Vol. 133: 253–261, 2019
https://doi.org/10.3354/dao03349

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published online April 11

MHC Class IIB gene polymorphisms associated with resistance/susceptibility to *Streptococcus agalactiae* in Nile tilapia *Oreochromis niloticus*

Feng-Ying Gao[1,2], Dong Zhang[1,2,3], Mai-Xin Lu[1,2,*], Jian-Meng Cao[1,2], Zhi-Gang Liu[1,2], Xiao-Li Ke[1,2], Miao Wang[1,2], De-Feng Zhang[1,2]

[1]Pearl River Fisheries Research Institute, Chinese Academy of Fishery Science, Guangzhou 510380, PR China
[2]Key Laboratory of Tropical & Subtropical Fishery Resource Application & Cultivation, Ministry of Agriculture, Guangzhou 510380, PR China
[3]National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai 201306, PR China

ABSTRACT: Genetic variation in the major histocompatibility complex (MHC) Class IIB was tested in Nile tilapia *Oreochromis niloticus*, and the association between the MHC IIB alleles and disease resistance was also studied. F_3 fry offspring (n = 1200) from 12 full-sib families were challenged with *Streptococcus agalactiae*, which caused significantly different mortalities in different Nile tilapia families (11.00–81.10%). Twenty fry (F_1) from each of the 12 families were selected to study the polymorphisms of the MHC Class IIB gene using PCR followed by cloning and sequencing methods. The results showed that the size of the amplified fragment was 770–797 bp. Thirty-seven sequences from 240 individuals revealed 22 different alleles, which belonged to 9 major allele types. Up to 63.58% of nucleotide positions were variable, while the proportion of the amino acid variable positions was up to 68.73%. According to the survival rate of offspring (F_3) from 12 full-sib families, we deduced that the alleles *Orni-DAB*0107*, *Orni-DAB*0201* and *Orni-DAB*0302* were highly associated with resistance to *S. agalactiae*, while the allele *Orni-DAB*0701* was associated with susceptibility to *S. agalactiae*. In addition, our previous study found that the allele *Orni-DAB*0201* was more frequently distributed in the disease-resistant groups. Therefore, the allele *Orni-DAB*0201* could be used as an *S. agalactiae* resistance-related MHC marker in molecular marker-assisted selective breeding programs for *S. agalactiae*-resistant Nile tilapia.

KEY WORDS: Nile tilapia · MHC Class IIB · *Streptococcus agalactiae* · Disease resistance

1. INTRODUCTION

The major histocompatibility complex (MHC) is a genetic region in the chromosome that consists of a series of closely linked loci; it encodes a class of cell surface transmembrane proteins that bind to T lymphocytes and present endogenous and exogenous antigens to the lymphocytes (Zhao et al. 2013). Two classes of MHC are found in fish (and most other jawed vertebrates with a few exceptions), i.e. MHC Class I and Class II molecules. MHC Class III is defined as a region in mammalian MHC, but it does not exist in fish. The MHC Class I molecule, consisting of 1 alpha chain and 1 β2-microglobulin, presents foreign peptides produced by the degradation of intracellular pathogens to cytotoxic $CD8^+$ T cells (Srisapoome et al. 2004). The MHC Class II molecule, consisting of 1 alpha chain and 1 beta chain, presents

*Corresponding author: mx-lu@163.com

foreign and endogenous peptides derived from extracellular pathogens to helper $CD4^+$ T cells (Kjøglum et al. 2006).

Since Hashimoto et al. (1990) reported the first MHC genes in carp, MHC Class I, Class IIA and Class IIB genes have been isolated and characterized in a large variety of fish species, including zebrafish (Sültmann et al. 1994), turbot (Zhang & Chen 2006), red sea bream (Chen et al. 2006), tongue sole (Xu et al. 2009) and Nile tilapia (Pang et al. 2013, Li et al. 2014). MHC Class I and II genes are found on the same linkage groups in all studied mammals; however, in teleosts, the MHC Class II genes are not linked to MHC Class I genes (Bingulac-Popovic et al. 1997, Naruse et al. 2000, Sambrook et al. 2002).

MHC genes are obvious candidates for use in breeding programs, as they have an important role in both the innate and adaptive immune response. Moreover, specific MHC alleles have been correlated with disease resistance in non-fish species, including chickens (Briles et al. 1983), mice (Medina & North 1998), sheep (Paterson et al. 1998) and humans (Hill et al. 1991). In fish, the association between the MHC polymorphisms and disease resistance has been investigated in Japanese flounder, turbot, tongue sole *Cynoglossus semilaevis*, common carp and grass carp (Zhang et al. 2006, Xu et al. 2008, 2010, Rakus et al. 2009, Du et al. 2011, 2012, Yu et al. 2013), as well as in salmonid species (Palti et al. 2001, Lohm et al. 2002, Grimholt et al. 2003, Kjøglum et al. 2006, 2008, Glover et al. 2007, Wynne et al. 2007, J. Yang et al. 2016). Recently, specific alleles of MHC Class IIB have been documented to correlate with some viral and bacterial diseases in orange-spotted grouper and pompano (M. Yang et al. 2016, Zhu et al. 2018). In orange-spotted grouper, the MHC IIB allele *EPCODBB*1001* was significantly associated with resistance to Singapore grouper iridovirus (M. Yang et al. 2016). In pompano, the *TO-DAB-01* allele was associated with immunity to *Photobacterium damselae*, and the *TO-DAB-04*, *TO-DAB-05* and *TO-DAB-10* alleles were associated with its sensitivity to *P. damselae* (Zhu et al. 2018).

