Mapping quantitative trait loci for principal components of bone measurements and osteochondrosis scores in a wild boar × Large White intercross

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Summary

Data on osteochondrosis and femur dimensions from 195 F2 pigs from a wild boar × Large White intercross were analysed with the aim of detecting quantitative trait loci (QTLs) for normal and disturbed bone formation. The information from numerous recorded traits was summarized by principal component analysis and analysed by least-squares interval mapping. An increase in the proportion of wild boar alleles across the genome increased length versus width of femur and reduced the prevalence of osteochondrosis. The presence of QTLs with an impact on femur dimensions was indicated on chromosomes 2, 4, 16 and 17 and on osteochondrosis on chromosomes 5, 13 and 15. A substantial effect of the chromosome 5 QTL calls for further studies within commercial populations to evaluate whether marker-assisted selection could be used to reduce the prevalence of osteochondrosis.

1. Introduction

Osteochondrosis in the pig is a generalized skeletal disease characterized by disturbed bone formation and cartilage retention (Reiland, 1978a). It affects young growing animals but the osteochondrotic lesions remain in the adult. Severe osteochondrosis is related to pain (Hill, 1990a), leg weakness and gait disturbances (Lundeheim, 1987), and results in economic losses mainly due to culling of pigs at many levels in the pig breeding industry (Hill, 1990a). Osteochondrosis is a multifactorial disease with a hereditary background. Heritability estimates for osteochondrosis usually fall within the range of 0.1 to 0.5 (e.g. Lundeheim, 1987; Stern et al., 1995). The disease occurs in high frequency in growing pigs in all commercial breeds, but the causes of it remain unclear. Many studies of associations between growth rate and osteochondrosis have been performed, the results most often showing weak and inconsistent correlations (Lundeheim, 1987; Nakano et al., 1987; Hill, 1990b; Stern et al., 1995; Uhlhorn et al., 1995). Selection for different degrees of leg weakness has been shown to result in alterations in bone length and width (Draper et al., 1992) and in angularity of joints (Draper et al., 1988). However, the correlated response on osteochondrosis was negligible in the same selection experiment (Goedegebuure et al., 1988).

Reports on the prevalence in wild boars (Klaessen, 1987) and in wild boar crosses have been contradictory (Vogel, 1976; Reiland, 1978b). However, we have found that the distribution and extent of osteochondrotic lesions were similar in an F2 intercross of European wild boar and Swedish Large White compared with the pure-bred Large White population (Uhlhorn et al., 1995). In the same F2 population, several quantitative trait loci (QTLs) have been identified that influence, for example, growth and fatness traits (Andersson et al., 1994; Knott et al., 1998), carcass composition and carcass length (Andersson-Eklund et al., 1998a) and immunological traits (Edfors-Lilja et al., 1998).

In the present investigation, we used recordings on osteochondrosis, specific femur measurements and a detailed linkage map aiming at identification of chromosome regions with QTLs for traits related to normal and disturbed bone formation.
2. Materials and methods

(i) Animals

The study comprised a three-generation pedigree, two European wild boars and eight Swedish Large White sows being the founders (Andersson et al., 1994). Four sires and 22 dams of the F1 generation were the parents of the 195 F2 animals. The experiment was performed in two batches comprising the first and second litter of the sows. To get larger full-sib families, most sows were mated to the same boar for both parities. The F2 animals were raised, with two feeding treatments in each batch, from 20 kg until slaughter at a live weight of at least 80 kg or at a maximum age of 190 days.

(ii) Marker map

The study was based on marker information from a comprehensive linkage map, including 236 markers with a total map length of about 2300 cM (Marklund et al., 1996). Markers were available on all of the 18 autosomes, with between five and 20 markers on each. The animals of all three generations were genotyped.

(iii) Phenotypic data

After slaughter, the femora and humeri from both sides of the carcass were removed, stripped of muscle, measured for a number of size parameters and examined for osteochondrotic lesions in the articular cartilage facing the knee and elbow joint as described by Uhlhorn et al. (1995). The phenotypic means and standard deviations of all measurements are given in Table 1.

