Identification of Genes Related to White and Black Plumage Formation by RNA-Seq from White and Black Feather Bulbs in Ducks

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Abstract

To elucidate the genes involved in the formation of white and black plumage in ducks, RNA from white and black feather bulbs of an F2 population were analyzed using RNA-Seq. A total of 2,642 expressed sequence tags showed significant differential expression between white and black feather bulbs. Among these tags, 186 matched 133 annotated genes that grouped into 94 pathways. A number of genes controlling melanogenesis showed differential expression between the two types of feather bulbs. This differential expression was confirmed by qPCR analysis and demonstrated that Tyr (Tyrosinase) and Tyrp1 (Tyrosinase-related protein-1) were expressed not in W-W (white feather bulb from white dorsal plumage) and W-WB (white feather bulb from white-black dorsal plumage) but in B-B (black feather bulb from black dorsal plumage) and B-WB (black feather bulb from white-black dorsal plumage) feather bulbs. Tyrp2 (Tyrosinase-related protein-2) gene did not show expression in the four types of feather bulbs but expressed in retina. C-kit (The tyrosine kinase receptor) expressed in all of the samples but the relative mRNA expression in B-B or B-WB was approximately 10 fold higher than that in W-W or W-WB. Additionally, only one of the two Mitf isofoms was associated with plumage color determination. Downregulation of c-Kit and Mitf in feather bulbs may be the cause of white plumage in the duck.

Introduction

Identification of genes controlling plumage color and their associated inheritance patterns are important topics in poultry science research. Plumage color control is essential for the uniform appearance of birds in the poultry industry. White plumage is the most favorable color for producers of meat-type commercial birds and is associated with loss of pigmentation and patterning, i.e., white spotting in both dogs and cattle [5–7] as opposed to hyperpigmentation, which in the Silky was recently shown that the higher expression of Mitf is a downstream effect of increased EDN3 expression [8]. Higher expression of Mitf, which is associated with hyperpigmentation, was observed in Silky Fowl [9]. A stop codon caused by a 2-bp deletion in exon 11 of Mitf was found to be responsible for the “silver” plumage color in Japanese quail [10]. Mitf expression can be regulated by Sf-Kit signaling and can itself activate the transcription of the Tyr genes [11,12]. c-Kit is required during the feather growth cycle for melanocyte activation in humans [13]. Mutations in c-Kit can cause coat color change in mammals [14–16]. Allele-specific genetic interactions between Mitf and c-Kit were also reported to affect melanocyte development in humans [17]. The expression pattern of c-Kit was investigated during embryonic development in chicken and quail [18,19]. Mutations in other genes were also found to be associated with plumage color in these systems. Gunnarsson et al. [20] reported an 8.3-kb deletion upstream of Sox10 that caused dark-brown plumage in chickens. Other genes, including Mc1r, Asip, and Pmel17, also contribute to plumage color [21–23]. Few recent studies have focused on the genetic mechanisms involved in duck plumage color formation. High-throughput genomic approaches are promising ways to identify genes and pathways involved in plumage color formation. Due to the unavailability of an assembled reference sequences, high-throughput expression tools...
have not been widely used in ducks, although one study used chicken microarrays for genome-wide expression analysis to identify genes related to sperm storage [24].

The white Laimheng is an egg-type duck and white Kaiya is a meat-type duck in South China. In our previous study, 80% of individuals in an F1 population from a Kaiya × Laimheng cross had a phenotype of grey plumage on their heads, wings, backs or tails, with a white belt running from neck to chest. The F2 population was segregated, individuals with white, black, and black-white plumage were found. We reported a new autosomal locus (designated T) that may control plumage color in ducks [25]. However, the identity and number of genes involved in plumage color control in these ducks is not clear.

