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Original research article

Transposon insertion in *pmel17* rewired skin and muscle transcriptomes in Mozambique tilapia

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ABSTRACT

The *pmel17* gene plays a crucial role in melanin pigmentation. Our previous studies showed that in Mozambique tilapia, a transposon inserted into the 3' untranslated region (3′ UTR) of *pmel17* resulted in the silencing of *pmel17* and led to the loss of melanin pigments (golden mutant phenotype). Additionally, the transposon insertion caused reduced growth performance and increased locomotion. In this study, to investigate the mechanisms underlying these phenotypic changes, we sequenced transcriptomes of the skin and muscle samples collected from wildtype and mutant tilapias. A total of 51 and 141 differentially expressed genes (DEGs) were identified in the skin and muscle transcriptomes, respectively. DEGs in the skin were primarily down-regulated in golden genotypes and associated with neural crest development and melanin pigmentation pathways. Besides these DEGs involved in the classic melanin pigmentation pathway of vertebrates, 14 DEGs were also observed to be related to melanogenesis. In muscle transcriptomes, there was an enrichment of GO terms associated with growth factors and cellular lipid catabolic processes. Specifically, DEGs related to growth factor binding exhibited a down-regulation, while those related to lipid metabolism showed an up-regulation in mutant genotypes. These findings agree with observed phenotypic changes. Furthermore, several DEGs associated with muscle function and mobility were up-regulated. Our study sheds light on how a single mutation in a gene can modulate multiple phenotypes by rewiring gene regulation networks. The research also provides valuable insights into the complex genetic mechanisms underlying the regulation of diverse phenotypic traits by a single gene.

1. Introduction

A single gene typically operates in a complicated gene regulation network and plays important roles in different stages of the development, growth, and functioning of living organisms (Davidson, 2010). Therefore, a single gene can take part in various biological processes and cause numerous phenotypic variations by influencing the associated regulatory network, a phenomenon known as pleiotropy (Alberts, 2017). Studies pertaining to gene regulatory networks are rapidly evolving, aiming to unravel the complexity of these processes (Mercatelli, Scalambra, Triboli, Ray, & Giorgi, 2020). In a gene regulation network, cascading interactions in a pathway are common, wherein a single gene can activate or inhibit the expression of multiple associated genes through diverse mechanisms, such as interactions with regulatory elements or the production of signalling molecules (Karlebach & Shamir, 2008; Mcginnis & Madden, 2004). Therefore, altering the expression pattern or function of a single gene in a regulatory network or cascading pathway can lead to the rewiring of the whole regulatory network and result in multiple phenotypic variations (Muntoni, Torelli, & Ferlini, 2003).

In our previous study, we found a natural recessive golden color mutant (loss of melanin) in Mozambique tilapia, *Oreochromis mossambicus* (Liu, Sun, Kuang, Wang, & Yue, 2022a). This mutation was due to the silencing of *pmel17*, resulting from a transposon insertion into the 3' UTR of this gene (Liu, Sun, Kuang, Wang, & Yue, 2022b). Other than the loss of melanin in the skin and eyes, mutant genotypes also showed reduced growth performance and increased locomotion. This is consistent with the data from another study where the mutant genotypes had significantly lower body weight, denser overall swimming trajectory and longer average swimming distance at four months old compared to wildtypes in the same family (Wang et al., 2023). *pmel17*, also known as the *silver* gene, encodes a premelanosome protein and is involved in the melanin polymerization in the pigment organelle, which is the melanosome of melanocytes (Theos, Truschel, Raposo, & Marks, 2005).

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Loss-of-function of this gene causes the disappearance of melanin granules in vertebrates (Liu et al., 2022b; Mcginnis & Madden, 2004; Watt, Van Niel, Raposo, & Marks, 2013). However, the mechanisms through which a gene which is associated with melanin pigmentation is implicated in the regulation of growth and locomotion remains unknown. We hypothesized that *pmel17* could modulate both the growth and locomotion regulatory pathways through its involvement in a coregulatory network or cascading pathway.

Transcriptome sequencing, also known as RNA sequencing (RNA-Seq), is a robust tool enabling the identification of potential candidate genes, pathways, and regulatory networks that are responsible for intricate phenotypic changes in organisms. This approach holds the potential to offer valuable molecular insights into the underlying mechanisms driving these changes (Wang, Gerstein, & Snyder, 2009). In this study, through transcriptome sequencing, we identified DEGs between the wildtype and golden mutant genotypes at the *pmel17* locus of Mozambique tilapia. Our first purpose is to identify candidate genes, pathways, and networks that are involved in the regulation of melanin pigmentation, growth performance, and locomotion. In addition, we aim to understand the underlying mechanisms of how *pmel17* rewires pathways or networks, which finally lead to decrease growth and increased locomotion besides reduced melanin pigmentation. This study enhances our understanding of the coregulatory network governing melanin pigmentation, growth performance, and locomotion.

2. Materials and methods

2.1. Statement on ethics approval

All fish handling in the study followed the guidelines set up by the Institutional Animal Care and Use Committee (IACUC) of Temasek Life Sciences Laboratory (TLL), Singapore (approval number: TLL (F)-20–003). This paper does not involve the use of any human data or tissue.

