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Identification of Disease Resistance Genes and CRISPR-Based Genome Editing in *Channa* spp.

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Abstract This study analyzed the hazards and immune response mechanisms of common diseases of snakehead fish in recent years (such as nocardia, *Aeromonas hydrophila*, viral hemorrhagic septicemia, etc.), and summarized the mining methods and functional research progress of key genes for disease resistance of snakehead fish, including screening of immune genes such as IL-17 and TRAF through whole genome scanning and transcriptomics. At the same time, the application status and advantages of CRISPR/Cas9 gene editing technology in aquaculture were discussed, such as efficient site-directed mutagenesis and introduction of exogenous antimicrobial peptide genes to enhance fish disease resistance. Through case analysis of the successful experience of disease-resistant gene editing in related fish (such as Atlantic salmon and catfish), this study prospected the potential path and results of disease-resistant gene editing breeding of snakehead fish, and discussed its ecological and ethical impacts (such as off-target effects, food safety and public acceptance, etc.), which is of great significance to improving aquaculture production and disease prevention and control, and also provides the latest theoretical basis and practical reference for disease-resistant breeding of snakehead fish.
Keywords Snakehead; Disease resistance gene; Immune mechanism; CRISPR/Cas9; Gene editing breeding

1 Introduction

Snakehead (*Channa argus*, etc., also known as mullet and money fish) has become one of the main farmed economic fish in Asia, especially in China, because of its delicious meat and rapid growth (Teng et al., 2024). However, the snakehead aquaculture industry is facing serious disease threats, with frequent outbreaks of various bacterial, parasitic and viral diseases, leading to large-scale deaths and economic losses (Qin, 2023). For example, nocardiosis is a chronic fish disease caused by *Nocardia seriolae*, which can cause abscesses and granulomas to form in snakehead carp, with a high mortality rate, causing a serious impact on the aquaculture industry. For another example, hemorrhagic septicemia caused by *Aeromonas* spp. is prone to outbreaks in high-density aquaculture environments. After infection, snakehead carp often show symptoms such as ulceration and bleeding, with a high acute mortality rate (Liu et al., 2021; Weng et al., 2024). In addition, in recent years, a rhabdovirus, the blackfish vesicular virus (SHVV), has been found in snakehead fish, which can cause large-scale hemorrhagic disease. So far, there is no effective vaccine or treatment (Qin et al., 2024).

Fish naturally have a certain immunity to infection, and the disease resistance varies greatly among different species and individuals, which is mainly determined by genetic factors. Mining the key genes that control disease resistance through genomic methods will help to clarify the molecular mechanism of host resistance to pathogen infection, and can be used as molecular breeding markers for breeding disease-resistant strains (Fraslin et al., 2020). In traditional breeding, disease resistance traits are often difficult to select directly, while gene mining can locate specific functional gene loci and improve breeding accuracy and efficiency (Zhu et al., 2024). For emerging aquaculture species such as snakehead fish, there is an urgent need to find molecular markers or genes related to disease resistance through gene mining. Mining the disease resistance genes of snakehead fish can not only deepen the understanding of the immune mechanism of fish, but also provide targets for breeding new disease-resistant strains, which is expected to reduce drug dependence and improve the survival rate of aquaculture.

Gene editing, represented by CRISPR/Cas9, the third-generation genome-directed modification technology, has brought revolutionary tools to aquatic animal breeding (Hallerman et al., 2021). Compared with traditional breeding, which requires multiple generations of hybridization and selection, gene editing can achieve precise genetic improvement in one generation of individuals, significantly shortening the breeding cycle (Zhu et al., 2024). The CRISPR/Cas9 system uses RNA-guided nucleases to produce mutations or knockouts at specific sites in target genes, thereby conferring or enhancing traits such as disease resistance. Unlike transgenic technology, CRISPR technology does not require the insertion of exogenous fragments into the genome, and can produce improved varieties that are "free of exogenous genes" (Roy et al., 2022). Gene editing tools such as CRISPR are rapidly being integrated into the field of aquaculture breeding with their advantages of high efficiency, precision and flexibility, providing unprecedented opportunities for breeding new disease-resistant varieties (Ferdous et al., 2022).

