A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the *IGF2* locus

We generated an intercross between the European Wild Boar and Large White domestic pigs and used this pedigree to map quantitative trait loci[1–4] (QTL). We previously reported a QTL on pig chromosome 2p with a moderate effect on muscle mass[4]. The conserved synteny between this region and human chromosome 11p suggested *IGF2* as a candidate for the QTL. We isolated a porcine BAC *IGF2* clone (253G10) and used it for FISH mapping, resulting in a consistent signal on the distal tip of chromosome 2p (band 2p1.7). A microsatellite is located in the *IGF2* 3′ UTR in mice (GenBank U71085), humans (GenBank S62623) and horse (GenBank AF020598). Direct BAC sequencing of the *IGF2* 3′ UTR revealed a microsatellite 800 bp downstream of the *IGF2* stop codon identical to a previously described microsatellite, *Swc9* (ref. 5). The *IGF2* microsatellite was found to be highly polymorphic, with three alleles among the two Wild Boar founders and another two among the eight Large White founders, and was fully informative in the intercross, as the breed and parent of origin could be determined for each allele in F_2 animals. Linkage analysis showed that *IGF2* maps 25 cM distal to *Sw256*, the end marker in our previous study[4] (Fig. 1). The *Swr2516*/*Sw2443* markers represent the end of the known linkage map; FISH mapping of *IGF2* indicated that they are close to the telomere.

We performed QTL analyses with the revised chromosome 2 map using a statistical model[2] testing for the presence of an imprinting effect as expected for *IGF2*. There was evidence for a paternally expressed QTL located at the distal tip of 2p (Fig. 1, Table 1). The QTL had large effects on lean meat content in ham and explained 30% of the residual phenotypic variance in the F_2 population. Large effects on the area of the longissimus dorsi muscle, heart weight and backfat thickness (subcutaneous fat) were also noted, as well as a moderate effect on one meat quality trait, reflectance value. The QTL had no significant effect on abdominal fat, birth weight, or growth, or weight of liver, kidney or spleen. The Large White allele was associated with larger muscle mass and reduced backfat thickness, consistent with the difference between this breed and the Wild Boar population. The imprinting effect observed for all affected traits suggested a single causative locus. Clear paternal expression was illustrated by the least-squares means, which fell into two classes following the population origin of the paternally inherited allele (Table 1). A highly significant segregation distortion (excess of Wild Boar-derived alleles) was found in the *IGF2* region (Table 1; χ^2=11.7, d.f.=2; P=0.003). The deviation was observed with all three distal markers and thus was not due to typing errors. The segregation distortion did not show an imprinting effect, as frequencies of the two reciprocal types of heterozygotes were identical (Table 1).

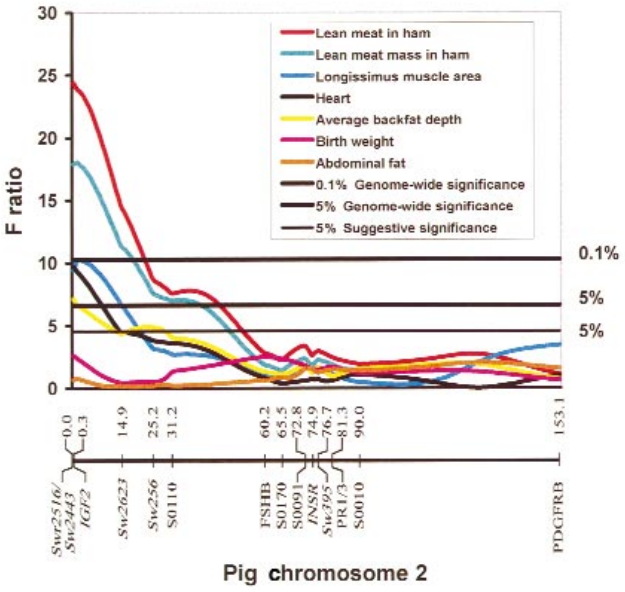

Fig. 1 Test statistic curves obtained in QTL analyses of chromosome 2 in a Wild Boar/Large White intercross. The graph plots the F ratio in a least-squares analysis[2], testing the hypothesis of a single QTL at a given position along the chromosome for the traits indicated. The marker map with the distances between markers in Kosambi centiMorgan is given on the X-axis. The horizontal lines represent genome-wide significant (P<0.001; P<0.05) and suggestive levels for the trait lean meat in ham; similar significance thresholds were obtained for the other traits.