This study is the first genome-wide expression analysis to use RNA-Seq to find differentially expressed genes related to black and white plumage color in ducks. A large number of genes was found to be differentially expressed between white and black feather bulbs. Our analysis found that important genes and pathways associated with pigment formation are differentially regulated between black and white feather bulbs. We further characterized the expression of a few key genes related to pigmentation.

Results

Overview of RNA-Seq Data

To maximize the coverage of duck feather bulb mRNA by RNA sequencing, libraries were constructed by pooling RNA isolated from 6 white feather bulbs (3 from white dorsal plumage and the other 3 from white-black dorsal plumage) as sample W-1 library, and 6 black feather bulbs (3 from black dorsal plumage and the other 3 from white-black dorsal plumage) as sample B-1 library. RNA-Seq yielded 5,000,000 raw reads from each library. Low-quality reads (i.e., tags containing only adaptors and ambiguously called bases (reads that has many Ns)) were removed, resulting in 4,887,399 and 4,867,376 clean tags, 217,133 and 235,874 of which were distinct (i.e., non-identical), from white and black feather bulbs, respectively. These distinct clean tags were mapped to 9,009 and 8,498 genes in Ensemble Gallus gallus databases, and 1,458 and 1,584 genes in an Anas platyrhynchos EST library for W-1 and B-1 libraries, respectively. In total, 10,467 and 10,082 distinct clean tags were mapped to genes, accounting for 4.43% and 4.64% of the total distinct clean tags in the white and black feather bulb RNA libraries, respectively. A summary of sequencing tags and matched genes is shown in Table 1.

Tag reads analysis showed that more than 83% of the tags were present in 1 to 5 reads, while less than 2% of the tags were present in more than 100 reads. Tags with different numbers of reads between black and white feather bulb libraries matched 5,240 annotated genes. Details of these genes and their related sequence counts in the W-1 and B-1 libraries are listed in Table S1.

Differentially Expressed Genes in White and Black Feather Bulbs

In this study, a rigorous algorithm was used to identify differentially expressed genes in the two samples based on "The significance of digital gene expression profiles" [26]. A total of 2,642 tags were found to be differentially expressed (DETs). Among these tags, only 186 mapped to annotated genes, yielding 133 differentially expressed genes (log2Ratio > 1, P < 0.01, FDR < 0.001) (see Tables S2 and S3) [26,27]. Compared to black feather bulbs, white feather bulbs showed 82 downregulated and 51 upregulated genes according to statistical criteria for raw reads and TMP (number of transcripts per million clean tags).

Gene Ontology Analysis of Differentially Expressed Genes

These 133 genes that are differentially expressed between white and black feather bulbs could be grouped into 94 pathways by gene ontology (GO) analysis. The pathways and differentially expressed genes between the two types of feather bulbs are shown in Table S4. Among the pathways, melanogenesis (c-KIT/Tyr/Tyrp1) and tyrosine metabolism (Tyr/Tyrp1) were directly related to bird plumage pigmentation. A summary of this pathway analysis is shown in Table 2. GO analysis also identified the MAPK (Mitogen-Activated Protein Kinase) signaling pathway, which can link the functions of KIT and Mitf. Differential expression of the Mitf isoforms is dependent on the activation of MEK1-ERK2 in the MAPK signaling pathway [28]. Interestingly, GO analysis also showed enrichment of pathways involving p53 signaling, apoptosis, Toll-like receptor signaling and immune function. Compared to unpigmented feather bulbs, pigmented feather bulbs have normal melanocytes, in which a series gene cooperated with each other to perform melanogenesis, melanin formation and transportation. Defects occurring in any part of this process may cause feather unpigmentation. The melanogenic pathway which involved in melanogenesis and Tyrosine metabolism was also enriched in the 94 pathways in GO analysis. In addition, we found that pigmented and unpigmented feather bulbs may have differences in many physiological and biochemical processes, including apoptosis, cell cycle, immune response, metabolism, and signaling transduction.