Tilapia are among the most important freshwater aquaculture species in the world. However, tilapia farming has been severely threatened since 2009 due to infection by *Streptococcus agalactiae* (Lu et al. 2010, Shoemaker et al. 2017a,b), the pathogen of streptococcosis (Shoemaker et al. 2017a). To date, an efficient means to prevent the disease has not been found, although breeding of new stock with enhanced disease resistance may provide a foundation for disease prevention. Screening and application of molecular markers in marker-assisted selection is a good approach for disease resistance breeding. In recent years, we carried out breeding of Nile tilapia for disease resistance and analyzed the molecular polymorphisms of the MHC IIA and MHC IIB genes in this species (Pang et al. 2013, Gao et al. 2014). Pang et al. (2013) identified MHC IIA (GenBank accession no. JN967619) and MHC IIB (JN967618) genes and investigated their cDNA polymorphisms. Gao et al. (2014) identified 7 alleles of the MHC IIB gene and found that the alleles *Orni-DAB*0201* and *Orni-DAB*0401* were significantly more prevalent in resistant individuals than in susceptible individuals, with 15.5 and 5.6% in resistant stock and 7.4 and 1.8% in susceptible stock, respectively; *Orni-DAB*0501* and *Orni-DAB*0601* were only found in resistant individuals. We found 25 MHC IIA alleles, among which *Orni-DAA*1101* was significantly associated with susceptibility to *S. agalactiae* (F. Y. Gao unpubl. data). We established a full-sib family of Nile tilapia and bred them to the F_3 generation. In this study, we assessed the relationship between MHC IIB gene molecular polymorphisms of F_1 and disease resistance of F_3 Nile tilapia, and laid a foundation for establishing molecular breeding technology for disease-resistant Nile tilapia.

2. MATERIALS AND METHODS

2.1. Fish families

Our laboratory team established 20 Gift strain Nile tilapia families in the Gaoyao fish farm of Pearl River Fisheries Research Institute (Guangzhou, China). Based on their disease resistance, 12 families were selected for research. Each family was kept in a separate tank. The fish were fed twice daily with a commercial diet (30% crude protein) at a rate of 3% of their body weight.

2.2. Challenge experiments

A total of 1224 offspring (F_3) from 12 full-sib families were used in the challenge experiment. Random samples of 102 fish from each family were divided into 3 replicate batches of 34 individuals. The body weight of the fish analyzed was 60–80 g. The test fish of each family were kept in 500 l tanks with a fresh water supply at 30–32°C with water flow throughout the experiment and were fed twice daily. Water quality parameters, including dissolved oxygen, pH and

ammonia nitrogen, were monitored during the experiment.

Streptococcus agalactiae (group B *Streptococcus*, capsular type Ia [ST7], beta-hemolytic) isolated by our laboratory (strain WC1535) was used in the challenge experiment. *S. agalactiae* was cultured at 37°C for 15 h until the density reached approximately 1×10^9 CFU ml^{-1}. The bacterial suspension was then diluted to 10^8, 10^7 and 10^6 CFU ml^{-1} in phosphate-buffered saline (PBS, pH 7.2). Twenty-five fish (F_1 generation individuals from all 12 of the assessed families) were used in each group (1 control group and 3 challenge groups) in the prechallenge experiment to determine the 50% lethal dose (LD_{50}). Bacterial suspension (0.1 ml) was injected intraperitoneally (i.p.) into each individual in the 3 challenge groups. The control group was injected with an equal amount of PBS. According to the prechallenge experiment, the LD_{50} was 1×10^7 CFU ml^{-1}, and this concentration was used for the formal experiment. For the challenge experiment in Nile tilapia, 102 fish (randomly selected and divided into 3 replicate batches of 34 individuals) from each family were inoculated by intraperitoneal injection of 0.1 ml *S. agalactiae* bacterial suspension (1×10^7 CFU ml^{-1}), while 102 individuals (randomly selected and divided into 3 replicate batches of 34 individuals) were i.p. injected with the same volume of PBS in the control group. The challenge trial lasted for 2 wk, and mortality was recorded for every family.

2.3. Sample collection and DNA isolation

To examine whether MHC Class IIB alleles are associated with resistance/susceptibility to *S. agalactiae*, fin samples of 20 F_1 individuals (the ancestors of the F_3 of the 12 families described in Section 2.2) from each family were collected in the challenge trials. A total of 240 caudal fin samples were preserved individually in 100% ethanol. Genomic DNA was extracted from tilapia fin samples using a HiPure Mollusc DNA Mini Kit (Magen) according to the manufacturer's instructions. The quality and concentration of DNA were assessed by agarose gel electrophoresis and measured with a Tiangen spectrophotometer. DNA was stored at −20°C for use.