2.2. Fish samples

The golden mutant phenotype (Fig. 1) in Mozambique tilapia was a recessive trait resulting from the insertion of a transposon into the 3' UTR of *pmel17* and subsequent silencing of its expression (Liu et al., 2022a, 2022b). The golden mutant (bb) showed a loss of melanin pigments in the skin and eye, reduced growth, and increased locomotion in comparison to the wildtype (BB). The fish with BB or bb genotypes used in this study are from a F_2 population. In brief, we crossed a wildtype (BB) with a golden Mozambique tilapia (bb) as F_0 and then crossed F_1 hybrids to obtain F_2 population (Wang et al., 2023). We identified fish with BB or bb genotypes by genotyping the *pmel17* locus using an insertion/deletion (InDel) marker developed in our previous study which was strictly linked with this gene (Liu et al., 2022a, 2022b). Skin and muscle samples were collected from four-month-old fish of both BB and bb genotypes for RNA extraction. The fish had an average body weight of 14.16 ± 3.97 g (BB) vs 12.26 ± 3.31 g (bb) at the same age (four months post hatch) with significant difference ($P < 0.01$, two-tailed *t*-test) and under the same culture conditions at the marine fish facility at the Temasek Life Sciences Laboratory, Singapore.

2.3. RNA extraction and sequencing

Total RNA was extracted from the skin and muscle tissues of three golden mutants (bb) and three wildtype individuals (BB), using TRIzol reagent (ThermoFisher Scientific, MA, USA) according to the manufacturer's protocol. RNA quality was examined using NanoDrop spectrophotometer (ThermoFisher, MA, USA) and electrophoresis using a 1% agarose gel. RNAseq libraries were constructed using the Illumina TruSeq RNA Library Prep Kit v2 (Illumina, CA, USA) according to the manufacturer's protocol. In brief, this process involved mRNA enrichment using poly-T oligo-attached magnetic beads, fragmentation of the enriched mRNA, cDNA synthesis, end repair, A-tailing, adapter ligation, and PCR enrichment. The libraries were quantified using the KAPA Illumina Library Quantification Kit (Roche, Switzerland). Finally, the libraries were sequenced with 2 × 75 bp pair-end reads on the Illumina NextSeq500 platform (Illumina, CA, USA).



Fig. 1. Phenotypes of wildtype and homozygous golden Mozambique tilapia.

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2.4. Identifying differentially expressed genes (DEGs)

Raw sequencing reads were processed and filtered using the program process_shortreads from the Stacks package (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011) to remove adaptors and low quality reads, following our previously established method (Wang et al., 2019). Clean reads were then aligned and mapped to the reference genome of Mozambique tilapia (unpublished) using the STAR aligner (Dobin & Gingeras, 2015) with default parameters. Only the uniquely mapped reads were extracted for further analyses. Uniquely mapped reads were counted against annotated gene features using HTSeq-count (Anders, Pyl, & Huber, 2015). To account for variations in sequencing depth among samples, transcript counts were normalized for detection of DEGs using DESeq2 (Love, Huber, & Anders, 2014). Transcripts with a low expression level, defined as having less than 1 count per million mapped reads (CPM), were excluded from further analysis. DEGs were determined based on a fold change (FC) cut-off of 2 and a significance cut-off of 0.05 after adjustment for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) correction (Thissen, Steinberg, & Kuang, 2002).

2.5. Analysing gene ontology and networks

Corresponding protein sequences of DEGs were extracted from the annotation files of the reference genome. These sequences were then mapped to the zebrafish protein database (Ensembl release 99) using BLASTP (Mcginnis & Madden, 2004) with an e-value threshold of 1E-10 to retrieve gene ontology (GO) accessions. DEGs from multiple samples were clustered using principal component analysis (PCA) and heatmap approaches based on normalized expression values. These analyses were performed using the ClustVis program (Metsalu & Vilo, 2015). Functional classification of the GO terms associated with the DEGs was carried out using REVIGO (Supek, Bošnjak, Škunca, & Šmuc, 2011) with zebrafish as a reference. To understand the interactions among different functional groups of the DEGs, network analysis based on the GO annotations was conducted using the program Metascape (Zhou et al., 2019) with reference to the zebrafish gene functioning network. Enrichment analyses were performed using default parameters.

2.6. Validating DEGs using quantitative PCR (qPCR)

The relative expression of randomly selected DEGs was examined using quantitative real-time PCR (q-RT-PCR) and compared with the mRNA sequencing data. Transcript sequences of selected DEGs were extracted from the annotated reference genome of Mozambique tilapia, and primers (Table S4) were designed using Primer3Plus (Untergasser et al., 2012). The housekeeping gene *beta-actin* was used as a reference for normalization, and qPCR reactions were conducted according to our previous study (Wang et al., 2014). Briefly, 2 μg of DNase-treated RNA were used to synthesize cDNA with the reverse transcriptase M-MLV (Promega, WI, USA). Real-time PCR was carried out in triplicates using the KAPA™ SYBR® FAST qPCR Kits (Kapa Biosystems, MA, USA) according to the manufacturer's protocol and in a BioRad CFX96 Real Time PCR Detection System (Bio-Rad, CA, USA). The $2^{-\Delta\Delta CT}$ method was employed to quantify the relative expression of individual genes. Pearson's correlation test was applied to examine the consistency between different data sets (Mukaka, 2012).

3. Results

3.1. RNA sequencing data

A total of 12 mRNA libraries from skin and muscle samples were constructed and sequenced, with each consisting of three wildtype (BB) individuals and three mutants (bb). The average number of clean reads throughout these mRNA libraries was 36.0 million (M), where the bb genotypes had a higher sequence coverage compared to the wildtype (BB) (with an average of ~40.4 M vs. 37.6 M in skin and ~36.3 M vs 29.8 M in muscle). In 11 samples, more than 82.2% of the clean reads were found to be uniquely mapped to the reference genome, while in one sample, 67.0% of the clean reads were uniquely mapped to the reference genome (unpublished) (Supplementary Table S1).

3.2. DEGs in the skin and muscle

A total of 51 DEGs were identified in the skin between the wildtype and golden mutant. Among these DEGs, 13 were upregulated and 38 were downregulated in the golden mutant (Fig. 2 A & B and Supplementary Table S2). These DEGs showed distinct expression patterns in contrast to the expression of background genes (Fig. 2 C). In muscle samples, a total of 141 DEGs were identified, within which 60 were up-regulated and 81 were down-regulated in the golden mutant (Fig. 2 D & E and Supplementary Table S3). Consistently, the expression patterns of these DEGs were also observed to be clearly different from those of the background genes (Fig. 2 F).

