15 s at 94°C, 30 s at the given annealing temperature, 30–90 s elongation at 72°C, and finished with a 10-min extension at 72°C. In touchdown protocols annealing temperature was decreased by 1°C at each cycle until reaching the final annealing temperature, which was then kept for the remaining cycles. The specific PCR conditions for each product accompany their description.

The reverse transcriptase (RT)-PCR reactions were performed in a 25-μl reaction (Invitrogen, SuperScript One-Step RT-PCR) with final magnesium concentrations of 2.2–3 mM. According to the manufacturer's requirements a 30-min cDNA synthesis step at 50°C was added prior to our usual touchdown PCR protocol (54 or 56 cycles; annealing temperature decreasing from 58°C to 44°C or to 42°C during the first 14 or 16 cycles; elongation time up to 90 s). If needed, a nested or semi-nested PCR on 2–10 μl of RT-PCR product was performed with appropriate primers (35–45 cycles; annealing temperature decreasing from 60°C to 50°C or from 58°C to 48°C during the first 10 cycles; elongation time of 30 or 45 s).

Primers and unincorporated dNTPs were removed from the PCR and RT-PCR reactions with Microcon YM-50 (Microcon) or ExoSAP-IT (Amersham Biosciences) prior to sequencing. Sequencing reactions were performed with Big Dye Terminator chemistry (ABI) in a 10-μl reaction with 1–6 μl purified PCR product, 2 μl Big Dye, and 1 μl dilution buffer. The program respected the Perkin Elmer temperature and time requirements and was run on a PE thermocycler (GeneAmp PCR system 9700). Sequencing products were purified with Sephadex technology (Amersham Pharmacia Biotech) and analyzed with an ABI 3730 automated sequencer. The DNA sequences were verified and aligned with Sequencher (Gene Codes), which was also used for identification of sequence variants.

Characterization of the Domestic Cat *TYR* Gene

Primers were designed with Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi; Rozen and Skaletsky 2000) to amplify and sequence the coding region of each of the five *TYR* exons, using conserved segments identified by alignment of homologous human (GenBank AP000720) and dog sequences (exon 1: AACN010607163 and gnl/ti/280428052, exon 2: AACN010219347, exon 3: AACN010256253, exon 4: AACN010747145 and gnl/ti/311207676, exon 5: AACN010234164; Table 1). Dog sequences were identified from wgs (whole genome sequence) reads by NCBI nucleotide-nucleotide BLAST (Altschul et al. 1997). Following initial sequencing, cat-specific primers were designed for more efficient and detailed sequencing of some segments (Table 1, in boldface).

Genomic DNA from four domestic cats differing in their albino phenotype (two exhibited wild-type coloration [American Shorthair: FCA938, Egyptian Mau breed: FCA3004], one siamese [Siamese breed: FCA559], and one burmese coloration [Burmese breed: FCA2221]) was amplified and sequenced for the complete coding region of each of the five *TYR* exons. The PCR reactions for the *TYR* exons were performed following a touchdown protocol (35–40 cycles; annealing temperature decreasing from 58°C to 48°C during the first 10 cycles; elongation time 30 s for exons 2 to 5, 90 s for exon 1).

Radiation Hybrid Mapping of *TYRP1*

Conserved primers (forward: AGACTCCCGACCCCA-CAG, reverse: ACCTGTGCCATGTGAGAAAA) spanning intron 2 of *TYRP1* were designed based on an alignment of available human genomic (AF001295) and mouse cDNA (X03687) sequences. PCR and sequencing with these primers, using standard conditions, was used for partial characterization of *TYRP1* intron 2 in the domestic cat. The obtained sequences were used to design cat-specific primers

Table 2. Primers for amplification of *TYRP1* coding region from cDNA. Cat-specific primers are in boldface

Position	Primer	Sequence
5′UTR	Forward	TCTCTACACAAAGGGCTGCA
5′UTR	**Forward**	**TCTGTTCTTCACTCTTGCTTCG**
+148	Reverse	CAGACAGTGGGGACAGGTCT
+458	**Forward**	**GGACAATGCACCCTCAGTTT**
+477	**Reverse**	**AAACTGAGGGTGCATTGTCC**
+736	Forward	CTCCCTTACTGGAATTTTGC
+773	**Forward**	**GTGACATTTGCACCGATGAC**
+823	Reverse	**TGGGGTCAAAGTTGCTTCTC**
+849	**Forward**	**TTCTCAATGGCGAGTGGTC**
+907	Reverse	AAAGGGTTCCCAGGGTATC
+1366	Forward	GCTCCAGACAATCTGGGATA
+1469	Reverse	ACCAGGGATAAAGCAGCAAC
3′UTR	**Reverse**	**TTCGACCAGGTGTTCTTGTG**

(forward: AGTTCTTCATGAGACTCTATGCATTT, reverse: GAGCAGTTGCTCCATAAATGTT) for radiation hybrid mapping of this gene. These new primers efficiently amplified a sequence tagged site (STS) of 209 bp in the domestic cat, with no product generated from the hamster background cell lines. The STS was amplified in the cat × rodent radiation hybrid panel (Murphy et al. 2000) following a touchdown protocol (10 μl reaction; 40 cycles; annealing temperature decreasing from 58°C to 48°C during the first 10 cycles; elongation time 45 s). The results were analyzed using RHMAP (Boehnke et al. 1991), and *TYRP1* was mapped relative to 572 other type I markers previously mapped (Menotti-Raymond et al. 2003b; Murphy et al. 1999, 2000).

Characterization of the Domestic Cat *TYRP1*

Cross-species PCR primers were designed to anneal within conserved areas of the noncoding 5′ and 3′ ends of the mRNA and within the coding region of *TYRP1* (Table 2). The conserved regions were determined based on an alignment of human, dog, cow, and goat *TYRP1* sequences (BC052608, AY052751, NM_174480, and AY291437, respectively). Subsequently, we designed cat-specific primers (Table 2, in boldface). Several primer combinations were used for RT-PCR. Nested or semi-nested PCR was performed on products with insufficient signal.

RT-PCR was performed on RNA obtained from two wild-type individuals (i.e., animals capable of synthesizing black pigments; Egyptian Mau breed: FCA232 and Domestic Shorthair breed: FCA508), one domestic cat of chocolate phenotype (Munchkin breed: FCA4507), and one of cinnamon phenotype (Abyssinian breed: FCA4510).

