Marine Biotechnology (2018) 20:666–675
https://doi.org/10.1007/s10126-018-9837-1

ORIGINAL ARTICLE

Mapping Genetic Loci for Quantitative Traits of Golden Shell Color, Mineral Element Contents, and Growth-Related Traits in Pacific Oyster (*Crassostrea gigas*)

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Received: 25 January 2018 / Accepted: 5 June 2018 / Published online: 22 June 2018

Abstract

Golden shell color and mineral content are important economic traits of Pacific oyster (*Crassostrea gigas*). In this study, we mapped a series of quantitative trait loci (QTLs) that control zinc (Zn) and magnesium (Mg) content, shell color and growth performance to two sex-averaged linkage maps from the FAM-A and FAM-B families. In total, ten QTLs were identified in seven linkage groups (LGs) in the FAM-B family, and seven QTLs were identified in four linkage groups in the FAM-A family. Two QTLs affecting the trait of golden shell color were identified in LG8 of the FAM-A and LG10 of the FAM-B families, which could explain 20.2 and 10.5% of the phenotypic variations, respectively. Two QTLs for Zn content were identified that could contribute to 17.9 and 34.44% of the phenotypic variations in FAM-A. Six QTLs for Zn and Mg contents were identified in four LGs (LG1, LG2, LG5, and LG9) in FAM-B, which explained 13.5–26.7% of the phenotypic variations. In addition, seven QTLs related to oyster growth were recognized in both FAM-A and FAM-B families accounting for 14.6–36.7% of the phenotypic variations. All of the DNA markers in QTL regions were blasted and 14 genes associated with above traits were identified. The mRNA expression of these genes was determined by quantitative RT-PCR. These QTLs and candidate genes could be used as potential targets for marker-assisted selection in *C. gigas* breeding.

Keywords Quantitative trait loci · *Crassostrea gigas* · Shell color · Zinc and magnesium element · Marker-assisted selection

Introduction

As an economically important aquatic species, Pacific oyster (*Crassostrea gigas*) is widely distributed throughout the coastal areas of Japan, China, and Korea. It has the highest production in farmed oyster of the world. In 2015, its global production reached 583,464 tons (FAO 2017). As a result of its importance, the success of *C. gigas* aquaculture is partially ascribed to selective breeding for larger size, rapid growth rate, appealing color of shell and mantle, and higher survival rate (Dégremont et al. 2007; Li et al. 2011a; Wang et al. 2012). With the increased application of molecular biotechnology, the marker-assisted selection (MAS) is more effective in accelerating the selective breeding program of *C. gigas*.

Quantitative trait locus (QTL) mapping based on the genotypic data provides an effective approach to identify potentially useful markers, and is essential for the MAS in genetic breeding (Massault et al. 2008). Particularly, constructing maps based on the expressed sequence tags (EST)-derived simple sequence repeats (SSRs) are of greater value in comparative mapping for evolutionarily distant organisms and locating QTL in linkage maps (Serapion et al. 2004; Kessuwan et al. 2016). QTL mapping studies using SSR have been successfully applied to bivalve mollusk in order to identify the genomic regions associated with various QTLs (Li et al. 2012;

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10126-018-9837-1) contains supplementary material, which is available to authorized users.

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Bai et al. 2015; Lu et al. 2013). To date, approximately 480 SSRs have been developed in *C. gigas* (Huvet et al. 2000; Sekino et al. 2003; Yu and Li 2007, 2008; Li et al. 2003; Qiu et al. 2008a, b; Wang et al. 2008; Yamtich et al. 2005; Qi et al. 2009; Yu et al. 2009; Li et al. 2009a, b, 2011b; Bai et al. 2011). However, these SSRs are not sufficient for SSR-based fine mapping studies. Moreover, several studies mainly based on single nucleotide polymorphisms (SNPs) and amplified fragment length polymorphism (AFLP) have mapped loci of *C. gigas* related to the growth traits (Guo et al. 2012), inbreeding depression (Plough and Hedgecock 2011), disease depression (Sauvage et al. 2010), and glycogen content and shell pigmentation (Zhong et al. 2014). Therefore, QTL for some other important traits based on SSR can be identified, and the loci could be implemented to benefit producers by MAS.

