IDENTIFICATION OF CHROMOSOMAL REGIONS ASSOCIATED WITH RESISTANCE/SUSCEPTIBILITY TO VHSV IN DOUBLED HAPLOID RAINBOW TROUT (*O. mykiss*): FIRST RESULTS

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Introduction

Viral haemorrhagic septicaemia (VHS), caused by Egtved rhabdovirus, is one of the diseases that constrain rainbow trout production in Europe. Different studies support the existence of genetic variation within trout populations for the susceptibility to the disease. The present study aimed at detecting quantitative trait loci (QTLs) affecting resistance in the species, as a first step for future prospects in selective breeding and increased knowledge of genetic architecture of disease resistance in fish.

Material and Methods

Selection of homozygous grand-parents for alternative resistance

Experimental fish originated from the experimental INRA SY strain. Mitotic homozygous gynogenetic rainbow trout were screened using an *in vitro* test for resistance, the VREFT value (viral replication in excised fin tissue), which we previously showed to be correlated with resistance to waterborne challenge against the virus (Quillet *et al.*, 2001). 'Resistant' (R) and susceptible (S) individuals were selected on the VREFT values, and their expected resistant/susceptible status was further checked by performing progeny testing.

Production of doubled haploid experimental progeny

Clonal F1 hybrids were produced by intercrossing the selected R and S parents. F1 females were then reproduced by mitotic gynogenesis, according to Ditere*at al*. (1993), in order to produce the experimental progeny. Two non related haplo-diploid families (F98 and F00) were produced in two different years and used in the present study. Experimental fish were reared under controlled conditions (recirculated tap water unit).

Phenotypic testing of resistance to VHSV

Waterborne challenge. Resistance tests to VSHV were conducted with waterborn challenges, according to Dorson *et al*. (1991) on 4 to 5-months old juveniles (mean weight: 1.2 to 1.6g). Infected fish were kept for 2h in a 5 x 10^4 pfu ml^{-1} virus suspension with vigorous aeration after stopping the water supply. For each family, 1100 to 1200 progeny, distributed into 9 or 10 replicated aquaria were challenged, and a control group was mock-infected. Mortality was monitored twice a day until a plateau was reached (34 days in F98 and 58 days in F00). Total mortality was 92.6% in F98 and 79% in F00. Dead fish were immersed in absolute ethanol for further DNA extraction. At the end of the period of survey, surviving fish were sacrificed (lethal dose of anaesthetics), and immersed in ethanol.

In vitro test for resistance. A sample of 300 F00 progeny not challenged against the virus was kept for further growth. When they were 16-months old, fish were anaesthetized, and individually PIT-tagged. Anal and/or pelvic fin were clipped and processed as described in Dorson and Torhy (1993) to perform a measure of replication in excised fin tissue (measure VREFT-1). Seven months later, a random sample of 100 fish were scored again (measure VREFT-2).

Genetic markers and source of DNA

Genetic markers. Microsatellites previously developed for salmonids were utilized. They were amplified with PCR conditions optimised for each microsatellite, and were run on polyacrylamide gels, using fluorescent labelled primers. Alleles were detected using a FM-BIO II, Hitachi fluorescence scanner. Microsatellites used to test association with resistance were chosen from linkage information in the families, in order to allow a even genome scan whenever possible.

Source of DNA. Selective genotyping was used to test association with challenge issue. In a first step, the earliest dead fish (10% of challenged progeny) and the survivors (all survivors in family F98, and 10% of the challenged fish in family in F00) were sampled from each aquarium (sample 1). Fish were examined for every chosen locus, and a test for association between genotype at the marker and challenge issue was performed. When the test was suggestive (P<20%), a second sample was examined (sample 2, including the next 10% to die, and 10% 'resistant' taken at the end of the mortality curve in F98, or among survivors in F00) in order to confirm the result.

Association with VREFT values was also tested in F00 family, for the most significant loci detected in challenge analysis (P<0.01). The test was performed for the two sets of measurements (VREFT-1 and VREFT-2).

Test for linkage and association studies

Simple association between allele at any given locus and challenge issue was assessed within each family using the χ^2-test (1dl). The null hypothesis is that there is no association between the genotype and the phenotype, and that the 2 parental alleles are equally represented in R and S progeny. A P value less than 0.05 was taken as significant at a point-wise level. A Bonferroni correction for the number of tested loci was used to calculate a genome-wise significant level (P<0.05 was taken as significant). A joined association study was then performed combining data from the 2 families. In that case, it was assumed that R grand-parents both transmitted the same allele coded 1, and that susceptible grand-parents both transmitted the same allele coded 0.

