BMC Genomics

Open Access

A time-course transcriptome analysis of gonads from yellow catfish (*Pelteobagrus fulvidraco*) reveals genes associated with gonad development

Dong Gao[1], Junrou Huang[1], Genmei Lin[1] and Jianguo Lu[1,2,3,4*]

Abstract

Background: The yellow catfish, *Pelteobagrus fulvidraco*, is a commercially important fish species. It is widely distributed in the fresh water areas of China, including rivers, lakes, and reservoirs. Like many other aquaculture fish species, people have observed significant size dimorphism between male and female yellow catfish and it shows a growth advantage in males.

Results: Here, at the first time, the time-course transcriptome was used to explore the various expression profiles of genes in different gonad developmental stages and genders. A total of 2696 different expression genes (DEGs) were identified from different stages. Based on these DEGs, 13 gonad development related genes were identified which showed time-specific or sex biased expression patterns.

Conclusion: This study will provide the crucial information on the molecular mechanism of gonad development of female and male yellow catfish. Especially, during the different gonad development stages, these 13 gonad development related genes exhibit various expression patterns in female and male individual respectively. These results could inspire and facilitate us to understanding the various roles of these genes play in different gonad development stages and genders.

Keywords: Time-course transcriptome, Sexual dimorphism, Gonad development

Background

Yellow catfish, *Pelteobagrus fulvidraco*, has become an important freshwater variety in Chinese aquaculture (cite China Fishery Statistical Yearbook 2018). Because of its tender flesh, few inter-muscular spines, and delicious flavor, this fish becomes much more popular in China.

Meanwhile, yellow catfish also has been regarded as an ideal model to research the sexual dimorphism, considering the significant different growth rates and maximum body sizes between female and male yellow catfish [1]. So, the studies of genetic mechanisms of sex dimorphism are crucial to yellow catfish. It is also benefited for yellow catfish aquaculture.

Gonad is the primary organ presenting morphological signs of sexual dimorphism. Thus, the studies of gonad development genes could provide valuable insights in studying sexual dimorphism [2]. To date, many gonad

*Correspondence: lujianguo@mail.sysu.edu.cn
[4] Pearl River Estuary Marine Ecosystem Research Station, Ministry of Education, Zhuhai 519000, China
Full list of author information is available at the end of the article

Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 2 of 12

development genes have been identified for several fish species, including *dmrt1* (double-sex and mab-3 related transcription factor 1) in half-tongue sole (*Cynoglossus semilaevis*), *sdy* (sexually dimorphic on the Y chromosome) in rainbow trout (*Oncorhynchus mykiss*), and *amhr2* (anti-Muellerian hormone receptor type II) in tiger pufferfish (*Takifugu rubripes*) [3].

For yellow catfish, although several gonad development genes have been detected by previous studies, such as *amhr2*, *gdf9* (growth and differentiation factor 9), *cyp1a1* (cytochrome P450 1A1), *cyp19a* (cytochrome P450 19A), *piwi* (P-element induced wimpy testis like) and *vasa* [4–6]. The performance of different gonad development genes in various gonad development stages is still ambiguous for yellow catfish.

In order to obtain the various expression patterns of gonad development genes during the entire gonad development stages of yellow catfish. A time-course gonadal transcriptome of yellow catfish was generated with high-through sequencing. The DEGs were identified among different comparison groups. The further annotation of gonad development DEGs may help us to illuminate the mechanism involved in gonad development and sexual dimorphism.

Results
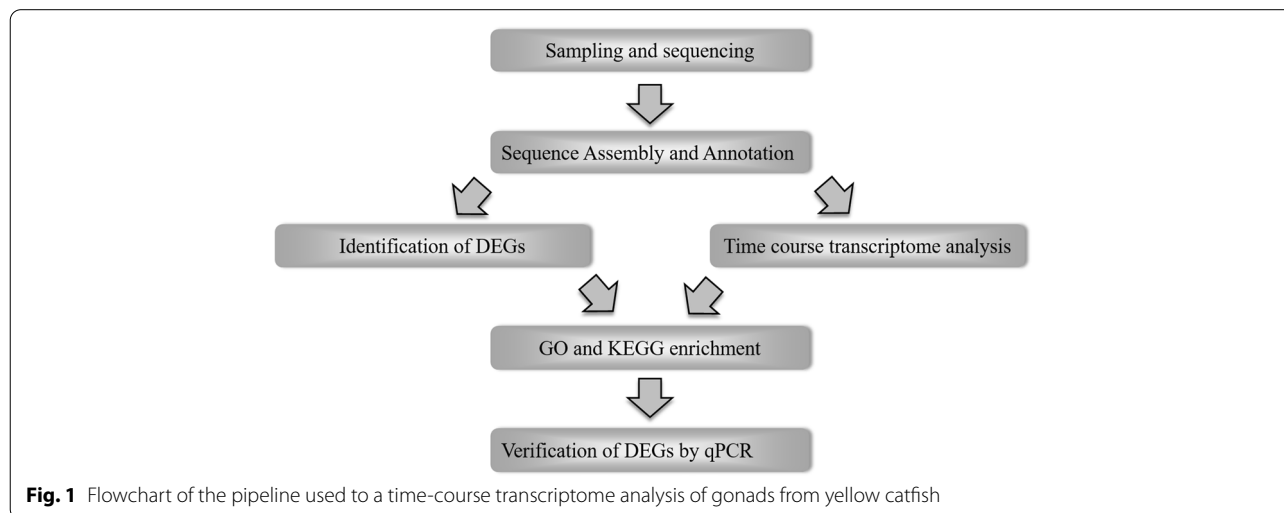
Overview of RNA-Seq results

In order to identify gonad development related genes in yellow catfish, the RNA-Seq was performed in all of sample groups. The data sets yielded from 8.54 Gbp to 16.38 Gbp and the average mapping rate for *P. fulvidraco* reference genome was 84.24%. The flowchart of processing steps in our pipeline is shown in Fig. 1. The sequencing results were listed in Table 1.

