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The genetics of brown coat color and white spotting in domestic yaks (*Bos grunniens*)

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Summary

Domestic yaks (*Bos grunniens*) exhibit two major coat color variations: a brown vs. wild-type black pigmentation and a white spotting vs. wild-type solid color pattern. The genetic basis for these variations in color and distribution remains largely unknown and may be complicated by a breeding history involving hybridization between yaks and cattle. Here, we investigated 92 domestic yaks from China using a candidate gene approach. Sequence variations in *MC1R*, *PMEL* and *TYRP1* were surveyed in brown yaks; *TYRP1* was unassociated with the coloration and excluded. Recessive mutations from *MC1R*, or p.Gln34*, p.Met73Leu and possibly p.Arg142Pro, are reported in bovids for the first time and accounted for approximately 40% of the brown yaks in this study. The remaining 60% of brown individuals correlated with a cattle-derived deletion mutation from *PMEL* (p.Leu18del) in a dominant manner. Degrees of white spotting found in yaks vary from color sidedness and white face, to completely white. After examining the candidate gene *KIT*, we suggest that color-sided and all-white yaks are caused by the serial translations of *KIT* (Cs_6 or Cs_{29}) as reported for cattle. The white-faced phenotype in yaks is associated with the *KIT* haplotype S^{wf}. All *KIT* mutations underlying the serial phenotypes of white spotting in yaks are identical to those in cattle, indicating that cattle are the likely source of white spotting in yaks. Our results reveal the complex genetic origins of domestic yak coat color as either native in yaks through evolution and domestication or as introduced from cattle through interspecific hybridization.

Keywords cattle, coat color, *KIT*, *MC1R*, *PMEL*, yak

Introduction

Most, if not all, domestic animals exhibit variable coat colors distinct from their wild ancestors because of intense human intervention, such as artificial selection and hybridization with other species for specific traits (Eriksson *et al.* 2008; Cieslak *et al.* 2011; Linderholm & Larson 2013). Coat color variation in domestic animals is an ideal model to study the genetic basis of phenotypic diversity found in wild and domestic animals, species adaptation and the evolutionary processes during domestication (Cieslak *et al.* 2011; Linderholm & Larson 2013).

The yak (*Bos grunniens*) is a long-haired bovid distributed on the Qinghai-Tibetan Plateau and adjacent highlands in Mongolia and Russia (Wiener *et al.* 2003). Yaks were domesticated 5000 years ago, the captive population exceeds 14 million animals (Zhang 1989; Wiener *et al.* 2003) at present, and only a small population survives in the wild. As an important symbol for Tibet and high-altitude environments, the yak has evolved genome adaptive traits for living at high altitudes and has become essential livestock providing meat, milk, hides, transportation and other necessities for Tibetans and other nomadic pastoralists (Zhang 1989; Wiener *et al.* 2003; Qiu *et al.* 2012). During the long history of yak rearing, breeders have set criteria (such as milk yield, meat quality and wool productivity) to selectively breed domestic yaks and have established the current breeds including 12 official yak breeds in China and five in India (Cai 1985; Pal *et al.* 1994). Several well-known yak breeds in China include the completely white Tianzhu breed and the black Jiulong and Maiwa breeds.

The yak has low productivity relative to other bovine species such as cattle (*Bos taurus*), from which it is estimated to have diverged about 4.9 million years ago (Qiu *et al.* 2012). To improve yak stock, interspecific hybridization between yaks and cattle has been

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introduced, either naturally, as documented in historical archives from the Zhou Dynasty of China (c. 1046–256 BC), or more recently via artificial insemination with improved breeds such as Holstein-Friesian and Highland cattle (Zhang 2000; Wiener *et al.* 2003). Although male hybrid offspring are infertile and active crossbreeding is generally restricted to F1 and F2 generations only, the practice is widespread across the Qinghai-Tibet Plateau and may have left genomic and phenotypic signatures in the yak.

Domestic yaks exhibit two major categories of fur variation regarding coat color and the distribution of color pigmentation. Black coloration is predominant in yaks and also known as the wild type (Fig. 1a), and a brown coat is a common variant (Fig. 1b,c). The second category of variation is an independent trait showing various degrees of pigmentation, from a solid-colored wild type to a partially pigmented coat, called 'white spotting'. Most of the pigmented fur in a white-spotted yak is either black or brown, yet the degree of spotting varies. Three typical patterns of white spotting in yaks include color sidedness, characterized by a white stripe along the spine and pigmented sectors on the flanks (Fig. 1d); white face, characterized by a white face and variable white patches on the torso (Fig. 1e); and completely white without pigmentation (Fig. 1f). The white spotting trait is epistatic, in that an all-white yak masks the phenotype of black or brown coat color from other loci.

Mammalian coat color is determined by the pigment melanin synthesized within melanosomes, specific organelles in the melanocyte. There are two types of melanin: the black to brown eumelanin and the red to yellow pheomelanin. The quantity and ratio of eumelanin to pheomelanin determine the color of mammalian skin, hair and eyes (Barsh 1996). Some attempts have been made to sequence *MC1R* coding regions in several yak breeds but have failed to find associations between *MC1R* genotypes and yak black/white phenotypes (Chen *et al.* 2009; Xi *et al.* 2012). Although the genetic basis of yak coat color has been poorly studied, previous work on cattle and hybridization records between cattle and yaks highlight several possible mechanisms underlying color variants found in yaks.

A review of cattle coat color genetics suggests at least three candidate genes for the brown coat color in yaks: (1) *Melanocortin 1 receptor* (*MC1R*), known as the bovine *E* locus, regulates the pigment syntheses switch between eumelanin and pheomelanin, and its null mutations are responsible for the recessive reddish color in Norwegian, Holstein, Highland and Galloway cattle (Klungland *et al.* 1995; Joerg *et al.* 1996; Schmutz & Dreger 2013); (2) *tyrosinase-related protein 1* (*TYRP1*) is involved in the eumelanin synthesis pathway and is associated with the

Figure 1 Domestic yaks displaying different coat color variations for the traits of white spotting and brown coloration. (a) Wild-type: +/+ at all loci; (b) brown: *MC1R* p.Gln34*/p.Gln34*, *PMEL* +/+; (c) brown: *MC1R* +/+, *PMEL* +/p.Leu18del; (d) color-sided: Cs_{29}/Wt_{29} or Cs_6/?; (e) white-faced: S^{wf}/?; (f) all-white: Cs_6/?.

dun brown coat color in Dexter cattle (Berryere *et al.* 2002); and (3) *premelanosome protein* (*PMEL*), an essential gene for melanosome development, determines the black, dun, silver dun, white/cream, red and yellow color in Highland cattle by interacting with *MC1R* (Schmutz & Dreger 2013).

