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Comparative Transcriptome Analysis of the Ovary and Testis in Noble Scallop (*Chlamys nobilis*)

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ABSTRACT

Noble scallop (*Chlamys nobilis*) is an economically important cultured marine bivalve shellfish common in southern China. Investigation on the molecular regulatory mechanisms of gonadal maturation in scallop is critical in the aquacultural industry. Here, gonads in maturing stage were obtained from noble scallops and sequenced using an Illumina high-throughput sequencer, producing 6.68 and 6.70 Gb of data for the ovary and testis, respectively. Reproduction-related genes, including vasa, nanos, and vitellogenin, and sex-determining genes, such as FoxL2, Dmrt, and sox9, were detected. Transcriptome comparison revealed 2,842 differentially expressed genes (DEGs), of which 591 exhibited biased expression in the ovary and 2,251 exhibited biased expression in the testis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of the DEGs were conducted. Results showed that GO terms and KEGG pathways related to protein glycosylation, fatty acid biosynthetic processes, hydrolase activity, and AMPK were enriched in the ovary, whereas those related to male organ formation and spermiogenesis were enriched in the testis. The glycosphingolipid biosynthesis pathway was identified for the first time in a mollusc testis.The present study provides the first transcriptomic analysis of *C. nobilis*, which will help clarify the molecular mechanisms of gonadal maturation.

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YHG designed the experiments. SGF collected sample, performed the experiment and analysed the data. YHX reviewed the manuscript.

Key words
***Chlamys nobilis*, Gonad transcriptome, Gonad maturation, Sex determination, Illumina**

INTRODUCTION

Sex is one of the most fundamental features of life. Sex determination and reproductive regulation have been extensively studied in mammals, insects, birds, and fish. Both genetics and the environment can influence sex determination (Zhang *et al*., 2014; Morishita *et al*., 2010). Molluscs are good animal models for studying sex determination and reproductive regulation due to their diverse modes of reproduction, including dioecy, hermaphroditism (e.g., bay scallop), and sex reversal (e.g., Pacific oyster). Studies on mollusc reproduction can provide insights into the molecular mechanisms of sex determination and reproductive regulation. Previously isolated and characterized reproduction-related genes include soxE (Santerre *et al*., 2014), beta-catenin (Santerre *et al*., 2014), FoxL2 (Naimi *et al*., 2009), vitellogenin (Zheng *et al*., 2012b), Dmrt2 (Shi *et al*., 2014), Dmrt5 (Shi *et al*., 2014), and 5-hydroxytryptamine receptors (HTR) (Wang, 2014).

In recent years, next generation sequencing technology has been widely used in genome and transcriptome analyses with low cost, high efficiency, and superior accuracy, with a growing number of reproduction-related genes found from such studies (Matsumoto *et al*., 2013; Zhang *et al*., 2014, 2019). More than 40 gene models were identified with high accuracy to encode reproduction-related genes reported for *P. fucata* and other molluscs (Matsumoto *et al*., 2013). Sex-related genes and pathways have also been identified in the transcriptomes of *Crassostrea hongkongensis* and *Patinopecten yessoensis* (Tong *et al*., 2015; Li *et al*., 2016b). In the Pacific oyster, certain genes, including SoxH, Foxl2, and Dsx, are thought to be involved in sex determination (Zhang *et al*., 2014). In *P. yessoensis*, FoxL2, Dmrt, and soxH are also considered to be key candidates for scallop sex determination/differentiation (Li *et al*., 2016b). Compared with model species, however, studies on bivalve sex determination genes and pathways are still limited.

The noble scallop (*Chlamys nobilis*) is an economically important and edible marine bivalve, which has been cultured in the Southern Sea of China since the 1980s (Zheng *et al*., 2012a). Recent research on *C. nobilis*

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has focused on genes involved in immunity (Yang *et al*., 2016; Zhang *et al*., 2016a) and reproduction (Zheng *et al*., 2012b; Shi *et al*., 2014) and on transcriptome sequencing for identification of candidate genes associated with carotenoid-based coloration (Liu *et al*., 2015). In this study, we employed Illumina sequencing technology and *de novo* assembly to obtain the transcriptome of the maturing ovaries and testes of *C. nobilis*. Many genes associated with sex determination and maturation were determined, and various differentially expressed genes (DEGs) between the ovaries and testes were identified. In addition, GO and KEGG enrichment analyses of DEGs demonstrated significant differences between the ovaries and testes. This study will be useful to clarify the molecular mechanisms of gonadal maturation. in *C. nobilis*.

