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Differential expression and alternative splicing analyses of multiple tissues reveal albinism-associated genes in the Wels catfish (*Silurus glanis*)

M.Y. Ozerov [a,b,c], K. Noreikiene [d,e], S. Kahar [d], M. Flajšhans [f], R. Gross [d], A. Vasemägi [a,d,*]

[a] Department of Aquatic Resources, Institute of Freshwater Research, Swedish University of Agricultural Sciences, 17893 Drottningholm, Sweden
[b] Biodiversity Unit, University of Turku, 20014 Turku, Finland
[c] Department of Biology, University of Turku, 20014 Turku, Finland
[d] Chair of Aquaculture, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 46, 51006 Tartu, Estonia
[e] Department of Botany and Genetics, Life Sciences Center, Vilnius University, 10257 Vilnius, Lithuania
[f] South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, 38925 Vodňany, Czech Republic

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ABSTRACT

Albinism is a widespread departure from a typical body colouration due to altered melanin production. The Wels catfish (*Silurus glanis*) is among the largest freshwater fish species in the world, and albino individuals occur both in the wild and in aquaculture. Here, we performed transcriptome-wide analysis of albino and normally pigmented *S. glanis* using four tissues (skin, dorsal fin, whole eye and liver) to identify genes associated with albinism by exploring patterns of differential expression (DE) and differential alternative splicing (DAS). Multi-tissue analyses revealed a large number of genes in skin ($n = 1355$) and fin ($n = 614$) tissue associated with the albino phenotype in *S. glanis*, while the number of DE genes in eye and liver tissues was lower ($n = 188$, $n = 189$, respectively). Several DE genes across multiple tissues were detected as the most promising candidates (e.g., *hsp4*, *hsp90b1*, *raph1*, *uqcrfs1*, *adcy*-family and *wnt*-family) potentially causally linked to the albino phenotype in Wels catfish. Moreover, our findings supported earlier observations of physiological differences between albino and normally pigmented individuals, particularly in energy metabolism and immune response. In contrast, there were only a few pigmentation-related genes observed among DAS genes (4 in skin, 2 in fin), the overlap between DAS and DE genes was low ($n = 25$) and did not include known pigmentation-related genes. This suggests that DAS and DE in Wels catfish are, to a large extent, independent processes, and the observed alternative splicing cases are probably not causally linked with albinism in *S. glanis*. This work provides the first transcriptome-wide multi-tissue insights into the albinism of Wels catfish and serves as a valuable resource for further understanding the genetic mechanisms of pigmentation in fish.

1. Introduction

Pigmentation plays an important role in the relationships between organisms and the environment and is often tightly linked with inter- and intra-specific communication, camouflage and mimicry, predation, thermoregulation, and protection from ultraviolet radiation (Cuthill et al., 2017). Animal pigmentation patterns generally depend on the location, arrangement and density of pigment cells (Kelsh, 2004). Colouration of birds and mammals is controlled by specific pigment cells, melanocytes, producing two types of melanin: eumelanin (black/brown) and pheomelanin (yellow/red). In contrast, at least six pigment cells, chromatophores, have been identified in fish: melanophores (black/brown), xanthophores (yellow), erythrophores (red), iridophores (iridescent, blue, silver or gold), leucophores (dull, whitish) and cyanophores (blue; Irion and Nüsslein-Volhard, 2019; Kelsh, 2004).

Pigmentation disorders cause deviations from normal body colouration, among which albinism is the most common, affecting the production of the polymeric pigment melanin (Garrod, 1923; Simeonov et al., 2013). Melanogenesis is a complex process that occurs inside melanosomes in melanocytes and involves multiple steps and enzymes.

* Corresponding author at: Department of Aquatic Resources, Institute of Freshwater Research, Swedish University of Agricultural Sciences, 17893 Drottningholm, Sweden.
E-mail address: anti.vasemagi@slu.se (A. Vasemägi).
https://twitter.com/snaudale (K. Noreikiene)

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>600 mutations linked to albinism have been discovered in key genes involved in the melanogenesis pathway (Baxter et al., 2019; Simeonov et al., 2013 http://www.ifpcs.org/albinism/). Thus far, seven recessive autosomal types of oculocutaneous albinism (OCA) caused by mutations in the *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *OCA5*, *SLC24A5* and *LRMDA* genes have been identified in humans (Jauregui et al., 2018). In addition, X-linked ocular albinism type 1 (OA1) can be caused by mutations in the *GPR143* gene, while syndromic forms of albinism, such as Hermansky–Pudlak syndrome (HPS), can be caused by mutations in the *HPS1*, *AP3B1*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, *DTNBP1*, *BLOC1S3*, and *BLOC1S6* genes. Furthermore, Chediak–Higashi syndrome (CHS) is caused by mutations in the *LYST* gene (Jauregui et al., 2018). However, in addition to the key genes directly linked to albinism, there are >650 genes that are known to affect pigmentation via a wide variety of cellular pathways (Baxter et al., 2019; Irion and Nüsslein-Volhard, 2019; http://www.ifpcs.org/colorgenes/).

Although rare, albino phenotypes have been described in all vertebrate lineages. For example, albino individuals have been found in many fish species, including channel catfish (*Ictalurus punctatus,* Bondari, 1984a; Zhang et al., 2019), grass carp (*Ctenopharyngodon idella,* Rothbard and Wohlfarth, 1993), medaka (*Oryzias latipes,* Koga et al., 1995), zebrafish (*Danio rerio,* Haffter et al., 1996), rainbow trout (*Oncorhynchus mykiss,* Boonanuntanasarn et al., 2004), Japanese flounder (*Paralichthys olivaceus,* Wang et al., 2007; Wang et al., 2017), yellow catfish (*Tachysurus fulvidraco*, Zou et al., 2015), oscar (*Astronotus ocellatus*, Wang et al., 2022), northern snakehead (*Channa argus,* Sun et al., 2023) and others. Few studies in fish have succeeded to link albinism to mutations in a single candidate gene. For example, albinism in cavefish (*Astyanax mexicanus*) was caused by deletions in the *oca2* gene (Protas et al., 2006). Similarly, a 99-bp deletion at the intron 2 and exon 3 junction, causing alternative splicing of the *hps4* gene was linked to albinism in channel catfish (Li et al., 2017; Zhang et al., 2019). Other resolved cases include a point insertion in the sixth exon of another member of the hps gene family, *hps5*, causing albinism in three-spined stickleback (*Gasterosteus aculeatus*, Hart and Miller, 2017). There have also been reports on the connection of albino phenotypes with an insertion of a transposable element into the *tyr* gene in medaka (Koga et al., 1995) and a non-synonymous mutation in the *slc45a2* gene in northern snakehead (Sun et al., 2023). Furthermore, several candidate genes potentially associated with albinism and variation in pigmentation of fish have been found using transcriptome analysis (e.g., Lee and Lee, 2020; Wang et al., 2017; Wang et al., 2022; Wu et al., 2022; Zou et al., 2015).