This study will introduce common diseases and their hazards in snakehead aquaculture, analyze the mechanistic basis of snakehead immune response, summarize the research status of snakehead disease-resistant genes in recent years, and focus on the application strategy of gene editing technology in snakehead disease-resistant breeding. By drawing on the successful cases of gene editing disease-resistant breeding of other fish (such as salmon and catfish), the inspiration for snakehead is analyzed. At the same time, the ecological safety, food safety and ethical supervision issues that need to be considered in the actual production of gene-edited snakehead are discussed, and the recommended paths for establishing a disease-resistant gene resource library, optimizing the editing platform and promoting industrialization are proposed. This study hopes to provide a comprehensive literature basis and forward-looking perspective for the molecular breeding of disease-resistant snakehead, and accelerate the breeding and application of new disease-resistant snakehead varieties.

2 Research Status of Common Diseases and Disease Resistance Genes of Snakehead Fish

2.1 Analysis of common diseases and hazards of snakehead fish

In the intensive farming environment of snakehead fish, high density and stress often lead to the rapid spread of pathogens, causing outbreaks of various diseases. Among bacterial diseases, nocardiosis and *Aeromonas hydrophila* are the most common. Nocardiosis is caused by infection with *N. seriolae*, which can invade the skin, muscles and internal organs of snakehead fish, forming a large number of white nodules (granulomas). Diseased fish often show emaciation and lethargy. Dissection shows that organs such as the liver and spleen are covered with granulomas (Figure 1) (Zhang et al., 2022; Teng et al., 2024).

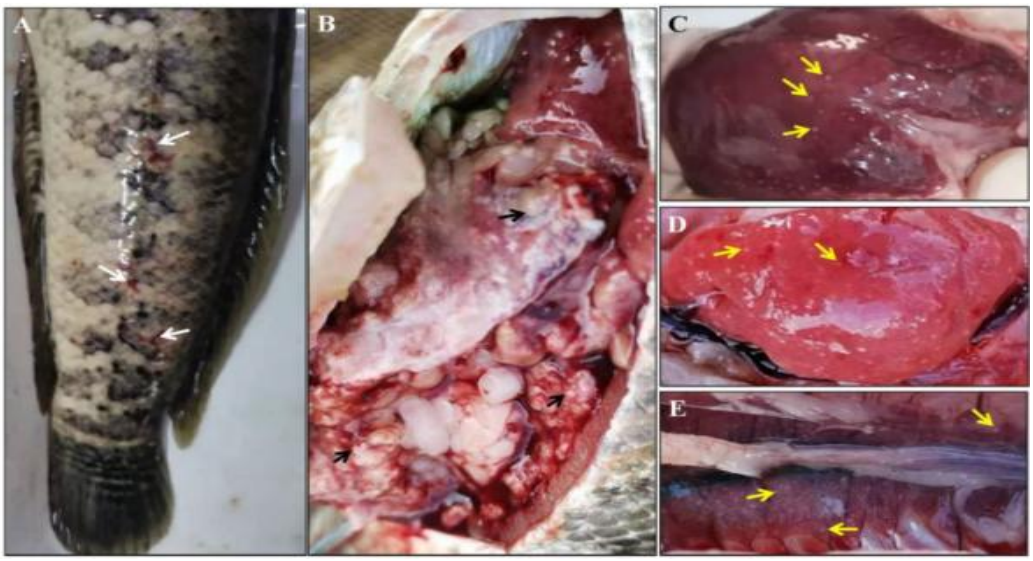

Figure 1 Clinical symptoms of snakehead infected with Nocardia (Adopted from Zhang et al., 2022)

Image Caption: (A) Clinical signs of diseased hybrid snakehead body surface. Appearance of abdominal cavity (B), liver (C), spleen (D) and kidney (E); The white arrows indicate superficial lesions, black arrows indicate granules of *N. seriolae*, and yellow arrows indicate the necrosis and granuloma (Adopted from Zhang et al., 2022)

The disease progresses slowly but has a high mortality rate. It has broken out in many farmed fish, including California bass, yellow catfish and hybrid snakehead fish, causing significant economic losses to the aquaculture industry. *Aeromonas hydrophila* is widely present in water bodies and is prone to multiply in large numbers when the water temperature rises or when organic matter is abundant. After being infected with *Aeromonas hydrophila*, snakehead fish will show obvious symptoms of bleeding and ulceration, often accompanied by ascites and gill anemia, with a rapid course of disease and rapid death (Liu et al., 2021).

