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Genetic variation at the growth hormone locus in a wild pig intercross; test of association to phenotypic traits and linkage to the blood group D locus

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Abstract A polymorphism in the TATA-box of the porcine growth hormone (*GH*) gene was analysed in a wild pig/Large White intercross, in which 129 markers had been scored previously. Linkage analyses demonstrated that the *GH* locus belonged to a linkage group on chromosome 12 together with a previously unassigned marker, the erythrocyte antigen D (*EAD*) locus. The linear order of this linkage group is *EAD-GH-S0096-S0090-S0106*-arachidonate12-lipoxygenase (*ALOX12*)-inhibin beta A (*INHBA*). The length of the linkage group was estimated at 93 cM (sex average). The effects of the *GH* genotype on growth and fat deposition traits were investigated using phenotypic data from the 191 F_2 animals. No significant effect of *GH* was detected, and we therefore conclude that this locus does not play a major role in defining the genetic differences between the wild and Large White pigs for these traits.

Key words Pig · Growth hormone gene · Growth QTL · EAD

Introduction

Several investigations have detected changes in the plasma level of growth hormone (GH) as a correlated response to selection for growth and for changes in carcass properties in the pig (Lund-Larsen and Bakke 1975; Althen and Gerrits 1976; Arbona et al. 1988). Injections of GH into growing pigs increase growth rate and the meat percent while they decrease fat accretion (e.g. Evock et al. 1988; Agergaard et al. 1991), and similar responses are seen in GH transgenic mice. Furthermore, associations of *GH* polymorphisms with quantitative traits have been detected in cattle (Høj et al. 1993) and mice (Winkelman and Hodgetts 1992). *GH* is thus a major candidate gene for controlling growth in mammals. For instance, several experiments have been carried out with the purpose of manipulating the expression of GH in transgenic pigs and to investigate the effect on growth and fatness traits (Pursel et al. 1989).

In the present study, the segregation of a polymorphism in the promoter region of the *GH* locus (Kirkpatrick et al. 1993) was analysed in an intercross between the wild pig and Large White domestic pigs. The purpose was to (1) provide a precise location of *GH* on the porcine linkage map and (2) to test for possible associations between the segregation at *GH* and variation in growth and fatness traits among 200 F_2 animals. Several quantitative traits have been measured in the pedigree and the detection of quantitative trait loci (QTLs) with large effects on growth and fatness was recently reported (Andersson et al. 1994).

Materials and methods

Animals

The parental generation of the pedigree consisted of two wild pig males and eight females of a Large White breed (Swedish Yorkshire). The F_1 generation comprised four sires and 22 dams which were intercrossed to produce 200 F_2-offspring. The traits measured in these animals were birth weight, growth rate in the intervals from birth to 30 kg, from 30 to 70 kg, and from birth to 70 kg, length of the small intestine at slaughter, abdominal fat as a percent of the total carcass weight, and back-fat thickness as the average of five measurements along the dorsal midline at the shoulder, last rib and loin. Birth weight was measured in 193 animals and data on the other traits were from 191 animals.

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Markers

Infromation on the 129 genetic markers previously typed in this pedigree are all given in Ellegren et al. (1994) except for S0090 which is described in Ellegren et al. (1993). The D blood group system is a two-allelic closed system (Saison et al. 1967), and the D blood group factors were detected by a direct agglutination test in microtiter plates.

The PCR for the double strand conformation polymorphism (DSCP)-test of variation in the *GH* TATA element included primers 1025(−118gaagcTTATCCATTAGCACATGCCTGCCAG-94)and +103L(+125CCCACGTCACCCTTCAGAACA+105). The PCR was carried out with 100μM of dNTP, 0.1 μM of each primer, 10–100 ng of genomic DNA, 2.5 μl of 10 × PCR buffer and 0.5 units of *Taq* polymerase in a final volume of 25 μl, overlaid by 30 μl of oil; the 10 × PCR buffer was 500 mM KCl, 100 mM Tris-Cl (pH 8.3 at room temperature), 15 mM $MgCl_2$, 0.1% Gelatin and 1% Triton X100. The PCR profile consisted of 30 cycles of: 95 °C for 45 s; 59 °C for 45 s; 76 °C for 1 min. Electrophoresis and allele designations were as previously described (Kirkpatrick et al. 1993).

