



Mutations of *TYR* and *MITF* Genes are Associated with Plumage Colour Phenotypes in Geese

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ABSTRACT: The polymorphism of microphthalmia-associated transcription factor (*MITF*) and tyrosinase (*TYR*) genes have been proposed to play a vital role in coat colour genesis in mammals, but their role remains ambiguous in geese at best. Here, we cloned and sequenced 1,397 bp coding region of *MITF* gene and a 588 bp fragment of *TYR* exon 1 for polymorphism analysis among 157 domestic geese showing three types of plumage colour. We detected a total of three SNPs (c.280T>C, c.345G>A, and c.369G>A) in *TYR* and six haplotypes (H1-H6). Among them, haplotypes H1, H2, H3, and H5 were significantly associated with white plumage trait of Zhedong White Geese. However, only diplotype H1H1 and H3H5 were significantly associated with white plumage trait of Zhedong White Geese ($p < 0.01$). We only detected one SNP (c.1109C>T) for *MITF* gene and found that genotype CT and TT were significantly associated with white plumage trait of Zhedong White Geese. Briefly, our study suggested an association between polymorphisms of *TYR* and *MITF* genes and the plumage colour trait in domestic geese. (**Key Words:** Goose, *TYR* and *MITF* Gene, SNP, Plumage Colour)

INTRODUCTION

The plumage, skin and eye colour of birds are mainly determined by the relative content and distribution of eumelanin and pheomelanin, which is produced by melanocytes present in the epithelium, iris, and feather follicles of birds. The eumelanin is brownish-black and the pheomelanin range of colour is from yellow to reddish-brown (Prota, 1980). The process of melanin biosynthesis is triggered by the binding of α -melanocyte stimulating hormone (α -MSH) to its receptor, melanocortin-receptor 1 (*MC1R*). The subsequent increasing level of cyclic AMP (cAMP) up-regulates the transcription of microphthalmia-associated transcription factor (*MITF*). Afterwards, *MITF*

activates the transcription of tyrosinase, which catalyzes the first step of melanogenesis (Smyth, 1990; Scherer and Kumar, 2010). The tyrosinase, encoded by *tyrosinase* (*TYR*) gene, is known to be the rate-limiting enzyme affecting the production of melanin pigment (Sánchez-Ferrer et al., 1995), which oxidates tyrosine to dihydroxyphenylalanine (DOPA) (Lerner and Fitzpatrick, 1950) and determines which type of melanin (eumelanin or pheomelanin) could be synthesized (Ito et al., 2000). After the report of (Oetting et al., 1985) that the C locus is the structural locus for tyrosinase in the fowl, the *TYR* gene was first cloned from chicken (Mochii et al., 1992). Previous studies have shown that mutations of the *TYR* gene are associated with albinism phenotype in many vertebrates, such as human (Fukai et al., 1995; Oetting, 2000), mouse (Yokoyama et al., 1990), rat (Blaszczyk et al., 2005), cat (Imes et al., 2006), rabbits (Aigner et al., 2000), cattle (Schmutz et al., 2004), chicken (Tobita-Teramoto et al., 2000; Sato et al., 2007; Liu et al., 2010).

MITF is a transcription factor and belongs to the basic-helix-loop-helix-leucine zipper (bHLHZip) protein family.

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Studies suggested that it regulates the melanogenesis through binding to a highly conserved M-box (GTCATGTGCT) and E-box (TCATGTG) motif upstream of the tyrosinase promoter, which strongly stimulates the tyrosinase promoter activities and promotes tyrosinase expression (Yasumoto et al., 1994; Bertolotto et al., 1996, 1998). Besides, it is also involved in the development of melanocytes, retinal pigment epithelium, mast cell and osteoclasts (Hodgkinson et al., 1993; Steingrímsson et al., 1994; Moore, 1995). Mutations of the *MITF* gene that have been described in several vertebrate species lead to coat colour dilutions, white spotting, or complete loss of pigmentation in mouse (Hodgkinson et al., 1993; Hughes et al., 1993; Yasumoto et al., 1998), cause Waardenburg syndrome (Variant, 1994) and Tietz syndrome (Amiel et al., 1998; Smith et al., 2000) in human with the characteristics of hearing loss and depigmentation of the hair and skin. Subsequent studies also reported that mutations in *MITF* are associated with white spotting coat colour in dogs (Andersson, 2009; Schmutz et al., 2009) and white plumage in Japanese quail (Minvielle et al., 2010).