Shell color is an important economic trait of oysters in the market. Appealing colors have a strong influence on the oyster price by attracting consumer preference. As an important phenotypic trait of bivalve species, shell color is highly variable, thus providing substantial numbers of variants for selection. In the Pacific oyster, true-breeding lines with different shell colors have been successfully developed over several generations (Ge et al. 2014). It has been shown that shell pigmentation is a quantitative trait (Brake et al. 2004; Evans et al. 2009). Although several studies have reported that the shell color is highly regulated by the genetic program (Hedgecock et al. 2006; Evans et al. 2009; Wan et al. 2017), the molecular mechanism of shell pigmentation in *C. gigas* remains unknown.

Pacific oyster is widely known for its rich contents of minerals such as Zinc (Zn) and magnesium (Mg). Zinc and magnesium are important minerals that play critical roles in maintaining normal metabolic and physiological conditions (Dato-Cajegas and Yakupitiyage 1996). Mineral deficiencies lead to broad spectrum of diseases in humans, and are considered to be a serious global challenge for human health (Yu et al. 2015). Thus, there is a strong interest in recognizing Zn and Mg as two economical traits in oyster aquaculture. However, the genetic basis determing the Mg and Zn contents remains obscure in *C. gigas*, and no studies have been conducted to identify the QTL for Zn and Mg contents in *C. gigas*.

In this study, we developed 480 new polymorphic SSRs that significantly enriched the resource of SSR markers for mapping QTLs of *C. gigas*. QTL regions related to golden shell color and mineral contents were located on two linkage maps of the FAM-A and FAM-B *C. gigas* families generated in our laboratory. Further, candidate genes for determing these economic traits were predicted. Collectively, data from these studies could facilitate the genetic breeding of *C. gigas* for desired economic traits.

Materials and Approaches

Mapping Population

Two F1 full-sib mapping families were developed with golden and white shell color variants in *C. gigas* (Fig. 1). The golden and white shell color variants have been successively selected for three generations for pure and stable shell color and fast growth. In May 2012, the FAM-A (white ♀× golden ♂) and FAM-B (golden ♀× white ♂) families were established by respective fertilization using eggs and sperms from the white and golden variants. Spat at 40 days of age were transported to a growing-out field in Weihai, Shandong province. In July 2013, 108 progeny from FAM-A and 96 progeny from FAM-B were sampled for analysis, respectively.

Trait Measurement

The growth-related traits including the total weight (TW), shell weight (SW), shell height (SH), and shell width (Swi) were evaluated and recorded in all offspring of both FAM-A and FAM-B families. The normality test was estimated by the Shapiro–Wilk test of the SPSS 16.0. The color change ΔE was calculated as described by Yam and Papadakis (2004) using the following formula:

$$\Delta E = \sqrt{\left(L^* - {L_0}^*\right)^2 + \left(a^* - {a_0}^*\right)^2 + \left(b^* - {b_0}^*\right)^2}$$

$$L^* = \frac{L}{255} \times 100;\quad a^* = \frac{240a}{255} - 120;\quad b^* = \frac{240b}{255} - 120.$$

Photoshop CS6 was utilized to measure the values of a (redness) and b (yellowness) color space as well as L (lightness). Values of L_0, a_0, and b_0 are used for the completely golden sample. The inductively coupled plasma-atomic emission spectrometer (VISTA-MPX, VARIAN, USA) was

Fig. 1 Representatives of two parents of the pacific oyster families with golden and white shell color used in the study

utilized to analyze the mineral elements. Mg and Zn contents were determined according to a method described by Hao et al. (2015). The result was expressed as mg/100 g dry weight.

SSR Markers

Microsatellite markers ($n = 960$) were used to test the genetic segregation in the two mapping families. These include 480 previously published SSR markers and 480 newly developed SSRs (Table S1). The procedure of developing SSR was done according to previously described by Li et al. (2009b). All SSR markers were screened in the four progenies and two parents initially. The polymorphic markers were genotyped subsequently in the two mapping families. PCR reactions were carried out in an ABI Veriti 96 well thermal cycler. PCR was conducted with a total volume of 10 μl with 0.8 μl of dNTP (2.5 mmol/l), 1 μl of 10 × reaction buffer, 0.05 μl of r*Taq* DNA polymerase (1 U, TaKaRa, Japan), 1 μl of each primer (2.5 μmol/l each), and 5.15 μl water, containing 1 μl genomic DNA (about 50 ng). The PCR program was 95 °C for 5 min and 35 cycles of 95 °C for 30 s, optimal annealing temperature for 30 s, 72 °C for 0.5 min, and finally 72 °C for 5 min. The PCR products were analyzed on a 6% denaturing polyacrylamide gel and visualized via silver-staining.

