The *IGF2*-intron3-G3072A substitution explains a major imprinted QTL effect on backfat thickness in a Meishan × European white pig intercross

BART J. JUNGERIUS*1, ANNE-SOPHIE VAN LAERE2, MARINUS F. W. TE PAS3, BERNARD A. VAN OOST4, LEIF ANDERSSON2,5 AND MARTIEN A. M. GROENEN1

1 Department of Animal Breeding and Genetics, Wageningen University, P.O. Box 40, 6700 AH Wageningen, The Netherlands
2 Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Sweden
3 Animal Sciences Group, Wageningen University and Research Centre, Lelystad, The Netherlands
4 Department of Laboratory Animal Sciences, Utrecht University, Utrecht, The Netherlands
5 Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

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Summary

A paternally expressed QTL for muscle growth and backfat thickness (BFT) has previously been identified near the *IGF2* locus on the distal tip of pig chromosome 2 (SSC2p) in three experimental F2 populations. Recently, a mutation in a regulatory element of the *IGF2* gene was identified as the quantitative trait nucleotide (QTN) underlying the major QTL effect on muscle growth and BFT in crosses between Large White and Wild Boar or Pietrain. This study demonstrates that the *IGF2* mutation also controls the paternally expressed QTL for backfat thickness in a cross between Meishan and European Whites. In addition, a comparison of QTL of backfat thickness measured by Hennessy grading probe (HGP) and by ultrasound measurement (USM) was made. In the USM analyses, the *IGF2* mutation explains the entire QTL effect on SSC2p, whereas in the HGP analysis the presence of a second minor QTL can not be excluded. Finally, this study shows that this particular *IGF2* mutation does not cause the paternally expressed QTL for teat number mapping to the same region of SSC2p as the BFT QTL.

1. Introduction

Several experimental crosses in pigs have been used to detect QTLs for a variety of production traits such as fatness and meat quality. For backfat thickness (BFT) significant QTL effects have been detected on 10 different chromosomes (reviewed by Bidanel & Rothschild, 2002). On the short arm of chromosome 2 (SSC2p) a paternally expressed QTL for muscle growth and BFT was identified in several experimental F2 populations. The QTL was first mapped near the *IGF2* locus on the distal tip of SSC2p using Large White × Wild Boar (Jeon et al., 1999) and Large White × Pietrain (Nezer et al., 1999) intercrosses. In a Meishan × European White intercross the maximum QTL peak was more proximal at 32 cM, even though a marker within the *IGF2* gene (*SWC9* at 2 cM) was highly informative (de Koning et al., 2000). However, *IGF2* was still within the QTL confidence interval. It was hypothesized that the observed difference in the QTL position was possibly caused by the presence of two neighbouring QTLs: one paternally expressed and one Mendelian additive QTL (Rattink et al., 2000). Because *IGF2* is known to be paternally expressed, it is the prime candidate gene underlying the paternally expressed QTL for BFT in the Meishan × White cross.

Recently, a mutation in a regulatory element of the *IGF2* gene was identified as the quantitative trait nucleotide (QTN) underlying the major QTL effect on muscle growth and BFT in the crosses between Large White and both Wild Boar and Pietrain (Van Laere et al., 2003). The mutation is of a single nucleotide substitution (G-A) at position 3072 in the third intron of *IGF2* and strong evidence for a causal relation with the observed QTL effect was provided. To analyse the contribution of this mutation to the observed QTL for BFT in the Meishan × European White cross...
cross, this population was genotyped for the QTN. In the same Meishan × European White population, a paternally expressed QTL for teat number (TN) was identified near SWC9 on the distal tip of the short arm of SSC2 (Hirooka et al., 2001). Because of the co-localization and the same mode of inheritance the QTL data for TN were reanalysed to test whether the IGF2-intron3 QTN may have pleiotropic effects on TN.