Table 1. RNA-Seq data summary and annotation results.

| RNA-Seq sample | Black feather bulb (B-1) | White feather bulb (W-1) |
|----------------|--------------------------|--------------------------|
| Total tags (raw data) | 5,000,000 | 5,000,000 |
| Clean tags | 4,887,399 | 4,867,376 |
| Total distinct clean tags | 217,133 | 235,874 |
| Mapping to gene | Reference | Chicken | Duck | Chicken | Duck |
| DCT | 6,498 | 1,584 | 9,009 | 1,458 |
| Total | 10,082 | 10,467 |
| % of TDCT | 4.64% | 4.43% |
| Total unknown DCT | 207,051 | 225,407 |
| % unknown of TDCT | 95.36% | 95.36% |

Note: DCT- distinct clean tag; TDCT- Total distinct clean tag; Chicken- Gallus gallus; Duck- Anas platyrhynchos.

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etc. It is a complicated regulation network. It could be that genes involved in several processes including melanogenesis and immunity are co-expressed. However, earlier studies showed that some of these pathways may be related to pigmentation [29], which is similar to our results. For example, up-regulation of genes in the Toll like receptor signaling pathway can be associated with melanocytes cell growth and melanogenesis [30]. Our result may provide further evidence for a relationship between TLRs and melanogenesis.

qPCR Confirmation of Differential Gene Expression in White and Black Feather Bulbs from Different Types of Ducks

To confirm the differential gene expression from the RNA-Seq data, we used qPCR to measure the expression of Tyr, Tyrp1, and c-Kit, three genes in the melanogenesis pathway, in four combinations of feather bulbs and plumage types: W-W (white feather bulb from white plumage), W-WB (white feather bulb from white-black plumage), B-B (black feather bulb from black plumage), and B-WB (black feather bulb from white-black plumage). The results showed that the two critical genes in the melanogenesis pathway, Tyr and Tyrp1, had almost no expression in white feather bulbs from either white dorsal plumage or white-black dorsal plumage (Figure 1). We also found that Tyr and Tyrp1 were normally expressed in black feather bulbs from either black dorsal plumage or white-black dorsal plumage. The expression or absence of expression of Tyr and Tyrp1 genes indicated the pigmentation status of the feathers, regardless of whether the feather is from ducks with white, black or white-black plumages. C-Kit expression was significantly different (P<0.01, Figure 2) between W-W and B-B, or B-WB, as well as between W-WB and B-B, or B-WB samples. In contrast, c-Kit expression showed no significant difference between W-W and W-WB or between B-B and B-WB samples. C-Kit mRNA expression is approximately 10-fold higher in B-B and B-WB compared to W-W or W-WB samples.

Expression Comparison of Mitf, Tyr, Tyrp1, c-Kit and Tyrp2 in Retinas and Feather Bulbs

The expression of Tyr and Tyrp1 is regulated by Mitf. However, we did not find differential expression of Mitf in the RNA-Seq data. To investigate whether Mitf showed the same expression pattern as Tyr and Tyrp1, we performed cloning and qPCR analysis of this gene. The results showed that two isoforms of Mitf, M and B, exist. The B isoform was expressed in both black and white feather bulbs, while the M isoform was only expressed in black feather bulbs, regardless of whether they were collected from ducks with pure black or black-white plumage (Figure 3). We also used qPCR to test the relative expression of Tyr, Tyrp1, c-Kit and Tyrp2 in retinas, another organ in which melanogenesis occurs. The results showed that all 4 genes were expressed in retinas (Figure 4). Moreover, Tyrp2 is expressed only in retinas; no expression was detected for this gene in feather bulbs (Figure 5).

Discussion

Until now, there has been no study using genome-wide expression analysis in duck feather bulbs by RNA-sequencing.