Nowadays, coat color is considered an important economic trait that caters to consumer preference. Geese are valued as much for their feathers when processed into clothing for warmth and fashion shows. However, little is known about the genetic basis of feather color. As a qualitative trait, the plumage colour of poultry is determined by the relative content and distribution of eumelanin and pheomelanin produced in melanocytes. The melanin synthesis is controlled by the sequential expression and interactions of genes (Scherer and Kumar, 2010). Taken together, the aforementioned reports have shown that the polymorphisms of *TYR* and *MITF* genes are the key factors for melanin synthesis and albino phenotype expression in mammals. However, the potential association between *MITF* and *TYR* genes and plumage colour phenotypes in geese has not been validated. The aim of the current study is to detect SNPs in *MITF* and *TYR* genes and explore their possible association with plumage colour phenotypes in

goose.

MATERIALS AND METHODS

Animals

Blood samples from 157 geese with three plumage colour phenotypes were collected within two breeds, including 41 gray plumage Landes (completely gray feathers at dorsum and breast), 42 spotted feather Landes (near black feathers at dorsum and breast), 74 Zhedong White Geese (pure white plumage). All samples were gathered from the Research Poultry Farm Veterinary Research Institute of Shanghai Academy of Agricultural Sciences where special staff raised the birds at the same level of nutrition and management in a poultry house. Meanwhile, skin hair-follicle tissues were collected from Sichuan white geese for RNA extraction and cDNA synthesis to clone the CDS (coding sequence) of *MITF* gene.

DNA extraction, sequencing and genotyping of *MITF* and *TYR* gene

The genomic DNA was prepared by a standard phenol/chloroform method to proceed the PCR amplification and sequencing of *TYR* and *MITF* genes to detect and analyze the sequence mutations. One pair of primers were designed by Oligo 6.0 and Primer 5.0 software based on the conserved sequence of exon 1 of *TYR* gene in *Gallus gallus* (NM_2046160, L46805), *Meleagris gallopavo* (XM_003203534) and *Coturnix coturnix* (AB024279). Another pair of primers were designed for sequencing of a partial coding sequence of *MITF* (Minvielle et al., 2010) (Table 1). A total volume of 50 µL PCR reaction mixture was used containing 25 µL 2×Taq Master Mix (TaKaRa Biotech Co. Ltd., Dalian, China), 100 ng genomic DNA, 2.5 µL of each primers and 17 µL ddH₂O. The 35 PCR cycles were carried out as follows: 94°C for 30 s, the respective annealing temperature of the primer pair for 45 s, 72°C for 1 min, preceded by a denaturizing step of 5 min at 94°C and followed by an extension step of 10 min

Table 1. Primers for *MITF* and *TYR* genotyping and cloning of *MITF*

Primers ¹	Sequence (5'-3')	Annealing temperature (°C)	Fragment (bp)
TYR-g-F ²	TGGGGAGCGTTCCAACAG	62	613
TYR-g-R ²	TGTTGGCCACCCATGTATTC		
MITF-g-F ³	AGCTCGGGCACATGGACT	63	280
MITF-g-R ³	GGAGAGGGTATCGTCCATCA		
MITF-C-1F ⁴	GCATCCTGGCGCTTCCAAGCCTC	70	1,107
MITF-C-1R ⁴	GAGCAAAGGCCGGTGGATGGCAC		
MITF-C-2F ⁴	GATGGACCCAGCCTTGC	60	797
MITF-C-2R ⁴	TGCTCCGCCTGCTACTC		
MITF-C-3F ⁴	CAGCAACGCACGAAGGA	58	593
MITF-C-3R ⁴	GGCTACCGATGAAGCACAAG		

