# Quantitative Trait Loci for Resistance to Heart- and Skeletal Muscle Inflammation in Atlantic salmon

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## Summary

Heart and skeletal muscle inflammation (HSMI) is a disease that causes substantial economic loss and animal welfare problems in farming of salmonids. In Atlantic salmon, outbreaks of the disease can cause up to 20 % mortality at affected sites, and morbidity is frequently close to 100 %, resulting in under-sized fish and poor product quality. Aiming at the identification of DNA markers to be used in marker-assisted selection (MAS), we performed a genome-wide association study (GWAS) on resistance to HSMI. Histopathology scores and CT-values for an infection-correlated gene were used as traits, and genotypes were obtained using a custom Affymetrix 50k SNP-chip for Atlantic salmon. The scan revealed that the trait is largely under the control of two major QTL, located on two chromosomes. The two QTL were responsible for more than 25 % of the phenotypic variation in histopathology scores, and almost 10 % of the phenotypic variation in CT-values, indicating a substantial potential for genetic improvement by means of MAS.

*Keywords: salmon, quantitative trait locus, heart and skeletal muscle inflammation*

## Introduction

Heart and skeletal muscle inflammation is a disease that causes substantial economic losses and animal welfare problems in farming of salmonids. In Atlantic salmon, outbreaks of the disease can cause up to 20 % mortality at affected sites, and morbidity is frequently close to 100 %, resulting in under-sized fish and poor product quality. The disease has been diagnosed in Norway, Scotland, Ireland, Chile, and Canada based on histopathological analysis of heart and muscle tissue (Biering & Garseth 2012, Olsen *et al.* 2015, Godoy *et al.*, 2016, Hjeltnes *et al.* 2016). The aetiological agent is believed to be a recently discovered virus called Piscine Reovirus (PRV) (Kongtorp *et al.* 2004; Palacios *et al*. 2010). The disease is primarily a problem during the first 6 months after transfer to sea cages, but outbreaks have also been recorded in freshwater hatcheries that use seawater in their production. A cure for HSMI has not yet been found, and commercial vaccines are not available. In the absence of other means for combatting HSMI, selective breeding could be used to create fish with increased resistance to the disease. Here, we wanted to investigate the potential for increasing resistance to the disease through Marker-Assisted Selection (MAS).

## Materials and Methods

### Challenge trial

The challenge trial was performed at VESO Vikan (Namsos, Norway). A total of 887 Atlantic

salmon (*Salmo salar*) smolts, from 507 full-sib groups (smolts per full-sib group: mean = 1.5, range = 1 - 4; smolts per sire: mean = 7.3, range = 2 - 12; smolts per dam: mean = 7.4, range = 1 - 13), having an average weight of 83 grams, were included in the test. Each smolt had been tagged with Passive Integrated Transponder (PIT) tags prior to testing. The challenge trial followed a cohabitant model, and was performed in sea water. Mortalities were registered daily until the test was terminated at 10 weeks post challenge. At this point all test fish were registered and sampled: From 240 fish, heart and skeletal muscle biopsies were taken and fixed in 10 % buffered formalin for subsequent histopathology.

**Histopathology**

Formalin-fixed samples were prepared for histological examinations by standard paraffin wax techniques and stained with haematoxylin and eosin (H&E stain).
Sections of cardiac and skeletal muscle tissue from individual fish were classified histologically based on the presence of mononuclear leukocyte infiltration and muscular degeneration and necrosis. The atrium, epicardium, compact and spongy layers of the ventricle and the endocardium were examined and evaluated. The findings were graded from 0-4 according to the following criteria: 0 = No pathological findings, 1 = Few focal lesions, slightly increased number of leukocytes, 3 = Several distinct lesions and moderate increase in numbers of leukocytes, 4 = Multifocal to confluent lesions and severe increase in number of leukocytes.

**Reverse transcription quantitative PCR (RT-qPCR)**

In order to provide an alternative (less expensive) phenotype analysis for HSMI infection or resistance, transcription levels of the Atlantic salmon *cluster of differentiation* 8 (*cd8*) gene was measured using reverse transcription quantitative PCR. Duplex real time PCR was performed using primers targeted against the Atlantic salmon *cd8* alpha chain and the housekeeping gene *elf1a*. Amplification was performed using QuantiTect Probe RT-PCR kit (Qiagen) on a Rotor-Gene Q 2 channel Real Time PCR Machine, 40 cycles of 95°C 15 sec and 60°C 60 sec.

