Ecological significance of G protein-coupled receptors in the Pacific oyster (*Crassostrea gigas*): Pervasive gene duplication and distinct transcriptional response to marine environmental stresses

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ABSTRACT

Marine ecosystems with ocean warming and industry pollution threaten the survival and adaptation of organisms. G protein-coupled receptors (GPCRs) play critical roles in various physiological and toxicological processes in vertebrates and invertebrates. The Pacific oyster (*Crassostrea gigas*) was widely used to study the adaptation of marine molluscs to coastal environments. In this work, we identified a total of 586 GPCRs in *C. gigas* genome. The *C. gigas* GPCRs were divided into five classes (including class A, B, C, E and F) with different degrees of expansion. Meta-analysis of multiple RNA-seq datasets revealed that transcriptional expression patterns of GPCRs in *C. gigas* were distinct in response to high temperature, salinity, air exposure, heavy metal, ostreid herpes virus 1 (OsHV-1) and Vibrio challenge. This work for the first time characterized the GPCR gene family and provided insights into the potential roles of GPCRs in adaptation of marine molluscs to stressful coastal environment.

1. Introduction

Marine ecosystems are the most valuable and vulnerable natural systems worldwide. The marine organisms, especially those inhabit in estuaries and coastal areas, are threatened by various environmental stresses, such as sea surface temperature rising resulted from climate change (Christensen et al., 2021), fluctuations in salinity due to freshwater input caused by precipitation (Du et al., 2021, 2019), air exposure according to the tidal cycle (Burge et al., 2014; Harvell et al., 2002; Kawabe et al., 2019), heavy metal levels increased by a parallel increase in wastewater runoff from nearby industries and terrestrial agriculture (Luo et al., 2018; Wong et al., 1981), virus such as OsHV-1 and bacteria such as *Vibrio* spp. (Barbosa Solomieu et al., 2015; He et al., 2015; Pernet et al., 2016). Considering the ecological and economic importance of marine molluscs (Lenihan et al., 2001; McLeod et al., 2019; Zhang et al., 2012), it is critical to understand the molecular mechanisms involved in the processes that protect them from environmental stresses.

G protein-coupled receptors (GPCRs), one of the largest families of transmembrane proteins, play critical roles in various physiological function (Bjarnadóttir et al., 2006; Broeck, 2001; Hanlon and Andrew, 2015; Li et al., 2014). The International Union of Pharmacology (IUPHAR) classification (class A, B, C, D, E, F) and GRAFS classification system including Glutamate (Class C/7tm_3), Rhodopsin (Class A/7tm_1), Adhesion (Class B/7tm_2), Frizzled (Class F/7tm_5) and Secretin (Class B/7tm_3) were frequently applied to sort out this superfamily both in vertebrates and invertebrates (Class D and E are unique to invertebrates) (De Francesco et al., 2017; Fredriksson et al., 2003; Yang et al., 2021). Although the overall repertoires of GPCRs have been characterized in mammals (Bjarnadóttir et al., 2006; Gloriam et al., 2007), chicken (Lagerström et al., 2006), amphibians (Ji et al., 2009), teleost fish (Sarkar et al., 2011), some invertebrates (Brody and Cravchik, 2000; Hanlon and Andrew, 2015; Hill et al., 2002; Kamesh et al., 2008; Kim et al., 2022b, 2022a) and fungus (Krishnan et al., 2012; Zheng et al., 2010), no such study was reported in marine bivalves yet. Accumulation of genomic resources allow us to conduct in-depth analysis of the mollusc GPCRs, which is helpful to understand the evolutionary relationship of GPCRs from vertebrates to invertebrates.

The Pacific oyster (*Crassostrea gigas*) is a preferred marine invertebrate model for our investigation considering its wide distribution in estuarine and intertidal zones and evolution status in invertebrates (Guo et al., 2008; Song et al., 2019; Zhang et al., 2016, 2012). The updated genome sequence of *C. gigas* has been released (Peñaloza et al., 2021)

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allowing for extensive characterization of GPCR gene families. Further, a great number of publicly available RNA-seq datasets in *C. gigas* provided abundant resources for studying the molecular mechanisms of oyster in response to multiple stresses (He et al., 2015; Zhang et al., 2012).

In this study, we identified a complete set of 586 GPCRs in *C. gigas* genome and classified them into 5 classes. Then we compared them with those in representative vertebrates and invertebrates, adding the information about GPCRs evolution in marine molluscs. Moreover, meta-analysis was conducted to determine transcriptional regulation patterns of GPCRs in *C. gigas* triggered by various environmental stresses. For the first time, this work provided an extensive characterization of GPCRs in *C. gigas* and provided insights into the potential roles of GPCRs in marine molluscs adaptation to coastal environment.

2. Materials and methods

2.1. Identification of GPCRs in C. gigas genome using HMMs

The *C. gigas* GPCRs homologs were identified based on HMMs (Hidden Markov models) method (Altschul, 1997). In brief, an initial dataset of GPCRs (CL0192) was derived from Pfam database (version 34.0) (Mistry et al., 2021). In this study, a total of 34 HMMs (Supplementary Table 1) were selected to scan the *C. gigas* protein sequence database (GCF_902806645.1) (Peñaloza et al., 2021) using hmmsearch from HMMER 3.3 package with an E-value lower than 1×10^{-10} to obtain the potential GPCR protein sequences (Friedrich et al., 2006). All the obtained sequences resulted from each HMMs were aligned using ClustalW2 (Larkin et al., 2007) and the alignments were subsequently used to construct oyster-specific HMMs using hmmbuild from HMMER 3.3 package. Finally, these HMMs were used to search against *C. gigas* protein sequence database (GCF_902806645.1) again with an *E*-value lower than 1×10^{-20} to obtain high-quality GPCR protein coding sequences (Gloriam et al., 2005).

2.2. Domain search

The predicted sequences were checked thoroughly using Conserved Domain Search Service (CD Search) (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?) to ensure their possession of conserved transmembrane region. In this work, the sequences with a number of 5, 6, or 7 TM domains were selected for further analysis (Ji et al., 2009; Käll et al., 2004).