The Wels catfish (*Silurus glanis*) belongs to the family Siluridae, order Siluriformes, and is an apex predator and one of the largest freshwater fish species in the world, native to wide areas of southern, central and eastern Europe (Copp et al., 2009; Kottelat and Freyhof, 2007). The wild type of *S. glanis* has a black to greenish-brown body with a pale yellow to white underside. The albino type of *S. glanis* has been frequently caught in Europe (Dingerkus et al., 1991) and is characterized by a white body and red eyes. In addition to a lack of pigmentation, albino Wels catfish have also been shown to express higher physiological and behavioural responses to stress stimuli than their normally pigmented conspecifics (Slavík et al., 2022). The Wels catfish is also a popular species in aquaculture, due to its highly-prized white boneless flesh, high carcass yield, feed utilization efficiency, and high growth rate, which is among the highest of any fish (Linhart et al., 2002; Jankowska et al., 2006; Adamek et al., 2015; Simeanu et al., 2022). The production of *S. glanis* has been growing during the recent decades, from 147 tons in 1991 to 2587 tons in 2021 (FAO, 2022). In addition, in some European countries the Wels catfish is also reared for stocking purposes (Cucherousset et al., 2018). Both normally pigmented and albino individuals are grown in fish farms; however, the high levels of aggression expressed by normally pigmented fish may reduce the well-being of less intensely pigmented conspecifics if they are kept together (Svitačová et al., 2023). Therefore, studying the molecular mechanisms of albinism in *S. glanis* is also of economic importance.

In this study, we performed transcriptome-wide analysis of albino and normally pigmented Wels catfish using four tissues (skin, dorsal fin, whole eye and liver) to i) identify transcripts that show differential expression (DE) between albino and normally pigmented catfish and ii) explore patterns of differential alternative splicing (DAS) potentially related to albinism. We also characterize molecular processes related to albinism and focus on the most consistent associations across tissues for both DE and DAS genes. Finally, we compare our findings with the known list of >650 pigmentation-associated genes (Baxter et al., 2019; Irion and Nüsslein-Volhard, 2019) to link the albinism-associated transcripts in *S. glanis* to a wider context.

2. Material and methods

2.1. Fish reproduction and maintenance

Both normally pigmented and albino catfish were obtained from the strains kept in captivity (in ponds) at Research Institute of Fish Culture and Hydrobiology (RIFCH) of the Faculty of Fisheries and Protection of Waters, University of South Bohemia (Vodňany, Czech Republic). The Hodonin strain and their offspring consisted of only wild type pigmented Wels catfish whereas the Albino strain and their offspring consisted exclusively of only albino catfish. The eggs of both types of *S. glanis* were fertilized in RIFCH (Vodňany, Czech Republic) on the 13th of June 2019, and fry hatched on the 16th of June 2019. The fry were further transported to the Chair of Aquaculture, Estonian University of Life Sciences (Tartu, Estonia), where they were grown together in the same tank at the experimental aquaculture facility starting on the 20th of June 2019. At the age of 110 days, the specimens were euthanized by an overdose of 99% 2-phenoxyethanol (5 ml/l) before sampling, and total length, weight and sex were recorded. Albino fish were slightly larger ($weight_{median}$ = 100.0 g, $length_{median}$ = 247.0 mm; $weight_{mean}$ = 102.7 ± 3.4 g, $length_{mean}$ = 246.0 ± 6.6 mm) than their normally pigmented conspecifics ($weight_{median}$ = 84.0 g, $length_{median}$ = 229.0 mm; $weight_{mean}$ = 98.7 ± 21.0 g, $length_{mean}$ = 237.0 ± 35.6 mm; Table S1); however, this difference was non-significant (non-parametric Mann–Whitney *U* test, both $P > 0.10$). In total, four tissues (skin, dorsal fin, whole eye and liver) were collected from each of the 5 normally pigmented (2 females and 3 males) and 5 albino catfish (2 females, 2 males, 1 of undetermined sex; Table S1). While skin, fin and whole eye tissues contain the highest number of pigment cells, liver is important organ involved in plethora of functions in vertebrates, including melanogenesis. Samples were immediately snap-frozen and stored at −80 °C. All sampled individuals were immature. Because of the unknown sex of one sampled albino catfish, it was excluded from further analyses (sample Slg8; Table S1).

The experimental aquaculture facility of Estonian University of Life Sciences (Tartu, Estonia), where the Wels catfish were grown, has been acknowledged by the Agriculture and Food Board of Republic of Estonia as fully meeting the requirements of the Minister of Rural Affairs' Regulation No. 53 (10.07.2017) for breeding and using of experimental animals and has been issued a Licence KL 1213 (28.12.2020). The requirements outlined in the Annex III (Requirements for establishments and for the care and accommodation of animals) and Annex IV (Methods of killing animals) Section B point 11 of the "Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purpose" were fully met. The authors have followed the principles of the 3Rs (Replacement, Reduction and Refinement) and have involved the minimum number of animals to produce statistically reproducible results.

2.2. RNA extraction, library preparation and sequencing

Before RNA isolation, tissues were mechanically crushed in liquid nitrogen using a steel.

mortar and pestle to produce a homogenized powder. Total RNA was

extracted from homogenized frozen tissues (skin, dorsal fin, whole eye and liver; ~ 15–20 mg each) using a NucleoSpin® RNA extraction kit (MACHEREY-NAGEL, Duren, Germany). The quality of the total RNA sample was evaluated using TapeStation 2200 (Agilent), and the sample concentration was measured with a Nanodrop ND-2000 instrument (Thermo Scientific™), resulting in $RIN_{mean} = 9.2 \pm 0.6$ and $concentration_{mean} = 251.6 \pm 167.6$ ng/μl. Sequencing libraries were prepared from 500 ng of total RNA using the TruSeq® Stranded mRNA library preparation kit (Cat# 20020595, Illumina Inc.), including polyA selection, and unique dual indices (Cat# 20022371, Illumina Inc.) according to the manufacturers' protocol (#1000000040498, Illumina Inc.). The library was sequenced using a NovaSeq 6000 instrument (2 × 150 bp read length) by the SNP&SEQ Technology Platform (Uppsala, Sweden).

2.3. Read quality control and mapping

Sequencing data were sorted by individual, and indexing adapters were removed at the sequencing facility. In total, from 108.08 M to 202.66 M reads were generated (mean = 146.37 M, median = 146.39 M; Table S1). FastQC ver. 0.11.8 (Andrews, 2017) was used to assess the quality of the reads. Illumina adapters and short (< 60 bp) and low-quality (average quality score < 25) reads were removed with fastp ver. 0.20 (Chen et al., 2018) using the following parameters: -g -w 12 -r —W 5 -M 25 –trim_front1 9 –trim_front2 9 –trim_tail1 2 –trim_tail2 2 -l 60, retaining from 85.45 M to 172.24 M reads (mean = 123.04 M, median = 123.29 M; Table S1). The filtered sequence reads were mapped to the *S. glanis* reference genome (Ozerov et al., 2020; GCA_014706435.1) using hisat2 ver. 2.1.0 (Kim et al., 2015; Kim et al., 2019) by applying default parameters, except for specifying strand-specific information (−-rna-strandness RF). Only the reads with the best match to their mapped location in the reference genome (primarily aligned) were extracted to bam files using Samtools ver. 1.10 (Li, 2011; —F 260) for subsequent analyses.