Bone measurements. A total of eight recordings were performed on the left femur: weight, total length, length of diaphysis, width at diaphysis and epiphysis, cortical thickness (two measurements) and angle between the axes of diaphysis and collum femoris.

Macroscopic examination of lesions. The left femur and humerus were used for a macroscopic examination of the presence and severity of osteochondrosis according to the routines of the Swedish pig progeny testing scheme. The findings from the medial femoral condyle and the medial humeral condyle were scored from 0 (best) to 5 (worst) according to Reiland et al. (1978a).

Radiographic examination of lesions. Serial sections, 5–7 mm thick, were cut through the distal femur and humerus of the right side. The sections were radiographed and radiologically examined. The extent of the radiolucent areas in the subchondral bone of the medial femoral condyle and of the medial humeral condyle was scored from 0 (best) to 3 (worst).

The phenotypic data were considered as two datasets: (a) linear bone measurements and (b) osteochondrosis scores. The between-sets correlations were low, the highest correlation being 0·20–0·25 between width at epiphysis and the four osteochondrosis variables. The within-sets correlations were considerably larger, with maximum correlations of

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<th>Table 1. Phenotypic means, standard deviations and principal component loadings of the measured traits</th>
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<tr>
<td>Mean</td>
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<tr>
<td><strong>Femur measurement</strong></td>
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<tr>
<td>Weight (g)</td>
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<td>Total length (mm)</td>
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<td>Length of diaphysis (mm)</td>
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<td>Width of diaphysis (mm)</td>
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<td>Width of epiphysis (mm)</td>
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<tr>
<td>Cortical thickness interior (mm)</td>
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<td>Cortical thickness exterior (mm)</td>
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<td>Collum angle (degrees)</td>
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<tr>
<td><strong>Osteochondrosis</strong></td>
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<tr>
<td>Radiographic score femur</td>
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<td>Radiographic score humerus</td>
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<tr>
<td>Macroscopic score femur</td>
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<td>Macroscopic score humerus</td>
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</table>

*a* Lesions scored from 0 (best) to 3 (worst).

*b* Lesions scored from 0 (best) to 5 (worst).
0.85 within the bone measurement set and 0.50 within the osteochondrosis set. We therefore decided to analyse the two sets of data separately.

(iv) Statistical analyses

Information from two datasets, with eight and four variables respectively, was used for QTL detection. We did not consider it biologically relevant to attempt to locate separate QTLs for each original variable as these to some extent were measurements of the same traits: size of femur and overall osteochondrosis burden. Instead, the primary data were gathered into a few, more informative and independent traits by a principal component analysis (SAS, Proc Princomp, 1989) of each set of variables. For dataset (a) the eigenvalues indicated that three components provided a good summary of the eight bone measurements, accounting for 80% of the standardized variance. The first component (PC1) explained 50% of the variance and showed approximately equal loadings on all input variables (Table 1). The second component (PC2) explained 18% of the standardized variance. PC2 had high positive loadings on length measurements and negative on width measurements, thus expressing length versus width of femur. The third component (PC3) had a high positive loading on angle between collum and diaphysis (Table 1) but explained only 12% of the variance in bone measurements.

For dataset (b) two principal components explained 73% of the standardized variance in radiographic and macroscopic osteochondrosis scores. The first component (PC1) had high loadings on all input variables (Table 1) and explained 48% of the standardized variance, whereas the second component (PC2) expressed the difference between elbow (humerus) and knee (femur) lesions. PC1 and PC2 were used as traits in the QTL analyses.

The marker genotypes were used to estimate the probabilities of the breed origin of each gamete at fixed 1 cM intervals through the genome for each F2 animal (Haley et al., 1994). These probabilities were used to calculate additive and dominance coefficients for a putative QTL at each position under the assumption that the QTL was fixed for alternative alleles in the two breeds. The trait values were then regressed onto these coefficients in intervals of 1 cM. The threshold values for suggestive (i.e. with expectation of one false positive result per genome scan) and significant linkage on a genome-wide level were set by permutation tests according to Knott et al. (1998).