Construction of Genetic Maps and QTL Analysis

The JoinMap4.0 software (Van Ooijen 2006) was used in constructing a sex-averaged linkage map for each family. This linkage map was subsequently utilized for QTL analysis. To obtain linkage groups (LGs) at a LOD threshold of 5.0 in FAM-A and 7.0 in FAM-B, separated markers were assigned on clusters. *P* values less than 0.05 from the interval analysis permutation test were regarded as indicator of an important QTL effect. QTL analysis for traits was performed with Map QTL version 6.0 (Van Ooijen 2009). Interval mapping (IM) method was employed to detect any significant association between marks and traits. Graphic representations of the linkage maps were formed by MapChart 2.2 (Voorrips 2002).

Candidate Gene Identification

To identify candidate genes, all SSR sequences in QTL regions were annotated using the NCBI database (http://www.ncbi.nlm.nih.gov/) against the sequence of *C. gigas*.

Quantitative RT-PCR Analysis

Total RNA from each sample was isolated using TRIzol reagent (Invitrogen, UK). Each sample was performed with three duplicates and included the gene *EFI* (Elongation factor I) as internal control. RT-qPCR was performed using LightCycler ® 480 SYBR Green I Master Kit (Roche, Germany) on LightCycler 480 real-time PCR instrument. RT-qPCR procedure was as described previously (Feng et al. 2015). The primers for RT-qPCR were listed in Table S2. The RT-qPCR data was calculated using the comparative Ct method (ΔΔCt).

Results

Phenotypes

A summary of originally observed phenotypes was listed in Table S3. Shapiro–Wilk test for the phenotype data indicated that the distribution of Mg in FAM-A and SH in FAM-B deviated significantly from the normality ($P < 0.05$), while those for the other phenotype data showed normal distribution ($P > 0.05$).

Microsatellite Genotyping

Among the 960-screened microsatellite markers, 289 markers were informative in FAM-A and 338 markers were useful in FAM-B. Seventy-one shared markers were assigned in both families.

Individual-Based Linkage Maps

The linkage grouping at LOD = 5 was obtained in FAM-A and LOD = 7 in FAM-B. On the sex-average map of FAM-A, the number of markers for each LG ranged from 5 to 34 (mean, 21) and covered 1104.8 cm. On the map of FAM-B, the number of markers for each LG ranged from 2 to 58 (mean, 29) and covered 1116.9 cm. The percentages of the genome covered by the two maps were 89.6 and 90.6%, respectively (Table 1).

QTL Analysis

The QTL of four growth-related characteristics including total weight (TW), shell weight (SW), shell height(SH), and shell weight (Swi) were scanned on a genome scale (Table 2). In FAM-A, four significant QTL (qSHA7-1, qSHA7-2, qTWA5, and qSWA5), controlling shell height (SH), total weight (TW), and shell weight (SW), were found on LG5 and LG7 explaining 16.8, 16, 36.7, and 31.3% of the observed respective phenotypic variations. Interestingly, two QTLs (qTWA5 and qSWA5) associated with total weight and shell weight in FAM-A were found in the same region near the marker X45 (Fig. 2). In FAM-B, QTL for SW and TW (qTWB4 and qSWB4) were also mapped to the same location on LG2, whereas QTL for Swi (qSwiB7) was mapped on LG7 (Fig. 2). No QTL was detected for the shell width in FAM-A and shell height in FAM-B, respectively.

Table 1 Number of markers and genetic length for sex-averaged linkage map in two families of *C. gigas*

LG	FAM-A			FAM-B		
	No. of marker	Total length (cm)	Average spacing (cm)	No. of marker	Total length (cm)	Average spacing (cm)
1	31	142.9	4.8	58	168.3	2.7
2	34	169.3	5.1	49	147.7	3.2
3	27	96.4	3.7	42	88.8	2.2
4	25	109.3	4.4	30	97.2	3.3
5	25	128.1	5.3	29	161.9	5.8
6	22	114.4	5.4	27	124.8	5.0
7	16	75.7	5.0	24	149.2	6.5
8	15	108.4	7.7	14	86.7	6.7
9	7	119.9	19.99	18	54.3	3.2
10	5	40.3	10.08	2	37.9	37.9
Total/average	207	1104.8	5.6	293	1116.9.	3.9
Genome coverage	89.6%			90.6%		