MATERIALS AND METHODS

Tissue collection

Eight healthy specimens of *C. nobilis* (N = 4 for each sex), averaging 51 mm in shell height, were purchased from an aquaculture farm in Shenzhen, Guangdong Province, China. The ovaries and testes were dissected and freezed in liquid nitrogen and stored at -80°C. A 5-mm thick section of gonad from each individual was cut and then fixed in 4% paraformaldehyde for 24 h.

Sample histology

Histological methodology followed that of Yan *et al*. (2010). After fixation in 4% paraformaldehyde, the gonad sections were dehydrated in graduated ethanol washes, and embedded in paraffin. Sections (6 µm thick) were prepared, and then stained with hematoxylin and eosin according to routine histological techniques. The sections were examined to assess the stage of gonadal development using a Nikon Eclipse CI microscope equipped with a Nikon DS-U3 image analyzing system (Nikon, Japan).

RNA extraction and cDNA library construction

Total RNA was isolated from each sample using Trizol Reagent (Invitrogen, Carlsbad, USA) following the manufacturer's protocols. Quantity and integrity of the RNA samples were comfirmed by 1% agarose gel and a 2100-Bioanalyzer (Agilent Technologies, USA).1µg of RNA from each ovary or testis were pooled, respectively.

Then cDNA was synthesized using the mRNA fragments as templates as usual and was sequenced by the oebiotech (Shanghai, China) using the Illumina HiSeq™ 2500 sequencing platform (Illumina, Inc, USA).

Sequence data analysis and assembly

Raw reads were processed by the NGS QC Toolkit (Patel *et al*., 2012). Low quality bases which the percentage of low quality bases (base quality≤20) and reads containing poly-N larger than 35bp were removed to obtain clean reads. *De novo* assembly of the clean reads was performed using the short reads assembling program Trinity (Grabherr *et al*., 2011). The TIGR Gene Indices Clustering (TGICL) tool was used to remove redundant sequences and perform further assembly (Pertea *et al*., 2003).

Sequence annotation

Unigenes were aligned with protein databases NR, Swiss-Prot, and KEGG using BLASTx alignment (e value < 10^{-5}). Gene names were assigned to each assembled sequence based on the best BLAST hit (highest score).

To annotate the assembled sequences with GO terms describing biological processes, molecular functions, and cellular components, the Nr BLAST results were imported into Blast2GO (Conesa *et al*., 2005; Götz *et al*., 2008). WEGO was used for GO functional classification of all unigenes (Ye *et al*., 2006). Additionally, pathway assignments were carried out based on the KEGG database.

Sequence mapping and differential expression analysis

Sequence reads were mapped to reference sequences using bowtie2 (Langmead and Salzberg, 2012) and eXpress. Expression levels for each unigene were measured with FPKM (Fragments Per Kb per Million Fragments) and input into the DEseq R package for differential expression analysis (Anders and Huber, 2013). Only genes with a false discovery rate (FDR) ≤ 0.05 were and $\log_2$ (fold change) values≥3 were selected as the thresholds, to identify differentially expressed genes (DEGs) between the testis (control) and ovary. GO and KEGG enrichment analyses of DEGs were performed using R based on hypergeometric distribution.

Quantitative RT-PCR validation

To validate the Illumina sequencing data, 11 DEGs between the ovary and testis were chosen for quantitative real-time PCR (qRT-PCR). All primers used were manufactured by Invitrogen (Shanghai, China) (Table I). The RNA samples used for qRT-PCR amplifications were the same as those used to construct the RNA-Seq library mentioned above. For each sample, quantification was performed using gDNA Eraser and the Prime-Script™ Reverse Transcriptase Reagent Kit with gDNA Eraser (TaKaRa, Japan). The β-actin of *C. nobilis* was used as an internal reference. The qRT-PCR was performed in triplicate for each sample using the CFX96 real-time PCR Detection System (Eppendorf, Germany) in a 25 µl reaction system, with the following components: 12.5 µl of 2 × SYBR Green Real-time PCR Master Mix (Takara, Japan),

Table I. Primers used in real-time PCR confirmation.

