1 **Skin transcriptome profiles associated with coat color in goat**

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13

14 **ABSTRACT**

15 Capra hircus, an economically important livestock, plays an indispensable
16 role in the world animal fiber industry. To identify additional genes that may
17 play important roles in coat color regulation, Illumina/Solexa high throughput
18 sequencing technology was used to catalog the global gene expression
19 profiles in the skin of three different coat colors goat (Lubei white goat (white),
20 Jining gray goat (gray) and Jianyang big ear goat (brown)). The RNA-Seq
21 analysis generated 83174342, 70222592 and 52091212 clean reads in white
22 skin, gray skin and brown skin, respectively, which provided abundant data for
23 further analysis. A total of 91 genes were differentially expressed between the
24 gray skin and white skin libraries, with 74 upregulated and 17 genes
25 downregulated. Between the brown skin and white skin libraries, there were 23

26 upregulated genes and 44 downregulated genes, while there were 33
 27 upregulated genes and 121 downregulated genes between the brown skin and
 28 gray skin libraries. To our surprise, *MC1R*, *MITF*, *TYR*, *KIT* and *KITLG* showed
 29 no significant difference in the skin of three different coat colors and the
 30 expression of *ASIP* was only detected in white skin and not in gray and brown
 31 skins. The expression of *PMEL*, *TRPM1*, *DCT*, *TYRP1* and *ELOVL3* was
 32 validated by real-time quantitative polymerase chain reaction (qPCR) and the
 33 results of the qPCR were consistent with the RNA-seq except the expression
 34 of *TYRP1* between the gray skin and white skin libraries. This study provides
 35 several candidate genes that may be associated with the development of
 36 different coat colors goat skin. More importantly, the fact that the *ASIP* gene
 37 was only detected in the white skin and not in the other dark skins and the
 38 *MC1R* gene showed no significant difference in expression between the three
 39 different coat colors goat is of particular interest for future studies that aim to
 40 elucidate their functional role in the regulation of skin color. These results will
 41 expand our understanding of the complex molecular mechanisms of skin
 42 physiology and melanogenesis in goat and provide a foundation for future
 43 studies.

44

45 **KEYWORDS:** goat skin; coat color; transcriptome; gene expression;
 46 melanogenesis

47

48 The domestic goat (*Capra aegagrus hircus*) is a subspecies of
 49 goat domesticated from the wild goat of southwest Asia and Eastern Europe.
 50 The goat is a member of the family Bovidae and is closely related to

51 the sheep as both are in the goat-antelope subfamily Caprinae. Goats have
52 long been used for their milk, meat, hair and skins throughout the world (Wang
53 *et al.* 2011a; Wang *et al.* 2011b; Xu *et al.* 2013).

54 *Capra hircus*, an economically important livestock, plays an indispensable
55 role in the world animal fiber industry. Fiber diameter, length and color are key
56 traits contributing to the economic value of goat and are determined by both
57 genetics (Bunge *et al.* 1996; Lamoreux *et al.* 2001) and other factors, including
58 the environment and certain drugs (Kidson and Fabian 1981; Dereure 2001;
59 Jablonski and Chaplin 2010; Sturm and Duffy 2012). Factors that determine
60 coat color in goat are becoming of increasing interest. White fleece holds
61 greatest economic value due to its ability to be dyed to virtually any color,
62 where as interest in natural colors is increasing due to the green revolution and
63 consumer preference for natural products.

64 In adult animals, both hair and skin color depend on pigment produced by
65 melanocytes at the base of the epithelium (Cieslak *et al.* 2011). Melanocytes in
66 mammals and birds produce two types of melanin, black to brown eumelanin
67 and yellow to reddish brown pheomelanin (Ito *et al.* 2000; Ito and Wakamatsu
68 2008). The basic coat colourations are defined by the ratio of the two pigments
69 eumelanin and pheomelanin (Cieslak *et al.* 2011).

70 In animals, melanic coloration is often genetically determined and
71 associated with various behavioral and physiological traits, suggesting that
72 some genes involved in melanism may have pleiotropic effects (Ducrest *et al.*
73 2008). At present, a large number of genes have been found to play
74 well-known roles in pigmentation and the analysis of these genes has
75 identified many single nucleotide polymorphisms (SNPs), e.g., *ASIP*, *MC1R*,

76 *TYR, TYRP1, DCT, MITF, KIT, KITLG, OCA2, SLC24A4, PMEL* (Brunberg *et*
77 *al.* 2006; Gutierrez-Gil *et al.* 2007; Sulem *et al.* 2007; Deng *et al.* 2009; Nan *et*
78 *al.* 2009; Duffy *et al.* 2010; Minvielle *et al.* 2010; Nicoloso *et al.* 2012; Hart *et al.*
79 2013; Fan *et al.* 2014; Li *et al.* 2014; Becker *et al.* 2015).

80 Coat color genes are good candidates for facilitation of trace ability of
81 animal breeds. Several previous studies have paid significant attention to the
82 coat color of animals and showed that this color is determined by the amount
83 and type of melanin produced and released by the melanocytes present in the
84 skin (Ito *et al.* 2000; Ito and Wakamatsu 2008). For example, recent
85 researches have demonstrated that *MC1R* and *ASIP* are known to be major
86 regulators of coat color in mice and *MC1R* and *ASIP* loci are functionally linked
87 to undesirable coat color phenotypes in sheep (Vage *et al.* 1999; Slominski *et*
88 *al.* 2004; Steingrimsson *et al.* 2006; Norris and Whan 2008). In addition,
89 tyrosinase-related protein 1 (*TYRP1*) is a strong positional candidate gene for
90 color variation in Soay sheep (Gratten *et al.* 2007). Recent studies have
91 combined SNP analysis and gene expression profiling to dissect the basis for
92 the piebald pigmentation phenotype in Merino sheep (Garcia-Gamez *et al.*
93 2011). It was reported that color variation was likely to stem from differences in
94 the expression levels of genes belonging to the melanocortin in the tawny owl
95 (Emaresi *et al.* 2013). Despite considerable knowledge of the genetic
96 regulation of coat color in some animals, the molecular and cellular
97 mechanisms regulating coat color in fiber-producing species, such as the goat,
98 are not completely understood. This information is critical not only to enhanced
99 basic understanding of regulation of melanogenesis, but also to the
100 identification of novel pharmacological and molecular genetics approaches to

101 regulate or select for coat color in fiber producing species.

102 RNA-Seq technology is a high-throughput sequencing platform allowing
103 us to detect transcripts with low abundance, identify novel transcript units, and
104 reveal their differential expression between different samples (Wang *et al.*
105 2009; Wilhelm and Landry 2009; Ozsolak and Milos 2011). To investigate the
106 different expression profiles of the genes involved in goat skin pigmentation
107 and gain insight into molecular mechanisms responsible for biochemistry of
108 skin and fibers (including pigmentation) in animals producing hair, we
109 investigated the transcriptome profiles in skin of goat of different coat colors
110 using high throughput RNA deep sequencing. This will enable us to
111 understand the molecular mechanisms involved in skin pigmentation and
112 provide a valuable theoretical basis for the selection of the excellent natural
113 colors trait.

114

115 **MATERIALS AND METHODS**

116

117 **Ethics Statement**

118 All of the animals were handled in strict accordance with good animal practices
119 as defined by the relevant national and/or local animal welfare bodies. The
120 experimental procedure was approved by the Animal Care and Use
121 Committee of Hebei Normal University of Science and Technology, China and
122 was performed in accordance with the animal welfare and ethics guidelines.