2.4. Differential expression (DE) analysis of sequence count data

Read counting was performed for exonic gene regions in a non-strand-specific manner with the GenomicFeatures ver. 1.46.5 and GenomicAlignments ver. 1.30.0 packages (Lawrence et al., 2013) in R 4.2.2 (R Core Team, 2021) using a .gff file containing catfish reference genome annotation records. To remove rare transcripts, genes with fewer than 10 raw reads per tissue in more than three individuals were excluded from further analyses. Fold changes (FCs) in transcript abundance between two groups of catfish (albino vs. normally pigmented) controlled for sex (design = ~ sex + group) were determined for each tissue with the DEseq2 package ver. 1.34.0 (Love et al., 2014) in R. Non-normalized read counts were used for DEseq2 analyses as its model internally corrects for library size. Given that small FCs may have important biological relevance and increased noisiness of FCs estimation for genes with low counts (Love et al., 2014), all genes with an adjusted *p* value ≤0.05 (Benjamini and Hochberg, 1995) were considered differentially expressed (DE) between albino and normally pigmented catfish. However, given that normally pigmented and albino catfish originated from different sources, it is possible that some of the DE genes are linked to the population of origin, rather than pigmentation.

2.5. Alternative splicing (AS) analysis

AS events were classified into five basic types: skipped exon (SE), mutually exclusive exon (MXE), retained intron (RI), alternative 3′ splice site (A3SS) and alternative 5′ splice site (A5SS). The AS events in albino and normally pigmented catfish were identified using the reads that mapped to splice junction (JC) outputs produced by rMATS turbo ver. 4.1.2 (Park et al., 2013; Shen et al., 2014) with default settings, except that read length was set to 118 bp (−-readLength 118) and variable read length was allowed to process reads with lengths other than that set by the read length parameter value (−-variable-read-length). Maser package ver. 1.12.1 (Veiga D, 2022) in R was used to exclude low-coverage splicing events (⟨10) and filter significant AS events ($\Delta\ \psi \geq 10\%$, FDR ≤ 0.05).

2.6. Gene ontology (GO) and protein–protein interaction (PPI) analysis

Human orthologue gene symbols were searched using complete gene names in the NCBI database. GO enrichment analysis of differentially expressed and alternatively spliced genes against all orthologous genes expressed in each tissue as a background was performed using the gprofiler package ver. 0.2.1 (Kolberg et al., 2020; Raudvere et al., 2019) in R. GO terms with a g:SCS threshold ≤0.05 were considered significant. To gain further insights into physical and functional associations among pigmentation- and melanogenesis-related DE genes (Table S2) at protein level a PPI network was generated using the Search Tool for the Retrieval of Interacting Genes ver. 12.0 (STRING; Szklarczyk et al., 2023) with default parameters. The network was visualized using Cytoscape ver. 3.10.0 (Shannon et al., 2003). The DE genes with the highest number of interactions were determined according to the degree of node, which was calculated using Network analyser in Cytoscape.

3. Results

3.1. Differential expression (DE) between albino and normally pigmented catfish

In total, 2062 genes were differentially ($P_{adj} \leq 0.05$) expressed between the albino and normally pigmented catfish across all tissues (Fig. 1, Table S2). The largest number of DE genes was observed in the skin ($n = 1355$), where 730 (53.9%) were up-regulated and 625 (46.1%) were down-regulated in albino catfish (Table 1). In fin tissue, the number of DE genes ($n = 614$) was more than two times lower than that in skin, with 298 (48.5%) and 316 (51.5%) genes being up- and down-regulated in albino catfish, respectively. The number of DE genes observed in eye ($n = 188$) and liver ($n = 189$) tissues was much lower than that in the skin and fin tissues. In the eyes of albino catfish, 86 (45.7%) and 102 (54.3%) genes were up- and down-regulated, respectively. In the liver, 79 (41.8%) and 110 (58.2%) genes showed up- and down-regulation in albino catfish, respectively (Table 1).

Among all DE genes, six were up-regulated in albino catfish in all four studied tissues (*inava*, *spns3*, *il6st*, *gna13*, *man2b2*, and *atg4d*; Fig. 2a; Table S2). In addition, 21 DE genes overlapped among the skin, fin and eye tissues. In total, 15 DE genes were consistently up-regulated and 5 were consistently down-regulated in the skin, fin and eye tissues of albino catfish. In contrast, only a few DE genes showed more complex expression patterns between tissues (e.g., *prss23,* up-regulation in fin but down-regulation in skin and eye tissues of albino catfish). Among the skin, fin and liver tissues, 11 DE genes overlapped, with 6 being up-regulated and 5 down-regulated in albino catfish. The number of overlapping DE genes among the skin, eye and liver tissues and among the fin, eye and liver tissues was 8 and 6, respectively (Fig. 2a; Tables S2, S3). The highest number of overlapping DE genes was observed between skin and fin ($n = 175$), followed by skin and eye ($n = 59$) and skin and liver ($n = 31$; Tables S2, S3) tissues. The numbers of overlapping DE genes in other tissue comparisons were lower and consisted of 27 genes between fin and eye, 23 between fin and liver and 9 between eye and liver tissues.

In total, 92 DE genes corresponded to 80 pigmentation-related human orthologues (https://www.ifpcs.org/colorgenes/; Baxter et al., 2019; Fig. 1; Table 1). The highest number of pigmentation-related DE genes was observed in skin ($n = 57$; 33 and 24 were up- and down-regulated in albino catfish, respectively) and fin (n = 31; 9 and 22 were up- and down-regulated in albino catfish, respectively) tissues. The number of pigmentation-related DE genes in other tissues was much lower ($n = 9$ in eye; $n = 13$ in liver; Table 1). The majority of