PVE, phenotypic variance explained

In FAM-A, two QTLs were identified on LG3 that were associated with varied Zn content (qZnA3-1 and qZnA3-2). These two QTLs might contribute to the phenotypic variance of 17.9 and 34.4%, separately. No QTL associated with Mg content could be identified in FAM-A. However, four QTLs were found to be associated with the Mg content in FAM-B on LG1 (qMgB1), LG2 (qMgB2), LG5 (qMgB5), and LG9 (qMgB9). Among them, qMgB2 showed the largest LOD (5.31), with 22.9% of the phenotypic variation, and qMgB9 presented the smallest LOD (2.95), which correlated with only 13.5% of the phenotypic variation. Interestingly, both qMgB2 and qMgB9 were also detected for QTL associated with Zn content (Fig. 2), accounting for 26.7 and 14.2% of the phenotypic variation, respectively.

QTLs correlated with shell color were found on LG8 in FAM-A and on LG10 in FAM-B (qSCA8 and qSCB10). In

Table 2 QTL identified in two families of *C. gigas*

Family	Trait	QTL name	LG	Flanking markers	Confidence interval (cm)	LOD threshold	Maximum LOD	PVE (%)
FAM-A	SH	qSHA7-1	G7	E549-Ceg329	20.7–29.8	3.1	3.6	16.8
		qSHA7-2	G7	E367-L70	37.8–38.8		3.7	16
	TW	qTWA5	G5	X51-Crgi13	89.3–93.6	3.2	5.94	36.7
	SW	qSWA5	G5	X51-X45	90.3–92.3	3.8	4.88	31.3
	Zn	qZnA3-1	G3	UCDCG192-UCDCG162	16.2–18.9	3.1	3.93	17.9
		qZnA3-2	G3	UCDCG164-E10	33.1–37.4		4.57	34.4
	ΔE	qSCA8	G8	E523-G221	8–14.2	3.1	3.23	20.2
FAM-B	Swi	qSwiB7	G7	E53-UCDCG 148	133.7–138.9	3.2	3.6	14.6
	TW	qTWB4	G4	CGE211-H34	47.3–47.9	3.3	3.67	15.8
	SW	qSWB4	G4	CGE211-H34	46.3–47.3	3.4	3.57	15.5
	Mg	qMgB1	G1	E563-G46	52.9–54.9	4.3	4.37	19.3
		qMgB2	G2	E531-G118	0–1	4.4	5.31	22.9
		qMgB5	G5	G130-UCDCG194	144.6–147.6	3.2	3.52	15.8
		qMgB9	G9	CGE32-H153	16.1–17.1	2.8	2.95	13.5
	Zn	qZnB2	G2	E531-G118	0–3	3.5	6.35	26.7
		qZnB9	G9	CGE9-H153	14.1–16.1	2.9	3.13	14.2
	ΔE	qSCB10	G10	E324-H104	18–26	2.3	2.36	10.5