2. Materials and methods

(i) Experimental population and phenotypes

A detailed description of the population used in this study was given previously (Janss et al., 1997a). Briefly, a cross between Meishan and Dutch Large White and Landrace lines was established for the detection of major genes (Janss et al., 1999, 2000; Rattink et al., 2000). Body weight and BFT were recorded on 1130 F2 pigs from 38 half-sib families shortly before slaughter. The ultrasound measurements (BFT-USM) were performed at two to four locations at one side of the spine using a Renco Lean-Meater (Renco, Minneapolis, MN, USA). After slaughter, carcass weight and the BFT, estimated using a Hennessy Grading Probe (BFT-HGP), were recorded on 774 pigs. In the Netherlands BFT and muscle content are routinely measured using the HGP between the third and fourth rib of the carcass, 6 cm from the spine. For 1173 F2 individuals, the TN (i.e. the number of morphologically normal teats) was scored.

(ii) Genotyping

Thirteen microsatellite markers located on SSC2 were used to obtain genotypes from the F2 animals, their F1 parents and the purebred Meishan grandparents as described by Rattink et al. (2000). Marker names and locations are indicated in Fig. 1. In addition, all 38 F1 boars and their parents were genotyped for the G-A substitution at position 3072 in the third intron of IGF2. Genotyping was performed by pyrosequencing (Pyrosequencing AB) as described by Van Laere et al. (2003).

(iii) Extrapolation of the IGF2 QTN to F2 individuals

The IGF2 QTN is located 12.2 kb upstream of SWC9 in the 2 cM interval between SW2443 and SWC9. The QTN genotypes of the F0 and F1 individuals were integrated with the genotypes of a subset of SSC2p microsatellites (i.e. SW2443, SWC9, SW256 and S0141 located at map position 1, 3, 26 and 40 cM respectively). For each family, parental haplotypes were determined using Simwalk2 (Sobel & Lange, 1996), based on the pedigree information and the inheritance of the microsatellite markers. Simultaneously, the QTN alleles were placed in these haplotypes. The QTN alleles and their parental origin in the F2 individuals were inferred with great confidence based on the segregation of the haplotype containing the QTN and the flanking markers.

(iv) QTL analyses

Statistical analyses were carried out as described in detail by de Koning et al. (1999, 2000). Briefly, the phenotypic data were adjusted for a number of systematic effects prior to the QTL analysis. The standard set of effects included in the model consists of sex, weight and a factor to correct for the facility where the measurements were performed (i.e. the five companies that provided the White lines).

Mendelian QTL analyses were based on the line cross concept (Haley et al., 1994), where the original breeds are assumed to be homozygous for different QTL alleles. This model has been extended to test for imprinting (Knott et al., 1998), but a separate test was needed to infer paternal or maternal expression. Therefore the model for imprinting was re-parameterized to test for the direct contribution of the paternally and maternally inherited effect (de Koning et al., 2000). This model facilitates discrimination between QTLs showing exclusive paternal expression, exclusive maternal expression or Mendelian expression.

Significance thresholds were determined empirically by permutation with at least 10,000 replicates (de Koning et al., 1999). The significance threshold was set at 5% genome-wise risk level (Lander & Kruglyak, 1995). Under all models used the information content is proportional to the variance of the estimators that are used in the regression analyses and has a maximum value of 1·125 (Knott et al., 1998). In this study, however, it is scaled to vary between 0 and 1.

QTL analyses were performed for three different traits: BFT-HGP, BFT-USM and TN. For each trait, three line-cross QTL analyses were performed each with a different subset of the population: (1) all 38 half-sib families; (2) the 20 families where the F1 sire was homozygous at the IGF2 QTN (G/G); (3) the 18 families with F1 sires heterozygous at the IGF2 QTN (A/G). The same 774 individuals were used for the analyses of both HGP and USM data. In addition, the BFT-HGP and the BFT-USM data were re-analysed using phenotypic data after correction by two alternative models: (1) the standard model but including the paternal allele of the IGF2-intron3-G3072A mutation as a systematic effect and (2) the standard model but omitting weight.
Fig. 1. QTL analyses on SSC2 for (A) BFT by Hennessy Grading Probe, (B) BFT measured by ultrasound measurement and (C) teat number. The thick continuous line represents the results from the segregating (A/G) families. The thin continuous line is from non-segregating (G/G) families. The ‘+’-marked line is a combined analysis of segregating and non-segregating families. Information content is represented by a grey dotted line. Horizontal lines indicate the genome-wise significant (continuous) and suggestive (dotted) significance thresholds. Marker names and positions are indicated at the top.
3. Results and discussion