123

124 **Goat skin sampling and total RNA extraction**

125 Housing and care of goat and collection of skin samples for use in the

described experiments were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals (http://www.cioms.ch/frame_1985_texts_of_guidelines.htm). The animals were locally anaesthetized with hydrochloridum (1.5 ml of 3%, i.h.), following the approval provided by the Animal Hospital of Hebei Normal University of Science and Technology to decrease the animal suffering. 3 Lubei white goats, 3 Jining gray goats and 3 Jianyang big ear goats were selected for sample collection from the goat farm in Laiwu, Shandong province, China. A piece of skin (8 mm in diameter) from the leg was collected via punch skin biopsy under local anesthesia and immediately placed in liquid nitrogen. Total RNA from the sample was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The RNA integrity was evaluated by gel electrophoresis and the RNA purity was checked by the ratio of OD260/OD280 and RIN value. RNA samples with RIN value greater than 7.5 and OD260/OD280 ratio greater than 1.75 were selected for goat sequencing.

141

142 **Library generation and sequencing**

Three RNA samples from white, gray and brown goat skin were pooled before mRNA isolation. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X) . First strand cDNA was synthesized using

151 random hexamer primer and M-MuLV Reverse Transcriptase (RNase H⁻).
 152 Second strand cDNA synthesis was subsequently performed using DNA
 153 Polymerase I and RNase H. Remaining overhangs were converted into blunt
 154 ends via exonuclease/polymerase activities. After adenylation of 3'ends of
 155 DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to
 156 prepare for hybridization. In order to select cDNA fragments of preferentially
 157 150~200 bp in length, the library fragments were purified with AMPure XP
 158 system (Beckman Coulter, Beverly, USA). Then 3 µl USER Enzyme (NEB,
 159 USA) was used with size-selected, adaptor-ligated cDNA at 37°C for 15 min
 160 followed by 5 min at 95 °C before PCR. Then PCR was performed with
 161 Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X)
 162 Primer. At last, PCR products were purified (AMPure XP system) and library
 163 quality was assessed on the Agilent Bioanalyzer 2100 system.

164 The clustering of the index-coded samples was performed on a cBot
 165 Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia)
 166 according to the manufacturer's instructions. After cluster generation, the
 167 library preparations were sequenced on an Illumina Hiseq 2000 platform and
 168 100 bp paired-end reads were generated.

169

170 **Reads mapping to the reference genome**

171 Reference genome and gene model annotation files were downloaded from
 172 genome website directly. Index of the reference genome was built using
 173 Bowtie v2.0.6 and paired-end clean reads were aligned to the reference
 174 genome using TopHat v2.0.9. We selected TopHat as the mapping tool for that
 175 TopHat can generate a database of splice junctions based on the gene model

176 annotation file and thus a better mapping result than other non-splice mapping
177 tools.

178

179 **Differential expression analysis**

180 The expression abundance of each assembled transcript was measured
181 through Reads Per Kilobase of exon model per Million mapped reads (RPKM).
182 Prior to differential gene expression analysis, for each sequenced library, the
183 read counts were adjusted by edgeR program package through one scaling
184 normalized factor. Differential expression analysis of two conditions was
185 performed using the DEGSeq R package (1.12.0). The P values were adjusted
186 using the Benjamini & Hochberg method. Corrected P-value of 0.005 and
187 log2(Fold change) of 1 were set as the threshold for significantly differential
188 expression.

189

190 **GO and KEGG enrichment analysis of differentially expressed genes**

191 Gene Ontology (GO) enrichment analysis of differentially expressed genes
192 was implemented by the Goseq R package, in which gene length bias was
193 corrected. GO terms with corrected Pvalue less than 0.05 were considered
194 significantly enriched by differential expressed genes. KEGG is a database
195 resource for understanding high-level functions and utilities of the biological
196 system, such as the cell, the organism and the ecosystem, from
197 molecular-level information, especially large-scale molecular datasets
198 generated by genome sequencing and other high-through put experimental
199 technologies (<http://www.genome.jp/kegg/>). We used KOBAS software to test
200 the statistical enrichment of differential expression genes in KEGG pathways.

201

202 **Real-time quantitative RT-PCR**

203 The expression of these genes was quantified by qRT-PCR using QuantiTect
 204 SYBR Green RT-PCR (Qiagen, Waltham, MA). Information regarding the
 205 primers of *PMEL*, *TRPM1*, *DCT*, *TYRP1*, *ELOVL3* used for the qPCR can be
 206 found in Table 1. *GAPDH* was used as housekeeping gene. Quantitative
 207 real-time PCR was performed in triplicate on the Stratagene iQ5 system. The
 208 20 µL PCR reaction included 10 µL SYBR Premix Ex Taq II (TaKaRa,
 209 Dalian, China), 0.5 µL specific forward primer, 0.5 µL reverse primer, 0.4 µL
 210 ROX reference dye, 0.5 µL diluted cDNA and 8.6 µL RNase free water. Cycling
 211 parameters were 95°C for 4 min, followed by 40 cycles of 95°C for 15 sec,
 212 56°C or 58°C for 30 sec and 72°C for 45 sec. Melting curve analyses were
 213 performed following amplifications. At the end of the cycles, melting
 214 temperatures of the PCR products was determined between 70°C and 90°C.
 215 The iQ5 software (Bio-Rad) was used for detection of fluorescent signals and
 216 melting temperature calculations. Quantification of selected mRNA transcript
 217 abundance was performed using the comparative threshold cycle (CT) method.
 218 The difference in abundance of mRNA for the genes was determined by
 219 analysis of variance.

220

221 **DATA AVAILABILITY**

222 Table S1 contains top 30 highly expressed genes in goat skin. Table S2 covers
 223 differentially expressed genes between the gray and white skin. Table S3
 224 covers differentially expressed genes between the brown and white skin. Table
 225 S4 covers differentially expressed genes between the brown and gray skin.

226 Table S5 contains the list of GO categories for DEGs between different coat
227 colors goat skin. Table S6 encompass KEGG pathway analysis for the gray
228 and white skin differentially expressed genes. Table S7 encompass KEGG
229 pathway analysis for the brown and white skin differentially expressed genes.
230 Table S8 encompass KEGG pathway analysis for the brown and gray skin
231 differentially expressed genes.

232

233 **RESULTS**

234

235 **Massively Sequencing and Aligning to the Reference Genome**

236 To maximise the coverage of the three different coat colors goat (Lubei white
237 goat (white), Jining gray goat (gray) and Jianyang big ear goat (brown)) skin
238 mRNA by RNA sequencing, RNA libraries were constructed by pooling RNA
239 isolated from different coat colors individuals as a sample library. These
240 RNA-Seq libraries generated over 54 million raw reads from each library. After
241 filtering the only adaptor sequences, those containing N sequences and low
242 quality sequences, the three RNA-Seq libraries still generated over 26 million
243 paired-end clean reads in each library, and the percentage of paired-end clean
244 reads among raw tags in each library ranged from 94.07% to 94.71% (Table 2
245 and Figure 1).

246 Of the total reads, more than 77% matched either to a unique or to
247 multiple genomic locations; the remaining were unmatched (Table 3), because
248 only reads aligning entirely inside exonic regions will be matched (reads from
249 exon-exon junction regions will not be matched). Of the total mapped, more
250 than 78% matched to exon and more than 11% matched to intron; the

251 remaning were matched the intergenic (Figure 2).