2.4. Primer design and polymerase chain reaction (PCR)

A pair of oligonucleotides as gene-specific primers, 2b-snp-sf (5′-CTG GGA TTT GGT ACA GAA ACG-3′) and 2b-snp-sr (5′-ATT AGA GTT CCT TCA GGC TG-3′), were designed according to the Nile tilapia MHC IIB genomic sequence (GenBank accession no. JN967618) and were used for amplifying partial sequences of Nile tilapia MHC IIB. The amplified fragments of the MHC IIB gene contained part of exon 2 and exon 4 and the complete intron 2, exon 3 and intron 3. PCR was performed in a total volume of 50 µl, which contained approximately 100 ng template DNA, 25 µl of Ex Taq Version 2.0 plus dye (TaKaRa), 0.4 µM forward primer and 0.4 µM reverse primer. PCR conditions were 94°C for 3 min; followed by 30 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 60 s; and a final extension at 72°C for 5 min. PCR was performed on a Lab Cycle Standard Plus. The PCR products were detected on 1% agarose gel.

2.5. Cloning and sequencing

PCR products were separated by electrophoresis on 1.5% agarose gels, and the desired DNA band was extracted using HiPure Gel Pure DNA Kits (Magen). The purified fragments were cloned into a PMD-19T vector (TaKaRa) according to the standard PMD-19T vector protocol (TaKaRa) and then transformed into *E. coli* DH5 Competent Cells (TaKaRa). Six positive clones from each individual were sequenced with an ABI 3730xl automated sequencer using the M13+/− primers (Genewiz).

2.6. Sequence analysis and statistical tests

All MHC IIB sequences were analyzed using DNASTAR 8.1 software. The polymorphism data of the sequences were analyzed with MEGA 7.0 and DNAsp 5.0 software. Statistical differences were determined by 1-way ANOVA followed by Duncan's multiple range test. Statistical analysis was performed with SPSS 23.0 software.

The new alleles were identified and named *Orni-DAB*0101* to *Orni-DAB*0901* on the basis of the rules reported by Gao et al. (2014). The abbreviation Orni refers to *Oreochromis niloticus*, and DAB refers to MHC Class IIB encoding genes. In the 4 digits following an asterisk, the first 2 digits refer to the major type (at least 5 amino acid substitutions), and the next 2 digits refer to the subtype (fewer than 5 amino acid substitutions) (Xu et al. 2008).

3. RESULTS

3.1. Disease resistance comparison

The first specific mortality due to injection of *Streptococcus agalactiae* appeared after 20 h, and each family had the highest mortality rates in the 24–48 h period. The entire challenge test lasted 2 wk, at which time the overall accumulated mortality reached 41.76%. The survival rates among the 12 test families ranged from 19 to 89%, which was determined on the basis of each family (Fig. 1). The dissolved oxygen concentrations in all tanks were 2–3 mg l^{-1}. The pH levels of water in all the tanks ranged from 7.5–8.2, and the ammonia nitrogen levels of water in all tanks ranged from 0.2–0.5 mg l^{-1}.

3.2. Sequence polymorphism within part of the MHC IIB gene

F_1 individuals (n = 240) from 12 families were analyzed. Six positive clones per individual were sequenced, and 1423 sequences were obtained. The size of the amplified fragment was 770–797 bp. Based on sequence alignment with Nile tilapia MHC IIB complete cDNA sequences (Pang et al. 2013), 774–797 bp fragments spanned the partial exon 2 (36 bp) and exon 4 (57 bp) of MHC IIB, complete exon 3 (214 bp), complete intron 2 (249–270 bp), and complete intron 3 (213–225 bp). These fragments revealed 22 different alleles, which belonged to 9 major allele types following established allele nomenclature methods (GenBank accession nos. MG882352–MG882373; Table A1 in the Appendix). A fragment of 307 bp containing partial exon 2, which encodes the β1 domain, complete exon 3 and partial exon 4, which encodes the β2 domain of the MHC IIB gene, were analyzed. The 307 bp fragment encodes 101 amino acids, which included the partial β1 domain (11 amino acids) and partial β2 domain (90 amino acids). The 22 different nucleotide sequences translated to 16 distinct amino acid sequences (Fig. 2).

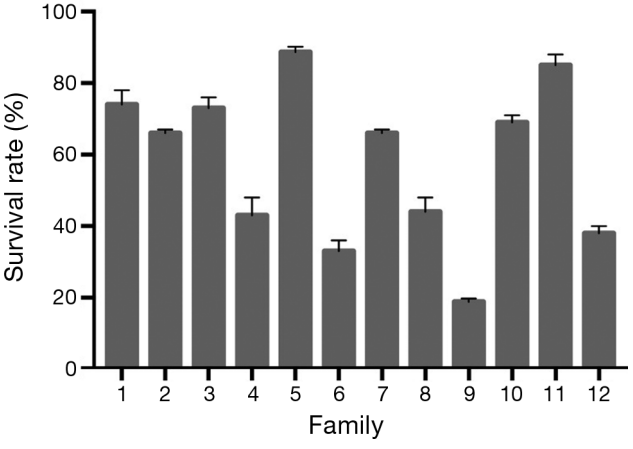

Fig. 1. Survival rates of F_3 of 12 Nile tilapia families after infection with *Streptococcus agalactiae*