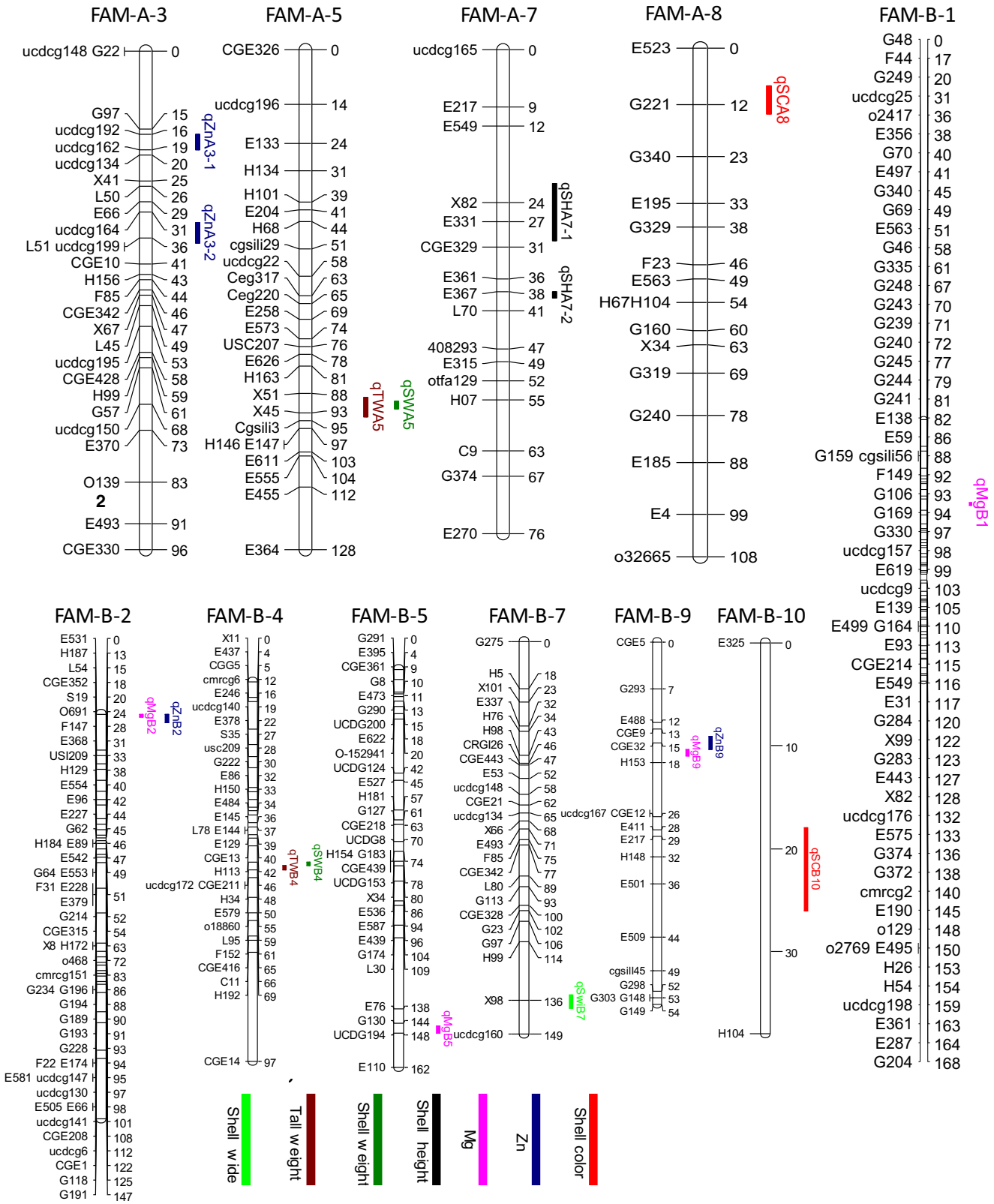

Fig. 2 Relationship of commercial traits on the linkage groups in two families. Marker names are shown on the left side of each linkage group, and physical distances are given on the right side

FAM-A, qSCA8 showed the largest LOD (3.23), illustrating 20.2% of the phenotypic variation. In FAM-B, qSCB10 had the largest LOD (2.36), contributing to 10.5% of the phenotypic variation.

Candidate Genes of Traits

In total, 35 marker sequences from 17 QTL regions were annotated. Fourteen genes were recognized in all QTL regions, in which six genes are associated with growth, six genes with Zn and Mg contents, and two genes on golden shell color (Table 3). Among the six genes with growth, *FBLN1*(fibulin-1-like) and *RBP1* (RNA-binding protein 1-like) were associated with both TW and SW. In addition, the gene *NMT2* (glycylpeptide N-tetradecanoyltransferase 2) was identified as a candidate gene regulating Zn and Mg contents.

The mRNA Expression of Candidate Genes

Three triplicate samples were collected at day 90 (G1) and 120 (G2) for RT-qPCR analysis of growth-related genes. As shown in Fig. 3, gene expression of *USP15* (ubiquitin carboxyl-terminal hydrolase 15) and *RBP1* was significantly decreased at day 90 than that at day 120 ($P < 0.05$; Fig. 3a, b), while *LIG1* (DNA ligase 1-like), *FBLN1*, *ARRB1* (beta-arrestin-1 isoform X2), and *ACTA1* (actin, alpha skeletal muscle) genes showed higher mRNA abundance in the samples at day 120 ($P < 0.05$; Fig. 3c–f).

The mRNA expression levels of Mg- and Zn-related genes were analyzed at two different stages (M1 and M2, Z1 and Z2) by RT-qPCR, respectively. The Mg content was significant higher in individuals at M2 (132.9–152.4 mg/g) than that in M1 (212.1–220.3 mg/g, $P < 0.05$). Moreover, the Zn content was significantly higher in individuals at Z2 (271.6–293.6 mg/g) than that in Z1 (132.9–152.4 mg/g, $P < 0.05$). All of these genes linked with Mg and Zn contents showed sufficient mRNA expression in M1 and Z1, when high Mg or Zn content was found ($P < 0.05$; Fig. 3g–m).

