A major QTL affects resistance to viral nervous necrosis in farmed European seabass

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Abstract

Viral nervous necrosis (VNN) presents a significant threat to the European seabass aquaculture industry, causing extensive losses due to mortality and impaired growth of infected fish. Selective breeding presents an opportunity to develop seabass strains with increased resistance, thus reducing the impact of the disease on the industry. Knowledge of the genetic architecture of the trait is important to inform selective breeding strategies, including use of genomic tools. In the current study the genetic basis of VNN resistance in European seabass was investigated through an extensive study based on nervous necrosis virus (NNV) challenge of juvenile seabass fish. The results highlighted moderate heritability (up to 0.39 ± 0.05) of VNN resistance in farmed seabass. A major QTL was identified on LG12 or Chromosome 13 and impacted VNN resistance when defined as binary survival or as days to death. This QTL explains up to 37% of the genetic variation in VNN resistance, and therefore has a significant value for marker-assisted and genomic selection to improve resistance in sea bass breeding programmes. Together, our results provide insight into the genetic architecture of resistance to VNN that could further be utilized for enhancing genomic selection for more resistant fish.

Introduction

European sea bass (*D. labrax*) is a fish species with major economic and cultural importance to the European aquaculture industry (Vandeputte *et al.* 2019). Viral nervous necrosis (VNN) disease causes significant losses to farmed sea bass production through mortalities and impaired growth of the infected fish (Barsøe *et al.* 2021). Despite the significant economic impact of this disease to Mediterranean aquaculture, there are limited vaccination, therapeutic, and biosecurity control measures. Genetics studies have revealed significant, moderate heritability of host resistance to VNN in different seabass populations (Palaiokostas *et al.* 2018; Faggion *et al.* 2021; Griot *et al.* 2021). Additionally, genomic regions or markers have showed significant association with VNN resistance in different European sea bass populations (Palaiokostas *et al.* 2018; Griot *et al.* 2021). However, information remains sparse on the genetic architecture of host resistance to VNN in different farmed populations. Furthermore, identification of genetic markers or regions involved in regulating VNN resistance enable marker-assisted selection or enhance the genomic selection for more resistant fish which is a sustainable route to reduce the negative economic and animal welfare impact of VNN. Therefore, in the current study, the aim was to characterise the genetic resistance to VNN in sea bass, and to identify specific genomic regions and markers associated with the trait.

Materials and Methods

Viral nervous necrosis challenge experiment and genotyping

Approximately 1,500 European seabass from a full factorial cross of 25 dams and 25 sires were challenged with nervous necrosis virus at a temperature of 25°C. These fish were produced by Valle Ca' Zuliani Società Agricola in April 2020 and were challenged at a weight ranging from 6 to 20 grams at the Istituto Zooprofilattico Sperimentale delle Venezie facilities under the authorization of the Italian Ministry of Health (authorisation code 975/2016-PR). Mortality was recorded twice a day for a period of 29 days as the primary phenotype for the genetic analyses, with days to death (a.k.a survival time) also used as a phenotype. The days to death phenotype was considered both in mortalities only, and in all animals where survivors were assigned a high value equivalent to the termination of the trial. A total of 1,066 fish (including the 50 parents and 1016 NNV challenged fish) were genotyped on the Thermo Fisher Scientific's Axiom 'MedFish' SNP array which contains circa 30K seabass SNPs (Penaloza *et al.* 2021).

Pedigree reconstruction and pedigree-based heritability estimation

Since the family structure was not known at the point of challenge, genotype data were used to reconstruct the pedigree. Circa 23,000 informative SNP marker genotypes were used to uniquely assign a dam and sire to each of the offspring using the APIS package (Griot *et al.* 2020) implemented in R, allowing for a Mendelian error rate of 5%. Subsequently, survival analyses were performed for the offspring for dam and sire families using the Survminer package (Kassambara *et al.* 2017) implemented in R. The reconstructed pedigree was used to fit a linear mixed animal model to estimate the heritability of VNN resistance in this population of farmed European sea bass using the ASReml software (Gilmour *et al.* 2015).