The 3′ end of the mRNA was obtained with a 3′ RACE KIT (Gibco BRL) following the manufacturer's requirements, adding a gene-specific primer extension step to favor the abundance of *TYRP1* transcript. Introns 2, 3, 4, 5, 6, and 7 were amplified via PCR (data not shown) and their size estimated with agarose analysis.

Further Characterization of the Domestic Cat *TYRP1* Sequence Variants

The 54 nucleotides corresponding to the sequence inserted at the boundary between exons 6 and 7 were aligned with a dog read corresponding to intron 6 to assess sequence similarity. Intron 6 (estimated via PCR and agarose analysis to be 6 kb long in the cat) was amplified in four individuals (Invitrogen elongase kit, following manufacturer's requirements) and partially sequenced to obtain information on the alternative splice sites. The 5′ end of cat intron 6 was subsequently compared to the 54-nt cDNA insertion using BLAST (Table 3). Primer pairs were designed surrounding the classic and the cryptic splice sites, and the *cinnamon* sequence variant (Table 3).

Determination of Intron–Exon Structure and Domains for *TYR* and *TYRP1* in the Domestic Cat

The genomic sequence of *TYR* was compared to known exons in the human and the presence of splice sites was verified at the predicted intron–exon boundaries. The mRNA sequence of *TYRP1* was compared to a genomic human sequence (AL138753) to determine the likely exon boundaries using BLAST (bl2seq). The position of the domains was determined with ScanProsite (www.expasy.org/tools/scanprosite) for the inferred amino acid sequences of cat TYR and TYRP1. The major domains are drawn on-scale on Figure 1.

Table 3. Genomic primers for amplification of *TYRP1* intron 6, and sequence variants. Cat-specific primers are in boldface

Study	Product	Forward primer (5′–3′)	Reverse primer (5′–3′)
Intron 6	Intron 6 Sequencing[a]	**ATGACCCTGCTATTCGAAGC** **TTTAAGTTGAAAGAGGAGAAGTGG**	**GGTAACTGGAGGCCAGAATG** **TGCAAATGTTCATTGGAAGC**
Genotyping	*chocolate* allele: 8C>G *cinnamon* allele: 298C>T	**TCTGTTCTTCACTCTTGCTTCG**[b] **ACCGTTGAGGCTCTGAGAAA**	CAGACAGTGGGGACAGGTCT[b] **GTCACAAGCAGCTCCTTTCC**
	chocolate allele: 1262+5G>A; donor splice sites	**GCCTTCACAATTTGGCTCAT**	**TTTGGGACATGTGGAAACAG**

[a] Used to obtain the complementary sequence of the 5′ and 3′ ends of intron 6.

[b] Primers used previously for cDNA amplification: Forward 5′UTR, reverse +148.

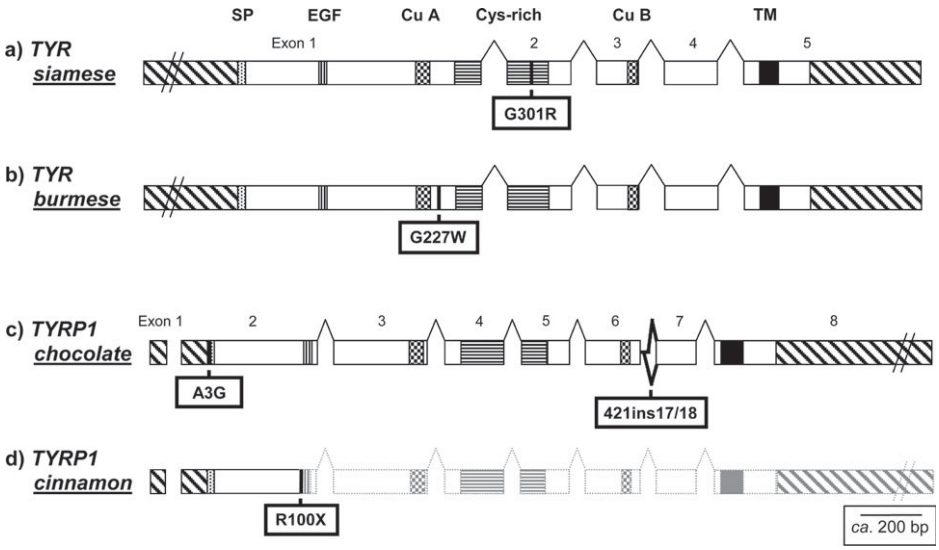

Figure 1. *TYR* alleles: (a) *siamese* and (b) *burmese*, and *TYRP1* alleles: (c) *chocolate* and (d) *cinnamon*. Gene structures for the *TYR* and *TYRP1* genes in the domestic cat with noncoding regions in dashed and the exons (5 and 8, respectively) as boxes. The exons and domains are drawn to scale; the intronic sizes are not available in the cat (in humans: *TYR* intron 1: 12 kb, intron 2: 36 kb, intron 3: 57 kb, intron 4: 10 kb, and *TYRP1* intron 1: .5 kb, estimated intronic sizes of *TYRP1* introns 2–7 in the cat based on PCR and agarose analysis: intron 2: 1 kb, intron 3: 3 kb, intron 4: 5 kb, intron 5: 3 kb, intron 6: 6 kb, intron 7: 1 kb). The domains are annotated: signal peptide (SP; dots), epidermal growth factor (EGF)-like domain (vertical lines), copper-binding domains A and B (CuA, CuB; squares), cysteine rich domain (Cys-rich; horizontal lines), transmembrane domain (TM; filled). Indication of the localization of the domestic cat amino acid sequence variants of (a) the *siamese* allele (TYR-G3301R), (b) the *burmese* allele (TYR-G227W), (c) the *chocolate* allele [TYRP1-A3G; TYRP1-421ins17/18] and (d) the *cinnamon* allele (TYRP1-R100X).