(i) Presence of the IGF2 wild-type and mutant alleles

The IGF2-intron3-G3072A mutation was successfully genotyped in 36 of the 38 F₁ sires, all 18 of their Meishan sires and 31 of their White dams. All Meishan F₂ sires were found to be homozygous for the wild-type allele (G/G). In the White F₂ dams both the wild-type and the mutant allele were represented although they were not equally distributed over the five commercial lines. All F₂ dams of the two lines of Dutch Landrace origin were found to be homozygous for the wild-type allele (G/G). In the White F₂ dams from the other three White lines the mutant (“lean” or A) allele was found with frequencies over 80%, but it was not fixed in either line. The contrast between absence of the mutant allele and high allele frequencies in all three lines where the mutation was present suggests that once the mutant allele is present within a population, it reaches high allele frequencies due to positive selection for lean growth. Two of 38 F₁ sires were not genotyped but both the parents were homozygous for the mutation and the genotypes of these F₁ sires were deduced. Overall, 20 F₁ boars were found to be G/G and 18 were scored as A/G (Table 1).

For the F₂ pigs, the genotype for the IGF2 mutation was not assayed directly, but was inferred based on segregation analysis of closely linked, flanking microsatellite markers. To do so, the IGF2 mutation genotypes of the F₂ and F₁ individuals were combined with the genotypes of the F₀, F₁ and F₂ individuals for four microsatellite markers. Subsequently, these data were used to reconstruct haplotypes that segregated in the population.

Because the IGF2 mutation is located in the 2 cM interval between microsatellite markers SW2443 and SWC9, a 2% recombination fraction in this marker bracket is expected. Recombinant haplotypes, however, were excluded from further analysis to prevent false allele assignments. Finally, a conclusive paternal allele for the IGF2 mutation was assigned for 726 F₂ pigs (94%). For the remainder, the observed segregation of microsatellites could only be explained by the introduction of recombinant haplotypes and they were therefore omitted. In total, 175 F₂ pigs inherited a mutant (A) allele from their sire and 551 inherited the wild-type (G) allele from their sire.

(ii) Phenotypic data

For 774 F₂ pigs in 38 half-sib families both live weight and carcass weight were recorded. In this dataset the correlation between live weight and carcass weight was 0.81. For the same 774 F₂ pigs both BFT-HGP and BFT-USM were measured. The correlation between BFT-HGP and BFT-USM was 0.74. The mean BFT-HGP was 22.0 mm. All F₂ individuals were grouped based on the SNP allele inherited from the father. The group of individuals which inherited an A allele from the paternal side had an average BFT-HGP of 20.8 mm whereas the individuals that inherited a paternal G allele had an average BFT-HGP of 22.4 mm (Table 2).

(iii) BFT QTL analyses and the IGF2 mutation

All 774 genotyped individuals were used in the analyses of BFT-HGP. The same 774 individuals were used in the analyses for BFT-USM to allow comparison of the results, although at the cost of reduced population size and lower F statistics. Data were also analysed using phenotypic data after correction by an alternative model in which the paternal IGF2 allele was included. The results of the QTL analyses with the paternal expression model are summarized in Table 3. QTL analyses following the Mendelian model were also performed, but in all occasions the F statistics were lower than with the paternal expression model (data not shown).

Initially, one QTL analysis for BFT-HGP was performed which included all 38 families. As expected, the outcome closely resembled the QTL analysis reported by de Koning et al. (2000). Since IGF2 is paternally expressed the QTL effect is expected to segregate only in families founded by F₁ sires that are

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Table 1. Distribution of genotypes at IGF2-intron3-nucleotide 3072 among F₁ sires in a Chinese Meishan × White intercross

<table>
<thead>
<tr>
<th>Line</th>
<th>Origin</th>
<th>G/G</th>
<th>A/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Great Yorkshire</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Large White</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Large White</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Dutch Landrace</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Dutch Landrace</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

* The sires are grouped by the white line origin of their mother.