¹ F = Forward; R = Reverse. ² Primers for genotyping *TYR*. ³ Primers for genotyping *MITF*. ⁴ Primers for cloning the CDS of *MITF*.

at 72°C. Subsequently, the PCR products of all 157 samples of *TYR* and *MITF* genes were purified with centrifugal columnar agarose gel DNA extraction kit (TianGen Biotech Co. Ltd., Beijing, China) and sequenced directly on ABI Prism 3700 DNA sequencer (Shanghai Biotech Co. Ltd., Shanghai, China).

RNA isolation, cDNA synthesis and CDS clone of *MITF*

Total RNA was isolated from skin hair-follicle tissues of Sichuan white goose using Trizol reagent (TakaRa Biotech Co. Ltd., Dalian, China). The quality of RNA was determined by the $A_{260/280}$ absorbance ratio (1.6-1.8) and the integrity of the 18S and 28S rRNA bands was checked on 1% formaldehyde agarose gel. Isolated RNAs were treated with 8 μ L DNase (Fermentas) at 37°C for 20 min and stored at -80°C. The cDNAs were synthesized using the ImProm-II Reverse Transcription System (TakaRa Biotech Co. Ltd., Dalian, China) according to the manufacturer's recommendation. The reaction was performed in a volume of 10 μ L, containing 5 \times PrimerScript Buffer, 10 mM of each dNTPs, 40 U/ μ L RNase Inhibitor, 2.5 μ M oligo-dT Primer. The reverse transcription was maintained at 37°C for 15 min, and 85°C for 5 s. The cDNA samples were stored at -80°C.

The primers for cloning *MITF* gene were designed by Oligo 6.0 and Primer 5.0 software according to the conserved mRNA sequence of *MITF* in *Gallus gallus* (NM_205029), *Meleagris gallopavo* (XM_003210180), *Coturnix coturnix* (AB005229) and *Taeniopygia guttata* (XM_002193131) (Table 1). PCR amplification was performed in a final volume of 50 μ L containing 25 μ L 2 \times Taq Master Mix (TaKaRa Biotech Co. Ltd., Dalian, China), 4 μ L cDNA template, 1.5 μ L of each primers and 19.5 μ L ddH₂O with the following cycling parameters: one cycle of 5 min at 94°C, 35 cycles of 30 s at 94°C, 45 s, 45 s at the

respective annealing temperature of each primer pair, 1 min at 72°C, and a final extension for 10 min at 72°C. Purified PCR products were cloned into pMD 18-T simple vector (TakaRa Biotechnology Co. Ltd., Dalian, China). The authenticity of the amplified fragment was verified by sequencing.

Data analysis

All sequences were spliced and aligned by DNASTAR package (DNASTAR Inc., Madison, WI, USA). We used MEGA 4.0 to export sequence variations. Haplotypes of the *TYR* gene were deduced by using the PHASE 2.0 program (Stephens et al., 2001). The potential association between the *TYR* and *MITF* alleles and plumage colours were evaluated by chi-square test for independence and which was performed on SAS V8.1 (SAS Institute Inc. Cary, NC, USA).

