Major Quantitative Trait Loci For Viral Nervous Necrosis Resistance In Atlantic Cod

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Introduction

Nodavirus has been reported to cause high mortalities in more than 20 marine fish species worldwide, with the same viral strains causing disease across species (Starkey, Ireland, Muir, et al. (2001); Munday, Kwang, and Moody (2002)). In Atlantic cod, the outbreaks of viral nervous necrosis (VNN) are typically chronic with moderate mortalities and have been observed in Scotland (Starkey, Ireland, Muir, et al. (2001), North America (Johnson, Sperker, Leggiadro, et al. (2002)) and Norway (Patel, Korsnes, Bergh, et al. (2007)). Studies on the additive genetic variation for resistance against the development of VNN have revealed extremely high heritability, with a value of 0.75 on the underlying scale (Ødegård, Kettunen Præbel, and Sommer (2010)). Given the lack of within-family selection for disease resistance traits in aquaculture species due to disease transmission risks, there is great potential for marker-assisted selection (MAS) or genomic selection to substantially improve genetic gain. MAS is now being successfully implemented at a major quantitative trait locus (QTL) for infectious pancreatic necrosis resistance in Atlantic salmon (Houston, Haley, Hamilton, et al. (2008); Moen, Baranski, Sonesson, et al. (2009)). This study aimed to detect QTL for resistance to VNN in a population of Atlantic cod within the national breeding program in Norway.

Material and methods

Mapping families and challenge test. The mapping population consisted of 20 full-sib families of around 100 individuals each challenge tested with nodavirus. The families in the current study were produced in March/April 2007, and had parents from the year-classes 2003 and 2004, representing the second generation of cod bred in captivity. Families were chosen for the mapping experiment based on the criteria of having parental tissue samples available, and overall intermediate mortality (higher likelihood of segregating QTL). At the day of challenge (17–21 d after tagging), fish were anaesthetized with Metacaine (0.7 g/l) and subjected to an intramuscular injection of 0.1 ml nodavirus suspension with a titre of 3×10^8 $TCID_{50}$ ml^{-1} in the anterior dorsal region. Mortality was recorded daily, and the experiment was terminated 35 days post-challenge.

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Genotyping. Selective genotyping was performed by sampling 13 survivors (or late mortalities) and the first 13 individuals to die in each family (representing a selective genotyping fraction of around 14% at each extreme). One hundred and sixty-one microsatellite markers were genotyped in multiplexes of four to eight markers. The lengths of the fluorescent PCR products were determined relative to the LIZ500 size standard (Applied Biosystems) on a 3730 DNA Analyzer (Applied Biosystems), using GeneMapper 4.0 (Applied Biosystems) software for allele calls. Genotype data was checked for pedigree errors and unlikely double recombinants.

Linkage mapping and QTL analysis. Sex-averaged linkage maps were produced using CRIMAP software. QTL detection was carried out using a linear regression interval mapping approach in GridQTL (Seaton, Hernandez, Grunchec, et al. (2006)). The binary trait dead/alive was analysed together with the number of survival days in the challenge. P-values were calculated for all trait-by-chromosome combinations with the significance of the peak F-statistic (putative QTL) estimated after 10,000 chromosome-wide permutation tests. A QTL was found to be genome-wide significant if the chromosome-wide significance level was smaller than 0.05 * 23, a Bonferroni correction based on the number of chromosomes in Atlantic cod. Correction for overestimation of QTL effects due to selective genotyping was performed by the method of Darvasi and Soller (1992). Confidence intervals (CI) were estimated for each genome-wide significant QTL using the bootstrap method and 10,000 iterations.

Results and discussion

One hundred and ten markers were found to be polymorphic, and mapped to 22 linkage groups covering a total of 834 cM (sex-averaged). Five genome-wide significant QTL were detected for resistance to VNN, explaining a total of 68% of the phenotypic variance for survival, after correction for the selective genotyping fraction (table 1). The same QTL were detected for both the binary dead/alive trait or survival days in challenge. These QTL were found to be segregating in 20% to 45% of the informative parents, suggesting potentially low selection pressure in the natural environment. The QTL on linkage group 1 had the largest within-family effect (table 2), and was mapped to a 19 cM confidence interval (figure 1).

