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ORIGINAL ARTICLE

Whole-Genome Re-sequencing and Transcriptome Reveal Oogenesis-Related Genes in Autotetraploid *Carassius auratus*

Chongqing Wang[1] · Huan Qin[1] · Chun Zhao[1] · Li Yang[1] · Tingting Yu[1] · Yuxin Zhang[1] · Xiang Luo[1] · Qinbo Qin[1] · Shaojun Liu[1]

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Abstract

Oogenesis involves a series of biochemical and physiological transformations and numerous regulated genes. The autotetraploid *Carassius auratus* (4nRR) originated from whole-genome duplication of *Carassius auratus* red var. (RCC), which produces diploid eggs through pairing of diploid-like chromosome during female meiosis. To explore the molecular mechanisms underlying oogenesis in 4nRR, we used the Illumina sequencing platform to characterize the ovaries of 4nRR and RCC. Transcriptome and whole-genome re-sequencing were performed to uncover the key genes and potential genetic mutations related to oogenesis. Each sample produced paired-end reads in the range of 66.97 to 98.36 million via Illumina HiSeq™ 2500. After comparing of the transcriptome profiles between the 4nRR and RCC, we uncovered 8562 differentially expressed genes (DEGs). The DEGs were enriched in oogenesis-related processes, including oogenesis, oocyte development, ubiquitin-mediated proteolysis, the signaling pathways of MAPK and calcium, and oocyte meiosis as investigated by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Additionally, whole-genome re-sequencing revealed 34,058,834 SNPs and 6,153,711 InDels, including 6,677,638 non-synonymous variations (SNPs) and 706,210 frame-shift InDels in the 8510 DEGs of 4nRR fish. Subsequently, whole-genome re-sequencing and transcriptomatic analyses revealed the genes that participate in oogenesis associated processes. Specifically, genes involved in ubiquitin-mediated proteolysis (SMURF1, UBE2I), calcium transport (CALM3, CAMK4), and meiosis (MAPK3, GRB2, CPEB1, CCNB2, YWHAE) were related to oogenesis in 4nRR. These findings enrich our understanding of oogenesis in the autopolyploid fish.

Keywords Oogenesis · Autotetraploid · Transcriptome · Whole-genome re-sequencing

Introduction

Polyploid, including allopolyploid and autopolyploid, is a heritable trait in which an organism possesses multiple sets of chromosomes (Peer et al. 2017). Polyploidization is widely used in agriculture to induce multiple beneficial phenotypic characteristics (He et al. 2011). For instance, artificial polyploidy has been utilized to induce new traits such as low seed setting, high disease resistance, fast growth, drought tolerance, altered flowering-time, and

✉ Qinbo Qin
30055776@qq.com

✉ Shaojun Liu
lsj@hunnu.edu.cn

Chongqing Wang
1436236021@qq.com

Huan Qin
1178373275@qq.com

Chun Zhao
3238431088@qq.com

Li Yang
1430404360@qq.com

Tingting Yu
768060178@qq.com

Yuxin Zhang
296984632@qq.com

Xiang Luo
2977286314@qq.com

[1] State Key Laboratory of Developmental Biology of Freshwater Fish, Engineering Research Center of Polyploid Fish Reproduction and Breeding of the State Education Ministry, College of Life Sciences, Hunan Normal University, Changsha 410081, Hunan, People's Republic of China

large organ size in both plants and animals (Osborn et al. 2003; Comai 2005; Yu et al. 2009; Weber et al. 2013; Pires et al. 2015). Among the polyploids, autotetraploid is particularly interesting. Generally, autotetraploids are believed to exhibit multivalent pairing during meiosis which disrupts normal chromosome segregations, generating less fertile progenies (Shahid et al. 2013). Interestingly, diploid like chromosome behavior have been observed in autotetraploids during meiosis. In contrast, however, autotetraploids undergo normal segregation of the chromosomes and subsequently normal gametes (Cifuentes et al. 2010; Le Comber et al. 2010). Given that female autotetraploid fish (4nRR) produce diploid eggs (Qin et al. 2014), it represents an ideal model for investigating the genetic basis of oogenesis in autotetraploid vertebrates. Recently, centromeric, 5S rDNA, Ag-NORs, and transcriptome methods have been used to uncover rapid genomic and genetic changes in 4nRR (Qin et al. 2019a, b). However, the molecular mechanism of gametogenesis, particularly oogenesis, is still poorly understood in the autotetraploid fish.

Oogenesis is a highly complex biological process essential to sexual reproduction. Oogenesis is characterized by mitotic cell division, two rounds of Meiosis (I and II), and one round of DNA synthesis to form an egg (Bowles and Koopman 2007). Oogenesis is even more complex at the molecular level, regulated through highly controlled processes of expression and interaction of numerous genes, proteins, and signaling pathways. For instance, The Figla, Lhx8, Nobox, Sohlh1, Sohlh2, Bax, Ahr, Gdf9, Pten, Scf, Bcl2, and Rps6 genes play important roles in follicular and oocyte development (Edson et al. 2009; Matzuk and Lamb 2008). On the other hand, Notch, Hippo, BMP, Wnt, and JAK pathways are involved in regulating oogenesis (Polesello and Tapon 2007; Xu and Gridley 2012; Antel and Inaba 2020). Recent advances in sequencing technology have facilitated the analysis of numerous molecular biological mechanisms in plants and animals. For instance, sequencing techniques have revealed gene expression variations and methylation associated with pollen fertility of autotetraploid rice (Li et al. 2018). Integration of the transcriptome and genome re-sequencing on its part identified genetic variations associated poor eggshell qualities (Zhang et al. 2015). Furthermore, RNA-seq-based transcriptome profiling of fish has successfully revealed the molecular mechanism underlying the development of fish ovum (Jia et al. 2018) and sex determination (Kamiya et al. 2012; Myosho et al. 2012; Yano et al. 2012), genes associated with gonadal development (Yu et al. 2018) and egg size (Wang et al. 2019). This underscores the potential value of high-throughput sequencing technologies in investigating the molecular determinants of oogenesis.