Association Studies between Genotypes and Coloration Phenotypes

Once candidate molecular variants were identified based on initial sequencing characterization of the *TYR* and *TYRP1* loci, we investigated their association with coloration phenotypes using a larger sample of individuals varying in their coat color. Exons 1 and 2 were amplified to determine the genotype of the individuals for the candidate sequence variants of the burmese and the siamese phenotype, respectively. Eighty-eight individuals were genotyped for *TYR*-901G>A in the *siamese* (c^s) allele association study (25 siamese phenotype, 20 burmese phenotype, 11 intermediate phenotype, 32 wild-type phenotype). Sixty-seven individuals were genotyped for *TYR*-679G>T in the *burmese* (c^b) allele association study (16 burmese phenotype, 15 siamese phenotype, 11 intermediate phenotype, 25 wild-type phenotype).

Eighty-six individuals were genotyped for the two candidate mutations of the *chocolate* (b) allele, *TYRP1*-8C>G and *TYRP1*-1262+5G>A (41 chocolate, 6 cinnamon, 39 wild-type phenotype). Seventy-six individuals were genotyped for *TYRP1*-298C>T in the *cinnamon* (b^l) allele association study (17 cinnamon, 27 chocolate, 32 wild-type phenotype). The primer pairs surrounding the candidate mutations for the cinnamon and the chocolate phenotype were used for genotyping. Each phenotypic group contained several breeds, which are listed in Tables 5 and 7. All reactions were performed according to the PCR conditions described previously.

The statistical significance was determined by Fisher's exact test for 2 × 2 contingency tables (GraphPad InStat program). The amino acid stretches containing the point mutations of the *chocolate* (*TYRP1*-A3G, *TYRP1*-1262+5G>A), the *siamese* (*TYR*-G301R), and the *burmese* (*TYR*-G227W) alleles were compared to other available sequences through NCBI protein query versus translated database blast (tblastn).

Results

Tyrosinase

We obtained the complete coding sequence (1587 bp) of the five *TYR* exons (AY959314–AY959318) from two domestic cats of wild-type (C/–) phenotype at the *C* locus (FCA232 and FCA938), one cat of siamese (c^s/c^s) coat pattern (FCA559), and one cat of burmese (c^b/c^b) coat pattern (FCA2221). Traditionally the siamese and burmese alleles are represented by the symbols c^s and c^b, respectively. Alignment of the full-length sequences of the three phenotypic variants led to the identification of five sequence variants in the *TYR* coding region (Table 4). Three variants were transitions and two transversions. Out of the five variants, only two alter the amino acid sequence. A nonsynonymous transition (*TYR*-901G>A) leading to the substitution of a glycine (G) by an

Table 4. *TYR* genotypes in four cats selected for alternative siamese/burmese phenotypes

		Sequence variants[a]					
		Exon 1			Exon 2		Exons 3, 4, 5
	Nucleotide:	216 G>T	450 C>T	679 G>T	901 G>A	930 G>A	No variation in coding sequence
Individual	Amino acid: Phenotype	T72T	V150V	G227W	G301R	R310R	
FCA0938	wild type	G/T	C/C	G/G	G/G	G/G	
FCA3004	wild type	G/T	C/C	G/G	G/G	G/G	
FCA0559	siamese	G/G	T/T	G/G	A/A	G/G	
FCA2221	burmese	G/G	C/C	T/T	G/G	A/A	

[a] Mutation nomenclature system as described in den Dunnen and Antonarakis (2000).

arginine (R) at codon position 301 was found in the siamese colored cat (Table 4, Figure 1a). A nonsynonymous substitution (*TYR*-679G>T) leading to the substitution of a glycine (G) by a tryptophan (W) at codon position 227 was found in the burmese-colored cat (Table 4, Figure 1b).

These mutations were further investigated with association studies. Genotyping of *TYR*-679G>A and *TYR*-901G>A was performed in individuals of different breeds exhibiting burmese phenotypes, siamese phenotypes, or a phenotype intermediate between siamese and burmese (Table 5). Control individuals (wild-type coloration) were used for each association study. Twenty-five siamese-colored cats of six different breeds were homozygous (A/A) at position 901, whereas 63 individuals of nonsiamese phenotype were either homozygous (G/G) or heterozygous (G/A) (Table 5). This result is in accordance with a recessive mode of inheritance of the *siamese* (c^s) allele ($P < .0001$).

Sixteen burmese-colored cats of three different breeds were homozygous (T/T) at position 679, while the 51 cats of non-burmese phenotype were either homozygous (G/G) or heterozygous (G/T) (Table 5). This result is in accordance with a recessive mode of inheritance of the *burmese* (c^b) allele ($P < .0001$). Eleven cats of intermediate phenotype were heterozygous at position 679 (G/T) and at position 901 (G/A), and no cat of other phenotypes had a heterozygous genotype at both positions (Table 5). This result is in accordance with a semi-dominant mode of inheritance of the *siamese* (c^s) and *burmese* (c^b) alleles ($P < .0001$).

TYRP1

A recent survey of microsatellite heterozygosity across 28 purebred cats ($n = 213$) showed high average heterozygosity for 22 microsatellite loci (He = 45–82%) (Menotti-Raymond,

Table 5. Association study of burmese, siamese, and intermediate phenotypes with sequence variants *TYR*-679G>T and *TYR*-901G>A

	679G>T[a]			901G>A[a]		
	G/G	G/T	T/T	G/G	G/A	A/A
burmese-colored[a] cats	0	0	16[c,d]	20[e]	0	0
burmese/siamese-colored[a,b] cats	0	11[f]	0	0	11[f]	0
siamese-colored[a] cats	15[g]	0	0	0	0	25[c,h]
wild type–colored[a] cats	23[i]	2[j]	0	28[k]	4[l]	0

[a] Breeders describe the burmese phenotype as ìsepiaì, the intermediate burmese/siamese phenotype as "mink," the siamese phenotype as "pointed," and the wild type phenotype as "solid."

[b] burmese/siamese: intermediate in color between burmese and siamese phenotype.

[c] *P*-values obtained by Fisher's exact test < .0001.

[d] Burmese ($n = 13$), Singapura ($n = 2$), Tonkinese ($n = 1$) [$N = 16$].

[e] Burmese ($n = 17$), Singapura ($n = 2$), Tonkinese ($n = 1$) [$N = 20$].

[f] Sphynx ($n = 1$), Tonkinese ($n = 10$) [$N = 11$].

