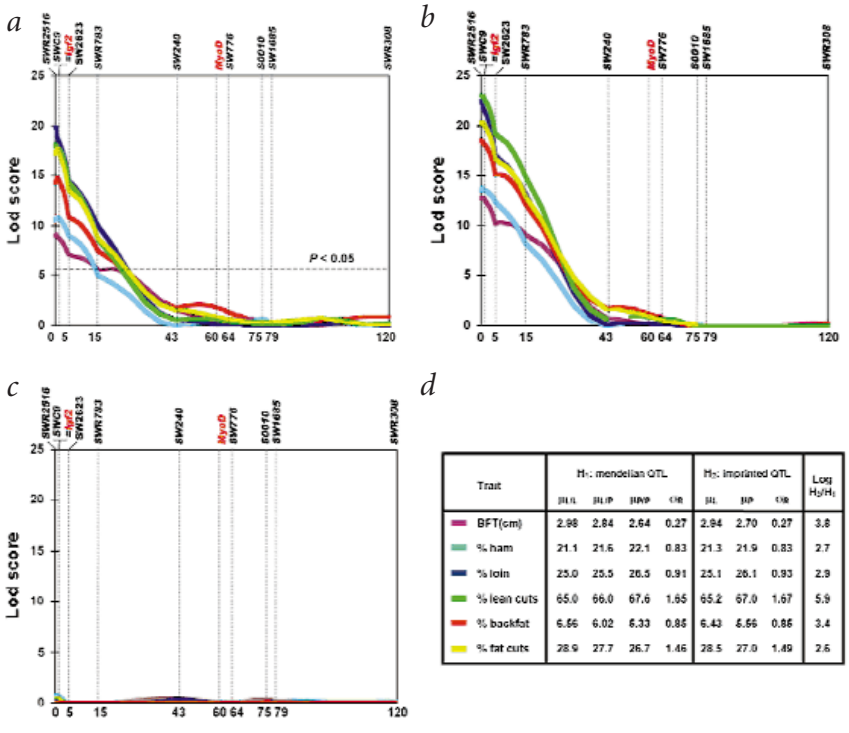
An imprinted QTL with major effect on muscle mass and fat deposition maps to the *IGF2* locus in pigs

We have generated an intercross between Large White and Piétrain pig strains yielding 1,032 F_2 offspring as described[1]. The Large White and Piétrain parental breeds differ in a number of economically important phenotypes. Piétrains are known for exceptional muscularity and leanness, whereas Large Whites show superior growth performance. We recorded 21 distinct phenotypes[1] measuring growth performance (5), body proportions (1), muscularity (5), fat deposition (6) and meat quality (4) on all F_2 offspring.

To map QTLs underlying the genetic differences between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F_2 individuals. Analysis of pig chromosome 2 using an ML multipoint algorithm revealed highly significant lod scores for three of five phenotypes measuring muscularity (percentage lean cuts, percentage ham, percentage loin) and three of six phenotypes measuring fat deposition (backfat thickness (BFT), percentage backfat, percentage fat cuts) at the distal end of the short arm of chromosome 2. Corresponding lod score curves and ML estimates for the three genotypic means as well as the residual variance are shown (Fig. 1). We obtained positive lod scores in the corresponding chromosome region for the remaining muscularity (percentage shoulder, conformation score) and obesity (percentage belly, percentage leaf fat, percentage jowl) phenotypes; however, they did not reach the experiment-wise significance threshold (α=5%). There was no evidence for an effect of the corresponding QTL on growth performance (including birth weight) or recorded meat quality measurements (muscle pH at 1 and 24 hours after slaughter). By subsequently analysing the remaining sample of 355 F_2 offspring, we confirmed the presence of a major QTL in this region (data not shown).