252

253 **Genes highly expressed in goat skin**

254 The top 30 genes most highly expressed in goat skin included genes of the
255 keratin family and ribosomal proteins (Table S1). The most highly expressed
256 gene in all three different coat colors goat skin was keratin associated protein
257 3-1. The other highly expressed genes in the different coat color goat skins
258 included, trichohyalin, transcript variant X1, keratin 71, keratin 5, keratin 25,
259 eukaryotic translation elongation factor 1 alpha 1, keratin 14, transcript variant
260 X1, keratin 27, keratin, type II microfibrillar, component 7C-like, collagen, type I,
261 alpha 1, uncharacterized LOC102168701, ribosomal protein L35, secreted
262 protein, acidic, cysteine-rich (osteonectin), ribosomal protein L13a. The list of
263 most highly expressed genes also included 2 unknown genes.

264

265 **Differentially expressed genes in different coat colors goat skin**

266 To identify the differentially expressed genes (DEGs) of different coat colors
267 goat skin, the differences in gene expression patterns were analysed for the
268 pairs of the gray skin and white skin, the brown skin and white skin, the brown
269 skin and gray skin. There were a total of 89 known genes and 2 novel genes
270 identified as differentially expressed in gray skin versus white skin, of which 17
271 were down-regulated and 74 (including 2 novel genes) were up-regulated in
272 skin from gray goat compared with skin from white goat (Figure 3 and Table
273 S2). Between the brown and white libraries, 67 genes (including 1 novel gene)
274 were differentially expressed, including 44 down-regulated (including 1 novel
275 gene) and 23 up-regulated genes (Figure 3 and Table S3). When we

276 compared the brown and gray libraries, 154 DEGs (including 6 novel genes)
 277 were found, with 121 down-regulated (including 5 novel genes) and 33
 278 up-regulated (including 1 novel gene) (Figure 3 and Table S4). This suggests
 279 that the differentiation of expressed genes between the brown skin and gray
 280 skin is larger than that between the gray skin and white skin, while a relatively
 281 smaller differentiation arises between the brown skin and white skin.

282

283 **Functional Classification Analysis**

284 For the GO analysis of the gray versus white skin, 1249, 349 and 607
 285 differentially expressed genes were grouped in biological process, cellular
 286 component and molecular function, respectively. Most of the differentially
 287 expressed genes were classified into two GO categories (single-organism
 288 metabolic process, oxidoreductase activity and catalytic activity; Figure 4 and
 289 Table S5). The majority of the GO terms including pigmentation do not appear
 290 to be significantly enriched in the differentially expressed genes. For the GO
 291 analysis of brown versus white skin, 505, 326 and 391 differentially expressed
 292 genes were grouped in biological process, cellular component and molecular
 293 function, respectively. Most of the differentially expressed genes were
 294 classified into three GO categories (oxidation-reduction process, extracellular
 295 region and structural molecule activity; Figure 4 and Table S5). The majority of
 296 the GO terms including pigmentation do not appear to be significantly enriched
 297 in the differentially expressed genes. For the GO analysis of brown versus
 298 white skin, 2368, 761 and 1100 differentially expressed genes were grouped in
 299 biological process, cellular component and molecular function, respectively.
 300 Most of the differentially expressed genes were classified into two GO

categories (oxidation-reduction process, single-organism metabolic process and oxidoreductase activity; Figure 4 and Table S5). The majority of the GO terms including pigmentation do not appear to be significantly enriched in the differentially expressed genes.

In order to validate the transcriptome sequencing results, we selected 5 genes at random for real time PCR to determine their relative expression in different coat color goat skin. These genes, identified as differentially expressed in different coat color goat skin based on transcriptome sequencing analysis. The results of the qPCR were consistent with the RNA-seq except the expression of *TYRP1* between the gray skin and white skin libraries (Figure 5).

311

312 **KEGG pathway analysis**

KEGG is a largely publicly available pathway-related database and is useful in searching for genes involved in metabolic or signal transduction pathways that were significantly enriched (Kanehisa *et al.* 2008). Of the 89 known genes differentially expressed in gray versus white goat skin, 40 had a specific KEGG pathway annotation. Of these KEGG pathway annotated genes, 3 were down-regulated in gray goat skin. These down-regulated genes are mainly involved in cysteine and methionine metabolism, melanogenesis, ribosome, metabolic pathways. Remaining KEGG pathway annotated genes were associated with 61 pathways including those functionally related to coat color in skin such as tyrosine metabolism (Table S6).

Between the brown and white goat skin, 23 DEGs with a KEGG pathway annotation were found and 14 were down-regulated in brown goat skin. These down-regulated genes were associated with 41 pathways including those

functionally related to coat color in skin such as melanogenesis. Remaining KEGG pathway annotated genes were associated with 31 pathways including those functionally related to coat color in skin such as melanogenesis, tyrosine metabolism (Table S7).

Between the brown and gray goat skin, 72 DEGs with a KEGG pathway annotation were found and 63 were down-regulated in brown goat skin. These down-regulated genes were associated with 98 pathways including those functionally related to coat color in skin such as tyrosine metabolism. Remaining KEGG pathway annotated genes were associated with 29 pathways including those functionally related to coat color in skin such as melanogenesis, tyrosine metabolism (Table S8).

Differential expression of known coat color genes

Approximately 171 cloned genes involved in different pathways controlling coat color formation have been identified in the mouse (<http://www.espcr.org/micemut/#cloned>). Those known coat color genes are routinely classified into six general functions including: Melanocyte development, Components of melanosomes and their precursors, Melanosome construction/protein routing, Melanosome transport, Eumelanin and pheomelanin and Systemic effects (Bennett and Lamoreux 2003). Expression of a total of 128 of aforementioned coat color genes was detected in goat skin in present studies, and 2 genes showed higher expression in gray goat skin versus white goat skin and 1 gene showed higher expression in white goat skin, 4 genes showed higher expression in brown goat skin versus white goat skin and 1 gene showed higher expression in white goat skin, 4 genes showed

351 higher expression in brown goat skin versus gray goat skin. Interestingly, the
 352 expression of *ASIP* (agouti signaling protein) were almost not detected in the
 353 gray skin and the brown skin, but there was high expression in the white goat
 354 skin for *ASIP*. *PMEL*, *DCT*, *TRPM1* and *TYRP1* were significantly up-regulated
 355 in brown goat skin (brown vs white, brown vs gray), but *DCT*, *TRPM1* and
 356 *TYRP1* showed no differences in expression between the gray and white skin
 357 samples and only *PMEL* was significantly up-regulated in gray goat skin
 358 versus white goat skin. *ELOVL3* was significantly up-regulated in gray goat
 359 skin (gray vs white, brown vs gray). However, there were no significant
 360 differences in the expression of *ELOVL3* between the brown and white skin
 361 samples. *MC1R*, *MITF*, *TYR*, *KIT* and *KITLG* showed no differences when
 362 comparing the three different coat color groups. The genes associated with
 363 oculocutaneous albinism (OCA) such as *HPS1*, *HPS3*, *HPS4*, *HPS5* and
 364 *HPS6* were expressed in goat skin but all of them did not show differential
 365 expression associated with coat color.