[g] Birman ($n = 1$), Ragdoll ($n = 1$), Siamese ($n = 13$) [$N = 15$].

[h] Balinese ($n = 1$), Birman ($n = 3$), Colorpoint Shorthair ($n = 1$), Javanese ($n = 1$), Ragdoll ($n = 2$), Siamese ($n = 17$) [$N = 25$].

[i] Abyssinian ($n = 13$), American Shorthair ($n = 1$), Chartreux ($n = 1$), Cornish Rex ($n = 2$), Egyptian Mau ($n = 2$), Ocicat ($n = 2$), Persian ($n = 1$), Somali ($n = 1$) [$N = 23$].

[j] Bombay ($n = 1$), Exotic Shorthair ($n = 1$) [$N = 2$].

[k] Abyssinian ($n = 13$), American Shorthair ($n = 1$), Bombay ($n = 1$), Chartreux ($n = 1$), Egyptian Mau ($n = 3$), Exotic Shorthair ($n = 1$), Havana Brown ($n = 1$), Korat ($n = 1$), Ocicat ($n = 4$), Persian ($n = 1$), Somali ($n = 1$) [$N = 28$].

[l] Cornish Rex ($n = 2$), Ocicat ($n = 2$) [$N = 4$].

Table 6. *TYRP1* genotypes in four cats selected for alternative chocolate/cinnamon phenotypes

		Sequence variants											
		Exon 2						Exon 3		Exon 4		Exon 6–7 boundary	Exon 7
	Nucleotide:	8 C>G	56 C>A	84 A>C	92 C>T	131 A>C	298 C>T	630 A>G	645 C>T	873 G>A	876 C>T	1262 ins51/54	1305 C>T
Individual	Amino acid: Phenotype	A3G	S19Y	R28S	A31V	D44A	R100X	E210E	H215H	E291E	S292S	421ins17/18	N453N
FCA0232	wild type	C/C	C/C	A/A	C/C	A/A	C/C	A/A	C/C	G/G	C/C	No	C/C
FCA0508	wild type	C/C	C/C	A/A	T/T	A/A	C/C	A/A	C/C	G/G	C/C	No	C/C
FCA4507	chocolate	G/G	C/A	A/C	C/C	A/A	C/C	G/G	T/T	A/A	T/T	Yes (51/54)	T/T
FCA4510	cinnamon	C/C	C/C	A/A	T/T	A/C	T/T	A/A	C/C	G/G	C/C	No	C/C

in press). Microsatellite FCA742 (Menotti-Raymond et al. 2003a) showed a high heterozygosity in 27 cat breeds (He = 75%) but a single allele size in the Havana Brown (He = 0%; $n = 10$) (Menotti-Raymond, in press), a breed that is fixed for the chocolate coat color. The fixed allele suggests that FCA742 might be linked to a genomic locus under selection in the Havana Brown. Using a comparative gene mapping approach (O'Brien et al. 1997), FCA742 was located in a genomic region corresponding to the human syntenic group containing *TYRP1* (Menotti-Raymond et al. 2003a), a candidate gene for the *B* locus.

We designed primers to generate an STS for *TYRP1*, which was amplified in a cat × rodent radiation hybrid panel relative to 572 Type I (coding) loci (Menotti-Raymond et al. 2003b; Murphy et al. 1999, 2000) and mapped on chromosome D4 at ~ 5 cM (based on a cR/cM ratio; Menotti-Raymond et al. 2003a) from FCA742. The tight linkage observed between *TYRP1* and FCA742 supported *TYRP1* as a candidate gene for the *B* locus in the domestic cat.

The complete coding sequences (1611 bp) of the *TYRP1* gene in the domestic cat (AY956310) was determined from cDNA generated from skin biopsies of two individuals that were wild-type (B/–) at the *B* locus (FCA232 and FCA508), one individual of chocolate (b/b) color (FCA4507), and one individual exhibiting cinnamon (b^l/b^l) color (FCA4510). Twelve variable sites were identified in the nucleotide sequence that codes for 537 amino acids of TYRP1 (Table 6): four transversions, seven transitions, and one insertion. The chocolate individual was homozygous for five unique synonymous substitutions and two protein-altering variants (Table 6). A transition (*TYRP1*-8C>G) led to the substitution of an alanine (A) by a glycine (G) at codon position 3 of the protein (Table 6, Figure 1c). The second variant was characterized by an insertion at position 1262; the cDNA sequence of the individual displayed two different length of insertion: 51 and 54 nucleotides (Table 6, Figure 1c). The cinnamon individual was homozygous for a C to T nucleotide substitution at position 298, which generates a stop codon at codon position 100 (Table 6, Figure 1d).

An association test was run for the *TYRP1*-C298T nonsense mutation in 17 cinnamon-colored cats of two different breeds and in 59 noncinnamon individuals (Table 7). All 17 individuals displaying the cinnamon phenotype, and only these, were homozygous (T/T) at position 298, which reflects perfect concordance with a recessive mode of inheritance of the *cinnamon* (b^l) allele of the *B* locus. In spite of the small sample size, the analysis performed with unrelated individuals alone showed very strong support for an association between the nonsense mutation and the cinnamon phenotype ($P < .0001$).

The mRNA insertion (*TYRP1*-1262ins51/54) was hypothesized to be induced by a missplicing event at the donor splice site of intron 6, given the insertion's localization at the exon 6–7 boundary and its similarity to the 5′ sequence of intron 6 in the dog (85.2% sequence identity, data not shown). Intronic sequence was obtained from the 5′ and 3′ ends of intron 6 in the cat. Segment identity of the insertion with the obtained 5′ end of cat intron 6 confirmed the missplicing hypothesis.

