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Sequence and expression analysis of the androgen receptor gene from Compact mouse (Brief Report)

Analyse der Sequenz und der Genexpression des Compact Maus Androgen-Rezeptor Gens (Brief Report)

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Background

The phenotype of hypermuscled Compact mouse is determined by the MstnCmpt-dl1Abc myostatin mutation and also by additional modifier genes, mapped to different chromosome regions (VARGA *et al.* 1997, SZABÓ *et al.* 1998, VARGA *et al.* 2003, VARGA *et al.* 2005). The androgen receptor gene (Ar) was considered to be a potential candidate gene on the basis of our mapping results and its function, as it is located in that region of the X chromosome, where the strongest modifier effect was detected in the males and because Ar was described earlier as a regulator of TGF-ß (CHIPUK *et al.* 2002). A similar regulation could thus also be assumed through the androgen response element of myostatin, a member of the TGF-ß superfamily (MA *et al.* 2001). The sex-influenced nature of the Compact phenotype (VARGA *et al.* 1997, BÜNGER *et al.* 2005) appeared to strengthen this hypothesis. In this study we analysed the coding sequence of the Ar locus in Compact mice and the expression of Ar mRNA by quantitative Real-Time PCR.

Procedures

Primer sequences

ANDR_1F: 5′-GCA GGA TAA GGG AAT TCG GTG-3′
ANDR_2F: 5′-TGG GAC CTT GGA TGG AGA AC-3′
ANDR_3R: 5′-GTC CCT GGT ACT GTC CAA ACG -3′
ANDR_4R: 5′-CCC ACC TTG TTC CCT TTC C-3′
ANDR_INTR_6R: 5′-TTG TTC TAT TGG GCG GGA GTC-3′
ANDR_7F: 5′-CTC CTC AAG CCC ACA TCA GA-3′
ANDR_8R: 5′-CTA CTA CAA CTT TCC GCT GGC T-3′
β-ACTIN_1F: 5′-TGC CGC ATC CTC TTC CTC-3′
β-ACTIN_1R: 5′-CCA CAG GAT TCC ATA CCC AAG-3′
GAPDH_HS_SY_F: 5′-GGC ATG GAC TGT GGT CAT GAG-3′
GAPDH_HS_SY_R: 5′-TGC ACC ACC AAC TGC TTA GC-3′

The Ar locus is approximately 168.40 Kb, therefore our analysis focused on the eight coding exons based on the mRNA sequence (GenBank acc. no. NM_013476). Fragments were amplified from skeletal muscle-derived cDNA (but it was partly successful:

ANDR_2F-ANDR_4R, containing the coding part of exons 2-8) and genomic DNA (ANDR_1F-ANDR_INTR_6R, containing the whole coding part of exon1) of hypermuscular Compact (Comp9 inbred strain) and normal muscled (*Mus musculus castaneus*, CAST/Ei) males (*n*=3/strain), then they were subjected to cloning (pGEM-T Easy Vector System I, Promega) and one clone/individual was sequenced (BigDye Terminator v3.1 kit, Applied Biosystems) on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems), using the vector-specific M13F, M13R and the labelled ANDR primers listed above (from 1F to 8R).

Ar expression was determined with relative quantification method from skeletal muscle RNA samples of 6-7 week old Comp9 and CAST/Ei males (*n*=3/strain). TRIzol (Invitrogen) reagent were used for total RNA isolation. Integrity evaluation and quantification was performed with Bioanalyzer 2100 (Agilent) and Nanodrop Spectrophotometer (Thermo Scientific). ProSTAR Ultra-HF RT-PCR System (Stratagene) were used for reverse transcription. The qPCR-s were performed according to the recommendation of ABI (two step PCR, 60°C annealing). In the reactions SYBR Green I dye, Gapdh (for endogen normalization) and Actb (for verification) primers for endogenous control amplification (Table 1), Ar gene specific primers (ANDR_2F and ANDR_3R) and dilutions of cDNA samples (1:1, 1:4, 1:16) were used. Three replicates were run on ABI 7000 Real Time PCR machine. For data analysis we used REST (relative expression software-tool), with endogen gene normalization and efficiency correction (PFAFFL *et al.* 2002).

Results

Comparing the Comp9 mouse Ar coding region to the consensus (strain C57Bl/6, Ensembl) and the CAST/Ei sequences, only the four known CAST/Ei SNPs were identified (rs31851337, rs29087626, rs31851336 and rs29085429) in the region while no Compact-specific mutations were found.

Table1
Relative expression of CAST/Ei and *Comp9 Ar* gene (CAST/Ei Ar expression is one unit)
Relative Expression des CAST/Ei und des Comp9 Ar Gens. (Expression von CAST/Ei Ar = 1)

	Comp9 Ar	Actb*	Gapdh*
relative expression	1.16	0.98	1.00
standard error	0.54	0.32	0.00
P-value	0.001	0.001	0.001

Data are from three 6-7 week old males/strain *endogenous control genes

The relative expression of Comp9 Ar was approximately one unit (expression of CAST/Ei Ar) and its *P*-value (calculated by REST-Pair Wise Fixed Reallocation Randomisation Test with normalisation by Gapdh) shows that this minor difference is significant (Table 1). Consequently the quantitative analysis of gene expression did not show remarkable differences between mRNA levels of Comp9 and CAST/Ei Ar in the male skeletal muscles.
According to these results, the androgen receptor gene does not seem to be a true X-linked modifier of the Compact phenotype. Our further aim is to narrow down the current wide interval using a special mapping population to be able to localise efficiently the putative modifier gene(s) in this chromosomal region.

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