366

367 **DISCUSSION**

368 Mammalian coat color exhibits a wide range of shades and is dictated by
 369 melanin production in melanocytes (melanogenesis). Melanogenesis involves
 370 a complex molecular regulation (Slominski *et al.* 2004). In order to understand
 371 the molecular mechanisms of coat color formation, previous studies have
 372 reported the global gene expression profiles in skin of sheep and chicken with
 373 white versus black skin through Illumina sequencing technology (Fan *et al.*
 374 2013; Zhang *et al.* 2015). Another study examined differences in gene

375 expression associated with black spots infleece of Corriedale sheep using
376 microarray technology (Penagaricano *et al.* 2012).

377 Our study offers new information related to gene expression profiles for
378 three different coat colors goat skin. Our data analysis was based on the NCBI
379 database of the goat reference genome (*Capra hircus* (assembly CHIR_1.0)).

380 To further investigate genes that may play important roles in goat skin,
381 particularly in fiber/coat pigmentation, over 200 million transcriptome sequence
382 reads were generated from different coat colors goat skin using high
383 throughput RNA deep sequencing. From these reads there were 24,086
384 known genes identified as expressed in goat skin, among which 89 were
385 differentially expressed in gray versus white goat skin, 66 were differentially
386 expressed in brown versus white goat skin and 148 were differentially
387 expressed in brown versus gray goat skin. It is acknowledged that study
388 design was not optimal due to limited biological replication because single
389 pooled samples (n=3 per coat color) were used in transcriptome sequencing
390 analysis and the same three samples from different coat colors goat skin were
391 used individually for quantitative real time PCR validation of the sequencing
392 results. Despite such limitations, results have significantly enhanced
393 understanding of goat skin transcriptome composition and potential
394 differences in gene expression associated with coat color that are foundational
395 to further study in the future.

396 Genes encoding for keratin family members and keratin associated
397 proteins, ribosomal proteins were among the most highly expressed genes
398 detected in goat skin. Of the top 30 highly expressed genes in goat skin, all 9
399 keratin family members and keratin associated proteins displayed no

400 difference between the three different coat colors goat skin. Hair keratins
 401 contain a much higher content of cysteine residues in their non-helical
 402 domains and thus form tougher and more durable structures via inter
 403 molecular disulfide bond formation (Mogosanu *et al.* 2014). Therefore, high
 404 expression of keratins is likely crucial for fleece strength. The ribosome is a
 405 central player in the translation system and its function is to decode the
 406 nucleotide sequence carried by the mRNA and convert it into an amino acid
 407 primary structure (Marshall *et al.* 2008). Abundant presence of ribosomal
 408 proteins in goat skin suggests the importance of high rates of protein
 409 translation in goat skin. In channel catfish skin, the expression of ribosomal
 410 proteins was high presumably due to higher levels of translational activities
 411 (Karsi *et al.* 2002; Patterson *et al.* 2003). It was also found that several
 412 collagen family members, particularly collagen, type I family members, were
 413 high expression in the three different coat colors goat skin. Collagen, in the
 414 form of elongated fibrils, is mostly found in fibrous tissues such as tendons,
 415 ligaments and skin (Zuber *et al.* 2015). Collagen has many medical uses in
 416 treating complications of the bones and skin. So high expression collagen may
 417 play important physiological function for skin. The expression of trichohyalin,
 418 transcript variant X1 was also high expression in the three different coat colors
 419 goat skin. Trichohyalin confers mechanical strength to the hair follicle inner
 420 root sheath and to other toughened epithelial tissues, such as the hard palate
 421 and filiform ridges of the tongue, by forming multiple complex crosslinks with
 422 itself and with other structural proteins (Steinert *et al.* 2003), therefore high
 423 expression trichohyalin plays important role in hair follicle development.

424 The GO and KEGG pathway analyses of differentially expressed genes

revealed that most were associated with the function of single-organism metabolic process, oxidoreductase activity and oxidation-reduction process ontology categories. Of particular interest in our dataset were pathways related to pigmentation and melanogenesis. In this study, in terms of the molecular function category, 3 significant genes were found to be involved in melanogenesis and 3 significant genes were found to be involved in tyrosine metabolism. All these results provide strong evidence that there is a significant difference in the level of melanogenesis between the different coat colors goat skin. However, further investigation is still needed to confirm the regulatory relationships of these genes.

Melanocortin-1 receptor (*MC1R*) is responsible for binding melanocyte stimulating hormone (MSH), which is expressed by stressed keratinocytes, and initiating the cascade of melanogenesis (Solano *et al.* 2006). *MC1R* has classically been considered to play a role in and be the irreplaceable target involved in regulating mammalian skin pigmentation and hair color (Roberts *et al.* 2006; Schaffler *et al.* 2006; Lalueza-Fox *et al.* 2007). Recently, a study found that there is no association between *MC1R* polymorphisms and plumagecoloration in the zebra finch (Hoffman *et al.* 2014) which is consistent with another study that was performed in leaf warblers (Macdougall-Shackleton *et al.* 2003). Another recently study also indicated that the coat color variation observed in rhesusmacaques (variation from light to dark) is unlikely to be due to differences in the expression levels of the *MC1R* gene. In a recent microarray experiment, no significant differences in the expression of *MC1R* were observed between the white skin and black spots of sheep (Penagaricano *et al.* 2012). There were no significant differences in the

450 expression of *MC1R* between the black-skinned and white-skinned Lueyang
451 chickens (Zhang *et al.* 2015). Similarly and to our surprise, *MC1R* showed no
452 significant differences in expression between the three different coat colors
453 goat used in this study. The specific function and role of *MC1R* in pigmentation
454 still requires further research.

455 The gene for agouti signaling protein (*ASIP*) is centrally involved in the
456 expression of coat-color traits in animals. Previous studies have shown that
457 the *ASIP* gene is responsible for the skin color of both white and black-coated
458 sheep (Norris and Whan 2008) and the *ASIP* CNV were association with coat
459 color in Tibetan sheep (Han *et al.* 2015). A mutation in *ASIP* causes the
460 black-and-tan pigmentation phenotype observed pigs (Drogemuller *et al.*
461 2006), A missense mutation in the agouti signaling protein gene (*ASIP*) is
462 associated with the no light points coat phenotype in donkeys (Abitbol *et al.*
463 2015). In our study, *ASIP* was found to show significantly different expression
464 levels in the gray-skinned goats versus the white-skinned goats and the
465 brown-skinned goats versus the white skinned goats, while *ASIP* was only
466 detected in the white skin and almostly not detected in the gray skin and brown
467 skin. The result that *ASIP* only was detected in the white skin and not in the
468 dark skins seems to be inconsistent with the previous study, which has shown
469 that the expression of the *ASIP* gene was also significantly greater in the
470 black-skinned compared to the white-skinned chickens (Zhang *et al.* 2015).
471 *ASIP* may play important role in the pigmentation of goat and further
472 investigations are still needed to confirm the mechanism of *ASIP*.

473 *TYRP1*, one of themembers of the tyrosinase family, is a I type membrane
474 bound protein that is expressed in both melanocytes and the retinal epithelium.