The white spotting gene in cattle has been mapped to chromosome 6 where the *v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog* gene (*KIT*) is located (Grosz & MacNeil 1999; Reinsch *et al.* 1999; Liu *et al.* 2009). Dominantly inherited color sidedness in cattle is determined by two alleles from serial translocations of *KIT* to chromosome 29 and 6: the first allele results from the translocation of a 492-kb *KIT*-encompassing segment from chromosome 6 to chromosome 29 (Cs_{29}), and a second allele is derived from the first by integration of fused 575-kb chromosome 6 and 29 sequences to the *KIT* locus on chromosome 6 (Cs_6) (Durkin *et al.* 2012). Color sidedness in domestic cattle has been dated to at least the Middle Ages and is presently segregating in several cattle breeds around the world. The overall similarity between white-spotted cattle and yaks suggests a correlation of domestic yak spotting phenotypes with the mutation or translocation of *KIT*. Interestingly, Durkin *et al.* (2012) found both *KIT* translocation alleles in color-sided domestic yaks, suggesting introgression from cattle through hybridization but requiring validation in a larger sample. In this study, we investigated the genetic basis of coat color variation in domestic yaks and proposed the likely origins of brown coloration and white spotting.

Materials and methods

Samples and DNA extraction

We obtained ear tissue samples from 92 domestic yaks from Ganzihe County, Qinghai Province, China, in May 2013. Samples were collected by owners with a v-cut ear notcher (Nasco Co.) and represented different yak stocks from four unrelated ranches. Each sampled animal was carefully examined and photographed, and coat color phenotypes were unambiguously identified. Pedigree and breeding histories associated with each animal were also interviewed and recorded. For the loci responsible for the brown coat color, samples from 60 wild-type (black or black-and-white) and 17 brown (brown or brown-and-white) yaks were collected. For the white-spotted trait, 31 solid-colored (wild type, brown or black), 24 white-faced, 20 color-sided and 15 all-white individuals were sampled. The details of yak coat color phenotypes are listed in Table S1.

Tissue samples were frozen directly and transported back to the laboratory in a liquid nitrogen dry shipper (MVE). Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (QIAGEN), following the manufacturer's instructions.

Candidate gene PCR and sequencing

In a pilot experiment, exons of the brown color candidate genes *MC1R*, *TYRP1* and *PMEL*, were amplified and sequenced in four black (wild type) and four brown yaks. The single exon of *MC1R* and the first exon of *PMEL* were further sequenced in another 56 black and 13 brown individuals. Primer sets for *MC1R*, *TYRP1* and *PMEL* (Table S2) were designed based on the latest cattle genome assembly (Btau_4.6.1), using PRIMER3 (http://frodo.wi.mit.edu/).

For the white-spotted trait, sequence variations were initially screened for all 21 exons of *KIT* in four solid-colored (wild type) and four white-faced yaks. To build haplotypes spanning the *KIT* gene in all white-faced and solid-colored individuals, five exons (exon 1, exon 3, exon 8, exon 15 and exon 21) along with their flanking regions were further sequenced in another 20 white-faced and 27 solid-colored yaks. Primers for *KIT* exons were adopted from Durkin *et al.* (2012, Table S2). Besides the coding regions, the first intron of *KIT* was also amplified and sequenced in 24 white-faced and 27 solid-colored individuals, with primers designed using PRIMER3 based on the latest cattle genome assembly (Btau_4.6.1). Haplotypes for *KIT* were then inferred for all 24 white-faced and 31 solid-colored yaks based on variations detected in the above-mentioned exonic and intronic sequences using PHASE (Stephens *et al.* 2001). *KIT* haplotypes of white-faced Hereford cattle were obtained from the latest cattle genome assembly (Btau_4.6.1) and compared with those of yaks.

All PCR and sequencing reactions for candidate genes' exons were conducted following procedures described previously (Xu *et al.* 2013). The potential effect of each nonsynonymous substitution on protein function was evaluated using multivariate analysis of protein polymorphism (MAPP; Stone & Sidow 2005). This method considers both physicochemical properties and evolutionary constraints, and a MAPP score >10 indicates a likely impact on protein function.

Amplification of intron 1 of *KIT* was performed with TaKaRa LA Taq® DNA polymerase and GC Buffer kit (TaKaRa), following the manufacturer's instructions.

Test for *KIT* serial translocations in domestic yaks

Serial translocations of *KIT* to chromosome 29 (Cs_{29} allele) and 6 (Cs_6 allele) were examined in 31 solid-colored and 59 white-spotted (20 color-sided, 15 all-white and 24 white-faced) yaks by amplifying the fusion points of each transposition. The primer sets and genotyping assay were adopted from Durkin *et al.* (2012).

Statistical test for genotype–phenotype associations

Tests of significance for association between genotypes (mutations or haplotypes) and yak coat color phenotype

were conducted using Fisher's exact test (http://vassarstats.net/textbook/ch8a.html). A dominant model was applied to test the association between *KIT* translocation mutations and the all-white and color-sidedness phenotypes and that between the *KIT* haplotypes and the white-faced phenotype. A multiple-gene inheritance model was used to test the association between *MC1R* and/or *PMEL* mutations and brown coat color, in which *MC1R* mutations were recessive and the *PMEL* mutation was dominant.

Results

MC1R and/or *PMEL* cause the brown coat color in domestic yaks

Exons of genes *TYRP1*, *MC1R* and *PMEL* were sequenced in both brown and black yaks. Three SNPs (one synonymous and two non-synonymous substitutions) were discovered in the coding sequence of *TYRP1*, but none were associated with the brown coat color in domestic yaks.

Six non-synonymous substitutions were identified from the 954-bp coding region of the *MC1R* gene in yaks, including one nonsense (p.Gln34*) and five missense substitutions (p.Met73Leu, p.Gln114Lys, p.Arg142Pro, p. Arg229His and p.Ala291Thr). Gln34 is located in the first extracellular domain of MC1R and p.Gln34* would introduce a premature stop codon truncating the protein, leading to loss of function of MC1R. MAPP analysis of the missense substitutions suggested that p.Met73Leu (score 13.5) and p.Arg142Pro (score 40.7) potentially affect the MC1R function, whereas the remaining three have no effect on MC1R (score <10, Table S3). Indeed, p.Gln114Lys and p.Ala291Thr represent previously reported polymorphisms found in yaks (Chen *et al.* 2009; Xi *et al.* 2012) and are not associated with brown coat pigmentation; Arg229 is a non-conserved amino acid residue site where a mutation is unlikely to impair MC1R's function. The Met73 residue is in the second transmembrane domain of MC1R and has been proven to be functionally important for the activation of MC1R in sheep (p.Met73Lys, Vage *et al.* 1999), suggesting that a p.Met73Leu substitution may affect MC1R's activation. Arg142 occurs in the second intracellular loop of MC1R with an essential role in maintaining MC1R's affinity for α-MSH (Schioth *et al.* 1999; Sanchez Mas *et al.* 2002), and thus, the p.Arg142Pro substitution likely reduces MC1R's affinity for α-MSH in a recessive mode.