RESULTS AND DISCUSSION

We obtained 588 bp fragments of exon 1 of *TYR* gene and 1,407 bp coding fragments of *MITF* gene in this study and the sequence data was deposited in GenBank under accession numbers of KF020633 and KF020632, respectively. Three SNPs (c.280T>C, c.345G>A and c.369G>A) were detected from *TYR* gene, among which c.345G>A and c.369G>A were only found in Zhedong White Geese and the c.280T>C was found in Zhedong White Geese and spotted plumage colour Landes, no polymorphism was detected in grey plumage colour Landes (Table 2). However, only one SNP (c.1109C>T) was detected in the *MITF* gene exclusively in Zhedong White Geese containing two alleles and deducing three genotypes (CC, CT, TT). All of these SNPs were synonymous and newly reported. As is known, the preference of tRNA binding degenerate codons generated by synonymous

Table 2. The genetic polymorphisms in *TYR* gene and *MITF* gene

Gene	SNP	Breed	Variant allele frequency ^a	Genotypes frequency ^a			HW ^b
				RR	RV	VV	
<i>TYR</i>	c.280T>C	Gray feather Landes	0.00	0.26 (41)	0.00 (0)	0.00 (0)	-
		Spotted feather Landes	0.04	0.25 (39)	0.02 (3)	0.00 (0)	0.81
		Zhedong White goose	0.10	0.38 (59)	0.10 (15)	0.00 (0)	0.33
	c.345G>A	Gray feather Landes	0.00	0.26 (41)	0.00 (0)	0.00 (0)	-
		Spotted feather Landes	0.00	0.27 (42)	0.00 (0)	0.00 (0)	-
		Zhedong White goose	0.18	0.39 (61)	0.00 (0)	0.08 (13)	7.81E-18
	c.369G>A	Gray feather Landes	0.00	0.26 (41)	0.00 (0)	0.00 (0)	-
		Spotted feather Landes	0.00	0.27 (42)	0.00 (0)	0.00 (0)	-
		Zhedong White goose	0.39	0.18 (29)	0.20 (32)	0.08 (13)	0.43
<i>MITF</i>	c.1109C>T	Gray feather Landes	0.00	0.26 (41)	0.00 (0)	0.00 (0)	-
		Spotted feather Landes	0.00	0.27 (42)	0.00 (0)	0.00 (0)	-
		Zhedong White goose	0.07	0.43 (68)	0.02 (3)	0.03 (4)	9.87E-10

R = Reference nucleotide; V = Variant nucleotide. ^a Frequency of each genotype. ^b Hardy-Weinberg test.

Table 3. The genotype distribution of *TYR* gene and the *MITF* gene

Gene	SNPs	Genotype	Frequency			χ^2 value	PIC ¹
			Gray feather	Spotted feather	White feather		
<i>TYR</i>	c.280T>C	CC	0.26	0.25	0.38	144.655	0.2118
		CT	0.00	0.02	0.10		
	c.345G>A	GG	0.26	0.27	0.39	157.000	0.2516
		GA	0.00	0.00	0.00		
		AA	0.00	0.00	0.08		
	c.369G>A	GG	0.26	0.27	0.18	33.832	0.3626
GA		0.00	0.00	0.20			
AA		0.00	0.00	0.08			
<i>MITF</i>	c.1109C>T	CC	0.26	0.27	0.43	126.774	0.1217
		CT	0.00	0.00	0.02		
		TT	0.00	0.00	0.03		

¹ PIC = Polymorphism information content.

mutations is not random in the process of gene translation and the synonymous mutation would affect and reduce the translation efficiency of gene, and subsequently affect the protein folding and function (Kimchi-Sarfaty et al., 2007; Komar, 2007). However, the genotypic milieu was not the reason for the occurrence of those SNPs, and the hypothesis of tRNA binding preference might explain why those synonymous mutations were only distributed in specific groups.

The *MITF* genotypes CT and TT were significantly associated with the white plumage trait of Zhedong White Geese ($p < 0.05$) which further verified that the mutation c.1109C>T affected the plumage color (Tables 2 and 3). While in most dogs an interspersed nucleotide element (SINE) insertion 3167 bp before the start codon of exon 1M in *MITF* plays an important role in coat color determination. Dogs homozygous for SINE had white markings that covered most of the body, while the heterozygous ones were solid colored or had minor white (Schmutz et al., 2009). The distinct patterns in geese and dogs provides a glimpse of the *MITF* association with coat color.