Table 1 Summary of the RNA-Seq data sets and the mapping ratios for all sample groups. Note: The sample names represent the different gonad development stages when the samples were collected. BG: before gonad differentiation; MG: male gonad differentiation; MGC: male gonad differentiation control; FG: female gonad differentiation; FGC: female gonad differentiation control; MAG: male after gonad differentiation; FAG: female after gonad differentiation

Sample name	Raw data (Gbp)	Clean data (Gbp)	Map rate (%)
BG	16.38	14.50	84.4
MG	10.46	9.26	83.8
MGC	8.54	7.56	83.1
FG	9.12	8.08	84.3
FGC	8.62	7.64	85.0
MAG	13.68	12.12	84.1
FAG	11.74	10.40	85.0
Total	78.54	69.56	–

Transcriptome assembly, annotation and Avaluation

Based on mapped reads, the time-coure transcriptome of *P. fulvidraco* was generated by Cufflinks. A total size of draft transcriptome was 271.25 Mbp including over 85,880 raw transcripts. In order to obtain a final assembly, two filter metrics were set, including the minor length is 150 bp and this transcript must express (FPKM >0) at least in a group. The final assembly consisted of 41,329 transcripts. These transcripts were annotated via searching against the Nr, GO and KEGG database respectively. There were 34,273 transcripts corresponding to 16,861 genes, with an E-value cutoff less than 1e-5. A total number of 29,303 transcripts were assigned at least one GO term, corresponding to 14,637 genes. 14,223 transcripts were annotated into 344 KEGG pathways (Additional

Fig. 1 Flowchart of the pipeline used to a time-course transcriptome analysis of gonads from yellow catfish

Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 3 of 12

file 1). The Benchmarking Universal Single-Copy Orthologs (BUSCO) was used to estimate transcriptome completeness using the actinopterygii_odb9 BUSCO set [7]. Among all of (4584) BUSCO core genes, 3694 (80.6%) BUSCO core genes were identified in the yellow catfish transcriptome which suggested that this assembly was good enough to subsequent analysis.

Identification of gonad development related DEGs

According to the method, 2696 DEGs were identified from all of these comparison groups. For the different ovary development stages, several gonad development related DEGs were detected in Fig. 2A-C and Table 2. For example, for BG vs. FG, 447 DEGs were up-regulated including *Aurka* (aurora kinase A), *cyp1a1*, *piwi*, *plk1* (polo-like kinase 1) and *vasa*. For FG vs. FGC, *Aurka*, *cyp1a1*, *org* (oogenesis-related isoform A), *piwi*, *plk1* and *vasa* were significantly highly expressed in female individual (FG). *Cyp1a1* was down-regulated in FG vs. FAG comparison group.

For the different testis development stages, *cyp1a1* was up-regulated in BG vs. MG. In the MG vs. MAG
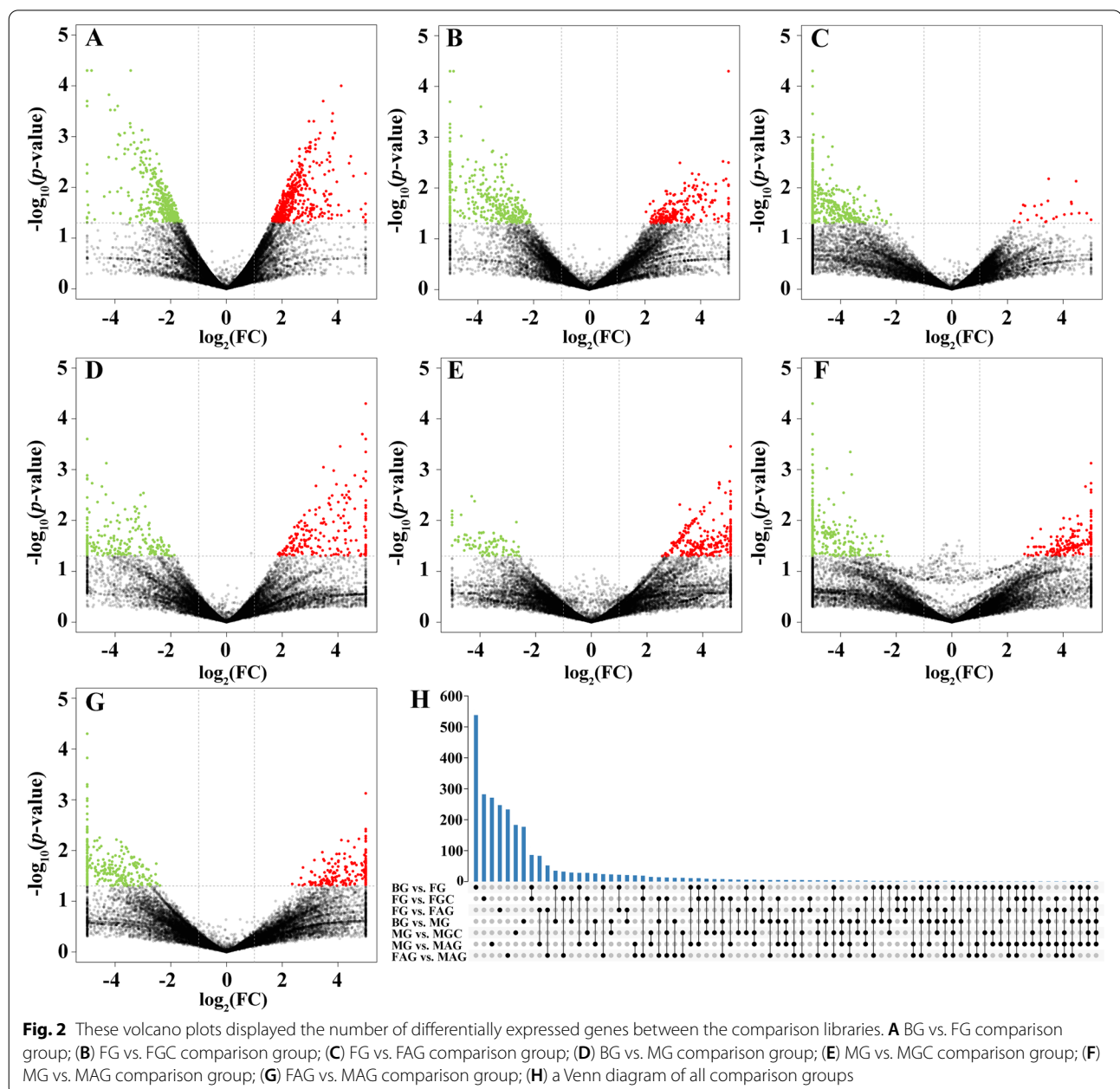

Fig. 2 These volcano plots displayed the number of differentially expressed genes between the comparison libraries. **A** BG vs. FG comparison group; (**B**) FG vs. FGC comparison group; (**C**) FG vs. FAG comparison group; (**D**) BG vs. MG comparison group; (**E**) MG vs. MGC comparison group; (**F**) MG vs. MAG comparison group; (**G**) FAG vs. MAG comparison group; (**H**) a Venn diagram of all comparison groups

Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 4 of 12

Table 2 Gonad development related DEGs in yellow catfish

Gene name	Description	Gene id	Comparison groups						
			BG vs. FG	FG vs. FGC	FG vs. FAG	BG vs. MG	MG vs. MGC	MG vs. MAG	FAG vs. MAG
amhr2	anti-Muellerian hormone type-2 receptor	XLOC_006098						↑	
aurka	aurora kinase A	XLOC_019110	↑	↓			↑		
bmp15	bone morphogenetic protein 15	XLOC_000571							↓
cyp1a1	cytochrome P450 1A1	XLOC_007208	↑	↓	↓	↑		↓	
gdf9	growth/differentiation factor 9-like	XLOC_003614							↓
org	oogenesis-related isoform A	XLOC_016409		↓					↓
piwi	piwi-like protein	XLOC_008581	↑	↓				↑	
plk1	polo-like kinase 1	XLOC_013011	↑	↓					
sox9	transcription factor Sox-9-A-like	XLOC_010029							↑
spata22	spermatogenesis-associated protein 22	XLOC_024282						↑	
tex11	testis-expressed sequence 11 protein	XLOC_012328						↑	
tex15	testis-expressed sequence 15 protein	XLOC_018159						↑	
vasa	ATP-dependent RNA helicase DDX4	XLOC_017737	↑	↓				↑	

comparison group, *amhr2*, *piwi* and *vasa* showed higher expressed level in MAG. On the contrary, *cyp1a1* showed higher expressed level in MG (Table 2).

Additionally, in the after gonad differentiation comparison group (FAG vs. MAG), *bmp15*, *gdf9* and *org* were activated in ovary and *sox9a* was activated in testis. In sum up, there are 13 gonad development related DEGs were found from these comparison among different groups (Table 2).

GO and KEGG enrichment analysis of sex-related DEGs
To better understand the possible functions involved in gonad development of yellow catfish, the DEGs from each comparison group were enriched with the GO database. As a result, several gonad development related genes were enriched into go terms. For example, *piwi* and *vasa* were enriched in germ plasm cellular part for MG vs. MAG comparison group (Additional files 2 and 3).

Correspondingly, the KEGG pathway enrichment analysis was performed. In BG vs. FG comparison group, *cyp1a1* was enriched in ovarian steroidogenesis pathway and *plk1* was enriched in cell cycle pathway. For FG vs. FGC group, *cyp1a1* was enriched in steroid hormone biosynthesis, *plk1* and *aurka* were enriched in oocyte meiosis. *Cyp1a1* was enriched in steroid hormone biosynthesis and ovarian steroidogenesis pathway for FG vs. FAG comparison group (Additional file 4).

Moreover, *cyp1a1* was enriched in steroid hormone biosynthesis pathway for BG vs. MG comparison group. *Aurka* was enriched in oocyte meiosis pathway for MG vs. MGC. *Cyp1a1* was also enriched in steroid hormone biosynthesis pathway for MG vs. MAG. *Bmp15*, *gdf9* and *org* were enriched in cytokine-cytokine receptor interaction pathway for FAG vs. MAG (Additional file 4).

Time-course analysis of expression profiles of genes
We further clustered genes which exhibited the similar expression profiles in the entire gonad development stages of female and male respectively. Then, these clustered genes were annotated and enriched by GO and KEGG database (Additional files 5 and 6).

For female, BG, FG, MGC and FAG were considered as four different ovary development stages. Seven clusters were obtained from these results (Fig. 3A). In profile 15, *aurka* was enriched in nuclear part (Additional files 5 and 7). In profile 17, *piwi* and *org* were enriched in intracellular; *vasa* was enriched in organelle and intracellular organelle (Additional files 5 and 7).

For male, BG, FGC, MG and MAG were considered as four different gonad development stages. Six clusters were obtained from these time-course transcriptome analysis results (Fig. 3B). In profile 7, *spata22* (spermatogenesis-associated protein 22), *tex11* (testis-expressed sequence 11 protein) and *tex15* (testis-expressed
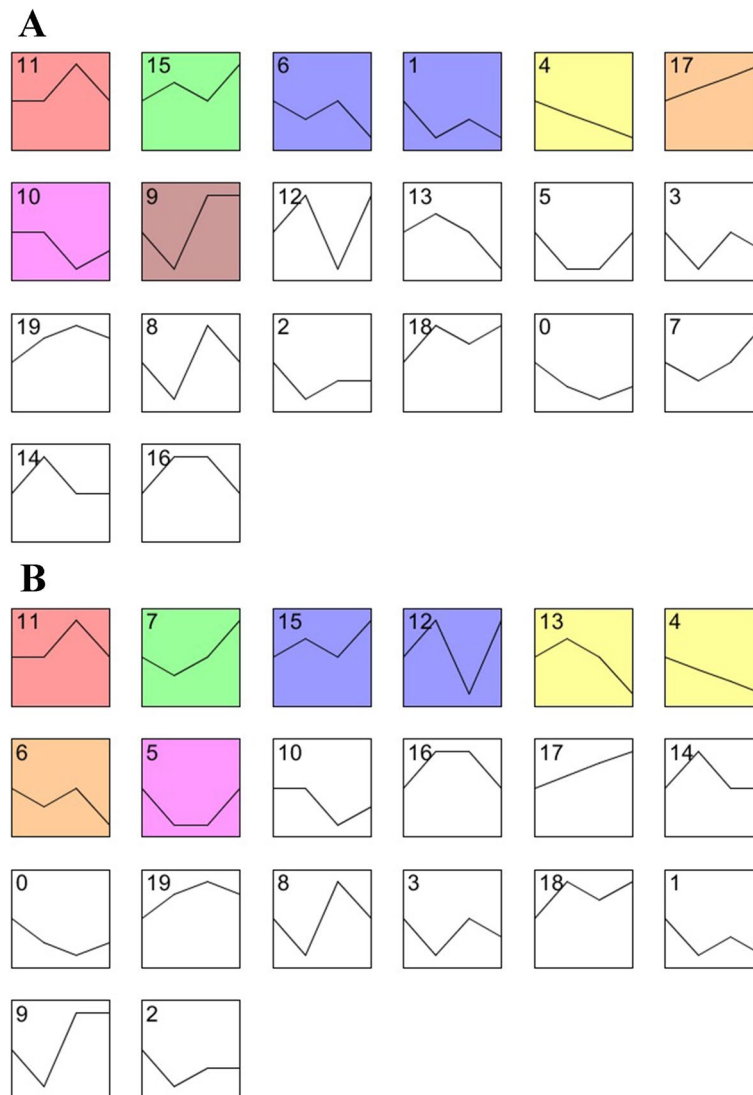
Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 5 of 12

Fig. 3 Gene clustering in different gonad development stages. Each box corresponds to a model expression profile. For each box, the left top number represents the profile number, the right bottom number represents the contained genes number. Colored profiles have a statistically significant number of gene assigned. **A** Expression pattern of female gonad development stages. **B** Expression pattern of male gonad development stages

sequence 15 protein) were enriched in meiotic cell cycle and reproduction biological process (Additional files 5 and 8).