We further verified the relative expression patterns of these DEGs using q-RT-PCR by randomly selecting four up-regulated and six down-regulated DEGs from either skin or muscle samples. The same RNA samples used for RNA-Seq were used for validation by qPCR. The relative expression patterns were consistent between RNA-Seq and q-RT-PCR data sets for these selected 10 DEGs (Fig. S1). The correlation between the transcriptome data (transcripts per million) and qPCR data sets (relative mRNA expression) assessed by Pearson's correlation coefficient was 0.874 ($P < 0.001$) (Fig. S1), indicating the reliability of the DEGs identified by RNA-Seq.

3.3. GO enrichment based on all identified DEGs

A total of 18 GO terms and/or pathways were significantly enriched, with the most significant two terms being related to melanosomes (GO:0042470) and kinesins (zebrafish pathway: R-DRE-983189) (Fig. 3 A). There were also some enriched GO terms related to growth and fatty acid metabolism, e.g., growth factor binding (GO:0019838). Only one protein-protein interaction network which was significantly enriched and primarily related to kinesins was identified. Within this network, almost all the DEGs showed down-regulation in the golden mutant except for *zic3* (Fig. 3 B).

3.4. Genes involved in melanin pigmentation patterns

Gene ontology and protein-protein interaction enrichment analyses of DEGs in skin transcriptomes identified seven significantly enriched GO terms (Fig. 4 A). The most significant enriched GO term was related to Neural crest development (Fig. 4 A). Melanocytes which differentiate into pigment-producing cells, originate from the neural crest. Consistently, GO enrichment in the category biological processes showed that most of the enrichments were related to melanin pigmentation (Fig. 4 B). We then mapped the identified DEGs to the melanin pigmentation pathways of vertebrates (Stuckert et al., 2019). We found that most of the DEGs were down-regulated, involved in the melanogenesis, and played roles downstream of the causal gene *pmel17* (Fig. 5). Interestingly, we found that several genes, e.g., *tyr*, *tyrp1,* and *ednrb,* were upstream of *pmel17*. These genes play important roles in the regulation of *pmel17* expression and melanin synthesis (Serre, Busuttil, & Botto, 2018). In addition to the known genes implicated in melanin synthesis, we conducted a comprehensive literature mining to explore the potential functions of the remaining DEGs. Our search found that certain DEGs were also potentially related to melanin pigmentation patterns, although they were not included in the above signalling pathway. These genes included the down-regulated *zgc:153031*, *hsdl2*, *ahnak*,

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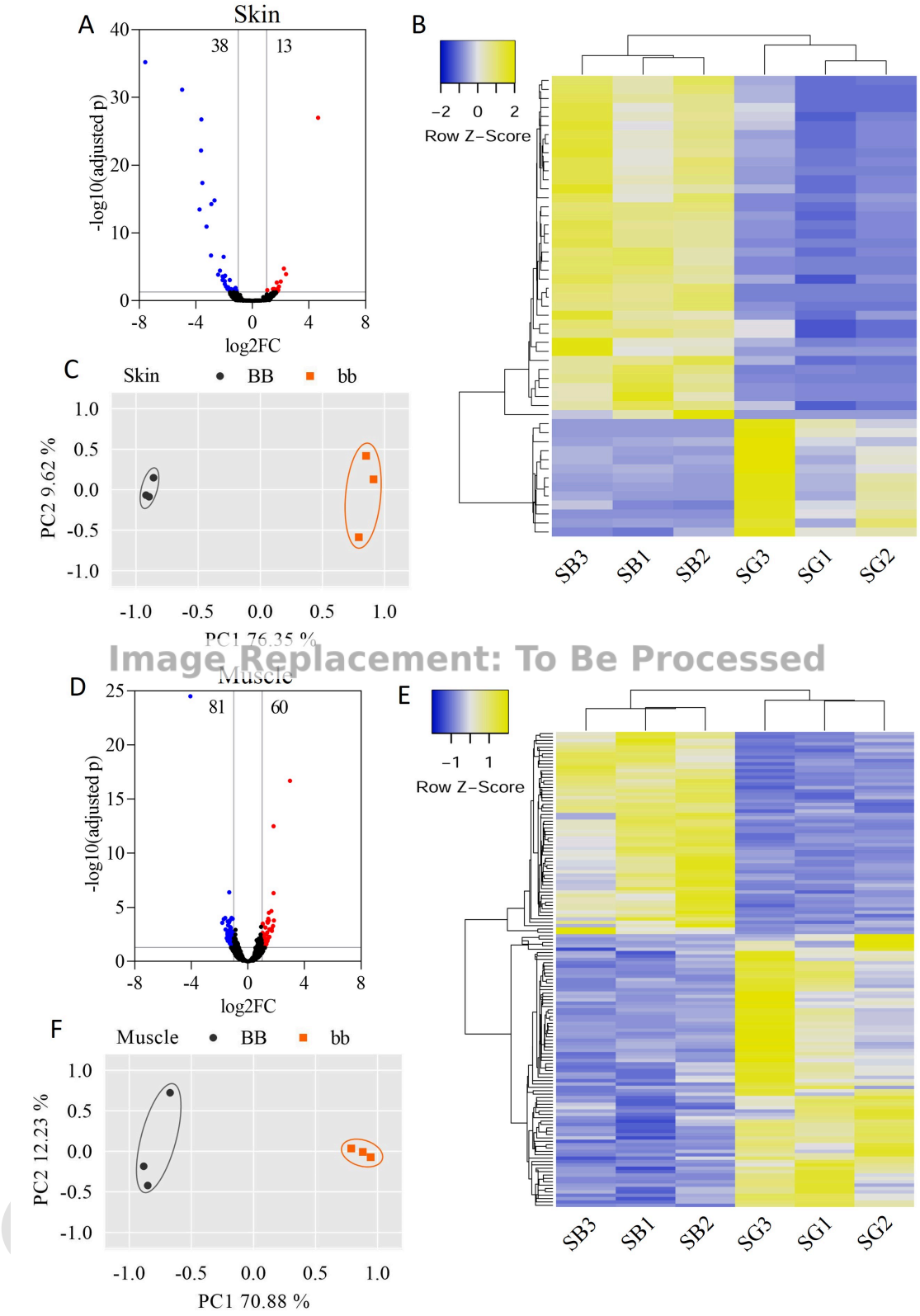

