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Genetic Variation in Resistance to Pancreas Disease in Atlantic Salmon.

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ABSTRACT: The viral disease Pancreas Disease (PD) is currently one of the most problematic diseases on Atlantic salmon farms. The aim of this study was to explore the genetic architecture of PD resistance. In a population of 5,558 PD-challenged Atlantic salmon fry from 218 full-sib families, the heritability of PD mortality was estimated to be ~0.5. Resistance QTL were mapped using a two-step approach, utilizing the disparity in recombination rates between the sexes in salmon. First, using a sparse SNP map and sire-based linkage analyses, one genome-wide significant QTL was mapped to chromosome 23, while two chromosome-wide significant QTL mapped to chromosomes 3 and 7. Second, a denser panel of SNPs on significant chromosomes and a dam-based analysis was used to confirm a QTL on chromosome 3 and estimate QTL positions. Further work to characterize these QTL is underway.

Keywords: pancreas disease; Atlantic salmon; QTL mapping

Introduction

Salmonids, particularly Atlantic salmon, are an increasingly important farmed species, as the demand for aquaculture products as a sustainable source of high protein and long chain fatty acids increases. However, with the intensification of aquaculture, an increase in the incidence of viral, bacterial and parasitic diseases has been reported (Soares et al. (2011)). Pancreas disease (PD), caused by the salmon pancreas disease virus (SPDV), is currently one of the most problematic diseases, causing high levels of morbidity and mortality on aquaculture farms (McLoughlin et al. (2002)). Management techniques to control or prevent the effects of PD have not been fully successful (Rodger and Mitchell (2007)).

The use of genomic techniques to selectively breed individuals with desired traits is increasingly gaining ground in aquaculture farming (e.g. Fuji et al. (2007), Houston et al. (2008), Ozaki et al. (2012)), particularly for disease resistance traits. Host genetic variation in PD resistance has been reported, with the heritability of PD resistance in farmed Atlantic salmon estimated at 0.21 (Norris et al. (2008)). Therefore, there is sufficient genetic variation to select for PD resistance. Selection, using genetic markers, is particularly useful for disease resistant traits, since the resistance status of an individual can be determined without exposing the individual to the pathogen. This not only improves fish welfare, but also allows selection of the most resistant fish at the individual rather than family level (Haley and Visscher (1998); Sonesson (2005)).

The aim of this study was to explore the genetic architecture of PD resistance, using Atlantic salmon fry as a challenge model. The main aims were to: (i) estimate the heritability of PD resistance in our study population and (ii) use a two-step approach which takes advantage of the difference in recombination rate between males and females to identify chromosomes harboring QTL for PD resistance, and localize the QTL on these chromosomes.

Materials and Methods

SPDV challenge and parentage assignment. Challenge experiments were carried out by Marine Harvest (MH). 5,558 fry from 218 full-sib (82 half-sib) families were challenged with a Norwegian strain of SPDV using a cohabitation challenge. Fry were reared and challenged in a single tank to eliminate tank effects. The experiment was terminated after 57 days, when mortalities were negligible. Each day, mortalities were collected from the tank and tissue samples were dated and stored in ethanol. At the end of the challenge, survivors were collected and tissue samples were stored in ethanol. Survivors were assigned back to parents using a panel of microsatellite markers by MH. DNA was extracted from mortalities using the Qiagen DNeasy Blood and Tissue kit (96 well format), and the parentage of mortalities was determined using a panel of 69 SNPs (Moen et al. (2008)) and three parentage assignment software (Anderson and Dunham (2005); Vandeputte et al. (2006); Taggart (2007)).