Herein, we performed whole-genome re-sequencing of 4nRR and RCC ova to unmask DNA sequence variations between the two organisms. In addition, whole-genome re-sequencing and mRNA-seq were performed simultaneously to determine the genes associated with oogenesis. Findings of this study deepen our understanding on the complex molecular events underlying oogenesis in 4nRR fish.

Materials and Methods

Ethical Approval

Protocol for this study was reviewed and approved by the Institute of Experimental Animals, Hunan Province, China. The fish were anesthetized using 100 mg/L MS-222 (Sigma-Aldrich, St Louis, MO, USA) before dissection.

Animals and Breeding

RCC and 4nRR were acquired from the State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, China. RCC (♀) and RCC (♂) were crossed during the reproductive season of April 2019. The fish were cultured in open pools (0.067 ha) with suitable pH (7.0–8.5), water temperature (22–24 °C), dissolved oxygen content (5.0–8.0 mg/L), and adequate forage.

Sample Collection and Preparation

Three 12-month-old females 4nRR and RCC fish were selected from either species and assessed for polyploidy using a cytometer (BD Biosciences, San Jose, CA, USA). Briefly, red blood cells were drawn from the caudal vein of each fish using heparinized syringes and re-suspended in staining solution (NIM-DAPI 731085) (NPE systems, Pembroke Pines, FL, USA) for 10 min. The total DNA content of each fish was then extracted and compared with that of RCC. Gonad tissues of the fish were harvested following anesthetization using 100 mg/L MS-222 (Sigma-Aldrich, St Louis, MO, USA) before surgically removed gonad tissue. Ovarian tissues were also harvested from both the 4nRR and RCC after euthanasia and stored in RNAlater (Qiagen) at – 80 °C.

mRNA Library Construction and Sequencing

Total ovarian RNA isolation was extracted using Trizol (Invitrogen) and thereafter treated with RNase Free DNase I (Dalian Takara Co. Limited, China). The concentration and quality of RNA were assessed using a NanoDrop-2000 spectrophotometer (Implen, Westlake Village, USA), whereas the RNA integrity assessed using agarose (1.0%) gel electrophoresis. cDNA was

synthesized from high-quality RNA of each sample before sequencing in high-throughput sequencing Illumina platform (Illumina HiSeq™ 2500). Paired-end libraries were constructed using a TruSeqTM RNA library prep kit (Illumina, San Diego, CA, USA) according to the manufacturer's protocol. Six cDNA libraries (3 RCC, 3 4nRR) were prepared via end-repair, 3′ end adenylation, and adapter ligation and enrichment.

Transcriptome Analysis

Adapter sequences and low-quality reads in the raw sequences were removed using the fastp v0.20.0 software (Chen et al. 2018). The quality of the pretreated data was assessed using the FastQC v0.11.9 software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The clean data was then aligned to the RCC reference sequence (DDBJ/EMBL/GenBank accession no. PRJNA289059) using the STAR software (Sahraeian et al. 2017). The transcription levels of genes were quantified based on the FPKM (Fragments Per Kilobase Million) using the Stringtie software (Pertea et al. 2016) and thereafter normalized using the Deseq2 R package (version 1.26.0). Only genes displaying a fold change ≥ 2 and a false discovery rate (FDR) < 0.05 were considered as differentially expressed genes (DEGs). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DEGs was implemented using the clusterProfiler R package (version 3.14.0).

Whole-Genome Re-Sequencing Analysis

Whole-genome re-sequencing data of 4nRR was downloaded from GenBank database with the accession number SRX2981275. The quality of the reads was further improved using the fastp v0.20.0 software (Chen et al. 2018) and assessed using the FastQC v0.11.9 software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). High-quality reads were aligned to the RCC reference genome sequence using the Burrows-Wheeler Aligner (BWA) software (Li and Durbin 2009). SNPs and InDels were identified using the freebayes software (Garrison and Marth 2012). SNPEFF software and the GTF file of RCC reference genome were applied in annotating SNPs and InDels (Cingolani et al. 2012).

