Searching For Functional Sequence Variation Of The Porcine *ADRB2* Gene

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Introduction

Beta-2 adrenergic receptors (AR) mediate metabolic actions of catecholamines, including glycogenolysis, proteolysis and lipolysis in skeletal muscle and adipose tissue (Nonogaki 2000, Navegantes et al. 2001). Accordingly, beta-2 AR stimulation or blockade affects pork quality in terms of pH, tenderness and color (Warriss et al. 1990, Du et al. 2005). In addition, treatment of pigs with synthetic beta-2 agonists stimulates accretion of muscle tissue at the expense of adipose tissue (reviewed by Beermann 2002). Factors influencing beta-2 AR signaling (e.g. receptor expression) thus might affect pork quality and carcass composition. We have previously shown that the porcine *Adrenergic Receptor Beta 2* (*ADRB2*) gene is a positional candidate for pork quality QTL on Ssc2. Moreover, we demonstrated that yet-unknown *cis*-acting sequence variation affects mRNA expression of *ADRB2* in skeletal muscle (*longissimus dorsi*, LD), and possibly also pork quality (Murani et al. 2009). In the present study we set out to analyze the *cis*-acting sequence variation of *ADRB2* and its effects on pork quality and carcass composition in more detail. We performed DNA-polymorphism screening and haplotyping of the proximal region (ca. 1 kb 5´ flanking, 2 kb transcribed, and 0.5 kb 3´ flanking), and determined haplotype-specific expression in LD muscle. The knowledge of the differences in haplotype structure and function will help us to pinpoint the causal *cis*-acting sequence variation.

Material and methods

DNA-polymorphism screening, genotyping, haplotype analysis. The screening for DNA-polymorphism was initially based on six animals showing different levels of allelic expression in the previous study (Murani et al. 2009). The target region was amplified in three overlapping PCR-fragments, cloned, and sequenced in order to facilitate molecular haplotyping. Based on this, potentially functional polymorphisms tagging different haplotypes (12 SNP depicted in Figure 1, and 1 VNTR) were selected and genotyped in a set of animals of different commercial breeds (German Landrace - LR, German Large White - LW, Pietrain - Pi) in order to explore the haplotype diversity in more detail. Haplotype inference was performed using the SAS software (Proc Haplotype). Newly identified haplotypes were sequenced. Haplotype genealogy was analyzed using the TCS1.21 software.

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Expression analyses. Allelic expression was measured using normalized sequencing essentially as described previously (Murani et al. 2009). Briefly, cDNA and the corresponding genomic DNA samples (German Landrace barrows) were directly sequenced on an ABI 3130 instrument and the sequence chromatograms were analyzed using the PeakPicker V0.5 software. Peak heights of the two polymorphic bases were normalized using heights of each three flanking reference peaks on either side. In addition, all allelic ratios obtained from cDNA samples were normalized by dividing with mean allelic ratio derived from genomic DNA within each amplification/sequencing run.

Statistical analyses. Allelic expression imbalance (AEI, i.e. difference between ratios obtained using cDNA and DNA) was tested using a two-tailed t-test with unequal variances in Microsoft Excel. The effect of haplotypes on AEI was tested using general linear model (SAS, Proc GLM). Least square means were compared by t-test and the significance adjusted by Tukey-Crammer correction. Association of haplotypes with pork quality and carcass composition was analyzed using the haplotype trend regression implemented in JMP Genomics software. Phenotypic data were pre-adjusted for systematic effects (fixed effect of farm, random effect of sire, for meat quality random effect of slaughter date and for carcass traits hot carcass weight as covariate) in SAS (Proc Mixed).

Results and discussion

Our previous study (Murani et al. 2009) already revealed high sequence variability of the 5´ flanking region of the porcine *ADRB2*. In order to perform the searching for the *cis*-acting sequence variation in a systematic way we first analyzed the haplotype structure of *ADRB2*. The polymorphism screening and haplotyping targeted the proximal region, because high-resolution eQTL mapping in humans revealed enrichment of *cis*-acting eQTL around transcription start and end sites (Veyrieras et al. 2008). We identified a total of 28 SNP (excluding SNP in a complex VNTR) and three indels, constituting seven haplotypes. In addition, two VNTR segregated in the target region. Haplotype frequencies in commercial breeds are summarized in Table 1. The evolutionary analysis of the haplotype sequencies revealed the presence of three major clades separated by long branch lengths (Figure 1).