Bidirectional chromosome painting established a correspondence between SSC2p and HSA11pter–q13 (refs 2,3). Two candidate genes map to this region in humans: *MYOD1* maps to HSA11p15.4 and *IGF2* maps to HSA11p15.5. A previously described amplified sequence polymorphism in the porcine *MYOD1* gene[4] segregated in our F_2 material, which we genotyped completely for this marker. We positioned *MYOD1* in the *SW240–SW776* interval by linkage analysis (odds>1,000), well outside the lod-2 drop-off support interval for the QTL (Fig. 1*a*). On the basis of a published porcine adult liver cDNA sequence[5], we designed primer pairs to amplify the entire *IGF2* coding sequence (containing 222 bp of 5′ leader and 280 bp of 3′ trailer sequences) from adult skeletal muscle cDNA. *IGF2* coding sequences were identical in both breeds, as well as to the published sequence. We did, however, find a G→A transition in the leader sequence, corresponding to exon 2 in human *IGF2* (Fig. 2). This single nucleotide polymorphism (SNP) was screened in an oligonucleotide ligation assay[6] (OLA), allowing us to genotype our pedigree material. These data showed that *IGF2* colocalizes with the *SWC9* microsatellite marker (θ=0%), coinciding with the most likely position of the QTL and well within the 95% support interval (Fig. 1). Subsequent sequence analysis demonstrated that the microsatellite marker *SWC9* is actually located in the 3′ UTR of *IGF2* (L. Andersson, pers. comm.). FISH analysis performed with a BAC clone containing porcine *IGF2* demonstrated the terminal location of this gene on SSC2p (ref. 7), making a more distal location of the identified QTL unlikely.

IGF2 therefore appeared to be a positional candidate for the observed QTL effect. In man and mouse, *IGF2* is imprinted and expressed exclusively from the paternal allele in several tissues[8]. We analysed skeletal muscle and liver cDNA from 10-week-old porcine fetuses heterozygous for the G→A transition and *SWC9* and showed that *IGF2* is imprinted in these tissues in the pig as well (Fig. 2). If *IGF2* is responsible for the observed effect and only the paternal *IGF2* allele is expressed, one can predict that: (i) the paternal allele transmitted by F1 boars (P or LW) will affect the phenotype of F2 offspring; (ii) the maternal allele transmitted by F1 sows (P or LW) has no effect on the phenotype of F2 offspring; and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared with a conventional



Trait	H1: mendelian QTL				H3: imprinted QTL			Log H3/H1
	μ1-μ2	μ1/P	μ/P	CR	μ1	μP	CR	
BFT(cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27	3.8
% ham	21.1	21.6	22.1	0.83	21.3	21.9	0.83	2.7
% loin	25.0	25.5	26.5	0.91	25.1	26.1	0.93	2.9
% lean cuts	65.0	66.0	67.6	1.65	65.2	67.0	1.67	5.9
% backfat	6.56	6.02	6.33	0.85	6.43	6.56	0.86	3.4
% fat cuts	28.9	27.7	26.7	1.46	28.5	27.0	1.49	2.6

Fig. 1 Lod score curves obtained in a Piétrain×Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions of the *IGF2* and *MYOD1* genes determined by linkage analysis, with respect to the microsatellite marker map, are shown. H_0 was defined as the null-hypothesis of no QTL, H_1 as testing for the presence of a mendelian QTL, H_2 as testing for the presence of a paternally expressed QTL and H_3 as testing for the presence of a maternally expressed QTL. ***a***, $\log_{10}(H_1/H_0)$; ***b***, $\log_{10}(H_2/H_0)$; ***c***, $\log_{10}(H_3/H_0)$. ***d***, Maximum likelihood phenotypic means for the different F2 genotypes estimated under: (i) a model of a mendelian QTL; and (ii) a model assuming an imprinted QTL and corresponding $\log_{10}(H_2/H_1)$ measured at the position of the *IGF2* locus.

mendelian model of a trimodal (1:2:1) F2 population. We adapted our QTL mapping programs (C.N. *et al.*; unpublished data) to test the corresponding hypotheses. H_0 was defined as the null hypothesis of no QTL, H_1 as testing for the presence of a mendelian QTL, H_2 as testing for the presence of a paternally expressed QTL and H_3 as testing for the presence of a maternally expressed QTL. Lod score curves corresponding with $\log_{10}(H_2/H_0)$ and $\log_{10}(H_3/H_0)$ are shown (Fig. 1*b*,*c*, respectively). We obtained significant lod scores when testing for the presence of a paternally expressed QTL, whereas there was no evidence for maternal transmission. The $\log_{10}(H_2/H_1)$ measured at the *IGF2* position (Fig. 1*d*) shows that the hypothesis of a paternally expressed QTL is significantly more likely than the hypothesis of a mendelian QTL for all examined traits ($2.6<\log_{10}(H_2/H_1)<5.9$).