The expression levels of color-related genes were determined by RT-qPCR in three golden shell (C1) and three white shell color (C2) samples. The *PTPPK* (receptor-type tyrosine-protein phosphatase kappa-like) and *KMT2D* (histone-lysine N-methyltransferase 2D) genes were mainly expressed in the golden shell color samples ($P < 0.05$; Fig. 3n, o).

Discussion

Linkage Mapping

At present, most of the published linkage maps for the Pacific oyster were established based on AFLP markers (Li and Guo 2004), SSRs (Hubert and Hedgecock 2004; Hubert et al. 2009; Plough and Hedgecock 2011), AFLPs and SSRs (Guo et al. 2012), SSRs and SNPs (Sauvage et al. 2010; Zhong et al. 2014), and SNPs (Hedgecock et al. 2015). In the study, we added more SSR markers to the linkage maps. Compared with the linkage map based on the AFLP or SNP markers, the linkage maps based on the SSR were easy to transfer between different laboratories. Therefore, it is beneficial to construct genetic linkage maps based on more SSR markers. As a results of these collective studies, the average marker interval in the linkage map were 5.6 and 3.9 cm, which was longer than

Table 3 Candidate genes identified in two families of *C. gigas*

Family	Trait	QTL name	LG	Flanking markers	Annotation	Gene
FAM-A	SH	qSHA7-1	G7	E549	Actin, alpha skeletal muscle	*ACTA1*
				X82	Ubiquitin carboxyl-terminal hydrolase 15	*USP15*
				CGE329	Beta-arrestin-1 isoform X2	*ARRB1*
		qSHA7-2	G7	L70	DNA ligase 1-like	*LIG1*
	TW	qTWA5	G5	X45	Fibulin-1-like	*FBLN1*
	SW	qSWA5	G5	X45	Fibulin-1-like	*FBLN1*
	Zn	qZn3-2	G3	ucdcg164	Putative per-hexamer repeat protein 5	*PHXR5*
		qAZn3-2	G3	ucdcg199	Aftiphilin isoform X1	*AFTPH*
	ΔE	qSCA8	G8	E523	Receptor-type tyrosine-protein phosphatase kappa-like	*PTPRK*
FAM-B	TW	qTWB4	G4	CGE211	RNA-binding protein 1-like	*RBP1*
	SW	qSWB4	G4	CGE211	RNA-binding protein 1-like	*RBP1*
	Mg	qMgB1	G1	E563	Cysteine dioxygenase type 1-like	*CDO1*
				G46	Hypothetical protein CGI_10000944/SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1	*SMARC*
		qMgB9	G9	H153	Glycylpeptide N-tetradecanoyltransferase 2	*NMT2*
	Zn	qZnB9	G9	CGE9	Heat shock protein 68-like	*HSPA*
				H153	Glycylpeptide N-tetradecanoyltransferase 2	*NMT2*
	ΔE	qSCB10	G10	H104	Histone-lysine N-methyltransferase 2D	*KMT2D*