In terms of viral diseases, snakehead fish vesicular virus (also known as snakehead fish rhabdovirus) has attracted attention in recent years. The virus belongs to the family of rhabdoviridae and can cause symptoms similar to carp spring virus disease, including lymphatic hemorrhage, abdominal distension, slow swimming, etc. Experiments have shown that after snakehead fish cells are infected with the virus, viral proteins use the host ubiquitin system to promote their own replication (Qin et al., 2024). In addition, snakehead fish is also susceptible to some parasites and fungi. For example, parasites such as wheelworms and ringworms can attach to the gills of snakehead fish, causing breathing difficulties and secondary infections. Myxosporidia caused by dinoflagellates also causes losses in the juvenile stage (Ferdous et al., 2022). Among fungal diseases, the most typical one is fulminant ulcerative syndrome (EUS) caused by invasive *Saprolegnia invadans*. After infection, snakehead fish develop large areas of ulcers and necrosis on the skin, with a high mortality rate. It is listed as a reportable disease by the World Organization for Animal Health (Qin, 2023).

2.2 Overview of immune response mechanism

As a teleost fish, the immune system of snakehead fish includes two major parts: innate immunity and adaptive immunity. It has both similarities and differences with higher vertebrates. Innate immunity is the first line of defense for snakehead fish against pathogens, and is composed of skin mucus barrier, phagocytes, lysozyme and complement (Figure 2) (Mokhtar et al., 2023). When pathogens invade, pattern recognition receptors (PRRs) in the innate immune system of fish, such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs), can recognize pathogen-associated molecular patterns (PAMPs) such as bacterial lipopolysaccharides and viral RNA, triggering downstream signaling pathways (Fraslin et al., 2020).

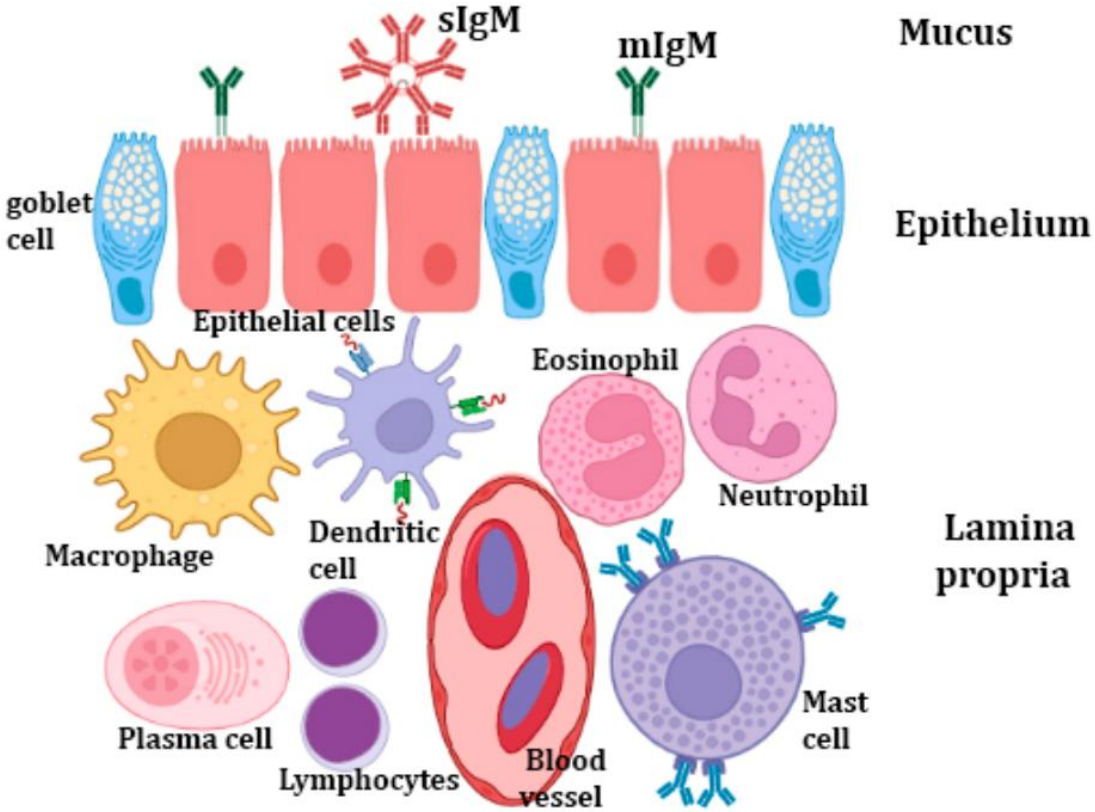

Figure 2 Schematic representation of the fish mucosal immunity (Adopted from Mokhtar et al., 2023)