475 *TYRP1* is involved in the distal eumelanin pathway and plays a role in
 476 stabilizing TYR, which is the critical and rate-determining enzyme in
 477 melanogenesis (Kobayashi *et al.* 1998). There existed a significant association
 478 between coat color and *TYRP1* in Soay sheep. In the free-living Soay sheep,
 479 coat color is either dark brown or light tawny color. The light phenotype is
 480 determined by homozygosity of a single recessive amino acid change (G→T
 481 transversion) at coding position 869 in the *TYRP1* gene (Gratten *et al.* 2007).
 482 This is consistent with studies in domestic sheep, where light coat color is
 483 known to be due to a decrease in the ratio of eumelanin to pheomelanin,
 484 relative to black coat color (Aliev *et al.* 1990). In Bionda dell'Adamello goat, the
 485 genotype frequency distribution of a non-synonymous SNP (G1112A)
 486 suggested a possible role of *TYRP1* in brown eumelanin goat coat colour
 487 (Nicoloso *et al.* 2012). The brown coat colour of Coppernecked goats is
 488 associated with a non-synonymous variant (p.Gly496Asp) at the *TYRP1* locus
 489 (Becker *et al.* 2015). In our study, *TYRP1* were significantly up-regulated in
 490 brown goat skin (brown vs white, brown vs gray) and *TYRP1* showed no
 491 differences in expression between the gray and white skin samples using high
 492 throughput RNA deep sequencing, but the subsequent validation were
 493 inconsistent with our transcriptome sequencing data. The results of the qPCR
 494 showed that *TYRP1* were significantly up-regulated in brown goat skin (brown
 495 vs white, brown vs gray) and had significantly higher expression in gray goat
 496 skin compared with white goat skin. These results still require additional
 497 research.

498 *PMEL* (*SILV* or *PMEL17*) is involved in melanosomal structure and acts as
 499 a scaffold in melanosomes by forming the proteolytic fibrillar matrix where

500 melanin is deposited (Theos *et al.* 2005). It is a prominent member of the
501 group of dilution genes. *SILV* mutations, which result in pigment dilution, have
502 been discovered in many animals (Kerje *et al.* 2004; Brunberg *et al.* 2006;
503 Clark *et al.* 2006; Gutierrez-Gil *et al.* 2007; Kuhn and Weikard 2007; Hellstrom
504 *et al.* 2011). In our study, *PMEL* were significantly up-regulated in brown goat
505 skin (brown vs white, brown vs gray) and had significantly higher expression in
506 gray goat skin compared with white goat skin. The results of our RNA-seq and
507 the subsequent validation were consistent.

508 *DCT* (*TYRP2*) is a melanogenic enzyme termed DOPAchrome
509 tautomerase, which isomerizes the pigmented intermediate DOPAchrome to
510 DHICA (5,6-dihydroxyindole-2-carboxylic acid) rather than to DHI
511 (5,6-dihydroxyindole) (Tsukamoto *et al.* 1992). Mutations of *DCT* were
512 associated with coat-color variation in mice (Jackson *et al.* 1992; Budd and
513 Jackson 1995). The mice deficient in *DCT* showed a diluted coat color
514 phenotype (Guyonneau *et al.* 2004). Mutations in dopachrome tautomerase
515 (*Dct*) affect eumelanin/pheomelanin synthesis (Costin *et al.* 2005). Sheep with
516 the GG genotype of *DCT* had significantly ($P < 0.05$) lower tyrosinase activity,
517 alkali-soluble melanin content, and ratio of eumelanin: total melanin than
518 sheep with GA and AA genotypes when measured across all investigated
519 samples (Deng *et al.* 2009). *DCT* did not show expression in duck feather
520 bulbs but expressed in retina (Li *et al.* 2012). Our RNA-seq and qPCR all
521 showed that *DCT* were significantly up-regulated in brown goat skin (brown vs
522 white, brown vs gray) and there were no significant differences in the
523 expression of *DCT* between the gray and white skin samples.

524 *TRPM1* is a member of the transient receptor potential (TRP) channel

525 family. Although the potential effects of *TRPM1* on pigmentation have not been
526 fully elucidated, it has been reported to be a potent regulator of pigmentation
527 (Oancea *et al.* 2009). Moreover, it is associated with a distinct leucistic white
528 phenotype, the so-called leopard complex spotting, which is found in some
529 horse breeds (Bellone *et al.* 2008; Wade *et al.* 2009). Interestingly, this
530 mutation is linked with night blindness in homozygous Appaloosa horses. In
531 homozygous leopard spotting horses, mRNA expression of *TRPM1* is
532 significantly downregulated in both skin and retina (Bellone *et al.* 2008; Bellone
533 *et al.* 2010). This gene is also involved in night blindness in humans (Van
534 Genderen *et al.* 2009; Koike *et al.* 2010). Our RNA-seq and qPCR all showed
535 that *TRPM1* were significantly up-regulated in brown goat skin (brown vs white,
536 brown vs gray) and there were no significant differences in the expression of
537 *TRPM1* between the gray and white skin samples.

538 *ELOVL3* encodes a protein that belongs to the GNS1/SUR4 family.
539 Members of this family play a role in elongation of long chain fatty acids to
540 provide precursors for synthesis of sphingolipids and ceramides. The
541 *Elovl3*-ablated mice displayed a sparse hair coat (Westerberg *et al.* 2004). Our
542 RNA-seq and qPCR all showed that *ELOVL3* were significantly up-regulated in
543 gray goat skin (gray vs white, brown vs gray) and there were no significant
544 differences in the expression of *ELOVL3* between the brown and white skin
545 samples.

546 *ASIP* was only detected in the white skin and not in the dark skins,
547 therefore *ASIP* may play important role in maintaining goat white coat color.
548 *PMEL*, *TYRP1*, *TRPM1* and *DCT* were significantly up-regulated in brown goat
549 skin compare with the gray and white color skin, so they may play important

550 role in the formation of goat brown coat color. In addition, *ELOVL3* were
 551 significantly up-regulated in gray goat skin compare with the brown and white
 552 color skin and there were no significant differences in the expression of
 553 *ELOVL3* between the brown and white skin samples. *PMEL* had significantly
 554 higher expression in gary goat skin compared with white goat skin and it was
 555 also found that *TYRP1* had significantly higher expression in gary goat skin
 556 compared with white goat skin by qPCR. *ELOVL3*, *PMEL* and *TYRP1* may be
 557 important for the goat gray coat color although the result of *TYRP1* qPCR were
 558 inconsistent with RNA-seq. *PMEL* may be a key factor in the pigmentation of
 559 the three different coat colors. These results still require additional research.

560

561 CONCLUSIONS

562 In summary, this is an original report of the transcriptome analysis of the
 563 skin from three different coat colors goat. The present study described and
 564 revealed a set differentially expressed known and novel genes found in goat
 565 skin that are potentially related to skin color and other physiological functions.
 566 The *MC1R* gene showed no difference in expression between the three
 567 diferent coat colors and the *ASIP* gene was only detected in the white skin,
 568 which are of particular interest for future studies to elucidate its functional roles
 569 in the regulation of skin color. *PMEL*, *TYRP1*, *TRPM1* and *DCT* may play
 570 important role in the formation of goat brown coat color and *ELOVL3*, *PMEL*
 571 and *TYRP1* may be important for the goat gray coat color. Results are
 572 foundational for future studies to potentially manipulate coat color via
 573 pharmacological and genetic approaches.