These data confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The identified chromosomal segment coincides with an imprinted domain documented in humans and mice, implicating the orthologous region in pigs. At least ten imprinted genes mapping to this domain have been documented (http://www.mgc.har.mrc.ac.uk/imprint-bin/imp-maps.pl), but only *IGF2* and *INS2* are paternally expressed. Although we cannot exclude that the observed QTL effect is due to an unidentified imprinted gene in this region, the effects of *IGF2* on myogenesis *in vitro* and *in vivo*[9] implicate this gene. Allelic variants of the *INS* VNTR have recently been associated with size at birth in man[10], and the same VNTR also affects levels of *IGF2* expression[11].

The observation of the same QTL effect in a Large White×Wild Boar intercross[7,12] suggests the existence of a series of three distinct functional alleles. Moreover, preliminary evidence based on marker-assisted segregation analysis points towards residual segregation at this locus in the Piétrain population (data not shown). Contrary to the findings of Jeon *et al.* in the accompanying paper[7], we found no evidence for a distorted segregation of SSC2p in our pedigree material.

The effects of the identified QTL on muscle mass and fat deposition are major and of the same magnitude as those reported for the *CRC* locus[1,13], although apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain 50% of the Piétrain versus Large White breed difference for muscularity and leanness. Variance analysis revealed no evidence for interaction between the newly identified QTL and the CRC locus (data not shown). The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits non-mendelian inheritance; we have previously shown that the *callipyge* locus is characterized by polar overdominance in which only heterozygous individuals inheriting the CLPG mutation from their sire express the double-muscling phenotype[14]. Understanding these parent-of-origin effects will allow for their optimal exploitation in breeding programs using marker-assisted selection.

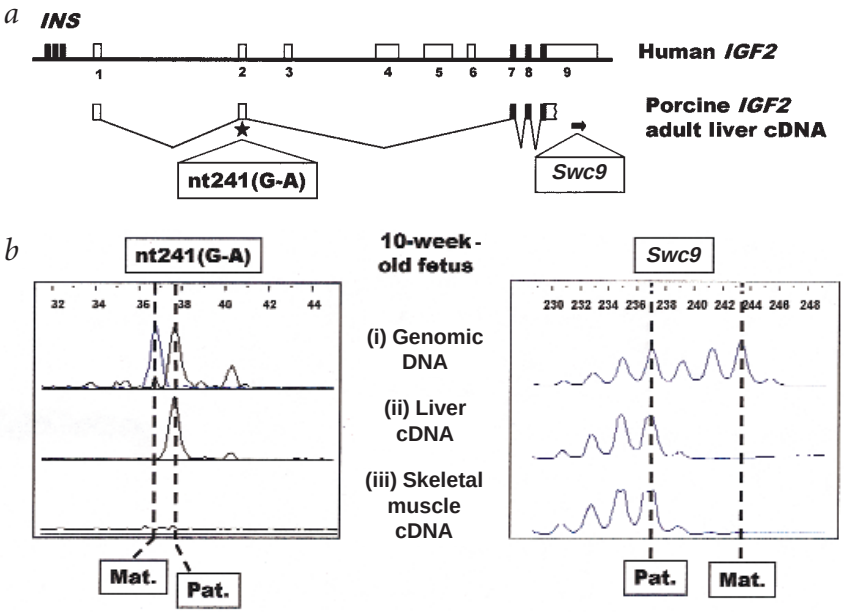


Fig. 2 Structure and expression of porcine *IGF2*. ***a***, Structure of human *IGF2* (ref. 6) with aligned porcine adult liver cDNA sequence[5]. The position of the *nt241(G-A)* transition and *Swc9* microsatellite are shown. ***b***, The corresponding markers were used to demonstrate the monoallelic (paternal) expression of *IGF2* in skeletal muscle and liver of 10-week-old fetuses. PCR amplification of the *nt421(G-A)* polymorphism and *Swc9* microsatellite from genomic DNA shows the heterozygosity of the fetus, whereas only the paternal allele is detected in liver cDNA (*nt421(G-A)* and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421(G-A)* in fetal muscle indicates the absence of mRNA including exon 2 in this tissue. Parental origin of the fetal alleles was determined from the genotypes of sire and dam (data not shown).

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