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Table 2. Mean backfat thickness of the F₂ individuals for both the Hennessy Grading Probe (BFT-HGP) and ultrasound measurements (BFT-USM) (raw data)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>BFT-HGP (SE)</th>
<th>BFT-USM (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>344</td>
<td>20.8 (0.33)</td>
<td>14.7 (0.22)</td>
</tr>
<tr>
<td>G</td>
<td>430</td>
<td>22.4 (0.26)</td>
<td>15.5 (0.17)</td>
</tr>
<tr>
<td>All</td>
<td>774</td>
<td>22.0 (0.20)</td>
<td>15.4 (0.13)</td>
</tr>
</tbody>
</table>

* Based on the IGF2 QTN allele inherited from the F₁ sire.
heterozygous A/G at the QTN, if this mutation is also underlying the major QTL effect in this cross. In a joint QTL analysis of the 20 families of which the F\textsubscript{1} sire was found to be homozygous for the \textit{IGF2} wild-type allele (G/G) the F statistic did not reach the genome-wise suggestive threshold along the entire chromosome. In a similar analysis of the 18 families of which the F\textsubscript{1} sire was found to be heterozygous for the mutation (A/G) the F statistic exceeded the genome-wise significant level for both BFT-HGP and BFT-USM (Fig. 1A, B). These results clearly demonstrate that the \textit{IGF2}-intron3-G3072A substitution splits the population in two groups: families segregating for a major QTL and families that do not. From this, it can be concluded that the \textit{IGF2} QTN explains the major part, if not all, of the observed paternally expressed QTL for BFT on SSC2 in this pedigree. This conclusion is supported by the differences in average BFT between individuals which inherited a G (22.4 mm) or an A (20.8 mm) from their father.

(iv) Additional QTL analysis and influence of measurement techniques

In addition to the analyses of BFT-HGP data, similar analyses were performed for BFT-USM (Fig. 1B). The results of the QTL analyses for BFT-HGP and for BFT-USM are highly consistent for analyses of the 18 families in which both alleles of the \textit{IGF2} SNP are segregating. Although the QTL profiles largely overlap in analyses including all 38 families, the best positions of the QTL peaks for BFT-HGP and for BFT-USM differ by almost 30 cM (Fig. 1A, B; dotted lines).

Based on previous analyses of the BFT-HGP QTL on SSC2 (de Koning et al., 2000; Rattink et al., 2000), it was suggested that the QTL identified in the Meishan × White cross could be different from the QTL reported by Nezer et al. (1999) and Jeon et al. (1999). A possible explanation for the observed difference in QTL position was that it might be caused by the presence of two neighbouring QTL: one paternally expressed and one Mendelian additive QTL (Rattink et al., 2000). Although no convincing evidence for the presence of a second QTL was found, its presence could not be excluded either. Since the mutation underlying the primary QTL in the region is now known, it is possible to incorporate the paternal \textit{IGF2} allele as a fixed effect and test for the presence of a second neighbouring QTL.

The QTL for BFT-USM showed no evidence for an additional (Mendelian) QTL contributing to the phenotype. However, a paternally expressed QTL around 40 cM was indicated in the analysis of the HGP data. Although this analysis is based on phenotypic data that were corrected for a deduced \textit{IGF2} genotype it exceeds the genome-wise significance threshold and the presence of an additional QTL can not be excluded. Because the possible second QTL is only observed with BFT-HGP, this would be a QTL for a characteristic that is measured exclusively by BFT-HGP and not by BFT-USM, e.g. the third layer of backfat. Total backfat consists of three layers of which the third (inner) layer develops at a later stage of growth and often is underdeveloped in European

Table 3. Summary of QTL results for pig chromosome 2 and backfat thickness under the paternal expression model