574

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585 586 LITERATURE CITED

- 587 Abitbol, M., R. Legrand and L. Turet, 2015 A missense mutation in the agouti signaling protein
588 gene (ASIP) is associated with the no light points coat phenotype in donkeys. *Genet*
589 *Sel Evol* 47: 28.
- 590 Aliev, G., M. Rachkovsky, S. Ito, K. Wakamatsu and A. Ivanov, 1990 Pigment types in
591 selected color genotypes of Asiatic sheep. *Pigment Cell Res* 3: 177-180.
- 592 Becker, D., M. Otto, P. Ammann, I. Keller, C. Drogemuller *et al.*, 2015 The brown coat colour
593 of Coppernecked goats is associated with a non-synonymous variant at the TYRP1
594 locus on chromosome 8. *Anim Genet* 46: 50-54.
- 595 Bellone, R. R., S. A. Brooks, L. Sandmeyer, B. A. Murphy, G. Forsyth *et al.*, 2008 Differential
596 gene expression of TRPM1, the potential cause of congenital stationary night
597 blindness and coat spotting patterns (LP) in the Appaloosa horse (*Equus caballus*).
598 *Genetics* 179: 1861-1870.
- 599 Bellone, R. R., G. Forsyth, T. Leeb, S. Archer, S. Sigurdsson *et al.*, 2010 Fine-mapping and
600 mutation analysis of TRPM1: a candidate gene for leopard complex (LP) spotting and
601 congenital stationary night blindness in horses. *Brief Funct Genomics* 9: 193-207.
- 602 Bennett, D. C., and M. L. Lamoreux, 2003 The color loci of mice--a genetic century. *Pigment*
603 *Cell Res* 16: 333-344.
- 604 Brunberg, E., L. Andersson, G. Cothran, K. Sandberg, S. Mikko *et al.*, 2006 A missense
605 mutation in PMEL17 is associated with the Silver coat color in the horse. *BMC Genet* 7:
606 46.
- 607 Budd, P. S., and I. J. Jackson, 1995 Structure of the mouse tyrosinase-related
608 protein-2/dopachrome tautomerase (Typr2/Dct) gene and sequence of two novel slaty
609 alleles. *Genomics* 29: 35-43.
- 610 Bunge, R., D. L. Thomas, T. G. Nash and C. J. Lupton, 1996 Performance of hair breeds and
611 prolific wool breeds of sheep in southern Illinois: wool production and fleece quality. *J*

612 Anim Sci 74: 25-30.

613 Cieslak, M., M. Reissmann, M. Hofreiter and A. Ludwig, 2011 Colours of domestication. Biol
614 Rev Camb Philos Soc 86: 885-899.

615 Clark, L. A., J. M. Wahl, C. A. Rees and K. E. Murphy, 2006 Retrotransposon insertion in
616 SILV is responsible for merle patterning of the domestic dog. Proc Natl Acad Sci U S A
617 103: 1376-1381.

618 Costin, G. E., J. C. Valencia, K. Wakamatsu, S. Ito, F. Solano *et al.*, 2005 Mutations in
619 dopachrome tautomerase (Dct) affect eumelanin/pheomelanin synthesis, but do not
620 affect intracellular trafficking of the mutant protein. Biochem J 391: 249-259.

621 Deng, W., Y. Tan, X. Wang, D. Xi, Y. He *et al.*, 2009 Molecular cloning, sequence
622 characteristics, and polymorphism analyses of the tyrosinase-related protein 2 /
623 DOPACHROME tautomerase gene of black-boned sheep (*Ovis aries*). Genome 52:
624 1001-1011.

625 Dereure, O., 2001 Drug-induced skin pigmentation. Epidemiology, diagnosis and treatment.
626 Am J Clin Dermatol 2: 253-262.

627 Drogemuller, C., A. Giese, F. Martins-Wess, S. Wiedemann, L. Andersson *et al.*, 2006 The
628 mutation causing the black-and-tan pigmentation phenotype of Mangalitza pigs maps
629 to the porcine ASIP locus but does not affect its coding sequence. Mamm Genome 17:
630 58-66.

631 Ducrest, A. L., L. Keller and A. Roulin, 2008 Pleiotropy in the melanocortin system, coloration
632 and behavioural syndromes. Trends Ecol Evol 23: 502-510.

633 Duffy, D. L., Z. Z. Zhao, R. A. Sturm, N. K. Hayward, N. G. Martin *et al.*, 2010 Multiple
634 pigmentation gene polymorphisms account for a substantial proportion of risk of
635 cutaneous malignant melanoma. J Invest Dermatol 130: 520-528.

636 Emaresi, G., A. L. Ducrest, P. Bize, H. Richter, C. Simon *et al.*, 2013 Pleiotropy in the
637 melanocortin system: expression levels of this system are associated with
638 melanogenesis and pigmentation in the tawny owl (*Strix aluco*). Mol Ecol 22:
639 4915-4930.

640 Fan, R., J. Xie, J. Bai, H. Wang, X. Tian *et al.*, 2013 Skin transcriptome profiles associated
641 with coat color in sheep. BMC Genomics 14: 389.

642 Fan, Y., P. Wang, W. Fu, T. Dong, C. Qi *et al.*, 2014 Genome-wide association study for
643 pigmentation traits in Chinese Holstein population. Anim Genet 45: 740-744.

644 Garcia-Gamez, E., A. Reverter, V. Whan, S. M. McWilliam, J. J. Arranz *et al.*, 2011 Using
645 regulatory and epistatic networks to extend the findings of a genome scan: identifying
646 the gene drivers of pigmentation in merino sheep. PLoS One 6: e21158.

647 Gratten, J., D. Beraldi, B. V. Lowder, A. F. McRae, P. M. Visscher *et al.*, 2007 Compelling
648 evidence that a single nucleotide substitution in TYRP1 is responsible for coat-colour
649 polymorphism in a free-living population of Soay sheep. Proc Biol Sci 274: 619-626.

650 Gutierrez-Gil, B., P. Wiener and J. L. Williams, 2007 Genetic effects on coat colour in cattle:
651 dilution of eumelanin and pheomelanin pigments in an F2-Backcross Charolais x
652 Holstein population. BMC Genet 8: 56.

653 Guyonneau, L., F. Murisier, A. Rossier, A. Moulin and F. Beermann, 2004 Melanocytes and
654 pigmentation are affected in dopachrome tautomerase knockout mice. Mol Cell Biol 24:
655 3396-3403.

656 Han, J. L., M. Yang, Y. J. Yue, T. T. Guo, J. B. Liu *et al.*, 2015 Analysis of agouti signaling
657 protein (ASIP) gene polymorphisms and association with coat color in Tibetan sheep
658 (*Ovis aries*). *Genet Mol Res* 14: 1200-1209.

659 Hart, K. L., S. L. Kimura, V. Mushailov, Z. M. Budimlija, M. Prinz *et al.*, 2013 Improved eye-
660 and skin-color prediction based on 8 SNPs. *Croat Med J* 54: 248-256.

661 Hellstrom, A. R., B. Watt, S. S. Fard, D. Tenza, P. Mannstrom *et al.*, 2011 Inactivation of
662 Pmel alters melanosome shape but has only a subtle effect on visible pigmentation.
663 *PLoS Genet* 7: e1002285.

664 Hoffman, J. I., E. T. Krause, K. Lehmann and O. Kruger, 2014 MC1R genotype and plumage
665 colouration in the zebra finch (*Taeniopygia guttata*): population structure generates
666 artefactual associations. *PLoS One* 9: e86519.

667 Ito, S., and K. Wakamatsu, 2008 Chemistry of mixed melanogenesis--pivotal roles of
668 dopaquinone. *Photochem Photobiol* 84: 582-592.