2.3. Phylogenetic analysis

The reference species used for phylogenetic analysis including chordates (*Homo sapiens, Gallus gallus, Takifugu rubripes, Xenopus tropicalis, Ciona intestinalis*), invertebrates (*Anopheles gambiae, Drosophila melanogaster, Aedes aegypti, Aedes albopictus, Culex quinquefasciatus, Musca domestica, Crassostrea virginica, Mizuhopecten yessoensis, Exaiptasia diaphana* and *Hydra vulgaris*) and eumycetozoa (*Dictyostelium discoideum*). The numbers of genes that was labeled in the phylogenetic tree were as described in previous studies (Brody and Cravchik, 2000; Fredriksson et al., 2003; Hill et al., 2002; Ji et al., 2009; Kamesh et al., 2008; Lagerström et al., 2006; Sarkar et al., 2011) and annotation in NCBI (https://www.ncbi.nlm.nih.gov/gene/). All of the protein sequences used for phylogenetic analysis (Supplementary Dataset 1 and Dataset 2) were retrieved from Uniprot (https://www.uniprot.org/uniprot/) and NCBI databases. Sequences were aligned using MAFFT (version 7) with default parameters (Katoh and Standley, 2013). Phylogenetic tree was generated using IQ-TREE v 2.1.2 (Nguyen et al., 2015). The best-fit substitution model was determined by ModelFinder (Kalyaanamoorthy et al., 2017) according to the Bayesian Information Criterion, which was implemented in IQ-TREE. The maximum likelihood (ML) method with 1000 bootstraps was performed to determine the significance of

branching. The phylogenetic trees were visualized with FigTree (Version v1.4.3) and iTOL v6.3 (Letunic and Bork, 2007).

2.4. Expression analysis of GPCRs based on RNA-seq meta-analysis

Publicly available RNA-seq datasets were retrieved from NCBI Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/Traces/sra) and CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) (https://db.cngb.org/cnsa/) (He et al., 2015; Li et al., 2022; Zhang et al., 2012). The accession numbers and sample description were provided in the Supplementary Table 2. Raw reads were initially cleaned using fastp software (Chen et al., 2018) and then mapped to the latest *C. gigas* genome (GCA_902806645.1) (Peñaloza et al., 2021) using HISAT2 (Kim et al., 2019). The number of aligned reads were counted using featureCounts (Liao et al., 2014). Differential expression analysis was performed using the DESeq2 (Love et al., 2014). Genes with $|\log_2 \text{FoldChange}| > 2.0$ and Bonferroni adjusted *p*-value <0.05 were determined as differentially expressed genes (DEGs).

2.5. Functional enrichment analysis

The gene ontology (GO) analysis was employed to determine the possible functions of differentially expressed GPCRs using the GOseq R packages. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of differentially expressed GPCRs was performed using KOBAS (2.0) software (Mao et al., 2005). GO terms and KEGG pathways with *q*-value <0.05 were assigned as significantly enriched.

2.6. Visualization

Expression profiles of GPCRs with their location on the chromosome were displayed using visualization tools of Circos (Krzywinski et al., 2009). Heatmap of DEGs was conducted using an online tool (https://jingege.shinyapps.io/shinyplots/). Comparison of differentially expressed GPCRs among different environmental stresses was visualized with the Venn diagram viewer (Bardou et al., 2014).

3. Results

3.1. Identification and characterization of GPCRs in C. gigas

A total of 586 GPCRs were identified in *C. gigas* genome (Supplementary Table 3). Among which, 64 genes with description of uncharacterized protein in NCBI were annotated as GPCRs in our study. Most of the genes were located on chromosomes with the remaining 28 GPCR genes identified from 23 scaffolds that were not anchored to the chromosomes. Notably, a large portion of the GPCRs were located in chromosome 10, account for 19 % of the total genes. Lengths of proteins encoded by these GPCRs ranged from 216 to 6279 amino acids, with the transcript length ranging from 651 to 19,974 nucleotides with or without 5′- and 3′- untranslated regions (UTRs). Exon numbers of these GPCRs mRNA differed from 1 to 106. The GPCRs were further classified into class A (454 genes), class B (107 genes), class C (18 genes), class E (1 gene) and class F (6 genes) according to the Conserved Domain Search in NCBI. All of the *C. gigas* GPCR members in each class contained the corresponding conserved domains. For example, the class A members contained the domain of "7tmA" or "7tm_classA", the class B members contained the domain of "7tmB" or "7tm_classB", and so on.