Estimating heritability. Successfully assigned parents and offspring were used to estimate the heritability of PD resistance, using ASReml (Gilmour et al. (2006)) with the following mixed model: $Y_{ijk} = \mu + Sire_i + Dam_{ij} + e_{ijk}$; where Y_{ijk} is the observed SPDV challenge outcome for individual k with sire i and dam j nested within the i^{th} sire; μ the population mean; and e_{ijk} the residual for individual k. Sire and Dam were fitted as random effects, with variances σ^2_s and σ^2_d, respectively, and the total additive genetic variance defined as $2(\sigma^2_s + \sigma^2_d)$. Given the binary nature of the data (0/1 for survived/died), the heritability was estimated on three scales: observed binary, logit link and probit link scales. Assuming a continuous underlying liability, the binary scale heritability was converted to the underlying liability scale using the formula (as per Falconer and Mackay (1996)): $h^2=h^2_{01}(1-p)/i^2p$. Also, the heritability of time to death was estimated using mortalities only: time to death was analyzed using the same mixed model as above, where Y_{ijk} is days post challenge.

PD resistance QTL mapping. QTL mapping was carried out using a two-step approach, as described by Hayes et al. (2006) and applied by Houston et al. (2008). We applied this approach as follows using 1,273 offspring (463 survivors, 810 mortalities) in 20 half-sib (55 full-sib) families showing intermediate levels of mortality (half-sib family size range: 41-90 offspring; half-sib family mortality range: 40-70%). Step 1: The sparse 69 SNP panel (spanning all 29 chromosomes) used in parentage assignment of

mortalities was also genotyped in the surviving offspring. The entire dataset was analyzed for within-sire segregation of QTL with PD survival/ mortality using the GridQTL software (Allen et al. (2012)) (www.gridqtl.org.uk). This identified three chromosomes containing significant QTL. A dam-linkage analysis with the same data identified another significant chromosome, totaling four chromosomes for further investigation. Step 2: A further 29 SNPs (from Lien et al. (2011) and Gonen et al. (in press)) were genotyped across these four chromosomes. Linkage maps using the denser SNP panel (36 SNPs, i.e. 29 SNPs plus 8 SNPs used in the sparse panel) were built using the Lep-MAP software (Rastas et al. (2013)) for each of these four chromosomes (Table 1). As females have a higher recombination rate, a dam-based linkage analysis was then conducted on these four chromosomes. This aimed to confirm and refine the QTL positions. The within-family variance explained by the QTL (PVE) was estimated as follows. For sire-linkage analysis: $h^2_{QTL}=4[1-(MSE_{full}/MSE_{red})]$, where MSE_{full} and MSE_{red} are the mean square errors for the models with and without the QTL, respectively. PVE was also estimated using both sire and dam models from step 1, as follows: $h^2_{QTL}=2\{[1-(MSE_{full}/MSE_{red})_{SIRE}] + [1-(MSE_{full}/MSE_{red})_{DAM}]\}$.

Table 1: SNP maps of significant PD resistance chromosomes.

Chromosome	No. of SNPs sparse analysis	No. of SNPs denser analysis	Dense SNP map length (cM)
3	1	12	135
4	2	6	77
7	3	7	65
23	2	11	71

Results

Challenge profile. PD mortalities began at ~5 days post-challenge and peaked at ~11 days post-challenge before falling to baseline levels by ~34 days post challenge (Figure 1). 2,102 survivors and 2,455 mortalities were successfully assigned parentage. Full-sib families with fewer than 15 assigned offspring were removed in order to have a sufficient number of offspring informative at the chosen markers, leaving 3,949 offspring in 72 half-sib (150 full-sib) families (2,367 mortalities, 1,582 survivors). The average mortality rate in these families was 61%, and ranged from 0-100% (Figure 2).

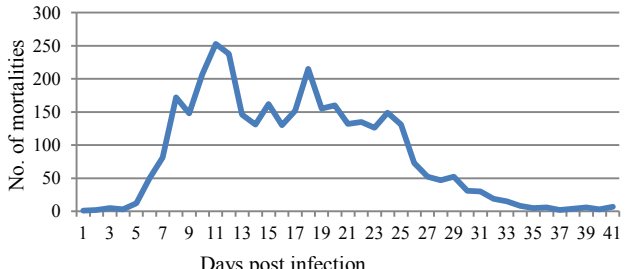

Figure 1: PD cl mber of mortalities observed per day.