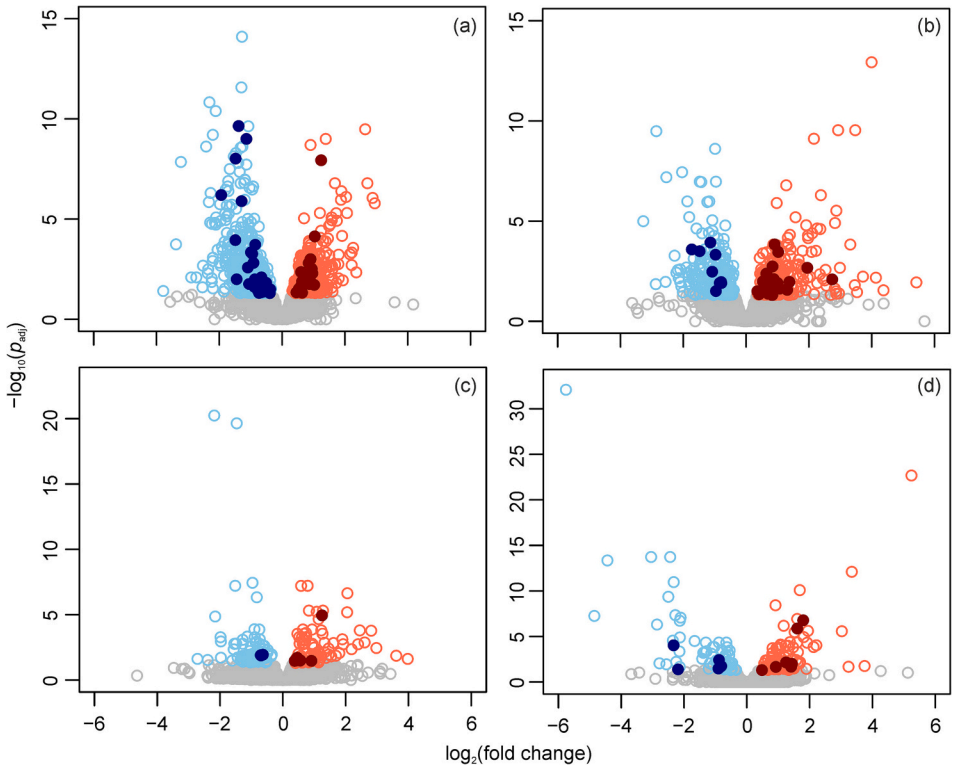

Fig. 1. Volcano plots showing differentially expressed genes between albino and normally pigmented *S. glanis* in (a) skin, (b) fin, (c) whole eye and (d) liver tissues. Up-regulated and down-regulated genes in albino fish are presented as unfilled red and light blue dots, respectively. Differentially expressed pigmentation-related genes are shown as filled dots. Non-significantly differentiated genes are shown as unfilled grey dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Summary statistics of DE genes between albino and normally pigmented types of *S. glanis* for four tissues.

Tissue	Total genes expressed	DE genes	DE genes down-regulated in albino	DE genes up-regulated in albino	Pigmentation-related DE genes down-regulated in albino	Pigmentation-related DE genes up-regulated in albino
skin	16,153	1355	625	730	24	33
fin	16,102	614	316	298	22	9
whole eye	17,507	188	102	86	6	3
liver	13,440	189	110	79	7	6

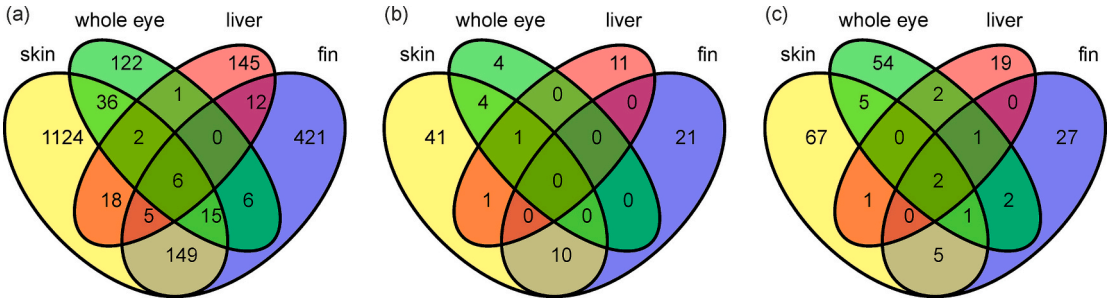

Fig. 2. Venn diagrams showing overlap among the studied tissues: (a) DE genes, (b) known pigmentation-related DE genes and (c) DAS genes between albino and normally pigmented *S. glanis*.

pigmentation-related DE genes overlapped between skin and fin ($n = 10$; Fig. 2b; Table S4), of which six (two copies of *atm*, atp*1a1*, *fmn1*, *erbb3* and *tenm3*) were up- and three (*hsp90b1*, *raph1* and *idh2*) were down-regulated in albino catfish. The expression pattern of a single pigmentation-related DE gene (*prdm1*) differed in two tissues, being up-regulated in skin and down-regulated in fin tissue of albino catfish. Four pigmentation-related DE genes overlapped between skin and eye tissues (two copies of *uqcrfs1*, *cop1*, and *idh2*), showing down-regulation in albino catfish (Table S4). The other two genes, *anxa2* and *cdk5,* were up-regulated in albino *S. glanis* skin and liver and skin, eye and liver tissues, respectively (Table S4). The PPI network analysis revealed 167 interactions among 64 pigmentation- and melanogenesis-related DE genes at the protein level in four tissues (Fig. 3). As expected, the majority of interactions were observed among DE genes in skin and eye tissues.