In addition, six haplotypes (H1 to H6) were obtained based on the three SNPs (c.280T>C, c.345G>A, and c.369G>A) of the *TYR* gene (Table 4), and the

corresponding diplotypes are displayed in Table 5. The results showed that the haplotypes H1, H2, H4, and H5 were only found in Zhedong White Geese. Particularly, haplotype H6 occurred in Zhedong White Geese and spotted Landes with the same frequency. Association analysis demonstrated that haplotypes H1, H2, H3, and H5 were significantly associated with the white plumage trait of Zhedong White Geese, whereas the H2 and H3 were significantly associated with gray and spotted plumage trait of Landes ($p < 0.01$). Furthermore, the diplotypes were only distributed in Zhedong White Geese, besides diplotype H3H3. Association analysis showed that diplotypes H1H1 and H3H5 were significantly associated with white plumage trait of Zhedong White Geese ($p < 0.01$). We can infer that the difference diplotypes of *TYR* exon 1 effect the plumage color determination. While in cats, three mutations (c.216G>T, c.450C>T, c.679G>T) were identified in exon 1 of *TYR* with one nonsynonymous and two synonymous mutations which showed strong association with *albino* locus (Schmidt-Küntzel et al., 2005). In Himalayan mice, strong association also been proved between a nonsynonymous substitutions in exon 4 of *TYR* and the *albino* locus (Kwon et al., 1989). Through different species, we can speculate that nucleotide mutation plays a crucial role in *TYR* on coat color determination.

Table 4. Distribution of the *TYR* gene haplotypes in three goose populations

Haplotype	No.	Frequency	Zhedong	Gray	Spotted
			White goose	feather Landes	feather Landes
H1(TAA)	13	0.0828	13	0	0
H2(TGA)	12	0.0764	12	0	0
H3(TGG)	122	0.7771	42	41	39
H4(CAG)	1	0.0063	1	0	0
H5(CGA)	3	0.0191	3	0	0
H6(CGG)	6	0.0382	3	0	3
Total	157	1.0000	74	41	42

Table 5. Distribution of diplotypes in different goose populations

Diplotype	No.	Frequency	Zhedong	Gray	Spotted
			white goose	feather Landes	feather Landes
H1H1	13	0.08442	13	0	0
H1H4	1	0.006494	1	0	0
H2H3	23	0.14935	23	0	0
H3H3	103	0.66883	23	41	39
H3H5	8	0.05195	8	0	0
H3H6	6	0.03896	6	0	3
Total	157	1.0000	74	41	42

As a complex trait, coat color is determined by a complex pathway system and multiple interactively patterns, further investigation would be helpful to further elucidate the molecular mechanism of plumage color formation. On the basis of our result in this study, we can speculated that, in geese, *TYR* and *MITF* genes play a vitally important role in plumage color determination.

