

Differential gene expression and SNP association between fast- and slow-growing turbot (*Scophthalmus maximus*)

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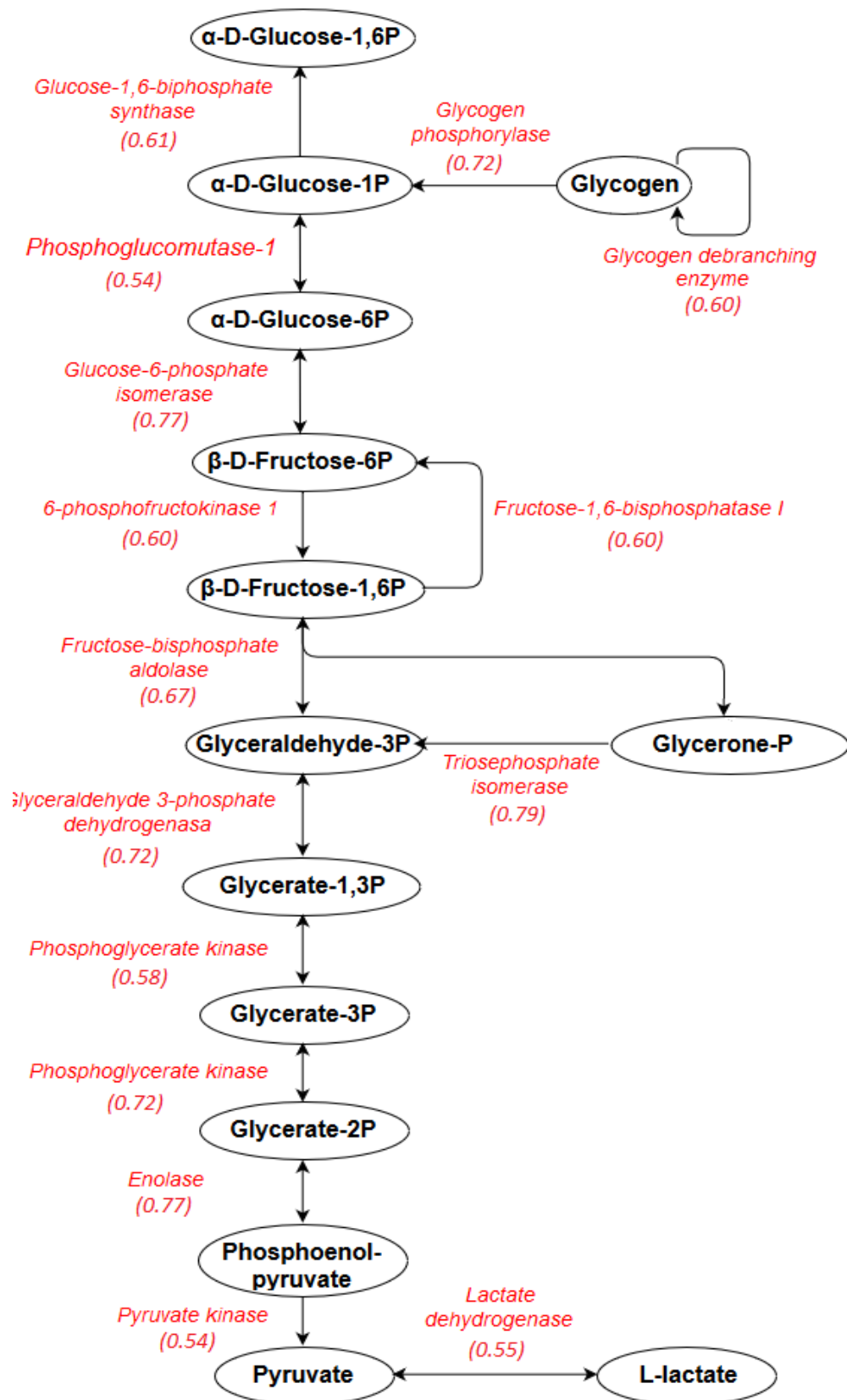
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Excel documents for Supplementary Datasets 1-3 are available as separate files.

Supplementary Figure 1. Glycolytic pathway enzyme-coding genes found up-regulated in turbot muscle and their fold change.