Adaptive immunity has also evolved in fish, but it has some unique features compared to mammals. Teleost fish have T lymphocytes and B lymphocytes, as well as corresponding immunoglobulins (Ig). Snakehead fish's B cells can secrete two types of antibodies, IgM and IgT. IgM mainly circulates in the blood, while IgT is a mucosal antibody unique to fish, concentrated in mucosal tissues such as the intestines and gills, and plays an important role in mucosal immunity (Liu et al., 2025). Snakehead fish's immune system can resist pathogens through the rapid response of innate immunity and the specific memory of adaptive immunity. A key factor affecting snakehead fish's disease resistance lies in the diversity and regulatory efficiency of its immune genes. There are significant differences in the intensity of immune responses among individuals with different genotypes (Fraslin et al., 2020; Liu et al., 2025)

2.3 Current status and existing problems of disease resistance gene research

In recent years, a series of progress has been made in the study of genes related to immunity and disease resistance in snakehead fish, but overall it is still in the stage of discovery and functional identification, with problems such as insufficient functional verification and unclear gene networks. In terms of candidate gene screening, researchers have mainly adopted two strategies: one is genome-level scanning, such as constructing a high-density genetic linkage map and locating QTLs for disease resistance traits, but there are few reports on the study of disease resistance QTLs in snakehead fish; the second is transcriptome or proteomics comparison, screening differentially expressed genes before and after infection. The latter has been applied in snakehead fish and its hybrids (Chen et al., 2018). For example, Zhang et al. (2022) used RNA sequencing to compare the spleen transcriptomes of healthy and nocardia-infected hybrid snakehead fish, and identified more than 3,500 differentially expressed genes, many of which are involved in innate immunity and inflammatory pathways.

In terms of the identification of core disease resistance genes, several specific genes have been reported to be closely related to snakehead fish immunity or disease resistance. Han et al. (2025a) identified all 7 members of the snakehead fish IL-17 cytokine family and their receptor genes, a total of 9 members, and found that snakehead fish IL-17 is highly conserved in evolution. When stimulated by Nocardia, especially IL-17C2, IL-17D, IL-17N and other subtypes are significantly upregulated in the gills and intestines of fish, suggesting that they play a key role in mucosal immune defense.

In terms of functional verification and mechanism of action, the current research on snakehead fish disease resistance genes is not deep enough, mostly staying at the level of identification and expression analysis, lacking direct functional evidence. On the one hand, because snakehead fish lacks mature experimental models and cell lines, gene knockout or overexpression experiments are difficult; on the other hand, the whole genome of snakehead fish has just been finely assembled (Sun et al., 2024), and functional research technology platforms such as gene editing are still under establishment.

3 Mining and Functional Study of Key Genes for Disease resistance in Snakehead Fish

3.1 Methods for screening candidate disease resistance genes

For non-model fish such as snakehead fish, screening of candidate disease resistance genes usually relies on large-scale omics analysis and comparative experiments. Whole genome scanning is a powerful means, such as constructing a family for linkage analysis or performing a genome-wide association study (GWAS) on a population to find gene loci associated with disease resistance phenotypes (Fraslin et al., 2020). Gene family screening is another strategy, which is to search and identify homologous sequences in the snakehead fish genome based on known immune-related genes in other species. For example, through sequence alignment, Han et al. (2025a) successfully identified all members of the IL-17 family in the snakehead fish genome and further analyzed their expression patterns. For another example, through Hidden Markov Model search, researchers found all gene sequences of the snakehead fish NLR family (Zhu et al., 2025). Traditional candidate gene methods have also played a certain role, such as directly selecting some key factors for snakehead fish sequence cloning and detection based on existing fish immunity knowledge.

3.2 Core disease resistance-related genes discovered

Cytokines play the role of signaling molecules in immune regulation. The IL-17 family members identified by Han et al. (2025a) are important proinflammatory cytokines, and their significant upregulation suggests that they play a key role in resisting bacterial infections. In particular, IL-17D and IL-17N subtypes increased dramatically in mucosal tissues when snakehead fish was infected with Nocardia, suggesting that they may recruit neutrophils and other antibacterial effects. In terms of signal transduction molecules, TNF receptor-associated factor (TRAF) is a connector molecule for many immune signaling pathways, such as TRAF6 involved in TLR and IL-1 receptor signals, and TRAF3 involved in antiviral RIG-I-like receptor signals (Chen et al., 2023). The eight snakehead fish TRAF genes cloned by Han et al. (2025b) provide a basis for studying the conservation and differences of these signaling pathways in fish immunity. Preliminary analysis showed that snakehead fish TRAF genes were widely expressed in different tissues and showed an upregulation trend after pathogen stimulation, suggesting that they were activated in the process of fish disease resistance.