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REFERENCES

- Aigner, B., U. Besenfelder, M. Müller, and G. Brem. 2000. Tyrosinase gene variants in different rabbit strains. *Mamm. Genome* 11:700-702.
- Amiel, J., P. M. Watkin, M. Tassabehji, A. P. Read, and R. M. Winter. 1998. Mutation of the *MITF* gene in albinism-deafness syndrome (Tietz syndrome). *Clin. Dysmorphol.* 7:17-20.
- Andersson, L. 2009. Genome-wide association analysis in domestic animals: A powerful approach for genetic dissection of trait loci. *Genetica* 136:341-349.
- Bertolotto, C., P. Abbe, T. J. Hemesath, K. Bille, D. E. Fisher, J. P. Ortonne, and R. Ballotti. 1998. Microphthalmia gene product as a signal transducer in cAMP-induced differentiation of melanocytes. *J. Cell Biol.* 142:827-835.
- Bertolotto, C., K. Bille, J. P. Ortonne, and R. Ballotti. 1996. Regulation of tyrosinase gene expression by cAMP in B16 melanoma cells involves two CATGTG motifs surrounding the TATA box: Implication of the microphthalmia gene product. *J. Cell Biol.* 134:747-755.
- Blaszczyk, W. M., L. Arning, K. P. Hoffmann, and J. T. Epplen. 2005. A tyrosinase missense mutation causes albinism in the Wistar rat. *Pigment Cell Res.* 18:144-145.
- Fukai, K., S. A. Holmes, N. J. Lucchese, V. M. Siu, R. G. Weleber, R. E. Schnur, and R. A. Spritz. 1995. Autosomal recessive ocular albinism associated with a functionally significant tyrosinase gene polymorphism. *Nat. Genet.* 9:92-95.
- Hodgkinson, C. A., K. J. Moore, A. Nakayama, E. Steingrímsson, N. G. Copeland, N. A. Jenkins, and H. Arnheiter. 1993. Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. *Cell* 74:395-404.
- Hughes, M. J., J. Lingrel, J. Krakowsky, and K. Anderson. 1993. A helix-loop-helix transcription factor-like gene is located at the mi locus. *J. Biol. Chem.* 268:20687-20690.
- Imes, D., L. Geary, R. Grahn, and L. Lyons. 2006. Albinism in the domestic cat (*Felis catus*) is associated with a tyrosinase (*TYR*) mutation. *Anim. Genet.* 37:175-178.
- Ito, S., K. Wakamatsu, and H. Ozeki. 2000. Chemical analysis of melanins and its application to the study of the regulation of melanogenesis. *Pigment Cell Res.* 13:103-109.
- Kimchi-Sarfaty, C., J. M. Oh, I. W. Kim, Z. E. Sauna, A. M. Calcagno, S. V. Ambudkar, and M. M. Gottesman. 2007. A "silent" polymorphism in the *MDR1* gene changes substrate specificity. *Science* 315:525-528.
- Komar, A. A. 2007. Silent SNPs: Impact on gene function and phenotype. *Pharmacogenomics* 8:1075-1080.
- Kwon, B. S. and R. Halaban, and C. Chintamaneni. 1989. Molecular basis of mouse Himalayan mutation. *Biochem. Biophys. Res. Commun.* 161:252-260.
- Lerner, A. B. and T. B. Fitzpatrick. 1950. Biochemistry of melanin formation. *Physiol. Rev.* 30:91-126.
- Liu, W., S. Chen, J. Zheng, L. Qu, G. Xu, and N. Yang. 2010. Developmental phenotypic-genotypic associations of tyrosinase and melanocortin 1 receptor genes with changing profiles in chicken plumage pigmentation. *Poult. Sci.* 89:1110-1114.
- Minvielle, F., B. Bed'hom, J. L. Coville, S.I. Ito, M. Inoue-Murayama, and D. Gourichon. 2010. The "silver" Japanese quail and the *MITF* gene: Causal mutation, associated traits and homology with the "blue" chicken plumage. *BMC Genet.* 11: 15.
- Mochii, M., A. Iio, H. Yamamoto, T. Takeuchi, and G. Eguchi. 1992. Isolation and characterization of a chicken tyrosinase cDNA. *Pigment Cell Res.* 5:162-167.
- Moore, K. J. 1995. Insight into the *microphthalmia* gene. *Trends Genet.* 11:442-448.
- Oetting, W., A. Churilla, H. Yamamoto, and J. Brumbaugh. 1985. C pigment locus mutants of the fowl produce enzymatically inactive tyrosinase-like molecules. *J. Exp. Zool.* 235:237-245.
- Oetting, W. S. 2000. The tyrosinase gene and oculocutaneous albinism type 1 (OCA1): A model for understanding the molecular biology of melanin formation. *Pigment Cell Res.* 13: 320-325.
- Prota, G. 1980. Recent advances in the chemistry of melanogenesis in mammals. *J. Invest. Dermatol.* 75:122-127.
- Sánchez-Ferrer, J. N. Rodríguez-López, F. García-Cánovas, and F. García-Carmona. 1995. Tyrosinase: a comprehensive review of its mechanism. *Biochim. Biophys. Acta. Protein Struct. Mol. Enzymol.* 1247:1-11.
- Sato, S., T. Otake, C. Suzuki, J. Saburi, and E. Kobayashi. 2007. Mapping of the recessive white locus and analysis of the tyrosinase gene in chickens. *Poult. Sci.* 86:2126-2133.
- Scherer, D. and R. Kumar. 2010. Genetics of pigmentation in skin cancer—A review. *Mutat. Res.* 705:141-153.
- Schmidt-Küntzel, A., E. Eizirik, S. J. O'Brien, and M. Menotti-Raymond. 2005. Tyrosinase and tyrosinase related protein 1 alleles specify domestic cat coat color phenotypes of the albino and brown loci. *J. Hered.* 96:289-301.
- Schmutz, S. M., T. G. Berryere, D. C. Ciobanu, A. J. Mileham, B. H. Schmitz, and M. Fredholm. 2004. A form of albinism in cattle is caused by a tyrosinase frameshift mutation. *Mamm. Genome* 15:62-67.
- Schmutz, S. M., T. G. Berryere, and D. L. Dreger. 2009. *MITF* and white spotting in dogs: a population study. *J. Hered.* 100:S66-S74.
- Smith, S. D., P. M. Kelley, J. B. Kenyon, and D. Hoover. 2000. Tietz syndrome (hypopigmentation/deafness) caused by mutation of *MITF*. *J. Med. Genet.* 37:446-448.
- Smyth, J. R. Jr. 1990. Genetics of plumage, skin and eye