Primers were designed to sequence the classic and cryptic donor splice sites of intron 6 in 31 chocolate-colored cats and 43 non-chocolate-colored cats (Table 3). In the classic splice site, in position +5 of intron 6, a G to A substitution was observed in the chocolate-colored individuals. The intronic sequence corresponding to the alternative splice sites lead to the identification of two partially overlapping cryptic splice sites that are present in chocolate as well as in non-chocolate-colored cats. Thus the two different lengths of insertion are not the result of two alternative alleles but of a stochastic event in which one or the other cryptic splice site is used when the classic donor splice site is nonfunctional. Analysis of the chromatogram corresponding to the sequencing reaction from the cDNA of the chocolate-colored individual revealed a similar height in the peaks corresponding to either insertion length, suggesting that both cryptic splice sites are similarly employed. The 5′ ends of the two alternative cryptic splice sites are located at a distance of 3 nucleotides from each other and lead to the addition of either 51 or 54 nucleotides to the mRNA. In both cases the reading frame is preserved, and 17 or 18 amino acids (GETFSYAFLRAQQGDRS[G]) are added to TYRP1, leading to a precursor protein composed of 554 or 555 amino acids, respectively.

An association study was performed for the two identified mutations (*TYRP1*-8C>G and *TYRP1*-1262+5G>A) on 41 chocolate-colored cats of nine different breeds and 45

Table 7. Association study of cinnamon and chocolate phenotypes with sequence variants *TYRP1*-298C>T, *TYRP1*-8C>G, and *TYRP1*-1262+5G/A

	TYRP1-298C>T			*TYRP1*-8C>G			*TYRP1*-1262+5G>A		
	C/C	C/T	T/T	C/C	C/G	G/G	G/G	G/A	A/A
cinnamon-colored cats	0	0	17[a,b]	6[c]	0	0	6[c]	0	0
chocolate-colored cats	26[d]	1[e]	0	0	3[f]	38[a,m]	0	3[f]	38[a,m]
wild type-colored cats	30[h]	2[i]	0	24[j]	15[k]	0	28[l]	11[m]	0

[a] *P*-values obtained by Fisher's exact test < .0001.

[b] Abyssinian ($n = 15$), Ocicat ($n = 2$) [$N = 17$]; 12 of the cinnamon-colored Abyssinians were excluded from the statistical analysis for being related.

[c] Abyssinian ($n = 4$), Ocicat ($n = 2$) [$N = 6$].

[d] Birman ($n = 2$), Burmese ($n = 7$), Cornish Rex ($n = 1$), Havana Brown ($n = 1$), Munchkin ($n = 1$), Ocicat ($n = 3$), Siamese ($n = 4$), Tonkinese ($n = 7$) [$N = 26$].

[e] Ocicat ($n = 1$) [$N = 1$].

[f] Ocicat ($n = 3$) [$N = 3$].

[g] Balinese ($n = 1$), Birman ($n = 4$), Burmese ($n = 6$), Cornish Rex ($n = 1$), Havana Brown ($n = 5$), Munchkin ($n = 1$), Ocicat ($n = 2$), Siamese ($n = 9$), Tonkinese ($n = 9$) [$N = 38$].

[h] Birman ($n = 1$), Bombay ($n = 1$), Burmese ($n = 6$), Chartreux ($n = 1$), Colorpoint Shorthair ($n = 1$), Domestic Shorthair ($n = 2$), Egyptian Mau ($n = 4$), Exotic Shorthair ($n = 1$), Javanese ($n = 1$), Ocicat ($n = 1$), Persian ($n = 1$), Ragdoll ($n = 1$), Siamese ($n = 2$), Singapura ($n = 1$), Sphynx ($n = 1$), Tonkinese ($n = 5$) [$N = 30$].

[i] Abyssinian ($n = 2$) [$N = 2$].

[j] Abyssinian ($n = 1$), Balinese ($n = 1$), Birman ($n = 1$), Bombay ($n = 1$), Burmese ($n = 2$), Chartreux ($n = 1$), Colorpoint Shorthair ($n = 1$), Cornish Rex ($n = 1$), Egyptian Mau ($n = 3$), Exotic Shorthair ($n = 1$), Ocicat ($n = 1$), Persian ($n = 1$), Ragdoll ($n = 2$), Siamese ($n = 2$), Singapura ($n = 2$), Somali ($n = 1$), Sphynx ($n = 1$), Tonkinese ($n = 1$) [$N = 24$].

[k] Burmese ($n = 4$), Javanese ($n = 1$), Korat ($n = 1$), Siamese ($n = 5$), Tonkinese ($n = 4$) [$N = 15$].

[l] Abyssinian ($n = 1$), Balinese ($n = 1$), Birman ($n = 1$), Bombay ($n = 1$), Burmese ($n = 5$), Chartreux ($n = 1$), Colorpoint Shorthair ($n = 1$), Cornish Rex ($n = 1$), Egyptian Mau ($n = 3$), Exotic Shorthair ($n = 1$), Korat ($n = 1$), Ocicat ($n = 1$), Persian ($n = 1$), Ragdoll ($n = 2$), Siamese ($n = 2$), Singapura ($n = 2$), Somali ($n = 1$), Sphynx ($n = 1$), Tonkinese ($n = 1$) [$N = 28$].

[m] Burmese ($n = 1$), Javanese ($n = 1$), Siamese ($n = 5$), Tonkinese ($n = 4$) [$N = 11$].

individuals of non-chocolate phenotype (Table 7). The *TYRP1*-8C>G substitution as well as a G to A substitution observed at position +5 of intron 6 were present in a homozygous state in 38 of the 41 chocolate-colored individuals (Table 7). Three individuals of Ocicat breed reported as chocolate-colored demonstrated a nonmutant genotype (Table 7). One of these individuals was heterozygous for both the *B* locus *chocolate* allele and the *cinnamon* allele, and the other two individuals could not be explained by our genotype (data not shown). The genotypes will be discussed in more detail in the Discussion. None of the 45 non-chocolate individuals were homozygous for either of the *B* locus mutations (Table 7). The association study analysis was consistent with a recessive mode of inheritance for a *chocolate* haplotype comprising both candidate mutations ($P < .0001$).

Discussion

We have characterized the gene structure of and common variation within the *C* and *B* loci in the domestic cat. *TYR* and *TYRP1* are part of the *tyrosinase* family and were found to share similar structure to that in other species. Six domains are represented within the coding region: the signal peptide (SP), an epidermal growth factor (EGF)-like domain, a cysteine (Cys)-rich domain, two copper-binding domains (CuA and CuB), and a hydrophobic transmembrane domain (TM) (Figure 1). Although TYRP1 contains copper binding domains, in vitro copper binding of TYRP1 has been questioned (Furumura et al. 1998).