Table 2. Digital differential expression analysis of c-Kit, Tyrp1, Tyr and Tyrp2 genes between black and white feather bulbs by RNA-Seq.

| Gene   | Raw-B-1 | Raw-W-1 | TPM-B-1 | TPM-W-1 | Log2ratio(W-1/B-1) | P-Value   | FDR       |
|--------|---------|---------|---------|---------|---------------------|-----------|-----------|
| c-Kit  | 160     | 16      | 32.74   | 3.29    | -3.31489            | 3.44E-31  | 5.00E-29  |
| Tyrp1  | 3250    | 0       | 664.89  | 0.01    | -16.021             | 0         | 0         |
| Tyr    | 184     | 0       | 37.85   | 0.01    | -11.8784            | 5.96E-56  | 3.48E-54  |
| Tyrp2  | 0       | 0       | 0       | 0       | 0                   | 0         | 0         |

Note: Raw-B-1, Raw data of black feather bulb expression; Raw-W-1, Raw data of white feather bulb expression; TPM-B-1, Normalized expression level of genes in black plumage feather bulb library; TPM-W-1, Normalized expression level of genes in white plumage feather bulb library; Log2ratio(W-1/B-1), Log2(multiples of differentially expressed genes); P-value corresponds to differential gene expression test; FDR (False Discovery Rate) is used to determine the threshold P-value in multiple tests and analyses by manipulating the FDR value [27].

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Our study offered new information related to gene expression profiles in black and white feather bulbs in the duck. The entire duck genomic sequence is not available; thus, our data analysis was based on the Ensemble *Gallus gallus* database and the *Anas platyrhynchos* EST database. Although duck and chicken coding sequences have high homology (up to 90% for many genes), using the *Gallus gallus* database to match duck sequences can be difficult, as most of the tags are from the 3'UTR of genes. Compared to more than 10 million tags, only 3,000 reference ESTs exist in the *Anas platyrhynchos* EST library, which is too few for annotation. In our study, only 133 genes were identified from 2,642 differentially expressed tags, while most tags did not match any annotated genes. The reasons may include: (1) The short tags were from 3'UTR of the mRNAs; (2) When using the chicken sequences as reference to annotate the genes, the tag will not be annotated to genes if there are 2-bp mismatches as the length of each tag is 17-bp. Thus, only a low proportion of differentially expressed tags could be matched to annotated genes. Also, this method is not sensitive enough to detect genes that are very weakly expressed. Fortunately, three genes in the melanogenesis pathway were identified, indicating that this pathway is crucial for duck plumage color determination.

Skin, coat, and feather color in mammals and birds are determined mainly by 2 melanosins, eumelanin and phaeomelanin [31]. In human, hair color (or lack thereof, i.e., white hair) is determined by whether the hair bulb has normal biosynthesis of melanin and its subsequent transfer from melanocytes to keratinocytes [32]. In colored feather bulbs, melanin can be synthesized at the first step as described by Korner [33]. For melanogenesis, Tyr, Tyrp1, and Tyrp2 were directly involved in the synthesis of melanins. In this study, Solexa sequencing showed low expression of *Tyr* and *Tyrp1* in white feather bulbs from ducks with pure white or white-black plumage, while these genes showed normal expression in black feather bulbs from ducks with pure black or white-black plumage. These results demonstrated that a lack of *Tyr* and *Tyrp1* expression led to a deficiency in the biosynthesis of melanin in white feather bulbs and is the direct cause of white duck plumage. Surprisingly, *Tyrp2* was not expressed in white or black feather bulbs but was expressed in retinas, indicating that this gene may be responsible for retinal pigmentation. This result is similar to that in human [34]. We cloned this gene from duck eye retina mRNA, but the mechanism of its restricted gene expression is not clear.

