CRISPR/CAS9-MEDIATED KNOCKOUT OF IL6 IMPAIRED DEFENSE SYSTEM AGAINST *E. piscicida* IN ZEBRAFISH

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ABSTRACT

Interleukin 6 (Il-6) is a multifunctional cytokine that plays a crucial role in the immune response and inflammation. It is a member of the interleukin family of cytokines, which are signaling proteins secreted by immune cells and certain other cell types that regulate a wide range of biological activities. In this study, we generated the *il6* knockout zebrafish using CRISPR/Cas9 system and investigated its absence and how the absence of *il6* affects the immune system. The *il6* gene of zebrafish is found a chromosome 19 and its transcript consists of 5 exons. The full-length cDNA sequence of zebrafish *il6* consists of 696 base-pair (bp) of ORF flanked by 5' and 3' sequences. Developmental expression analysis showed that *il6* was expressed from early developmental stages. In tissue distribution in adult zebrafish, the highest expression was detected in the ovary, followed by the testis and spleen. To generate the mutants, a we targeted the sgRNA binding site within exon 2, resulting in a 7 bp deletion in the target area and the introduction of an early stop codon. The downstream gene analysis in 7 days embryos of wild type (WT) and *il6*$^{(-/-)}$ zebrafish revealed that there is no significant difference in *stat3* expression. The expression of *socs3*, *mpx*, and *mpeg* was upregulated and the expression of *tnf-α1*, *il-1β* was down regulated in *il6*$^{(-/-)}$ mutant compared to WT zebrafish. To assess the functional consequences, we performed an *Edwardsiella piscicida* (*E. piscicida*) immersion challenge using WT and *il6* ablated zebrafish. We examined mortality of WT and *il6*$^{(-/-)}$ zebrafish caused from *E. piscicida* infection at different concentrations. The *il6* ablated fish displayed increased susceptibility to bacterial infection and exhibited a higher mortality rate than WT fish, irrespective of the bacterial concentration. Furthermore, downstream gene expression analysis highlighted distinctive patterns in the absence of *il6* during bacterial infection. In summary, our study reveals the critical role of *il6* in antibacterial defenses, shedding light on its importance of *il6* in the immune responses against infection.