Genes	Primer sequences (5'-3')	Amplicon length (bp)	Source
Sox11	F:AGTTGTTGACGGAGACTGAGCG R:TTTACCCACTGGTTTGGCCTTT	218	This study
Sox2	F:ATGAAGGAGCATCCCGATTACA R:CAGTCGGCATTCCGGGTAA	81	This study
Dmrt-4	F:GAGAGTTCAGACGACTATCC R:GGTGTTGAGGATCGAGATT	94	This study
Vitellogenin	F:AAAGGCTGATATGAAAGACCGAC R:GCGTTGGTGGATTTTGTGAC	104	Zheng *et al*., 2012
Nanos-like protein 1	F:TCAAGGACACCCACGGAAAGG R:GAATGGACAGTGTCGGAGCGTA	107	This study
5-HT receptor	F:GTCACGGTAAATGGAGGCGAACA R:GGCAGCCAACAGGCGATAAA	76	This study
Sox9	F:TCCTCCACCAAGATGACAACAAACT R:CATGGGTACAAGGGACCAGTCG	227	This study
Sox2B	F:TATTTGGTGCCGTGTTGCC R:GGTGTCGGTAGTGATCTTCTGGT	137	This study
Vasa	F:AGTGGTGACCGTGGATGCTTT R:GGTGCTGGAGGGATGTATGGA	100	This study
FoxL2	F:GTTTGCCGTCGCCTCACTA R:CGCAATGTTGGCTGTTCTGG	125	This study
Estrogen receptor	F:CCCTGGACCCGTGGATTATGT R:TTTATTCATGCCGACTTCGTAGCA	81	This study
β-actin	F:CAAACAGCAGCCTCCTCGTCAT R:CTGGGCACCTGAACCTTTCGTT	94	Zheng *et al*., 2012

0.5 µl of each primer (10 µM), 1.0 µl of cDNA, and 10.5 µl of RNase-free water. The PCR program was as follows: 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 30 s. To assess the specificity of the PCR amplification, a melting curve was obtained at the end of the reaction, and a single peak was observed. Data were quantified using the $2^{-\Delta\Delta CT}$ method. The amplification efficiencies of the target and reference genes were verified and found to be approximately equal. All qRT-PCR data were analyzed using SPSS 19.0. Differences between means were considered significant at the 95% confidence level ($P < 0.05$). The results were expressed as means ± standard errors.

RESULTS

Histological analysis of gonads

Histology of the sampled *C. nobilis* gonads revealed that both the female ovaries and male testes were at the maturing stage. The ovaries were filled with oocytes and the testes were filled with sperm (Fig. 1).

Table II. Summary statistics of *C. nobilis* gonad transcriptome sequencing.

	Female	Male
Raw reads	46,404,820	46,527,700
Raw bases	5,800,602,500	5,815,962,500
Clean reads	46,404,820	46,527,700
Clean bases	5,800,127,180	5,815,485,557
Valid ratio (base)	99.99%	99.99%
Q30 (%)	94.86%	95.00%
GC content (%)	42.00%	43.00%

Sequence analysis and assembly

As shown in Table II, a total of 92,932,520 raw sequence reads were generated (46,404,820 and 46,527,700 reads from the ovary and testis, respectively). After stringent quality checking and data clean-up, the two sequence datasets consisted of clean reads only. The number of clean read is same as that of raw read, but with a different number of bases. The valid data rate was 99.99%, which proved that high-quality bases were obtain

Table III. Assembly statistics of *C. nobilis* gonad transcriptome.

	All	>=500 bp	>=1000 bp	N50	Total length	Max length	Min length	Average length
Unigene	73812	41944	21830	1806	79176755	50665	301	1072

Table IV. Top 10 genes with the greatest difference in expression in *C. nobilis* male and female gonads.

