Genet. Res., Camb. (2004), **84**, *pp.* 95–101. *With* 1 *figure.*
DOI: 10.1017/S0016672304007098 Printed in the United Kingdom

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 LAERE[2], MARINUS F. W. TE PAS[3], BERNARD A. VAN OOST[4], LEIF ANDERSSON[2,5] AND MARTIEN A. M. GROENEN[1]

[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*

(*Received 26 February 2004 and in revised form 10 August 2004*)

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 F_2 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 *IFG2* 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 F_2 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

* Corresponding author. Tel: +31 317 484622. Fax: +31 317483929. e-mail: bart.jungerius@wur.nl

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*., 1997*a*). Briefly, a cross between Meishan and Dutch Large White and Landrace lines was established for the detection of major genes (Janss *et al*., 1997*b*) and for QTL analyses (de Koning *et al*., 1999, 2000; Rattink *et al*., 2000). Body weight and BFT were recorded on 1130 F_2 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 of these F_2 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 F_2 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 F_2 animals, their F_1 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 F_1 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* F_2 *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 F_0 and F_1 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 F_2 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 F_1 sire was homozygous at the *IGF2* QTN (G/G); (3) the 18 families with F_1 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.

https://doi.org/10.1017/S0016672304007098 Published online by Cambridge University Press