**Genotyping**

All fish were genotyped using a custom Axiom®SNP genotyping array from Affymetrix (San Diego, CA, USA), containing 56,177 SNPs. The SNPs on the chip had been identified through Illumina paired-endsequencing of 30 animals from the AquaGen breeding nucleus, using a draft reference genome for Atlantic salmon as reference. The SNP-chip was genotyped, and genotypes were called, using software and best practices provided by Affymetrix.

**Genome-wide association study (GWAS)**

Each genotyped SNP was assayed individually for its effect on the two traits. When the phenotype was histopathology score, the linear mixed model for each SNP was $y = mean + animal + geno + error$, where $y$ = histopathology score for the animal in question; *mean* = the overall mean across all animals; *animal* = the additive genetic value of the animal in question (random effect); *geno* = genotype at the SNP for the animal in question (random regression; coded as 0, 1, and 2 for one homozygous genotype, the heterozygous genotype, and the other homozygous genotype, respectively); error = residual error. When the

phenotype was cd8-qPCR, the linear mixed model for SNP *i* was $y = mean + plate + animal + geno + error$, where $y$ = histopathology score; *mean* = the overall mean across all animals; *plate* = fixed effect of the sample plate on which the RNA sample corresponding to the animal in question were located, and other terms were as defined above. The linear mixed model equations were solved using the software DMU (Jensen *et al.* 1997). The likelihood ratio test (LRT) statistic was used in order to compare likelihoods under the null hypothesis (without *geno* effect), with the one under the alternative hypothesis (with *geno* effect).

## Results

The two traits were strongly correlated (Pearson correlation coefficient ± standard error = -0.91 ± 0.16). The correlation coefficient's negative sign shows that high histopathology scores (severe degrees of pathology) correlate to low cd8-qPCR values (signifying high concentrations of cd8 mRNA and hence high levels of viral infection). High concentrations of *cd8* mRNA correlate to low resistance because they indicate that the animal has mounted an immune response to HSMB virus present in the body as a result of infection.

The GWAS results revealed that histopathology scores are strongly affected by a quantitative trait locus (QTL) on Atlantic salmon chromosome A (Figure 1). The most significant tag-SNP had a p-value of $1.9*10^{-13}$ and explained 38 % of the genetic variation and 26 % of the phenotypic variation. The GWAS results further revealed that cd8-qPCR values are strongly affected by a QTL located within the same region of the same chromosome (Figure 1). The most significant tag-SNP of this QTL had a p-value of $2.3*10^{-8}$ and explained 47 % of the genetic variation and 5 % of the phenotypic variation. Another QTL for cd8-qPCR was found on chromosome B (Figure 1). The best tag-SNP for this QTL had a p-value of $8.6*10^{-8}$ and explained 42 % of the genetic varioation and 5 % of the genetic variation. The best tag-SNPs for the latter QTL were, on inspection, also found to be nominally (but not genome-wide) significant for histopathology scores. The lack of genome-wide significant effects on histopathology scores, for SNPs on chromosome B, could possibly be explained by the lower number of observations on histopathology score relative to cd8-qPCR, and by a low minor-allele frequency observed at the chromosome B QTL.

In summary, we conclude that both traits are likely to be affected by a QTL on chromosome A and by a QTL on chromosome B. Since both traits are proxys for HSMI-resistance, MAS on the two loci could lead to improved resistance to HMSI. The two QTL are currently being fine-mapped, with an aim to identify candidate causative genes and -mutations. The QTL is being implemented in AquaGen's production of Atlantic salmon eggs as of the 2017/2018 season.