ID	Genes	M/F (FPKM)	P-value (ovary-testis)
Male up-regulated genes			
comp62793_c0_seq2	Sperm motility kinase X-like	13424.20	0.000877499
comp60994_c0_seq1	Testis-specific serine/threonine-protein kinase 1	9304.39	0.000289122
comp68313_c0_seq1	Kelch-like protein 10	7501.98	0.000164084
comp60179_c0_seq1	E3 ubiquitin-protein ligase MARCH3	5508	0.000702734
comp60764_c0_seq1	Potassium voltage-gated channel protein Shaw	3429.44	0.000026587
comp69860_c0_seq1	Hemicentin-1	2574.88	0.000973017
comp40556_c0_seq1	Sperm protein	1518.80	0.000973017
comp64884_c0_seq1	Fascin	1206.79	0.000641185
comp61013_c0_seq1	Low-density lipoprotein receptor-related protein 12	1074.58	0.009628182
comp54687_c0_seq1	cAMP and cAMP-inhibited cGMP 3	1071.41	0.000490393
Female up-regulated genes			
comp60048_c0_seq1	Sarcoplasmic calcium-binding proteins II	1157.044118	0.094002299
comp62792_c1_seq1	Deleted in malignant brain tumors 1 protein-like	807.1372549	0.093875806
comp74276_c0_seq3	Titin	320.6800826	0.329993152
comp60317_c0_seq2	Caveolin-1-like	236.754902	0.136813623
comp73049_c2_seq1	MAM and LDL-receptor class A domain-containing protein 1-like	206.6078431	0.063965965
comp54366_c0_seq1	Fatty acid synthase-like	180.1588235	0.234334085
comp53233_c0_seq1	GTP-binding protein Rhes-like	137.6715686	0.077940042
comp71473_c0_seq1	MAM and LDL-receptor class A domain-containing protein 1-like	124.9294118	0.084507302
comp71132_c0_seq1	Low-density lipoprotein receptor-related protein 2-like	123.143986	0.80498201
comp63224_c0_seq1	Alkyl/aryl-sulfatase BDS1-like	102.2399077	0.180469613

Genes above and below the line are up-regulated in males and females, respectively.

during sequencing. Detailed sequencing and assembly results are shown in Table III. All reads were deposited in the Short Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) with the accession number SRX3049257 and SRX3055267.

A total of 73,812 unigenes were assembled from the ovary and testis transcriptomes (Table III). The N50 length was 1,806 bp and the average length was 1,072 bp. The number of contigs above 500 bp was 41,944, accounting for 57%. In addition, 21,830 unigenes (30%) were greater than 1,000 bp. The assembly program produced a substantial number of high-quality long sequences.

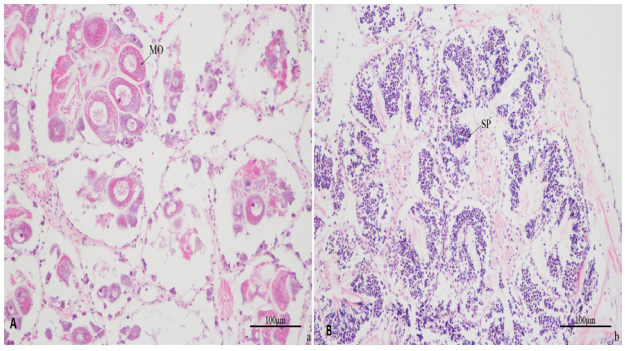

Fig. 1. Mature gonad stage in female (A) and male *C. nobilis* (B). MO, mature oocyte; SP, sperm.

Table V. Top 10 genes specifically expressed in *C. nobilis* male and female gonads.

ID	Genes	Male (FPKM)	P-value
Male			
comp47962_c0_seq1	Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit C-like	1396.36	4.82E-06
comp57762_c0_seq1	GTPase IMAP family member 7-like	872.17	4.91E-06
comp70989_c1_seq5	DC-STAMP domain-containing protein 1-like isoform X1	856.55	4.97E-06
comp56505_c0_seq4	Von Willebrand factor D and EGF domain-containing protein	590.00	7.95E-06
comp71598_c0_seq3	Transient receptor potential cation channel subfamily M member 8-like	234.21	0.000101621
comp63617_c0_seq1	Glutathione S-transferase omega	217.48	0.000132248
comp54517_c0_seq1	43 kDa receptor-associated protein of the synapse-like isoform X1	118.22	0.001344454
comp20654_c0_seq1	Radial spoke head protein 3 homolog B-like	110.42	0.001736294
comp50734_c0_seq1	Sodium- and chloride-dependent taurine transporter-like	107.07	0.001945348
comp46754_c0_seq2	Zinc finger protein 862-like	103.72	0.002185756
Female			
comp76189_c0_seq1	Calmodulin-like	440.24	2.43E-05
comp64460_c0_seq11	Gamma-butyrobetaine dioxygenase-like isoform X2	313.82	6.20E-05
comp63277_c0_seq1	B1 bradykinin receptor-like	305.75	6.72E-05
comp22346_c0_seq1	Vitellogenin	303.95	6.84E-05
comp61921_c0_seq1	DBH-like monooxygenase protein 1	232.22	0.000165541
comp55274_c0_seq3	Transposon Ty3-I Gag-Pol polyprotein	220.57	0.000197763
comp60101_c0_seq1	Serine/threonine-protein kinase mos-like	208.02	0.000242767
comp71662_c0_seq5	Solute carrier family 22 member 21-like	184.70	0.000371409
comp59103_c1_seq3	Sulfotransferase 1A2-like	167.67	0.000530388
comp51636_c1_seq1	Long-chain fatty acid CoA ligase 1	164.08	0.000574806