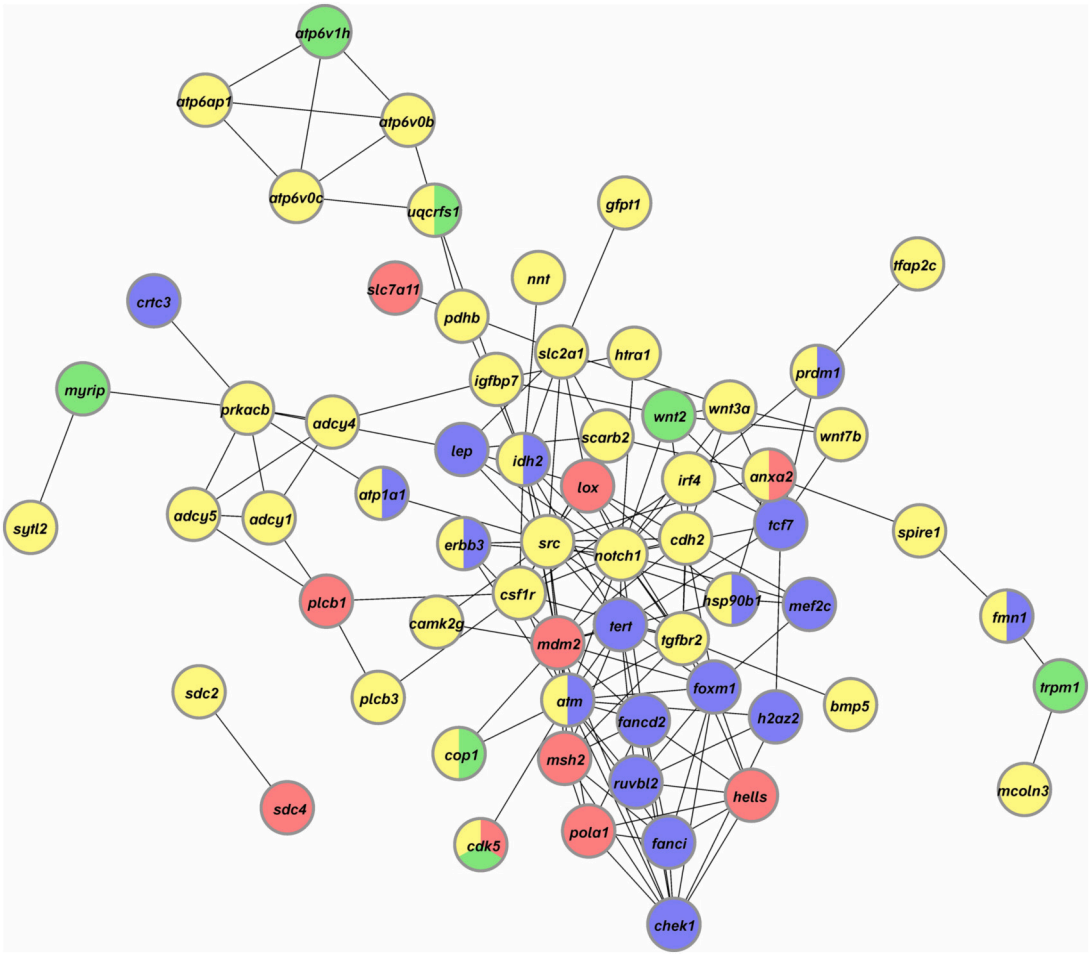

Fig. 3. PPI network of pigmentation- and melanogenesis-related DE genes in *S. glanis*. Nodes represent proteins and lines represent interactions between proteins. Yellow, purple, green and red filling of the nodes show DE genes in skin, fin, whole eye and liver tissues, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Further analysis showed that *notch1*, *src*, and *atm* have the highest number of interactions (degree = 19, 17 and 17, respectively), indicating their potential involvement in albinism of *S. glanis*.

3.2. Functional enrichment analysis of DE genes

Gene Ontology (GO) analysis indicated significant enrichment of GO terms for DE genes in skin and fin tissues and overlapping DE genes between skin and fin tissues. On the other hand, no significant GO enrichment was observed for DE genes in the liver and whole eye or for other tissue combinations. DE genes in skin tissue were enriched for 72 biological process (BP), 46 cellular component (CC) and 20 molecular function (MF) GO terms (Fig. 4; Table S5). The most significant terms included aerobic electron transport chain (BP GO:0019646), oxidative phosphorylation (BP GO:0006119), mitochondrial protein-containing complex (CC GO:0098798), mitochondrial inner membrane (CC GO:0005743), electron transfer activity (MF GO:0009055) and oxidoreduction-driven active transmembrane transporter activity (MF GO:0015453). DE genes in fin tissue were enriched for 12 BP GO terms, 8 CC GO terms and 1 MF GO term (Fig. 4, Table S5). The most significant were defence response (BP GO:0006952), immune response (BP GO:0006955), proteasome complex (CC GO:0000502), external side of plasma membrane (CC GO:0009897) and antigen binding (MF GO:000382). DE genes overlapping between skin and fin tissues showed enrichment for 24 BP GO terms and one MF GO term, which were mostly related to immune processes (Table S5). The most significant terms were immune response (BP GO:0006955), biological process involved in interspecies interaction between organisms (BP GO:0044419) and antigen binding (MF GO:000382). Interestingly, the majority of DE genes common to skin and fin tissues with GO term enrichment were up-regulated in albino catfish.

3.3. Differential alternative splicing (DAS) between albino and normally pigmented catfish

In total, 21,892 alternative splicing (AS) events were identified across four tissues in the albino and normally pigmented *S. glanis* transcriptome datasets (Table 2). These AS events were distributed in 6731 genes with an average of 3.25 AS events per gene. Only the skipped exon (SE) and mutually exclusive exon (MXE) types of AS events were observed, of which SE was most common (20,714; 94.6%), followed by MXE (1178; 5.4%). The highest number of differential alternative splicing (DAS) events ($\Delta\ \psi \geq 10\%$, FDR ≤ 0.05) was observed in skin ($n = 91$) and eye ($n = 73$) tissues, whereas in fin and liver, the number of significant DAS events was lower ($n = 39$ and $n = 25$, respectively; Table 2). In general, increased exon inclusion levels in all four tissues were observed more often in normally pigmented rather than in albino catfish (Table 2). However, the observed DAS events, except *ddx31*, did not show perfect separation of albino and normally pigmented individuals, suggesting that the majority of detected DAS events are likely not causally linked to albinism (Fig. 5).

The number of DAS genes between albino and normally pigmented catfish across multiple tissues was low and varied from 2 to 8 (Fig. 2c; Table S3). Two genes, *ddx31* and *trim10*, showed significant SE events in