Quantitative PCR Analysis

The expression of 9 DEGs (SMURF1, UBE2I, CALM3, CAMK4, MAPK3, GRB2, CPEB1, CCNB2, and YWHAE) based on RNA-seq analyses was validated using quantitative real-time PCR (qRT-PCR) with the QuantStudio®5 Real-Time PCR Systems (Applied Biosystems, USA). Sequences for the corresponding target genes were retrieved from NCBI. Primers (Table S6) specific to the target genes were designed using the Primer 5.0 software. cDNA was synthesized based on Transcriptor First Strand cDNA Synthesis Kit (Roche) using 1 µg of the isolated RNA. qRT-PCR was performed using the SYBR Green dye (Bio-Rad, Hercules, CA, USA). Each 10 µl of qRT-PCR reaction mixture contained 5 µl SYBR Green, 20 µM primers, and 1 µl cDNA (1:10 dilution). The qRT-PCR was performed under the following conditions: initial denaturing at 95 °C for 2 min, subsequent denaturation through 40 cycles at 95 °C for 15 s, and annealing at 60 °C for 30 s. Each experiment was performed in triplicate. The relative expression level of mRNA was assessed based on using the $2^{-\Delta\Delta Ct}$ equation, with *β-actin* was used as an internal control gene. Data were analyzed statistically using SPSS (v22.0) software (SPSS Inc., Chicago, IL, USA). Comparison between groups was performed with Student's *t* test. Statistical significance was set at $p < 0.05$.

Data Availability Statement

The mRNA-seq data for 4nRR and RCC were submitted to the NCBI SRA (SRR12533875, SRR12533876, SRR12533877, SRR12533878, SRR12533879, and SRR12533880).

Results

Identification of DEGs Between 4nRR and RCC

Illumina sequencing platform generated 68.83 G of sequence data. After trimming the raw data, 452.71 million clean reads were obtained, with the clean reads/raw reads rate ranging from 98.56 to 98.74% in six samples. About 76.33% of the reads were mapped in our material by aligning reads against RCC genome sequence (S1 Dataset). Transcriptome data indicated that there was a significant correlation between the three biological replicates in RCC and 4nRR (Fig. 1). Exactly 8562 DEGs were identified between 4nRR and RCC, in which 4815 genes were upregulated and 3747 genes were downregulated (Fig. 2; S2 Table), demonstrating that ovary genes were differentially expressed in two groups.

Functional Annotation of DEGs

To further comprehend the biological processes and molecular pathways, DEGs were subjected to GO term and KEGG pathway enrichment analysis using the clusterProfiler R package. Among the GO function categories, oogenesis (GO:

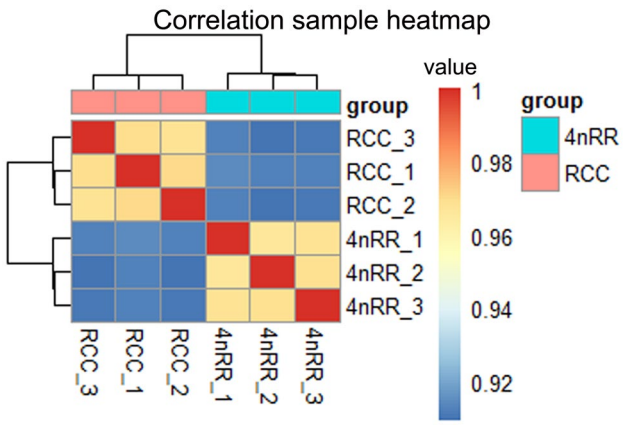

Fig. 1 Heatmap of the correlation analysis of samples in RCC and 4nRR. RCC_1, RCC_2, and RCC_3, samples from *Carassius auratus* red var. (RCC); 4nRR_1, 4nRR_2, and 4nRR_3, samples from the autotetraploid *Carassius auratus* (4nRR)

0,048,477) and oocyte development (GO: 0,048,599) were directly related to oogenesis (Table 1). The outcome of KEGG enrichment analysis indicates that DEGs were enriched in the ubiquitin-mediated proteolysis (KO: 04,120), the pathways of MAPK signaling (KO: 04,013) and calcium signaling (KO: 04,020), and oocyte meiosis (KO: 04,114) (Table 2). These molecular processes are thought to be closely associated with oogenesis in the ovary of female 4nRR.

Whole-Genome Re-sequencing of 4nRR

Whole-genome re-sequencing generated about 819 million raw reads for 4nRR fish with clean read percentage of 92.86%. In the end, 91.79% of high-quality reads were mapped on to the RCC reference genome (S3 Table). Mapping revealed 34,058,834 SNPs in 4nRR fish, corresponding to approximately 20.83 SNP/kb. Approximately 47.72% of SNPs were detected in exon region and 30.82% in intergenic regions. Meanwhile, 10.75% and 10.71% of SNPs were found to occur in upstream and downstream regions, respectively (S4 Table). In addition, we found 6,153,711 InDels in 4nRR fish, corresponding to approximately 3.77 InDels/kb. Overall, 44.21% of InDels were detected in exon region, whereas 33.58% of the genetic alterations were found in intergenic regions. The rest, 11.25% and 11.96%, were found to occur in upstream and downstream regions, respectively (S4 Table).

Whole-Genome Re-Sequencing and Transcriptome Analysis Uncovered Key Genes Associated with Oogenesis

A total of 8562 DEGs between 4nRR and RCC were identified by using RNA-seq analysis. Whole-genome re-sequencing analysis uncovered 34,058,834 SNPs and 6,153,711 InDels in the 4nRR fish. Through the combined analysis of RNA-seq and whole-genome re-sequencing, 6,677,638 instances of non-synonymous (SNPs) and 706,210 frame-shift mutations (InDels) were identified in 8510 DEGs (S5 Dataset) and contain ubiquitin-mediated proteolysis-related genes (SMURF1, UBE2I), calcium signaling pathway–related genes (CALM3, CAMK4), and meiosis-related genes (MAPK3, GRB2, CPEB1, CCNB2, YWHAE) (Table 3).