Previous studies on coat or feather color mainly focused on the effects of nucleotide deletion, mutation, or insertion in single genes. Schmidt [35] found that a single point mutation in exon 1 of the mouse tyrosinase gene caused the dark-eyed albino phenotype. Tobita-Teramoto et al., [3] reported that a six-nucleotide deletion in the tyrosinase coding sequence caused chickens to be albino. Additionally, in chickens, a retroviral insertion in intron 4 of the tyrosinase gene, leading to a lack of exon 5, which encodes the carboxy-terminal membrane spanning domain, caused the recessive white phenotype [4,36,37]. In this study, our results showed that the duck *Tyr* gene was expressed normally in retinas from ducks with either black or white plumage. All the ducks in this study had normal, dark retinas. Thus, the *Tyr* gene has normal function due to white or black plumage, but the expression in white feather bulbs was suppressed. The genes that inhibit *Tyr* expression could be responsible for plumage color in this population.

It was reported that *Mitf* is a member of the bHLH-leucine zipper transcription factor family and played an important role in the development of retinal cells, mast cells, osteoclasts and melanocytes [38]. Alleles of *Mitf* have been associated with coat color in dogs [7] and mice [39,40], as well as with plumage color in quail [41]. Minvielle [10] demonstrated that a 2-bp deletion in exon 11 of *Mitf* caused white plumage in Japanese quail. In this study, in contrast to Japanese quail, the *Mitf*-M isofrom did not show expression in white plumage feather bulbs, although both *Mitf*-M and *Mitf*-B isoforms were normally expressed in retinas and black plumage feather bulbs. The difference in *Mitf*-M expression between white and black feather bulbs in this study suggests that *Mitf*-M is involved in determining feather pigmentation in the duck through either cis or trans acting regulatory elements as opposed to non-synonymous coding variants like in the quail. The expression pattern of the duck *Pmel17* gene is the same as *Mitf*-M, *Tyr*, *Tyrp1*, *Pmel17* and the maintenance of human hair pigmentation [50] have been widely studied in various models.
Nucleotide deletions from introns [14] and copy-number variation [51–53] of c-Kit were associated with pig coat color. C-Kit mutations in horses were associated with white coat color [16,54–56], and exon skipping in the c-Kit gene in horses causes a Sabino spotting pattern [57]. Furthermore, c-Kit is a candidate for white spotting in cats [58], and the c-Kit signaling pathway is involved in post-developmental processes of mature cells [59]. Taken together, the c-Kit gene plays a critical role in animal coat color and is specifically associated with ‘white’. In this study, we found there was no significant difference in c-Kit expression in retinas from ducks with white or black plumage. W-W, B-B, W-WB, and B-WB feather bulbs all showed expression of the c-Kit gene, although the expression level in black feather bulbs was 10-fold higher than that in white feather bulbs. In white feather bulbs, this basal level of c-Kit expression may be able to maintain cell proliferation and differentiation but is not sufficient to promote pigmentation, although there is no in vivo confirmation work. In contrast, c-Kit expression in black feather bulbs is 10 times higher than that in white feather bulbs, allowing for cell proliferation and differentiation as well as maintenance of postnatal melanogenesis.

It is possible that the lower level of c-Kit expression in the white feather bulb is a downstream consequence of few or no active melanocytes in the feather bulb, but not a genetic lesion at the c-Kit locus.

Conclusion

Plumage color variation in Kaiya-Liancheng F2 ducks was determined by whether melanin can be synthesized in the feather bulb. Our results provide solid evidence on some of the functional players in feather pigmentation in the duck, e.g. upregulation of c-Kit and Mitf in black feather bulbs may be responsible for black plumage formation.

Materials and Methods

Experimental Animals

The genetic background of experimental ducks was described by Gong et al., [25]. Three white plumage ducks, three black plumage ducks and three white-black plumage ducks were randomly selected from a population of the Kaiya-Liancheng F2
RNA-Seq, Data Mining and Gene Ontology Analysis

The approved permit number for this study is “HBAC20091138”. Congress, and the ethics committee of Huazhong Agricultural University, P. R. China. The approved permit number for this study is “HBAC20091138”.