669 Ito, S., K. Wakamatsu and H. Ozeki, 2000 Chemical analysis of melanins and its application
670 to the study of the regulation of melanogenesis. *Pigment Cell Res* 13 Suppl 8:
671 103-109.

672 Jablonski, N. G., and G. Chaplin, 2010 Colloquium paper: human skin pigmentation as an
673 adaptation to UV radiation. *Proc Natl Acad Sci U S A* 107 Suppl 2: 8962-8968.

674 Jackson, I. J., D. M. Chambers, K. Tsukamoto, N. G. Copeland, D. J. Gilbert *et al.*, 1992 A
675 second tyrosinase-related protein, TRP-2, maps to and is mutated at the mouse slaty
676 locus. *EMBO J* 11: 527-535.

677 Kanehisa, M., M. Araki, S. Goto, M. Hattori, M. Hirakawa *et al.*, 2008 KEGG for linking
678 genomes to life and the environment. *Nucleic Acids Res* 36: D480-484.

679 Karsi, A., D. Cao, P. Li, A. Patterson, A. Kocabas *et al.*, 2002 Transcriptome analysis of
680 channel catfish (*Ictalurus punctatus*): initial analysis of gene expression and
681 microsatellite-containing cDNAs in the skin. *Gene* 285: 157-168.

682 Kerje, S., P. Sharma, U. Gunnarsson, H. Kim, S. Bagchi *et al.*, 2004 The Dominant white,
683 Dun and Smoky color variants in chicken are associated with insertion/deletion
684 polymorphisms in the PMEL17 gene. *Genetics* 168: 1507-1518.

685 Kidson, S. H., and B. C. Fabian, 1981 The effect of temperature on tyrosinase activity in
686 Himalayan mouse skin. *J Exp Zool* 215: 91-97.

687 Kobayashi, T., G. Imokawa, D. C. Bennett and V. J. Hearing, 1998 Tyrosinase stabilization
688 by Tyrp1 (the brown locus protein). *J Biol Chem* 273: 31801-31805.

689 Koike, C., T. Numata, H. Ueda, Y. Mori and T. Furukawa, 2010 TRPM1: a vertebrate TRP
690 channel responsible for retinal ON bipolar function. *Cell Calcium* 48: 95-101.

691 Kuhn, C., and R. Weikard, 2007 An investigation into the genetic background of coat colour
692 dilution in a Charolais x German Holstein F2 resource population. *Anim Genet* 38:
693 109-113.

694 Lalueza-Fox, C., H. Rompler, D. Caramelli, C. Staubert, G. Catalano *et al.*, 2007 A
695 melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals.
696 *Science* 318: 1453-1455.

697 Lamoreux, M. L., K. Wakamatsu and S. Ito, 2001 Interaction of major coat color gene
698 functions in mice as studied by chemical analysis of eumelanin and pheomelanin.
699 *Pigment Cell Res* 14: 23-31.

Li, M. H., T. Tiirikka and J. Kantanen, 2014 A genome-wide scan study identifies a single nucleotide substitution in ASIP associated with white versus non-white coat-colour variation in sheep (*Ovis aries*). *Heredity* (Edinb) 112: 122-131.

Li, S., C. Wang, W. Yu, S. Zhao and Y. Gong, 2012 Identification of genes related to white and black plumage formation by RNA-Seq from white and black feather bulbs in ducks. *PLoS One* 7: e36592.

MacDougall-Shackleton, E. A., L. Blanchard, S. A. Igodura and H. L. Gibbs, 2003 Unmelanized plumage patterns in Old World leaf warblers do not correspond to sequence variation at the melanocortin-1 receptor locus (MC1R). *Mol Biol Evol* 20: 1675-1681.

Marshall, R. A., C. E. Aitken, M. Dorywalska and J. D. Puglisi, 2008 Translation at the single-molecule level. *Annu Rev Biochem* 77: 177-203.

Minvielle, F., B. Bed'hom, J. L. Coville, S. Ito, M. Inoue-Murayama *et al.*, 2010 The "silver" Japanese quail and the MITF gene: causal mutation, associated traits and homology with the "blue" chicken plumage. *BMC Genet* 11: 15.

Mogosan, G. D., A. M. Grumezescu and M. C. Chifiriuc, 2014 Keratin-based biomaterials for biomedical applications. *Curr Drug Targets* 15: 518-530.

Nan, H., P. Kraft, D. J. Hunter and J. Han, 2009 Genetic variants in pigmentation genes, pigmentary phenotypes, and risk of skin cancer in Caucasians. *Int J Cancer* 125: 909-917.

Nicoloso, L., R. Negrini, P. Ajmone-Marsan and P. Crepaldi, 2012 On the way to functional agro biodiversity: coat colour gene variability in goats. *Animal* 6: 41-49.

Norris, B. J., and V. A. Whan, 2008 A gene duplication affecting expression of the ovine ASIP gene is responsible for white and black sheep. *Genome Res* 18: 1282-1293.

Oancea, E., J. Vriens, S. Brauchi, J. Jun, I. Splawski *et al.*, 2009 TRPM1 forms ion channels associated with melanin content in melanocytes. *Sci Signal* 2: ra21.

Ozsolak, F., and P. M. Milos, 2011 RNA sequencing: advances, challenges and opportunities. *Nat Rev Genet* 12: 87-98.

Patterson, A., A. Karsi, J. Feng and Z. Liu, 2003 Translational machinery of channel catfish: II. Complementary DNA and expression of the complete set of 47 60S ribosomal proteins. *Gene* 305: 151-160.

Penagaricano, F., P. Zorrilla, H. Naya, C. Robello and J. I. Urioste, 2012 Gene expression analysis identifies new candidate genes associated with the development of black skin spots in Corriedale sheep. *J Appl Genet* 53: 99-106.

Roberts, D. W., R. A. Newton, K. A. Beaumont, J. Helen Leonard and R. A. Sturm, 2006 Quantitative analysis of MC1R gene expression in human skin cell cultures. *Pigment Cell Res* 19: 76-89.

Schaffler, A., J. Scholmerich and C. Buechler, 2006 The role of 'adipotropins' and the clinical importance of a potential hypothalamic-pituitary-adipose axis. *Nat Clin Pract Endocrinol Metab* 2: 374-383.

Slominski, A., D. J. Tobin, S. Shibahara and J. Wortsman, 2004 Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiol Rev* 84: 1155-1228.

Solano, F., S. Briganti, M. Picardo and G. Ghanem, 2006 Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. *Pigment Cell Res* 19:

744 550-571.

745 Steinert, P. M., D. A. Parry and L. N. Marekov, 2003 Trichohyalin mechanically strengthens

746 the hair follicle: multiple cross-bridging roles in the inner root sheath. *J Biol Chem* 278:

747 41409-41419.

748 Steingrimsdottir, E., N. G. Copeland and N. A. Jenkins, 2006 Mouse coat color mutations:

749 from fancy mice to functional genomics. *Dev Dyn* 235: 2401-2411.

750 Sturm, R. A., and D. L. Duffy, 2012 Human pigmentation genes under environmental

751 selection. *Genome Biol* 13: 248.

752 Sulem, P., D. F. Gudbjartsson, S. N. Stacey, A. Helgason, T. Rafnar *et al.*, 2007 Genetic

753 determinants of hair, eye and skin pigmentation in Europeans. *Nat Genet* 39:

754 1443-1452.

755 Theos, A. C., S. T. Truschel, G. Raposo and M. S. Marks, 2005 The Silver locus product

756 Pmel17/gp100/Silv/ME20: controversial in name and in function. *Pigment Cell Res* 18:

757 322-336.

