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Family-specific differences in growth rate and hepatic gene expression in juvenile triploid growth hormone (GH) transgenic Atlantic salmon (*Salmo salar*)

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ABSTRACT

Growth hormone transgenic (GHTg) Atlantic salmon (*Salmo salar*) have enhanced growth when compared to their non-transgenic counterparts, and this trait can be beneficial for aquaculture production. Biological confinement of GHTg Atlantic salmon may be achieved through the induction of triploidy (3N). The growth rates of triploid GH transgenic (3NGHTg) Atlantic salmon juveniles were found to significantly vary between families in the AquaBounty breeding program. In order to characterize gene expression associated with enhanced growth in juvenile 3NGHTg Atlantic salmon, a functional genomics approach (32K cDNA microarray hybridizations followed by QPCR) was used to identify and validate liver transcripts that were differentially expressed between two fast-growing 3NGHTg Atlantic salmon families (AS11, AS26) and a slow-growing 3NGHTg Atlantic salmon family (AS25); juvenile growth rate was evaluated over a 45-day period. Of 687 microarray-identified differentially expressed features, 143 (116 more highly expressed in fast-growing and 27 more highly expressed in slow-growing juveniles) were identified in the AS11 vs. AS25 microarray study, while 544 (442 more highly expressed in fast-growing and 102 more highly expressed in slow-growing juveniles) were identified in the AS26 vs. AS25 microarray study. Forty microarray features (39 putatively associated with fast growth and 1 putatively associated with slow growth) were present in both microarray experiment gene lists. The expression levels of 15 microarray-identified transcripts were studied using QPCR with individual RNA samples to validate microarray results and to study biological variability of transcript expression. The QPCR results agreed with the microarray results for 12 of 13 putative fast-growth associated transcripts, but QPCR did not validate the microarray results for 2 putative slow-growth associated transcripts. Many of the 39 microarray-identified genes putatively associated at the transcript expression level with fast-growing 3NGHTg salmon juveniles (including APOA1, APOA4, B2M, FADSD6, FTM, and GAPDH) are involved in metabolism, iron homeostasis and oxygen transport, and immune- or stress-related responses. The results of this study increase our knowledge of family-specific impacts on growth rate and hepatic gene expression in juvenile 3NGHTg Atlantic salmon. In addition, this study provides a suite of putative rapid growth rate-associated transcripts that may contribute to the development of molecular markers [e.g. intronic, exonic or regulatory region single nucleotide polymorphisms (SNPs)] for the selection of GHTg Atlantic salmon broodstock that can be utilized to produce sterile triploids of desired growth performance for future commercial applications.

1. Introduction

In the past few years, due to decreasing wild fish stocks (Myers et al., 1997; Myers and Worm, 2003) and increasing demand of fish for human consumption, there has been a large expansion of the aquaculture industry (Naylor and Burke, 2005; Liu and Sumaila, 2008; Bostock et al., 2010). For this industry to meet increased demands, it is important to develop technologies that can enhance aquaculture production of marine finfish such as Atlantic salmon (*Salmo salar*). Growth hormone (GH) transgenes can greatly enhance the growth rate of commercially important fish species including salmonids (e.g. Atlantic salmon and coho salmon [*Oncorhynchus kisutch*] [Devlin et al., 1994; Du et al., 1992]), thereby significantly shortening their production cycles (Fletcher et al., 2004). Although GH transgenic (GHTg) fish are potentially attractive technology products for the continuing development of the aquaculture industry, there are requirements to be addressed before GHTg fish can become commercially viable. One of the conditions for commercial cultivation of

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transgenic salmon is biological confinement of the integrated transgene. Among the efforts that have been made in confinement of farmed fish, triploidization (which renders the fish reproductively sterile) is an effective approach to reduce the genetic impacts of unwanted gene flow (Cotter et al., 2000).

In GHTg salmonids, elevated GH expression modifies a number of biochemical pathways mostly through GH-mediated signaling, resulting in changes in feeding behavior (e.g. Abrahams and Sutterlin, 1999; Devlin et al., 1999), metabolism (e.g. Cook et al., 2000; Rise et al., 2006; Leggatt et al., 2009), and development (e.g. Devlin et al., 2000). It has been demonstrated that the growth performance of rainbow trout (*Oncorhynchus mykiss*) in response to GH transgenesis (using the construct *OnMTGH1*) is largely determined by genetic background, as the integrated gene construct enhanced the growth rate in a slow-growing wild strain, but it had very little impact on the growth rate of a fast-growing domesticated (i.e. selectively bred) strain (Devlin et al., 2001). Furthermore, a recent transcriptomic study showed that both domestication and GH-transgenesis modified a set of common pathways involved in growth regulation (e.g. protein synthesis, cell/tissue structure, energy production, metabolism) in coho salmon muscle and liver tissues (Devlin et al., 2009). It is important to note that, due to a putative whole-genome duplication event and subsequent partial re-diploidization in the salmonid lineage, modern salmonid genomes are considered to be pseudotetraploid (Allendorf and Thorgaard, 1984; Davidson et al., 2010). However, to improve the readability of this paper, we will refer to the wild-type Atlantic salmon genome as diploid.