Three feather bulbs from the same individual were pooled as one sample. The white feather bulbs from white back duck were marked as W-W, whereas the black feather bulbs from black duck back were marked as B-B. White feather bulbs from white-black duck back were marked as W-WB and black feather bulbs from white-black duck back were marked as B-WB, respectively. The four feather bulb types are shown in Figure 6. All research involving animals were conducted according to the regulation (No. 5 proclaim of the Standing Committee of Hubei People’s Congress) approved by the Standing Committee of Hubei People’s Congress, and the ethics committee of Huazhong Agricultural University, P. R. China. The approved permit number for this study is “HBAC20091138”.

**Table 3. Primers used in Semi-RT-PCR and qPCR.**

| Gene | Primers | Sequence (5’-3’) | Size (bp) | AT | Function |
|------|---------|-----------------|-----------|----|----------|
| β-actin | β-actin-F | AACTGGAGTACAATGGAGAGA | 189 | 60°C | Semi-RT PCR & qPCR |
| | β-actin-R | ATGCCTGGGTATGGAAGGT |
| c-Kit | c-Kit-F | GCTGATTGTCGCGATAGT | 151 | 60°C | qPCR |
| | c-Kit-R | TTGGCACCCTGTGAAGAAG |
| Tyr | EY-F1425 | TTACATGGCCTTATTCC | 182 | 60°C | qPCR |
| | E4NR | CAATATGCGCTGCCAAAC |
| Tyrp1 | F1250 | AATGAGATTTTGTATCG | 208 | 60°C | qPCR |
| | R1419 | ACTGATGTTTGCAGAGG |
| Tyrp2 | F1496 | CATCATATGCAAGTGTGCC | 228 | 60°C | qPCR |
| | R1703 | AGCAAGAAGCCGCTAAGGG |
| Mitf-B | BF383 | CCCAGTTCATCGAGCAGAGT | 268 | 60°C | Semi-RT-PCR |
| | MBR20 | CACCAGGCAGCCAGCAGTAC |
| Mitf-M | MF156 | TGCAATGGATATGCTTCC | 226 | 60°C | qPCR |
| | MBR20 | CCAGGCGGATGACATGGAGAAG |

Note: AT = Annealing temperature.

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To confirm the differential expression of genes revealed by RNA-Seq, the expression of genes in the melanogenesis pathway, including c-Kit, Tyr, Tyrp1, and Tyrp2 was measured because they are directly involved in melanin biosynthesis. β-actin was used as a reference control. qPCR analysis was performed on Roche lightcycler® 480, using lightcycler® 480 SYBR Green I master detection reagents (Roche Diagnostics 11367523). All reactions were performed in triplicate within each PCR assay and under the same cycling conditions: denaturation at 95°C for 3 min, followed by 40 cycles of amplification (95°C for 20 s, 60°C for 20 s, and 72°C for 20 s) with a single acquisition of fluorescence at the end of the extension step. Melting curve analysis was performed over a range of 55°C to 95°C to verify single product generation at the end of the assay. qPCR data analysis was performed with the Light Cycler analysis software. Relative quantification analyses were performed in EXCEL using the comparative CT method. Comparisons between qPCR data sets were made with Student’s t-test. Differences were considered significant if P<0.05. Further, semi-RT-PCR measurements of Tyrp2 gene expression in retinas and feather bulbs from ducks with different plumage types were also conducted. All primers information is shown in Table 3.

**Supporting Information**

Table S1 The information of genes that expressed in duck feather bulbs that have different reads.

(XLS)

Table S2 Differentially expressed tags between white and black plumage feather bulb libraries.

(XLS)

Table S3 Differentially expressed genes between white and black plumage feather bulb libraries.

(XLS)
Table S4 Gene Ontology analysis of the differentially expressed genes.

Author Contributions
Performed the experiments: SL. Analyzed the data: CW WY. Wrote the paper: SL. Conceived the study: SL SZ YG. Collected the duck hair bulb samples and RNA extraction: CW WY. Edited the manuscript: SL.