Verification of DEGs by RT-qPCR

Seven DEGs were randomly selected for RT-qPCR validation to verify the RNA-Seq results of time-course gonadal transcriptome. The primers used for these genes are listed in Table 3.

The expression profiles of these selected genes obtained from RT-qPCR corresponded to the RNA-Seq results (Fig. 4).

Discussion

In this work, a time-course gonadal transcriptome of yellow catfish was constructed with Illumina HiSeq 2000 sequencing technology. These results provided the valuable genetic resources to understand the mechanisms of gonad development and sexual dimorphism. A total of 2696 DEGs were obtained. Then, these DEGs were annotated and enriched by GO and KEGG databases. Time-course analysis were employed to detect the genes with similar expression profiles in female and male entire gonad development stages. Finally, 13 gonad development related genes were obtained from different gonad

Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 6 of 12

Table 3 PCR primers used in this study

Genes	Sequence (5′-3′)	Product size (bp)	Gene_id
amhr2	GGCGAGGACGGTTTTCA AAGAGGACAGGCGTTGGTT	222	XLOC_006098
aurka	CAAACCCCAGCCAGCAC GCTTCCAAACTTTCCCTTCC	199	XLOC_019110
bmp15	ACTTCTCACGCTGGCTCAA CCCAACCAAGATCACGGA	151	XLOC_000571
cyp1a1	CGCTCATTCGCAAACATC GGTTTCCGCTGCCCAC	271	XLOC_007208
gdf9	GGGAGAAATCCATTCCAACC CGGAAGTCATAGAGGTCGC	192	XLOC_003614
plk1	TAAGATTGGTGACTTCGGTTTG TGTGCCTGGGAATGGTGTA	251	XLOC_013011
vasa	TTGCCAAATCAGGATACGAA TGCCACCATAAACCACGA	300	XLOC_017737

development stages of female and male yellow catfish (Table 2).

Cyp1a1 gene

Cyp1a1, a cytochrome P450 monooxygenase, involved in the metabolism of various endogenous substrates, including fatty acids, steroid hormones and vitamins [8–11]. Exhibits high catalytic activity for the formation of hydroxyestrogens from estrone (E1) and 17β-estradiol (E2) (Additional file 9). In this study, compare with BG, *cyp1a1* was up-regulated in early gonad differentiation stage (Additional file 9). Then, this gene was enriched in ovarian steroidogenesis (for female) and steroid hormone biosynthesis pathway (for male). These results suggested that *cyp1a1* may play a role in early gonad development through metabolizes steroid sex hormones (E1 and E2) in the gonad, and finally induces spermatogenesis and oogenesis.

Piwi and *vasa* genes

Additionally, the time-specific expression patterns of *piwi* and *vasa* were detected in BG vs. FG and MG vs. MAG comparison group. In details, the two gonad development related genes showed highly expression levels in the period of ovary differentiation (in FG) and testis after differentiation (in MAG) respectively. *Piwi* controls the

number of primordial germ cells (PGCs) via protecting maternal mRNA from decay and adult germ stem cell division in Drosophila [12]. In zebrafish, *piwi* is required for maintaining germ cells, because loss of *piwi* leads to germ cell loss by apoptosis [13]. For medaka, *piwi* is required not only for determining the PGC number but also for controlling PGC migration [14]. *Vasa* (ATP-dependent RNA helicase DDX4) gene is a RNA helicase of the DEAD-box helicase family, which plays a crucial role in germ cell formation. *Vasa* is localized specifically in germline cells, indicating a particular function in gonads [15]. Differential expression of a *vasa* homolog in the gonads of tilapia (*Oreochromis niloticus*) during gametogenesis suggests a possible role for *vasa* in regulating the meiotic progression of male and female germ cells [16]. So, time-specific expression patterns of *piwi* and *vasa* might be suggested that these two genes played more important roles in both ovary differentiation and testis maintain.

Female-biased genes

According to our results, *aurka* was activated in early stage of ovary differentiation (Fig. 5A). Aurka, which is a centrosome-localized serine/threonine kinase crucial for cell cycle control, plays an important role in mitosis [17] and also regulates the meiotic cell cycle of Xenopus oocytes [18]. Meanwhile, *plk1*, which could be activated by *aukra*, was also up-regulated in FG (Fig. 5E). It has multiple regulatory roles in the cell cycle, including the control of cell cycle progression into mitosis [19]. These two genes were enriched in oocyte meiosis suggesting that *aurka* and *plk1* may play roles in proliferation and differentiation during the early stage of ovary development.

In FAG vs. MAG comparison group, *bmp15* and *gdf9* displayed the significant highly expression only in FAG (Fig. 5B and C). *Bmp15* and *gdf9* are two closely related members of the TGF-β superfamily who can regulate granulosa cell development [20–24]. Specifically, zebrafish *bmp15* mutants can initially develop as females. But during the juvenile stage, their sex revert to fertile males [21]. In our results, *bmp15* and *gdf9* had significantly higher expression levels in female after gonad differentiation. It is suggested that *bmp15* and *gdf9* may perform functions in maintaining the sexual phenotype of female yellow catfish.