Fig. 2. Identifying differentially expressed genes (DEGs) in skin and muscle transcriptomes between wildtype and golden Mozambique tilapia. **A**, the expression patterns of DEGs identified in the skin by DESeq2, where the down- and up-regulated DEGs in golden tilapia are highlighted with blue and red, respectively. The number of DEGs in each category is denoted. **B**, Heatmap relationship among control (wildtype) and experimental (golden) skin samples based on differentially expressed genes (DEGs), revealed by hierarchical clustering. **C**, relationship among control (wildtype) and experimental (golden) skin samples based on differentially expressed genes (DEGs), revealed by Principal component analysis (PCA). **D**, the expression patterns of DEGs identified in the muscle by DESeq2, where the down-

Fig. 2.—*continued*
and up-regulated DEGs in golden tilapia are highlighted with blue and red, respectively. The number of DEGs in each category is denoted. **E**, Heatmap relationship among control (wildtype) and experimental (golden) muscle samples based on differentially expressed genes (DEGs), revealed by hierarchical clustering. **F**, relationship among control (wildtype) and experimental (golden) muscle samples based on differentially expressed genes (DEGs), revealed by Principal component analysis (PCA).

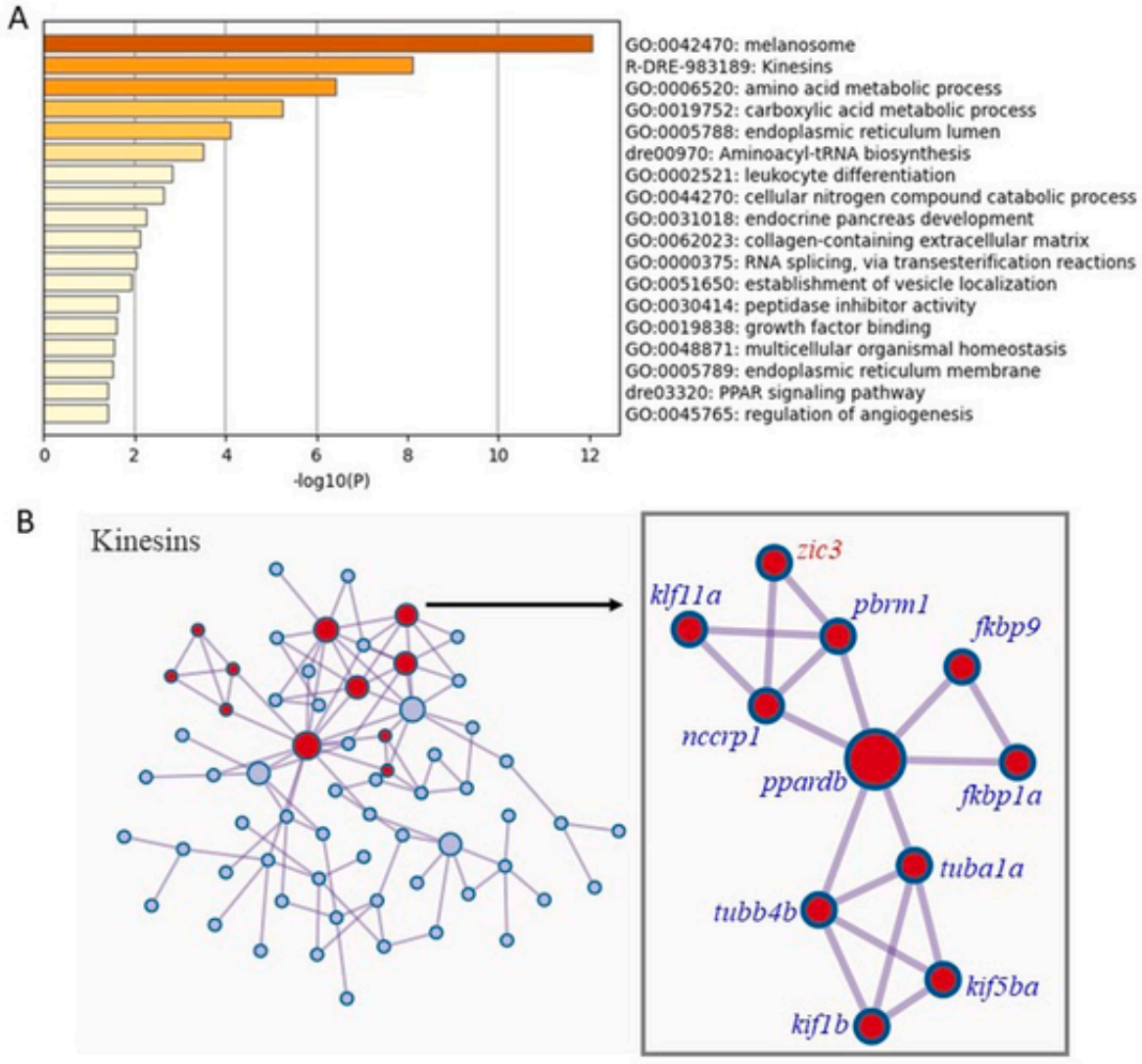

Fig. 3. Gene ontology and protein-protein interaction enrichment analysis of all the DEGs of both skin and muscle transcriptomes. A, the enriched GO terms at the significance level of 0.05. B, the enriched protein-protein interaction network related to the GO term Kinesins. DEGs in the network are highlighted with gene names, where red and blue indicate up- and down-regulation, respectively.

titin, *kif5ba*, *marcksl1b*, *viml*, *lepr*, *ppardb*, *cldn11a,* and up-regulated *ecel1*, *calphotin*, *cx47.1*, and *tbx5a* (Fig. 5).