Genes above and below the line are specifically expressed in males and females, respectively.

Table VI. Real-time PCR confirmation of DEGs between the ovary and testis.

Sequence ID	Genes	Fold-change Ovary/Testis	Real-time PCR Ovary/Testis
comp31527_c0_seq1	sox11	3.30	4.54±1.27
comp44628_c0_seq2	sox2	12.32	8.23±2.58
comp5019_c0_seq1	dmrt-4	0.40	0.42±0.12
comp52532_c1_seq1	vitellogenin	2392.10	12126.21±5034.37
comp52668_c0_seq2	nanos-like protein 1	2.87	3.00±0.95
comp60205_c0_seq1	5-HT receptor	36.18	11.39±2.58
comp63638_c0_seq1	sox9	2.17	2.11±0.87
comp67199_c0_seq1	soxB2	4.96	8.35±4.92
comp67725_c0_seq1	vasa	2.25	3.53±1.48
comp73569_c0_seq3	foxL2	57.15	58.08±15.35
comp82439_c0_seq1	estrogen receptor	Inf	Inf

Functional annotation

For validation and annotation of assembled unigenes, BLASTx alignment between unigenes and protein databases, including Nr, Swiss-Prot and KEGG, was conducted, with an E value threshold of 10^{-5} (Supplementary Table S1). The best alignment results were used to annotate protein function and determine the sequence direction of the unigenes. The results indicated

that 23,535 unigenes (31.89%) were annotated to known proteins in the nr database. Furthermore, 59.95% of the unigenes were matched with *C. gigas*. In addition, 17,220 unigenes (23.33%) were similar to known proteins in the Swiss-Prot database.

We found that 15,714 annotated unigenes (66.77%) were assigned to one or more sub-categories of GO terms. Among the 160,580 GO terms, 76,442 (47.60%), 63,203 (39.36%), and 20,935 (13.04%) were involved in biological progresses, cellular components, and molecular functions, respectively. For biological processes, genes involved in cellular processes (GO: 0009987) and single-organism processes (GO:0044699) were highly represented. Regarding molecular functions, binding (GO: 0005488) was the most represented GO term, followed by catalytic activity (GO: 0003824). Cells (GO: 0005623) and cell parts (GO: 0044464) were the most represented categories for cellular components.

Unigene KEGG pathway analysis was carried out as an alternative approach for functional categorization and annotation. In total, 6,483 unigenes were divided into six major categories covering metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, and human diseases. Among these annotated unigenes, 4,521 were classified into metabolism, including carbohydrate metabolism (808 sequences) and amino acid metabolism (686 sequences); 1,910 were grouped into genetic information processing, including translation (783 sequences), folding, sorting, and degradation (640 sequences), transcription (236 sequences), and replication and repair (251 sequences); 3,491 were assigned to environmental information processing; 2,208 were assigned to cellular processes; and 5,823 were classified into organismal systems.

Differential expression analysis

Differential expression analysis revealed that 2,842 sex-biased unigenes (including up-regulated genes and specifically expressed unigenes) were significantly differentially expressed between the ovaries and testes (FDR ≤ 0.05, Supplementary Table S2). Among them, 591 unigenes exhibited biased expression in the ovary and 173 unigenes were expressed specifically in the ovary. A total of 2,251 testis-biased unigenes were identified from the transcriptome and 965 unigenes were exclusively expressed in the testis. Due to the lack of genomic information for *C. nobilis*, a large fraction of DEGs (63.69%) could not be annotated, although they might contain novel genes important for gonadal differentiation and development. However, some well-known reproduction-related DEGs were detected. For example, dmrt2 and TSSK1 were isolated in testis-biased unigenes, and HTR, Fox2L, nanos,

