Evidence For An Additional Functional Polymorphism Within The Porcine *IGF2* Gene

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Introduction

The Insulin-like growth factor (IGF) system is an evolutionarily conserved regulatory network essentially comprising the two growth factors IGF-I and IGF-II and their cell surface receptors IGF-1R and IGF-2R as well as a number of binding proteins (IGFBP 1-6). IGF-I is one of the important factors regulated by the pituitary growth hormone (GH) influencing growth, differentiation, cell proliferation and apoptosis. Varying levels of IGF-I expression can be observed in most tissues during the entire lifespan of an organism. The expression level IGF-II on the other hand is independent from GH and its role is more confined to fetal development and growth. The *IGF2* gene is subject to genomic imprinting with only the paternal allele being expressed.
The sum of anabolic and mitogenic effects mediated by the IGF-system makes the contributing genes likely to influence relevant growth and performance traits in livestock species. In the pig, a major QTL for muscle growth, fat deposition and heart size was mapped in the vicinity of *IGF2* on porcine chromosome (SSC) 2 (Nezer et al., 1999; Jeon et al., 1999; De Koning et al., 2000; Jungerius et al., 2004; Thomsen et al., 2004). The QTL explains up to 30% of the phenotypic variance for muscle growth and up to 20% for backfat size, respectively. A causative quantitative trait nucleotide (QTN) was identified by Van Laere et al. (2003). The mutation is located within a regulatory structure in intron 3 (*in3G3072A*) of the gene affecting the binding of a repressing nuclear factor and thus leading to postnatal IGF-II expression. The objective of the current study was to confirm the QTL in a large Piétrain x (Large White x Landrace) F_2 resource population and to test for additional genetic variation explaining phenotypic variance beyond the described QTN *in3G3072A*.

Material and methods

Animals and Phenotypes. A three-generation resource pedigree comprising 2741 F_2 animals was established based on 5 purebred Piétrain boars and 10 Large White, Landrace and crossbred sows. Large F_2 fullsib families were produced by repeated full sib mating of F_1 animals. Comprehensive phenotypic data was recorded including carcass and meat quality traits. Within this study we analysed eight body composition traits (Figure 1). All traits were measured after routine slaughter according to the performance testing directive of the central association for German pig production (Zentralverband der Deutschen Schweineproduktion). Fat and meat characteristics were measured using the IR reflection stab-in probe Fat-O-

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Meat'er (Carometec A/S, Herlev, Denmark) between the third and second last ribs. The meat proportion was calculated according to a standard formula ("Bonner Formula").

Marker Data. Four microsatellite markers (SW2443, SWC9, SW2623 and SW256) spanning a 20cM interval on SSC2 including *IGF2* were genotyped in all animals. The polymorphism *in3G3072A* was genotyped in the founder animals, the F_1 generation and 715 F_2 animals. Perfect cosegregation between the QTN and the adjacent microsatellite marker SWC9 located only 13 kb apart within the 3'-UTR of the gene was observed. This allowed us to infer the QTN genotype of 1380 additional F_2-animals and to determine the parental origin of the QTN alleles in 1592 F_2 animals.

Statistical analyses. The QTL analyses were carried out according to Haley & Knott (1992) using the QTL mapping application GridQTL (Seaton et al., 2006). Significance thresholds were derived empirically by permutation test with 10,000 permutations as implemented in GridQTL according to Churchill & Doerge (1994). The QTL analysis was performed in two steps starting with the entire set of genotyped animals. In the second step, the QTL analysis was performed in two subsets being alternatively homozygous at the QTN to eliminate this polymorphism as a source of variation. The statistical model included the fixed effects of sex, stable and family and the weight as a covariate.