(See figure on next page.)
Fig. 4 Validation of the expression patterns both in RNA-Seq (red) and RT-qPCR (blue). **A** BG vs. FG, **(B)** FG vs. FGC, **(C)** FG vs. FAG, **(D)** BG vs. MG, **(E)** MG vs. MGC, **(F)** MG vs. MAG, **(G)** FAG vs. MAG. Gene relative expression: $\log_2$(fold change). RT-qPCR fold changes is normalized by changes in beta-actin values. The averages of three relative quantities of biological replications were used in a two-tailed student's t test with a 95% confidence level ($P < 0.05$) to determine the gene expression significance
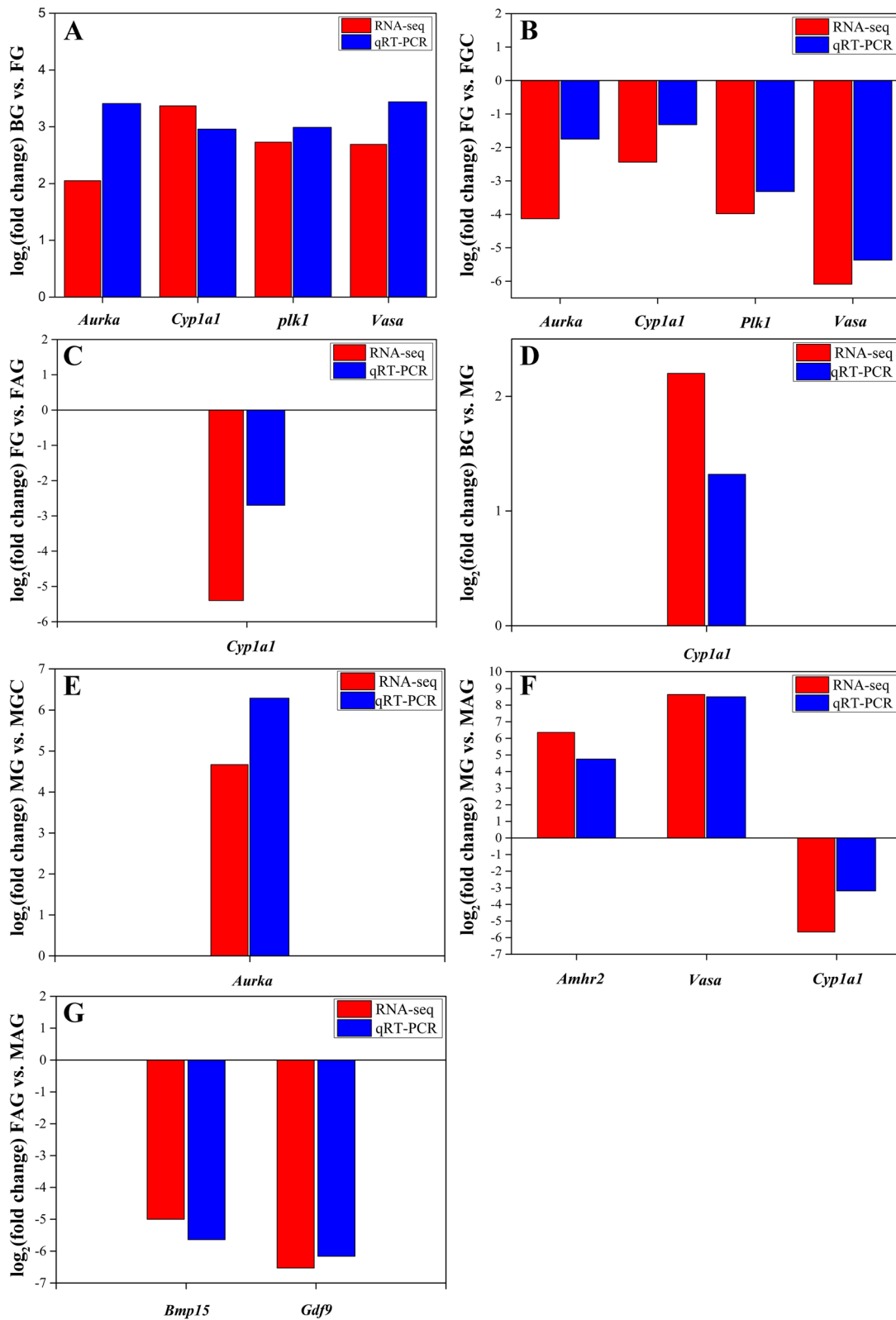
Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 7 of 12

Fig. 4 (See legend on previous page.)

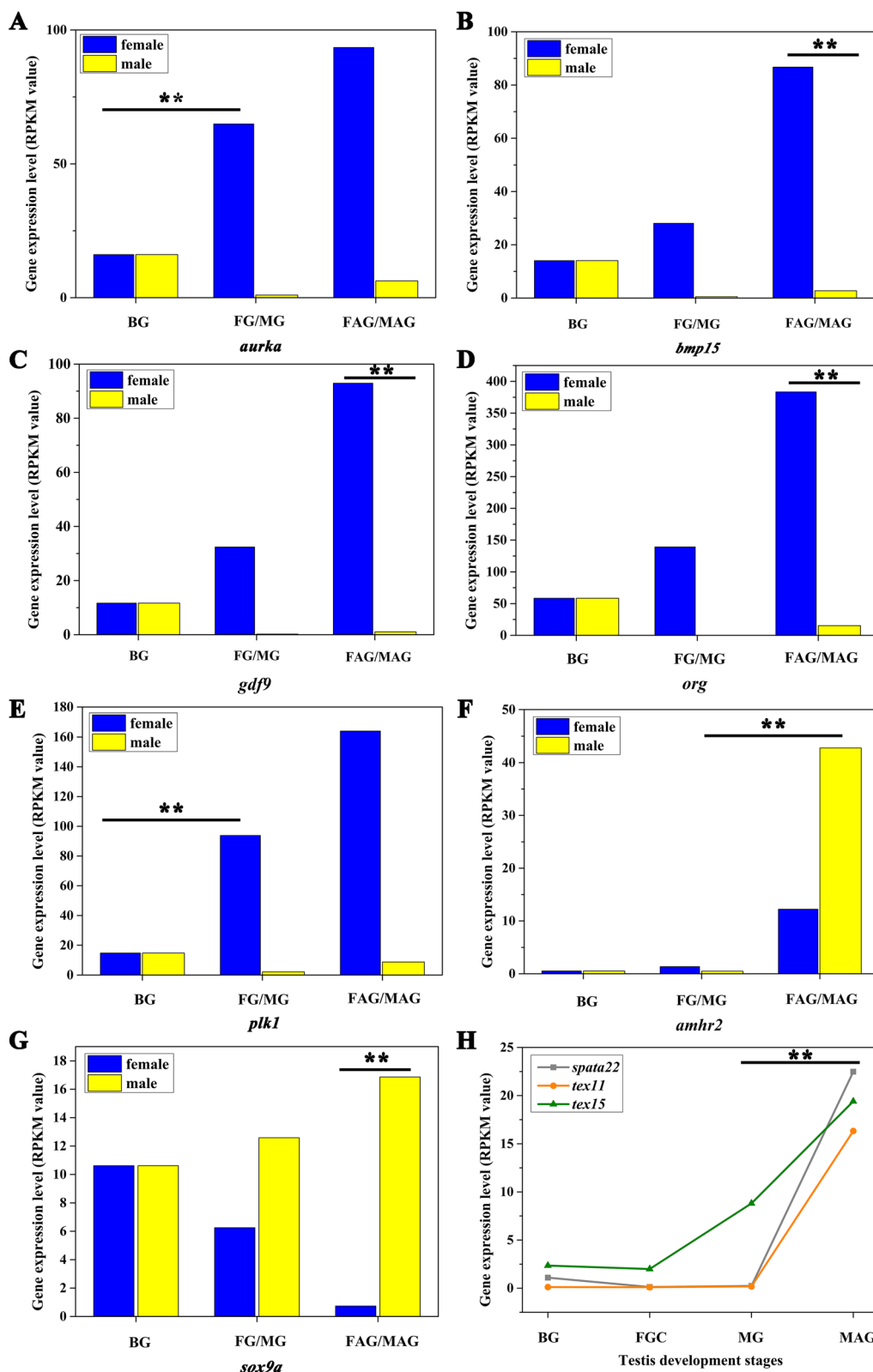Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 8 of 12