METHODS

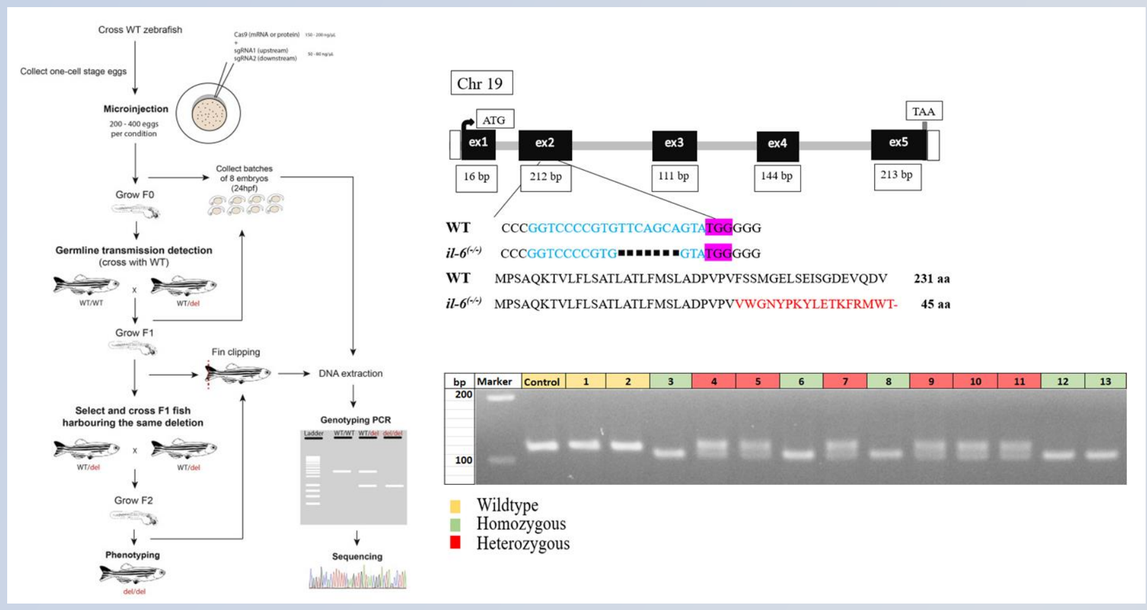

Figure 1. Schematic diagram of *il6*-knockout zebrafish establishment using CRISPR/Cas9 system.

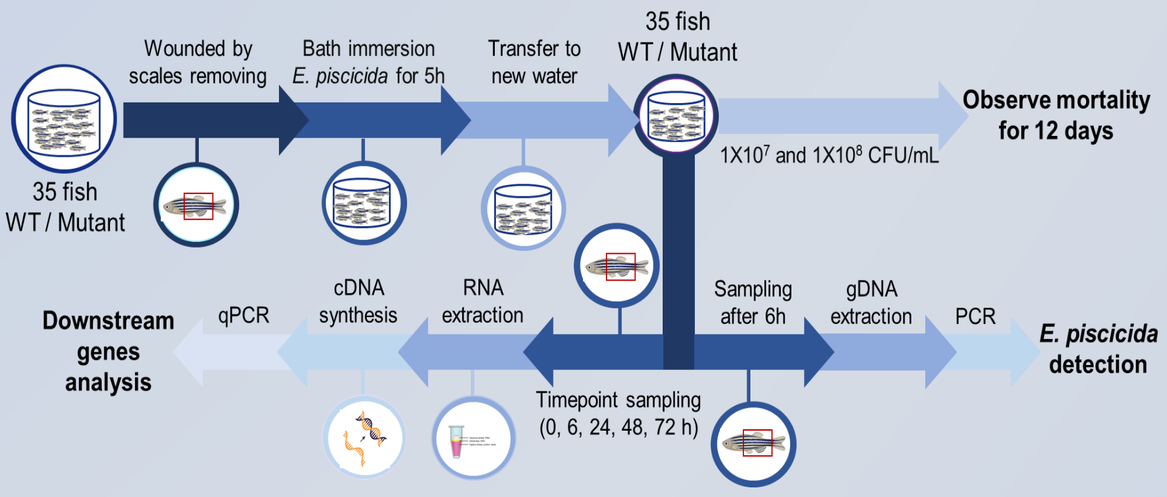

Figure 2. Schematic diagram of artificial *E. piscicida* infection experiment progress in zebrafish.

RESULTS

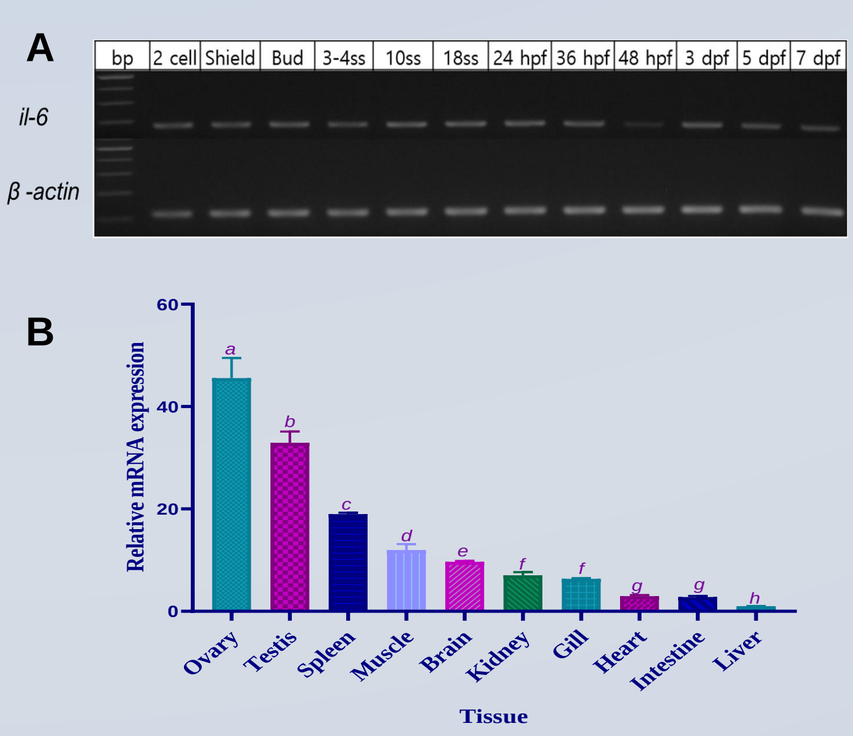

Figure 3. Expression analysis of *il6* during embryonic developmental stage (A) and adult tissue distribution (B) of zebrafish. (A) The mRNA expression of *il6* was detected ubiquitously in all developmental stage by RT-PCR. (B) Tissue specific mRNA expression analysis of *il6* in adult zebrafish by qRT-PCR. Expression fold changes are indicated relative to the transcript level detected for liver tissues with respect to the gene.

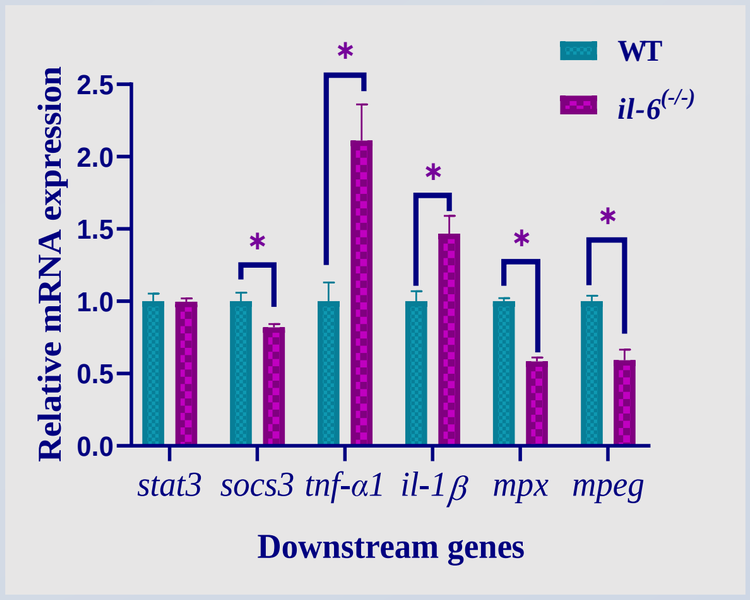

Figure 4. Gene expression comparison in WT and *il6* knockout larvae at 7 dpf. The expression of *il6* related genes (*stat3, socs3, tnf-α1, il-1β, mpx,* and *mpeg*) was analyzed by qRT-PCR. Error bars represent SD (n = 3). Statistical significances between WT and *il6* knockout mutant were calculated by Student's t-test and indicated using an asterisk (*, P < 0.05).

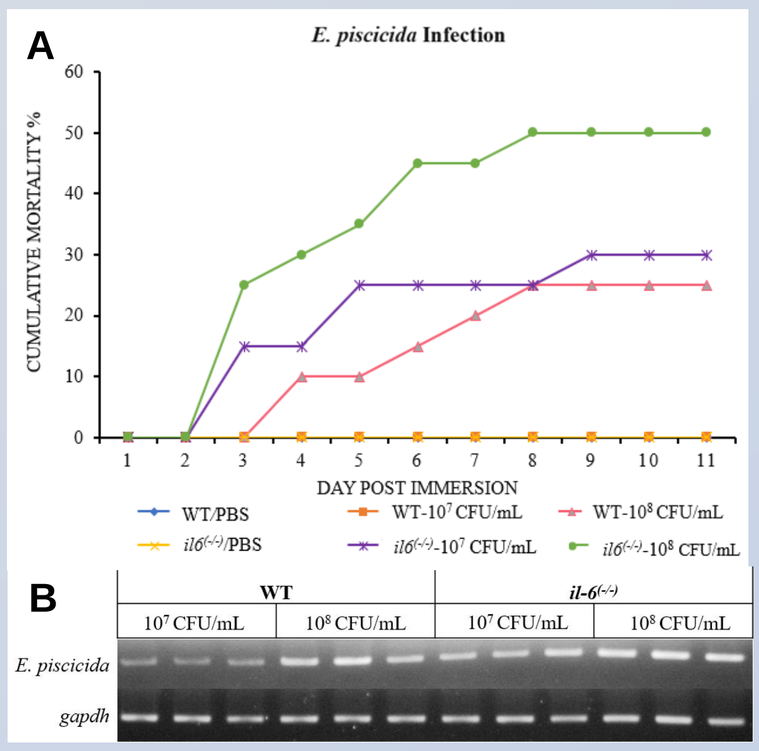

Figure 5. Comparative analysis of mortality (A) and bacterial load (B) in WT and *il6*-knockout zebrafish upon *E. piscicida* infection. Infection experiments were performed by immersion in two concentrations (10^7, 10^8 CFU/mL) of *E. piscicida* after creating wounds by removing scales. *Il6* knockout zebrafish exhibited increased susceptibility to bacterial infection and higher mortality rates compared to WT zebrafish.

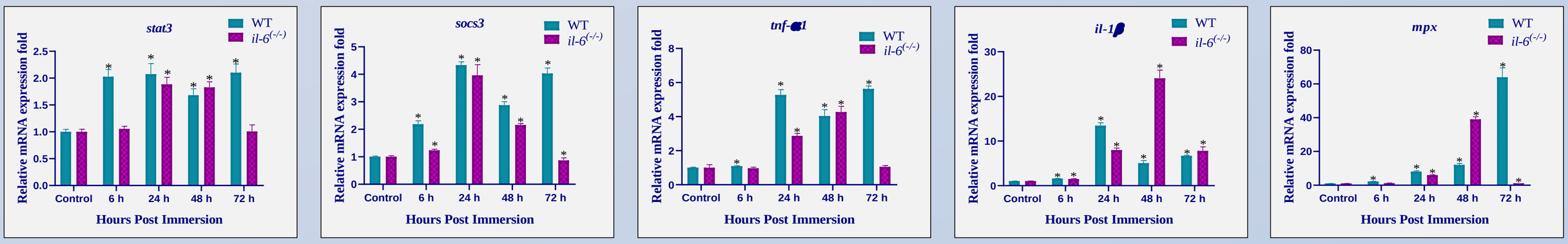

Figure 6. Transcriptional analysis of *il6* related genes in WT and *il6*-knockout zebrafish upon *E. piscicida* infection. In the infection experiment, in the same manner as the mortality comparison experiment, scales were removed to create a wound, and *E. piscicida* infection was induced by immersion. The internal organs (liver, kidney, intestine, and spleen) were sampled at 6, 24, 48, and 72 hours post infection, and RNA was extracted and cDNA was synthesized for analysis. The relative mRNA expression of *stat3, socs3, tnf-α1, il-1β,* and *mpx* were detected by qRT-PCR. The relative mRNA expression folds of the non-infected group of WT and *il6*-knockout zebrafish were considered as their respective basal expression levels. Error bars represent SD (n = 3) (*, P < 0.05)

CONCLUSION

- The absence of *il6* led to a significant increase in mortality rates during *E. piscicida.*
- *Il6* deficiency resulted in altered regulation of specific genes involved in immune signaling, cytokine production, and pathogen recognition.
- In aggregate, our results concluded the pivotal role of *il6* in antibacterial defense, providing insight into the significance of *il6* in the immune responses against Pathogen infection.

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