Validation of DEGs by qRT-PCR

Nine DEGs were selected for validating RNA-seq data using qRT-PCR. The $\log_2$ fold-change from quantitative real-time PCR (qRT-PCR) results was compared with the RNA-seq results (Table 4). The mRNA expressions profiling based on qRT-PCR was comparable to that of RNA-seq data analysis ($p < 0.05$) (Fig. 3), indicating that RNA-seq data were reliable.

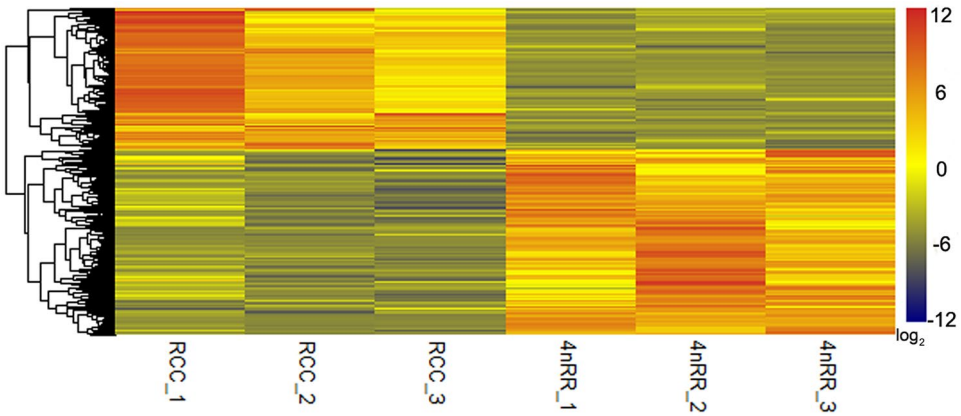

Fig. 2 Detailed hierarchical clustering of 8,562 DEGs in 4nRR ovaries. The colors and numbers ($\log_2$ fold change) indicate changes in expression levels. RCC_1, RCC_2. RCC_3, ovary samples from *Carassius auratus* red var. (RCC); 4nRR_1, 4nRR_2, 4nRR_3, ovary samples from autotetraploid *Carassius auratus* (4nRR)

Table 1 Enriched Gene Ontology (GO) terms associated with oogenesis in autotetraploid differentially expressed genes

Gene symbol	Log_2 (RCC/4nRR radio)	Gene symbol	Log_2 (RCC/4nRR radio)	Gene symbol	Log_2 (RCC/4nRR radio)
Oogenesis (GO: 0048477)					
UAP1L1	− 3.63	BMP2	1.81	CT	− 4.76
YWHAE	1.90	MSX2	− 3.57	PTBP1	1.46
BMP15	1.30	CYCB	1.49	SMAD3	− 2.26
AKAP1	1.16	CTNNB1	1.45	MED19	1.07
RBFOX3	1.40	IRX4	− 6.63	SMAD4	− 1.25
FSCN2	1.58	MSN	− 1.21	DLG1	− 1.65
PPP2CA	2.36	CYP2J2	1.41	CYP2E1	− 4.39
PANK1	− 2.75	PI4KA	− 2.34	GLI2	1.11
ELMO1	1.64	RPS6KA2	− 1.93	CASP3	− 2.19
STK24	− 3.01	RPS6KB1	− 1.18	PRKACB	− 2.16
TUSC3	− 1.11	MEF2C	− 1.61	RGS14	− 1.82
HORMAD2	1.14	DLL1	1.88	ADCY6	− 1.07
MEF2C	− 1.72	RXRG	1.21	PAQR7	− 1.32
CAPN1	5.71	ITPR1	− 1.27	FOXO6	− 1.56
RBM25	− 5.48	HIF1A	− 2.31	MACF1	− 2.91
CYP303A1	− 1.17	PAQR7	4.69		
BMP2	1.81	YWHAE	1.90	PTBP1	1.46
Oocyte development (GO: 0048599)					
BMP15	1.30	CYCB	1.49	SMAD3	− 2.26
CTNNB1	1.45	PI4KA	− 2.34	RPS6KA2	− 1.93
STK24	− 3.01	PRKACB	− 2.16	DLL1	1.88
ADCY6	− 1.07	PAQR7	4.69	ITPR1	− 1.27
FOXO6	− 1.56	MACF1	− 2.91		

Discussion

The major drawback of polyploidy is reduced fertility in species that reproduce sexually. Oogenesis is a critical sexual reproduction process that impacts on fertility. Oogenesis is regulated through numerous molecular processes, which underlines the need to dissect these processes in polyploid organisms. Herein, transcriptome profiling revealed significance difference in gene expression between 4nRR and RCC. Moreover, whole-genome re-sequencing revealed numerous gene mutations in the 4nRR genome.