Supplementary Table 1. RNA-Seq and RT-qPCR results obtained for selected growth-related transcripts in turbot

Gene Name	RNA-Seq		qPCR								
	FG vs SG		FG VS SG (Pooled Families)		FG (unpooled) vs SG (unpooled)						
	Log2 FC ¹	P value	Log2 FC	Conf. Int. 95% ²	FG_GROUP	FG VS SG_group1		FG VS SG_group2		FG VS SG_group3	
						Log2 FC ¹	Conf. Int. 95% ²	Log2 FC ¹	Conf. Int. 95% ²	Log2 FC ¹	Conf. Int. 95% ²
Creatine Kinase-m	-0.595 -0.466	0.000338934 0.003091596	-0.6634	-0.3674 1.8428	FG_G1	1.3750 *	1.6973 0.7886	-0.2319	-	-0.3876	-
					FG_G2	1.6825 *	1.9713 1.2358	-0.2931	0.0533 -1.4226	-0.5299	-0.0885 -
					FG_G3	0.4100	0.7448 -0.1931	-1.5660 *	2.8639	-1.8037 *	-1.3425 -
Creatine Kinase-c	0.668428	0.001505129	0.8094 *	0.84124 0.5376	FG_G1	0.9292 *	1.0609 0.7369	0.3689 *	0.4526 0.1559	0.0707	0.3220 -0.2323
					FG_G2	1.6573 *	1.7951 1.3869	1.0994 *	1.1932 0.8151	0.7939 *	1.0479 0.4203
					FG_G3	1.1517 *	1.3240 0.8836	0.5925 *	0.7347 0.2083	0.2912	0.5613 -0.0740
TGFβ1	-0.724 -0.557	0.003362231 0.004350885	-0.4204	-0.1937 0.9522	FG_G1	-0.3354	-	0.0064	0.2059 -0.1880	-0.5441	-0.3162 -
					FG_G2	-0.1374	-	0.2050	0.3770 -0.0345	-0.3458	-0.1400 -
					FG_G3	-0.3702	-	-0.0292	0.2033 -0.2357	-0.5793	-0.3234 -
PYGM	0.717	0.000180384	1.2452 *	1.3281 0.8638	FG_G1	1.8625 *	1.9722 1.5277	1.0330 *	1.1533 0.6198	0.6509 *	0.8633 0.2199
					FG_G2	1.8594 *	2.0277 1.4741	1.0271 *	1.2061 0.5671	0.6439 *	0.8893 0.1670
					FG_G3	2.1288 *	2.2991 1.7809	1.2938 *	1.4733 0.8743	0.9094 *	1.1548 0.4750
GHR2	-1.013	0.000198749	-1.3102 *	-1.1052 2.0203	FG_G1	-0.3948	-	-1.1284	-	-1.7442	-
					FG_G2	-0.5656 *	1.0469	-1.3615 *	1.8436	-2.2343 *	3.1082
					FG_G3	-0.0268	-	-0.7955	-	-1.4680	-
LDH	0.541	0.001156074	0.8411 *	0.9472 0.4035	FG_G1	-0.2747	0.6891	-1.0835 *	1.7566	-1.9723 *	3.1229
					FG_G2	-0.3645	-	-1.0879	-	-1.6892	-
					FG_G3	-0.5207 *	0.7495	-1.3110 *	1.7793	-2.1771 *	3.0405
LDH	0.541	0.001156074	0.8411 *	0.9472 0.4035	FG_G1	1.2533 *	1.4590 0.9072	1.0260 *	1.2116 0.8190	-0.0298	0.2204 -0.3025
					FG_G2	1.3525 *	1.5493 1.0001	1.1250 *	1.3007 0.9101	0.0696	0.3130 -0.2103
					FG_G3	1.4326 *	1.7280 0.5977	1.2054 *	1.4891 0.4992	0.1489	0.4728 -0.2634

* Significant statistical differences determined for ddCts p < 0.05

1 FC: fold change

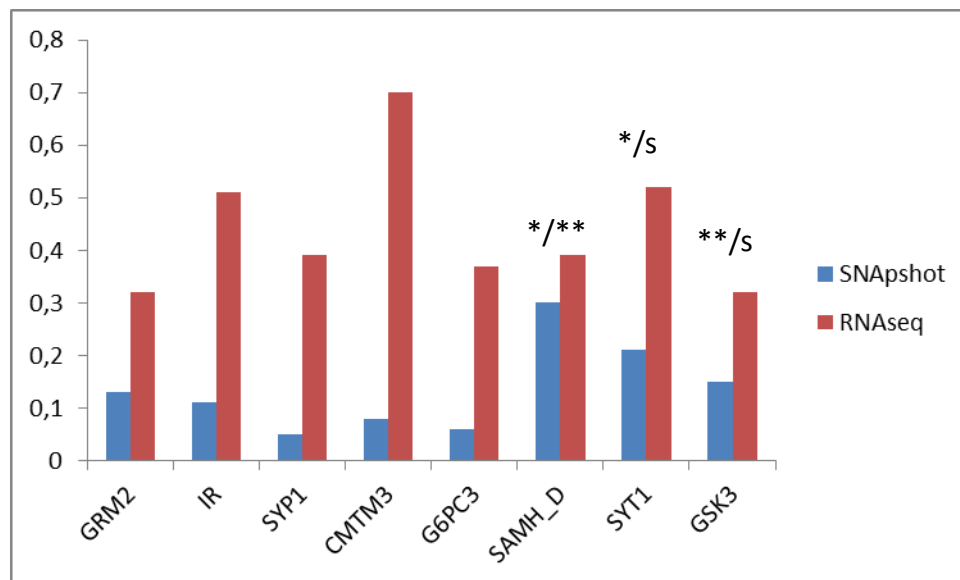
2 Confidence interval

Supplementary Table 2: SNaPshot validation of the allele frequency differences between fast- and slow-growing turbot. A) p-values and significance of the genotypic and allelic differentiation ; B) Graphical representation of allele frequency differences between SNaPshot and RNAseq pool results

A)

Gene	Genotypic G-test p-value	Allelic G-test p-value
SAMHD1	0.0341	0.0079
SYT1 synaptotagmin 1	0.0386	0.0532
GSK3 glycogen synthase kinase 3	0.0094	0.1063
IR insulin receptor	0.4046	0.4095
SYP1 synaptophysin protein 1	0.8241	0.8346
GRM2 glutamate receptor 2	0.1599	0.1702
CMTM3 Cklf-like marvel transmembrane domain-containing protein 3	0.4833	0.4921
G6PC3 glucose-6-phosphatase 3	0.6849	0.6705

B)



P-value: genotypic/allelic significance: (s) suggestive <0.10, (*) 0.05, (**) 0.01

Supplementary Table 3. Primers used for real-time PCR

Gene	Primer Forward	Primer Reverse
LDH	ACATCGTCAAGTACAGCCCC	GCTTGACGGGTGGATGTTGA
PYGM	GTCGTCTTGCTGCCTGTTTC	CCTCTTCAACCTGCCAACCA
GHR2	TTCAACGAGAACCACACGGA	AGTTGAGGTTGACCGGAGGA
CKs	TCTGGGCTACATCCTGACCT	ACGCCATCAACCACCATCTG
CKm	AATGAGGAGGACCACACCAG	AATGAGGAGGACCACACCAG
TGF β 1	TCGCTTCCCGTTTCATCACT	CCATGCTTTGCTCATTCCCG

Supplementary Table 4: Primers used for the SNaPshot analysis

	Forward¹	Reverse¹	SNaPshot¹²
Multiplex 1			
Insulin receptor IR	ACAGTGGACTCTTTGGCGTG	CTTGTTCCAAACCACCGTCC	gactgaACGGTGACCACTGTCTTCAG
Glutamate receptor GRM2	GGGAACGACCGTTTCGAAGG	TGTGAAGCAGACAATGTGAAGT	gactgactgaTCACATCCTGGGCTTCACGT
Myosin heavy chain MHC	TTCTTAGCCTTGTCCTCTGC	CATTGAAATCAAGCATTGTTGGTGACA	TGGATCTGGACCAGGTCA ³
Synaptophysin protein 1 SYT1	AAATGTGAAGCCACGCCCAT	CGTCCACGACGAGGTAAAGG	gactgactgacTAAGTAGATTGACATGTATGG
Multiplex 2			
Cklf-like-marvel-transmembrane domain-containing protein 3 CMTM	GGAGGAACTGGATCCTCTGTA	CAGAATCGTCTGAGACACTGAAT	gactgactgaAGCATTAAAGGGTGAAACACA
SAMHD1	GATGTCTCTGCGGGTGATGA	TTCAGTGTTCAGGGTGTGCT	gactgactgactgacTACTTGGCAGGACAGCTTGTA
Synaptotagmin 1 SYT1	CCAAATACAAGCTGGGCTCC	GGGATGGTCGTATTTTGGCCA	gactgactgactGTAATTATGTGTCTTTATGTGTATAGTT
Glucose-6-phosphatase 3 G6PC3	CGTCTGTTTCCTCAGGCAGT	AGAACAGGTAGGGCGAGACT	GGTGTCTCGCTGGGTTC
Singleplex			
Glycogen synthase kinase 3 GSK3	CAGGTTCTGGGGACACCAAC	TGTTTAACGGGAACCCAGCA	gactTGTGGGAATTTGAACTCTGTGTA

1 all primers are in 5'→ 3'.

2 in lower case are added sequences that allowed multiplexing.

3 in red, failed SNaPshot primer for minisequencing.