References
1. Ducret AJ, Keller L, Roulin A (2008) Pleiotropy in the melanocortin system, colouration and behavioural syndromes. Trends In Ecology & Evolution 23: 502–510.
2. Lancaster FM (1963) The inheritance of plumage colour in the common duck. Bibliotheca genetica XIX. p. 317–404.
3. Tohita-Terramoto T, Jang GY, Kino K, Salter DW, Brumback J, et al. (2000) Autozygous albino chicken mutation (ca/ca) deletes hexamericotide (delta-GACTGGTG) at a copper-binding site of the tyrosinase gene. Poult Sci 79: 4650.
4. Chang CM, Coville JL, Coquerelle G, Gourionch D, Oulhmoud A, et al. (2006) Complete association between a retrolental insertion in the tyrosinase gene and the recessive white mutation in chickens. BMC Genomics 7: 19.
5. Fontanesi L, Scotti E, Russo V (2011) Haplotype variability in the bovine MITF gene and association with piebaldism in Holstein and Simmental cattle breeds. Animal Genetics 42: 782–789.
6. Hayes BJ, Fryce J, Chamberlain AJ, Bowman PJ, Goddard ME (2010) Genetic architecture of color traits and accuracy of genomic prediction: coat color, milk-fat percentage, and type in Holstein cattle as contrasting model traits. PLoS Genet 6: e1001139.
7. Karlsson EK, Baranowska I, Wade CM, Salmon HN, Zody MC, et al. (2007) Efficient mapping of mendelian traits in dogs through genome-wide association. Nature Genetics 39: 1321–1329.
8. Dohooi B, Molin AM, Rubin CJ, Johansso AM Strømslien I, et al. (2011) A complex genomic rearrangement involving the endodrin 3 locus causes dermal hyperpigmentation in the chicken. PLoS Genet 7: e1002412.
9. Liu Y, Zhu X, Yang L, Li J, Lian Z, et al. (2011) Expression and network analysis of genes related to melanocyte development in the Silky Foot and White Leghorn embryos. Molecular Biology Reports 38: 1433–1441.
10. Minvielle F, Bed’Hom B, Coville JL, Ito S, Inoue-Murayama M, et al. (2010) The “silver” Japanese quail and the MITF gene: causal mutation, associated haplotype and homozygotes with the “blue-egg” trait. BMC Genet 11: 15.
11. Tsujimura T, Morii E, Nozaki M, Hashimoto K, Moriyama Y, et al. (1996) Involvement of transcription factor encoded by the mi locus in the expression of c-kit receptor tyrosine kinase in cultured mast cells of mice. Blood 98: 1225–1233.
12. Hou L, Panthier JJ Arheimer H (2000) Signaling and transcriptional regulation in the neural crest-derived melanocyte lineage: interactions between KIT and MITF. Development 127: 5379–5389.
13. Borchjaerven NV, Klibaniz M, Longley M, Borchjaerven VA, Gilchrest BA (2001) SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. Febslet Journal 15: 645–658.
14. Fontanesi L, D’Alessandro E, Scotti E, Liotta L, Crovetti A, et al. (2010) Genetic heterogeneity and selection signature at the KIT gene in pigs showing different pigmentation unit. Faseb Journal 15: 645–658.
15. Haase B, Brooks SA, Schlumbaum A, Azor PJ, Bailey E, et al. (2007) Allelic heterogeneity at the equine KIT locus in dominant white (W) horses. PLoS Genet 3: e195.
16. Chen B, Chen Y, Li H, Wang J, Shen J, et al. (2010) Allele-specific genetic interactions between Mitf and Kit affect melanocyte development. Pigment Cell Melanoma Res 23: 441–447.
17. Lecoin I, Lahav R, Martin FH, Tillet MA, Le Douarin NM (1995) Steel and c-kit in the development of avian melanocytes: a study of normally pigmented birds and of the hypopigmented mutant silkie fowl. Dev Dyn 203: 100–118.
18. Niwa T, Mochi N, Nishikawa S (2002) Pmel17 pigmentation and expression of its regulatory genes during quail development–histochemical differentiation and transdifferentiation of chicken pigmented epithelial cell. Molecular Biology Of The Cell 12: 3451–3464.
19. Nishikawa S, Kusakabe M, Yoshinaga K, Ogawa M, Hayashi S, et al. (1991) In vivo manipulation of coat color formation by a monoclonal anti-c-kit antibody: two distinct waves of c-kit-dependency during melanocyte development. Embryo Journal 10: 2111–2118.
20. Yoshida H, Hayashi S, Shiuve LD, Yamamura K, Nishikawa S, et al. (1996) Neural and skin cell-specific expression pattern, referred by steel factor regulatory sequence in transgenic mice. Dev Dyn 207: 222–232.
21. Koi L, Aronza A, Takayama H, Maima F, Ponzetto C, et al. (1999) Hepatocyte growth factor/scatter factor-MET signaling in neural crest-derived melanocyte development. Pigment Cell Melanoma Res 12: 233–241.
22. Kurin K, Nishido M, Shinozaki H, Takada K, Yamazaki H, et al. (2005) Suppression of progressive loss of coat color in microphalina-vitiligo mutant mice. Journal of Investigative Dermatology 125: 538–544.