All 59 black individuals carried at least one copy of the wild-type *MC1R* allele, whereas seven (~40%) of the 17 brown individuals were homozygous or compound heterozygous for one or both of the two *MC1R* variants, p.Gln34* and p.Met73Leu (Table 1). *MC1R* p.Arg142Pro was found in only one black yak heterozygous for the mutation in our samples, and its association with brown coloration requires further validation. In conclusion, p.Gln34*, p.Met73Leu and p.Arg142Pro may potentially affect the function of MC1R and are considered putative mutations for the brown color in yaks. However, 10 of 17 brown yaks carried no or only one *MC1R* mutant allele (+/+ or +/p.Gln34*; Table 1), implying that brown variants in domestic yaks could be caused by other loci besides *MC1R*.

Table 1 Correlation ($P = 2.62E-17$) between coat color pigmentation and mutations of *MC1R* and *PMEL* in 76 domestic yaks.

Coat color	*n*	*MC1R*[1]	*PMEL*[2]
Black (Wild type)	33	+/+	+/+
	21	+/p. Gln34*	+/+
	4	+/p.Met73Leu	+/+
	1	+/p.Arg142Pro	+/+
Brown	5	p.Gln34*/p.Gln34*	+/+
	1	p.Gln34*/p.Met73Leu	+/+
	1	p.Gln34*/p.Gln34*	+/p.Leu18del
	6	+/+	+/p.Leu18del
	4	+/p.Gln34*	+/p.Leu18del

[1]+ in this column designates the wild-type allele of *MC1R* in yaks.
[2]+ in this column designates the wild-type allele of *PMEL* in yaks.

Fourteen SNPs were detected in exons of *PMEL*, within which five were non-synonymous substitutions. None of the amino acid changes caused by these SNPs seemed to have a significant impact on the protein's function due to the non-conserved nature of these residues. However, a three-base pair deletion (c.50_52del) discovered in the first exon of *PMEL* appeared to account for the yak's brown coat color in the rest of the individuals. The c.50_52del caused a deletion of a leucine residue (p.Leu18del) from the signal peptide of PMEL. Residue Leu18 is conserved in vertebrates, suggesting an important role in maintaining PMEL function; therefore, p.Leu18del might affect PMEL and subsequent pigment biosynthesis. The same p.Leu18del deletion mutation has been reported in Highland and Galloway cattle, yielding a yellow and dun coat color in a semidominant inheritance pattern (Schmutz & Dreger 2013). All of the 10 brown yak individuals whose coat colors were not associated with the three recessive mutations found in *MC1R* were heterozygous for the *PMEL* allele exhibited by p.Leu18del, whereas all 59 black individuals were homozygous for the wild-type *PMEL* allele (Table 1). Cattle bearing the *PMEL* genotype exhibited by p.Leu18del/p.Leu18del displayed a white/cream or silver dun coat color (Schmutz & Dreger 2013), and no such yak individual homozygous for *PMEL* genotyped exhibited by p.Leu18del was found in our sample.

Taken together, the brown coat color phenotypes of domestic yaks sampled from ranches in Qinghai are explained by *MC1R* and *PMEL* alleles on solid coat color ($P = 2.62E-17$), including three SNPs in *MC1R* and/or one deletion in *PMEL* (Table 1). There is no apparent difference in coat color between the brown individuals caused by the *MC1R* alleles or *PMEL* allele. In particular, the only brown individual (QHYA0074, a white-faced brown female) carrying two copies of a null allele at *MC1R* (p.Gln34*/

p.Gln34*) and one copy of a mutant allele (+/p.Leu18del) at *PMEL* (Table 1) shows no observable difference in coat color from other brown yaks.

White spotting in yaks either is caused by serial translocations of KIT or is associated with KIT haplotypes from domestic cattle

We detected the presence of cattle alleles (Cs_6 and Cs_{29}) caused by serial translocations of *KIT* in white-spotted but not solid-colored yaks. All white ($n = 15$) or color-sided ($n = 20$) individuals were homozygous or heterozygous for at least one type of the two alleles, or both. None of the 31 solid-colored individuals carried any Cs_6 or Cs_{29} alleles (Table 2), suggesting the same serial *KIT* translocations in cattle also cause all white and color sidedness in domestic yaks in a dominant manner ($P = 1.56E\text{-}19$). The background fur pigmentation in a color-sided yak varies by individual and is determined by independent loci, showing no association with genotypes of the Cs_6 and Cs_{29} alleles. All-white individuals with different genotypes of the Cs_6 and Cs_{29} alleles showed no observable phenotypic difference in coat color. However, neither Cs_6 nor Cs_{29} alleles were identified in any of the 24 white-faced yaks (Table 2).

To determine whether the *KIT* gene is responsible for white-face coloring in domestic yak, exons of *KIT* were sequenced in four white-faced and four solid-colored yaks. Seven SNPs were detected in the coding region, only one of which was a non-synonymous substitution, resulting in a p.Met195Thr change. Met195 is not a conserved residue among mammals, and the MAPP analysis also suggested that p.Met195Thr does not likely affect KIT's function (Table S3). Therefore, we excluded the possibility that the white face in yaks is caused by changes in the coding region of *KIT*.

We examined the first intron of the *KIT* gene, reported as a regulatory element of *KIT* in mouse (Cairns *et al.* 2003), and checked whether any genome structural changes such as large indels may be responsible for the white-faced phenotype in yaks. However, no obvious length polymorphism was observed between white-faced and solid-colored individuals.

Table 2 Correlation ($P = 1.56E\text{-}19$) between all-white and color-sided phenotypes in yaks and genotypes of alleles derived from serial translocations of *KIT*.

