Expression analysis of the irak1 gene in Siniperca chuatsi against Aeromonas hydrophila and SNP development

Yu-xun Zhang, Jin-hua Gao , Xiao-li Yao, Yu-fei Liu , Gazahegn Wakjira Yadata , Jin-liang Zhao *, Yan Zhao *

Abstract

The interleukin-1 receptor-associated kinase 1 (*irak1*) gene is a key regulator in the innate immunity of fish. In this study, bioinformatics analyses of *irak1* from *Siniperca chuatsi* were performed, and quantitative real-time PCR was used to examine the expression profiles of *irak1* following *Aeromonas hydrophila* infection. Spatial localization of *irak1* mRNA and *Aeromonas hydrophila* DNA was examined using double-label in situ hybridization. Single nucleotide polymorphism (SNP) sites in *irak1* were identified by Sanger sequencing, and their association with resistance to *Aeromonas hydrophila* was analyzed. The results showed that the full-length of *irak1* was 2,264 bp with a Death domain and a PKC domain. Following infection with *Aeromonas hydrophila*, *irak1* was significantly upregulated in the head kidney, spleen, and liver of *Siniperca chuatsi* ($P < 0.05$). In the liver, *irak1* mRNA and *Aeromonas hydrophila* exhibit a high degree of spatial overlap. Four SNP loci were successfully genotyped. Both SNP2 and SNP4 were significant loci. However, SNP2 did not fit the Hardy-Weinberg equilibrium (HWE). Taken together, it is concluded that the *irak1* gene played an immune regulatory role in resistance to *Aeromonas hydrophila*. The SNP4 locus showed a highly significant association with resistance to *Aeromonas hydrophila* and could be a potential molecular marker, but its specific function requires further investigation.

Highlight

1.The disease-resistant SNP locus (g.31029300 G > T) of *irak1* gene was first found in *Siniperca chuatsi*.
2. The mRNA of *irak1* gene was co-localized with *Aeromonas hydrophila* DNA.
3.The *irak1* gene plays an immunoregulatory role in resistance to *Aeromonas hydrophila* infection.

Table.1 Association analysis between SNP sites and A. hydrophila resistance traits in irak1 gene

Name	Genotype	SG	RG	χ2	P value	Name	Genotype	SG	RG	χ2	P value
	AA	14	13	2.305	0.316		TT	25	27	1.314	0.518
SNP1	AT	8	4			SNP3	CT	7	9		
	TT	16	22				CC	6	3		
	TT	13	11	7.829	0.02*		TT	2	4	11.369	0.003**
SNP2	CT	14	25			SNP4	GT	7	20		
	CC	11	3				GG	29	15		

The χ^2 value for SNP1 and SNP3 were 2.305 ($P = 0.316$) and 1.314 ($P = 0.518$), respectively. while those for SNP2 and SNP4 were 7.829 ($P = 0.02$) and 11.369 ($P = 0.003$), respectively.

Results and discussion

- **Multiple sequence alignment and phylogenetic tree construction of the S. chuatsi IRAK1 protein**