3.2. Phylogenetic analysis of GPCRs in C. gigas

The *C. gigas* GPCRs were roughly divided into 5 classes based on HMM and conserved domain research. In order to obtain a clearer view of the GPCRs, we produced phylogenetic tree using the protein sequences (Fig. 1). In general, most of these receptors showed clear cluster
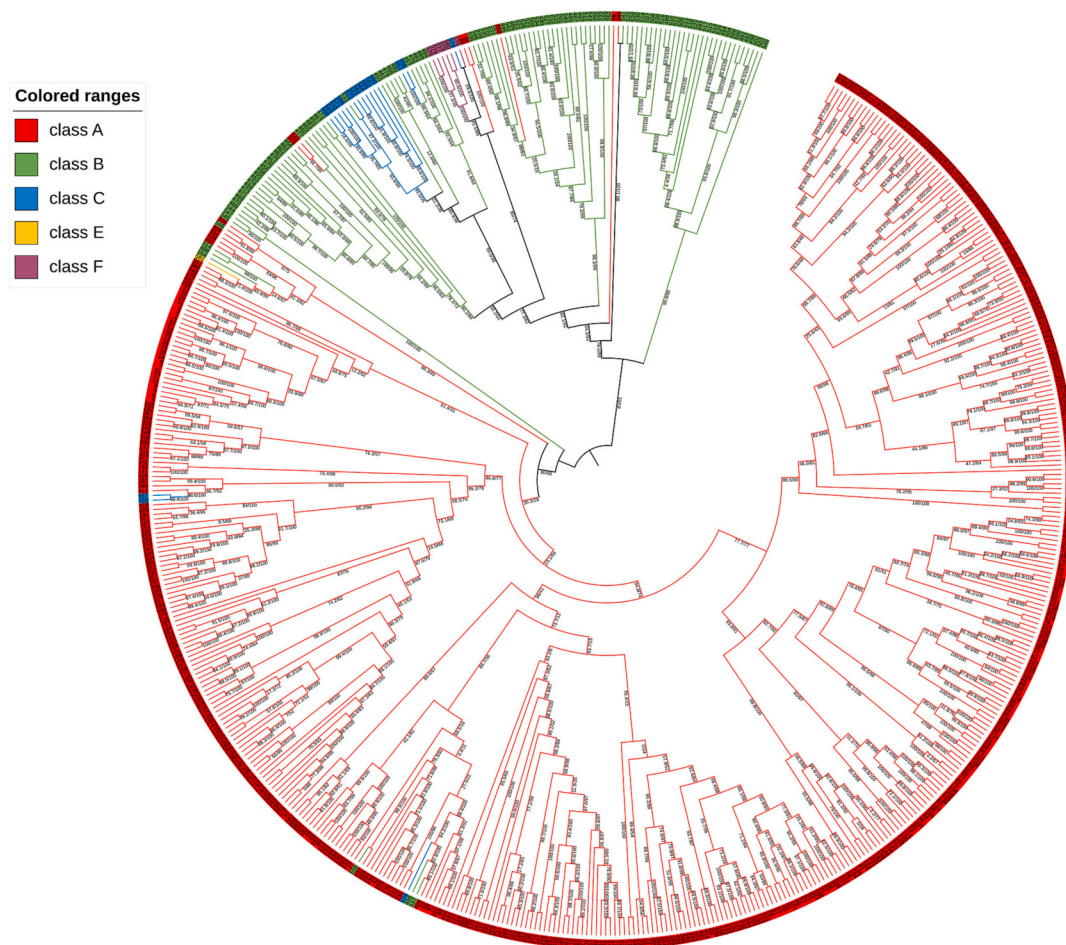
Fig. 1. Phylogenetic analysis of GPCRs in *C. gigas*. The branches of the phylogenetic tree were colored according to the divisions of GPCR classes. The maximum likelihood tree was constructed with 1000 bootstraps. Best-fit model (LG + F + R9) was chosen according to the Bayesian information criterion to create the tree. The symbol code of each member was mentioned in Supplementary Table 3.

with their homologs of corresponding classes. The classification based on the phylogenetic tree was essentially consistent with the result that was determined based on HMM and conserved domain. Phylogenetic analysis of the GPCR family is very challenging because of its big size and diversity, thus some GPCRs failed to cluster to corresponding branches.

3.3. Comparison of GPCRs among species

The member and quantity of GPCRs were compared between *C. gigas* and several other representative species, including *Homo sapiens, Gallus gallus, Takifugu rubripes, Xenopus tropicalis, Ciona intestinalis, Anopheles gambiae* and *Drosophila melanogaster* (Supplementary Table 4). The class A accounts for the major portion of all GPCR members in *C. gigas*, which is consistent with that in other representative species except for *Drosophila melanogaster*. Notably, the number of *C. gigas* GPCRs in class B was greater than that in other representative species. Interestingly, we identified one GPCR gene in class E in *C. gigas*, which was not present in any other species under investigation. In contrast, we did not identify any *C. gigas* 7tm_6 and 7tm_7 classes of GPCRs, which were only reported in arthropods (Supplementary Table 4). To better understand evolutionary relationship of the GPCRs among species, we further constructed a phylogenetic tree of GPCRs from diverse species including chordates and invertebrates. As shown in Fig. 2, GPCRs were grouped into seven well-separated clusters corresponding to their classification, in which, the class E, 7tm_6 and 7tm_7 comprised sequences only from invertebrates. The *C. gigas* GPCRs were well separated from those in

chordates and other invertebrates (arthropoda and cnidaria).