TYR

Temperature-dependent variation in pigmentation could be caused by thermosensitivity of TYR itself, by temperature-dependent affinity of mutant TYR for an inhibitory molecule as described for the mouse H420R TYR (Kidson and Fabian 1981), or by temperature-dependent trafficking of TYR as described for the human R422G TYR (Berson et al. 2000). Decrease in glycosylation (Halaban et al. 1988) in addition to changes in secondary structure was suggested to affect temperature sensitivity of TYR (Kwon et al. 1989) but is not supported by potential TYR glycosylation sites (Spritz et al. 1990).

The TYR-G301R substitution associated with the siamese coat pattern takes place in the Cys-rich domain of TYR (Figure 1a) and leads to the replacement of a nonpolar glycine (G) by a positively charged arginine (R). Although the domain is characterized as cysteine rich, other amino acids might play critical roles. A change in charge is consistent with a functional change and might modify the secondary structure of the protein. The glycine residue at position 301 of cat TYR is conserved across vertebrates as well as in the ascidian *Halocynthia roretzi* (data not shown); it is therefore likely to be the ancestral amino acid and to have been maintained by selective constraints. The substitution might

affect the enzymatic efficiency, the trafficking, or the interaction with an inhibitory molecule. Any of these changes can occur directly or indirectly, through modification of the secondary structure of TYR.

The TYR-G227W amino acid substitution associated with the burmese coat pattern is located between CuA and the Cys-rich domain (Figure 1b). A small, nonpolar glycine (G) is substituted by a larger, nonpolar tryptophan (W); introduction of the aromatic ring of tryptophan might create spatial constraints and thereby influence the activity of TYR. The glycine residue at position 227 of cat TYR is conserved across vertebrates, including *Xenopus* and fish (i.e., *Ictalurus punctatus*; data not shown); it is therefore likely to be the ancestral amino acid and to be maintained by selective pressure. As with the *siamese* (c^s) allele, the substitution of the *burmese* (c^b) allele might lead to a direct or indirect modification of the activity of TYR.

Given the more extreme color variation in the siamese phenotype as compared with the burmese phenotype, the TYR-G301R substitution of the *siamese* (c^s) allele is expected to influence the activity of TYR more than the TYR-G227W substitution found in the *burmese* (c^b) allele. It could be argued that a change in charge of an amino acid residue is more likely to affect the structure of the protein than a change in size, which is expected to influence primarily the structure of the neighboring amino acids.

TYRP1

The missplicing event caused by a *TYPR1*-1262+5G>A substitution in the splice donor site of intron 6 leads to the use of an alternative splice site 51 or 54 nucleotides downstream of the classic donor position. Although adenine has been previously reported at nucleotide position +5, it is rarely used and has only been observed in 7% of functional donor splice sites (Shapiro and Senapathy 1987).

The use of the cryptic splice sites in the chocolate-colored cats leads to the addition of the amino acid sequence GETFSYAFLRAQQGDRS(G) between CuB and the hydrophobic TM (Figure 1c). No known domain is disrupted by the insertion. However, the addition of 17 or 18 amino acids is likely to lead to changes in the three-dimensional structure of TYRP1, thereby partially impairing its enzymatic activity, that is, the production of lighter pigments (brownish) than the wild type (black).

The nonsynonymous *TYRP1*-8C>G mutation found in the chocolate-colored cat leads to the substitution of an alanine by a glycine at position 3 of the signaling peptide (Figure 1). The 24 amino acids of the signaling peptide are cleaved off the precursor protein (Alberts et al. 2002) and are hence not involved with catalytic function. However, a change in the signaling peptide could influence the targeting of the protein. Although no glycine residue has been reported at this position in other species (horse, dog, human, mouse, cow, goat; data not shown), the A3G substitution is conservative (both are small and nonpolar) and therefore not expected to induce major changes. Also signal peptides do not have stringent amino acid requirements (von Heijne 1998). The presence of five additional sequence variants unique to the chocolate-colored individual (Table 6) suggests an extended haplotype encompassing both sequence variants. To determine the causative mutation, functional studies would be required.

In our association study, three chocolate-colored individuals exhibited a non-chocolate genotype (Table 7). One of these individuals was heterozygous for the *chocolate* allele [*TYRP1*-8C>G; *TYRP1*-1262+5G>A] as well as for the *cinnamon* allele (*TYRP1*-298C>T), and might be a compound heterozygous containing no *wild type* allele. We expect the coat color of a b/b^l individual to be of lighter coloration than a chocolate (b/b) phenotype, but given the fact that the color is only present in the spots of the Ocicat, the nuance might have been too subtle to be detected. The two other cats that were heterozygous for the *chocolate* allele did not carry a *cinnamon* allele. The particularly dark coloration of one of the cats (personal communication from its owner) pointed to the potential presence of an alternative *brown* allele. A darker coat color variant in the mouse, cordovan, was attributed to a difference in expression level of *tyrp1* (Jackson et al. 1990). The fact that both cats of unexplained phenotype were of the same breed (Ocicat) and that 38 chocolate-colored cats of 9 domestic cat breeds share the [*TYRP1*-8C>G; *TYRP1*-1262+5G>A] allele (Table 7) suggests that a new mutation in *brown*, or possibly a nonlinked modifier locus, may contribute to a chocolate-like phenotype in the Ocicat breed.

The single base pair change (*TYRP1*-298C>T) found in the cinnamon individual leads to a triplet coding for a premature stop codon (TGA) at position 100, instead of a triplet coding for cysteine (CGA). The premature stop codon results in a truncated precursor protein of 99 amino acids instead of 537 amino acids in the functional TYRP1 (Figure 1d). The truncated peptide lacks functional domains including the two copper-binding domains, the Cys-rich and EGF-like domains as well as the hydrophobic TM that are conserved in the tyrosinase family (Figure 1d). The absence of the known functional domains likely causes loss of function of the truncated protein product. The decrease of pigment intensity in the alleles of the *B* locus, from wild type (black) to chocolate to cinnamon, is consistent with the severity of expected effect of a modified allele (*chocolate*) and a loss of function allele (*cinnamon*) on wild type enzyme activity.