Genomic Structural Reprogramming Influences Fertility in 4nRR

Besides inducing gene mutation, polyploidization often alters gene expression at the transcription level (Li et al. 2018). In the present study, transcriptomatic analysis revealed more than 8500 DEGs between 4nRR and RCC, as well as numerous SNPs and InDels in the 4nRR genome. GO analysis and KEGG prediction revealed the DEGs participated in several different pathways such as oogenesis, oocyte development, and oocyte meiosis, all essential in oogenesis. These findings demonstrate the presence of genetic

Table 2 Enriched pathways associated with oogenesis in autotetraploid differentially expressed genes (DEGs). KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase

KEGG pathway	KEGG ID	No. input DEGs	No. background DEGs	*p* value
Ubiquitin-mediated proteolysis	ko04120	30	243	0.99
MAPK signaling pathway	ko04013	36	154	0.36
Calcium signaling pathway	ko04020	112	391	0.034
Oocyte meiosis	ko04114	41	213	0.85

Table 3 Important mutant DEGs in autotetraploid fish

ID	Gene name	Non-synonymous SNPs	Frame-shift InDels
Caur.EVM.23910-RA	SMURF1	820	191
Caur.EVM.12624-RA	UBE2I	28	6
Caur.EVM.14685-RA	CALM3	188	34
Caur.EVM.09201-RA	CAMK4	677	155
Caur.EVM.08031-RA	MAPK3	312	84
Caur.EVM.32217-RA	GRB2	408	72
Caur.EVM.20450-RA	CPEB1	516	124
Caur.EVM.29117-RA	CCNB2	49	4
Caur.EVM.33167-RA	YWHAE	85	10

alterations in 4nRR, a known polyploid, which may influence transcription of proteins and even the phenotype. Meanwhile, SNPs and InDels are genetic alteration that increase genomic polymorphisms and may result in diploid-like chromosome pairing during meiosis. This phenomenon further reduces the rates of homologous recombination. Multivalent frequency inhibits formation of gametes in new autopolyploid, and high rates of aneuploidy correlate with high multivalence at metaphase I during meiosis (Andreuzza and Siddiqi 2008; Lloyd and Bomblies 2016). Therefore, the normal generation of gametes in polyploids may be linked low multivalency. In 4nRR, the bivalent frequency in meiosis may be facilitated by genomic structural reprogramming, generating diploid eggs.

Mutations in Oogenesis-Related DEGs Influence Fertility in 4nRR

Table 4 highlights transcriptome and whole-genome re-sequence analyses of the DEGs associated with oogenesis in 4nRR. DEGs regulating ubiquitin-mediated proteolysis may participate in oogenesis in 4nRR. The ubiquitin proteasome pathway, most common in eukaryotic cells, regulates non-lysosomal protein degradation (Gao et al. 2014). The ubiquitin proteasome pathway also participates in the regulation of numerous cellular activities including cell growth, apoptosis, and differentiation. Meanwhile, oogenesis is dependent on the ubiquitin-proteasome system (Hinnant et al. 2017), which plays an essential role in cell growth by regulating the mitotic cycle through timely degradation of cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors (Karabinova et al. 2011). Therefore, ubiquitin and ubiquitin-like proteins are generally highly expressed in ovary tissues. In this study, we found mutations and in SMURF1, the gene that encodes smad ubiquitination regulatory factor 1. In addition, compared with RCC, the expression of SMURF1 was substantially up-regulated in 4nRR fish. The same was observed for ubiquitin conjugating enzyme E2 I gene (UBE2I) in 4nRR. In general, mutation and corresponding over-expression of SMURF1 and UBE2I in the ovary of 4nRR fish promotes hydrolysis of peripheral peptides, which in turn regulates oogenesis in the fish (Fraune et al. 2010).

DEGs associated with calcium transport could be necessary for oogenesis in 4nRR. Being an important trace element in organisms, calcium ions regulate several metabolic processes (Clapham 2007). Ca^{2+}-mediated transport process is driven by numerous complex calcium ion transport systems located at the cell organelles and cell membranes of cells (Kim et al. 2007). Researches show that calcium also is an integral component of the signal transduction pathway that activates and regulates oogenesis. Calcium signaling pathway is a critical regulatory component of meiosis during oogenesis (Lefèvre et al. 1997). In the present study, calmodulin 3 encoding gene (CALM3) and calcium/calmodulin-dependent protein kinase 4 encoding gene (CAMK4) in 4nRR were mutated and displayed disparate expression from those in RCC after polyploidization. In summary, CALM3 and CAMK4 may regulate calcium ion concentration. Given the role in regulating meiosis, CALM3 and CAMK4 may regulate oogenesis in 4nRR through Ca^{2+} associated pathways.

DEGs associated with meiosis might be important for oogenesis in 4nRR. Meiosis is a unique type of cell division process that generates gametes (sperm and egg) for sexual reproduction. Biologically, meiosis is a complex process that is regulated by numerous genes. Our analyses uncovered 5 meiosis-associated genes (MAPK3, GRB2, CPEB1, CCNB2, YWHAE) related to oogenesis. Compared with RCC, these genes were mutated and showed different expressions in 4nRR, which might explain the production of diploid eggs in 4nRR. MAPK3, an abundant protein in vertebrate oocytes, activates various transcription factors and as well mediates phosphorylation of specific proteins, suggesting it may be involved in inducing meiosis (Prochazka and Blaha 2015). On the other hand, GRB2 is a cytosolic protein that participates in organogenesis, growth,

Table 4 qRT-PCR validation of the selected genes. The data shows $\log_2$ fold-change of the relative mRNA expression level in the ovaries of RCC/4nRR from RNA-seq and qRT-PCR