Linkage analysis

The linkage analysis was performed using CRIMAP, version 2.4 (Green et al. 1990). A lod score (Z) above 3 was used as a criterion for significant linkage. After the construction of linear maps using the option BUILD, the CHROMPIC option was employed to identify putative double recombinants. The genotype data for these animals were re-checked to exclude the possibility that a double recombinant reflected typing errors. Recombination fractions were converted to centiMorgan (cM) by means of the Kosambi mapping function.

Statistical analyses

The effect of *GH* genotypes on growth and fatness traits was evaluated with least square analyses using the GLM procedure of SAS (SAS Institute Inc. 1988). The effect of sex, family, and parity of the animal was used in the statistical model for all traits. The effect of a feeding treatment was included for all traits except growth to 30 kg. The following co-variates were also included: exact weight at start of period for growth rate from rate from 30 to 70 kg, exact weight at end of period for growth rate to 30 kg and 70 kg, and weight at slaughter for both fatness traits.

Results and discussion

Polymorphism in the *GH* promoter region

A number of polymorphisms have been described at the porcine *GH* locus (Kirkpatrick and Huff 1991; Nielsen and Larsen 1991; Kirkpatrick 1992; Kirkpatrick et al. 1993; Larsen and Nielsen 1993). Several of these were tested in the P-generation of the family material investigated here, and the DSCP test for a sequence variation in the TATA-box was chosen for further analysis since it was the most informative one. For this polymorphism, the two wild pig males were both homozygous *1/1* while the Large White founder females were either homozygous *2/2* or heterozygous *1/2*. The observed heterozygosity in the F_1 generation was 69%. The F_1 matings, as regards the *GH* TATA polymorphism, were either *1/2* × *1/2* or *1/2* × *1/1*. There was no significant deviation from the expected Mendelian segregation.

Linkage analyses

The *GH* locus was tested for linkage against the 129 genetic markers previously scored in this pedigree (Ellegren et al. 1993, 1994). As expected on the basis of the previous in situ assignments (Thomsen et al. 1990; Yerle et al. 1993; Chowdhary et al. 1994), *GH* could be firmly assigned to the chromosome-12 linkage group, previously comprising *S0083*, *S0090*, *S0096*, *S0106*, *ALOX12*, and *INHBA* (Ellegren et al. 1993, 1994). *GH* showed significant lod scores against *S0083* ($Z = 16.2$; $\theta = 0.09$) and *S0096* ($Z = 25.0$; $\theta = 0.16$). In addition, *GH* showed significant linkage to one of the previously unassigned markers, *EAD* ($Z = 3.1$; $\theta = 0.24$).

A linear map for chromosome 12 was constructed using the BUILD option of CRIMAP. The building of the linear map was initiated by setting the order *GH-S0096-S0106* fixed on the basis of previous in situ hybridization data (see Fig. 1). The location of all remaining loci, except *S0083*, could be set with confidence ($Z > 3$ in favour of the given order; Fig. 1). The information on the new flanking markers (*GH* and *EAD*) revealed that there were apparent typing errors for *S0083* which could not be resolved. However, the order *GH-S0083-S0090* was established in the USDA (Rohrer et al. 1994) and the PiGMaP-consortium (Archibald et al. 1995) linkage maps.

After the construction of a linear map for chromosome 12, this information was used to further evaluate the statistical support for the assignment of *EAD* to this chromosome. This multipoint analysis resulted in a lod score of 4.8 in favour of *EAD* being located distal to *GH* when compared with *EAD* being unlinked to chromosome-12 markers. The blood group locus D (*EAD*) was one of the few markers (7 out of 128) which could not be assigned to a linkage group in our recent report of a primary linkage map based on the Swedish pedigree (Ellegren et al. 1994). This finding excluded *EAD* from large parts of the porcine genome and suggested that it is located at one of the ends of one of the previously established linkage groups or at one of the two chromosomes (17 and 18) lacking linkage groups at that time. The mapping of *EAD* to the distal part of chromosome 12p in this study thus provides a plausible location. Consequently, the two-point lod score of 3.1 and the