3.5. Genes involved in growth and locomotion

Enrichment analyses identified 14 significantly enriched GO terms and protein-protein interaction pathways being identified at significance level of 0.05. Notably, the diversity of the enriched terms and pathways in the muscle transcriptome was found to be greater compared to that observed in the skin transcriptome (Fig. S3, Figs. S2 and S4). Within these enriched GO terms, we found the terms: growth factor binding (GO:0019838) and cellular lipid catabolic process (GO:0044242) were more likely related to growth performance (Table 1). Among the DEGs identified, three were found to be associated with growth factor binding. Specifically, in the muscle tissue of golden genotypes (bb) compared to the wildtype (BB), two of these genes, namely glypican 1 b (*gpc1b*) and growth factor binding protein 5 b (*igfbp5b*), exhibited downregulation. Notably, the gene insulin-like growth factor binding protein 1 a (*igfbp1a*) which has previously been reported to impact growth, metabolism, and development through the inhibition of IGF activity (Lee, Giudice, Conover, & Powell, 1997), showed up-regulation within the muscle tissue of golden genotypes. Above all, the expression patterns of all three genes in golden genotypes were in line with growth retardation. In addition to the previously mentioned DEGs, several genes were found to be associated with the cellular lipid catabolic process in the muscle tissue of the golden genotypes. These genes included beta-carotene oxygenase 2 a (*bco2a*), glutaryl-CoA dehydrogenase, mitochondrial (*gcdh*) and carnitine palmitoyltransferase 1 b (*cpt1b*). Interestingly, these DEGs exhibited up-regulation in the muscle of golden genotypes. These genes play crucial roles in fatty acid metabo-

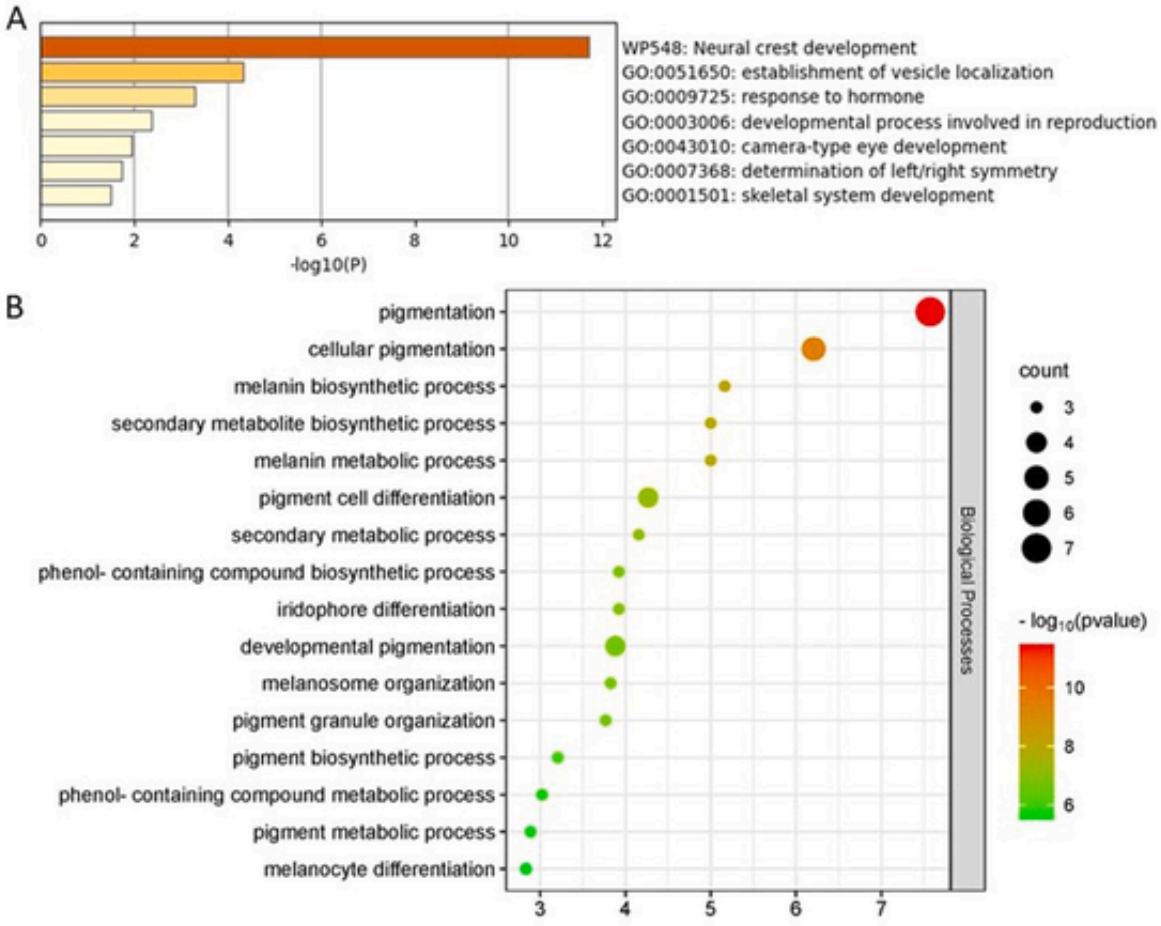

Fig. 4. Gene ontology analysis of the DEGs in skin transcriptome. A, the enriched GO terms at the significance level of 0.05. B, the enriched GO terms in the category of Biological Processes at the significance level of 0.05.