	SMURF1	UBE2I	CALM3	CAMK4	MAPK3	GRB2	CPEB1	CCNB2	YWHAE
RNA-seq	− 1.13	− 1.79	− 1.31	1.33	− 3.11	− 1.79	4.74	1.65	1.90
qRT-PCR	− 2.49	− 1.00	− 1.94	1.57	− 2.35	− 1.70	4.85	2.01	7.37

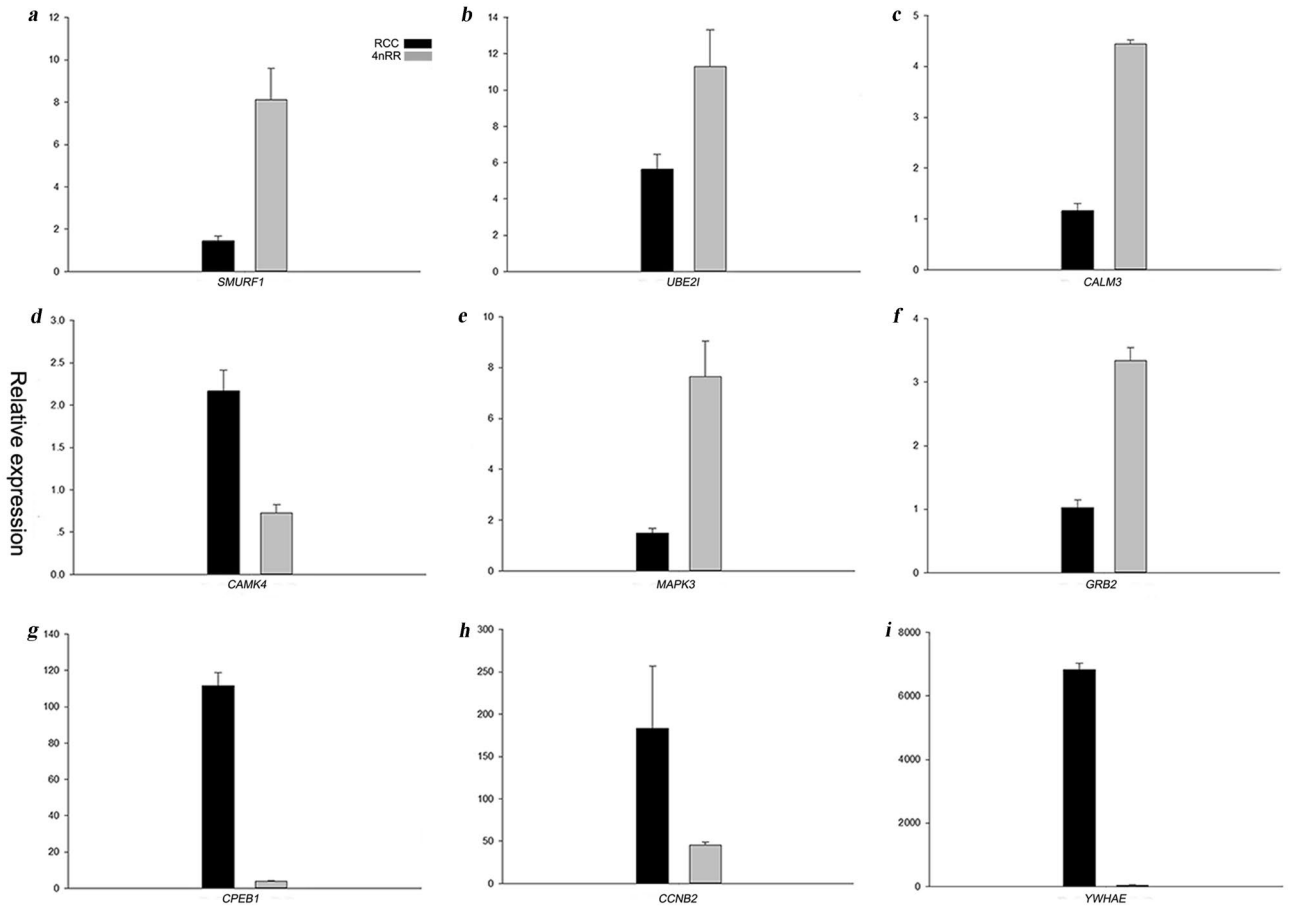

Fig. 3 qRT-PCR analysis of 9 differentially expressed genes in ovaries of the *Carassius auratus* red var. (RCC) and autotetraploid *Carassius auratus* (4nRR). **a** smad ubiquitination regulatory factor 1 (SMURF1); **b** ubiquitin conjugating enzyme E2 I (UBE2I); **c** calmodulin 3 (CALM3); **d** calcium/calmodulin dependent protein kinase IV (CAMK4); **e** mitogen-activated protein kinase 3 (MAPK3); **f** growth factor receptor bound protein 2 (GRB2); **g** cytoplasmic polyadenylation element binding protein 1 (CPEB1); **h** cyclin B2 (CCNB2); **i** tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon (YWHAE). Comparative analysis reveals significant differences in gene expression ($p<0.05$) ($n=3$ for each group)

and development as well as induction of meiosis in oocytes (Cailliau et al. 2001). CPEB1 on its part is associated with Cyclin B syntheses and induction of meiosis (Nishimura et al. 2010). CCNB2 mediates phosphorylation independent regulation of chromosome separation in mammalian oocytes (Daldello et al. 2019). YWHAE, a family of highly conserved acidic polypeptides, is also thought to induce meiosis (Meng et al. 2013). Therefore, it was possible that variation in meiosis-related genes is associated with oogenesis in 4nRR fish.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10126-021-10018-7.