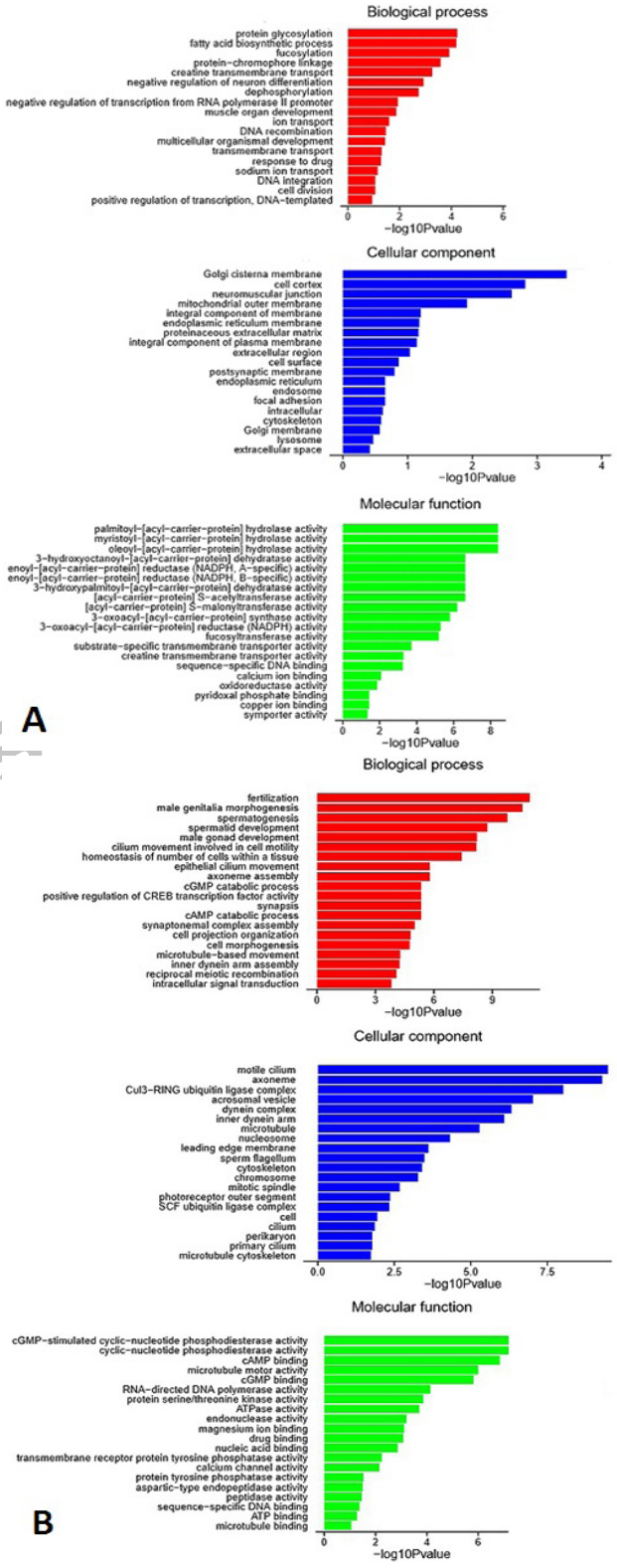

Fig. 2. Gene ontology (GO) annotation of ovary-biased genes (A) and testis-biased genes (B).

vitellogenin, and sox2 were detected in ovary-biased unigenes (Supplementary Table S2). Tables IV and V show the top 10 up-regulated and specifically expressed genes in the testis and ovary of *C. nobilis*, respectively.

We detected 600 DEGs in several GO terms. Among them, 369 were detected in 1,195 testis-biased terms and 231 were detected in 964 ovary-biased terms (Supplementary Tables S3, S4). For the ovary-biased genes, GO terms related to protein glycosylation, fatty acid biosynthetic processes, hydrolase activity, Golgi cisternae membrane, and cell cortex were significantly enriched (Fig. 2a, Supplementary Table S4). Most testis-biased GO enrichment terms were related to male organ formation and spermiogenesis, such as fertilization, male genitalia morphogenesis, spermiogenesis, motile cilium, axoneme, cGMP activity, cAMP binding, and ATPase activity (Fig. 2b, Supplementary Table S4).