Phenotype	$Cs_6/?^1$ Cs_{29}/Wt_{29}	$Cs_6/?$ Wt_{29}/Wt_{29}	Wt_6/Wt_6 Cs_{29}/Wt_{29}	Wt_6/Wt_6 Wt_{29}/Wt_{29}
All-white	3	12	0	0
Color-sided	3	9	8	0
White-faced	0	0	0	24
Solid-colored	0	0	0	31

Wt_6, wild-type allele at the *KIT* locus on chromosome 6; Wt_{29}, wild-type allele from the region on chromosome 29 without translocation of *KIT* from chromosome 6.

[1]The PCR assay proposed by Durkin *et al.* (2012) was not able to distinguish the homozygous or heterozygous status of Cs_6 allele.

Table 3 Association ($P = 4.76E\text{-}9$) between white-faced phenotypes and *KIT* haplotypes in yaks based upon four indels and 11 SNPs spanning 47 kb of *KIT*.

Phenotype	S^+/S^+	S^{wf}/S^+	S^{wf}/S^{wf}
Solid-colored	24	7	0
White-faced	0	14	10

Haplotypes of *KIT* from all sequenced regions, including intron 1, exon 1, exon 3, exon 8, exon 15 and exon 21, were constructed for 31 solid-colored and 24 white-faced yaks. Two haplotypes were detected based on 15 variable sites including four indels and 11 SNPs spanning 47 kb of genome (Table S4). The major allele (designated as S^+) in solid-colored yaks is a unique haplotype found only in yaks and designated as the wild type; the minor allele (designated as S^{wf}) is identical to that of white-faced Hereford cattle (*Bos taurus*, Btau_4.6.1). An association analysis indicated that S^{wf} was associated with the white-faced phenotype of yaks in a dominant manner ($P = 4.76E\text{-}9$) (Table 3).

Discussion

Genetic basis of coat color variation in domestic yaks

MC1R is the first gene known to be associated with cattle coat color and is well known for its role in regulating the switch between eumelanin and pheomelanin biosynthesis pathways in mammals. Loss of function in MC1R fails to activate downstream eumelanogenesis and leads to exclusive pheomelanin synthesis (Barsh 1996). Therefore, loss-of-function mutations in MC1R usually cause red or yellow coat/hair color, as evidenced in human, mouse, horse, cattle, pig, rabbit, dog and cat (Robbins *et al.* 1993; Klungland *et al.* 1995; Marklund *et al.* 1996; Kijas *et al.* 1998; Everts *et al.* 2000; Rees 2000; Fontanesi *et al.* 2006; Peterschmitt *et al.* 2009). However, this is not the case in domestic yaks, as *MC1R*-associated brown coloration indicates the production of a certain amount of eumelanin. In this study, we have characterized one premature stop codon in *MC1R* (p.Gln34*) that leads to loss of function in MC1R and is associated with brown coat color in yaks. In six brown yaks homozygous for p.Gln34*, although pheomelanin was greatly increased, eumelanin was still produced in some parts of the body, indicating other genes or pathways could be involved in activating eumelanogenesis.

Vage *et al.* (1999) reported p.Met73Lys in MC1R in dominant black sheep. There is pharmacological evidence that p.Met73Lys constitutively activates MC1R and directs melanin synthesis to eumelanin, which seems to contradict our observation that a mutation at the same position, p.Met73Leu, causes brown coat color in a recessive manner

in yaks. However, lysine (KLys) and leucine (Leu) residues have distinct physicochemical properties, in that lysine is positively charged and polar, whereas leucine is hydrophobic and nonpolar. Therefore, it may not be surprising to predict that p.Met73Leu found in yaks affects the function of MC1R in a way different from p.Met73Lys in sheep.

Here, p.Arg142Pro was extremely rare within the three MC1R mutations, and the only individual carrying the allele for this mutation was heterozygous for p.Arg142Pro, making it difficult to validate its association with brown coloration. Even though the functional importance of Arg142 suggests that p.Arg142Pro may impair MC1R, further studies with larger sample sizes are needed to validate the association between the MC1R mutation p.Arg142Pro and brown coat color in yaks.

Mutations in *PMEL* have been reported to cause hypopigmentation in mouse, dog, horse, cattle, chicken and zebra fish (Kwon *et al.* 1995; Kerje *et al.* 2004; Schonthaler *et al.* 2005; Brunberg *et al.* 2006; Clark *et al.* 2006; Schmutz & Dreger 2013), but its precise function remains controversial (Theos *et al.* 2005). Brunberg *et al.* (2006) found that mutations in *PMEL* affected black or dark brown colors only and suggested involvement in eumelanin synthesis exclusively, with no or little effect on the pheomelanin pathway. By contrast, Schmutz and Dreger (2013) discovered a dose-dependent dilution effect of *PMEL* mutations (c.50_52del or p.Leu18del) on coat color in Highland and Galloway cattle. Heterozygotes for the *PMEL* deletion mutation (+/p.Leu18del) experience only a reduction in eumelanin, showing yellow or dun coat color, yet homozygous cattle (p.Leu18del/p.Leu18del) have a silver dun or white/cream coat, evidence of the inhibitory effect on both eumelanin and pheomelanin pigmentation. We found a large portion (10 of 17, or 60%) of brown yaks were caused by the same *PMEL* deletion reported in Highland and Galloway cattle and confirmed the eumelanin dilution effect of *PMEL* deletion mutation. However, given that all domestic yaks with the *PMEL* deletion were heterozygotes in this study, the phenotypic effect of the homozygous genotype (p.Leu18del/p.Leu18del) at the *PMEL* locus on yaks requires further study. In addition, yaks carrying either *MC1R* mutations (homozygous or compound heterozygous) or the *PMEL* deletion mutation show indistinguishable brown color, which is different from the case in cattle (Schmutz & Dreger 2013).

Durkin *et al.* (2012) reported that the serial translocation of *KIT* from chromosome 6 to chromosome 29 (Cs_{29} allele) and repartition to chromosome 6 (Cs_6 allele) were responsible for color sidedness in domestic cattle (nine breeds) and yaks. Brenig *et al.* (2013) confirmed the presence of the Cs_{29} allele in White Galloway and White Park cattle and suggested a dose-dependent effect of Cs_{29}, in which heterozygous (Wt_{29}/Cs_{29}) individuals exhibited variable degrees of pigmented spots on the white body trunk, whereas homozygous (Cs_{29}/Cs_{29}) individuals produced no pigmentation on the body trunk.