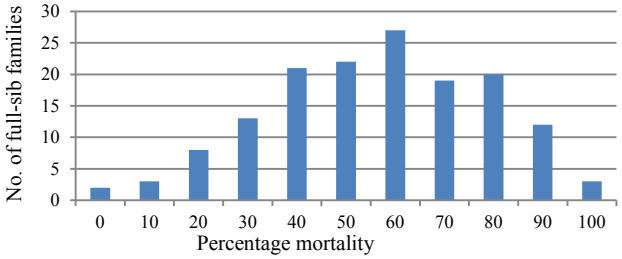

Figure 2: Histogram of full-sib family mortality rates.

Estimating heritability. The heritability of resistance to PD was estimated at ~0.5 (Table 2), and this estimate was consistent across the different models. This is high for a disease resistance trait and suggests considerable genetic variation in PD resistance in this population. The time to death amongst mortalities was not heritable in our population.

Table 2: Estimated heritabilities for PD resistance.

Method	Heritability (±SE)
Observed binary scale	0.34 (±0.05)
Underlying liability scale	0.55
Probit-link scale	0.54 (±0.07)
Logit-link scale	0.46 (±0.06)

PD resistance QTL mapping. Sire-based analyses utilizing low recombination identified three chromosomes (3, 7 and 23) with significant QTL and chromosome 23 reached genome-wide significance (determined using 50,000 permutations). Dam-linkage analysis using the same map confirmed the QTL on chromosome 3, and identified another QTL on chromosome 4. Dam-based linkage analyses with the denser map confirmed and mapped the QTL on chromosomes 3 and 4 to the ends of the chromosomes, at positions 135 cM and 74 cM respectively. Numbers of sires and dams segregating for each QTL and the percentage of variation explained by each QTL are shown in Table 3.

Table 3: Number of QTL segregating sires and dams, and PVE estimations.

Chromosome	No. of S[1]. Sires	No. of S. Dams	PVE (%) (sire)	PVE (%) (sire + dam)
3	3	8	7.6	9.2
4	0	6	NS	6.3
7	3	5	7.4	5.5
23	2	6	8.3	4.8

NS – not significant; [1]S. = segregating.

Discussion

Using a cohabitation method of challenge in Atlantic salmon fry, this study has explored the genetic architecture of resistance to infection with SPDV. We demonstrated considerable genetic variation in PD resistance in a large dataset, with the heritability estimate (~0.5) being much higher than for most disease traits. Notably, this estimate is higher than that of Norris et al. (2008) (0.21) which looked at a natural SPDV challenge in

farmed Atlantic salmon smolts. The difference in life stage between the two studies (fry vs. smolts), as well as the challenge model (tank vs. natural challenge) and the lower mortalities obtained in the natural challenge may explain the difference in heritability observed.

The very low recombination rate across much of the genome in male Atlantic salmon results in tight linkage between markers. This is advantageous for QTL mapping experiments, since a sparse marker panel can be used to identify chromosomes with QTL by tracking inheritance from males. QTL positions can be refined using a dam-linkage analysis with a denser marker panel, genotyped only on chromosomes which were significant in the sparse SNP analysis, thus reducing genotyping costs.

With the sparse SNP map, this study identified four chromosomes with at least chromosome-wide significant QTL. The genome-wide significant QTL on chromosome 23 was segregating in only two sires, albeit with large effect (estimates of effect in mortality proportion: -0.71±0.16 and -0.48±0.17). This is consistent with a relatively rare variant with a large effect on PD resistance segregating in this commercial population. To support this, two (of 55) dams reached significance in the full-sib family QTL analyses, and both were estimated to have a large within-family effect. However, the overall dam-based analysis for chromosome 23 did not reach significance. The QTL on chromosome 3 was replicated with the denser panel in a dam analysis, providing some validation of the result for this chromosome.