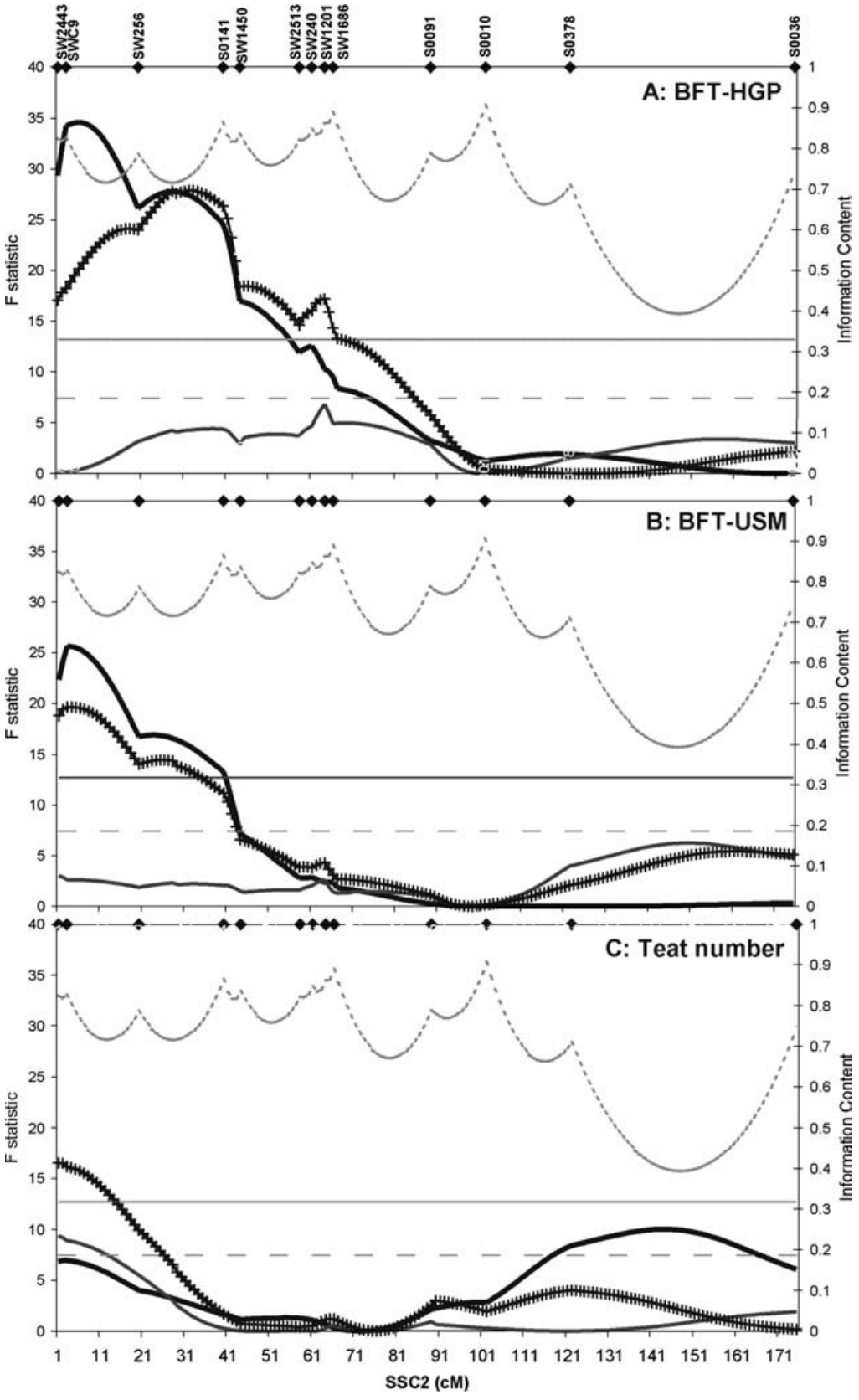

Fig. 1. QTL analyses on SSC2 for (*A*) BFT by Hennessy Grading Probe, (*B*) BFT measured by ultrasound measurement and (*C*) teat number. The thick continuous line represents the results from the segregating (A/G) families. The thin continuous line is from non-segregating (G/G) families. The '+'-marked 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.

https://doi.org/10.1017/S0016672304007098 Published online by Cambridge University Press

Table 1. *Distribution of genotypes at IGF2-intron3-nucleotide 3072 among F_1 sires in a Chinese Meishan × White intercross*

Line	Origin[a]	G/G	A/G
1	Great Yorkshire	3	5
2	Large White	1	6
3	Large White	1	7
4	Dutch Landrace	7	0
5	Dutch Landrace	8	0
Total		20	18

[a] The sires are grouped by the white line origin of their mother.

Table 2. *Mean backfat thickness of the F_2 individuals for both the Hennessy Grading Probe (BFT-HGP) and ultrasound measurements (BFT-USM) (raw data)*

Group[a]	Number	BFT-HGP (SE)	BFT-USM (SE)
A	344	20·8 (0·33)	14·7 (0·22)
G	430	22·4 (0·26)	15·5 (0·17)
All	774	22·0 (0·20)	15·4 (0·13)

[a] Based on the *IGF2* QTN allele inherited from the F_1 sire.

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 F_1 sires, all 18 of their Meishan sires and 31 of their White dams. All Meishan F_0 sires were found to be homozygous for the wild-type allele (G/G). In the White F_0 dams both the wild-type and the mutant allele were represented although they were not equally distributed over the five commercial lines. All F_0 dams of the two lines of Dutch Landrace origin were found to be homozygous for the wild-type allele (G/G). In the F_0 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 F_1 sires were not genotyped but both the parents were homozygous for the mutation and the genotypes of these F_1 sires were deduced. Overall, 20 F_1 boars were found to be G/G and 18 were scored as A/G (Table 1).

For the F_2 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 F_0 and F_1 individuals were combined with the genotypes of the F_0, F_1 and F_2 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 F_2 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 F_2 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 F_2 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 F_2 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 F_2 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 phenotyped 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 F_1 sires that are

https://doi.org/10.1017/S0016672304007098 Published online by Cambridge University Press

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

Measurement[a]	Population	*n*	Best position	F ratio	QTL effect[c]	Marker bracket[d]
BFT-HGP	All	774	32	27·9***	0·04	*SW2443–S0091*
	AG	344	6	34·6***	0·09	*SW2443–SW240*
	GG	430	64	6·8	0·02	
BFT-USM	All	774	4	19·6**	0·02	*SW2443–S0141*
	AG	344	3	25·6**	0·07	*SW2443–S0141*
	GG	430	150	6·3	0·01	
BFT-HGP[b]	All	774	40	13·1*	0·02	
BFT-USM[b]	All	774	8	7·6	0·01	

[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 [b]corrected by standard model including the paternal allele of *IGF2*.
[c] Fraction of observed variation explained by the QTL.
[d] Marker bracket where the F statistic exceeded genome-wise significance level.
* $P<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$ (based on tabulated F values after 10 000 permutations).