In this study, we confirm that Cs_6 and Cs_{29} are responsible for color sidedness in domestic yaks and also propose the causal effect of Cs_6 and Cs_{29} on the all-white phenotype in yaks. No obvious difference was observed among color-sided individuals carrying either one type of the *Cs* allele or both, suggesting Cs_6 and Cs_{29} equally affected pigmentation without an additive effect due to coexistence. The 15 all-white individuals were Cs_6 carriers, but because the current PCR assay (Durkin *et al.* 2012) cannot differentiate the homozygous from heterozygous status of the Cs_6 allele, the precise genotype of these white individuals was unknown. However, the facts that breeding between white yaks consistently produces all-white offspring and the Tianzhu yak breed is fixed with all-white coat color indicate a homozygous status at the mutant locus. We hypothesize that all-white individuals in this study are homozygous for the Cs_6 allele. It is highly likely that a Cs_{29}/Cs_{29} yak is white as well, although larger samples are necessary for validation. In conclusion, we suggest that heterozygotes for Cs_6 and/or Cs_{29} may have caused color sidedness and homozygosity results in the all-white phenotype in domestic yaks.

The origin of coat color variation in domestic yaks

Crossing of yaks to cattle has been common to improve yak stock, with the earliest records dating back 3000 years ago (Zhang 1989, 2000; Wiener *et al.* 2003). The F1 offspring of crossing a yak and local Chinese yellow cattle (*Bos taurus*) is named the Chinese yakow (Xi *et al.* 2012). More recently, Western breeds, such as Holstein-Friesian, Simmental, Hereford, Angus, Limousin, Charolais and Highland cattle, were also used in the hybridization program (Zhang 2000; Wiener *et al.* 2003). Crossbreeding practices improved productivity of domestic yaks (i.e., better heat tolerance and enhanced milk yield) but introduced unexpected traits, such as coat color variants.

Durkin *et al.* (2012) suggested that the cattle color-sided alleles of *KIT* translocation (Cs_6 and Cs_{29}) were introduced into the domestic yak population through interspecific hybridization. In this study, we proved that Cs_6 and Cs_{29} are responsible for the color-sidedness and white phenotypes of domestic yaks, providing further evidence to support the origin of color-sidedness (and its related) phenotypes in yaks via hybridization with cattle.

Our data also demonstrated the association of the *KIT* allele S^{wf} with the white-faced phenotype in yaks. Intriguingly, S^{wf} was identical to the *KIT* haplotype found in Hereford cattle (data from genome assembly Btau_4.6.1), the coat color of which is typically white face. Therefore, we propose the white-faced phenotype in domestic yak is also derived from hybridization with cattle.

The brown coat color of domestic yaks in our sample is caused by *PMEL* and *MC1R* mutations. The *PMEL* mutation (p.Leu18del) found in yaks is identical to that of Highland

cattle, a breed that has been well documented in crossbreeding records, suggesting the role of hybridization in introducing genetic and phenotypic variation from cattle to yaks.

By contrast, the three *MC1R* mutations (p.Gln34*, p.Met73Leu and p.Arg142Pro) identified from yaks in this study have not been reported in cattle. The three classical bovine *MC1R* alleles, the wild-type E^+ allele for producing both eumelanin and pheomelanin, the E^D (p.Leu99Pro) allele for dominant black and the *e* allele (p.Tyr155*) for recessive red coat color, were not retrieved from our samples, consistent with previous surveys (Chen *et al.* 2009; Xi *et al.* 2012). Furthermore, local records have reported occasional sightings of 'yellow yaks' in wild yak populations, which may represent the same brown pigmentation in the study and indicate its natural existence (Zhang 1989; Wiener *et al.* 2003). We therefore suggest that the *MC1R* mutations accounting for brown coat color in domestic yaks are natural polymorphisms inherited from their wild ancestor or derived during the process of domestication.

In summary, we have determined the genetic basis of the major coat color variants in domestic yaks and proposed multiple genetic origins of such variation either naturally from yaks or artificially from cattle through interspecific hybridization. The brown coat color is caused by mutations of either *MC1R* or *PMEL*, the all-white and color-sided phenotypes are caused by the serial translocations of *KIT* and the white-faced phenotype is strongly associated with the *KIT* haplotype S^{wf}. Except for *MC1R*, all causal mutations found in *PMEL* and *KIT* of domestic yaks are identical to those in cattle, indicating the genetic origins of brown color and white spotting from cattle/yak crossbreeding. Brown color variants caused by *MC1R* are native of yaks; however, whether these were natural polymorphisms in pre-domesticated wild yaks or originated from the process of domestication requires further work.

Accession numbers

Sequences of *MC1R*, *PMEL*, *TYRP1* and *KIT* from domestic yaks discussed in this study have been deposited in NCBI GenBank under the accession numbers KF790926–KF790935.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. List of yaks ($N = 92$) used in the study with corresponding coat color phenotypes and genetic variants.

Table S2. Primers for candidate gene amplification and sequencing.

Table S3. MAPP analysis for the missense mutations found in yak *MC1R* and *KIT*.

Table S4. The *KIT* haplotypes found in domestic yaks from this study.

Table S1. List of yaks (n = 92) used in the study with corresponding coat color phenotypes and genetic variants.

		Coat Color Phenotypes		Genotypes or Haplotypes				Gene Segments Sequenced				
Animal ID	**Sex**	**white spotting**	**brown**	***MC1R***	***PMEL***	***KIT***	***KIT* trans-locations**	***MC1R***	***PMEL***	***TYRP1***	***KIT***	**Owner[5]**
QHYA0001	M	all-white[4]	N/A	N/A	N/A	N/A	$Cs_6/?$	N/A	N/A	N/A	N/A	Mucuo Jian, Dayu
QHYA0002	F	color-sided	wild-type[2]	+/p.Q34*	+/+	N/A	$Cs_6/?$	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai
QHYA0003	M	wild-type[1]	wild-type	+/p.Q34*	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0004	F	color-sided	wild-type	+/p.Q34*	+/+	N/A	$Cs_{29}/+$	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai
QHYA0005	M	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exons 1-11	exons 1-7	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0006	F	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0007	F	wild-type	wild-type	+/p.Q34*	N/A	S^+/S^+	+/+	exon 1	N/A	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0008	M	wild-type	wild-type	+/p.Q34*	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0009	F	wild-type	wild-type	+/p.Q34*	+/+	S^+/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0010	F	wild-type	wild-type	+/+	+/+	S^+/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0013	F	color-sided	wild-type	+/+	+/+	N/A	$Cs_{29}/+$	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai
QHYA0014	F	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exons 1-11	exons 1-7	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai

		Coat Color Phenotypes		Genotypes or Haplotypes				Gene Segments Sequenced				
Animal ID	**Sex**	**white spotting**	**brown**	***MC1R***	***PMEL***	***KIT***	***KIT* trans-locations**	***MC1R***	***PMEL***	***TYRP1***	***KIT***	**Owner[5]**
QHYA0015	F	wild-type	wild-type	+/p.Q34*	+/+	S^{+}/S^{+}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0016	F	wild-type	wild-type	+/p.Q34*	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0017	F	color-sided	wild-type	+/p.Q34*	+/+	N/A	Cs_{29}/+	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai
QHYA0018	M	white-faced	wild-type	+/p.Q34*	+/+	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0019	F	wild-type	wild-type	+/+	+/+	S^{+}/S^{wf}	+/+	exon 1	exons 1-11	exons 1-7	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0020	M	white-faced	brown	p.Q34*/p.Q34*	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0021	M	wild-type	wild-type	+/+	+/+	S^{+}/S^{+}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0022	M	white-faced	wild-type	+/p.Q34*	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0023	F	all-white	N/A	N/A	N/A	N/A	Cs_{6}/?	N/A	N/A	N/A	N/A	Shijia Yang, Gahai
QHYA0024	F	color-sided	brown	p.Q34*/p.M73L	+/+	N/A	Cs_{6}/?	exon 1	exons 1-11	exons 1-7	N/A	Shijia Yang, Gahai
QHYA0025	F	wild-type	wild-type	+/+	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0026	F	all-white	N/A	N/A	N/A	N/A	Cs_{6}/?	N/A	N/A	N/A	N/A	Shijia Yang, Gahai
QHYA0027	F	color-sided	wild-type	+/p.M73L	+/+	N/A	Cs_{6}/?	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai
QHYA0028	M	all-white	N/A	N/A	N/A	N/A	Cs_{6}/?	N/A	N/A	N/A	N/A	Shijia Yang, Gahai

		Coat Color Phenotypes		Genotypes or Haplotypes				Gene Segments Sequenced				
Animal ID	**Sex**	**white spotting**	**brown**	***MC1R***	***PMEL***	***KIT***	***KIT* trans-locations**	***MC1R***	***PMEL***	***TYRP1***	***KIT***	**Owner[5]**
QHYA0029	M	white-faced	wild-type	+/+	+/+	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0030	F	white-faced	wild-type	+/+	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1-21, partial intron 1	Shijia Yang, Gahai
QHYA0031	F	white-faced	wild-type	+/+	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1-21, partial intron 1	Shijia Yang, Gahai
QHYA0032	M	white-faced	wild-type	+/+	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0033	M	wild-type	wild-type	+/p.Q34*	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0034	F	white-faced	wild-type	+/+	+/+	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0035	F	white-faced	wild-type	+/+	+/+	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0036	F	white-faced	wild-type	+/+	+/+	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0037	F	color-sided	wild-type	+/p.Q34*	+/+	N/A	Cs_{29}/+	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai
QHYA0038	M	white-faced	wild-type	+/+	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0039	M	color-sided	wild-type	+/p.M73L	+/+	N/A	Cs_{6}/?	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai
QHYA0040	M	color-sided	wild-type	+/+	+/+	N/A	Cs_{6}/?, Cs_{29}/+	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai

		Coat Color Phenotypes		Genotypes or Haplotypes				Gene Segments Sequenced				
Animal ID	**Sex**	**white spotting**	**brown**	***MC1R***	***PMEL***	***KIT***	***KIT* trans-locations**	***MC1R***	***PMEL***	***TYRP1***	***KIT***	**Owner[5]**
QHYA0041	F	wild-type	wild-type	+/p.Q34*	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0042	M	wild-type	wild-type	N/A	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0043	M	wild-type	wild-type	+/p.Q34*	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1-21, partial intron 1	Shijia Yang, Gahai
QHYA0044	F	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0045	M	wild-type	wild-type	+/p.Q34*	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1-21, partial intron 1	Shijia Yang, Gahai
QHYA0046	F	wild-type	wild-type	+/p.Q34*	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0047	F	wild-type	wild-type	+/p.Q34*	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0048	F	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0049	M	wild-type	wild-type	+/p.M73L	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1-21, partial intron 1	Shijia Yang, Gahai
QHYA0050	F	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0051	F	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai

		Coat Color Phenotypes		Genotypes or Haplotypes				Gene Segments Sequenced				
Animal ID	**Sex**	**white spotting**	**brown**	***MC1R***	***PMEL***	***KIT***	***KIT* trans-locations**	***MC1R***	***PMEL***	***TYRP1***	***KIT***	**Owner[5]**
QHYA0052	F	ambiguous[3]	wild-type	+/p.Q34*	+/+	N/A	+/+	exon 1	exon 1	N/A	N/A	Shijia Yang, Gahai
QHYA0053	M	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0054	M	wild-type	wild-type	+/p.R142P	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shijia Yang, Gahai
QHYA0055	F	wild-type	wild-type	+/+	+/+	S^+/S^+	+/+	exon 1	exon 1	N/A	exon 1-21, partial intron 1	Shijia Yang, Gahai
QHYA0056	F	all-white	N/A	N/A	N/A	N/A	$Cs_6/?$, $Cs_{29}/+$	N/A	N/A	N/A	N/A	Rijie Duo, Gahai
QHYA0057	M	white-faced	brown	+/+	+/p.L18del	S^+/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Rijie Duo, Gahai
QHYA0058	F	color-sided	brown	+/+	+/p.L18del	N/A	$Cs_{29}/+$	exon 1	exon 1	N/A	N/A	Rijie Duo, Gahai
QHYA0059	F	white-faced	brown	+/+	+/p.L18del	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Rijie Duo, Gahai
QHYA0060	F	color-sided	brown	+/+	+/p.L18del	N/A	$Cs_6/?$	exon 1	exon 1	N/A	N/A	Rijie Duo, Gahai
QHYA0061	M	color-sided	wild-type	+/+	+/+	N/A	$Cs_6/?$	exon 1	exon 1	N/A	N/A	Rijie Duo, Gahai
QHYA0062	M	white-faced	wild-type	+/+	+/+	S^+/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1-21, partial intron 1	Rijie Duo, Gahai
QHYA0063	F	color-sided	wild-type	+/+	N/A	N/A	$Cs_{29}/+$	exon 1	N/A	N/A	N/A	Rijie Duo, Gahai
QHYA0064	NA	white-faced	wild-type	+/+	+/+	S^+/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1-21, partial intron 1	Rijie Duo, Gahai
QHYA0065	F	color-sided	wild-type	+/+	+/+	N/A	$Cs_6/?$	exon 1	exon 1	N/A	N/A	Rijie Duo, Gahai
QHYA0066	F	color-sided	wild-type	+/+	+/+	N/A	$Cs_6/?$, $Cs_{29}/+$	exon 1	exon 1	N/A	N/A	Rijie Duo, Gahai
QHYA0067	F	white-faced	brown	+/+	+/p.L18del	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Rijie Duo, Gahai
QHYA0068	F	white-faced	brown	+/+	+/p.L18del	S^+/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Rijie Duo, Gahai