The use of genomic techniques to select and breed for disease resistant fish have become increasingly popular in aquaculture. In Atlantic salmon, QTL for diseases such as infectious pancreatic necrosis (Houston et al. (2008); Moen et al (2009)), infectious salmon anaemia (Moen et al. (2007)) and resistance to the parasite *Gyrodactylus salaris* (Gilbey et al. (2006)) have been identified, as well as single gene effects, such as PPARG polymorphisms influencing furunculosis resistance (Sundvold et al. (2010)). In common with this study, chromosomes 3, 4 and 23 have been shown to have QTL influencing *G. salaris* resistance, suggesting that these chromosomes may contain genes involved in a general innate immune response.

Conclusions

We estimated a high (~0.5) heritability for PD resistance using a cohabitation challenge model. Sire- and dam-linkage analyses identified four chromosomes with QTL significantly influencing the outcome of infection with SPDV, two of which were refined using a denser SNP mapping panel. We now aim to: (i) characterize these QTL in order to better understand factors influencing PD resistance, and (ii) identify markers in linkage disequilibrium with the QTL, potentially enabling marker-assisted selection.

Acknowledgments

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Literature Cited

Allen, J., Scott, D., Illingworth, M. et al. (2012). Digital Research 2012, September 10-12, 2012 Oxford, UK.

Anderson, E. C., and Dunham, K. K. (2005). Mol. Ecol. Notes 5:459-461.

Fuji, K., Hasegawa, O., Honda, K. et al. (2007). Aquaculture 272:291-295.

Falconer, D. S., and Mackay, T. F. C. (1996). Introduction to quantitative genetics. 4th ed London: Longmann.

Gilbey, J., Verspoor, E., Mo, T. A. et al. (2006). Dis. Aquat. Org. 71:119-129.

Gilmour, A. R., Gogel, B. J., Cullis, B. R. et al. (2006). ASReml User Guide Release 2.0 VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Gonen, S., Lowe, N. L., Cezard, T. et al. (in press). BMC Genomics.

Haley, C. S., and Visscher, P. M. (1998). J. Dairy Sci. 81:85-97.

Hayes, B. J., Gjuvsland, A., Omholt, S. (2006). Heredity 97:19-26.

Houston, R. D., Haley, C. S., Hamilton, A. et al. (2008). Genetics 178:1109-1115.

Lien, S., Gidskehaug, L., Moen, T. et al. (2011). BMC Genomics 12.

McLoughlin, M. F., Nelson, R. N., McCormick, J. I. et al. (2002). J. Fish Dis. 25:33-43.

Moen, T., Baranski, M., Sonnesson, A. K., et al. (2009). BMC Genomics 10.

Moen, T., Hayes, B., Baranski, M. et al. (2008). BMC Genomics 9.

Moen, T., Sonesson, A. K., Hayes, B. et al. (2007). BMC Genetics 8.

Norris, A., Foyle, L., Ratcliff, J. (2008). J. Fish Dis. 31:913-920.

Ozaki, A., Araki, K., Okauchi, M. et al. (2012). Bull. Fish. Res. Agency 35:31-37.

Rastas, P., Paulin, L., Hanski, I. et al. (2013). Bioinformatics 29:3128-3134.

Rodger, H., and Mitchell, S. (2007). J. Fish Dis. 30:157-167.

Soares, S., Green, D. M., Turnbull, J. F. et al. (2011). Aquaculture 314:7-12.

Sonesson, A. K. (2005). Genet. Sel. Evol. 37:587-599.

Sundvold, H., Ruyter, B., Ostbye, T. K. et al. (2010). Fish Shellfish Immunol. 28:394-400.

Taggart, J. B. (2007). Mol. Ecol. Notes 7:412-415.

Vandeputte, M., Mauger, S., Dupont-Nivet, M. (2006). Mol. Ecol. Notes 6:265-267.