KEGG pathways related to metabolism of fatty acid, glycan amino acid, hormones, and mineral absorption were enriched in the ovary-biased genes (Fig. 3a), whereas those related to meiosis, insulin resistance, glycosphingolipid biosynthesis, and adherens junction were enriched in the testis-biased genes (Fig. 3b).

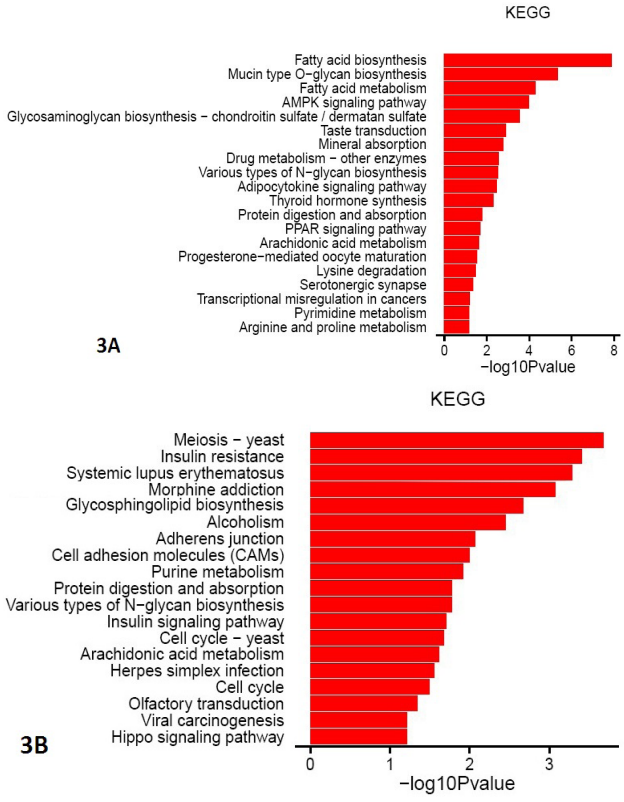

Fig. 3. KEGG annotation of ovary-biased genes (A) and testis-biased genes (B).

Quantitative RT-PCR validation

To validate the expression profiles obtained from Illumina sequencing analysis, 11 genes were chosen for quantitative RT-PCR. Of these, seven matched the Illumina sequencing results (Table VI). Although the other four genes did not perfectly match the sequencing data, they still showed the same differential expression tendencies, as revealed by RNA-Seq. In general, the qRT-PCR results confirmed that the Illumina data were credible.

DISCUSSION

In this study, the transcriptome of the *C. nobilis* testis and ovary in the maturing stage were sequenced by RNA-Seq technology. Based on the transcriptome data, genes involved in germline development, gonad development, oocyte maturation, and fertilization were identified. Vasa, nanos, and piwi play important roles in germline development. Vasa is a key determinant in germline formation and in gonad differentiation in eukaryotes, and can be used to track germ cell specification, migration, and differentiation (Gallardo *et al*., 2007; Wessel *et al*., 2014; Xu *et al*., 2014). Nanos plays an important role in primordial germ cell development (Tsuda *et al*., 2003), whereas piwi deficiency can result in complete depletion of germline stem cells and sterility in both males and females (Lin and Spradling, 1997). Vitellogenin (Vtg), vitellogenin receptor (Vtgr), and estrogen receptor are involved in gonad development. In most oviparous animals, Vtg is a large multidomain apolipoprotein that serves as a precursor of the major egg yolk protein vitellin (Matsumoto *et al*., 2013). In *Argopecten purpuratus*, vitellogenin mRNA expression is specifically expressed in the mature ovary (Boutet *et al*., 2008), which is in accordance with our results (Tables IV and VI). In addition, VgR is responsible for binding circulating Vg and transporting it into oocytes through endocytosis (Tufail and Takeda, 2009). Estrogen receptor regulates the transactivation of estrogen target genes, such as teleost Vtg (Matsumoto *et al*., 2013). HTR is involved in the induction of oocyte maturation (Wang and He, 2014). 17-beta-hydroxysteroid dehydrogenases (17-beta-HSDs) are important enzyme in sex steroid metabolism (Moeller and Adamski, 2009). Some 17-beta-HSDs including 17-beta-HSD6, 17-beta-HSD8, 17-beta-HSD10, 17-beta-HSD11, 17-beta-HSD12 and 17-beta-HSDs14 were found in this study. The substrate of these HSDs are androgens and estrogens. Zona pellucida sperm-binding protein is useful in fertilization (Aagaard *et al*., 2010).