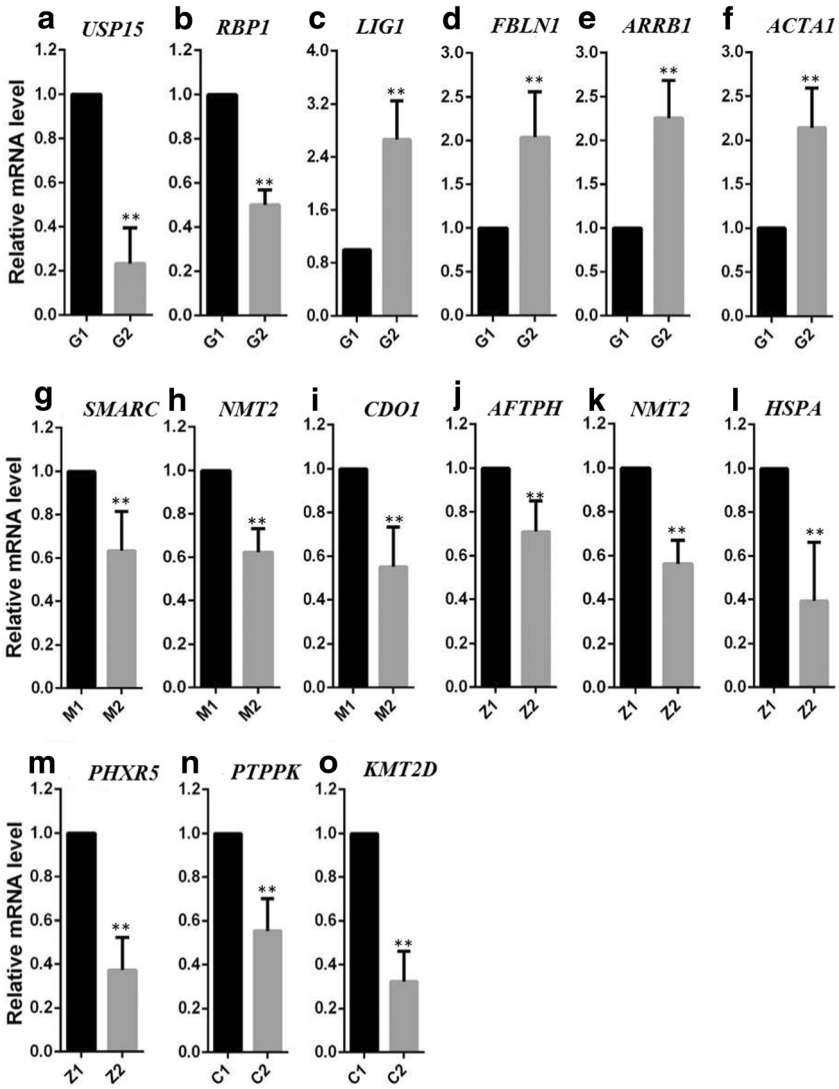

Fig. 3 The mRNA expression levels of 14 genes examined by RT-qPCR. Expression levels were normalized to EF I and presented as relative expression to controls (mean ± SD). *$P < 0.05$; ** $P < 0.01$

some published linkage maps, while smaller than the minimal size (10–20 cm) for QTL mapping (Massault et al. 2008). Therefore, our newly established linkage maps are useful for detecting QTL of beneficial traits in *C. gigas.*

Mapping Growth Traits

QTLs for principal components of the growth traits have been reported in *C. gigas* (Zhong et al. 2014; Guo et al. 2013). However, the QTL functional regions for single growth trait were not investigated. In this study, we analyzed the independent QTL related to the growth traits in shell formation. The analysis showed that four QTL of size-related traits were located on LG5 and LG7 in FAM-A and LG4 and LG7 in FAM-B, which accounted for 14.6–36.7% of the phenotypic variance, respectively. These data suggest that the size-related traits of *C. gigas* may be controlled by multiple QTL loci or regions. This finding was in agreement with a previous report by Guo et al. (2013) suggesting that the growth traits might be influenced by many loci with small subtle effects. It is well accepted that most economic beneficial traits in domestic animals (e.g., growth) were quantitative that were influenced by numerous genes on different genomic regions (Goddard and Hayes 2009).

Interestingly, the same QTL for growth was detected for TW and SW in both families. Multifactorial QTLs for growth traits have been observed in common carp (*Cyprinus carpio* L.), Asian seabass (*Lates calcarifer*), Atlantic salmon (*Salmo salar*), and Zhikong Scallop (*Chlamys farreri*) (Jin et al. 2012; Baranski et al. 2010; Xia et al. 2013; Jiao et al. 2014), suggesting that the loci may have pleiotropic effects on the growth traits. Pleiotropy based on some key factors controlling various traits through diverse metabolic pathways might contribute to multifactorial QTLs (Jin et al. 2012). It is generally believed that when closely linked markers in multifactorial QTL regions were utilized in MAS, several of the traits

could get improved at the same time. Hence, the cluster QTL finding will assist the selective breeding of *C. gigas*.

The markers related to growth traits were annotated in genes involved in both physiological process and biochemical reactions, such as *ACTA1*, *LIG1*, and *FBLN1*. *ACTA1* is a member of the actin family which is highly conserved during evolution and plays a vital role in cell cytoskeleton organization, cell integrity, and motility. *LIG1* is critical for DNA replication, recombination as well as DNA repair. *FBLN1* is important in cell adhesion and cell migration within extracellular matrix. We showed that different mRNA expression of these three genes tended to occur at day 120 rather than at day 90. These results suggest that the three proteins may play key roles in tissue growth, implying potential important QTL targets in *C. gigas* breeding.