3.4. Transcriptional regulation of GPCRs in C. gigas in response to environmental stresses

To explore the role of GPCRs involved in response to environmental stress, publicly available RNA-seq datasets of *C. gigas* challenged by seven types of biotic and abiotic environmental stresses, including high temperature, high salinity, low salinity, air exposure, heavy metal, OsHV-1 and Vibrio, were analyzed. The transcriptional expression changes of the *C. gigas* GPCRs were found after exposure to various environmental stresses (Fig. 3 and Supplementary Table 5). Notably, the expression of GPCRs located on the chromosome 6, 7 and 9 showed similar patterns among stresses. While the expression of GPCRs located on the chromosome 1 and 10 showed obviously different patterns among stresses. Moreover, most GPCRs were highly induced in *C. gigas* in response to OsHV-1 challenge except for those located on the chromosome 1 and 5. A total of 185 differentially expressed GPCRs ($|\log_2$FoldChange$| > 2.0$, *p*-value <0.05) were identified in *C. gigas* in response to various stresses (Supplementary Table 6). The expression profiles of the DEGs in such conditions were obviously different (Fig. 4). The induced GPCRs were generally different among stresses and tended to be induced in a stress-specific manner. The number of differentially expressed *C. gigas* GPCRs were also different in response to various stresses and most stress-specific GPCRs were observed under OsHV-1 challenge with a total of 41 genes (Fig. 5). Notably, these stress-specific DEGs were mainly involved in class A and class B
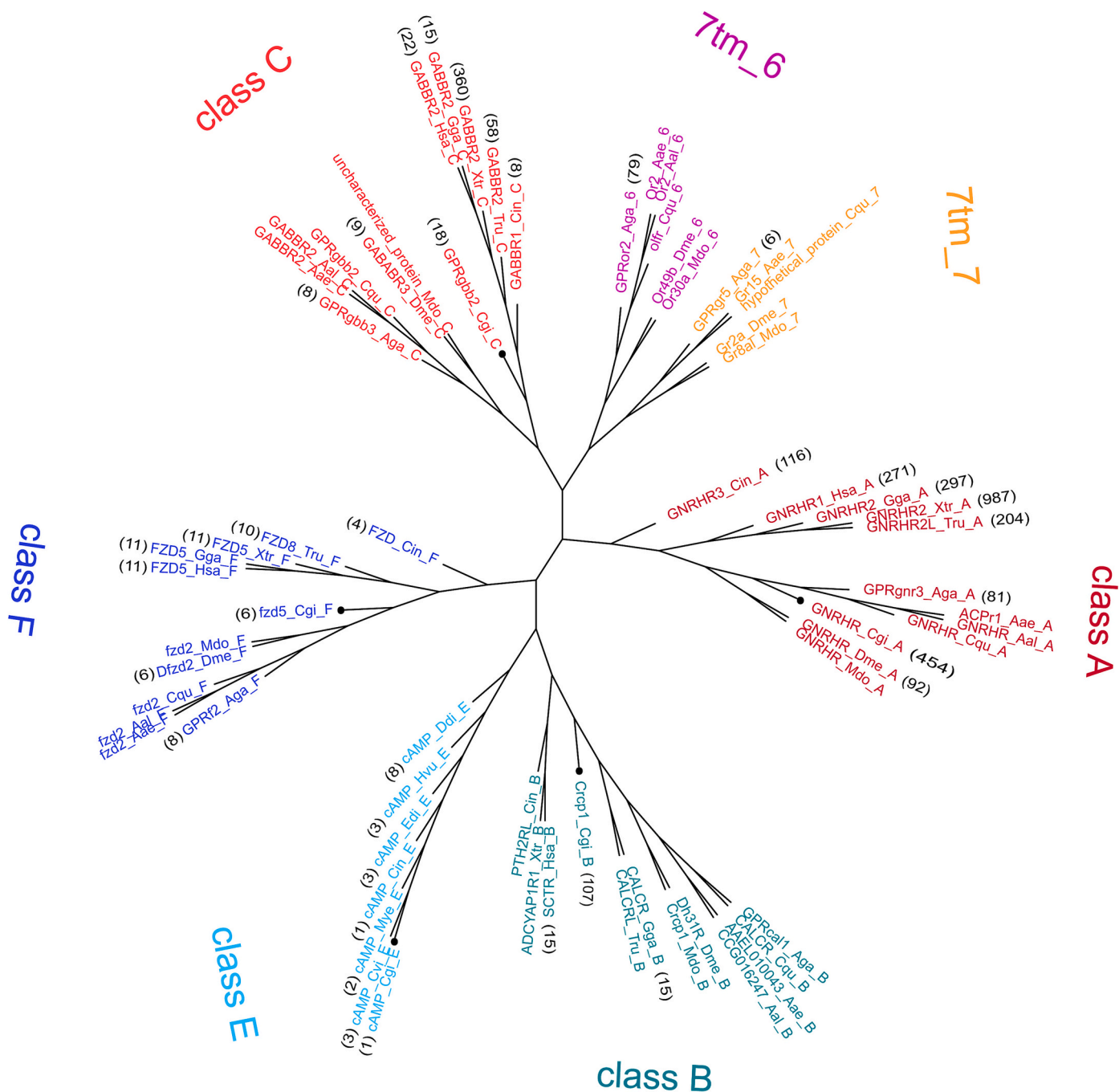
Fig. 2. Phylogenetic tree of GPCRs from *C. gigas* and other eukaryotic species. The number of genes was labeled in the phylogenetic tree. The position of each class was represented by one gene along with its orthologous genes in representative species. The maximum likelihood tree was constructed with 1000 bootstraps. GPCRs from different classes were differently colored. Best-fit model (WAG+F + R5) was chosen according to the Bayesian information criterion to create the tree. The *C. gigas* genes were marked with black dots. Abbreviations: Hsa, *Homo sapiens*, Gga, *Gallus gallus,* Tru, *Takifugu rubripes*, Xtr, *Xenopus tropicalis*, Cin, *Ciona intestinalis*, Aga, *Anopheles gambiae*, Dme, *Drosophila melanogaster*, Aae, *Aedes aegypti*, Aal, *Aedes albopictus*, Cqu, *Culex quinquefasciatus*, Mdo, *Musca domestica*, Cvi, *Crassostrea virginica*, Mye, *Mizuhopecten yessoensis*, Edi, *Exaiptasia diaphana*, Hvu, *Hydra vulgaris*, Ddi, *Dictyostelium discoideum*, Cgi, *Crassostrea gigas*.

(Supplementary Table 7). These results may imply the potential roles of the specific GPCRs with certain functional domains in response to environmental stresses in *C. gigas*.