The polymorphism values were calculated by MEGA 7.0 and DnaSP 8.1 software. A total of 522 variable nucleotide sites were detected in this experiment, including 338 parsimony-informative sites. Up to 63.58% nucleotide positions were variable, while the proportion of the amino acid variable positions reached 68.73%. The average nucleotide diversity (π) value was 0.020, and the haplotype diversity (Hd) was 0.942. The average number of nucleotide differences (k) was 14.529, and the theta value per site (Theta-W) was 0.0951. Fig. 3 shows the spatial distribution of the nucleotide diversity in the analyzed fragment of the MHC IIB gene. The peak appeared upstream of the sequences, and most of the Theta-W values were between 0.08 and 0.1.

3.3. Allele distributions in the 12 Nile tilapia families

Table 1 shows the number of alleles per individual and the comparative individual number. We sequenced approximately 6 clones ind.$^{-1}$, and discovered 1–4 alleles ind.$^{-1}$. We found that 34.16% of individuals had 2–4 MHC IIB alleles (Table 1). Of the 12 families, 10 contained more than 2 alleles; 60% of individuals in family 7 had 3–4 alleles, and 55% of individuals in family 9 had more than 2 alleles, whereas individuals from family 8 and family 12 had only 1 allele.

In total, 22 different alleles were identified from the 240 individuals, and their distribution numbers in each family are displayed in Table 2. The allele numbers were distributed unequally, with some alleles present at a low frequency or only existing in 1 family. We found 7 different alleles in family 7 and 5 different alleles in family 4. The *Orni-DAB*0101* allele was distributed in all 12 families (60.8–100%), and *Orni-DAB*0301* existed in 9 families (1.7–16.9%). *Orni-DAB*0201* was detected only in families 4 and 7 (13.6 and 21.7%, respectively), *Orni-DAB*0302* was found only in family 3 (15%), and *Orni-DAB*0701* was found only in families 9 and 11 (11.0 and 1.7%, respectively) (Table 2).

Fig. 2. Putative amino acid sequences for MHC IIB alleles of Nile tilapia; the putative peptide binding region is indicated with asterisks. Yellow: amino acid (a.a) residue present in 100% of sequences; blue: a.a. residue present in >50% of sequences; green: a.a. residue present in 10–50% of sequences

3.4. Association between alleles and resistance to *S. agalactiae*

Alleles with a low frequency (<1%) have no value as molecular markers, and these alleles were excluded from the distribution analysis when analyzing the association between alleles and resistance to disease. Here, 9 alleles were used for distribution analysis (Table 2). Varied distribution frequencies of alleles in each family were observed. For example, *Orni-DAB*0201* was significantly more frequent in individuals of family 7 (22%) than in individuals of family 4 (14%) among all families ($p < 0.01$), and the survival rate in the offspring of family 7 (66%) was significantly greater than in the offspring of family 4 (43%). *Orni-DAB*0107* was present only in family 7 ($p < 0.05$), while the survival rate in the offspring of family 7 reached 66%. The frequency of *Orni-DAB*0302* in family 3 was only 15%, while the survival rate in the offspring of family 3 reached 73%. The frequency of *Orni-DAB*0701* in family 11 was significantly lower than in family 9 ($p < 0.01$), while the survival rate was

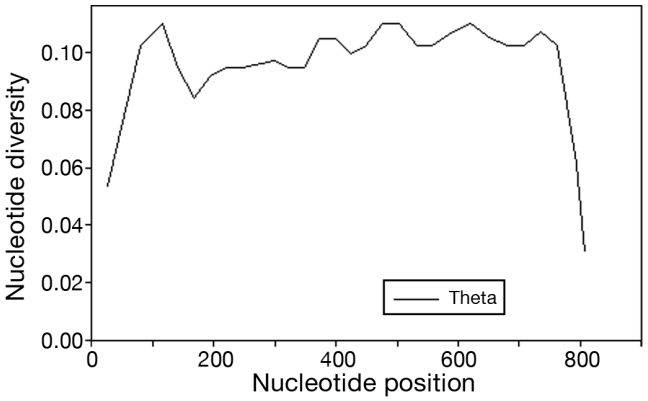

Fig. 3. Nucleotide diversity of the MHC IIB genes of the 22 alleles denoted by Theta-W. Sliding window length: 50; step size: 25

Table 1. Number of MHC Class IIB *Orni-DAB* alleles per fish in each Nile tilapia family and their corresponding individual numbers. Each family consisted of 20 individuals

Family	1 allele	2 alleles	3 alleles	4 alleles
1	14	5	1	0
2	19	1	0	0
3	12	5	2	1
4	5	9	4	2
5	16	2	2	0
6	18	1	0	1
7	2	6	11	1
8	20	0	0	0
9	7	2	9	2
10	9	9	1	1
11	16	1	3	0
12	20	0	0	0
Total	158	41	33	8

significantly higher in family 11 (85%) than in family 9 (18.9%). The frequencies of *Orni-DAB* 0301* were significantly different in some families, but the survival rate did not differ significantly in the offspring of those families. We therefore deduced that *Orni-DAB* 0107*, *Orni-DAB* 0201* and *Orni-DAB* 0302* are highly associated with resistance to *S. agalactiae* in Nile tilapia, while *Orni-DAB* 0701* is associated with susceptibility to *S. agalactiae*.