Mapping Mineral Element Traits

As essential mineral elements for all living organisms, Mg and Zn are important to the maintenance of life. QTLs for mineral contents have been detected in various plants, such as milled rice, wheat, and maize (Yu et al. 2015; Gong et al. 2016; Jin et al. 2015). However, no study has been reported on QTL mapping for traits of mineral contents in aquatic animals. In FAM-A, two QTL regions of Zn content were mapped on LG3, while QTL of Mg content could not be detected. This differs from the results obtained in FAM-B. The family-specific QTL could result from fixation of QTL and statistical sampling in some families (Melchinger et al. 1998). Another factor accounting for different QTL regions could come from the mapping parents. During artificial selective breeding, the homozygous QTL allele might be fixed in the mapping parent, where no segregation could occur. Furthermore, the differences could be caused by possible false positives, which might result from several factors such as small sample sizes and statistical power (Mackay et al. 2009).

In FAM-B, it was noted that the two QTLs (qMgB2 and qMgB9) found for magnesium content had the same location as the two qZnB2 and qZnB9 involved in zinc content. Because QTLs involved in Zn and Mg contents were found on the same region, it indicates that Zn and Mg might share a common genetic mechanism for cooperative uptake and utilization. Similar result was also reported from the QTL analysis of Zn and Mg contents in wheat (Gong et al. 2016). This may be due to the use of same nutrient transporters in transporting several different mineral elements. In this study, six genes were identified as being associated with the variation of Zn and Mg concentration. Among the six genes, *NMT2* is significant because it has been implicated in membrane trafficking and protein transportation. Moreover, *NMT2* showed the high levels of mRNA expression at M1 and Z1 stages with high Mg or Zn content. Therefore, it is tempting to speculate that *NMT2* might be responsible for Zn and Mg transport and regulation of their accumulation.

QTL Analysis of Golden Shell Color Polymorphism

In *C. gigas*, it has been demonstrated that the allele for golden shell color was dominant over the white shell color (Ge et al. 2015a, b). Similar phenomenon has been observed for the shell color traits in Pacific lion-paw (*Nodipecten subnodosus*) and bay scallop (*Argopecten irradians*) (Petersen et al. 2012; Qin et al. 2007). However, some of the colors cannot be adequately explained on the basis of the simple Mendelian mechanism. In the noble scallop (*Chlamys nobilis*), the appearance of three colors could be explained by a one-locus three-allele model (Winkler et al. 2001). In Chilean scallop (*Argopecten purpuratus*), two loci were identified that determine five color strains (Zheng et al. 2013).

In FAM-B, we found a QTL for the golden shell color trait on LG8, located between two SSR loci (E325 and H104). Interestingly, in FAM-A, H104 was also mapped to LG10, which contained QTL associated with the golden shell color trait. In addition, our studies here showed that the golden shell color QTL in two families could explain 20.2 and 10.5% of the phenotypic variation. It has been suggested that altering shell color of Pacific oysters during selective breeding could be caused by the fixation of major genes, followed by more sustained and gradual response on polygenes (Evans et al. 2009).

Our studies further demonstrated that the flanking marker E523 in FAM-A was located within the *PTPRK* gene. *PTPRK* is a member of the protein tyrosine phosphatase (PTP) family, which removes phosphate moieties from tyrosine residues on other proteins. The result of RT-qPCR indicated that *PTPPK* was consistently up-regulated in golden *C. gigas*. Thus, *PTPPK* may play an important role in golden coloration in *C. gigas*, although it is not clear how *PTPPK* is involved in shell color formation. Our data also revealed that the flanking marker H104 in FAM-B was closely linked to the gene coding for *KMT2D*, which represents a specific tag for epigenetic transcriptional activation (www.uniprot.org/uniprot/o14686). In addition, *KMT2D* showed a higher mRNA abundance in golden *C. gigas*. It has been suggested that *KMT2D* might be associated with epigenetic regulation of golden shell color. Further studies are needed to determine if other loci are involved in the determination of golden shell color in this species.

In summary, we have mapped QTLs associated with golden shell color, Mg and Zn contents, and growth traits across the two linkage mappings. Seventeen QTLs for the above traits and 14 associated genes were found in these two families, which could have potential application for marker-assisted selection in *C. gigas* breeding.

Funding Information This work was supported by the grants from the National Natural Science Foundation of China (31772843), the Fundamental Research Funds for the Central Universities (201762014), Taishan Scholars Seed Project of Shandong, and the Industrial Development Project of Qingdao City (17-3-3-64-nsh).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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