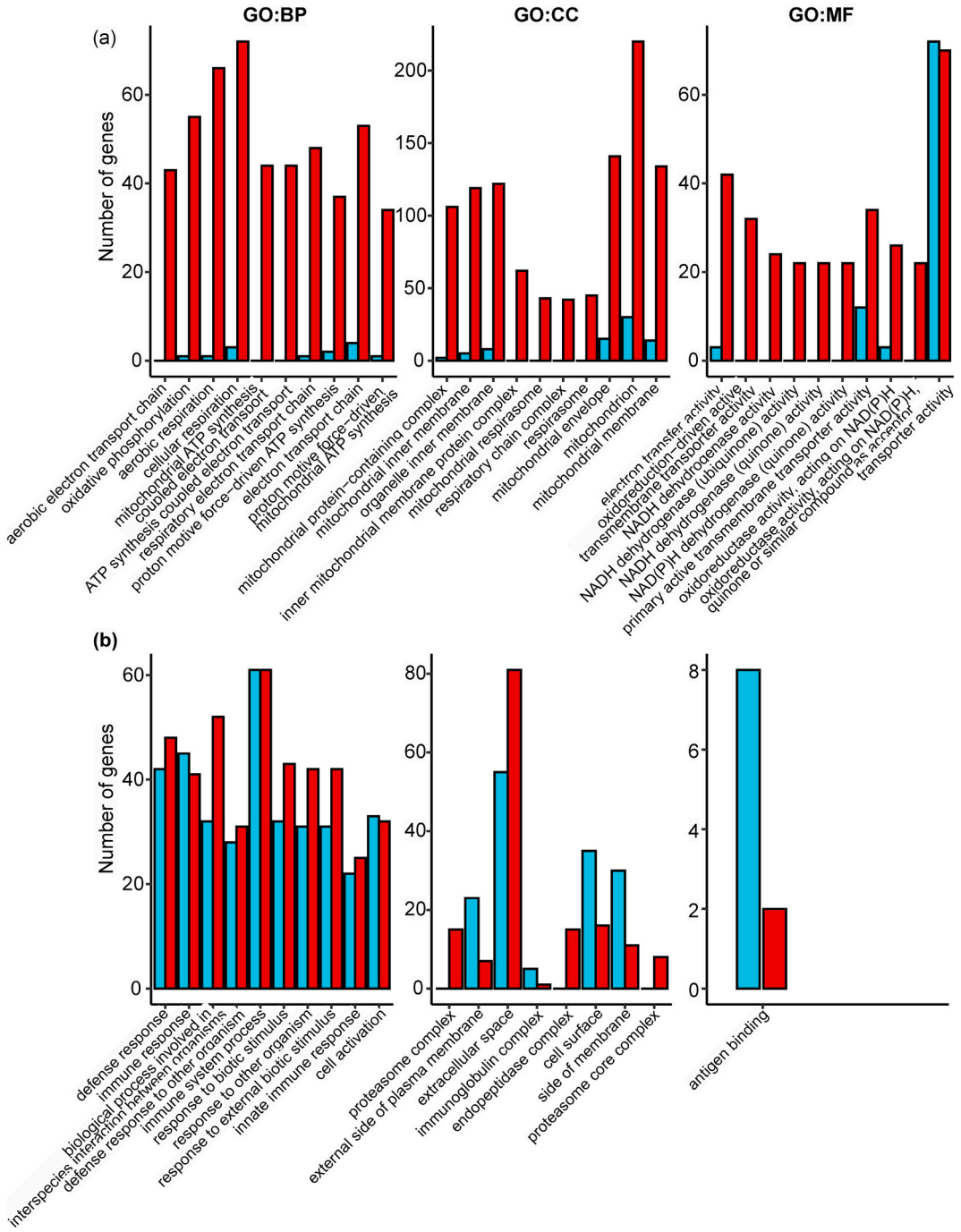

Fig. 4. Top 10 GO classifications of the DE genes observed in (a) skin and (b) fin tissues. Vertical bars indicate the number of up-regulated (red) and down-regulated (cyan) genes in the albino type of *S. glanis* with corresponding GO terms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

all four tissues (Fig. 5). Among those, *trim10* was also significantly up-regulated in the skin of albino catfish (Table S2). The highest number of overlapping DAS genes ($n = 8$) was observed between skin and fin tissues and for skin and eye tissue combinations (Table S3). In contrast to DE genes, there were only a few pigmentation-related genes observed among DAS genes: four in skin (*sytl2*, *pkn2*, *mycbp2*, *plxnb2*) and two in fin (*sgpl1*, *igsf11*) tissues, and none of the GO terms were enriched among DAS genes. Furthermore, the overlap between DAS and DE genes was low (n = 25) and did not include known pigmentation-related genes, indicating that DAS and DE are, to a large extent, unrelated processes, and albinism in Wels catfish is not driven by alternative splicing.

4. Discussion

We performed whole-transcriptome analyses using four different tissues to identify molecular pathways and potential candidate genes associated with albinism in *S. glanis*. Based on DE analyses, we uncovered a large set of genes (> 2000) associated with the albino phenotype, which included nearly a hundred known pigmentation-related genes, of which 15 showed highly consistent expression patterns across multiple tissues. However, we were not able to pinpoint a single causative mutation and gene underlying albinism in *S. glanis.* Instead, we discovered that multiple biological and molecular processes, such as energy metabolism and immune response, differ between albino and normally

Table 2
Summary of alternative splicing events (DAS) between albino and normally pigmented types of *S. glanis* for four tissues.

Tissue	Alternatively spliced event	Total alternative splicing events	Filtered events: $\|\Delta\ \psi\| \geq 10\%$; FDR ≤ 0.05	Increased inclusion alternatively spliced levels in albino	Increased inclusion alternatively spliced levels in normally pigmented
skin	SE	4688	80	28	52
	MXE	237	11	7	4
fin	SE	6860	35	17	18
	MXE	401	4	1	3
whole eye	SE	6290	70	23	47
	MXE	353	3	2	1
liver	SE	2876	22	8	14
	MXE	187	3	3	0

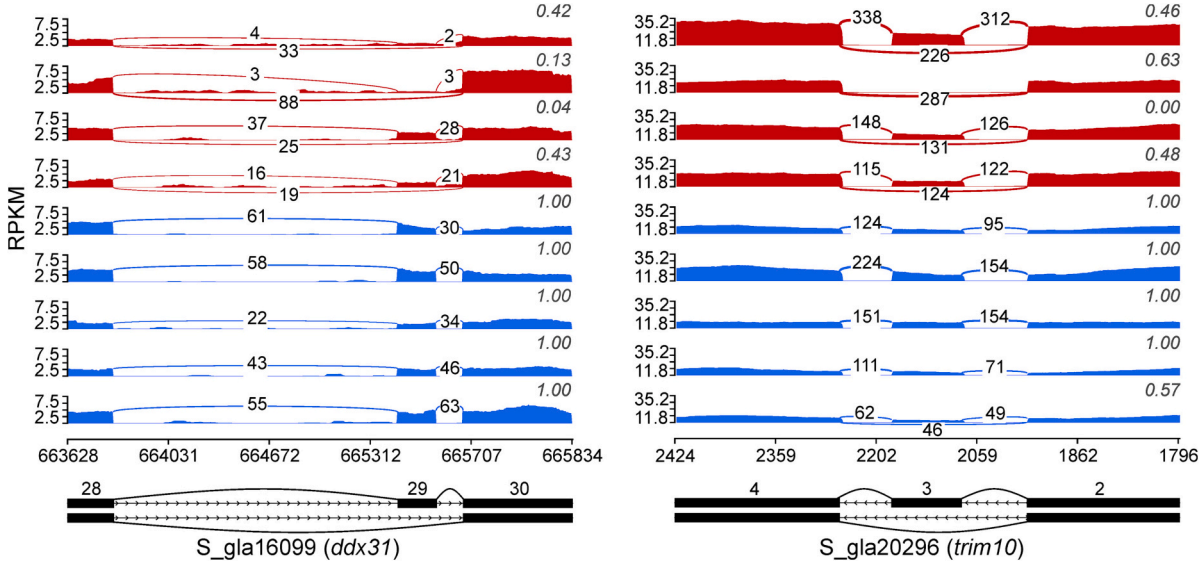

Fig. 5. Sashimi plots showing differential exon and splice junction usage during exon skipping between albino (red) and normally pigmented (blue) *S. glanis* at the S_gla16099 (*ddx31*) and S_gla20296 (*trim10*) genes in skin tissue. Genomic locations are shown on the X-axis, and the transcription intensity, measured as RPKM (reads per kilobase per million mapped reads), is presented on the Y-axis. The arcs connecting exons indicate junction reads. The numbers of junction reads are shown on top of the arcs. The grey numbers in italics on the right indicate inclusion level, i.e., the proportion of normalized read counts in the neighbourhood of the respective exon, which indicates a splicing event, where the exon was included in the final processed mRNA transcripts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pigmented catfish. In addition, DAS analysis identified a small number of genes that differ between normally pigmented and albino catfish, suggesting that albinism is largely independent of alternative splicing. Below, we discuss the main findings, overlaps among tissues, molecular processes and the most promising candidates linked with albinism in Wels catfish.