The marker genotypes were also used to calculate the probability that an F2 animal had inherited the wild boar alleles at fixed positions in the genome. The proportion of wild boar alleles averaged over the genome was then calculated from these probabilities. The effect of the proportion of wild boar alleles was tested as a regression for the whole genome.

In addition to the regressions on the additive and dominance coefficients, or the proportion of wild boar alleles, the statistical models included the fixed effects and covariates that were significant in the analysis of at least one principal component within the dataset. The analyses of osteochondrosis traits included sex and full-sib family and the analyses of bone measurement traits included sex, full-sib family and feeding regime within batch as well as live weight at the end of the test period. In the analyses, cofactors were added for all unlinked QTLs that were significant at least at the suggestive level in a preliminary analysis without cofactors.

3. Results

An increase in total proportion of wild boar alleles did not have an overall effect on femur dimensions in general (PC1) when a correction was made to the same live weight of the animals (P = 0.80). However, there were QTLs on chromosome 4 and 16 at which the wild boar allele reduced femur size (Table 2). Both of these QTLs showed complete dominance for the wild boar allele. The length versus width measures (PC2) significantly increased with an increased proportion of wild boar alleles across the genome (P < 0.0001). This was largely explained by significant QTLs with positive additive effects of the wild boar allele for PC2 on chromosomes 2 and 17. The QTLs explained between 6.5% and 11.3% of the residual F2 variance of PC1 or PC2 (Table 2).

An increase in proportion of wild boar alleles across the genome significantly improved the general osteochondrosis status (PC1, P = 0.05). There were QTLs on chromosomes 5 and 13 at which the wild boar allele reduced the prevalence of osteochondrosis and both QTLs showed overdominance. The QTL effect on chromosome 5 reached genome-wide significance and explained 10.6% of the residual F2 variance of PC1, whereas the QTL on chromosome 13 explained 7.8%. For PC2, expressing the difference in osteochondrosis between elbow and knee joints, there was no effect of the proportion of wild boar alleles (P = 0.60). However, QTLs with different effects on PC2 were indicated on chromosomes 5 and 15 (Table 2). Tables with results from all chromosomes and traits can be obtained from the authors.

4. Discussion

In the present study we had information on many related measurements that were made to achieve a good overall status of two complex traits: femur dimension and osteochondrosis burden. As we were more interested in the traits than in each measurement
per se, we found it appropriate to gather the information into a few composite traits. To do this we used principal component analysis as suggested by James (1991) and elaborated in a single-marker multi-trait analysis by Weller et al. (1996). To our knowledge, principal component analysis has not been used before in multiple marker mapping of QTLs. It definitely has some advantages in analyses of mapping experiments in which numerous traits are recorded. As in the present study, the original variables are often highly correlated and can be replaced by a few highly informative, independent variables without losing much information. We have previously reported the results from some preliminary analyses of the four separate osteochondrosis scores of the same material (Andersson-Eklund et al., 1998b). The existence of single QTLs was indicated in that study for at least one of the four measurements on chromosomes 1, 5 and 13, but the statistical evidence for the QTL effects was weaker than in the present study. Furthermore, the present analyses of principal components did not violate the assumptions of normally distributed residuals in the regression analysis, which some analyses of separate measurements did.

As the principal components are uncorrelated, the results can readily be interpreted on a multi-trait basis, i.e. the significance thresholds can be corrected for the number of traits or the number of significant results can be directly compared to the expectations. The fact that the variables are uncorrelated is also interesting for the interpretation of the results. A significant QTL that has been identified for two different uncorrelated traits is indicative of two linked QTLs affecting the two traits (Weller et al., 1996). This could be the case with the QTLs on chromosome 5 affecting PC1 sub 0 and PC2 sub 0 in the present study, assuming that the traits are also genetically uncorrelated. The QTLs were located on the same chromosome region and the additive effect of the wild boar allele reduced the incidence of osteochondrosis.

However, the QTL for PC1 sub 0 had a large, significant overdominant effect, whereas the wild boar QTL allele for PC2 sub 0 had a recessive effect (Table 2). The differences in dominance effects and the fact that the two traits are uncorrelated support the interpretation that two different linked QTLs, where one affects general osteochondrosis status and the other affects the relation between elbow and knee joint lesions, are located on chromosome 5.