Fig. 5 Sexual biased genes and clustered sex-related genes were identified from this study. **A-E** These histograms showed the female-biased sex-related genes. **F-G** These two male-biased sex-related genes were displayed by histograms. **H** *spata22*, *tex11* and *tex15* show increasing expression from FGC to MAG

Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 9 of 12

Male-biased genes

Amhr2 and *sox9a* displayed the significant male-biased expression in male yellow catfish testis and they were up-regulated in MAG (Fig. 5F-G). Amh (anti-Muelle-rian hormone) is the gonadal hormone responsible for the regression of Mullerian ducts in male fetuses during mammalian embryogenesis [25, 26]. Amh signals through the *amhr2* to regulate the differentiation and growth of target cells in mammals [27]. In fish, an *amhr2* mutation results in a male-to-female sex change in XY medaka suggested that *amhr2* is involved in sex determination [28]. Our laboratory have observed highly expression of *sox9a* in 1-year-old and 2-year-old yellow catfish testis. The present results consist with our previous studies which indicated that these two genes are not only important to testis differentiation but also are crucial to the maintenance and development of testis.

The clustered genes

Additionally, from the results of STEM, we found that *spata22*, *tex11* and *tex15* showed increased expressions from FGC to MAG (Fig. 5H). *Spata22* is required early in meiotic prophase in both male and female germ cells [29]. *Tex11* and *tex15* display testis-specific expression which is required for meiotic recombination [30, 31].

Conclusions

Thanks for published high quality chromosome-level yellow catfish reference genome, a time-course gonadal transcriptome analysis was performed with cufflinks for yellow catfish to explore the mechanisms of gonad development and sexual dimorphism. A final assembly which consists of 41,329 transcripts was generated and annotated. GO and KEGG enrichment analysis and STEM cluster analysis revealed that all of 13 gonad development related genes were enriched in multiple go terms and pathways including meiotic cell cycle, reproduction biological process, ovarian steroidogenesis, steroid hormone biosynthesis, and oocyte meiosis pathway. These gonad development related genes showed various expression patterns. For example, *cyp1a1* was up-regulated in gonad differentiation stages and enriched in ovarian steroidogenesis (in FG) or steroid hormone biosynthesis pathway (in MG). These results indicated that *cyp1a1* may play a role in early gonad development through metabolizes steroid sex hormones (E1 and E2) in the gonad, and finally induces spermatogenesis and oogenesis. Moreover, *piwi* and *vasa* showed highly expression levels in ovary differentiation stage (FG) and after testis differentiation stage (MAG) respectively. It is suggested that these two genes may play an important role in ovary differentiation and testis maintaining. These results provided a reference for subsequent research on the mechanism of sexual dimorphism and sex control of yellow catfish.

Materials and methods

Samples and RNA extraction

Depended on previous studies, in yellow catfish, the gonad differentiation was observed at 13dph (day post-hatch) and 54 dph in females and males respectively [32, 33]. To compare the gene expression patterns of different gonad stages, a total of 42 full-sibling offsprings were sampled at before gonad differentiation (3dph), gonad differentiation (13dph for female and 54dph for male), and after gonad differentiation (90dph) respectively. Additionally, at female gonad differentiation stage, male individuals were collected into a control group (FGC). Similarly, at male gonad differentiation stage, female individuals were collected into a control group (MGC). All details of sampled groups were listed in Table 4.

The sex of each individual was confirmed by sex-linked marker as described previously [34]. Then, for each group, gonad tissues were sampled and pooled from 6 individuals to obtain enough total RNA and avoid individual variations. Total RNAs were extracted by Trizol method with manufacturer's protocol (Ambion). The concentration and quality of each RNA sample were examined using a NanoDrop-2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The RNA integrity was checked by ethidium bromide staining of 28S and 18S ribosomal bands on a 1% agarose gel.

Table 4 Summary of sampling and grouping from different gonad development stages

Group	Gonad differentiation Stage	Individuals number	Gender of individuals
BG	before gonad differentiation	6	undifferentiated
FG	female gonad differentiation	6	female
FGC	female gonad differentiation	6	male
MG	male gonad differentiation	6	male
MGC	male gonad differentiation	6	female
FAG	after gonad differentiation	6	female
MAG	after gonad differentiation	6	male

Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 10 of 12

Libraries construction and sequencing

Equal amounts of high-quality RNA samples were used to synthesize cDNA libraries. Briefly, mRNAs were purified from total RNAs and used as templates to synthesize the first-strand and the second-strand of cDNAs, according to the protocol of Super Script Double-Stranded cDNA Synthesis kit (Thermo Fisher Scientific, MA, USA). cDNAs were cut into short fragments following the TruSeq RNA sample preparation guide. After end repair and the addition of poly (A), the short fragments were ligated with sequencing adapters and enriched by PCR amplification to construct the cDNA library templates. Finally, transcriptome sequencing was performed using the Illumina HiSeq 2000 platform, which generated about 100-bp paired-end (PE) raw reads.