In this study, 591 unigenes exhibited biased expression in the ovary, of which 173 were expressed specifically in the ovary. A total of 2,251 testis-biased

unigenes were identified from the transcriptome, with 965 unigenes exclusively expressed in the testis. Our results showing more testis-biased than ovary-biased unigenes agree with previous findings for *P. yessoensis* (Li *et al*., 2016b) and *P. olivaceus* (Zhang *et al*., 2016b), but differ from that of *C. gigas*, in which more genes were found in the ovary (Zhang *et al*., 2014).

Genes that exhibited the greatest differential or specific expression in the gonads are listed in Tables IV and V, respectively. Potassium voltage-gated channel protein was highly expressed in the testis, which is the first time this has been found in a mollusc. In mammals, potassium voltage-gated ion channel is present in the testis and spermatozoa (Jacob *et al*., 2000). K^+ channel activation is a primary event leading to acrosome reactions in mammalian spermatozoa (Benoff, 2009). TSSK1 is one member of Tssk family. The serine/threonine protein kinase catalytic (S-TKc) domain of TSSK1 is highly conserved in animals. TSSK1 is expressed when spermatids differentiate into morphologically mature spermatozoa during spermiogenesis, which highlights its important role in normal male reproduction (Xu *et al*., 2008). TSSK1 is only transcribed in the male gonads of *Atrina pectinata* and is highest in the mature testis (Li *et al*., 2016a), as found in our results (Table IV).

Nutrients and energy reserves are key factors in supporting oocyte development. Glycogens, lipids, and proteins play important roles in the reproductive processes of many bivalves. Glycogens are an important energy source for gonad development in clam (Li *et al*., 2011; Yan *et al*., 2010) and oyster (Zeng et al., 2010). Lipids and proteins are accumulated as vitellin, which is a major organic component of oocytes in female gonads. In male gonads, lipids and proteins are energy sources during spermatogenesis after glycogen breakdown (Li *et al*., 2011; Bi *et al*., 2016). These above results were further shown in the transcriptome level findings in our study. GO terms and KEGG pathways related to protein glycosylation, fatty acid biosynthetic processes, and hydrolase activity were enriched in the *C. nobilis* ovary (Figs. 2 and 3). Our results are agreement with previous transcriptome research in ovary. For example, in the *Haliotis rufescens* ovary, a high percentage of transcripts are associated with metabolism and catalytic and enzymatic activity (Valenzuela-Muñoz *et al*., 2014); In *P. yessoensis*, ovary-biased genes are enriched in the glycan-related pathway, which suggests active glycan biosynthesis and metabolism (Li *et al*., 2016b); Here, the AMPK signaling pathway was identified in the *C. nobilis* ovary, which was reported in a mollusc ovary for the first time. The AMPK pathway is involved in controlling cellular energy homeostasis and, at the whole animal level, in regulating energy balance and food intake (Proszkowiec-Weglarz *et al*., 2016). Furthermore, AMPK plays a vital role in ovary development through mediating glucose, insulin-like growth factor-1(IGF-1), follicle stimulating hormone (FSH), and adiponectin (Ratchford *et al*., 2007; Tosca *et al*., 2007; Kayampilly *et al*., 2009; Lu *et al*., 2008; Chen *et al*., 2006).

In the *C. nobilis* testis, GO terms were enriched in male organ formation and spermiogenesis. Some KEGG pathways were similar to those of other molluscs. For example, meiosis is significantly enriched in *C. gigas* and *P. yessoensis* testes (Zhang *et al*., 2014; Li *et al*., 2016b), and the insulin signaling pathway has been identified in *C. hongkongensis* (Tong *et al*., 2015). The glycosphingolipid biosynthesis pathway was first identified in a mollusc testis in this study. Glycosphingolipids are ubiquitous molecules composed of a lipid and carbohydrate moiety. Glycosphingolipids have been isolated and identified in other animal sperm, including that of humans and sea urchins (Ritter, 1987; Ijuin *et al*., 1996), and are critical for sperm activation in *C. elegans* (Dou *et al*., 2012). Further studies on DEGs associated with GO terms and pathways are needed to reveal the different molecular mechanisms that exist between ovarian and testicular maturation processes.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20190125080146

Statement of conflict of interest

The authors have declared no conflict of interest.

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