4. DISCUSSION

As one of the members of the MHC family, MHC IIB plays important roles in the innate and adaptive immune systems. Previous studies found that the MHC IIB cDNA and genome have many polymorphisms (Pang et al. 2013, Gao et al. 2014). In the current study, the association between the MHC IIB alleles and disease resistance of 12 Nile tilapia full-sib families was investigated.

Table 2. Frequency of 9 MHC Class IIB alleles (>1%) in each of 12 Nile tilapia families (20 ind. family^{-1}, 6 clones ind.$^{-1}$). Asterisks in 'Frequency' column indicate a significant differences in the distribution frequency of the allele within each of the 12 families ($^{*}p < 0.05$, $^{**}p < 0.01$)

Family	Allele(s) found	Number	Frequency
1	*Orni-DAB* 0101*	108	0.92
1	*Orni-DAB* 0301*	8	0.07**
2	*Orni-DAB* 0101*	118	0.99
3	*Orni-DAB* 0101*	80	0.67**
3	*Orni-DAB* 0301*	19	0.16**
3	*Orni-DAB* 0302*	18	0.15**
4	*Orni-DAB* 0101*	84	0.71
4	*Orni-DAB* 0201*	16	0.14**
4	*Orni-DAB* 0301*	12	0.10**
4	*Orni-DAB* 0305*	2	0.02
4	*Orni-DAB* 0306*	2	0.02
5	*Orni-DAB* 0101*	107	0.91
5	*Orni-DAB* 0301*	9	0.08**
6	*Orni-DAB* 0101*	116	0.97
6	*Orni-DAB* 0301*	2	0.02
7	*Orni-DAB* 0101*	73	0.61
7	*Orni-DAB* 0201*	26	0.22**
7	*Orni-DAB* 0301*	9	0.08**
7	*Orni-DAB* 0107*	3	0.03*
7	*Orni-DAB* 0102*	2	0.02
7	*Orni-DAB* 0305*	2	0.02
7	*Orni-DAB* 0306*	2	0.02
8	*Orni-DAB* 0101*	118	1.00
9	*Orni-DAB* 0101*	83	0.70
9	*Orni-DAB* 0301*	20	0.17**
9	*Orni-DAB* 0701*	13	0.11**
10	*Orni-DAB* 0101*	98	0.82
10	*Orni-DAB* 0301*	18	0.15**
11	*Orni-DAB* 0101*	110	0.92
11	*Orni-DAB* 0301*	6	0.05*
11	*Orni-DAB* 0701*	2	0.02
12	*Orni-DAB* 0101*	115	1.00**

A total of 1423 sequences from 240 individuals of 12 families revealed 22 different alleles (π = 0.020, Hd = 0.942, k = 14.529). Among them, there were 15 new alleles. The association between the MHC IIB polymorphisms and disease resistance ability has been reported in many fish species. In tongue sole, 88 MHC IIB alleles were found in 1618 sequences of 8 families (Du et al. 2011). Thirty-seven MHC IIB alleles were found in 6 families of turbot (Du et al. 2012). In orange-spotted grouper, a total of 33 MHC IIB alleles were identified from 40 high-susceptibility and 40 high-resistance individuals (M. Yang et al.

2016). Zhu et al. (2018) identified 43 different MHC IIB alleles from *Trachinotus ovatus*. In the current study, 22 MHC IIB alleles were identified in 240 individuals of 12 Nile tilapia families. The polymorphism of Nile tilapia in the current study was lower than in other reported fish species. This may be because, in our study, the amplified MHC IIB gene fragment only contained part of the β1 domain. This domain has high polymorphism as an antigen recognition region. It may also be the nature of the species, or it may be related to the evolution of Nile tilapia.

Several hypotheses have been presented to explain the reason for MHC gene polymorphisms, including heterozygosity, overdominant selection, frequency-dependent selection and positive selection (Doherty & Zinkernagel 1975, Takahata & Nei 1990, Slade & McCallum 1992). The 22 MHC IIB alleles identified in the current study were distributed unequally in the 12 families. Some alleles appeared only in a specific family. For example, alleles *Orni-DAB*0107* and *Orni-DAB*0102* only appeared in family 7; *Orni-DAB*0201*, *Orni-DAB*0305* and *Orni-DAB*0306* were only present in family 4 and family 7; *Orni-DAB*0302* only existed in family 3; and *Orni-DAB*0701* only appeared in family 9 and family 11. The 22 alleles of MHC IIB in each family showed frequency-dependent selection, thus maintaining the diversity of the MHC gene. The phenomenon of frequency-dependent selection of MHC IIB alleles may be due to the different genetic backgrounds of different families, or the result of pathogen-driven selection (Grimholt et al. 2003).