The role of *IGF2* in prenatal development is well documented[6,7]. We now demonstrate a role for the *IGF2* region in postnatal development as well. Our approach is particularly powerful for the detection of QTLs with parent-of-origin–specific effects, as multiple alleles (or haplotypes) are segregating and we were able to deduce whether a heterozygous F_2 animal received the Wild Boar allele from the F_1 male or female. The segregation of an imprinted QTL may be overlooked in human studies or in intercrosses between inbred rodent populations due to experimental design or statistical treatment of data. Our results have bearings on future analyses of association between genetic polymorphism in the *insulin-IGF2* region, multifactorial traits and diseases in humans[8–9], inbred rodent models and meat-producing farm animals.

One impetus for generating an intercross between the domestic pig and its wild ancestor was to map and identify major loci that have responded to selection. The *IGF2*-linked QTL and the *FAT1* QTL on chromosome 4 (refs 1,2) are the loci with the greatest effect on body composition and fatness segregating in our Wild Boar intercross. The *IGF2* QTL controls primarily muscle mass, whereas *FAT1* has major effects on fat deposition, including abdominal fat, a trait not affected by the *IGF2* QTL (Fig. 1). A model including both QTLs explains as much as 33.1% of the variance for percentage of lean meat in ham, 31.3% for the percentage of lean meat plus bone in back and 26.2% for average back-fat depth (Table 1). This is a gross deviation from the underlying assumption in the classical infinitesimal model in quantitative genetics theory that quantitative traits are controlled by an infinite number of loci, each with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations is controlled by a few loci, selection should quickly fix QTL alleles with large effects, leading to a selection plateau. Nevertheless, this is not typically seen in animal breeding programs or selection experiments in which substantive, persistent, long-term selection responses are generally obtained, provided that the effective population size is reasonably large[10]. A possible explanation is that QTL alleles controlling a large proportion of genetic differences between two populations may be due to several consecutive mutations in the same gene or in several closely linked genes. There are indications

Table 1 • QTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross[a]

Trait	F ratio[b]		Map position[c]	Percent of F_2 variance[d]	Least-squares means[e]			
	QTL	Imprinting			W^P/W^M n=62	W^P/L^M n=43	L^P/W^M n=43	L^P/L^M n=30
Body composition traits:								
Lean meat in ham, %	24.4***	19.1***	0	30.6	63.6[f]	64.2[f]	66.4[g]	67.3[g]
Lean meat mass in ham, kg	18.1***	16.8***	1	24.3	4.69[f]	4.72[f]	4.94[g]	5.02[g]
Lean meat + bone in back, %	12.2**	9.6**	0	17.4	66.3[f]	66.7[f]	69.3[g]	70.8[g]
Longissimus muscle area, cm^2	10.3**	4.8*	1	15.4	31.9[f]	33.0[f]	34.5[g]	35.2[g]
Fatness traits:								
Average backfat depth, mm	7.1*	8.7**	0	10.4	27.2[f]	27.7[f]	25.5[g]	24.7[g]
Weight of internal organs:								
Heart, gram	9.7**	11.4***	0	14.4	226[f]	225[f]	238[g]	244[g]
Meat quality traits:								
Reflectance value, EEL	5.7	6.1*	1	8.1	18.6[f]	18.4[f]	21.8[g]	19.7[f]