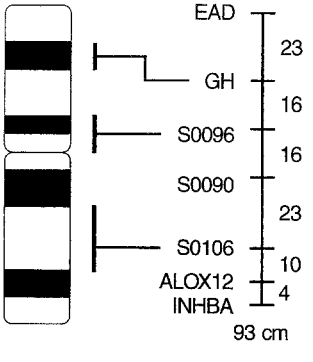

Fig. 1 A linkage map of pig chromosome 12 along with a drawing of the G-banded chromosome. Map distances are given as Kosambi cM. The *vertical bars* indicate the position of three markers that have been physically assigned by in situ hybridization

Table 1 Least-square means (± SE) for growth and fatness traits according to *GH* TATA genotypes in a cross between the European wild pig and the domestic Large White pig. The F-statistic and *P*-values for tests of differences among genotypes are also given

Trait	*GH* TATA genotype			F	*P*
	1/1 ($n=73$)	1/2 ($n=98$)	2/2 ($n=26$)		
Birth weight (kg)	1.29 ± 0.04	1.34 ± 0.04	1.37 ± 0.05	1.86	0.16
Growth from birth to 30 kg (g/day)	233.8 ± 4.3	233.6 ± 3.8	235.1 ± 4.3	0.02	0.98
Growth from birth to 70 kg (g/day)	361.2 ± 4.7	366.9 ± 4.1	367.6 ± 8.2	0.49	0.61
Growth from 30 kg to 70 kg (g/day)	605.2 ± 11.5	635.0 ± 10.1	631.2 ± 20.3	2.08	0.13
Length of small intestine (m)	17.5 ± 0.21	17.4 ± 0.18	18.0 ± 0.36	1.38	0.25
Abdominal fat (%)	2.32 ± 0.07	2.27 ± 0.06	2.43 ± 0.12	0.66	0.52
Average back-fat (mm)	26.6 ± 0.49	25.9 ± 0.43	26.6 ± 0.85	0.83	0.44

multipoint lod score of 4.8 are sufficient statistical evidence for an assignment of *EAD* to the chromosome-12 linkage group. This study thus provides the first assignment of *EAD* to the pig linkage map.

Association to quantitative traits

The design of the wild pig/Large White intercross and the informative polymorphism at the *GH* locus typed in this study allow us to address the important question of whether a significant proportion of the response to the selection for lean growth in the domesticated pig can be attributed to the *GH* locus. No statistically significant association between the *GH* TATA polymorphism and the variation in quantitative traits among F_2 animals was revealed (Table 1). There was only a weak tendency for the *TATA2* allele, which originated exclusively from the Large White founder females, to be associated with higher growth rate and longer small intestine, in line with differences between the domesticated and wild pig (Andersson et al. 1994).

Thus, we conclude that no major effect of the *GH* locus on growth and fatness traits could be detected. However, we cannot exclude the possibility that *GH* has an effect which is too small to reach statistical significance in an experiment of this rather restricted size (200 F_2 animals). It is clear that the effect, if any, of the *GH* locus on growth and fatness traits is small compared with the QTLs for these traits recently mapped to porcine chromosome 4 using the same pedigree (Andersson et al. 1994). The results of the present study are consistent with the previous QTL analysis where we did not find any significant effects on growth and fatness traits on chromosome 12. However, that study was not fully informative for the *GH* region as the closest marker (*S0083*) was about 10 cM away from *GH*.

It is well documented that the plasma levels of GH in pigs have changed as a correlated response to selection for growth and leanness (Lund-Larsen and Bakke 1975; Althen and Gerrits 1976; Arbona et al. 1988). If this reflects the mechanism by which selection acted on the animal, then *GH* and other loci with products influencing the release of GH would be candidate QTLs. Here, we investigated the *GH* locus directly, but no significant effects were detected. As no effect of the regions around the *IGF1* and *GHR* loci were detected in Andersson et al. (1994), other genes influencing the plasma GH levels, e.g., somatostatin and growth hormone releasing factor, are logical next candidates.