		Coat Color Phenotypes		Genotypes or Haplotypes				Gene Segments Sequenced				
Animal ID	**Sex**	**white spotting**	**brown**	***MC1R***	***PMEL***	***KIT***	***KIT* trans-locations**	***MC1R***	***PMEL***	***TYRP1***	***KIT***	**Owner[5]**
QHYA0069	F	white-faced	wild-type	+/+	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Rijie Duo, Gahai
QHYA0070	F	all-white	N/A	N/A	N/A	N/A	$Cs_{6}/?$	N/A	N/A	N/A	N/A	Rijie Duo, Gahai
QHYA0071	F	all-white	N/A	N/A	N/A	N/A	$Cs_{6}/?$, $Cs_{29}/+$	N/A	N/A	N/A	N/A	Rijie Duo, Gahai
QHYA0072	M	color-sided	brown	p.Q34*/p.Q34*	+/+	N/A	$Cs_{29}/+$	exon 1	exons 1-11	exons 1-7	N/A	Rijie Duo, Gahai
QHYA0073	F	color-sided	brown	p.Q34*/p.Q34*	+/+	N/A	$Cs_{6}/?$	exon 1	exon 1	N/A	N/A	Rijie Duo, Gahai
QHYA0074	F	white-faced	brown	p.Q34*/p.Q34*	+/p.L18del	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Rijie Duo, Gahai
QHYA0075	F	white-faced	wild-type	+/p.Q34*	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Rijie Duo, Gahai
QHYA0076	F	white-faced	wild-type	+/+	+/+	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Rijie Duo, Gahai
QHYA0077	F	color-sided	wild-type	+/p.Q34*	+/+	N/A	$Cs_{6}/?$, $Cs_{29}/+$	exon 1	exon 1	N/A	N/A	Rijie Duo, Gahai
QHYA0078	F	all-white	N/A	N/A	N/A	N/A	$Cs_{6}/?$	N/A	N/A	N/A	N/A	Shengfu Li, Gahai
QHYA0079	F	all-white	N/A	N/A	N/A	N/A	$Cs_{6}/?$	N/A	N/A	N/A	N/A	Shengfu Li, Gahai
QHYA0080	F	all-white	N/A	N/A	N/A	N/A	$Cs_{6}/?$	N/A	N/A	N/A	N/A	Shengfu Li, Gahai
QHYA0081	M	all-white	N/A	N/A	N/A	N/A	$Cs_{6}/?$	N/A	N/A	N/A	N/A	Shengfu Li, Gahai
QHYA0082	M	all-white	N/A	N/A	N/A	N/A	$Cs_{6}/?$	N/A	N/A	N/A	N/A	Shengfu Li, Gahai
QHYA0083	F	all-white	N/A	N/A	N/A	N/A	$Cs_{6}/?$	N/A	N/A	N/A	N/A	Shengfu Li, Gahai
QHYA0084	F	wild-type	wild-type	+/+	+/+	S^{+}/S^{+}	+/+	exon 1	exons 1-11	exons 1-7	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Shengfu Li, Gahai
QHYA0085	M	color-sided	wild-type	+/+	+/+	N/A	$Cs_{29}/+$	exon 1	exon 1	N/A	N/A	Rijie Duo, Gahai
QHYA0086	F	wild-type	brown	+/p.Q34*	+/p.L18del	S^{+}/S^{+}	+/+	exon 1	exons 1-11	exons 1-7	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Yucai Da & Hedong Yong, Dayu

		Coat Color Phenotypes		Genotypes or Haplotypes				Gene Segments Sequenced				
Animal ID	**Sex**	**white spotting**	**brown**	***MC1R***	***PMEL***	***KIT***	***KIT* trans-locations**	***MC1R***	***PMEL***	***TYRP1***	***KIT***	**Owner[5]**
QHYA0087	M	all-white	N/A	N/A	N/A	N/A	$Cs_6/?$, $Cs_{29}/+$	N/A	N/A	N/A	N/A	Yucai Da & Hedong Yong, Dayu
QHYA0089	M	all-white	N/A	N/A	N/A	N/A	$Cs_6/?$	N/A	N/A	N/A	N/A	Yucai Da & Hedong Yong, Dayu
QHYA0090	F	white-faced	brown	p.Q34*/p.Q34*	+/+	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Yucai Da & Hedong Yong, Dayu
QHYA0091	F	white-faced	brown	p.Q34*/p.Q34*	+/+	S^{wf}/S^{wf}	+/+	exon 1	exons 1-11	exons 1-7	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Yucai Da & Hedong Yong, Dayu
QHYA0092	F	white-faced	brown	+/p.Q34*	+/p.L18del	S^{wf}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Yucai Da & Hedong Yong, Dayu
QHYA0093	F	ambiguous[3]	brown	+/p.Q34*	+/p.L18del	N/A	+/+	exon 1	exon 1	N/A	N/A	Yucai Da & Hedong Yong, Dayu
QHYA0094	F	wild-type	brown	+/p.Q34*	+/p.L18del	S^{+}/S^{wf}	+/+	exon 1	exon 1	N/A	exon 1, exon 3, exon 8, exon 15, exon 21, partial intron 1	Yucai Da & Hedong Yong, Dayu
QHYA0095	F	color-sided	wild-type	+/p.M73L	+/+	N/A	$Cs_6/?$	exon 1	exon 1	N/A	N/A	Mucuo Jian, Dayu

1. The wild type of the *white spotting* loci refers to a solid-background coat color.
2. The wild type of brown phenotype refers to the presence of black coat color, including solid black and black-and-white coat color.
3. Individuals QHYA0052 and QHYA0093 were ambiguous for the *white spotting* phenotypes and excluded from genotyping for *KIT*.
4. The yak has all-white coat color at the *white spotting* locus that masks the brown phenotype, thus the brown candidate genes were not sequenced.
5. All yak farms are located in Ganzihe, Haibei County, Qinghai Province, China.

Table S2. Primers for candidate gene amplification and sequencing.