758 Tsukamoto, K., I. J. Jackson, K. Urabe, P. M. Montague and V. J. Hearing, 1992 A second

759 tyrosinase-related protein, TRP-2, is a melanogenic enzyme termed DOPAchrome

760 tautomerase. *EMBO J* 11: 519-526.

761 Vage, D. I., H. Klungland, D. Lu and R. D. Cone, 1999 Molecular and pharmacological

762 characterization of dominant black coat color in sheep. *Mamm Genome* 10: 39-43.

763 van Genderen, M. M., M. M. Bijveld, Y. B. Claassen, R. J. Florijn, J. N. Pearing *et al.*, 2009

764 Mutations in TRPM1 are a common cause of complete congenital stationary night

765 blindness. *Am J Hum Genet* 85: 730-736.

766 Wade, C. M., E. Giulotto, S. Sigurdsson, M. Zoli, S. Gnerre *et al.*, 2009 Genome sequence,

767 comparative analysis, and population genetics of the domestic horse. *Science* 326:

768 865-867.

769 Wang, L., J. Fan, M. Yu, S. Zheng and Y. Zhao, 2011a Association of goat (*Capra hircus*)

770 CD4 gene exon 6 polymorphisms with ability of sperm internalizing exogenous DNA.

771 *Mol Biol Rep* 38: 1621-1628.

772 Wang, P. Q., L. M. Deng, B. Y. Zhang, M. X. Chu and J. Z. Hou, 2011b Polymorphisms of the

773 cocaine-amphetamine-regulated transcript (CART) gene and their association with

774 reproductive traits in Chinese goats. *Genet Mol Res* 10: 731-738.

775 Wang, Z., M. Gerstein and M. Snyder, 2009 RNA-Seq: a revolutionary tool for

776 transcriptomics. *Nat Rev Genet* 10: 57-63.

777 Westerberg, R., P. Tvrdik, A. B. Unden, J. E. Mansson, L. Norlen *et al.*, 2004 Role for

778 ELOVL3 and fatty acid chain length in development of hair and skin function. *J Biol*

779 *Chem* 279: 5621-5629.

780 Wilhelm, B. T., and J. R. Landry, 2009 RNA-Seq-quantitative measurement of expression

781 through massively parallel RNA-sequencing. *Methods* 48: 249-257.

782 Xu, T., X. Guo, H. Wang, F. Hao, X. Du *et al.*, 2013 Differential gene expression analysis

783 between anagen and telogen of *Capra hircus* skin based on the de novo assembled

784 transcriptome sequence. *Gene* 520: 30-38.

785 Zhang, J., F. Liu, J. Cao and X. Liu, 2015 Skin transcriptome profiles associated with skin

786 color in chickens. *PLoS One* 10: e0127301.

787 Zuber, M., F. Zia, K. M. Zia, S. Tabasum, M. Salman *et al.*, 2015 Collagen based

788 polyurethanes-A review of recent advances and perspective. Int J Biol Macromol 80:
789 366-374.
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791 **Figure captions.**

792 **Figure 1. Classification of total raw reads pairs of different coat colors goat skin.** After
793 filtering the only adaptor sequences, containing N sequences and low quality sequences, the
794 three RNA-Seq libraries still generated over 26 million clean reads pairs in each library, and
795 the percentage of clean reads among raw tags in each library ranged from 94.07% to 94.71%.

796 **Figure 2. Percent of reads mapped to genome regions.** The reads mapped to genome
797 regions including exon, intron and intergenic.

798 **Figure 3. The numbers of DEGs between the three different coat colors goat skin.**
799 Between the gray skin and white skin libraries, there were 74 upregulated genes and 17
800 downregulated genes; Between the brown skin and white skin libraries, there were 23
801 upregulated genes and 44 downregulated genes, while there were 33 upregulated genes and
802 121 downregulated genes between the brown skin and gray skin libraries.

803 **Figure 4. GO functional analysis of DEGs based on RNA-Seq data.** The results were
804 summarised the most enriched GO terms in three main categories: biological process, cellular
805 component and molecular function.

806 **Figure 5 The qPCR validation of differentially expressed genes in the three different**
807 **coat colors goat skin.** Abundance of target genes was normalized relative to abundance of
808 *GAPDH* gene. Bars in each panel represent the mean $\pm$ standard error (n = 3).
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Figure 1

Figure 2

Figure 3

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832 **Figure 4**

Figure 5

846 **Table captions**

847 **Table 1. Information regarding the specific primers used for the qPCR.**

848 **Table 2. Quality Control of the Sequencing Data**

849 **Table 3. Summary of read numbers based on the RNA-Seq data from different coat**
 850 **colors goat skin.**

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872 **Table 1**

Primer	Sequences (5'→3')	GenBank accession number	Product Length (bp)
<i>PMEL</i>	Forward: AGAGACTACCATTGCCTGAAA	XM_005680385.1	126
	Reverse: TCCTTGGGTTGGGAAGAG		
<i>TRPM1</i>	Forward: GTGTCTGGAATTACAATGGAAG	XM_005695175.1	131
	Reverse: CTAGATGACCAAGATGGAGCT		
<i>DCT</i>	Forward: CACCCTTAACGGAGACACA	XM_005687699.1	106
	Reverse: TAGGTCAGAACTACGGCTTG		
<i>TYRP1</i>	Forward: TCTTGTCTGATTCGTGCCA	NM_001285727.1	114
	Reverse: AGACTGATTAGGGTTATGGATT		
<i>ELOVL3</i>	Forward: GTCGGTATCCTGGCTTAT	XM_005698356.1	168
	Reverse: TCACTGGCTCTTGGTCTT		
<i>GAPDH</i>	Forward: AGTTCCACGGCACAGTCAAG	AJ431207.1	117
	Reverse: ACTCAGCACCAGCATCACC		

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888 **Table 2**

Sample name	Raw reads	Clean reads	clean bases	Error rate(%)	Q20(%)	Q30(%)	GC content(%)
White_1	44207310	41587171	5.2G	0.04	94.95	89.92	51.36
White_2	44207310	41587171	5.2G	0.04	93.45	87.66	51.35
Gray_1	37218908	35111296	4.39G	0.04	94.79	89.62	51.39
Gray_2	37218908	35111296	4.39G	0.04	93.28	87.37	51.45
Brown_1	27499268	26045606	3.26G	0.04	94.99	89.99	51.51
Brown_2	27499268	26045606	3.26G	0.04	93.48	87.74	51.50

889 Raw reads: the numbers of original data; Clean reads: the numbers of after filtering the original

890 data; Error rate: Phred score, Qphred = $-10\log_{10}(e)$; Q20、Q30: the percent of the bases of

891 Qphred>20 or 30 in the total bases.

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908 **Table 3**

Sample_name	White	Gray	Brown
Total reads	83174342	70222592	52091212
Total mapped	65032149 (78.19%)	54279674 (77.3%)	40999750 (78.71%)
Multiple mapped	1350169 (1.62%)	1123310 (1.6%)	833097 (1.6%)
Uniquely mapped	63681980 (76.56%)	53156364 (75.7%)	40166653 (77.11%)
Non-splice reads	39477742 (47.46%)	33330204 (47.46%)	25547588 (49.04%)
Splice reads	24204238 (29.1%)	19826160 (28.23%)	14619065 (28.06%)

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