lism, particularly in reducing body adiposity (Amengual et al., 2011) and facilitating the transport of long-chain fatty acids into the mitochondria, thereby promoting energy production (Kölker, Koeller, Okun, & Hoffmann, 2004; Mcgarry & Brown, 1997). The potential functions of the remaining DEGs were also investigated through literature mining. We found nine up-regulated genes in the muscle of golden mutant in contrast to the wildtype, which were revealed to be related to muscle functions (Table 2). These DEGs included motor protein genes involved in muscle contraction and movement, e.g., myosin heavy chain b (*myhb*) and myosin light chain, phosphorylatable, and fast skeletal muscle b (*mylpfb*). The other up-regulated DEGs related to the regulation of growth and development, energy homeostasis, and mobility included angiopoietin-like 4 (*angptl4*) and transcription factor SMAD family member 6 b (*smad6b*).

4. Discussion

Transcriptome sequencing is an effective approach for the identification of differentially expressed genes (DEGs), reconstruction of gene regulation pathways and networks, thereby offering valuable insights into the mechanisms of phenotypic innovation (Ozsolak & Milos, 2011). In this work, we studied the transcriptomes of the skin and muscle samples of golden mutant and wildtype Mozambique tilapias. We identified DEGs between the two genotypes. The consensus observed between the RNA-seq, and qPCR data sets indicates a high level of confidence in the DEGs identified through RNA-seq. Gene ontology and protein-protein interaction network enrichment analyses of these DEGs identified genes, regulation pathways, and networks that are related to the phenotypic changes of the golden mutant. These data provide valuable information for understanding genetic mechanisms by which silencing of *pmel17* leads to loss of melanin pigments, reduced growth, and increased locomotion.

4.1. Melanism related genes beyond melanin pigmentation pathway

The identification of DEGs related to melanism in this study provides valuable insights into the underlying molecular pathways. The significant enrichment of the GO term “Neural crest development” highlights the association between melanin pigmentation and the developmental processes of neural crest cells, which have been well-established in previous studies (Adameyko et al., 2009; Kelsh, 2004; Mayor & Theveneau, 2013). This finding suggests that melanin production is intricately linked to the proper development and differentiation of neural crest cells. Furthermore, our analysis reveals that the majority of the DEGs are downstream of the causal gene *pmel17* and involved in the Eumelanin production pathway. *pmel17* encodes a scaffolding fibrils protein that optimize sequestration and condensation of the pigment melanin, and is essential for the normal maturation of melanosomes and normal deposition of the melanin pigment therein (Watt et al., 2013). Therefore, our finding aligns with the established melanin pigmentation pathway in vertebrates. The genes *tyr*, *tyrp1*, and *ednrb*, identified as upstream regulators of *pmel17*, are well-known for their crucial roles in the regulation of *pmel17* gene expression and melanin synthesis. Their involvement in pigmentation and coloration patterns has been extensively studied in various organisms and highlighted the importance of these genes in determining the intensity and

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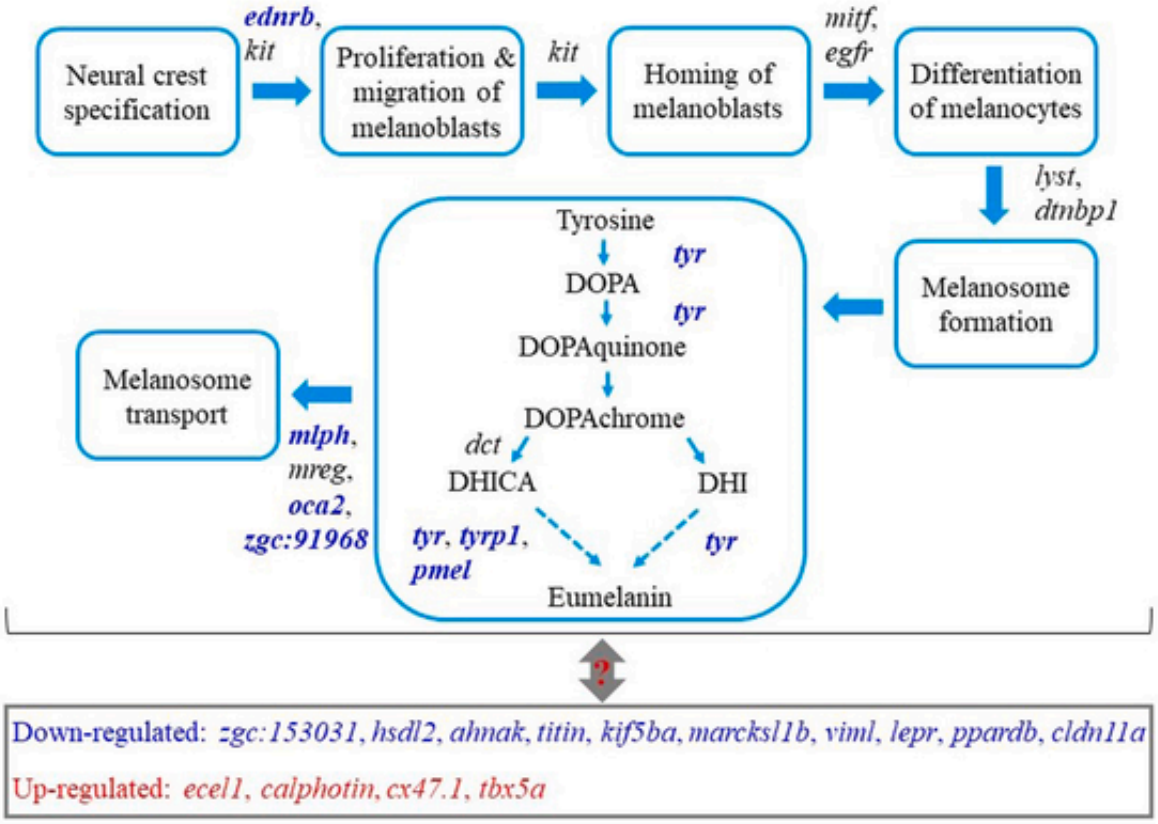

Fig. 5. Differentially expressed genes (DEGs) involved in melanin pigmentation pathway of vertebrates. DEGs involved in the pathway are highlighted in bold. Genes that are related to melanin pigmentation but not involved in the above pathway are shown in the bottom box. Up- and down-regulated DEGs are highlight in red and blue, respectively.

Table 1
Differentially expressed genes that are related to growth and lipid metabolisms identified in muscle between golden and wildtype of Mozambique tilapia.

Gene ID	log2FC	*P* (FDR)	Regulation	Gene name
Growth factor binding (GO:0019838)				
OmoY00013606	−1.24	3.25E-03	Down	glypican 1 b (*gpc1b*)
OmoY00008803	−1.05	1.88E-02	Down	insulin-like growth factor binding protein 5 b (*igfbp5b*)
OmoY00030094	1.33	8.24E-03	Up	insulin-like growth factor binding protein 1 a (*igfbp1a*)
Cellular lipid catabolic process (GO:0044242)				
OmoY00027565	1.66	2.22E-05	Up	beta-carotene oxygenase 2 a (*bco2a*)
OmoY00007787	1.40	2.59E-04	Up	glutaryl-CoA dehydrogenase, mitochondrial (*gcdh*)
OmoY00004660	1.16	2.15E-02	Up	carnitine palmitoyltransferase 1 b (*cpt1b*)

Table 2
Differentially expressed genes that are related to muscle function identified in muscle between golden and wildtype of Mozambique tilapia.

Gene ID	log2FC	*P* (FDR)	Regulation	Gene name
OmoY00016321	1.83	1.64E-04	Up	myosin, heavy chain b (*myhb*)
OmoY00005602	1.49	3.19E-05	Up	transmembrane protein 120 B (*tmem120b*)
OmoY00024115	1.41	9.25E-03	Up	myosin light chain, phosphorylatable, fast skeletal muscle b (*mylpfb*)
OmoY00009624	1.31	2.43E-02	Up	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF b (*obscnb*)
OmoY00013768	1.24	8.10E-03	Up	angiopoietin-like 4 (*angptl4*)
OmoY00025111	1.09	2.02E-02	Up	SMAD family member 6 b (*smad6b*)
OmoY00025987	1.05	1.98E-02	Up	delta-like 2 homolog (*dlk2*)
OmoY00026460	1.03	2.11E-03	Up	GTP binding protein 2 b (*gtpbp2b*)
OmoY00019529	1.02	2.39E-03	Up	SET and MYND domain containing 2 b (*smyd2b*)

distribution of pigmentation in different tissues and species (Bennett & Lamoreux, 2003; Boonanuntanasarn, Yoshizaki, Iwai, & Takeuchi, 2004; Braasch, Liedtke, Volff, & Schartl, 2009; Mao et al., 2023 a). The regulation of upstream genes by *pmel17* could occur through various mechanisms, including feedback loops or interactions with other genes in the regulatory network. The exact mechanisms involved in the regulation of upstream genes by *pmel17* are still unknown or require further investigation. Gene regulatory networks can be complex, with interconnected pathways and feedback loops, and understanding the precise interactions between genes and their regulators is an ongoing area of research. In addition to these genes, we conducted a literature mining search to explore the potential functions of the remaining DEGs. Interestingly, we discovered that some of these DEGs are associated with pigmentation patterns, although they do not belong to the traditional melanin pigmentation pathway observed in vertebrates. These genes include down-regulated genes of *zgc:153031*, *hsdl2*, *ahnak*, *titin*, *kif5ba*, *marcksl1b*, *viml*, *lepr*, *ppardb*, *cldn11a* and up-regulated genes of *ecel1*, *calphotin*, *cx47.1*, *tbx5a*. These genes are likely involved in pigmenta-

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tion patterns. For example, zgc:153031 (Dihydrofolate reductase, *dhfr*) catalyses the production of 5,6,7,8-tetrahydrobiopterin (BH4) in the xanthophores (Danish-Daniel et al., 2023-a); *hsdl2* likely plays a role in retinal pigment epithelium (Kobayashi, Kobayashi, Ueda, & Honda, 1997); and *lepr* is also suggested to be involved in the leptin-melanocortin pathway in skin and hair pigmentation (Kanti et al., 2021). By comparing our findings with other studies on pigmentation patterns (Ducrest, Keller, & Roulin, 2008; Hoekstra & Nachman, 2003; Mao et al., 2023 a), we underscore the importance of our work in expanding the current understanding of melanin synthesis and regulation. The enrichment of GO terms related to melanin pigmentation, along with the identification of genes both in the known pigmentation pathway and beyond, contributes to a more comprehensive picture of the molecular mechanisms underlying pigmentation processes.