The association between MHC genetic polymorphisms and disease resistance or susceptibility has been reported in many teleosts. In brook charr *Salvelinus fontinalis*, the *Safo-DAB*0101* allele was associated with resistance to *Aeromonas salmonicida* (Croisetière et al. 2008). In common carp, genotype E of MHC IIB was associated with resistance to cyprinid herpesvirus-3 (CyHV-3), and genotypes B, H and J were associated with susceptibility to CyHV-3 (Rakus et al. 2009). The polymorphisms of grass carp MHC IIB were associated with resistance to *Flavobacterium columnare* (Yu et al. 2013). Allele *Scma-DBB1*02* of turbot MHC IIB was associated with resistance to *Edwardsiella tarda*, and *Scma-DBB1*10* was associated with susceptibility to *E. tarda* (Du et al. 2012). In tongue sole, 5 alleles were associated with susceptibility to *Vibrio anguillarum*, and 4 alleles were associated with resistance to *V. anguillarum* (Du et al. 2011). Zhu et al. (2018) reported that in *Trachinotus ovatus*, allele *TO-DAB-01* was associated with immunity to *Photobacterium damselae*, and the alleles *TO-DAB-04*, *TO-DAB-05* and *TO-DAB-10* were associated with sensitivity to *P. damselae*. Alleles *EPCO-DBB*1001* of grouper MHC IIB could be used as a disease resistance-related MHC marker in the molecular marker-assisted selection breeding program of grouper (M. Yang et al. 2016). In the current study, we found that the alleles *Orni-DAB*0107*, *Orni-DAB*0302* and *Orni-DAB*0201* were associated with resistance to *S. agalactiae,* and *Orni-DAB*0701* was associated with susceptibility to *S. agalactiae*. Our previous study showed that the MHC IIB alleles *Orni-DAB*0201* and *Orni-DAB*0401* had a high frequency in the disease-resistant population (Gao et al. 2014). These results indicate that allele *Orni-DAB*0201* can be used as a molecular marker for disease resistance breeding in Nile tilapia.

We found that the F_3 of family 5 and family 7 had higher survival rates after infection with *S. agalactiae*. However, no *S. agalactiae* resistance-related alleles (*Orni-DAB*0107*, *Orni-DAB*0201* and *Orni-DAB*0302*) were found in the F_1 individuals of these 2 families. This may be because the MHC IIB alleles in these 2 families are interlinked with other disease-resistance genes, rather than representing a disease-resistant allele of the MHC gene itself. It may also be because the 2 families have other MHC IIB alleles that are associated with *S. agalactiae* resistance (Rakus et al. 2009). The results also showed that F_1 individuals of family 9 possess more MHC IIB alleles. However, this family exhibited the lowest survival rate of F_3 individuals (18.90%). The distribution frequency of the *S. agalactiae* susceptibility-related allele *Orni-DAB*0701* in family 9 was 11%. This may be because individuals with this allele are more sensitive to *Streptococcus*, which caused the high mortality of family 9 after *S. agalactiae* infection. Our experiment demonstrated that a pathogen has the potential to place intense selection pressure on particular MHC alleles (Lohm et al. 2002). However, the results presented in this study were obtained from only 1 pathogen infection, and it is likely that the fitness of different alleles differs for different pathogens and shifts over time (Croisetière et al. 2008).

In summary, our study showed that MHC IIB of Nile tilapia has many polymorphisms, and there are at least 2 MHC IIB loci in Nile tilapia. Associations between specific MHC IIB alleles and resistance/susceptibility to *S. agalactiae* were observed. Alleles *Orni-DAB*0107*, *Orni-DAB*0302* and *Orni-DAB*0201* were associated with *Streptococcus* resistance, and allele *Orni-DAB*0701* was associated with *Streptococcus* susceptibility. These alleles can be used as molecular markers for disease resistance

breeding of Nile tilapia. Families with high survival could be used as the dominant group for the selection of strains resistant to *S. agalactiae* in the future.

Acknowledgements. This work was financially supported by the China Agriculture Research System (No. CARS-46), science and technology planning project of Guangdong Province (2016A020210022) and a grant from the National Natural Science Foundation of China (Grant 31402295).

LITERATURE CITED

Bingulac-Popovic J, Figueroa F, Sato A, Talbot WS and others (1997) Mapping of *Mhc* class I and class II regions to different linkage groups in the zebrafish, *Danio rerio*. Immunogenetics 46:129–134

Briles WE, Briles RW, Taffs RE, Stone HA (1983) Resistance to a malignant lymphoma in chickens is mapped to subregion of major histocompatibility (B) complex. Science 219:977–979

Chen SL, Zhang YX, Xu MY, Ji XS, Yu GC, Dong CF (2006) Molecular polymorphism and expression analysis of MHC II *B* gene from red sea bream (*Chrysophrys major*). Dev Comp Immunol 30:407–418