*P<0.05. **P<0.01. ***P<0.001. [a]Only the traits for which the QTL peak was in the *IGF2* region (0–10 cM) and the test statistic reached the nominal significance threshold of F=3.9 are included. [b]QTL is the test statistic for the presence of a QTL under a genetic model with additive, dominance and imprinting effects (3 d.f.), whereas 'Imprinting' is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. We used genome-wide significance thresholds (estimated by permutation[2]) for the QTL test and nominal significance thresholds for the imprinting test. [c]In cM from the distal end of 2p; *IGF2* is located at 0.3 cM. [d]The reduction in the residual variance of the F_2 population effected by inclusion of an imprinted QTL at the given position. [e]Means and standard errors estimated at *IGF2* by classifying the genotypes according to the population and parent of origin of each allele. *W* and *L* represent alleles derived from the Wild Boar and Large White founders, respectively; superscript 'P' and 'M' represent paternal and maternal origin, respectively. [f,g]Significantly different at least at the 5% level; most vary at the 1% or 0.1% level.

that new mutations may also contribute substantially to long-term selection responses[11]. We propose that the presence of multiple alleles and multiple consecutive mutations may be common at major trait loci under selection for many generations in domestic animals, crops or natural populations. Our recent characterization of the *KIT* (ref. 12) and *MC1R* (ref. 13) loci, which control coat-colour variation in pigs, supports this hypothesis. In this context, the identification of a third allele at the *IGF2*-linked QTL (ref. 14) is notable. Our results also have important practical implications for the pig breeding industry. The practice of breeding individual males with many females may in fact favour the selection of alleles at paternally expressed QTLs.

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Jin-Tae Jeon[1], Örjan Carlborg[1], Anna Törnsten[1], Elisabetta Giuffra[1], Valerie Amarger[1], Patrick Chardon[2], Lena Andersson-Eklund[1], Kjell Andersson[1], Ingemar Hansson[3], Kerstin Lundström[3] & Leif Andersson[1]

[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala Biomedical Center, Box 597, S-751 24 Uppsala, Sweden. [2]Institut National de la Recherche Agronomique, LMRA-INRA-CEA, 78352 Jouy-en-Josas, France. [3]Department of Food Science, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden. Correspondence should be addressed to L.A. (e-mail: leif.andersson@bmc.uu.se).

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QTL influencing autoimmune diabetes and encephalomyelitis map to a 0.15-cM region containing *Il2*

Insulin-dependent diabetes in NOD mice and mouse experimental autoimmune encephalomyelitis (EAE) are the major disease models for human type I diabetes and multiple sclerosis, respectively. In recent genetic analyses, a number of quantitative trait loci (QTL) controlling susceptibility to these autoimmune diseases have been identified[1–4]. Some QTL overlap between the two diseases, raising the possibility that there may be disease genes common to diabetes and EAE (ref. 5). Given the large number of QTL identified and the large QTL support interval sizes attained in the genetic segregation analyses used, we investigated whether this overlap was due to a shared 'autoimmunity gene' or merely to the coincidental grouping of two unrelated genes. Among the diabetes QTL mapped so far in NOD mice, two overlap with QTL that we identified in EAE (ref. 1). One of these QTL, in the medial region of chromosome 3, has been further analysed through the generation of congenic mice and was found to be composed of QTL *Idd3*, *Idd17*, *Idd10* and *Idd18* (refs 6,7). We were able to more precisely examine the effect of the diabetes QTL on EAE using these NOD congenic lines, which carry diabetes-resistance alleles at one or more of these QTL (refs 6,7).

NOD mice are susceptible to both insulin-dependent diabetes, which develops spontaneously, and EAE, which can be induced by immunization with myelin antigens[8]. NOD mice and NOD lines congenic with B6.PL-*Thy1*^{a}/*Cy* (abbreviated B6) for regions of chromosome 3 were tested for their susceptibility to EAE by immunizing with a peptide from myelin oligodendrocyte glycoprotein (MOG 35–55). Congenic lines carrying B6-derived regions encompassing *Idd3* (NOD.B6-