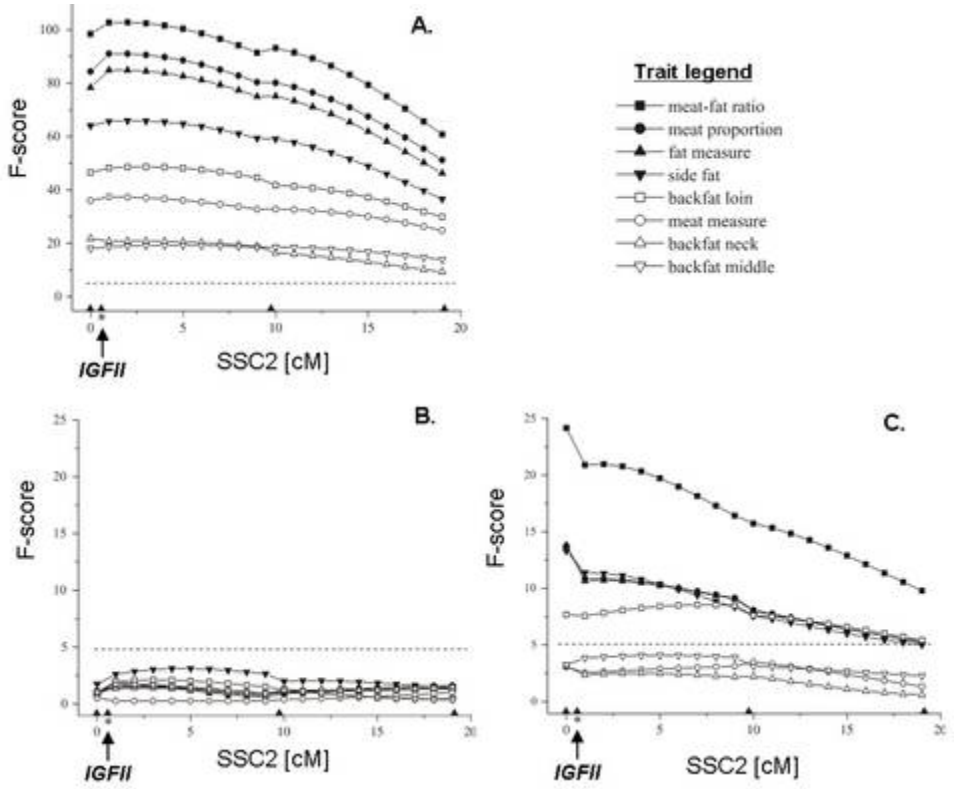

Figure 1: Test statistics for the complete set of animals (A.) and the subsets of animals alternatively homozygous at the QTN (B. GG, C. AA). Marker positions are shown on the abscissa. Dashed lines depict the significance threshold for α=0.01.

Results and discussion

The Piétrain founder boars were found to be homozygous for the desireable QTN allele (A), while most of the sows were homozygous for the alternative allele (G). The initial QTL analysis using the complete set of 2404 genotyped F_2-animals revealed highly significant QTL influencing all analysed traits (Figure 1).
The estimated QTL effects (additive, dominance and parent-of-origin) of the two selected traits meat proportion (mean 54.0% ± 3.92%) and fat measure (mean 19.1mm ± 4.05mm) are presented in Table 1. These traits stand exemplarily for the effect on body composition described for this locus by Nezer et al. (1999), Jeon et al. (1999), De Koning et al. (2000), Jungerius et al. (2004) and Thomsen et al. (2004). A clear parent-of-origin dependent effect was detected and due to the known parental origin of the QTN alleles we were able to confirm the expected paternal expression.

Table 1: Estimated QTL effects with standard errors for the exemplary traits meat proportion [%] and fat measure [mm]. The left column shows the effects estimated in the complete set of animals, the right column shows the values for the homozygous AA-subset.

	Complete		**Subset AA**	
	Estimated effect±S.E.		**Estimated effect±S.E.**	
	meat proportion	**fat measure**	**meat proportion**	**fat measure**
additive	1,28±0,101	-1,37±0,109	1,11±0,391	-1,12±0,416
dominance	-0,17±0,144	0,20±0,157	-0,24±0,566	0,38±0,602
parent-of-origin	1,29±0,122	-1,25±0,130	1,82±0,488	-1,94±0,518

In order to test for the presence of genetic variation beyond the described in3G3072A mutation, two subsets of animals alternatively homozygous for this QTN were analysed. This approach was only possible due to the extremely large resource population. While no QTL could be detected in the group homozygous for the G allele (n=568), a highly significant QTL was detctable in the subset homozygous for the desireable allele A (n=489) (Figure 1).
These results indicate the presence of a further polymorphism within or adjacent to the *IGF2* gene affecting the analysed traits beyond the known effect of the in3G3072A mutation.
As the Piétrain boars are completely homozygous for the A allele, the subset of animals homozygous GG do exclusively carry *IGF2* haplotypes derived from the white breeds. However, two of the founder sows are heterozygous, meaning that within the subset homozygous for the A-allele all possible combinations of Piétrain and Large White / Landrace haplotypes do occur. Excluding all descendants of the two heterozygous sows from the analysis, no significant QTL remains within the AA subset as well. This suggests the presence of a polymorphism alternatively fixed in Piétrain and the White breeds.

Conclusion

The objective of the current study was to test for additional genetic variation at the porcine *IGF2* locus explaining phenotypic variance beyond the described QTN *in3G3072A*. We were able to confirm the presence of a QTL with a pronounced parent-of-origin effect on several carcass and body composition traits. Furthermore, we were able to show the presence of a putative additional polymorphism within or adjacent to the *IGF2* gene affecting the analysed traits beyond the known effect of the in3G3072A mutation. This polymorphism is likely to be alternatively fixed within the founder lines. Further analyses of the *IGF2* gene within our resource population will probaly allow the identification of this mutation.

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