3.5. Functional enrichment analysis of differentially enriched GPCRs in C. gigas

To explore the biological significance of differentially expressed GPCRs in *C. gigas* in response to various environmental stresses, all of the DEGs were used to perform the GO and KEGG enrichment analysis. A total of 410, 621, 576, 230, 621, 639, 898 and 563 GO terms were significantly enriched respectively in *C. gigas* under heat stress, low-salinity stress, high-salinity stress, air exposure stress, heavy metal stress, OsHV-1 challenge and vibrio challenge (*q*-value <0.05) (Supplementary Table 8). The top30 GO terms showed that the DEGs were significantly enriched in the molecular function (MF) of G protein-coupled peptide receptor activity and peptide receptor activity and biological process (BP) of phospholipase C-activating G protein-coupled receptor signaling pathway in *C. gigas* under all the analyzed stress conditions (Supplementary Table 9). Analysis of stress-specific GO terms
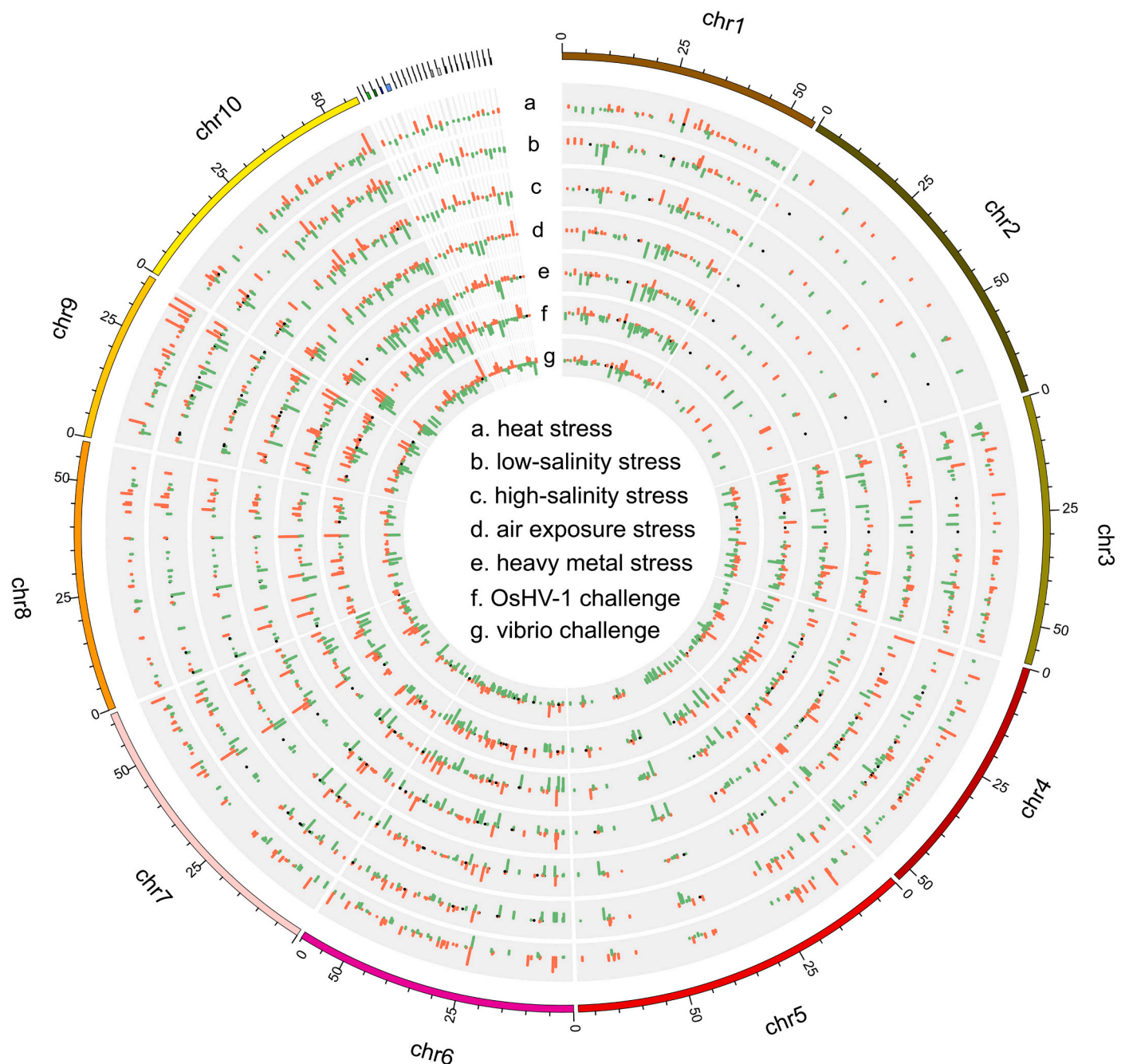
Fig. 3. Expression profiles of GPCRs in *C. gigas* after exposure to various environmental stresses. The histogram in the circos represented the fold change of *C. gigas* GPCRs after stress. The induced genes ($|\log_2 \text{FoldChange}| > 0$) were labeled in red while suppressed gene ($|\log_2 \text{FoldChange}| < 0$) were labeled in green. Most of the GPCRs were located on 10 chromosomes (chr) with the remaining 28 GPCRs located on 23 scaffolds. (a) heat stress (30 °C for 24 h). (b) low-salinity stress (salinity of 15 ‰ for 7 days). (c) high-salinity stress (salinity of 40 ‰ for 7 days). (d) air exposure stress (7 days). (e) heavy metal (Cd) stress (12 h). (f) OsHV-1 challenge (24 h). (g) vibrio challenge (12 h). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

revealed that the BP related to 'regulation of vasoconstriction' was enriched under heat stress and OsHV-1 challenge, the BP related to 'response to water deprivation' was enriched under low-salinity stress, the MF involved in 'calcitonin binding' was enriched under heavy metal stress, the BP related to 'regulation of digestive system process and gastric acid secretion' was enriched under pathogen challenge. The significantly enriched KEGG pathways (q-value <0.05) were similar among various stress conditions (Table 1). The G protein-coupled receptors and calcium signaling pathway were enriched under all stress conditions.

4. Discussion

Marine organisms living in coastal zones are vulnerable to environmental challenges such as ocean warming, pollutants, extreme precipitation events, tidal fluctuation and pathogenic vibrio and virus in the aquatic environment (Des et al., 2021; Friedman et al., 2005; Lacoste et al., 2001; Lowe et al., 2017; Luo et al., 2018; Pickering et al., 2017; Vezzulli et al., 2013). Marine bivalves are considered as good bioindicator species in ecotoxicology and environmental risk assessment programs (Bayen et al., 2007; Des et al., 2021; Ding et al., 2020; Dutta et al., 2018; Tian et al., 2021). As sessile and filter-feeding marine
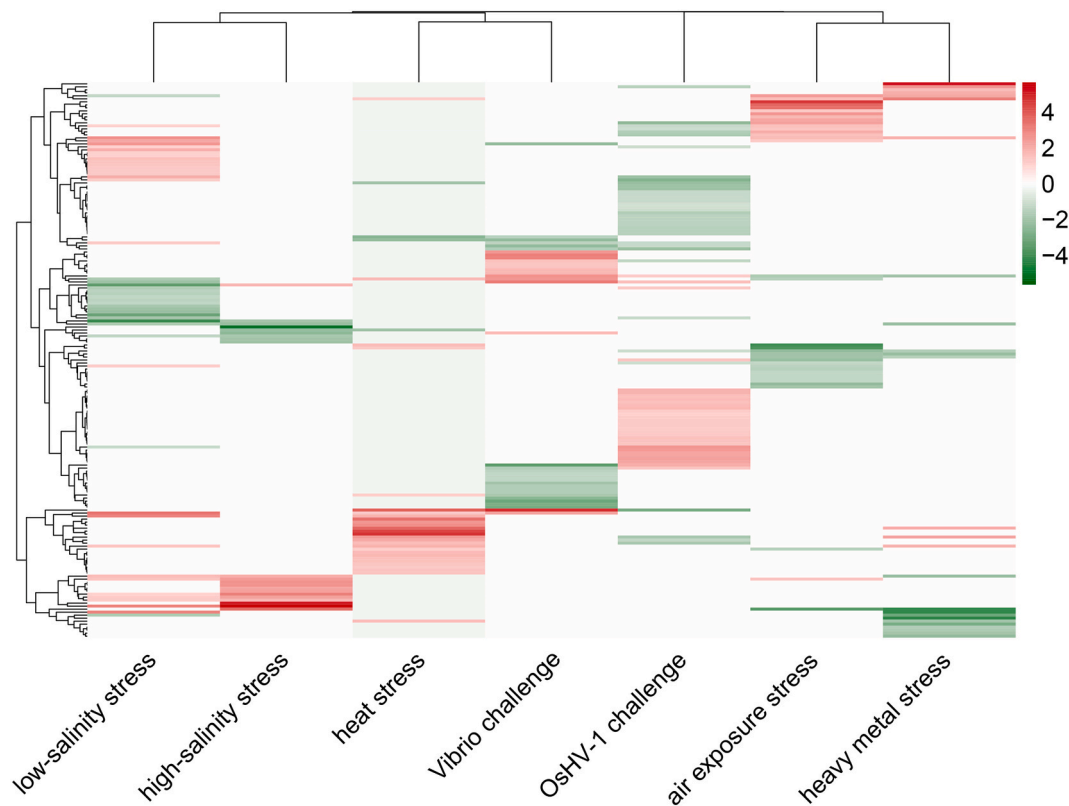
Fig. 4. Differentially expressed GPCRs in *C. gigas* in response to various environmental stresses. Fold change of differentially expressed GPCRs ($|\log_2 \text{FoldChange}| > 2$, *p*-value <0.05) was represented in the heatmap. The color shown an overview of the numeric differences. Genes that were highly induced were labeled in red and suppressed were labeled in green. The scale of fold change was marked right. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

organism, oyster is sensitive to ocean environmental changes, making them an excellent model in discovering functional genes in adaptation of molluscs to natural habitats (Bayen et al., 2007; Clark et al., 2013; Li et al., 2017, 2018; Lowe et al., 2017). The GPCR superfamily is one of the most abundant families of membrane proteins, which plays critical roles in various physiological and toxicological processes via translating signals into cellular functions (Broeck, 2001; Hanlon and Andrew, 2015; Kiselyov et al., 2003; Yang et al., 2021). The GPCR gene family has been summarized and functionally analyzed in many marine organisms (Cardoso et al., 2016; Clark et al., 2010; Cummins and Degnan, 2010; Gloriam et al., 2005; Jiang et al., 2022; Kim et al., 2022a, 2022b; Klein et al., 2021; Li et al., 2016; Marquet et al., 2020; Saunders et al., 2000). However, there is no report on the integral landscape of GPCRs in marine molluscs. Here, we conducted identification, characterization and expression profiling of GPCRs to understand their potential roles in response to environmental stresses in *C. gigas*.

The GPCR subfamilies were greatly expanded in different degrees in *C. gigas* genome. In this study, we identified a complete set of 586 GPCRs in *C. gigas* genome which were classified into 5 classes (class A, B, C, E, F) according to similarity of sequence and conserved domain (Liu et al., 2021). The classification of GPCRs was further confirmed by phylogenetic analysis. Only one class E (cAMP receptor) member was found in *C. gigas* and it was clustered with the same class of other invertebrates. The cAMP receptor was predicted to be the ancestor of the main families (class A, B and F) and it was present only in invertebrates and lost in the vertebrates (Krishnan et al., 2012; Lilly et al., 1988; Nordstrom et al., 2011). It's reported that cAMP system played a wide and central role in nonmammalian vertebrates and invertebrates, especially in bivalves in response to environmental stresses (Fabbri and Capuzzo, 2010). Here, the presence of cAMP receptor of GPCR gene family in *C. gigas* with distinct difference compared to vertebrates may indicate its special roles

in adaptation to the marine environment in the oyster. The class A emerged from the cAMP receptor family in an event close to the split of Opisthokonts and then expanded greatly in Metazoans (Krishnan et al., 2012). In this study, the class A is the largest subfamily of GPCRs in *C. gigas* with 454 members flowed by class B with 107 members and class C with 18 members, which was generally consistent with the GPCRs distribution in reference species. The gene expansion may imply the potential mechanism of GPCRs in response to environmental stresses (Mu et al., 2018; Pavey et al., 2010; Zhang et al., 2012).