In terms of anti-infective effector molecules, some molecules that directly intervene in bactericidal and insecticidal activities have also been identified. For example, there are multiple antimicrobial peptide genes in the snakehead fish genome, such as β-defensin and hepcidin. Mucosal immunity-related genes are also worth mentioning, including the aforementioned polyimmunoglobulin receptor (pIgR) (Xu et al., 2025). snakehead fish pIgR is highly expressed in the intestinal mucosa and can bind to IgM/IgT and transport it to the surface. After infection, the expression of pIgR further increases, which helps to transport IgT antibodies generated in the mucosa to the infection site to neutralize pathogens. This shows that the immunity mediated by snakehead fish mucosal antibodies is similar to the IgA mechanism of mammals and is an important component of disease resistance.

3.3 Gene function verification strategy

Traditional functional research methods include gene knockout, overexpression, gene mutation association analysis, etc. In model organisms such as zebrafish, gene knockout lines can be constructed to directly observe changes in mutant resistance to infection. However, for snakehead, there has been a lack of efficient gene manipulation methods, and functional verification mainly relies on in vitro cell experiments and heterologous models. One strategy is to overexpress or knock down candidate snakehead genes in bacteria or cell lines to evaluate their effects on pathogen growth. In recent years, CRISPR/Cas9 has been successfully used to functionally verify some important fish disease resistance genes. For example, the Nae1 gene of Atlantic salmon was knocked out in cell lines by CRISPR, and a significant decrease in IPNV replication was observed, thus confirming the role of the Nae1 gene (Pavelin et al., 2021). This method can also be used for snakehead research.

With the development of snakehead primary cell and embryo manipulation technology, researchers can try to construct snakehead gene knockout lines or knockout individuals. Fortunately, Zhao et al. (2021) have reported the first successful case of snakehead gene editing: they used CRISPR/Cas9 to knock out the myostatin gene of spotted snakehead (*Channa maculata*) and cultivated genetic mutant fish with significantly accelerated growth. Gene overexpression and gene injection are also commonly used strategies. For example, the candidate gene mRNA is injected into the fertilized eggs of snakehead fish through microinjection, so that the gene is highly expressed in the fry stage, and then the effect is tested by pathogen challenge.

4 Application of Gene Editing Technology in Improving Disease Resistance of Snakehead Fish

4.1 Current status of CRISPR/Cas system and aquatic application

CRISPR/Cas9 has simple design, low cost and high editing efficiency, and has become the preferred method for aquatic genome editing (Roy et al., 2022; Zhu et al., 2024). The application of gene editing in the aquatic field started with the model fish zebrafish and some economic fish. Around 2015, zebrafish successfully achieved CRISPR-mediated multi-site editing for the first time, laying the foundation for subsequent fish applications. Since then, gene editing attempts have been carried out in a number of farmed fish species, including Nile tilapia, carp, crucian carp, catfish, salmon, etc. (Zhu et al., 2024). So far, gene editing research has been reported in more

than 20 species of fish, covering multiple target traits such as growth, muscle, pigment, reproduction and disease resistance (Ferdous et al., 2022). Specifically for disease resistance, CRISPR technology has shown unique advantages: it can directly knock out negative regulatory genes that restrict disease resistance, or accurately repair/introduce favorable mutations, thereby significantly improving the disease resistance of fish (Wang and Cheng, 2023). For species such as snakehead, the introduction of CRISPR technology will open a new path for rapid improvement of disease resistance.

4.2 Key technical paths for gene editing in snakehead

To apply CRISPR/Cas9 technology to snakehead and achieve effective gene editing, it is necessary to overcome the challenges of its reproductive biology and early embryo manipulation and establish a complete set of technical paths. (1) Obtain fertilized eggs and fertilize in vitro. Snakehead gametes can be obtained through artificial induction of spawning, and fertilized eggs can be obtained by in vitro fertilization. Due to the small diameter of snakehead eggs and the tough egg membrane, attention should be paid to the fertilization time window and the maintenance of egg activity. (2) Microinjection of editing elements: Cas9 protein or mRNA encoding it is mixed with the designed gRNA to form an editing reagent, which is injected into the embryonic cells of snakehead fertilized eggs under a microscope using a microneedle (Ou et al., 2023). (3) Embryo hatching and screening: The injected fertilized eggs are hatched at a suitable temperature and develop into F0 generation fry. For those with a high mutation rate, they can be cultured to maturity (Figure 3) (Roy et al., 2022). (4) Establishing homozygous mutant lines: Individuals carrying germline mutations in the F0 generation are mated as broodstock, and some homozygous mutant offspring individuals can be obtained in the F1 generation. For snakehead carp, similarly, 1 to 2 generations of breeding are required to obtain stable knockout or knock-in lines (Zhao et al., 2021). (5) Phenotypic verification: After obtaining homozygous gene-edited snakehead carp, disease resistance can be evaluated. (6) Expansion and evaluation. If the gene editing effect is ideal, the breeding population can be expanded under isolation conditions to evaluate its production traits such as growth and reproduction, as well as environmental adaptability. Snakehead carp is a fast-growing fish that can reach the evaluation body length within a few months.