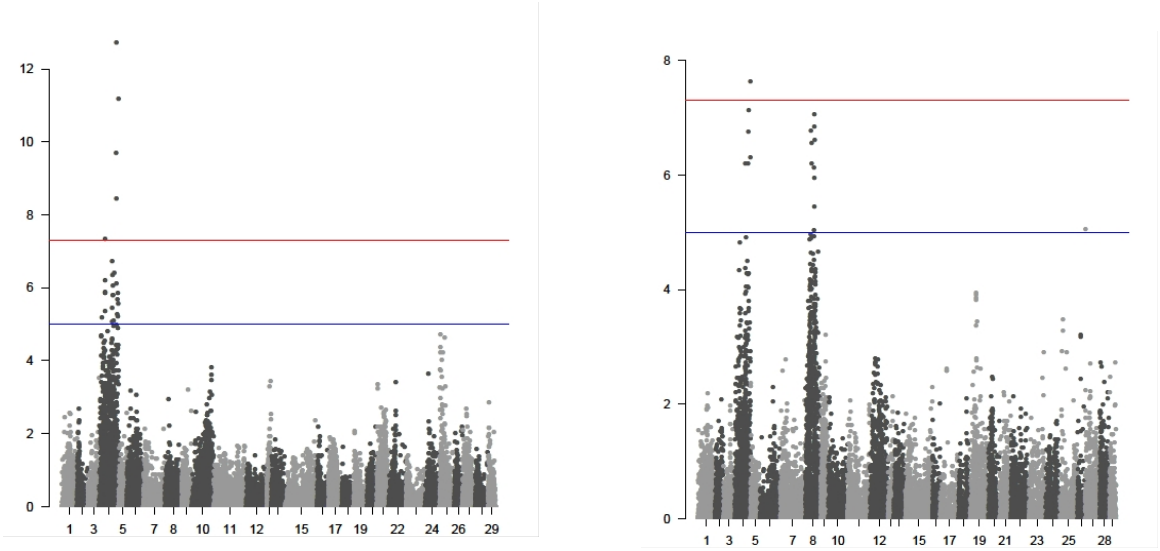


*Figure 1. GWAS for histopathology scores and cd8 Ct-values*. Chromosome numbers have been randomized. Y-axes values = -log10(p-value)

## List of References

Biering, E. & Å.H. Garseth, 2012. Heart and skeletal muscle inflammation (HSMI) of farmed Atlantic salmon (Salmo salar L.) and the associated Piscine reovirus (PRV). ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. Leaflet No. 58. 6 pp.

Godoy M.G., M.J.T. Kibenge, Y. Wang, R. Suarez, C. Leiva, F. Vallejos & F.S.B. Kibenge, 2016. First description of clinical presentation of piscine orthoreovirus (PRV) infections in salmonid aquaculture in Chile and identification of a second genotype (Genotype II) of PRV. Virology Journal 13: 98.

Hjeltnes B., C. Walde, B. Bang Jensen & A. Haukaas (ed.), 2016. The Fish Health Report 2015. The Norwegian Veterinary Institute 2016.

Jensen J., E. A. Mäntysaari, P. Madsen & R. Thompson, 1997. Residual maximum likelihood estimation of (co) variance components in multivariate mixed linear models using average information, Jour Ind Soc Ag Statistics 49, 215-236.

*Kongtorp R.T., A. Kjerstad, T. Taksdal, A. Guttvik & Falk K. (2004) Heart and skeletal muscle inflammation in Atlantic salmon, Salmo salar L.: a new infectious disease. J Fish Dis. 2004, 27: 351-358.*

Olsen A.B., M. Hjortaas, T. Tengs, H. Hellberg, and R. Johansen, 2015. First Description of a New Disease in Rainbow Trout (*Oncorhynchus mykiss* (Walbaum)) Similar to Heart and Skeletal Muscle Inflammation (HSMI) and Detection of a Gene Sequence Related to Piscine Orthoreovirus (PRV). PLoS ONE 10: e0131638.

*Palacios G., M. Løvoll, T. Tengs, M. Hornig, S. Hutchison, J. Hui, R.T. Kongtorp, N. Savji, A.V. Bussetti, A. Solovyov, A.B. Kristoffersen, C. Celone, C. Street, V. Trifonov, D.L. Hirschberg, R. Rabadan, M. Egholm, E. Rimstad & W.I. Lipkin, 2010. Heart and skeletal muscle inflammation of farmed salmon is associated with infection with a novel reovirus. PLoS One 5: e11487-10.*