Expression of genes was closely linked to the physiological processes (Harrison et al., 2012). The expression of GPCRs were characterized in eukaryotic organisms under stress or pathological conditions (Cao et al., 2020; Horgue et al., 2022; Li et al., 2014; Mojib and Kubanek, 2020; Yadav and Tuteja, 2011). The expression profiles of GPCRs in *C. gigas* as revealed by transcriptome analysis were different among various stresses including temperature, salinity, air exposure, heavy metal (Cd), OsHV-1 challenge and vibrio challenge, which may imply the involvement of GPCRs in oyster in response to environmental stress. Devastating mortality events of oyster caused by pathogens have been reported previously and the interaction between host and pathogens are extremely complex (Friedman et al., 2005; Guo and Ford, 2016). The GPCR gene family is reported as a fundamental component of invertebrate innate immunity to resist pathogens infection (Reboul and Ewbank, 2016). It has been reported that a great number of GPCRs were induced in *C. gigas* after OsHV-1 infection (He et al., 2015). The involvement of GPCRs in neuroendocrine-immune regulatory network in scallops was also reported (Song et al., 2015). In this present study, the greatest number of differentially expressed GPCRs were found in *C. gigas* under OsHV-1 challenge, which supported the results of previous study with some newly identified GPCRs (He et al., 2015). These results may suggest that the selective regulation of functional GPCRs play
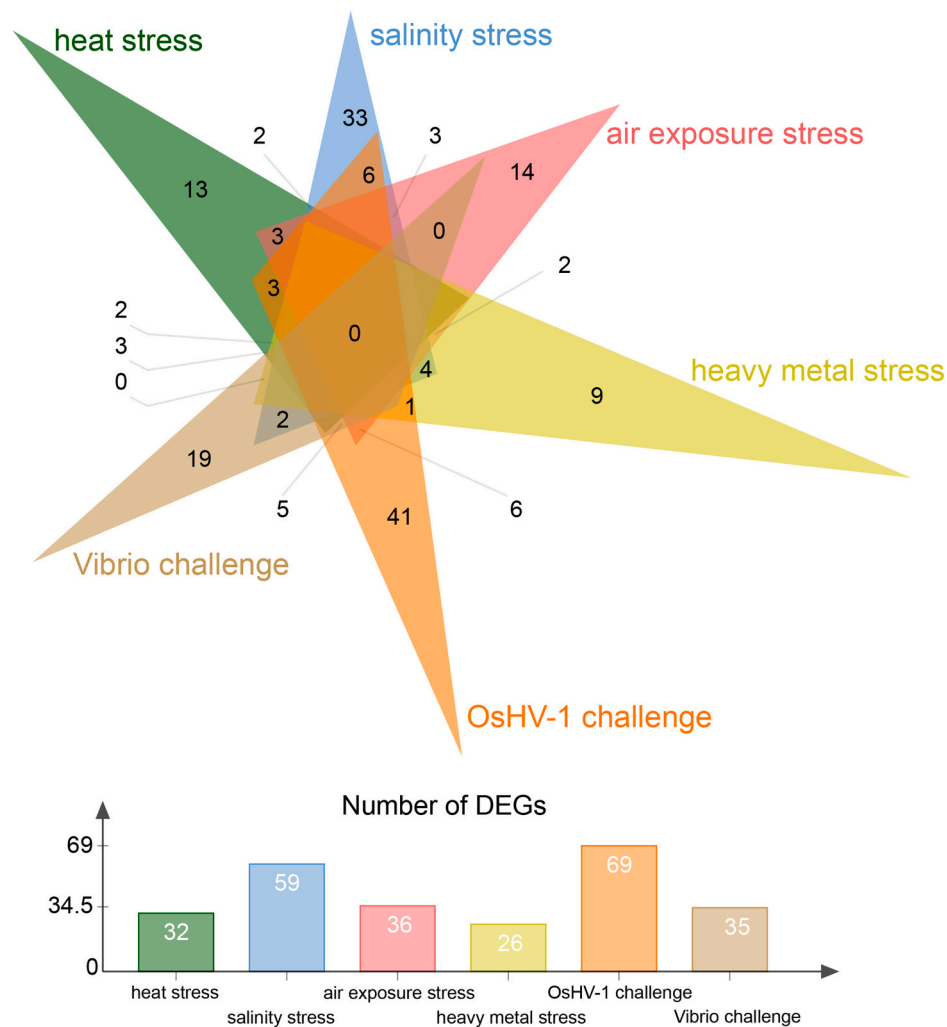
Fig. 5. Comparison of differentially expressed GPCRs in *C. gigas* among various environmental stresses. The number of differentially expressed GPCRs in *C. gigas* under each stress condition were displayed in the histogram. The two sets of low and high salinity stress were merged to create a single salinity set.

potential roles in oysters in response to environmental stresses.

The stress-specific GPCRs involved in response to environmental stresses were mainly found in class A and class B. There was a possible involvement of the class A in sensory detection flowing chemical stimulation and neuroendocrine system and the class B in the regulation of digestive system where is in charge of food and energy conversion in molluscs (Cummins and Degnan, 2010; Herpin et al., 2004; Klein et al., 2021). Therefore, it is reasonable to presume that the expression of GPCRs in class A and class B in *C. gigas* may imply a functional evolution corresponding to their living habitat, since the energy metabolism and neuroendocrine regulation are important for marine organisms to adapt to environments (Dong and Zhang, 2016; Li et al., 2017; Sokolova et al., 2012). The Class C GPCRs play important roles in many physiological processes such as synaptic transmission and calcium homeostasis (Wu et al., 2014). It has been reported that the orthosteric sites of the metabotropic glutamate receptors (mGlu) GPCR subtypes were the most highly conserved throughout evolution and strictly selected for ligands with amino acid-like structures. The mGlu GPCRs were specifically regulated in *C. gigas* under OsHV-1 challenge. These results may imply the potential mechanism for activation of GPCRs under OsHV-1 challenge. Furthermore, these stress-specific GPCR members that may play potential roles in response to corresponding stresses should be taken into consideration as candidates for further analyzation.