Amplicon	Size(bp)	Forward primer (5'-)	Reverse primer (5'-)	Reference
Tyrp1 ex1	785	ACGTGCCTCGGTCTCTACAC	GAGTTCCATGCAGGACTGTG	this study
Tyrp1 ex2	723	TTCACATGGCAGATGTTTTCA	ATGTGGCCATGTCTCATGC	this study
Tyrp1 ex3	605	GGGAGCATTTTAAACAAAAGCA	GGGAATTCCAAATCCTAGTTGTC	this study
Tyrp1 ex4	568	TGTGTCCTCATACCCCCTCT	TCAATTCAGAGCACCAGTTTTG	this study
Tyrp1 ex5	580	TCCCAATATAATTACTGCTTTAGACTT	GCTGAGTTTGCAAAAAGCAT	this study
Tyrp1 ex6	547	TGTCTATTAACAAGGTGTCTTTGACAT	AAAGGTAACAAGTTTGATTTTGGAA	this study
Tyrp1 ex7	606	TGTCAAGTGCCTCGAACAAC	TGTGGTTTTTATGATTCAAATGC	this study
Mc1r ex1_1	1052	AGTTGAGCAGGACCCTGAGA	AGGACGATGAGCGAGAGGT	this study
Mc1r ex1_2	702	CCTGATGGCCGTCCTCTAC	ACTGCCCTGGCCTCACAG	this study
Pmel ex1	150	GTCTTTGGTTGCTGGAAGGA	GCAACCCCAAATTCACACTT	this study
Pmel ex2-3	498	CAGACACTGTTGTCCCCTGA	GAAGGAGGCGGAGAGGTACT	this study
Pmel ex4-5	850	GTATTCCTGGAGCCCCTCTC	CTCTTTCCCCTTTCCTGTCC	this study
Pmel ex6	812	AGCTTGGGTTGGTTGAAAAA	TGCCCCACCTCTATGAACTC	this study
Pmel ex7-8	600	TTGCCTCTTTAATCCTCTCCTC	ATGCAGGCTTCCTTGGGTAG	this study
Pmel ex9-10	627	AGTGTCCCTTCCCAGATTCC	GTAAAGGAGTGGGAGCAGGA	this study
Pmel in10-ex11	841	CAGCTTTGTTGCTTGCATCT	CTTGCTCTCCACCTGGGTAA	this study
Kit in1	1966	TTGATCCTGCTGAGCATCTG	AGCTGCCCAGCTATTTATGG	this study
Kit ex1	928	CTGGGCTCAGCCTTCTACC	TCCTGAAAGACTCGCAGCTC	Durkin *et al.* (2012)
Kit ex1-short[1]	596	CTCGAAAGAACAGGGGTCAG	CCTCACAAAAGCAGCCCTAA	this study
Kit ex2	743	GGAAACTTGACCCCGTTGTA	CATACCCGAAGCCACTATGC	Durkin *et al.* (2012)
Kit ex3	500	CCGAAAGGCAACGTCTTAGAT	ATTTTGAGGCTGGGAGAACC	Durkin *et al.* (2012)
Kit ex4	516	CATGGCTGAGGAAAAATGGT	GTGCTATGCAATGGGGAAAT	Durkin *et al.* (2012)
Kit ex5	630	GCACTGCAGAGAATTTGGAA	TTGCTTTTGTGCTCTGGTTG	Durkin *et al.* (2012)
Kit ex6	533	TCTTTCCGTTTCATTCTGCTG	AGCCCCAAACTTCCTTCTGT	Durkin *et al.* (2012)
Kit ex7	624	GAGGCTGAACAGAGGACCAG	TCATGTGGTCAGCGAATTGT	Durkin *et al.* (2012)
Kit ex8	786	GGAGCTTCAGCATCTTCACC	TCTACCTGCAGGCTGGAAAT	Durkin *et al.* (2012)
Kit ex9	342	CCGATGCCTTCAGTTGATTT	GCCAGTGATGGAATGGACTT	Durkin *et al.* (2012)
Kit ex10-11	459	TGGAGGTGAGAGGTGTTGTG	CTAAAGGCAATGCGATGTGA	Durkin *et al.* (2012)
Kit ex12-13	458	CCACCACCACCATTTATTCC	CCATTTGGGTCAAAATCCTG	Durkin *et al.* (2012)
Kit ex14	862	CTGACCCCTAATCAGGCAGA	GCCTTTCCCATGTTCCCTAT	Durkin *et al.* (2012)
Kit ex15	562	ATAGCCTGCCTCTCACATGC	CAGTGACAACACCACCAAGG	Durkin *et al.* (2012)
Kit ex16	378	TTCAGCACCTTCCTGTCCTT	TCAAGCGACACTCTGCATTC	Durkin *et al.* (2012)
Kit ex17	567	GGCACCGAATGGTTTAAATG	TTCTCCTGCTGTGACCTTCA	Durkin *et al.* (2012)
Kit ex18-19	508	TTGGATCTTTTGTGCTTCCA	GCGACCGAAATAACATTTGC	Durkin *et al.* (2012)
Kit ex20	494	GTAAGGGCCCAGATGTCCTT	CCAAGAGAATGGAGGTCCTG	Durkin *et al.* (2012)
Kit ex21	791	CATTCCAGCAGAAAAGCACA	TTTCCGCATCAAGGGATAAG	Durkin *et al.* (2012)

1.Primers for *Kit* ex1-short was re-designed to amplify a shorter fragment spanning the full exon 1 of *Kit*, according to the sequence of *Kit* ex1, which includes both exon 1 and flanking regions.

Table S3. MAPP analysis for the missense mutations found in yak *MC1R* and *KIT*.

Gene	Mutation	MAPP score[1]
MC1R	p.M73L	13.49
	p.Q114K	2.96
	p.R142P	40.70
	p.R229H	2.98
	p.A291T	8.62
KIT	p.M195T	8.33

1. A MAPP score > 10 suggests a likely impact of the mutation on protein function.

Table S4. The *KIT* haplotypes found in domestic yaks from this study.

	Intron 1									Exon 3	Intron 3	Intron 8		Intron 15	Intron 20
Position[1]	**72744124**	**72744159**	**72744160**	**72744162**	**72744209**	**72744255**	**72744262**	**72744283**	**72744334**	**72748070**	**72748262**	**72777273**	**72777285**	**72784776**	**72791146**
S^{wf}	C	T	G	A	C	A	A	A	C	A	…..	G	T	TTC	T
S^{+}	T	G	A	G	T	G	G	G	T	G	TTCTC	.	.	…	G

1. Nucleotide coordinates follow bovine chr6 on *btau_4.6.1*