4.1. Candidate genes associated with albinism in Wels catfish

The highest number of DE genes between albino and normally pigmented catfish was observed in skin, followed by fin tissue. This was anticipated, given that these two tissues are most exposed to environmental factors, including ultraviolet radiation, and that the highest concentration of pigment cells is observed in the skin (Rosdahl and Rorsman, 1983). The skin and fin tissues also showed the highest number of overlapping DE genes ($n = 175$), the majority of which (96%) showed similar expression patterns (Table S2). Furthermore, we observed several known pigmentation-related genes among DE genes in Wels catfish. For example, we found significant down-regulation of the *hps4* gene in the skin tissue of albino catfish. *Hps4* is involved in melanosome biosynthesis, mutations in which cause Hermansky–Pudlak syndrome type 4, an autosomal recessive disorder of oculocutaneous albinism, in humans (Anderson et al., 2003). In line with our observations in albino catfish, patients with mutations in the *hps4* gene show decreased amounts of the protein product (Carmona-Rivera et al., 2011; Nazarian et al., 2008). Furthermore, a deletion in the *hps4* gene has been shown to cause albinism in channel catfish (*I. punctatus*, Li et al., 2017; Zhang et al., 2019) and *Xenopus laevis* (Fukuzawa, 2021). However, in contrast to Zhang et al. (2019), who reported differential alternative splicing of the *hps4* gene between albino and normally pigmented channel catfish, we did not find differences in alternative splicing patterns between albino and normally pigmented *S. glanis*. Nevertheless, our results hint the potential involvement of *hps4* in the albinism of *S. glanis*.

In addition, a number of DE genes between normal and albino catfish show expression patterns that are consistent with pigmentation disturbances in other species. For instance, the down-regulation of *hsp90b1* observed in albino catfish in the present study was in line with perturbed melanin synthesis and blocked late melanosome maturation in mice with knocked down *hsp90b1* gene (Zhang et al., 2014). Similarly, *raph1*, which knockout causes decreased melanocyte numbers in mice (Law et al., 2013), was down-regulated in albino *S. glanis*. Furthermore, a 3′UTR modification of *uqcrfs1* in mice have shown to cause a change in hair colour from dark to grey in homozygote individuals, with a markedly decreased protein product (Garcia et al., 2008). This implies a potential association of *uqcrfs 1* with the albino phenotype, given its down-regulation in albino catfish. Finally, the up-regulation of *erbb3* in albino catfish is in line with earlier findings that *erbb3* signalling

negatively regulates melanocyte differentiation and pigmentation (Buac et al., 2009).

On the other hand, several pigmentation-related DE genes showed also unexpected expression patterns in Wels catfish. For example, *atp1a1* and *fmn1* were up-regulated in albino catfish, whereas knockdown of these genes impairs the normal melanosome distribution of melanocytes in mice (Alzahofi et al., 2020; Booth et al., 2014). Similarly, *anxa2* and *cdk5* were up-regulated in albino *S. glanis*, while in mice, knockdown of these genes reduced melanin production (Delevoye et al., 2016; Dong et al., 2017). *idh2* and *cop1* were down-regulated in albino catfish; however, it has been demonstrated that *idh2* deficiency enhances melanin synthesis in mice (Park et al., 2018), and high levels of *cop1* protein reduce melanoma in mice (Kappelmann-Fenzl et al., 2019; Migliorini et al., 2011). Therefore, these pigmentation-related genes require further work to disentangle their specific role in albinism of Wels catfish.

4.2. Melanogenesis pathway

Among the genes involved in the melanogenesis pathway, we found several adenylate cyclase members (*adcy5* and *adcy1*) to be down-regulated in the skin of albino catfish, whereas the *adcy4* gene showed up-regulation in the skin but down-regulation in the fin tissue of albino specimens (Table S2). It was previously demonstrated that silencing of *adcy* genes decreased melanogenesis by 30–35% in zebrafish (Motiani et al., 2018), thus implying the potential role of these genes in albinism in *S. glanis*. We also found several wnt-genes to be up-regulated in albino catfish (wnt*3a* and *wnt7b* in skin, *wnt2* in eye tissue), which play a critical role in melanocyte development (D'Mello et al., 2016). This is in line with observations in albino mouse embryos showing higher *wnt2b* gene expression in the retinal pigment epithelium. Similarly, *wnt3a* inhibits the proliferation of melanocytes in mice (Guo et al., 2012). Moreover, given that alteration of melanogenesis in the retinal pigment epithelium is associated with fewer retinal ganglion cells, activation of wnt signalling in mice reduced the number of retinal ganglion cells (Iwai-Takekoshi et al., 2018), corroborating the role of up-regulation of wnt genes in albino catfish. On the other hand, the expression patterns of several genes involved in the melanogenesis pathway were not fully consistent with observations in other vertebrates (e.g., D'Mello et al., 2016; Hoekstra, 2006; Hubbard et al., 2010). For example, *prkacb*, a gene involved in the protein kinase C-dependent pathway, which also regulates melanogenesis, was up-regulated in albino catfish skin, suggesting that the cAMP-dependent or melanocortin pathway is likely not involved in regulating pigmentation in *S. glanis*, as in Virginia opossum (*Didelphis virginiana*, Nigenda-Morales et al., 2018). We also observed up-regulation of *plcb3, camk2g* and *tcf7* in albino catfish, implying that these genes most likely do not drive the albinism in *S. glanis*, as their activation promotes melanin production (D'Mello et al., 2016). Similarly, down-regulation of *plcb1* in the liver of albino catfish does not support its role in albinism of *S. glanis*, since up-regulation of this gene has been shown to promote inhibition of melanin production in murine melanoma cells (Bourhim et al., 2021). Taken together, our findings show that among several DE genes involved in the melanogenesis pathway, the members of *adcy* and *wnt* gene families are the strongest candidates potentially associated with albinism in *S. glanis*.

4.3. Albinism, energy metabolism and the immune system

To our surprise, we did not observe significant enrichment of GO terms related to melanogenesis or melanocyte functioning among DE genes detected in skin or fin tissues. Instead, the most significant GO terms observed for DE genes in skin were linked to electron and energy transport, whereas DE genes in fin tissue and common DE genes in both tissues were enriched for immune-related processes. Furthermore, among the six DE genes that overlapped across all four studied tissues and showed up-regulation in albino catfish, four were involved in the immune response, namely, *inava* (Chang et al., 2021), *il6st* (Tanaka et al., 2014), *gna13* (Healy et al., 2016) and *atg4d* (Zhang et al., 2016), while the remaining two were associated with ion transport functions: *spns3* (Jacobsson et al., 2007) and *man2b2* (Venkatesan et al., 2009).