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Performed the experiments: SL. Analyzed the data: CW WY. Wrote the paper: SL. Conceived the study: SL SZ YG. Collected the duck hair bulb samples and RNA extraction: CW WY. Edited the manuscript: SL.
50. Hachiya A, Sriririyawong P, Kobayashi T, Nagasawa A, Yoshida H, et al. (2009) Stem cell factor-KIT signalling plays a pivotal role in regulating pigmentation in mammalian hair. Journal of Pathology 218: 30–39.
51. Giuffra E, Tornsten A, Marklund S, Bongcam-Rudloff E, Chardon P, et al. (2002) A large duplication associated with dominant white color in pigs originated by homologous recombination between LINE elements flanking KIT. Mammalian Genome 13: 569–577.
52. Johansson MM, Chaudhary R, Hellmen E, Hoyheim B, Chowdhary B, et al. (1996) Pigs with the dominant white coat color phenotype carry a duplication of the KIT gene encoding the mast/stem cell growth factor receptor. Mammalian Genome 7: 822–830.
53. Seo BY, Park EW, Ahn SJ, Lee SH, Kim JH, et al. (2007) An accurate method for quantifying and analyzing copy number variation in porcine KIT by an oligonucleotide ligation assay. BMC Genet 8: 81.
54. Haase B, Brooks SA, Tozaki T, Burger D, Poncet PA, et al. (2009) Seven novel KIT mutations in horses with white coat colour phenotypes. Animal Genetics 40: 623–629.
55. Marklund S, Moller M, Sandberg K, Andersson L (1999) Close association between sequence polymorphism in the KIT gene and the roan coat color in horses. Mammalian Genome 10: 283–288.
56. Terry RR, Bailey E, Bernoco D, Cothran EG (2001) Linked markers exclude KIT as the gene responsible for appaloosa coat colour spotting patterns in horses. Animal Genetics 32: 98–101.
57. Brooks SA, Bailey E (2005) Exon skipping in the KIT gene causes a sabino spotting pattern in horses. Mammalian Genome 16: 893–902.
58. Cooper MP, Fretwell N, Bailey SJ, Lyons LA (2006) White spotting in the domestic cat (Felis catus) maps near KIT on feline chromosome B1. Animal Genetics 37: 163–165.
59. Motro B, van der Kooy D, Rossant J, Reith A, Bernstein A (1991) Contiguous patterns of c-kit and steel expression: analysis of mutations at the W and Sl loci. Development 113: 1207–1221.