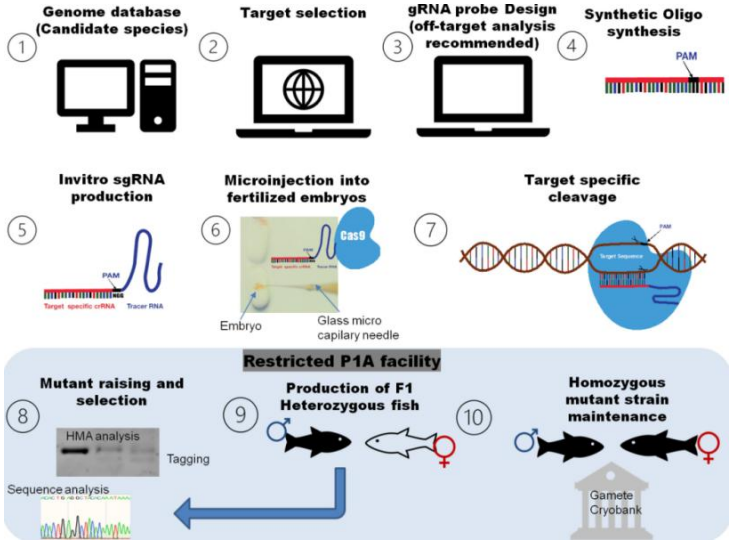

Figure 3 A simple roadmap of general methodology for CRIPSR/Cas genome editing in aquaculture and fisheries (Adopted from Roy et al., 2022)

Image Caption: The target gene has to be selected after searching the genome database of candidate species. The sgRNA has to be designed with the help of sgRNA-designing tools, and then, the sgRNA oligo has to be synthesized. For target-specific cleavage, the sgRNA and cas9 mixture needs to be delivered to the newly fertilized embryo at a one-cell stage by microinjection or similar methods. The final step is the assessing the genome-editing results and application stage that includes mutagenesis analysis, the selection of mutants, crossing with wild population and production of a specific mutant line, the evaluation of CRISPR-induced mutation associated phenotyp(s), and the establishment of new varieties with improved values in aquaculture (Adopted from Roy et al., 2022)

4.3 Potential targets for gene editing for disease resistance

Editing targets for improving disease resistance can be divided into two categories: one is to enhance genes related to immune defense, and the other is to weaken genes related to susceptibility pathways. For the former, the disease resistance of fish can be improved by gene editing knock-in or activation (Gutási et al., 2023). For example, it is possible to consider introducing exogenous antimicrobial peptide genes into the snakehead genome so that it can express broad-spectrum antimicrobial substances at high levels when infected. This is similar to the idea of introducing antimicrobial peptide genes into crocodiles in catfish (Ferdous et al., 2022). For the second type of target, it is to knock out some negative regulatory factors or pathogen receptors to reduce pathogen infection and pathogenic processes. For example, the receptor genes required for viruses to enter host cells in snakehead fish can be knocked out first. If a virus receptor on the cell membrane of snakehead fish is found, it will be difficult for the virus to invade after knocking it out, thereby greatly improving the antiviral ability. Similar cases have been achieved in grass carp (Zhu et al., 2024). In addition, it is possible to consider knocking out genes in the immune suppression pathway. The normal functions of certain genes can inhibit immune responses or promote pathogen escape, and knocking them out can remove the "brakes" on immunity.