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References

Agergaard N, Oksbjerg N, Sörensen MT (1991) Influence of exogenous growth hormone on homeostasis in finishing pigs. In: Proc 6th Int Symp Protein Met Nutr Herning 9–14 June 1991, Research Centre Foulum, National Institute of Animal Science, Denmark, pp 194–197

Althen TG, Gerrits RJ (1976) Pituitary and serum growth hormone levels in Duroc and Yorkshire swine genetically selected for high and low backfat. J Anim Sci 42:1490–1497

Andersson L, Haley CS, Ellegren H, Knott S, Johansson M, Andersson K, Andersson-Eklund L, Edfors-Lilja I, Fredholm M, Hansson I, Håkansson J, Lundström K (1994) Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science 263:1771–1774

Arbona JR, Marple DN, Russell RW, Rahe CH, Mulvaney DR, Sartin JL (1988) Secretory patterns and metabolic clearance rates of porcine growth hormone in swine selected for growth. J Anim Sci 66:3068–3072

Archibald AL et al. (1995) The PiGMAP consortium linkage map of the pig (*Sus scrofa*). Mamm Genome 6:157–175

Chowdhary BP, Thomsen PD, Harbitz I, Landset M, Gustavsson I (1994) Precise localization of the genes for glucose phosphate isomerase (GPI), calcium release channel (CRC), hormone sensitive lipase (LIPE) and growth hormone in pigs using non-radioactive in situ hybridization. Cytogenet Cell Genet 67:211–214

Ellegren H, Johansson M, Chowdhary BP, Marklund S, Marklund L, Bräuner-Nielsen P, Edfors-Lilja I, Gustavsson I, Juneja RK, Andersson L (1993) Assignment of 20 microsatellite markers to the porcine linkage map. Genomics 16:431–439

Ellegren H, Chowdhary BP, Johansson M, Marklund L, Fredholm M, Gustavsson I, Andersson L (1994). A primary linkage map of the porcine genome reveals a low rate of genetic recombination. Genetics 137:1089–1100

Evock CM, Etherton TD, Chung CS, Ivy RE (1988) Pituitary porcine growth hormone (pGH) and a recombinant pGH analog stimu-

late pig growth performance in a similar manner. J Anim Sci 66:1928–1941

Green P, Falls K, Crooks S (1990) Documentation for CRIMAP, version 2.4. Washington University School of Medicine, St. Louise

Höj S, Fredholm M, Larsen NJ, Nielsen VH (1993) Growth hormone gene polymorphism associated with selection for milk fat production in lines of cattle. Anim Genet 24:91–95

Kirkpatrick BW (1992) *Hae*III and *Msp*I polymorphisms are detected in the 2nd intron of porcine growth hormone gene. Anim Genet 23:180–181

Kirkpatrick BW, Huff BM (1991) Detection of insertion polymorphisms in 5'flank and 2nd intron of the porcine growth hormone gene. Anim Genet 22:192–193

Kirkpatrick BW, Huff BM, Casa-Carrillo E (1993) Double-strand DNA conformation polymorphisms as a source of highly polymorphic genetic markers. Anim Genet 24:155–161

Larsen NJ, Nielsen VH (1993) *Apa*I and *Cfo*I polymorphisms in the porcine growth hormone gene. Anim Genet 24:71

Lund-Larsen TR, Bakke H (1975) Growth hormone and somatomedin activities in lines of pigs selected for rate of gain and thickness of backfat. Acta Agric Scand 25:231–234

Nielsen VH, Larsen NJ (1991) Restriction fragment length polymorphism at the growth hormone gene in pigs. Anim Genet 22:291–294

Pursel VG, Pinkert CA, Miller KF, Bolt DJ, Campbell RG, Palmiter RD, Brinster RL, Hammer RE (1989) Genetic engineering of livestock. Science 244:1281–1288

Rohrer GA, Alexander LJ, Keele JW, Smith TP, Beattie CW (1994) A microsatellite linkage map of the porcine genome. Genetics 136:231–245

Saison R, Rasmusen BA, Hradecky J (1967) Da, a new factor in a new blood group system in pigs. Can J Genet Cytol 9:794–798

SAS Institute Inc. (1988) SAS/STAT Users Guide, Release 6.03 Edition, Cary, North Carolina

Thomsen PD, Fredholm M, Christensen K, Schwerin M (1990) Assignment of the porcine growth hormone gene to chromosome 12. Cytogenet Cell Genet 54:92–94

Winkelmann DC, Hodgetts RB (1992) RFLPs for somatotropic genes identify quantitative trait loci for growth in mice. Genetics 131:929–937

Yerle M, Lahbib-Mansais Y, Thomsen PD, Gellin J (1993) Localization of the porcine growth hormone gene to chromosome 12p11–p14. Anim Genet 24:129–131