Association between genotype and VREFT was tested using one-way Anova performed on log-transformed VREFT values (comparison of mean values of progeny having inherited alternative allele).

Results

Genomic regions associated with response to challenge

Twenty-nine out the 31 linkage groups of the species could be scanned. The mean number of markers examined per linkage group was 2.8 in F98 and 3 in F00.

Table 1. Significant microsatellites for association with challenge issue in the two experimental families

Marker	LG[a]	Point-wise P value (P<0.05)	Experiment -wise level significance
Family F98			
OmyFGT18/1 TUF	II	0.025	
Ocl1UW	XI	0.012	
Omy7DIAS		0.006	
Omy14INRA	XV	0.015	
OMM1131	XXVII	0.028	
Ots108SS1	XXXIX	0.033	
Ssa124NVH	XXXI[b]	<0.0001	YES
Family F00			
OmyFGT18/1 TUF	II	0.009	
Omy18 INRA	VIII	0.002	
Ssal14DU	XIV	0.027	
Omy77DU	XVI	0.037	
OMM1013		0.016	
OMM3000	XXVI	0.024	
OMM1053	XXXI[c]	<0.0001	YES
OMM1080		<0.0001	YES
Joined analysis			
OmyFGT18/1 TUF	II	0.0004	YES
Ocl1	XI	0.023	
Omy7DIAS		0.002	
OMM1030	XII	0.034	
OMM1034	XIX	0.049	
OMM1023	XXII	0.039	

[a]: the linkage group (LG) are numbered according Nichols et al. (2003). Synteny was inferred from common microsatellites with published maps (Sakamoto *et al*., 2000; Nichols *et al*., 2003). In a number of linkage groups, linkage in females was inferred from published information on male linkage maps.
[b]: locus not yet mapped in the family at time of impression. Position inferred from unpublished data (Danzmann, R. Guyomard, pers. comm.)
[c] : putative location (lod =1.4, loci informative in one family only for the construction of the map). OMM1080 and OMM1053 totally linked.

Family F98. Analysis of sample 1 revealed 31 suggestive loci out of 92 analysed. After analysis of sample 2, 7 loci located on 6 different linkage groups remained significant, of which one was significant at the experiment-wise level (Ssa124NVH). The allele of the R grand-parent at that locus was inherited by 71% of the resistant fish.
Family F00. Twenty-four loci out of 86 examined were suggestive in sample 1. After analysis of sample 2, 8 loci located on 6 different linkage groups remained significant at a point-wise level, of which two were significant (OMM1080, OMM1053, totally linked). For these loci, 87% of survivors inherited the allele of the R grand-parent.
Joined analysis. Three new putative linkage groups were detected. Association of LG II loci with challenge issue was confirmed (Table 1).

Genomic regions associated with VREFT values in family F00

The effect of the allelic origin on the values of VREFT was tested for 3 markers (Table 2). The two clustered markers (OMM1053 and OMM1080) displayed high association with VREFT-1. Fish that carried the allele from the R grand-parent exhibited lower VREFT values, an evidence for a higher level of resistance. Yet, the effect of the couple of loci was found to be not significant on VREFT-2 values.

Table 2. Test for association with VREFT values in family F00 at the 2 dates of measurement (Values are the probabilities of allelic effect in the Anova model)

Marker	LG[a]	VREFT-1	VREFT-2
OmyFGT18/1 TUF	II	ns	ns
Omy18INRA	VIII	ns	ns
OMM1053	XXXI[b]	<0.0001	ns
OMM1080		<0.0001	ns

[a]: labelled as in Table 1.
[b] : putative location (lod =1.4, loci informative in one family only for the construction of the map). OMM1080 and OMM1053 totally linked.

Discussion - Conclusion

The study supports the existence of several chromosomal regions of trout genome associated with resistance to VHSV (maximum of 13 putative regions). Two linkage groups (LGII and LGXXXI) were highly significant, and common to both families. Associations with markers expected to belong to LG XXXI were found to be particularly tight, although VREFT values gave some inconsistent results, which question the biological and immunological significance of the trait. The finding of DNA markers associated with resistance can help in marker assisted selection, and is a step towards a better knowledge of disease resistance mechanisms in fish, and future development of efficient vaccines or therapeutics.

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