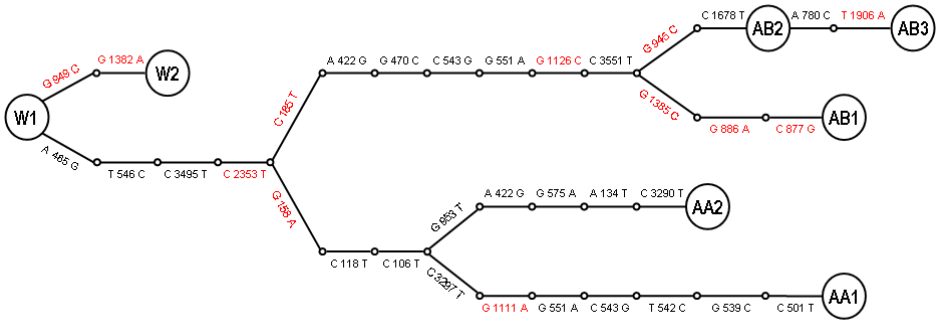

Figure 1: Genealogy of haplotypes of the porcine *ADRB2* segregating in LR, LW and Pi (genotyped SNP are highlighted in red)**.**

Table 1: Haplotype frequencies (%) of porcine *ADRB2* in LR, LW and Pi

		Haplotype						
Breed		W1	W2	AA1	AA2	AB1	AB2	AB3
LR	(n=307)	0.8	51.0	23.5	0.2	16.1	8.1	0.3
LW	(n=25)	0.0	64.0	28.0	0.0	0.0	0.0	8.0
Pi	(n=30)	0.0	55.0	20.0	13.3	11.7	0.0	0.0

We reasoned that the large inter-clade distances might be an indication of admixture. In 18th and 19th centuries Asian genetics was introgressed into European breeds, and it has been shown by Ramirez et al. (2009) that the proportion of Asian alleles in the genetic pool of European commercial breeds is substantial. Hence to trace the origin of the haplotypes we genotyped Vietnamese Muong Khuong (MK), Chinese Meishan (M) and European Wild Boar (WB) samples for SNP tagging different clades. The results are summarized in Table 2. Differential distribution of alleles between the European Wild Boar and the Asian breeds, especially that of SNP C 2353 T, strongly supports the hypothesis that haplotypes belonging to the clades AA and AB originate from Asian pigs.

Table 2: Frequencies of the derived alleles (%) of porcine *ADRB2* in WB, MK and M

		Tagged branch/SNP			
		W2	AA+AB	AA	AB
Breed		G 1382 A	C 2353 T	G 158 A	C 185 T
WB	(n=25)	58	0	0	0
MK	(n=25)	2	98	41	57
M	(n=4)	0	100	25	75

In our previous study we demonstrated AEI of the SNP c.1056C>T (here C 2353 T) in the LD muscle, evidencing that haplotypes from the Asian-derived clades AA and AB show higher expression of *ADRB2* compared to haplotypes from the W clade. The relatively wide range of AEI we observed (1.01–2.13) led us to hypothesize that there might exist differences in haplotype-specific expression of *ADRB2* not only between, but also within different clades. Using AEI data from the previous study we got evidence that the haplotype AB2 shows intermediate expression between the haplotype W2, and haplotypes AB1 and AA1 respectively (Figure 2A). This pattern, i.e. W2<AB2<AA1~AB1, was confirmed by measuring allelic expression of *ADRB2* in LD muscle using an independent transcribed SNP (Figure 2B). Obviously multiple DNA-polymorphisms affect the mRNA expression of the porcine *ADRB2* in *cis*. In the analysis of the effects of *ADRB2* haplotypes on pork quality (pH, color, conductivity, drip loss) and carcass composition (lean content, back fat thickness, loin area) we focused on the German Landrace population where we described the haplotype-specific expression. This revealed association of the haplotype W2 with initial pH of loin, which corresponds with our previous analysis using SNP.

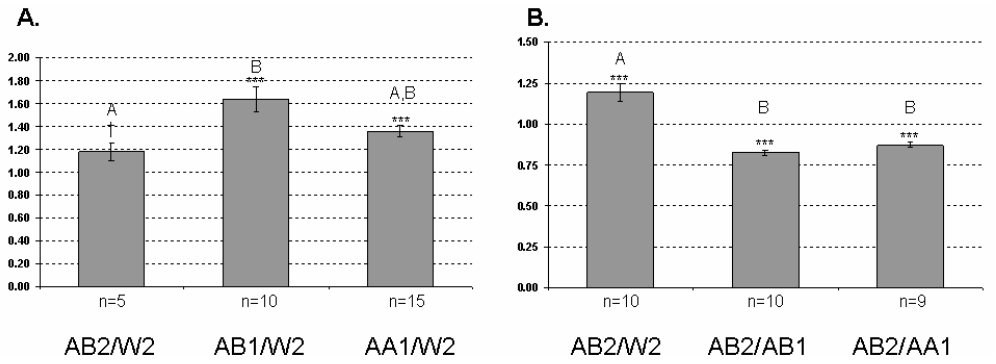

Figure 2: Haplotype-specific expression of the porcine *ADRB2* in LD muscle.
A. Allelic expression measured using SNP C 2353 T (allelic ratio T/C). **B.** Allelic expression measured using SNP C 1678 T (allelic ratio T/C). Each bar shows the mean ± standard error. *** indicates AEI significant at $p<0.01$, † indicates tendential AEI $p<0.1$. Haplotype combinations with different letters (A,B) show differences in AEI significant at $p<0.01$.

Conclusion

In the present study we demonstrated strikingly high structural and functional diversity of the porcine *ADRB2*. Detailed dissection of the molecular basis of the functional differences and association analysis in additional populations is needed to ascertain the effects of *ADRB2* on pork quality and carcass composition.

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