- pigmentation in chickens. In: Poultry Breeding and Genetics (Ed. R. D. Crawford). Elsevier, Amsterdam, Holland. pp. 109-168.
- Steingrímsson, E., K. J. Moore, M. L. Lamoreux, A. R. Ferré-D'amaré, S. K. Burley, D. C. S. Zimring, L. C. Skow, C. A. Hodgkinson, H. Arnheiter, and N. G. Copeland. 1994. Molecular basis of mouse microphthalmia (*mi*) mutations helps explain their developmental and phenotypic consequences. *Nat. Genet.* 8:256-263.
- Stephens, M., N. J. Smith, and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. *The Am. J. Human Genet.* 68:978-989.
- Tobita-Teramoto, T., G. Jang, K. Kino, D. Salter, J. Brumbaugh, and T. Akiyama. 2000. Autosomal albino chicken mutation (*ca/ca*) deletes hexanucleotide (-deltaGACTGG817) at a copper-binding site of the tyrosinase gene. *Poult. Sci.* 79:46-50.
- Variant, W. S. 1994. A gene for Waardenburg syndrome type 2 maps close to the human homologue of the microphthalmia gene at chromosome 3p12-p14. 1. *Nat Genet.* 7:509-512.
- Yasumoto, K.-I., K. Yokoyama, K. Shibata, Y. Tomita, and S. Shibahara. 1994. Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene. *Mol. Cell. Biol.* 14:8058-8070.
- Yasumoto, K. I., S. Amae, T. Uono, N. Fuse, K. Takeda, and S. Shibahara. 1998. A big gene linked to small eyes encodes multiple *Mitf* isoforms: many promoters make light work. *Pigment Cell Res.* 11:329-336.
- Yokoyama, T., D. W. Silversides, K. G. Waymire, B. S. Kwon, T. Takeuchi, and P. A. Overbeek. 1990. Conserved cysteine to serine mutation in tyrosinase is responsible for the classical albino mutation in laboratory mice. *Nucl. Acids Res.* 18:7293-7298.