Association of corticotropin-releasing hormone gene variation with performance and meat quality traits in commercial pig lines

E. Muráni*, S. Ponsuksili†, K. Schellander‡ and K. Wimmers*

*Research Unit Molecular Biology, and †Research Group Functional Genomics, Research Institute for the Biology of Farm Animals (FBN), 18196 Dummerstorf, Germany. ‡Animal Breeding and Husbandry Group, Institute of Animal Science, University of Bonn, 53115 Bonn, Germany

Summary

The porcine corticotropin-releasing hormone (CRH) gene is a functional–positional candidate for quantitative trait loci on porcine chromosome 4 with major effects on growth and carcass composition. In addition, the central role of CRH in the neuroendocrine response to stress implicates the CRH gene as a functional candidate for meat quality. Association of a single nucleotide polymorphism (SNP) in the promoter region of the porcine CRH gene (g.233C>T) with several growth, carcass and meat quality traits was examined using more than 2000 individuals from four commercial lines: German Landrace (LR), Pietrain (Pi), German Large White × German Landrace (F1) and the German commercial fattening pig cross of Pietrain × F1 (PiF1). Significant association of the CRH SNP was found with feed conversion ratio in the PiF1 line, with carcass length in the LR line and with lean content in the F1, LR and Pi lines. Moreover, significant association with meat colour was found in the Pi and LR lines; however, the effects were in opposite directions. The presented results indicate that sequence variation in the porcine CRH gene has no major effect on growth and carcass composition in commercial pig lines, although it may significantly contribute to variation in meat quality. The g.233C>T SNP may be in incomplete linkage disequilibrium with causal mutations and/or exhibit effects in the context of DNA variation at other interacting loci.

Keywords: carcass traits, corticotropin-releasing hormone, meat quality, pig, single nucleotide polymorphism.

Glucocorticoids and catecholamines, which are released by the two main stress-responsive systems [hypothalamo-pituitary–adrenal (HPA) and sympathoadrenal (SA) respectively], govern energy-mobilizing processes such as proteolysis, lipolysis and glycogenolysis and hence affects growth, carcass composition and pork quality (Shaw et al. 1995; Foury et al. 2005; Yoshioka et al. 2005). The observations that breeding for enhanced lean growth in pigs has led to functional changes in the HPA axis and the levels of cortisol in improved breeds are lower than in unimproved breeds underscore the important role of stress hormones in the determination of pig performance (Weller et al. 1998; Hari & Pliska 2005). Considerable variation in stress hormone levels have been shown not only between breeds, but also within breeds (Wimmers et al. 2002b).

Corticotropin-releasing hormone (CRH), a 41-amino-acid neuropeptide, is a key coordinator of the HPA and SA systems (Chrousos 1998). Recently, we mapped the porcine CRH gene to a quantitative trait loci (QTL) region on chromosome 4 with major impact on growth and body composition (Wimmers et al. 2002a; Murani et al. 2006). Moreover, we found a highly significant association of a single nucleotide polymorphism (SNP) in the porcine CRH gene (c. +83G>A; DQ358705) with growth and carcass traits in the Duroc × Berlin Miniature Pig (DUMI) F2 resource population (Murani et al. 2006). Thus, the CRH gene is a promising functional and positional candidate for stress susceptibility, growth, carcass composition and meat quality in the pig. The aim of the present study was to further investigate the effect of the porcine CRH gene on growth, carcass and meat quality traits using an association analysis in several commercial pig lines.

Samples and phenotypic records were obtained from castrates of the German Landrace (LR) and German Large White × German Landrace (F1) dam lines; from sows of the Pietrain (Pi) sire line; and from sows of the German commercial fattening pig cross Pietrain × F1 (PiF1), all

Address for correspondence

K. Wimmers, FBN-Dummerstorf, Wilhelm-Stahl-Allee, 2,18196 Dummerstorf, Germany.
E-mail: wimmers@fbn-dummerstorf.de

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maintained and performance tested at the Frankenforst Research Farm at the University of Bonn. The pigs were slaughtered at a commercial slaughterhouse where an AutoFOM device (SKF Technology, Herlev, Denmark) was used in the online carcass-grading routine. Of the carcass traits measured by AutoFOM, carcass (cLEANPa) and belly (bLEANPa) lean content were included in the analysis. Additional traits were measured according to the German performance test directives (ZDS 2004) and included average daily gain on test, feed conversion ratio (FCR), carcass length, loin muscle area (LMA), average backfat thickness (BFT), carcass lean content estimated using new ‘Bonner formula’ (cLEANPe), belly lean content estimated using ‘Gruber formula’ (bLEANPe), pH of loin 1-h post-mortem (PH1LD), conductivity of loin 24 h post-mortem (CON24LD) and meat colour (MCOLOR). Feed intake was measured per pen on a sibpair basis; therefore, FCR was computed for sibpairs rather than for individual pigs.

Genomic DNA was isolated from ear notches using the NucleoSpin-96 Tissue kit (Macherey-Nagel, Düren, Germany). Because the c.+33G>A SNP did not segregate in commercial lines, a C>T SNP located in the 5′-flanking region of the porcine CRH gene (g.233C>T; DQ358705) was genotyped using a single-strand conformation polymorphism assay as described by Murani et al. (2006). In silico analysis indicated that g.233C>T is likely a regulatory SNP because it occurs in an evolutionary conserved motif and eliminates predicted, physiologically relevant transcription factor-binding sites (Murani et al. 2006). The wild-type C allele segregated with similar frequency (about 0.6) in LR, F1 and PiF1 lines, whereas in the Pi line, the C allele occurred with lower frequency (0.47; Table 1). In all lines the genotype distribution was in Hardy–Weinberg equilibrium. The allele distribution in the PiF1 line significantly deviated (P < 0.0001) from expected when comparing the Pi and F1 as parental lines to the PiF1 line. The higher than expected frequency of the C allele may indicate that there was selection in favour of mating partners carrying the C allele. In addition to the g.233C>T SNP, the RYR1 mutation, which is responsible for malignant hyperthermia syndrome and is associated with pale, soft and exudative meat (Sellier 1998), was genotyped in the Pi and PiF1 lines. The mutant allele segregated in the Pi and PiF1 lines with a frequency of 0.56 and 0.25 respectively. The mutation was absent in the LR and F1 lines.