<table>
<thead>
<tr>
<th>Measurement\textsuperscript{a}</th>
<th>Population</th>
<th>n</th>
<th>Best position</th>
<th>F ratio</th>
<th>QTL effect\textsuperscript{c}</th>
<th>Marker bracket\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFT-HGP</td>
<td>All</td>
<td>774</td>
<td>32</td>
<td>27.9***</td>
<td>0.04</td>
<td>SW2443–S0091</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>344</td>
<td>6</td>
<td>34.6***</td>
<td>0.09</td>
<td>SW2443–SW240</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>430</td>
<td>64</td>
<td>6.8</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>BFT-USM</td>
<td>All</td>
<td>774</td>
<td>4</td>
<td>19.6**</td>
<td>0.02</td>
<td>SW2443–S0141</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>344</td>
<td>3</td>
<td>25.6**</td>
<td>0.07</td>
<td>SW2443–S0141</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>430</td>
<td>150</td>
<td>6.3</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>BFT-HGP\textsuperscript{b}</td>
<td>All</td>
<td>774</td>
<td>40</td>
<td>13.1*</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>BFT-USM\textsuperscript{b}</td>
<td>All</td>
<td>774</td>
<td>8</td>
<td>7.6</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} BFT-HGP, backfat thickness by Hennessy Grading Probe; BFT-USM, backfat thickness by ultrasound measurement correction with the standard model std (weight + sex + facility), except \textsuperscript{b}corrected by standard model including the paternal allele of \textit{IGF2}.

\textsuperscript{c} Fraction of observed variation explained by the QTL.

\textsuperscript{d} Marker bracket where the F statistic exceeded genome-wise significance level.

\* \(P<0.05\), ** \(P<0.01\), *** \(P<0.001\) (based on tabulated F values after 10000 permutations).
commercial lines. Very thin third-layer fat might be difficult to detect by BFT-USM. Consequently, in some cases BFT-USM only represents the thickness of two layers of fat, while BFT-HGP measures the total amount of fat in all three layers.

Another difference between the BFT-HGP and the BFT-USM QTL analyses is the correction for body weight: carcass weight is used for BFT-HGP and live weight for BFT-USM. To eliminate the effect of the difference between carcass weight and live weight, the QTL were re-analysed using phenotypic data that were corrected using a model that did not include body weight. Although for both BFT-HGP and BFT-USM the simplified model (excluding weight) led to a reduced F statistic the F values exceeded the genome-wise significant threshold in all analyses. The patterns of the QTL did not change substantially by omitting the correction for weight, or by correcting BFT-HGP for live weight and BFT-USM for carcass weight (data not shown). This finding suggests that the difference in positioning of the peak is not caused by the difference in body weight measurement.

Finally, the shift in QTL position between BFT-HGP and BFT-USM might be explained by a ghost QTL at position 40 cM. This ghost QTL might be the result of the differences in F-values at the IGF2 locus between BFT-HGP and BFT-USM for the non-segregating families. In these families the F-value is absolutely zero for BFT-HGP but around 5 for BFT-USM (Fig. 1A, B; thin continuous lines).

Despite the observed differences, both BFT-HGP and BFT-USM appear to be equally efficient in picking up the major QTL effect in segregating families.

(v) Teat number QTL analyses and the IGF2 mutation

A total of 1173 F2 individuals in 38 half-sib families have previously been applied to perform a QTL scan for TN resulting in the identification of a paternally expressed QTL at the distal tip of SSC2p (Hirooka et al., 2001). A QTL with an effect on TN was observed both in the analyses of the 20 families of which the F1 sire was found to be homozygous for the IGF2 wild-type allele (G/G) and in the analyses of the 18 families of which the F1 sire was found to be heterozygous for the mutation (A/G) (Fig. 1C). This shows that the IGF2-intron3-G3072A substitution does not have a pleiotropic effect on TN.

In conclusion, this study demonstrates that the previously described IGF2-intron3-G3072A substitution (Van Laere et al., 2003) IGF2 mutation also causes the paternally expressed QTL for backfat thickness in the cross between Meishan and European Whites. Although differences between QTL for BFT-HGP and BFT-USM exist, both methods are caused by the same mutation and both methods are equally efficient at detecting the observed QTL, although in the BFT-HGP analysis the presence of a second minor QTL (at 40 cm) can not be excluded. The QTL for teat number identified on SSC2 in the Meishan × European White cross (Hirooka et al., 2001) was not affected by the previously described IGF2 mutation. Thus, another mutation in the IGF2 region may cause this effect on teat number.

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References