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Data Availability All data generated or analyzed during this study are included in this paper [and its supplementary information files] and Figshare (10.6084/m9.figshare.13489575).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

Ethics Approval The fish treatments were reviewed and approved by the Institute of Experimental Animals, Hunan Province, China. And all fish were deeply anesthetized using 100 mg/L MS-222 (Sigma-Aldrich, St Louis, MO, USA) before dissection.

References

Andreuzza S, Siddiqi I (2008) Spindle positioning, meiotic nonreduction, and polyploidy in plants. PLoS Genet 4:e1000272

Antel M, Inaba M (2020) Modulation of cell-cell interactions in Drosophila oocyte development. Cells 9:274

Bowles J, Koopman P (2007) Retinoic acid, meiosis and germ cell fate in mammals. Development 134:3401–3411

Cailliau K, Browaeys-Poly E, Broutin-L'Hermite I, Nioche P, Garbay C, Ducruix A, Vilain JP (2001) Grb2 promotes reinitiation of meiosis in Xenopus oocytes. Cell Signal 13:51–55

Clapham DE (2007) Calcium signaling. Cell 131:1047–1058

Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890

Cifuentes M, Grandont L, Moore G, Chèvre AM, Jenczewski E (2010) Genetic regulation of meiosis in polyploid species: new insights into an old question. New Phytol 186:29–36

Cingolani P, Platts A, Wang Coon Nguyen Wang Land Lu Ruden IMTTSJXYDM (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. Fly 6:80–92

Comai L (2005) The advantages and disadvantages of being polyploid. Nat Rev Genet 6:836–846

Daldello EM, Luong XG, Yang CR, Kuhn J, Conti M (2019) Cyclin B2 is required for progression through meiosis in mouse oocytes. Development 146:dev172734

Edson MA, Nagaraja AK, Matzuk MM (2009) The mammalian ovary from genesis to revelation. Endocr Rev 30:624–712

Fraune S, Augustin R, Anton-Erxleben F, Wittlieb J, Gelhaus C, Klimovich VB, Samoilovich MP, Bosch TC (2010) In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. Proc Natl Acad Sci USA 107:18067–18072

Gao C, Aqie K, Zhu J, Chen G, Xu L, Jiang L, Xu Y (2014) MG132 ameliorates kidney lesions by inhibiting the degradation of Smad7 in streptozotocin-induced diabetic nephropathy. J Diabetes Res 2014:918396

Garrison E, Marth G (2012) Haplotype-based variant detection from short-Read sequencing. Quant Biol

He J, Shahid MQ, Chen Z, Chen X, Liu X, Lu Y (2011) Abnormal pmc microtubule distribution pattern and chromosome behavior resulted in low pollen fertility of an intersubspecific autotetraploid rice hybrid. Plant Syst Evol 291:257–265

Hinnant TD, Alvarez AA, Ables ET (2017) Temporal remodeling of the cell cycle accompanies differentiation in the *Drosophila germline*. Dev Biol 429:118–131

Jia Y, Nan P, Zhang W, Wang F, Zhang R, Liang T, Ji X, Du Q, Chang Z (2018) Transcriptome analysis of three critical periods of ovarian development in Yellow River carp (*Cyprinus carpio*). Theriogenology 105:15–26

Kamiya T, Kai W, Tasumi S, Oka A, Matsunaga T, Mizuno N, Fujita M, Suetake H, Suzuki S, Hosoya S, Tohari S, Brenner S, Miyadai T, Venkatesh B, Suzuki Y, Kikuchi K (2012) A trans-species missense SNP in Amhr2 is associated with sex determination in the tiger pufferfish, *Takifugu rubripes* (fugu). PLoS Genet 8:e1002798

Karabinova P, Kubelka M, Susor A (2011) Proteasomal degradation of ubiquitinated proteins in oocyte meiosis and fertilization in mammals. Cell Tissue Res 346:1–9

Kim JS, Park SJ, Kwak KJ, Kim YO, Kim JY, Song J, Jang B, Jung CH, Kang H (2007) Cold shock domain proteins and glycine-rich RNA-binding proteins from *Arabidopsis thaliana* can promote the cold adaptation process in Escherichia coli. Nucleic Acids Res 35:506–516

Le Comber SC, Ainouche ML, Kovarik A, Leitch AR (2010) Making a functional diploid: from polysomic to disomic inheritance. New Phytol 186:113–122

Lefèvre B, Nagyova E, Pesty A, Testart J (1997) Acquisition of meiotic competence is related to the functionality of the phosphoinositide/calcium signaling pathway in the mouse oocyte. Exp Cell Res 236:193–200

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760

Li X, Yu H, Jiao Y, Shahid MQ, Wu J, Liu X (2018) Genome-wide analysis of DNA polymorphisms, the methylome and transcriptome revealed that multiple factors are associated with low pollen fertility in autotetraploid rice. PLoS One 13:e0201854

Lloyd A, Bomblies K (2016) Meiosis in autopolyploid and allopolyploid *Arabidopsis*. Curr Opin Plant Biol 30:116–122

Matzuk MM, Lamb DJ (2008) The biology of infertility: research advances and clinical challenges. Nat Med 14:1197–1213

Meng J, Cui C, Liu Y, Jin M, Wu D, Liu C, Wang E, Yu B (2013) The role of 14-3-3ε interaction with phosphorylated Cdc25B at its Ser321 in the release of the mouse oocyte from prophase I arrest. PLoS One 8:e53633

Myosho T, Otake H, Masuyama H, Matsuda M, Kuroki Y, Fujiyama A, Naruse K, Hamaguchi S, Sakaizumi M (2012) Tracing the emergence of a novel sex-determining gene in medaka. Oryzias luzonensis Genetics 191:163–170

Nishimura Y, Kano K, Naito K (2010) Porcine CPEB1 is involved in Cyclin B translation and meiotic resumption in porcine oocytes. Anim Sci J 81:444–452

Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, Lee HS, Comai L, Madlung A, Doerge RW, Colot V, Martienssen RA (2003) Understanding mechanisms of novel gene expression in polyploids. Trends Genet 19:141–147

Peer YVD, Mizrachi E, Marchal K (2017) The evolutionary significance of polyploidy. Nat Rev Genet 18:411–424

Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT. StringTie and Ballgown Nat Protoc 11:1650–1667

Pires JC, Zhao J, Schranz ME, Leon EJ, Osborn TC (2015) Flowering time divergence and genomic rearrangements in resynthesized brassica polyploids (*Brassicaceae*). Biol J Lin Soc 82:675–688

Polesello C, Tapon N (2007) Salvador-warts-hippo signaling promotes Drosophila posterior follicle cell maturation downstream of notch. Curr Biol 17:1864–1870

Prochazka R, Blaha M (2015) Regulation of mitogen-activated protein kinase 3/1 activity during meiosis resumption in mammals. J Reprod Dev 61:495–502

Qin Q, Cao L, Wang Y, Ren L, Liu Q, Zhou Y, Wang C, Qin H, Zhao C, Liu S (2019) Rapid genomic and genetic changes in the first generation of autotetraploid lineages derived from distant hybridization of Carassius auratus red var. (♀) × Megalobrama amblycephala (♂). Mar Biotechnol 21:139–149

Qin Q, Liu Q, Wang C, Cao L, Zhou Y, Qin H, Zhao C, Liu S (2019b) Molecular organization and chromosomal localization analysis of 5S rDNA clusters in autotetraploids derived from *Carassius auratus* red var. (♀)×*Megalobrama amblycephala* (♂). Frontiers in Genetics 10:437

Qin Q, Wang Y, Wang J, Dai J, Xiao J, Hu F, Luo K, Tao M, Zhao C, Liu Y, Liu S (2014) The qutotetraploid fish derived from hybridization of *Carassius auratus* red var. (Female)×*Megalobrama amblycephala* (Male). Biol Reprod 91:93, 1–11

Sahraeian S, Mohiyuddin M, Sebra R, Tilgner H, Afshar PT, Au KF, Bani Asadi N, Gerstein MB, Wong WH, Snyder MP, Schadt E, Lam HYK

(2017) Gaining comprehensive biological insight into the transcriptome by performing a broad-spectrum RNA-seq analysis. Nat Commun 8:59

Shahid MQ, Li Y, Saleem MF, Naeem M, Wei C, Liu X (2013) Yield and yield components in autotetraploid and diploid rice genotypes (indica and japonica) sown in early and late seasons. Aust J Crop Sci 7:632–641

Wang Y, Zhang M, Qin Q, Peng Y, Huang X, Wang C, Cao L, Li W, Tao M, Zhang C, Liu S (2019) Transcriptome profile analysis on ovarian tissues of autotetraploid fish and diploid eed crucian carp. Front Genet 10:208

Weber GM, Wiens GD, Welch TJ, Hostuttler MA, Leeds TD (2013) Comparison of disease resistance between diploid, induced-triploid, and intercross-triploid rainbow trout including trout selected for resistance to flavobacterium psychrophilum. Aquaculture 410–411:66–71

Xu J, Gridley T (2012) Notch signaling during oogenesis in *Drosophila melanogaster*. Genet Res Int 2012:648207

Yano A, Guyomard R, Nicol B, Jouanno E, Quillet E, Klopp C, Cabau C, Bouchez O, Fostier A, Guiguen Y (2012) An immune-related gene evolved into the master sex-determining gene in rainbow trout Oncorhynchus mykiss. Curr Biol 22:1423–1428

Yu L, Xu D, Ye H, Yue H, Ooka S, Kondo H, Yazawa R, Takeuchi Y (2018) Gonadal transcriptome analysis of Pacific abalone Haliotis discus discus: identification of genes involved in germ cell development. Mar Biotechnol 20:467–480

Yu Z, Haage K, Streit VE, Gierl A, Ruiz RA (2009) A large number of tetraploid *arabidopsis thaliana* lines, generated by a rapid strategy, reveal high stability of neo-tetraploids during consecutive generations. Theor Appl Genet 118:1107–1119

Zhang Q, Zhu F, Liu L, Zheng C, Wang D, Hou Z, Ning Z (2015) Integrating transcriptome and genome re-sequencing data to identify key genes and mutations affecting chicken eggshell qualities. PLoS One 10:e0125890