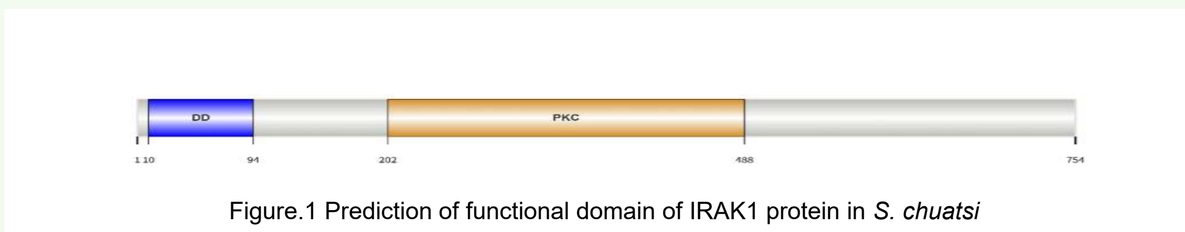

Figure.1 Prediction of functional domain of IRAK1 protein in *S. chuatsi*

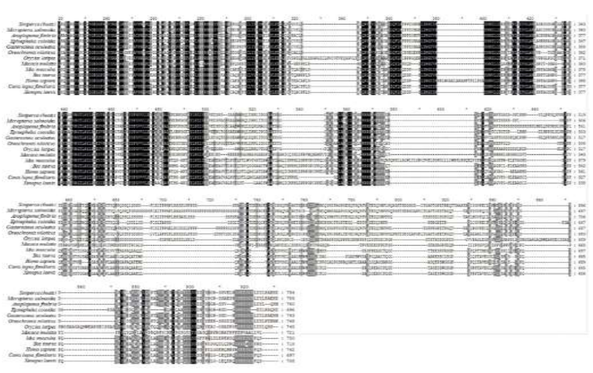

Figure.2 Multiple alignment of amino acid sequences of IRAK1

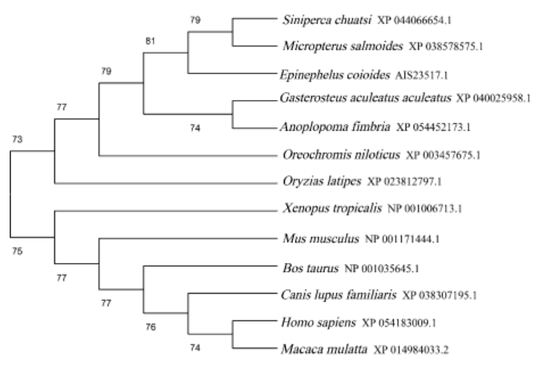

Figure.3 The phylogenetic tree analysis of IRAK1

The phylogenetic tree divided the 13 animal species into four clades. The IRAK1 protein has two domains, namely PKC and Death domains. By comparing the amino acid sequences of IRAK1 in 13 species, it was found that the PKC and Death domains of the IRAK1 protein are relatively conserved in evolution.

- **Expression of irak1 gene in different tissues of healthy S. chuatsi and in the head kidney, spleen, liver and muscle following infection with A. hydrophila**

The *irak1* gene was expressed in seven tissues of the mandarin fish, with expression levels decreasing in the following order: liver, stomach, spleen, gut, gill, head kidney, and muscle.

Following infection of mandarin fish with *A. hydrophila*, the expression of the *irak1* gene in liver, spleen and head kidney showed a significant upregulation over time. The significant upregulation of the *irak1* gene in the head kidney (6 hpi) and spleen (6 hpi) occurred earlier than in the liver (36 hpi).