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_1 sire was found to be homozygous for the *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_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 *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 *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 *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 *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 *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

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 life 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 F_2 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 F_1 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 F_1 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.

We thank Henk Bovenhuis, Richard Crooijmans, Bart Ducro, Piet de Groot, Dirk-Jan de Koning, Jan van der Poel, Annemieke Rattink and Maria Siwek for their assistance, help and suggestions.

References

Bidanel, J. P. & Rothschild, M. (2002). Current status of quantitative trait locus mapping in pigs. *Pig News and Information* **23**, 39N–54N.

de Koning, D. J., Janss, L. L., Rattink, A. P., van Oers, P. A., de Vries, B. J., Groenen, M. A., van der Poel, J. J., de Groot, P. N., Brascamp, E. W. & van Arendonk, J. A. (1999). Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (*Sus scrofa*). *Genetics* **152**, 1679–1690.

de Koning, D. J., Rattink, A. P., Harlizius, B., van Arendonk, J. A., Brascamp, E. W. & Groenen, M. A. (2000). Genome-wide scan for body composition in pigs reveals important role of imprinting. *Proceedings of the National Academy of Sciences of the USA* **97**, 7947–7950.

Haley, C. S., Knott, S. A. & Elsen, J. M. (1994). Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* **136**, 1195–1207.

Hirooka, H., de Koning, D. J., Harlizius, B., van Arendonk, J. A. M., Rattink, A. P., Groenen, M. A. M., Brascamp, E. W. & Bovenhuis, H. (2001). A whole-genome scan for quantitative trait loci affecting teat number in pigs. *Journal of Animal Science* **79**, 2320–2326.

Janss, L. L., van Arendonk, J. A. & Brascamp, E. W. (1997*a*). Bayesian statistical analyses for presence of single genes affecting meat quality traits in a crossed pig population. *Genetics* **145**, 395–408.

Janss, L. L., van Arendonk, J. A. & Brascamp, E. W. (1997*b*). Segregation analyses for presence of major genes affecting growth, backfat, and litter size in Dutch Meishan crossbreds. *Journal of Animal Science* **75**, 2864–2876.

Jeon, J. T., Carlborg, O., Tornsten, A., Giuffra, E., Amarger, V., Chardon, P., Andersson-Eklund, L., Andersson, K., Hansson, I., Lundstrom, K. & Andersson, L. (1999). A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. *Nature Genetics* **21**, 157–158.

Knott, S. A., Marklund, L., Haley, C. S., Andersson, K., Davies, W., Ellegren, H., Fredholm, M., Hansson, I., Hoyheim, B., Lundstrom, K., Moller, M. & Andersson, L. (1998). Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs. *Genetics* **149**, 1069–1080.

Lander, E. & Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics* **11**, 241–247.

Nezer, C., Moreau, L., Brouwers, B., Coppieters, W., Detilleux, J., Hanset, R., Karim, L., Kvasz, A., Leroy, P. & Georges, M. (1999). An imprinted QTL with major

effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. *Nature Genetics* **21**, 155–156.

Rattink, A. P., De Koning, D. J., Faivre, M., Harlizius, B., van Arendonk, J. A. & Groenen, M. A. (2000). Fine mapping and imprinting analysis for fatness trait QTLs in pigs. *Mammalian Genome* **11**, 656–661.

Sobel, E. & Lange, K. (1996). Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *American Journal of Human Genetics* **58**, 1323–1337.

Van Laere, A. S., Nguyen, M., Braunschweig, M., Nezer, C., Collette, C., Moreau, L., Archibald, A. L., Haley, C. S., Buys, N., Tally, M., Andersson, G., Georges, M. & Andersson, L. (2003). A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature* **425**, 832–836.