The impact of triploidization on the growth performance of teleost fish has been intensively investigated (reviewed in Tiwary et al., 2004). It has been hypothesized that somatic growth of reproductively sterile triploid fish is expected to increase as their energy contribution to gonad development is greatly reduced (Felip et al., 2001; Piferrer et al., 2009). However, in Atlantic salmon, previously published studies are equivocal regarding the impact of triploidization on growth performance. For example, lower survival and growth rate (McGeachy et al., 1995) and higher incidence of deformity (O'Flynn et al., 1997; Benfey, 2001) have been observed in triploid Atlantic salmon in comparison to their diploid counterparts, while accelerated growth rate in triploid Atlantic salmon also has been reported (Galbreath et al., 1994; Oppedal et al., 2003). In addition, other studies have found that variability in growth performance is consistently higher among triploid Atlantic salmon families than among their diploid counterparts (Friars et al., 2001; Johnson et al., 2007). These observations suggest that triploidization can be an additional source of variability that, along with genetic diversity, further complicates the study of the genetic/molecular basis of growth rates in these fish. It is therefore of great interest to select diploid broodstock that are optimal for triploid production based on the performance of their triploid offspring. The use of microarray technology has previously identified changes in the transcriptome that are associated with various biological processes in fish, including growth (e.g. Rise et al., 2006; Devlin et al., 2009; Tymchuk et al., 2009), development (e.g. Gallagher et al., 2008; Xu et al., 2011), response to pathogens (e.g. Rise et al., 2004), environmental stressors (e.g. Hampel et al., 2010), and different diets (e.g. Morais et al., 2011). Therefore, in the current study we utilized microarrays in a broad survey to test the hypothesis that candidate molecular biomarkers (i.e. differentially expressed genes) could be associated with fast somatic growth in juvenile triploid GH transgenic (3NGHTg) Atlantic salmon. In this study, a functional genomics approach was used to identify and validate genes that differ in hepatic transcript expression level between 3NGHTg Atlantic salmon families and may be associated with juvenile growth performance (with growth rate assessed during a 45-day period of juvenile development). The constitutive hepatic transcriptomes of two families of fast-growing 3NGHTg Atlantic salmon juveniles were compared to that of a family of slow-growing 3NGHTg Atlantic salmon juveniles in two independent microarray comparisons using pooled RNA samples and the ~32,000 gene (32K) microarray platform generated by the consortium for Genomic Research on All Salmonids Project (cGRASP) (Koop et al., 2008). Fifteen microarray-identified transcripts with growth-relevant functional annotations were selected for quantitative reverse transcription-polymerase chain reaction (QPCR) studies using individual RNA samples from the three families included in the functional genomics study in order to validate the microarray results and to assess the biological variability of transcript expression. We anticipate that putative growth-associated genes identified in this functional genomics study will be suitable candidate genes for the development of molecular markers (SNPs) to be used in the selection of GHTg salmon broodstock that will give rise to fast-growing sterile triploids. Similar candidate gene-based approaches have been successful in cattle and swine to improve traditional selective breeding schemes, where genes differentially expressed between phenotypes of interest (e.g. marbling, body composition, disease resistance and birth weight) contain SNPs associated with these traits (Ponsuksili et al., 2005; Sasaki et al., 2009; Liu et al., 2011; Sugimoto et al., 2012).

2. Materials and methods

2.1. Fish husbandry and family selection

All experiments were conducted following the Canadian Council on Animal Care guidelines and were approved by Animal Care Committees of both AquaBounty Canada and Memorial University of Newfoundland. In this study, 19 families of GHTg Atlantic salmon were obtained by fertilization of eggs from a non-transgenic female Atlantic salmon with milt from a hemizygous (i.e. having one copy of the GH transgene) *AquAdvantage*® (AquaBounty Canada, Fortune, Souris, PE, Canada) male. The non-transgenic Atlantic salmon were derived from St. John River strains. All of the transgenic individuals descended from a single founder, which was created through microinjection of the opAFP-GHc2 construct containing the regulatory region of the ocean pout (*Macrozoarces americanus*) antifreeze protein (AFP) gene and the open reading frame of the Chinook salmon (*Oncorhynchus tshawytscha*) growth hormone (GH) gene (Butler and Fletcher, 2009) into fertilized eggs of wild Atlantic salmon harvested from Newfoundland rivers. For each family, one-half volume of the fertilized eggs was treated with hydrostatic-pressure (9500 psi) for a period of 5 min at 300 degree-minutes post-fertilization to induce triploidy (O'Flynn et al., 1997). The eggs were incubated in stacked incubation trays (10 L) until hatch, when the yolk-sac fry were transferred to individual tanks in the early rearing area. The yolk-sac fry were first cultured in a flow-through system, and then moved to a recirculation system when they reached ~1 g in weight. Fish of different ploidy were cultured separately in combi-tanks (500 L for early sac-fry or 1500 L for later stages). Density in the tanks was maintained at <1 kg m^{-3}. Fish were reared with 24 hour light and fed to satiation with a commercial salmon diet of appropriate composition and pellet size for the different developmental stages (e.g. Skretting Nutra ST, Nutra Fry & Optiline [1.0–4.0 mm], Vancouver, BC, Canada). The water temperature was maintained at 13 °C and dissolved oxygen was kept >90% of air saturation.

Approximately 95 days after the onset of first feeding (May 20–26, 2009), 50 fish from each family were assessed for weight, length, and visible deformities. Based on this assessment, three families with larger body sizes and three families with smaller body sizes were selected from the initial 19 families. At the initial time point (t_1: July 27–29, 2009), 18 randomly selected fish from each of these six selected families were tagged with passive integrated transponder (PIT) tags. These fish then were assessed for weight, length, and visible deformities (Supplemental Fig. S1 A and B) before being evenly distributed into three 500 L tanks (6 fish from each of the 6 PIT-tagged families for a total of 36 fish per tank). Approximately 45 days later (t_2: