

Evolutionary expression differences of creatine synthesis-related genes: Implications for skeletal muscle metabolism in fish

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Table S1. Gene expression of *SLC6A8* in tissues of different species, determined by RNA-Seq.

Species	Reference gene	Length (bp)	CPM kidney	CPM liver	CPM muscle
Rat	NM_017348.2	3910	400.8	39.9	161.4
Sheep	ERR489264	3164	19.2	1.6	6.4
Human	NM_005629.3	3580	57.5	0.4	53.5
Rainbow trout	XM_021566996.1/	6458/	12.3/	5.9/	340.8/
	XM_021566943.1	2668	4.6	2.0	0.4
Zebra mbuna	XM_004568184.3: 1277-3295	2019	37.4	2.8	17.0
Pike	XM_013132722.2	3431	0.9	0.1	4.0

Table S2. Sequences used for the deduction of degenerated primers.

Fish species	Gene		
	<i>GATM</i>	<i>GAMT</i>	<i>CKM</i>
<i>Anoplopoma fimbria</i>	GAJJ01047113	BT082179	
<i>Chaenocephalus aceratus</i>			AY161316, AY161314
<i>Haplochromis burtoni</i>	XM_005943275	XM_005934883	
<i>Maylandia zebra</i>	XM_004543456	XM_004564826	
<i>Neolamprologus brichardi</i>		XM_006792039	
<i>Oncorhynchus mykiss</i>	HG315738	HG315739	HG315740
<i>Oreochromis mossambicus</i>			AY034097
<i>Oreochromis niloticus</i>		XM_003449022	
<i>Oryzias latipes</i>	XM_004066917	XM_004067912	
<i>Pundamilia nyererei</i>	XM_005753030	XM_005730241	
<i>Takifugu rubripes</i>	XM_003969870	XM_003974307	
<i>Xiphophorus maculatus</i>		XM_005800850	

Table S3. Primers used in this study.

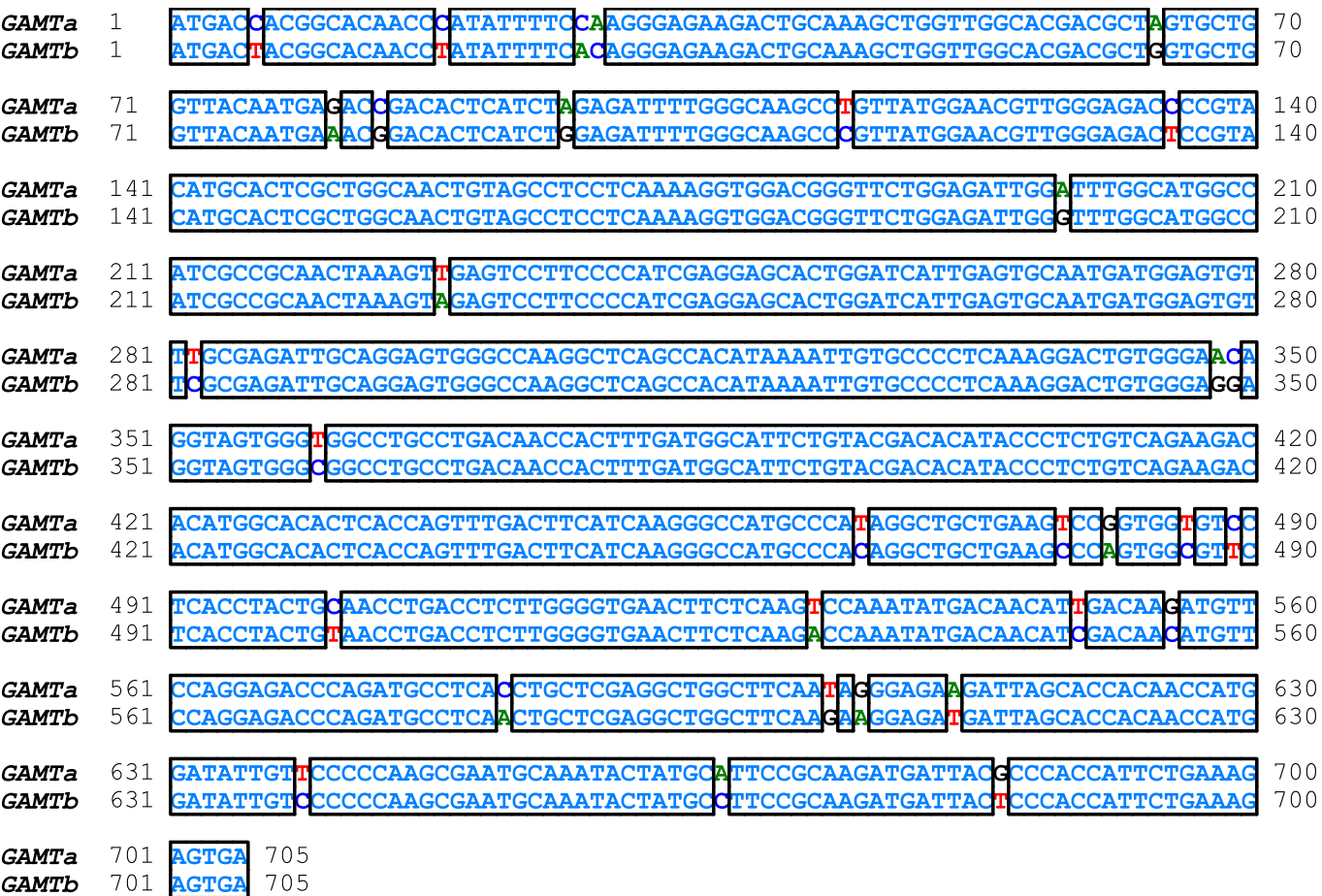
Species	Gene	Forward Primer 5'-3'	Reverse Primer 5'-3'
<i>Pisces</i> ¹	<i>GATM</i>	GGMATGTATGCKGCCATGC	CGCATCCAYTCAATYCCC
	<i>GAMT</i>	AGGAGCACTGGATCATYGAR	TTRTTGAAGGAATAGTATTTGCATTC
	<i>CKM</i>	TGRTCTGGGTGAAYGAGGA	CTSTCCCTTCTCCAKCTTCTTCT
<i>Corgonous maraena</i>	<i>GATM</i>	GCTGTACGATCAGGAGTACCC	CATCCACTCAATCCCCATGTAG
	<i>GAMTa</i>	GTGGTGTCCCTCACCTACTGC	TTTGCAATTCGCTTGGGGGAAC
	<i>GAMTb</i>	CTGAAGCCCAGTGGCGTTCT	GGTTGTGGTGCTAATCATCTCC
	<i>CKM</i>	CGTCTGCAGAAGCGTGGCAC	TGCTGTGATGGCCTCTCCC
	<i>EEF1A1</i>	CCTCCACTTGGTTCGTTTCG	CGCAGGATGTAGGGCAGCAGA
<i>Clupea harengus</i>	<i>RPL9</i>	ACCACATCAACCTGGAACCTCA	CGCATCTTGTAAACGGAAACC
	<i>RPL32</i>	CAGGCGGTTTAAGGGTCAG	ATCTCAGCAGCATGGGTC
	<i>GATM</i>	ACCATGTCTGATGAGCTGTATGA	TGTAACCTGACTCCTCTGTACAA
	<i>GAMT</i>	CAACCACTTTGACGGCATTCTG	ATTTACCCTTCAGCAGCTCTCC
	<i>CKM</i>	GCAAAAGATTGAGGCCATCTTCA	TGCAGACGCAGCCTTTTCAGG
<i>Perca fluviatilis</i>	<i>EEF1A1</i>	GGCTTCAACATTAAGAACGTGTC	GGTGCAATAGCCAGCACTGATC
	<i>RPL8</i>	TAAGGGAATCGTGAAGGACATCA	TGGGCCTTCTTGCCACAGTAG
	<i>GATM</i>	AAACGGTGCCAAATGGACCACT	CTCGAATGAAGTCAGCAGCATC
	<i>GAMT</i>	CAGACTTCAACATCCTGTGAGC	TCGTCCCTCTGAAGGGCCTTT
	<i>CKM</i>	GCGTTGGCCTGAAGAAGATTGA	TCTCCTCGAACTTGGCGTGTG
<i>Sander lucioperca</i>	<i>EEF1A1</i>	GGAAATTCGTCTGTGGATACG	GGGTGGTTCAGGATGATGAC
	<i>ACTB</i>	ACCTTCTACAACGAGCTGAGAGTT	AGTGGTACGACCAGAGGGCATACT
	<i>GATM</i>	CACTATGGCCGATGAGCTGTAT	ATTTGTAACCTGACTCCTCTGGA
	<i>GAMT</i>	TCGCCCCAGGAGGTCAGGTT	TCCCACACTTCCAGATAACCAC
	<i>CKM</i>	CCCACGGAGGCGGTGTCCA	ACGAGCATCTGGGCTACATCC
<i>Mus musculus</i>	<i>RPL8</i>	GTTATCGCCTCTGCCAC	ACCGAAGGGATGCTCAAC
	<i>EF1A</i>	ACATGCTGGAGACCAGTGAGAA	TCCAATACCGCGATTTTGTAGA
	<i>GATM</i>	TACTCCTCCAACACCAGTCATC	CTGATACCCAGCTTCTCAAACAT
	<i>GAMT</i>	TTTCGCGCCCGCGAGGACT	AGACCCGCCCCCTCTGGA
	<i>CKM</i>	ACGTGAAGCTGGCGAACCTGA	CTGTTCTGACTTCGGATGAGCC
<i>Bos taurus</i>	<i>RPL38</i>	CCGGCGGAAGGATGCCAAGT	ACTGCCAAACCCGGGGGTAG
	<i>EEF2</i>	CCGGAACATGTCAAGTCATCGC	GATGGCGGTGGATTTGATTGTG
	<i>GATM</i>	ACTCCTCCAACACCAATCATCC	ACTGATACCCAGCTTTTCAAACAT
	<i>GAMT</i>	CACTTTGATGGGATCCTGTACG	TGGTGGTGATGTCTGAATACTTG
	<i>CKM</i>	CCGTGGCATCTGGCACAATGA	CGGGTGGCCGGCTTTCTTGA
<i>Sus scrofa</i>	<i>EIF3K</i>	CCAGGCCACCAAGAAGAAC	GACATGGCAGATAAAATTTTCGG
	<i>MTG1</i>	CTGGCTGACTTCCCTTCTCTAC	CCGTCAGCACCTTCACCTTC
	<i>GATM</i>	CCTGATTTTGAGTCTACGGGTTT	CCCACGGCGGAAGTAGTCTTT
	<i>GAMT</i>	TGCAATGACGGCGTCTTCCAG	CCTCAGACAGCGGGTACGTG
	<i>CKM</i>	AGAACCCTCAAGGGTGGAGACG	CTTGAACCTCTCCCGTCAGGCT
	<i>TOP2B</i>	ATGATGCTGGTGGCAAACACTC	TTATGAGAAGCCTCCCGCACAT
	<i>HSPCB</i>	GCACTCACAGTTCATAGGTTATC	ATCTGAGCCACATCTTCAATCT
	<i>YWHAZ</i>	GTACTGTCTCTTTTGAAAAAGTTC	TGATTTCAAAAAGCTTCTGGTATG

¹degenerated primers for various fish species

Table S4. Sources for RNA-Seq data and reference sequences.

Species	RNA-Seq data			Reference sequence		
	kidney	liver	muscle	GATM	GAMT	CKM
<i>Anolis carolinensis</i>	SRR579557	SRR391651	SRR579559	XM_003230613	XM_003230422	XM_008125032
<i>Balaena mysticetus</i>	SRR1685390	SRR1685415	SRR1685388	XM_007169721	XM_007176625	XM_007168131
<i>Balaenoptera acutorostrata</i>	SRR919295	SRR919296	SRR922171	XM_007169721	XM_007176625	XM_007168131
<i>Bos taurus</i>	ERR514720	ERR514717	ERR514718	NM_001045878	NM_001038544	NM_174773
<i>Callithrix jacchus</i>	SRR1758983	SRR1758984	SRR1758987	XM_002753420	XM_002761531	XM_008988218
<i>Callorhinchus milii</i>	SRR513760	SRR514105	SRR514104	XM_007905550	XM_007898733	XM_007885227
<i>Esox lucius</i>	SRR1228724	SRR1228725	SRR1228726	XM_010895950	NM_001304086	XM_010873709
<i>Ficedula albicollis</i>	ERR168754	ERR168726	ERR168764	XM_005052064	XM_005060155	XM_005062927
<i>Gallus gallus</i>	SRR594503	SRR594504	SRR594506	NM_204745	XM_001234062	NM_205507
<i>Homo sapiens</i>	SRR1957192	SRR1957193	SRR1957201	NM_001482	NM_000156	NM_001824
<i>Latimeria chalumnae</i>	DRR002308	SRR387384	DRR002313	XM_005989627	XM_005999865	XM_005991595
<i>Maylandia zebra</i>	SRR385837	SRR385834	SRR385833	XM_004543456	XM_004564826	XM_004569613
<i>Mus musculus</i>	SRR1158575	SRR1158583	SRR1158599	NM_025961	NM_010255	NM_007710
<i>Oncorhynchus mykiss</i>	ERR324378	ERR324372	ERR324368	HG315738	HG315739	HG315740
<i>Oreochromis niloticus</i>	SRR391680	SRR391688	SRR391702	XM_003458201	XM_003449022	XM_003456381
<i>Ovis aries</i>	ERR489260	ERR489264	ERR489244	XM_004010649	XM_012116137	XM_012190548
<i>Pan troglodytes</i>	SRR1758924	SRR1758924	SRR1758930	XM_001163621	XM_009434279	XM_001163660
<i>Rattus norvegicus</i>	SRR594431	SRR594432	SRR594434	NM_031031	NM_012793	NM_012530
<i>Saimiri boliviensis</i>	SRR500947	SRR500946	SRR500943	XM_003928903	XM_010332444	XM_003940241
<i>Sus scrofa</i>	ERR209000	ERR208927	ERR208926	NM_001128442	XM_003353975	NM_001129949
<i>Xenopus laevis</i>	SRR1187000	SRR514105	SRR1187003	NM_001086230	NM_001087223	NM_001086604

A)



B)

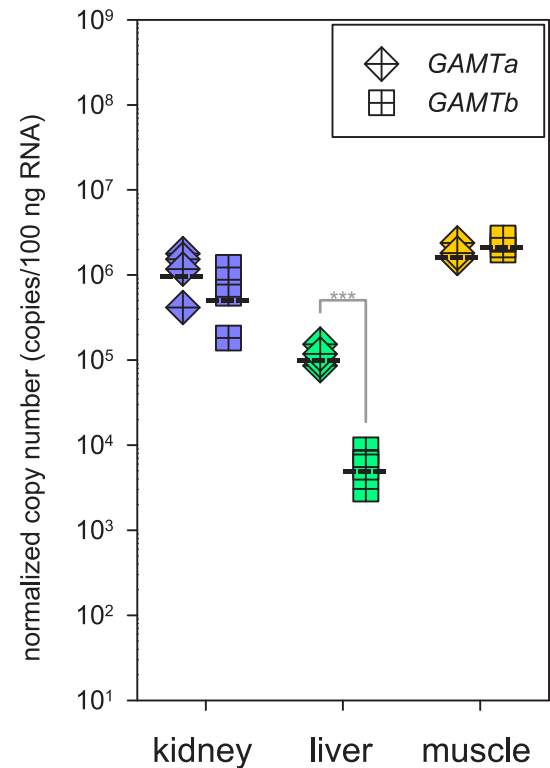


Figure S1. *GAMTa* and *GAMTb* of maraena whitefish. A) Sequence alignment of the coding regions of both *GAMT* variants. Identical bases are shadowed blue. B) Expression levels of both variants in kidney, liver and muscle. Values for *GAMTa* are the base for the whitefish *GAMT*-values in Fig. 3C. Asterisks mark significant differences between groups (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

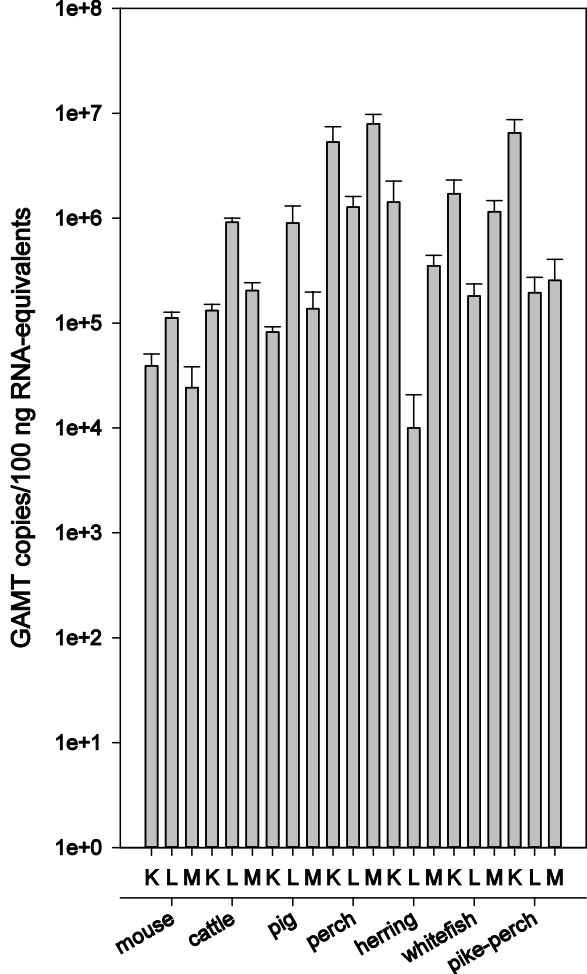
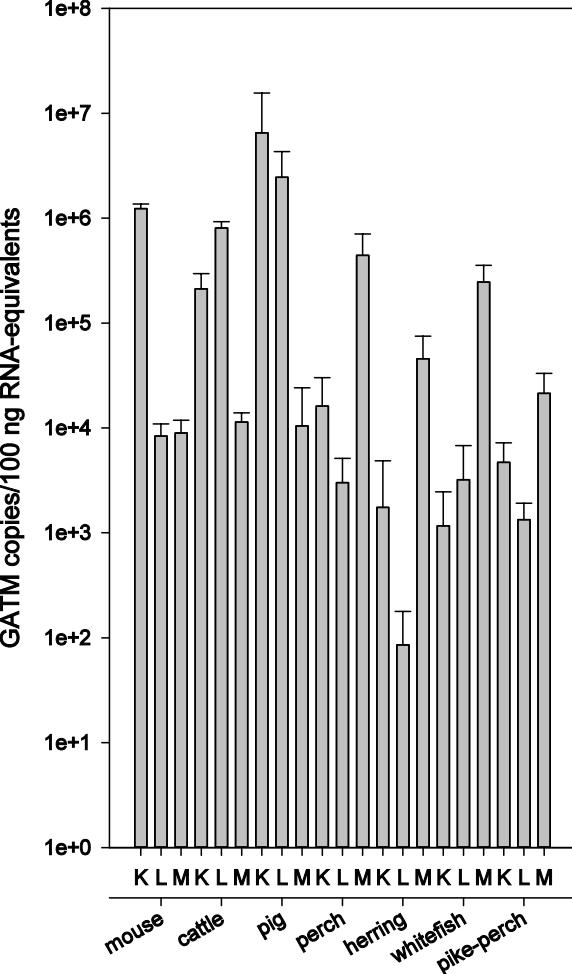


Figure S2. Absolute copy number of *GATM* and *GAMT*.

Copies of *GATM* or *GAMT* per 100 ng RNA-equivalent in the reaction. The copy numbers were directly calculated from ct values and the respective absolute standard curves and were normalized to the input of RNA into the cDNA reaction. No normalization to reference genes was applied here.

K=kidney, L=liver, M=muscle. Values are means + standard deviation