5 Case Study and Prospect of Gene-Edited Snakehead Breeding for Disease Resistance

5.1 Analysis of successful cases of gene editing for disease resistance in related fish

Although there are no publicly reported cases of disease-resistant gene editing breeding for snakehead, some successful experiences of other aquatic fish can be used for reference. In farmed salmon, infectious pancreatic necrosis (IPN) was once a devastating viral disease, but it has been successfully controlled using traditional breeding and modern genomic methods. The key is the discovery of a major QTL on chromosome 26 of salmon that determines the fish's resistance to IPNV (Pavelin et al., 2021). Inspired by this, the team is trying to introduce the natural disease-resistant mutant Nae1 allele into susceptible salmon strains through CRISPR. American researchers creatively adopted the "enhanced defense" strategy to improve catfish's resistance to bacterial pathogens. Professor Dunham's team used CRISPR/Cas9 to insert the alligator-derived antimicrobial peptide gene Cath into catfish embryos and knocked it into the catfish growth hormone receptor gene site (Ferdous et al., 2022; Ye et al., 2025). This study proves that the idea of cross-species introduction of disease-resistant genes combined with gene editing is feasible, which not only improves the disease resistance of the fish itself, but also ensures biosafety from an ecological perspective.

5.2 Prediction of potential successful cases of disease-resistant gene editing in snakehead fish

Nocardia is one of the top killers of snakehead fish farming. It is conceivable to improve the resistance of snakehead fish to this disease through gene editing. One feasible solution is to edit both the infection strategy of Nocardia and the immune response of snakehead fish. There is currently no prevention and treatment for the rhabdovirus SHVV, and gene editing can provide an innovative approach (Michael et al., 2024). It is possible to consider editing the antiviral natural immune pathway of snakehead fish to quickly eliminate the virus. For example, using CRISPR activation technology (CRISPRa) to continuously activate the expression of type I interferon genes or downstream interferon-stimulated genes (such as Mx and PKR) in snakehead fish, the fish is in a "warning" state, and once the virus invades, its replication can be inhibited. Parasitic problems such as wheelworms in snakehead aquaculture are also expected to be alleviated through gene editing. One strategy is to make the surface of snakehead fish (skin, gills) form characteristics that are not conducive to the attachment of parasites. On the other hand, it can also enhance the immune recognition ability of snakehead fish to parasite antigens. With the improvement of CRISPR multiple editing efficiency reported in current literature (successful editing of 45 sites at a time has been achieved in zebrafish, etc.), this "one-step multi-target" solution is technically feasible (Hallerman et al., 2021; Ferdous et al., 2022). If the produced snakehead fish can show excellent survival rate in both bacterial and viral attack tests, it will be a revolutionary new strain.

6 Risk Assessment and Ethical Considerations of Disease Resistance Gene Editing

6.1 Ecological safety issues

While gene editing snakehead fish improves disease resistance, it also raises concerns and discussions about the ecological environment. Snakehead fish itself is a top predator fish, and once it escapes to non-local waters, it has

a strong ability to invade the ecology. If edited snakehead fish with stronger disease resistance or even faster growth are cultivated, if they are not managed well and enter the wild environment, they may have a more competitive advantage than ordinary snakehead fish, thus causing a greater impact on the local ecology (Robinson et al., 2024). In addition, the impact on other species needs to be considered. Edited snakehead fish may affect the predator-prey relationship through the food chain, or hybridize with closely related species to produce environmental consequences. Considerations from the perspective of ecological diversity: Large-scale promotion of disease-resistant edited fish varieties may lead to reduced genetic diversity in farmed populations. Because these varieties have artificially selected dominant alleles, farmers may tend to use a single strain, thereby reducing the diversity of snakehead culture germplasm (Roy et al., 2022). Low diversity poses risks when encountering environmental changes or new diseases.

6.2 Food safety and market acceptance

Any gene-edited animal used for food production must ensure that its edible parts are safe for human consumption and recognized by consumers. In terms of food safety, the scientific community generally believes that if gene-edited animals do not introduce exogenous DNA, their products are essentially no different from traditional breeding products (Roy et al., 2022). For edited snakehead, a case-by-case assessment is required. If the change is only to knock out a snakehead gene, the resulting fish meat does not contain new substances and is theoretically as safe as ordinary snakehead. If the editing introduces exogenous genes (such as antimicrobial peptides), the presence of exogenous proteins in the fish meat and the risk of allergies need to be assessed. Alligator antimicrobial peptides are small molecule polypeptides that may be inactivated during cooking and degraded in the digestive system. They generally do not cause toxicity or allergies (Puthumana et al., 2024). In terms of market and public acceptance, this is a more complicated issue than scientific safety. Many consumers still have doubts or resistance to "genetically modified foods." Although gene editing is different from GMOs, many countries have adjusted their regulations to exclude gene-edited products that do not contain exogenous DNA from GMO regulation, but it will take time for the public to understand it.