In summary, we report the identification of five sequence variants associated with coat color variation in the domestic cat. Two nonsynonymous mutations were characterized in *TYR*: *TYR*-901G>A showed complete association with the siamese phenotype (Table 5), and *TYR*-679G>T was completely associated with the burmese phenotype (Table 5). Three mutations were characterized in *TYRP1*: *TYRP1*-298C>T, leading to a stop codon, showed complete association with the cinnamon phenotype, and a nonsynonymous substitution and a missplicing event showed near complete association with the chocolate phenotype. These results contribute to the understanding of the molecular basis of the melanogenic pathway and coat color variation in domestic cats.

Appendix I. List of domestic cats used in the study. The individuals are classified according to their phenotypes at the brown and albino loci in column four and five, respectively. The inferred genotype is given for each individual for the loci for which phenotypic information was available in columns six to eleven. Symbols as according to Robinson 1991

FCA	Breed	Sex	*brown* locus	*albino* locus	brown	albino	dilute	agouti	silver	orange
302	Burmese	F	chocolate	burmese	b/b	c^b/c^b	D/-	a/a	i/i	o/o
305	Burmese	M	chocolate	burmese	b/b	c^b/c^b	D/-	a/a	i/i	o
313	Burmese	?	chocolate	burmese	b/b	c^b/c^b	D/-	a/a	i/i	o/o, o
430	Burmese	F	chocolate	burmese	b/b	c^b/c^b	D/-	a/a	i/i	o/o
2219	Burmese	F	chocolate	burmese	b/b	c^b/c^b	D/-	a/a	i/i	o/o
2222	Burmese	M	chocolate	burmese	b/b	c^b/c^b	D/-	a/a	i/i	o
2475	Burmese	F	chocolate	burmese	b/b	c^b/c^b	D/-	a/a	i/i	o/o
2487	Tonkinese	M	chocolate	burmese	b/b	c^b/c^b	d/d	a/a	i/i	o
308	Burmese	?	wild type	burmese	B/-	c^b/c^b	D/-	a/a	i/i	o/o, o
435	Burmese	F	wild type	burmese	B/-	c^b/c^b	D/-	a/a	i/i	o/o
2221	Burmese	M	wild type	burmese	B/-	c^b/c^b	d/d	a/a	i/i	o
2482	Burmese	M	wild type	burmese	B/-	c^b/c^b	D/-	a/a	i/i	o
2485	Burmese	F	wild type	burmese	B/-	c^b/c^b	D/-	a/a	i/i	o/o
2842	Burmese	F	wild type	burmese	B/-	c^b/c^b	D/-	a/a	i/i	o/o
2860	Burmese	M	wild type	burmese	B/-	c^b/c^b	D/-	a/a	i/i	o
2822	Singapura	F	wild type	burmese	B/B	c^b/c^b	D/D	A/A	i/i	o/o
2867	Singapura	F	wild type	burmese	B/B	c^b/c^b	D/D	A/A	i/i	o/o
360	Burmese	F		burmese		c^b/c^b		a/a		
487	Burmese	F		burmese		c^b/c^b		a/a		
520	Burmese	?		burmese		c^b/c^b		a/a		
642	Burmese	F		burmese		c^b/c^b		a/a		
1965	Tonkinese	F	chocolate	intermediate	b/b	c^s/c^b	D/-	a/a	i/i	o/o
2483	Tonkinese	M	chocolate	intermediate	b/b	c^s/c^b	d/d	a/a	i/i	o
2506	Tonkinese	F	chocolate	intermediate	b/b	c^s/c^b	D/-	a/a	i/i	o/o
2507	Tonkinese	M	chocolate	intermediate	b/b	c^s/c^b	D/-	a/a	i/i	o
2508	Tonkinese	M	chocolate	intermediate	b/b	c^s/c^b	D/-	a/a	i/i	o
2553	Tonkinese	F	chocolate	intermediate	b/b	c^s/c^b	D/-	a/a	i/i	o/o
2555	Tonkinese	F	chocolate	intermediate	b/b	c^s/c^b	d/d	a/a	i/i	o/o
2662	Tonkinese	F	chocolate	intermediate	b/b	c^s/c^b	d/d	a/a	i/i	o/o
2398	Sphynx	F	wild type	intermediate	B/-	c^s/c^b	D/-	a/a	i/i	o/o
323	Tonkinese	?	wild type	intermediate	B/-	c^s/c^b	D/-	a/a	i/i	o/o, o
2554	Tonkinese	M	wild type	intermediate	B/-	c^s/c^b	d/d	a/a	i/i	o
2671	Tonkinese	F	wild type	intermediate	B/-	c^s/c^b	d/d	a/a	i/i	o/o
2672	Tonkinese	F	wild type	intermediate	B/-	c^s/c^b	D/-	a/a	i/i	o/o
2732	Tonkinese	F	wild type	intermediate	B/-	c^s/c^b	D/-	a/a	i/i	o/o
2827	Tonkinese	M	wild type	intermediate	B/-	c^s/c^b	d/d	a/a	i/i	o
2829	Tonkinese	F	wild type	intermediate	B/-	c^s/c^b	D/-	a/a	i/i	o/o
2527	Balinese	M	chocolate	siamese	b/b	c^s/c^s	D/-	a/a	i/i	o
2512	Birman	F	chocolate	siamese	b/b	c^s/c^s	D/-	a/a	i/i	o/o
2528	Birman	F	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o/o
2567	Birman	M	chocolate	siamese	b/b	c^s/c^s	D/-	A/-	i/i	o
2670	Birman	M	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o
2264	Siamese	M	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o
2383	Siamese	M	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o
2557	Siamese	F	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o/o
2616	Siamese	F	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o/o
2734	Siamese	F	chocolate	siamese	b/b	c^s/c^s	D/-	a/a	i/i	o/o
2735	Siamese	M	chocolate	siamese	b/b	c^s/c^s	D/-	a/a	i/i	o
2756	Siamese	F	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o/o
2765	Siamese	F	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o/o
2854	Siamese	F	chocolate	siamese	b/b	c^s/c^s	d/d	a/a	i/i	o/o
2582	Balinese	F	wild type	siamese	B/-	c^s/c^s	d/d	a/a	i/i	o/o
2669	Birman	M	wild type	siamese	B/-	c^s/c^s	D/-	a/a	i/i	o
2658	Colorpoint Shorthair	M	wild type	siamese	B/-	c^s/c^s	d/d	a/a	i/i	o
2581	Javanese	F	wild type	siamese	B/-	c^s/c^s	d/d	A/-	i/i	o/o
2394	Ragdoll	M	wild type	siamese	B/-	c^s/c^s	D/-	a/a	i/i	o
2420	Ragdoll	F	wild type	siamese	B/-	c^s/c^s	d/d	a/a	i/i	o/o
2600	Siamese	F	wild type	siamese	B/-	c^s/c^s	D/-	a/a	i/i	O/o
2651	Siamese	M	wild type	siamese	B/-	c^s/c^s	D/-	a/a	i/i	o
2749	Siamese	F	wild type	siamese	B/-	c^s/c^s	D/-	a/a	i/i	o/o