The differentially expressed GPCRs involved in multiple stresses were also identified. Although there was no differentially expressed

GPCRs shared by all types of environmental stresses, a number of GPCRs were identified to play potentially critical roles in multiple stress conditions. Moreover, cell senses and responds to environment changes through GPCRs and signal cascades including Ca^{2+} signal transduction. The convergence of downstream pathways on common signaling and transcriptional mechanisms integrates diverse GPCR effects and may provide a path to overcome redundancy (Haak et al., 2020). As previously reported, the increased expression of different GPCRs in the absence of others may imply that the functional redundancy and compensatory functions exist among GPCRs (dos Reis et al., 2019). In our study, common GO terms and KEGG pathways enriched with differentially expressed GPCRs were identified in *C. gigas* under various environmental stresses. It has been reported that there was an association of temperature, salinity and disease outbreaks in molluscs (Guo and Ford, 2016; Lowe et al., 2017; Zhang et al., 2016). GPCRs involved in oxidative stress, desiccation stress, food intake and growth, energy metabolism in insects and molluscs were widely reported in insects and molluscs (Balbi et al., 2019; Cardoso et al., 2016; Klein et al., 2021; Li et al., 2016; Liu et al., 2021). Such cellular reactions were commonplace in oysters under environmental stresses, such as heat stress, heavy metal stress, salinity stress, tidal fluctuation and pathogen infection (Barbosa Solomieu et al., 2015; Li et al., 2017, 2022; Lowe et al., 2017; Tian et al., 2021; Zhang et al., 2016). Therefore, our results imply that these GPCRs could be well orchestrated to play critical roles in stress responses. Considering the mechanisms of GPCRs in regulation of downstream

Table 1
KEGG pathways of differentially expressed GPCRs in *C. gigas* in response to environmental stresses.

Stress condition	KEGG pathway	*q*-Value
Heat stress	G protein-coupled receptors	1.38E-24
	Neuroactive ligand-receptor interaction	2.64E-12
	Vascular smooth muscle contraction	0.000130667
	Calcium signaling pathway	0.000787018
	Endocrine and other factor-regulated calcium reabsorption	0.011725382
	Autoimmune thyroid disease	0.018716356
Low-salinity stress	G protein-coupled receptors	1.26E-42
	Neuroactive ligand-receptor interaction	6.36E-18
	Calcium signaling pathway	3.04E-09
	Gastric acid secretion	0.00010655
	Vascular smooth muscle contraction	0.001122511
	Renin secretion	0.007615364
High-salinity stress	G protein-coupled receptors	4.09E-21
	Neuroactive ligand-receptor interaction	1.62E-06
	Olfactory transduction	0.002157514
	Vascular smooth muscle contraction	0.007718685
	Relaxin signaling pathway	0.016171041
Air exposure stress	G protein-coupled receptors	3.87E-34
	Neuroactive ligand-receptor interaction	3.82E-16
	Calcium signaling pathway	1.18E-05
	Renin secretion	0.002210273
	Vascular smooth muscle contraction	0.002318237
	Taste transduction	0.011363899
	Salivary secretion	0.015110952
	Gastric acid secretion	0.015278579
	Inflammatory mediator regulation of TRP channels	0.015278579
Heavy metal stress	G protein-coupled receptors	1.25E-28
	Neuroactive ligand-receptor interaction	1.95E-16
	Vascular smooth muscle contraction	5.05E-05
	Endocrine and other factor-regulated calcium reabsorption	0.000161556
	Parathyroid hormone synthesis, secretion and action	0.001412832
	Cushing syndrome	0.002385985
	Renin secretion	0.004521766
	Inflammatory mediator regulation of TRP channels	0.005095599
	Relaxin signaling pathway	0.008469043
	Calcium signaling pathway	0.011888109
	Human cytomegalovirus infection	0.011888109
	Adrenergic signaling in cardiomyocytes	0.017842671
OsHV-1 challenge	G protein-coupled receptors	1.81E-61
	Neuroactive ligand-receptor interaction	9.65E-37
	Calcium signaling pathway	1.07E-09
	Gastric acid secretion	2.99E-08
	Renin secretion	2.14E-07
	Inflammatory mediator regulation of TRP channels	3.89E-07
	Human cytomegalovirus infection	1.21E-05
	Endocrine and other factor-regulated calcium reabsorption	0.003850107
	Cocaine addiction	0.006193008
	Parathyroid hormone synthesis, secretion and action	0.030551558
Vibrio challenge	G protein-coupled receptors	6.18E-33
	Neuroactive ligand-receptor interaction	8.30E-17
	Inflammatory mediator regulation of TRP channels	0.000129024
	Calcium signaling pathway	0.000700653
	Gastric acid secretion	0.001101394
	Renin secretion	0.00890108
	Autoimmune thyroid disease	0.009881022
	Relaxin signaling pathway	0.014073537
	Human cytomegalovirus infection	0.023940603

signaling cascades, we speculated that the identified functional GPCRs mediated the complex transcriptomic response to stresses and contributed to the sophisticated adaptations of *C. gigs* to a sessile life in the highly stressful marine environment.

5. Conclusion

In this study, we identified a complete set of 586 GPCRs in *C. gigas* genome, adding the information about GPCRs evolution in marine molluscs. The *C. gigas* GPCRs were divided into five classes (including class A, B, C, E and F) with different degrees of expansion in comparison with other species. Expression analysis of the *C. gigas* GPCRs using multiple RNA-seq datasets suggested their distinctive expression patterns in response to various marine environmental stresses, including temperature, salinity, air exposure, heavy metal (Cd), OsHV-1 challenge and vibrio challenge. Identification of stress-specific differentially expressed GPCRs suggested their specific roles under certain stress conditions. The pervasive gene expansion and distinct transcriptional regulation of GPCRs implied an important role in adaptation to the complicated ocean system in the oyster. This work for the first time characterized the GPCR gene family and provided insights into the potential roles of GPCRs in adaptation of marine molluscs to stressful coastal environment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.114269.

CRediT authorship contribution statement

Huiru Fu: Investigation, Formal analysis, Writing – original draft. **Jing Tian:** Investigation. **Chenyu Shi:** Investigation. **Qi Li:** Supervision, Resources. **Shikai Liu:** Supervision, Resources, Project administration, Writing – review & editing.

Declaration of competing interest

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

Data accessibility statement

The datasets used in this study are publicly available in the databases of NCBI and CNGBdb. Raw sequences can be found with NCBI accession numbers of PRJNA146329, PRJNA282703 and PRJNA756710, and CNGBdb accession number of CNP0003351.

Acknowledgements

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