Sequence assembly and annotation

Raw reads were cleaned by removing the adaptor-containing sequences and low-quality reads. All clean reads were aligned and mapped using Tophat [35] to the published yellow catfish reference genome [36]. These mapped reads were subsequently used by Cufflinks [37] to generate non-redundant set of transcript contigs representing the transcriptome of yellow catfish. Cuffnorm was run on each group reads separately to estimate transcript abundance (read counts). Cuffdiff was then performed to differential expression analysis of 1) BG vs. FG; 2) BG vs. MG; 3) FG vs. FGC; 4) MG vs. MGC; 5) FG vs. FAG; 6) MG vs. MAG; and 7) FAG vs. MAG respectively. The considered thresholds of DEGs including *p*-value $\leqq$ 0.05 and |log$_2$ (fold change)| $\geq$ 1.

All assembled transcripts were annotated using several popular publicly available databases, including NCBI non-redundant protein sequences (Nr), Gene Ontology (GO) database [38] and Kyoto Encyclopedia of Genes and Genomes (KEGG) database [39] by using BLASTX [40] with a cut-off of $E \leq$ 1e-5, OmicsX, and KOBAS respectively. Combined with the results of annotation and enrichment of DEGs, two criteria were used to identify the gonad development related DEGs in yellow catfish: 1) the DEGs were enriched (p-value ≤0.05) in gonad related GO terms or KEGG pathways, such as ovarian steroidogenesis, reproduction biological process; 2) the DEGs were enriched in GO terms or KEGG pathways and reported by previous gonad development studies, such as *gdf9 and vasa*.

Gene expression during the developmental stages of gonad

Depended on the transcript abundance results produced by Cuffnorm, Short Time-series Expression Miner (STEM) [41] were employed to cluster these genes which exhibited the similar expression profiles in entire gonad development stages (from before gonad differentiation to after gonad differentiation stage) in female and male respectively. Genes were considered as having similar expression patterns which were clustered into a same cluster. The clustered profile with FDR value ≤0.05 were regarded as a valid cluster.

Real-time PCR verification

Seven randomly selected DEGs were validated by real-time quantitative PCR (RT-qPCR) to prove the reliability of RNA-seq results. The PrimerScript TM RT regent Kit with gDNA Eraser was used to cDNA synthesis following the manufacturer's instructions. The primer information for these genes are available in Table 3. *β-actin* was employed as the endogenous reference gene. The qPCR mixture consisted of 10 µl of 2 × Master Mix, 1 µl of cDNA, 0.5 µl of each primer and 8 µl of ddH$_2$O under the following conditions to collect fluorescence: denaturation at 95 °C for 30 min, followed by 40 cycles of amplification at 95 °C for 5 s and 60 °C for 40 s. To establish the melting curve of the PCR product, the reaction was performed at 95 °C for 10 s, 60 °C for 60 s, and 95 °C for 15 s, after which it was slowly heated from 60 °C to 99 °C. The relative expression levels of target genes in each sample were individually determined using the 2$^{-\Delta\Delta CT}$ method [42].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08651-0.

Additional file 1: Table S1. Nr, GO and KEGG annotation results of the transcriptome.

Additional file 2: Fig. S1. Bubble plot for the GO enrichment results of DEGs.

Additional file 3: Table S2. GO enrichment results of the DEGs.

Additional file 4: Table S3. KEGG enrichment results of the DEGs.

Additional file 5: Table S4. GO enrichment results of the clustered genes which exhibit the similar expression profiles in female or male entire gonad development stages.

Additional file 6: Table S5. KEGG enrichment results of the clustered genes which exhibit the similar expression profiles in female or male entire gonad development stages.

Additional file 7: Table S6. Bubble plot for the GO enrichment results of gene clusters in female gonad development stages.

Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 11 of 12

Additional file 8: Fig. S2. Bubble plot for the GO enrichment results of gene clusters in male gonad development stages.

Additional file 9: Fig. S3. Steroid hormone biosynthesis pathway. The heat map shows the expression of cyp1a1 (log2 (FPKM)) in different gonad development stages.

Availability of data and materials
All datasets from the Illumina sequencing platform can be found in the Short Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) under accession number (PRJNA668564).

Declarations

Ethics approval and consent to participate
All procedures in our study including the handling and treatment of fish were approved by the Ethic Committee on the Care and Use of Laboratory Animals of Sun Yat-Sen University, Guangzhou, China.

Consent for publication
Not applicable.

Competing interests
The authors declare that we don't have any commercial or associative interest that represents a conflict of interests in connection with the work submitted.

Author details
[1] School of Marine Sciences, Sun Yat-Sen University, Zhuhai 519082, China. [2] Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519080, China. [3] Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering, Guangzhou 510275, Guangdong, China. [4] Pearl River Estuary Marine Ecosystem Research Station, Ministry of Education, Zhuhai 519000, China.