Table 1: Summary of genome-wide significant QTL detected

Linkage group	Markers[a]	Position (cM)	F	P-value	PVE[b]	No seg pars[c]
1	14 (51)	40	4.55	<0.0001	18.2	8 (40)
6	5 (71)	9	2.29	0.0003	7.2	8 (39)
18	3 (46)	0	4.89	<0.0001	19.7	14 (31)
19	2 (0)	0	3.69	<0.0001	14.2	6 (28)
20	2 (9)	0	2.63	<0.0001	9.0	10 (29)

[a] Number of markers mapped to linkage group (map distance in cM)
[b] Percentage of phenotypic variance explained by QTL after correction for selective genotyping
[c] Number of parents segregating for the QTL (number of informative parents)

Table 2: Allele substitution effects for the QTL on LG1 and segregation data for the nearest microsatellite in QTL heterozygous parents

				Survivors[c]		Mortalities[c]	
Parent	ASE[a]	S.E.	ABS(t)[b]	Allele A	Allele B	Allele A	Allele B
P6	0.94	0.18	5.34	1	11	12	0
P41	0.81	0.18	4.57	3	10	13	0
P1320	0.65	0.18	3.64	4	9	10	2
P1315	0.78	0.19	4.01	3	10	12	1
P13	0.43	0.22	1.96	3	9	11	2
P1274	1.01	0.17	5.77	0	13	12	1
P1114	1.02	0.18	5.62	0	13	12	1
P1113	0.46	0.20	2.25	3	10	8	5

[a] Allele substitution effect
[b] Absolute t-value
[c] Allele counts in phenotypic extremes for nearest informative microsatellite to QTL peak

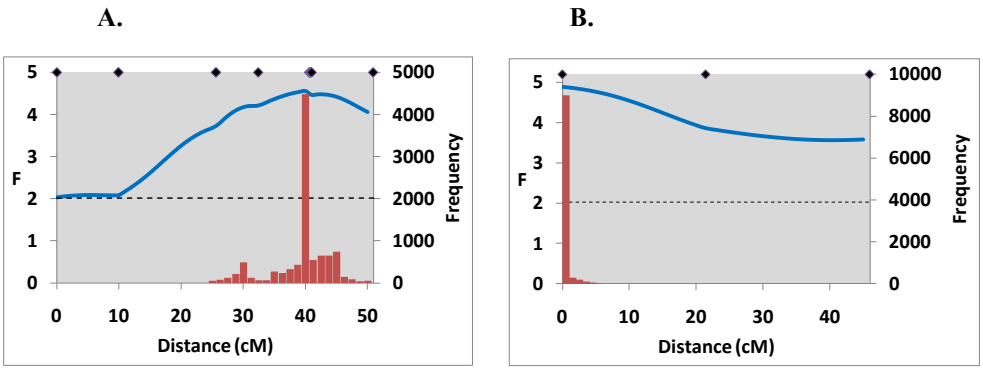

Figure 1: F-statistic profile (blue line) and QTL position bootstrap frequencies (red bars) for (A) LG1 and (B) LG18. Genome-wide significance threshold is indicated by the horizontal broken line. Marker positions are indicated by black diamonds on the upper x axis.

Conclusion

These results show that major QTL explaining a large portion of the phenotypic variance for VNN resistance are segregating in Norwegian farmed Atlantic cod, and are in agreement with other QTL studies for viral resistance in aquaculture species where loci of large effect are reported (Fuji, Kobayashi, Hasegawa, et al. (2006); Houston, Haley, Hamilton, et al. (2008); Moen, Baranski, Sonesson, et al. (2009)). The magnitude of these QTL, and the inherent breeding and phenotypic recording structuring in Atlantic cod farming mean that marker-assisted selection at these QTL could be relatively rapidly implemented to increase genetic gain. Through the use of a high-density SNP resource currently in development, these QTL will be validated and fine-mapped in a large mapping population.

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