Appendix I. Continued

FCA	Breed	Sex	*brown* locus	*albino* locus	brown	albino	dilute	agouti	silver	orange
2757	Siamese	F	wild type	siamese	B/-	c^s/c^s	D/-	a/a	i/i	o/o
2759	Siamese	M	wild type	siamese	B/-	c^s/c^s	D/-	a/a	i/i	o
2760	Siamese	M	wild type	siamese	B/-	c^s/c^s	D/-	a/a	i/i	o
2761	Siamese	M	wild type	siamese	B/-	c^s/c^s	d/d	a/a	i/i	o
559	Siamese	M		siamese		c^s/c^s				
2770	Cornish Rex	M	chocolate	wild type	b/b	C/-	D/-	a/a	i/i	o
770	Havana Brown	?	chocolate	wild type	b/b	C/-		a/a	i/i	o/o, o
771	Havana Brown	?	chocolate	wild type	b/b	C/-		a/a	i/i	o/o, o
779	Havana Brown	?	chocolate	wild type	b/b	C/-		a/a	i/i	o/o, o
780	Havana Brown	?	chocolate	wild type	b/b	C/-		a/a	i/i	o/o, o
2591	Havana Brown	M	chocolate	wild type	b/b	C/-	D/-	a/a	i/i	o
4507	Munchkin	M	chocolate	wild type	b/b	C/-	D/-	a/a	i/i	o
2583	Ocicat	F	chocolate	wild type	b/b	C/-	D/-	A/-	i/i	o/o
2667	Ocicat	M	chocolate	wild type	b/b	C/-	D/-	A/-	I/-	o
2704	Ocicat	M	chocolate	wild type	b/b	C/-	D/-	A/-	i/i	o
2837	Ocicat	M	chocolate	wild type	b/b	C/-	d/d	A/-	i/i	o
2919	Ocicat	M	chocolate	wild type	b/b	C/-	D/-	A/-	I/-	o
1902	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
1907	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
2396	Abyssinian	M	cinnamon	wild type	b^l/b^l	C/-	d/d	A/A	i/i	o
2515	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
2674	Abyssinian	M	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o
4510	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
4703	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
4704	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
4705	Abyssinian	M	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o
4706	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
4707	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
4708	Abyssinian	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o/o
4709	Abyssinian	M	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o
4710	Abyssinian	M	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o
4711	Abyssinian	M	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o
4712	Abyssinian	M	cinnamon	wild type	b^l/b^l	C/-	D/-	A/A	i/i	o
2247	Ocicat	F	cinnamon	wild type	b^l/b^l	C/-	D/-	A/-	I/-	o/o
2400	Ocicat	M	cinnamon	wild type	b^l/b^l	C/-	d/d	A/-	i/i	o
1903	Abyssinian	F	wild type	wild type	B/-	C/-	d/d	A/A	i/i	o/o
1904	Abyssinian	M	wild type	wild type	B/-	C/-	D/-	A/A	i/i	o
2430	Abyssinian	M	wild type	wild type	B/-	C/-	D/-	A/A	i/i	o
2783	Abyssinian	F	wild type	wild type	B/-	C/-	D/-	A/A	i/i	o/o
2785	Abyssinian	F	wild type	wild type	B/-	C/-	D/-	A/A	i/i	o/o
938	American Shorthair	F	wild type	wild type	B/-	C/-				
2556	Bombay	?	wild type	wild type	B/-	C/-	D/-	a/a	i/i	o/o, o
2471	Chartreux	F	wild type	wild type	B/-	C/-	d/d	a/a	i/i	o/o
2402	Cornish Rex	M	wild type	wild type	B/-	C/-	D/-	a/a	I/-	o
508	Domestic Shorthair	F	wild type	wild type	B/-	C/-	D/-	a/a	i/i	o/o
800	Domestic Shorthair	F	wild type	wild type	B/-	C/-			i/i	
2486	Exotic Shorthair	M	wild type	wild type	B/-	C/-	D/-	A/-	I/-	o
2530	Korat	M	wild type	wild type	B/-	C/-	d/d	a/a	I/-	o
232	Egyptian Mau	F	wild type	wild type	B/-	C/-	D/-	A/-	i/i	o/o
2503	Egyptian Mau	M	wild type	wild type	B/-	C/-		A/-	i/i	o
2560	Egyptian Mau	F	wild type	wild type	B/-	C/-	D/-	a/a	I/-	o/o
2655	Egyptian Mau	F	wild type	wild type	B/-	C/-	D/-	A/-	i/i	o/o
2752	Egyptian Mau	M	wild type	wild type	B/-	C/-	D/-	A/-	i/i	o
2607	Ocicat	M	wild type	wild type	B/-	C/-	D/-	A/-	i/i	o
2490	Oriental Shorthair	F	wild type	wild type	B/-	C/-	D/-	a/a	I/-	o/o
2861	Persian	F	wild type	wild type	B/-	C/-	D/-	a/a	i/i	o/o
2525	Somali	M	wild type	wild type	B/-	C/-	D/-	A/A	i/i	o
3004	Egyptian Mau	M		wild type		C/-				o
640	Abyssinian	M		wild type		C/-		A/A		o
653	Abyssinian	M		wild type		C/-		A/A		o
1905	Abyssinian	M		wild type		C/-		A/A		
1906	Abyssinian	F		wild type		C/-		A/A		oo

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