6.3 Potential impact of off-target effects and imbalanced repair mechanisms

The safety of gene editing technology itself is also an issue that must be taken seriously, of which off-target effects and imbalanced repair mechanisms are two major concerns. Off-target effects refer to the possibility that CRISPR/Cas9 may also produce mutations at other locations in the genome outside the target site. If these mutations fall on key genes, they may cause unexpected traits or health problems (Zhu et al., 2024). In addition to off-target effects, another concern is the imbalance of DNA repair mechanisms. After Cas9 produces double-strand breaks, cells repair the breaks through non-homologous end joining (NHEJ) or homologous recombination (HDR). NHEJ is prone to indel mutations, while HDR can be accurately repaired when there is a template. Large-scale gene editing may trigger stress of DNA damage response in cells, especially in the early stages of embryonic development, when a large number of cells undergo repair at the same time, which may lead to abnormal embryonic development or cell aging.

7 Application Prospects and Future Research Directions

7.1 Establish a database of disease resistance traits and a functional gene resource library for snakehead fish

In order to better utilize gene editing to improve the disease resistance of snakehead fish, it is necessary to consolidate basic research and establish a complete disease resistance phenotype and genotype database. Specifically, on the one hand, it is necessary to collect disease resistance phenotypic data of different snakehead fish strains and individuals through large-scale breeding experiments, including survival rate, morbidity, and degree of lesions under different pathogens. In conjunction with genotyping, disease resistance-related genetic markers can be located. This is actually similar to the family challenge test in traditional breeding, but it should be upgraded to a database containing genomic information (Fraslin et al., 2020). On the other hand, existing and future snakehead fish genome and transcriptome data should be integrated to establish a special "snakehead fish disease resistance gene database". It includes the identified snakehead fish immune-related gene sequences, expression profiles, functional annotations, literature evidence, etc. There are fish immune gene databases abroad

(such as iFish ency, etc.), and researchers can create a more targeted sub-library for snakehead fish. Furthermore, whole-genome selective breeding experiments can be carried out to associate molecular markers with disease resistance and assist in the selection of disease-resistant parents (Zhu et al., 2024).

7.2 Building a stable gene editing technology platform

To truly promote the application of the technical path of snakehead gene editing, it is necessary to develop it into an efficient, low-cost, and scalable stable technology platform. In terms of technical optimization, the efficiency and accuracy of CRISPR editing need to be improved. A supporting detection and screening system should also be developed (Ferdous et al., 2022). If the edited snakehead fry are to be mass-produced in the future, it is necessary to quickly identify which embryos/juveniles have successfully undergone the required mutations. Another thing to pay attention to is off-target monitoring. Perhaps the whole genome of the breeding fish can be quickly tested through third-generation sequencing, and then the algorithm can be used to determine the impact of potential off-target mutations and screen out unqualified individuals. In addition to the technology itself, platform construction also includes personnel training and standard establishment. At the same time, operating specifications and standards should be formulated. For example, the survival rate of embryo injection should be what, the off-target rate detection standard, the population breeding generation requirements, etc. Only with unified standards can the quality of edited fish produced by different batches and different institutions be guaranteed to be consistent.

7.3 Promotion path from laboratory to industrialization

The real application of gene-edited disease-resistant black fish in aquaculture production requires a series of transformation steps from laboratory results to commercial products. The first is pilot testing and strain selection. After obtaining the ideal disease-resistant edited black fish strain in the laboratory, it is necessary to conduct pilot breeding tests in an environment close to actual breeding conditions. This test usually lasts for one breeding cycle to fully evaluate the effect. If the edited strain performs well, it can enter the new variety approval process (Okoli et al., 2022). Only after passing the approval can it be promoted as a legal variety. The next step is large-scale breeding. Like traditional fish species, edited black fish needs to establish a great-grandparent-grandparent-parent breeding system to ensure the stability and supply of germplasm. Industrialization also needs to solve cost and efficiency issues. At present, the cost of laboratory-scale gene editing operations is relatively high (large manpower investment and high screening losses). As the technology matures, these costs are expected to decrease. On the other hand, market promotion needs to consider the acceptance of farmers, and public publicity and popular science should also keep up. Through media reports on the results of demonstration sites and experts' interpretation of the safety of fish, society will gradually accept this new thing and pave the way for the market. Finally, consider the international market. Snakehead fish is not only consumed domestically, but also exported to neighboring countries.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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