Croisetière S, Tarte PD, Bernatchez L, Belhumeur P (2008) Identification of MHC class IIβ resistance/susceptibility alleles to *Aeromonas salmonicida* in brook charr (*Salvelinus fontinalis*). Mol Immunol 45:3107–3116

Doherty PC, Zinkernagel RM (1975) Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. Nature 256:50–52

Du M, Chen SL, Liu YH, Liu Y, Yang JF (2011) MHC polymorphism and disease resistance to *Vibrio anguillarum* in 8 families of half-smooth tongue sole (*Cynoglossus semilaevis*). BMC Genet 12:78

Du M, Chen SL, Liu YH, Niu BZ, Yang JF, Zhang B (2012) MHC polymorphism and disease-resistance to *Edwardsiella tarda* in six turbot (*Scophthalmus maximus*) families. Chin Sci Bull 57:3262–3269

Gao FY, Pang JC, Lu MX, Zhu HP, Ke XL (2014) Major histocompatibility complex MHC IIB gene polymorphism and its association with resistance/susceptibility to *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*). Zhongguo Nongxue Tongbao 30:76–78

Glover KA, Grimholt U, Bakke HG, Nilsen F, Storset A, Skaala Ø (2007) Major histocompatibility complex (MHC) variation and susceptibility to the sea louse *Lepeophtheirus salmonis* in Atlantic salmon *Salmo salar*. Dis Aquat Org 76:57–65

Grimholt U, Larsen S, Nordmo R, Midtlyng P, Kjoeglum S (2003) MHC polymorphism and disease resistance in Atlantic salmon (*Salmo salar*); facing pathogens with single expressed major histocompatibility class I and II loci. Immunogenetics 55:210–219

Hashimoto K, Nakanishi T, Kurosawa Y (1990) Isolation of carp genes encoding major histocompatibility complex antigens. Proc Natl Acad Sci USA 87:6863–6867

Hill AVS, Allsopp CEM, Kwiatkowski D, Anstey NM and others (1991) Common West African HLA antigens associated with protection from severe malaria. Nature 352: 595–600

Kjøglum S, Larsen S, Bakke HG, Grimholt U (2006) How specific MHC class I and class II combinations affect disease resistance against infectious salmon anaemia in Atlantic salmon (*Salmo salar*). Fish Shellfish Immunol 21: 431–441

Kjøglum S, Larsen S, Bakke HG, Grimholt U (2008) The effect of specific MHC class I and class II combinations on resistance to furunculosis in Atlantic salmon (*Salmo salar*). Scand J Immunol 67:160–168

Li JP, Gao FY, Lu MX, Cao JM, Zhu HP, Ke XL, Liu ZG (2014) cDNA isolation, polymorphism analysis, and tissue expression of MHC I of Nile tilapia (*Oreochromis niloticus*). J Fish Sci China 21:1134–1145

Lohm J, Grahn M, Langefors A, Andersen Ø, Storset A, von Schantz T (2002) Experimental evidence for major histocompatibility complex-allele-specific resistance to a bacterial infection. Proc R Soc B 269:2029–2033

Lu MX, Li J, Ye X, Deng GCh, Jiang XY, Tian YY, Lai CL (2010) Identification and characterization of *Streptococcus agalactiae* isolated from tilapia cultured in Guangdong and Hainan provinces. Microbiol China 37: 766–774

Medina E, North RJ (1998) Resistance ranking of some common inbred mouse strains to *Mycobacterium tuberculosis* and relationship to major histocompatibility complex haplotype and *Nramp1* genotype. Immunology 93: 270–274

Naruse K, Fukamachi S, Mitani H, Kondo M and others (2000) A detailed linkage map of medaka, *Oryzias latipes*: comparative genomics and genome evolution. Genetics 154:1773–1784

Palti Y, Nichol KM, Waller JE, Parsons JE, Thorgaard GH (2001) Association between DNA polymorphism tightly linked to MHC class II genes and IHN virus resistance in backcrosses of rainbow and cutthroat trout. Aquaculture 194:283–289

Pang JC, Gao FY, Lu MX, Ye X, Zhu HP, Ke XL (2013) Major histocompatibility complex class IIA and IIB genes of Nile tilapia *Oreochromis niloticus*: genomic structure, molecular polymorphism and expression patterns. Fish Shellfish Immunol 34:486–496

Paterson S, Wilson K, Pemberton JM (1998) Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population. Proc Natl Acad Sci USA 95: 3714–3719

Rakus KŁ, Wiegertjes GF, Adamek M, Siwicki AK, Lepa A, Irnazarow I (2009) Resistance of common carp (*Cyprinus carpio* L.) to Cyprinid herpesvirus-3 is influenced by major histocompatibility (MH) class II *B* gene polymorphism. Fish Shellfish Immunol 26:737–743

Sambrook JG, Russell R, Umrania Y, Edwards YJ, Campbell RD, Elgar G, Clark MS (2002) Fugu orthologues of human major histocompatibility complex genes: a genome survey. Immunogenetics 54:367–380

Shoemaker CA, Xu DH, García JC, LaFrentz BR (2017a) Capsular typing of *Streptococcus agalactiae* (Lancefield group B streptococci) from fish using multiplex PCR and serotyping. Bull Eur Assoc Fish Pathol 37:190–197