Genotype data processing, genomic heritability estimation, and genome-wide association study (GWAS) analyses

Genotype data were filtered using Plink (Purcell *et al.* 2007) to exclude SNPs with minor allele frequencies < 5%, SNPs with missing genotype rate > 5%, and SNPs that strongly ($p < 1E-4$) deviated from Hardy Weinberg equilibrium. Additionally, individuals with high genotype missing rate (> 10%) were also excluded from the analysis. All of the animals that passed the QC filters were retained, and the final dataset contained genotype data for 24,740 informative SNP markers. GWAS was performed on 990 fish which had high quality phenotype and genotype data. Phenotype records (i.e. binary survival and days to death) were firstly adjusted to account for population substructure using the first two principal components that explained 77.5% of the variation between the animals. The heritability of resistance to VNN was estimated using the genomic relationships, and an equivalent mixed linear model to that described above. GWA analyses were used to assess the association between SNP marker genotypes and the corrected VNN resistance phenotype data using the GCTA software (Yang *et al.* 2011). GWAS results were then visualized using Manhattan plots using the qqman package implemented in R (Turner 2014).

Results

Parentage assignment analysis revealed that every parent (sires and dams) in the study population contributed a significant number of progeny with moderate degree of variability. Survival analyses of the families revealed marked differences in mortality levels within and between families for both dam and sire parents over the challenge study time. In general, moderate and significant heritability was found for the three VNN resistance traits (Table 1).

Table 1. Heritability estimates of VNN resistance in farmed seabass.

VNN resistance definition	**Pedigree based h^2 ± SE**	**Genomic h^2 ± SE**
Binary survival	0.38 ± 0.10	0.37 ± 0.05
Days to death (mortalities and survivors)	0.23 ± 0.08	0.39 ± 0.05
Days to death (mortalities only)	0.06 ± 0.10	0.21 ± 0.08

A total of 528, 578 and 42 markers were identified as significantly (FDR < 0.05) associated with binary survival, days to death (all animals), and days to death (mortalities only), respectively. Additionally, 92, 104 and 23 markers, respectively, showed suggestive (FDR < 0.1) association with the same traits. For all the three traits, most (78 to 95%) of the significant SNP markers were located on LG12 (also denoted chromosome 13, Figure 1a, b and c), indicating presence of a major QTL on this chromosome. For all the three traits, AX-371028009 was the most significant SNP marker associated with each of the traits and explained 12.7%, 14.0% and 6.3% of the phenotypic variation in VNN resistance when defined as binary survival, days to death (all animals), or days to death (mortalities only), respectively. This equates to 37.0%, 36.0% and 29.8% of the additive genetic variance of the same traits, respectively. Indeed this SNP demonstrated additive genetic effect on VNN resistance in seabass with the homozygous genotype GG associated with the highest (81% survival rate) level of resistance, while the AA genotype associated with the lowest (30.7% survival rate) resistance(Figure 1d). This SNP is located within the 26th intron of the EML5 (EMAP Like 5) gene. EML5 encodes for the Echinoderm microtubule associated protein like 5, which is involved in regulating cytoskeleton rearrangement (O'Connor et al. 2004).