Association between the g.233C>T SNP and phenotypic variation was analysed using a general mixed model (PROC Mixed, SAS v. 8.2; SAS Inc., Cary, NC, USA). In addition to the fixed effect of the g.233C>T genotype, the model included random effect of sire, random effect of slaughter date for meat quality traits and slaughter weight as a covariate for carcass traits and slaughter age for growth traits. The RYR1 genotype was included as a fixed effect in the Pi and PiF1 lines. Because the RYR1 mutation has an established effect on the HPA-axis activity (Weaver et al. 2000), an interaction between the CRH and RYR1 genotypes was also tested. However, the interaction term did not reach significance (P < 0.05) for any of the traits and was, therefore, excluded from the model. Because the FCR was available only for sibpairs, individual genotype effect was substituted in the model by genotype of a sibpair when the genotypes were identical between the sibpairs. Sibpairs with different genotypes were omitted from the analysis. Least square mean values for the porcine CRH genotypes were compared by a t-test, and the P-values were adjusted by a Tukey–Kramer correction.

Results of the association analysis are summarized in Table 2. Only traits for which association was found in at least one population are shown. The presented significance values are nominal P-values not adjusted for multiple testing, which inflates the risk of finding false positives. However, there is a lack of suitable methods for multiple-test adjustments, and routinely used methods like the Bonferroni correction are overly conservative because they do not take into account correlation between traits. Seven of the 48 (four populations × 12 traits) tests were found to be significant at the nominal 5% level compared with two tests that could be expected by chance alone, indicating that majority of the associations are most likely true positives.

Analysis of growth traits revealed a significant association with FCR in the PiF1 line (P < 0.05), evidencing better feed conversion in the C-allele carriers. This association might explain the suggestion of selection for the C allele in the PiF1 line. The better feed conversion of the C-allele carriers was not accompanied by an effect on daily gain, indicating that these animals might have lower feed intake. With regard to carcass composition, the AutoFOM traits had some significant associations: whole cLEANPa in the F1 line (P < 0.05) and bLEANPa in the LR (P < 0.1), F1 (P < 0.05) and Pi (P < 0.05) lines. Carriers of the T allele tended to produce carcasses and bellies with higher lean content. However, none of the linear carcass measurements (BFT and LMA) or lean content estimates based on these measurements (cLEANPa and bLEANPa) were significantly associated with the SNP, although trends similar to those with the AutoFOM traits were observed (data not shown). In the LR line, animals carrying the T allele had significantly

Table 1 Genotypic frequencies of the g.233C>T single nucleotide polymorphism in the promoter of the porcine corticotropin releasing hormone gene.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Commercial lines</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LR</td>
<td>F1</td>
<td>Pi</td>
<td>PiF1</td>
</tr>
<tr>
<td>CC</td>
<td>0.32</td>
<td>0.35</td>
<td>0.23</td>
<td>0.34</td>
</tr>
<tr>
<td>CT</td>
<td>0.49</td>
<td>0.47</td>
<td>0.47</td>
<td>0.50</td>
</tr>
<tr>
<td>TT</td>
<td>0.19</td>
<td>0.18</td>
<td>0.30</td>
<td>0.16</td>
</tr>
<tr>
<td>No. animals tested</td>
<td>372</td>
<td>594</td>
<td>360</td>
<td>1040</td>
</tr>
</tbody>
</table>

LR, German Landrace; F1, German Large White × German Landrace; Pi, Pietrain; PiF1, Pietrain × F1.
longer carcasses than animals carrying the C allele ($P = 0.01$).

The most pronounced of all effects was found on meat colour in the Pi line ($P < 0.01$), where carriers of the T allele produced paler meat. This result is in line with the widely accepted and important role of pre-slaughter stress and stress response in the determination of pork quality (Shaw et al. 1995; Hambrecht et al. 2004). An association with meat colour was also found in the LR line ($P < 0.05$); however, the effects were opposite compared with the Pi line. Strikingly, no association with pH or conductivity was found in either line. This finding indicates that the effect on meat colour might rely on cortisol-induced muscle proteolysis and/or a shift in muscle fibre composition rather than on glycogenolysis (Yoshioka et al. 2005). Wimmers et al. (2006) mapped a QTL for proportion of fast twitch glycolytic fibres to a region on chromosome 4 coincident with the porcine CRH gene location. On the other hand, pH and conductivity measurements generally have lower heritability than meat colour (Sellier 1998); they are, to a larger extent, influenced by environmental factors. Because the g.233C>T SNP is likely regulatory, its effect may be strongly dependent on the environmental effects that could mask an association with pH and conductivity. In addition, the lack of consistent associations across all four lines and the opposing direction of effects on meat colour indicate that the effect of the g.233C>T SNP may also be dependent on haplotype context and/or on interaction with genetic background. Incomplete linkage disequilibrium between the g.233C>T SNP and DNA variation at another locus should also be considered. Functional characterization of the g.233C>T SNP and of different haplotypes of the porcine CRH gene are now required to provide insight into the mechanism of the effect of the porcine CRH gene variation and to provide proof of causality.

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References