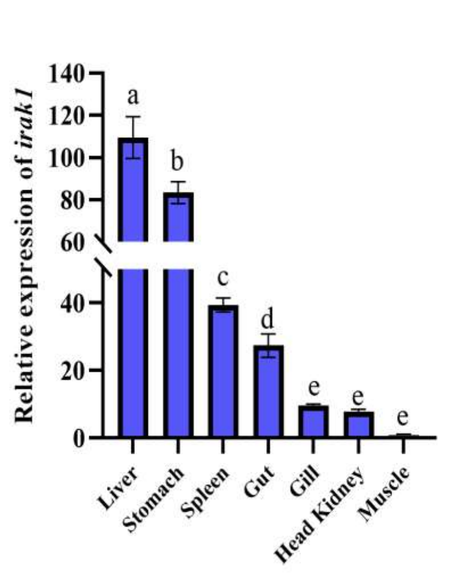

Figure.4 Expression of *irak1* gene in different tissues of *S. chuatsi*

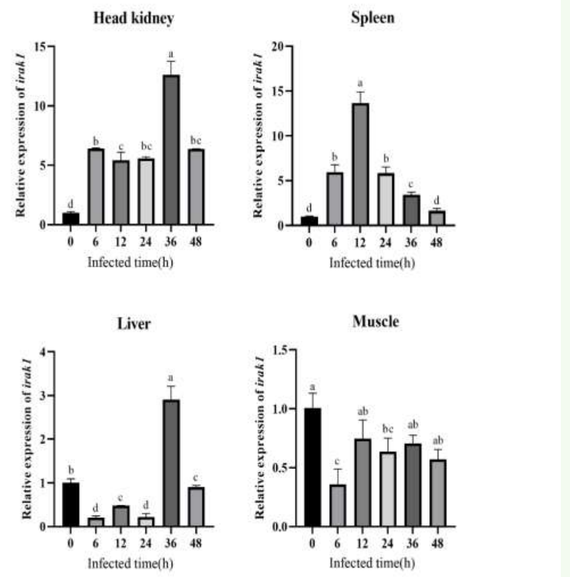

Figure. 5 Expression of *irak1* gene in *S. chuatsi* after infection with *A. hydrophila*

- **Spatial localization of *irak1* mRNA and *Aeromonas hydrophila* DNA**

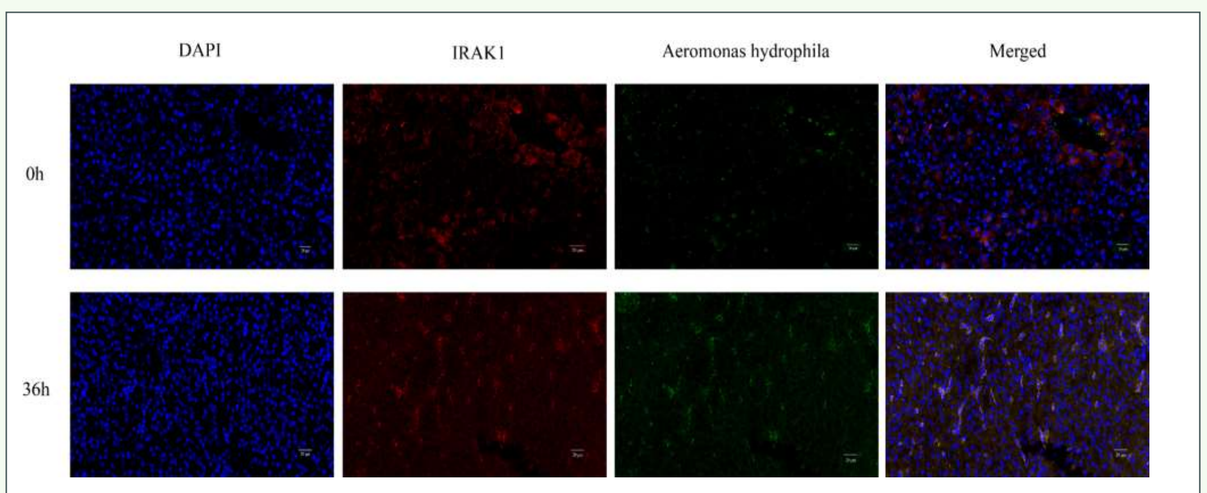

Figure.6 The location of *irak1* mRNA and *A. hydrophila* DNA in the liver of *S. chuatsi*, scale bar = 20 μm

Dual-label in situ hybridization results showed that *irak1* mRNA exhibited a high degree of overlap with *A. hydrophila* DNA locations in liver of mandarin fish at 36 hpi, further confirming the role of the *irak1* gene in mandarin fish resistance to *A. hydrophila*.

Conclusion

The *irak1* gene is expressed in muscle, head kidney, gut, liver, spleen, stomach and gills, with the highest expression in the liver. Following infection with *A. hydrophila*, *irak1* expression is significantly up-regulated, contributing to resistance against *A. hydrophila* invasion. The SNP4 locus shows a highly significant association with resistance to *A. hydrophila* and could be a potential molecular marker, but its specific function requires further investigation.

National Demonstration Center for Experimental Fisheries Science Education (Shanghai Ocean University), Shanghai 201306, China
Key Laboratory of Freshwater Aquatic Genetic Resources, Ministry of Agriculture and Rural Affairs, Shanghai Ocean University, Shanghai 201306, China
Shanghai Collaborative Innovation Center for Cultivating Elite Breeds and Green-culture of Aquaculture animals, Shanghai, 201306, China