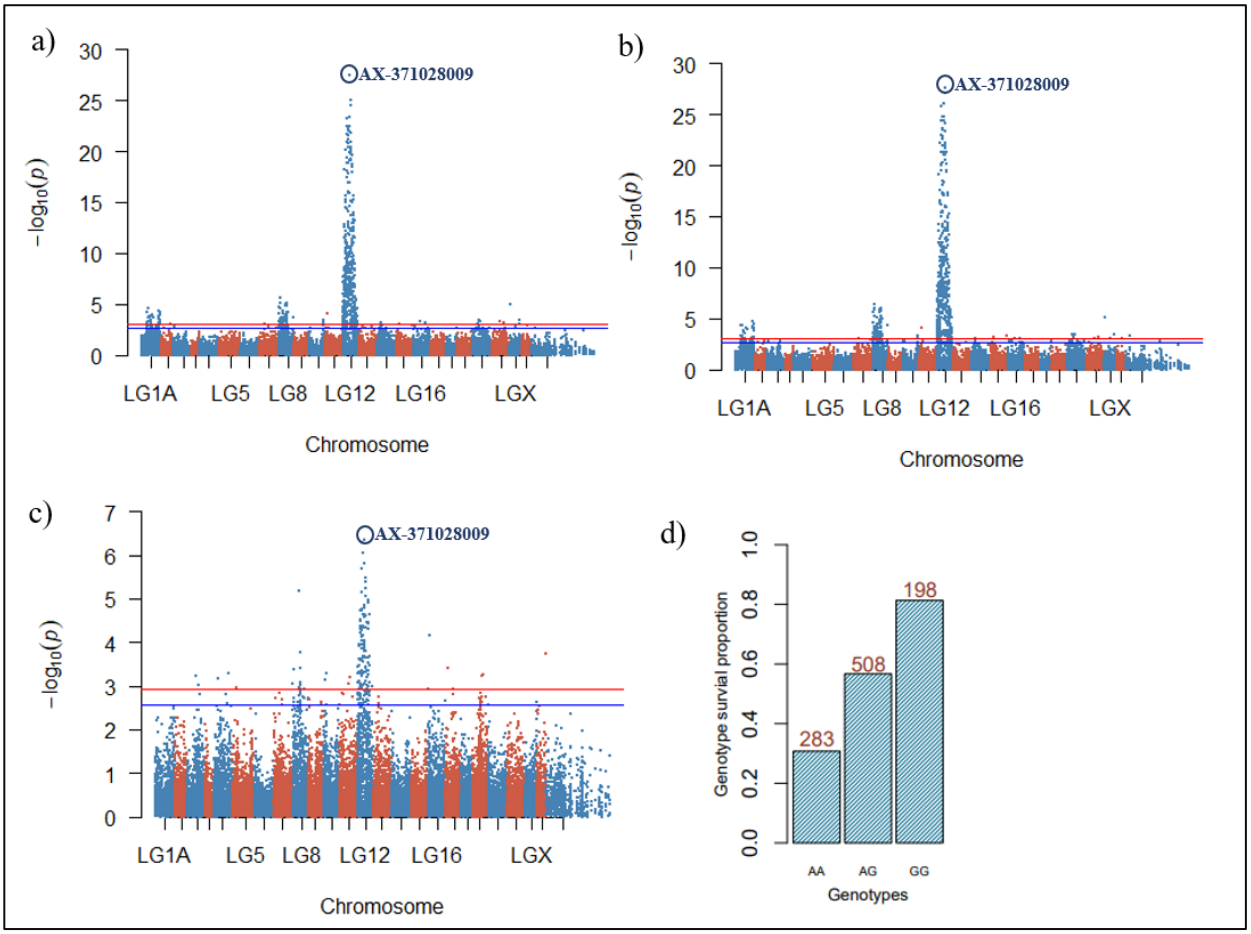

Figure 1. Manhattan plot summarizing genome-wide association study results for VNN resistance in European seabass defined as; a) binary survival, b) days to death for both mortalities and surviving fish, and c) days to death with mortalities; and d) bar plot showing survival proportion of individuals from the three genotype classes (AA, AG and GG) for the most significant SNP marker (AX-371028009), the numbers individuals with each of the three genotypes are provided above each bar in the plot.

Discussion

The moderate heritability estimates of VNN resistance observed in the current study concur with the moderate to high heritability estimates of VNN resistance that have been reported in other farmed European seabass populations (Palaiokostas et al. 2018; Faggion et al. 2021; Griot et al.

2021). Together, these results highlight the potential for selective breeding as a viable as a sustainable approach to improve resistance and therefore reduce economic losses due to disease outbreaks on seabass farms. The GWAS results presented herein are also in agreement with the recent discovery of a major QTL for VNN resistance on LG12 in different European seabass subpopulations (Griot *et al.* 2021) where the QTL explained up to 9.2% of the total genetic variability. Palaiokostas et al. (2018) did not identify such a major QTL on LG12, however they identified a SNP marker on LG12 that was significantly associated to VNN resistance and accounted for 4% of the total additive genetic variance (Palaiokostas et al. 2018). Therefore, the current study confirms the presence of a major VNN resistance locus on LG12 that can be utilized for breeding of more resistant seabass populations. Further analyses are underway using whole genome resequencing data for all animals, combined with gene expression comparison of resistant and susceptible animals, with a view to identifying the causative gene and variant for this major QTL.

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