4.2. Genes related to growth and locomotion

In muscle transcriptomes, we found that significant enrichment of GO terms, protein-protein interaction pathways, and networks were related to growth performance and locomotion. The down-regulation of genes involved in growth factor binding, such as glypican 1 b (*gpc1b*) and insulin-like growth factor binding protein 5 b (*igfbp5*), suggests a potential decrease in growth performance in the golden mutant. On the other hand, the up-regulation of genes involved in cellular lipid catabolic processes, such as beta-carotene oxygenase 2 a (*bco2a*) and carnitine palmitoyltransferase 1 b (*cpt1b*), suggests an accelerated metabolism of fatty acids, potentially leading to increased mobility and decreased growth performance (Horowitz & Klein, 2000). Furthermore, we identified several other up-regulated genes that were associated with muscle function, regulation of growth and development, energy homeostasis, and mobility. These genes include myosin heavy chain b (*myhb*), myosin light chain, phosphorylatable, fast skeletal muscle b (*mylpfb*), angiopoietin-like 4 (*angptl4*), and transcription factor SMAD family member 6 b (*smad6b*). In this group, *myhb* is a key component of muscle contraction and is responsible for generating force and movement in skeletal muscles (Bottinelli, 2001). The *mylpfb* gene plays a role in the regulation of muscle contraction and contributes to the fast-twitch muscle fiber phenotype (Wang et al., 2007). The *angptl4* gene is involved in angiogenesis, which is the formation of new blood vessels, and also has roles in lipid metabolism and energy homeostasis (Carbone et al., 2018). Lastly, *smad6b* is a regulator of gene expression and is involved in various signalling pathways, including those related to muscle development and differentiation (Imamura et al., 1997). The up-regulation of these genes suggests their potential involvement in the observed phenotypic variations related to muscle performance and locomotion in the golden mutant. Their roles in muscle contraction, mobility regulation, angiogenesis, and signalling pathways indicate their importance in muscle function and mobility. This provides additional evidence for the altered molecular processes underlying the phenotype of the golden mutant. Through a comprehensive exploration of the molecular mechanisms governing growth, mobility, and muscle functions, our study substantially contributes to the advancement of knowledge in this field. However, it is essential to acknowledge that further research is required to fully ascertain the specific roles of these genes in the context of muscle function and mobility.

4.3. Rewiring of gene pathways by a single gene bring about multiple phenotypic changes

The rewiring of gene regulation networks resulting from a single gene variation can have profound effects on phenotypic innovations. In our study, we observed multiple phenotypic changes along with extensive alterations in the skin and muscle transcriptomes of the Mozambique tilapia golden mutant. These alterations were due to an insertion of a transposon in the *pmel17* locus. This transposon insertion silenced *pmel17* and resulted in the alteration of the gene regulation pathways and regulatory networks related to the gene, leading to multiple phenotypic changes. "A gene for speed", *actn3*, a notable example of a monogenic influence on multiple phenotypic characteristics, has been linked to the regulation of diverse phenotypic traits, such as muscle fiber composition, muscle strength, endurance performance, susceptibility to muscle injury, and even susceptibility to specific diseases. These associations arise from the multifunctionality of the gene within related gene regulation pathways and regulatory networks(Ma et al., 2013; Pickering & Kiely, 2017). All these data highlight that variation of a single gene can generate multiple phenotypes by affecting its related gene regulatory pathways and networks.

4.4. Why pmel17 can regulate pigment, growth, and locomotion

The involvement of *pmel17* in multiple pathways related to pigment, growth, and mobility can be explained by its association with neural crest cell development. Neural crest cells have a remarkable capacity to differentiate into various cell types. They play a crucial role in the development of not only pigmentation-related structures but also numerous other systems, including the musculoskeletal, cardiovascular, and nervous systems (Bronner & Ledouarin, 2012; Le Douarin & Dupin, 2003; Le Douarin & Kalcheim, 1999). As pigmentation genes, including *pmel17*, are expressed in neural crest-derived cells, they can influence not only pigmentation, but also other developmental processes mediated by neural crest cells (Baxter & Pavan, 2003). For instance, genes downstream of *pmel17* in the pigment synthesis pathway may also have roles in growth and locomotion due to their expression in tissues derived from neural crest cells (Mort, Jackson, & Patton, 2015). Additionally, the genes involved in neural crest development have been linked to growth and locomotion (Mayor & Theveneau, 2013), suggesting that the neural crest-related pathways and networks have broader implications beyond pigmentation. Therefore, the connections between pigmentation genes, neural crest development, and various phenotypic traits such as growth and locomotion are not surprising. The shared involvement of these genes and pathways highlights the interplay between pigmentation and other aspects of development, further emphasizing the multi-faceted nature of genes, like *pmel17*, in shaping the phenotype through neural crest-related mechanisms.

These findings support the notion that even a single genetic variation can have far-reaching consequences through the rewiring of gene regulation networks. Understanding the mechanisms by which gene regulatory networks are rewired by a single gene is crucial for unravelling the underlying genetic basis of complex phenotypic innovations.

5. Conclusion

This work contributes to the reconstruction of the melanin pigmentation pathway, identification of novel genes, pathways, and regulatory networks governing melanism, growth, and mobility. Our discoveries offer valuable insights not only into the genes and pathways influencing pigmentation, growth, and locomotion, but also shed light on the mechanisms through which a single gene can govern multiple phenotypes by reconfiguring the associated gene regulatory network. Further exploration is warranted to gain a comprehensive understanding of the functional implications of the identified DEGs. Functional validation techniques, such as knockdown or overexpression experiments (Yang, Yu, Tay, & Yue, 2022), will be instrumental in unravelling the precise roles of these genes in pigmentation, growth, locomotion, and muscle functions. Overall, our research emphasizes that altering the expression pattern of a single gene can lead to the rewiring of gene regulatory networks and, consequently, trigger diverse effects on phenotypic innovations.

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Consent for publication

Not applicable.

Data availability

The transcriptome data have been deposited in the DDBJ database with the accession number Bioproject no. PRJDB16155.

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CRediT authorship contribution statement

Fei Sun: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Le Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Gen Hua Yue:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aaf.2023.12.002.

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