Shoemaker CA, Lozano CA, LaFrentz BR, García JC and others (2017b) Additive genetic variation in resistance of Nile tilapia (*Oreochromis niloticus*) to *Streptococcus iniae* and *S. agalactiae* capsular type Ib: Is genetic resistance correlated? Aquaculture 468:193–198

Slade RW, McCallum HI (1992) Overdominant vs. frequency-dependent selection at MHC loci. Genetics 132: 861–864

Srisapoome P, Ohira T, Hirono I, Aoki T (2004) Cloning, characterization and expression of cDNA containing major histocompatibility complex class I, IIa and IIb genes of Japanese flounder *Paralichthys olivaceus*. Fish Sci 70:264–276

Sültmann H, Mayer WE, Figueroa F, O'Huigin C, Klein J (1994) Organization of *Mhc* class II B genes in the zebrafish (*Brachydanio rerio*). Genomics 23:1–14

Takahata N, Nei M (1990) Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. Genetics 124:967–978

Wynne JW, Cook MT, Nowak BF, Elliott NG (2007) Major histocompatibility polymorphism associated with resistance towards amoebic gill disease in Atlantic salmon (*Salmo salar* L.). Fish Shellfish Immunol 22:707–717

Xu TJ, Chen SL, Ji XS, Tian YS (2008) MHC polymorphism and disease resistance to *Vibrio anguillarum* in 12 selective Japanese flounder (*Paralichthys olivaceus*) families. Fish Shellfish Immunol 25:213–221

Xu TJ, Chen SL, Ji XS, Sha ZX (2009) Molecular cloning, genomic structure, polymorphism and expression analysis of major histocompatibility complex class IIA and IIB genes of half-smooth tongue sole (*Cynoglossus semilaevis*). Fish Shellfish Immunol 27:192–201

Xu TJ, Chen SL, Zhang YX (2010) MHC class IIa gene polymorphism and its association with resistance/susceptibility to *Vibrio anguillarum* in Japanese flounder (*Paralichthys olivaceus*). Dev Comp Immunol 34: 1042–1050

Yang J, Liu Z, Shi HN, Zhang JP, Wang JF (2016) Association between MHC II beta chain gene polymorphisms and resistance to infectious haematopoietic necrosis virus in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792). Aquacult Res 47:570–578

Yang M, Wei J, Li P, Wei S, Huang Y, Qin Q (2016) MHC polymorphism and disease resistance to Singapore grouper iridovirus (SGIV) in the orange-spotted grouper, *Epinephelus coioides*. Sci Bull (Beijing) 61:693–699

Yu H, Tan S, Zhao H, Li H (2013) MH-DAB gene polymorphism and disease resistance to *Flavobacterium columnare* in grass carp (*Ctenopharyngodon idellus*). Gene 526:217–222

Zhang YX, Chen SL (2006) Molecular identification, polymorphism, and expression analysis of major histocompatibility complex class IIA and B genes of turbot (*Scophthalmus maximus*). Mar Biotechnol 8:611–623

Zhang YX, Chen SL, Liu YG, Sha ZX, Liu ZJ (2006) Major histocompatibility complex Class IIB allele polymorphism and its association with resistance/susceptibility to *Vibrio anguillarum* in Japanese flounder (*Paralichthys olivaceus*). Mar Biotechnol 8:600–610

Zhao M, Wang Y, Shen H, Li C, Chen C, Luo Z, Wu H (2013) Evolution by selection, recombination, and gene duplication in MHC class I genes of two Rhacophoridae species. BMC Evol Biol 13:113

Zhu K, Yu W, Guo H, Zhang N, Jiang S, Zhang D (2018) The polymorphisms of MHC II β gene of *Trachinotus ovatus* and their association with resistance/susceptibility to *Photobacterium damsela* [sic]. Aquaculture 485:160–165

Appendix

Table A1. MHC IIB alleles of Nile tilapia and their corresponding GenBank accession numbers

Allele	Accession no.
Orni-DAB 0101*	MG882352
Orni-DAB 0102*	MG882353
Orni-DAB 0103*	MG882354
Orni-DAB 0104*	MG882355
Orni-DAB 0105*	MG882356
Orni-DAB 0106*	MG882357
Orni-DAB 0107*	MG882358
Orni-DAB 0108*	MG882359
Orni-DAB 0201*	MG882360
Orni-DAB 0202*	MG882361
Orni-DAB 0203*	MG882362
Orni-DAB 0204*	MG882363
Orni-DAB 0205*	MG882364
Orni-DAB 0206*	MG882365
Orni-DAB 0301*	MG882366
Orni-DAB 0302*	MG882367
Orni-DAB 0401*	MG882368
Orni-DAB 0501*	MG882371
Orni-DAB 0601*	MG882370
Orni-DAB 0701*	MG882369
Orni-DAB 0801*	MG882372
Orni-DAB 0901*	MG882373

Editorial responsibility: Catherine Collins, Aberdeen, UK

Submitted: June 29, 2018; Accepted: January 19, 2019
Proofs received from author(s): March 29, 2019