The observed enrichment of energy and electron transport GO terms is in line with knowledge of albinism and melanogenesis (D'Mello et al., 2016; King et al., 2001). For example, several studies have revealed significant differences in metabolism between albino and normally pigmented individuals (e.g., Bondari, 1984a, 1984b; King et al., 2001; Silverstone and Mendelsohn, 1983) and an important role of ion transport and calcium (Ca^{2+}) ions in melanogenesis (e.g., Bellono and Oancea, 2014; Wiriyasermkul et al., 2020). Furthermore, higher movement activity and ventilatory frequency in response to stress have been reported in albino *S. glanis* compared to their normally pigmented conspecifics (Slavík et al., 2022). The same study showed higher levels of haemoglobin, erythrocytes, neutrophil granulocytes, glucose and lactate in the blood of albino Wels catfish, indicating higher haematological and biochemical responses to stress in albino individuals (Slavík et al., 2022). Thus, the enrichment of DE genes related to energy and electron transport in *S. glanis* corroborates earlier findings on metabolic differences between albino and normally pigmented fish. The observed strong enrichment of GO terms related to the immune system also corroborates the earlier findings, which report compromised immune response in albino patients, particularly with syndromic forms of albinism (e.g., Chediak-Higashi, Griscelli, and Hermansky-Pudlak syndromes; Dotta et al., 2013; Stinchcombe et al., 2004). Therefore, a number of differentially expressed immune-related genes discovered in our study indicate that albinism and immune system functions are also interlinked in *S. glanis*.

4.4. DAS between albino and normally pigmented catfish

Despite a large number of splicing events (> 20,000) detected in our transcriptome data, differential alternative splicing (DAS) between the albino and normally pigmented catfish was observed in only ~1% of AS events. Furthermore, we detected only skipped exon (SE) and mutually exclusive exon (MXE) cases, while retained intron (RI), alternative 3′ splice site (A3SS) and alternative 5′ splice site (A5SS) events were not observed in our dataset. There are several non-exclusive explanations to this. First, based on the recent study by Mehmood et al. (2020), current alternative splicing data analysis tools may result strikingly different results across different data sets in vertebrates. Yet, based on preliminary analyses of two pipelines (HISAT2 + rMATS ver. 4.1.2 vs. STAR + rMATS ver. 4.2.0), we did observe highly similar results (data not shown). Second, the performance of alternative splicing analysis tools also depends on many factors, including sample size and sequencing depth. Given that our sample sizes for most within-tissue comparisons were quite moderate, this may have affected the results. Third, given that A3SS, A5SS and RI events are typically less frequent than exon-skipping (SE), it is possible that the number of supporting reads was insufficient for reliable detection of former AS events. Additional comparative studies are clearly needed to shed light on prevalence and variability of various AS events in teleosts.

Our analyses revealed that the largest number of DAS genes was observed in the skin ($n = 81$), followed by the whole eye ($n = 67$), fin ($n = 38$) and liver ($n = 25$). Two DAS genes were consistently observed across all four tissues: *ddx31* and *trim10*. The former belongs to the DEAD-box helicase family, encoding proteins involved in ATP-dependent RNA unwinding required for a number of cellular processes, such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly. *trim10* is a member of the trim family, members of which play important roles in cellular processes such as antiviral immunity, cell proliferation, and apoptosis. However, given that all DAS genes (except *ddx31*) showed non-perfect segregation between albino and normally pigmented catfish, it is likely that the majority of detected DAS events are not causally linked to albinism. In

addition, similar to other studies (Li et al., 2016; Jacobs and Elmer, 2021), the low number of overlapping DAS and DE genes observed in *S. glanis* indicates that alternative splicing and differential gene expression are to a large extent independent and complementary processes.

4.5. Study limitations

Here, we provided first transcriptome-wide perspective on gene expression and alternative splicing patterns in Wels catfish attempting to link the molecular processes with albinism. However, similar to many RNAseq studies, this work has limitations. Firstly, we did not include a qPCR-based validation of the results. Historically, the need for a validation of genome-scale expression results was recognised when carrying out microarray analyses (Coenye, 2021). However, RNA-seq experiments typically do not suffer from the same issues as microarrays and there are a number of studies showing high concordances between RNA-seq and qPCR methodologies (e.g. Everaert et al., 2017). Furthermore, the question about feasibility of qPCR validation emerges when hundreds or even thousands of genes show differential expression between treatments. For example, we observed altogether 1355, 614, 189 and 189 DEGs in skin, fin, liver and eye tissue, which makes individual validation of all DE genes impractical. In addition, it is not obvious how informative, in terms of the overall level of false positives, such small-scale qPCR validation experiments would be. Therefore, since our main findings are drawn by combining information from multiple tissues and DEGs sharing similar function or molecular process, our conclusions are expected to be robust. Secondly, this study did not employ additional functional assays to confirm that the differences in gene expression translate to a change at different levels of biological complexity. Thus, we lack information on how the observed differences in mRNA abundance translate into protein levels, and eventually to variation in physiological and pigmentation traits. However, the absence of functional assay should not be considered a major weakness of this study since the genetic basis of pigment cell development and melanin pathway in vertebrates is fairly well understood (Hoekstra, 2006; Kelsh et al., 2009; McNamara et al., 2021). Thirdly, it is possible that some expression differences between normally pigmented and albino catfish reflect genetic divergence between two strains rather than physiological processes linked to pigmentation. For example, based on analyses of twenty microsatellite markers, Gross et al. (*in prep*) found the reduced genetic diversity of albino catfish compared to normally pigmented strains. However, despite strong effect of random genetic drift, the albino catfish still grouped together with normally pigmented catfish from Hodonin and Vodnany strains in the genetic distance-based dendrogram when compared to the wild or farmed fish of Baltic origin (Gross et al. *in prep*). Thus, while genome-wide differences between albino and normally pigmented catfish from Hodonin strain certainly exist, the presence of large number of differentially expressed genes (DEGs) in skin ($n = 1355$) and fin ($n = 614$) in contrast to eye ($n = 188$) and liver ($n = 189$) suggests that the majority of observed DEGs in the former two tissues are likely associated with albinism rather than with general population divergence. This inference is based on the assumption that random genetic drift would generate approximately similar number of DEGs in different tissues.

5. Conclusions

Current multi-tissue RNA-seq analyses revealed a large number of genes co-vary with albinism in *S. glanis,* including several well-known candidates that cause pigmentation loss in other species. In contrast, only a few genes showed differential alternative splicing between albino and normally pigmented individuals, indicating that albinism is largely independent of alternative splicing. Our differential expression patterns also corroborate earlier observations of physiological differences between albino and normally pigmented individuals, particularly in relation to energy metabolism and immune response. Overall, this work provides the first transcriptome-wide insights into the albinism of *S. glanis* and will serve as an important step towards understanding the genomic and transcriptomic mechanisms of pigmentation in teleosts.

CRediT authorship contribution statement

M.Y. Ozerov: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **K. Noreikiene:** Writing – review & editing, Methodology, Data curation. **S. Kahar:** Writing – review & editing. **M. Flajšhans:** Writing – review & editing, Resources, Funding acquisition. **R. Gross:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **A. Vasemägi:** Writing – review & editing, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Transcriptome reads of *S. glanis* skin, dorsal fin, whole eye and liver tissues are available in the NCBI SRA (SRR24510184 – SRR24510223) as a part of BioProject PRJNA971623. https://dataview.ncbi.nlm.nih.gov/object/PRJNA971623?reviewer=aio1gccikv5amerd7p45ah3aeg

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Appendix A. Supplementary data

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