References

1. Mei J, Gui J-F. Sexual size dimorphism, sex determination, and sex control in yellow catfish. Sex Control Aquacult. 2018. Chapter 24:495–507.
2. Siegfried KR. In search of determinants: gene expression during gonadal sex differentiation. J Fish Biol. 2010;76(8):1879–902.
3. Kamiya T, Kai W, Tasumi S, Oka A, Matsunaga T, Mizuno N, et al. A trans-species missense SNP in Amhr2 is associated with sex determination in the tiger pufferfish, Takifugu rubripes (fugu). PLoS Genet. 2012;8(7):e1002798.
4. Chen X, Mei J, Wu J, Jing J, Ma W, Zhang J, et al. A comprehensive transcriptome provides candidate genes for sex determination/differentiation and SSR/SNP markers in yellow catfish. Mar Biotechnol (NY). 2015;17(2):190–8.
5. Lu J, Luan P, Zhang X, Xue S, Peng L, Sun X. Gonadal Transcriptomic Analysis of Yellow Catfish (Pelteobagrus fulvidraco): Identification of Sex-related Genes and Genetic Markers. Physiol Genomics. 2014;46:798–807.
6. Lu J, Zheng M, Zheng J, Liu J, Liu Y, Peng L, et al. Transcriptomic analyses reveal novel genes with sexually dimorphic expression in yellow catfish (Pelteobagrus fulvidraco) brain. Marine Biotechnol (New York, NY). 2015;17(5):613–23.
7. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31(19):3210–2.
8. Kisselev P, Schunck W-H, Roots I, Schwarz D. Association of CYP1A1 polymorphisms with differential metabolic activation of 17beta-estradiol and estrone. Cancer Res. 2005;65(7):2972–8.
9. Li D, Wang M, Cheng S, Zhang C, Wang Y, Zhang W, et al. CYP1A1 based on metabolism of xenobiotics by cytochrome P450 regulates chicken male germ cell differentiation. In Vitro Cell Dev Biol Anim. 2017;53(4):293–303.
10. Sowers MR, Wilson AL, Kardia SR, Chu J, McConnell DS. CYP1A1 and CYP1B1 polymorphisms and their association with estradiol and estrogen metabolites in women who are premenopausal and Perimenopausal. Am J Med. 2006;119(9, Supplement 1):S44–51.
11. Xu H, Zhang X, Ye Y, Li X. Bisphenol a affects estradiol metabolism by targeting CYP1A1 and CYP19A1 in human placental JEG-3 cells. Toxicol Vitro. 2019;61:104615.
12. Lin H, Spradling AC. A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the drosophila ovary. Development. 1997;124(12):2463.
13. Houwing S, Kamminga LM, Berezikov E, Cronembold D, Girard A, van den Elst H, et al. A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in zebrafish. Cell. 2007;129(1):69–82.
14. Li M, Hong N, Gui J, Hong Y. Medaka piwi is essential for primordial germ cell migration. Curr Mol Med. 2012;12(8):1040–9.
15. Hartung O, Forbes MM, Marlow FL. Zebrafish vasa is required for germ-cell differentiation and maintenance. Mol Reprod Dev. 2014;81(10):946–61.
16. Kobayashi T, Kajiura-Kobayashi H, Nagahama Y. Differential expression of vasa homologue gene in the germ cells during oogenesis and spermatogenesis in a teleost fish, tilapia, *Oreochromis niloticus*. Mech Dev. 2000;99(1):139–42.
17. Jeon H-Y, Lee H. Depletion of Aurora-A in zebrafish causes growth retardation due to mitotic delay and p53-dependent cell death. FEBS J. 2013;280(6):1518–30.
18. Sarkissian M, Mendez R, Richter JD. Progesterone and insulin stimulation of CPEB-dependent polyadenylation is regulated by Aurora a and glycogen synthase kinase-3. Genes Dev. 2004;18(1):48–61.
19. Medema RH, Lin C-C. Yang JC-H: polo-like kinase 1 inhibitors and their potential role in anticancer therapy, with a focus on NSCLC. Clin Cancer Res. 2011;17(20):6459.
20. Clelland E, Kohli G, Campbell RK, Sharma S, Shimasaki S, Peng C. Bone morphogenetic Protein-15 in the zebrafish ovary: complementary deoxyribonucleic acid cloning, genomic organization, tissue distribution, and role in oocyte maturation. Endocrinology. 2006;147(1):201–9.
21. Dranow DB, Hu K, Bird AM, Lawry ST, Adams MT, Sanchez A, et al. Bmp15 is an oocyte-produced signal required for maintenance of the adult female sexual phenotype in zebrafish. PLoS Genet. 2016;12(9):e1006323.
22. Liu L, Ge W. Growth differentiation factor 9 and its spatiotemporal expression and regulation in the zebrafish Ovary1. Biol Reprod. 2007;76(2):294–302.

Gao *et al. BMC Genomics* 2022, **23**(Suppl 1):409

Page 12 of 12

23. McGrath-Morrow S, Esquela-Kerscher A, Lee S. Oocyte specific expression of growth differentiation factor-9. Mol Endocrinol (Baltimore, Md). 1995;9:131–6.

24. Dube JL, Wang P, Elvin J, Lyons KM, Celeste AJ, Matzuk MM. The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. Mol Endocrinol. 1998;12(12):1809–17.

25. Behringer RR, Cate RL, Froelick GJ, Palmiter RD, Brinster RL. Abnormal sexual development in transgenic mice chronically expressing Müllerian inhibiting substance. Nature. 1990;345(6271):167–70.

26. Pfennig F, Standke A, Gutzeit H. The role of Amh signaling in teleost fish - Multiple functions not restricted to the gonads. Gen Comp Endocrinol. 2015;223:87–107.

27. Behringer RR, Finegold MJ, Cate RL. Müllerian-inhibiting substance function during mammalian sexual development. Cell. 1994;79(3):415–25.

28. Morinaga C, Saito D, Nakamura S, Sasaki T, Asakawa S, Shimizu N, et al. The hotei mutation of medaka in the anti-Mullerian hormone receptor causes the dysregulation of germ cell and sexual development. Proc Natl Acad Sci U S A. 2007;104(23):9691–6.

29. La Salle S, Palmer K, O'Brien M, Schimenti JC, Eppig J, Handel MA. Spata22, a novel vertebrate-specific gene, is required for meiotic progress in mouse germ cells. Biol Reprod. 2012;86(2):45.

30. Boroujeni PB, Sabbaghian M, Totonchi M, Sodeifi N, Sarkardeh H, Samadian A, et al. Expression analysis of genes encoding TEX11, TEX12, TEX14 and TEX15 in testis tissues of men with non-obstructive azoospermia. JBRA Assist Reprod. 2018;22(3):185–92.

31. Yang F, Eckardt S, Leu NA, McLaughlin KJ, Wang PJ. Mouse TEX15 is essential for DNA double-strand break repair and chromosomal synapsis during male meiosis. J Cell Biol. 2008;180(4):673–9.

32. YJCJoZ H. Sex differentiation in *Pelteobagrus fulvidraco*. Chin J Zool. 2008;43:103–8.

33. Li-Na P, Jian-Guo LU, Pei-Xian L, Yan-Long GE, Shu-Qun X, Xiao-Feng Z, et al. Observation on Gonadal Differentiation in Yellow Catfish *Pelteobagrus fulvidraco*. Chin J Fisheries. 2014;27(3):48–51.

34. Dan C, Mei J, Wang D, Gui J-F. Genetic differentiation and efficient sex-specific marker development of a pair of Y- and X-linked markers in yellow catfish. Int J Biol Sci. 2013;9(10):1043–9.

35. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics. 2009;25(9):1105–11.

36. Gong G, Dan C, Xiao S, Guo W, Huang P, Xiong Y, et al. Chromosomal-level assembly of yellow catfish genome using third-generation DNA sequencing and Hi-C analysis. Giga Sci. 2018;7(11):giy120.

37. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010;28(5):511–5.

38. Young MD, Wakefield MJ, Smyth GK, Oshlack A. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol. 2010;11(2):R14.

39. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, et al. KEGG for linking genomes to life and the environment. Nucleic Acids Res. 2008;36(suppl_1):D480–4.

40. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–10.

41. Ernst, Jason, and Ziv Bar-Joseph. "STEM: a tool for the analysis of short time series gene expression data". BMC bioinformatics. 2006;7(1):1-11.

42. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25(4):402–8.

Publisher's Note