A possible disadvantage of using principal components in QTL analyses is that the magnitudes of the estimated effects are difficult to interpret directly in terms of traits. However, genome scans for QTLs are the first step in the process of identification of QTLs. The knowledge gained must be confirmed in other populations and the QTLs must be pinpointed by fine mapping or searches for candidate genes. The proportion of the variance that each QTL explains is more relevant for comparisons over traits and populations than the absolute magnitude of the estimated effects and therefore more interesting at the stage of genome scans. In the case of an interest in the effects

<table>
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<tr>
<th>Femur measurements</th>
<th>Reduction in F2 variance</th>
<th>Additive effect</th>
<th>Dominance effect</th>
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<tbody>
<tr>
<td><strong>F-ratio</strong></td>
<td><strong>Map position</strong></td>
<td><strong>chr</strong></td>
<td><strong>cM</strong></td>
</tr>
<tr>
<td>PC1 sub 0</td>
<td>61</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>:</td>
<td>58</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>PC2 sub 0</td>
<td>9.7*</td>
<td>17</td>
<td>87</td>
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- **Only effects that are significant at least on the suggestive level are included in the table.** The 5% significance thresholds obtained from permutation tests for the F-ratio in the interval mapping vary between 8.7 and 9.2 for the different traits and the suggestive levels (i.e. with expectation of one false positive result per genome scan) vary between 4.5 and 5.9.
- **Map position** is the one giving the highest test statistic (F-ratio) on that chromosome (Chr) estimated in centimorgans (cM) from the proximal end as defined by Marklund et al. (1996).
- **The percentage reduction in residual variance of the F2 population due to the inclusion of a QTL at the given position.**
- **Additive effect of a QTL defined as a deviation of animals homozygous for the wild boar allele from the mean of the two homozygotes; estimates given with standard error.**
- **Dominance effect of a QTL defined as the deviation of animals heterozygous for the wild boar allele from the mean of the two homozygotes; estimates given with standard error.**
on the original variables, these can either be estimated in separate analyses at the positions of identified QTLs, or they can be resolved by a reverse transformation, i.e. by multiplying the inverse of the eigenvector matrix by the vector of effects (Weller et al., 1996). We used reverse transformation to resolve the effects of the significant QTL on chromosome 5 on the original four osteochondrosis measurements. The effects of principal components 3 and 4 were replaced with zeros as these variables were disregarded in the analyses due to their low explanatory power. The additive effect of the wild boar allele was small for all traits (−0.2 to 0.0), whereas the dominance effect varied considerably between original traits (−0.4 to 0.6). For reasons of comparison and in order to obtain standard errors of the estimates, direct analyses of the original variables were performed at the locations of the two QTLs that reached genome-wide significance levels. A significant dominance effect for macroscopic scores on femur was found at the location of the significant QTL for PC1<sub>1</sub> (Table 3). The dominance effect was estimated to be −0.75, which agrees relatively well with the resolved dominance effect of −0.42 for the same trait, i.e. the overdominance effect was detected by both methods of analysis. There were no significant QTLs for the other three osteochondrosis scores, but they all contributed to the estimated effects on PC1<sub>1</sub>. Analysis of the position of the significant QTL for PC2<sub>1</sub> revealed significant effects only for the two measurements of femur length (Table 3).

All suggestive QTLs for the bone measurement traits (PC1<sub>1</sub> and PC2<sub>1</sub>) and for the trait expressing general osteochondrosis status (PC1<sub>0</sub>) had relatively large negative estimates of dominance effects. We have not found this in previous studies of related traits such as body length and femur weight (Andersson-Eklund et al., 1998a) in the same population. Neither did we have any expectations of large dominance effects based on quantitative genetic studies of these traits. The dominance effects, and especially the overdominance effect found on chromosome 5 which implies that heterozygous animals had a lower prevalence of osteochondrosis than both types of homozygotes, could in part explain the inconsistency in results between genetic studies performed on osteochondrosis in different crossbreeding materials.

Osteochondrosis is caused by disturbed enchondral ossification, i.e. disturbed bone formation from cartilage in the young growing animal. The most important hormones involved in the regulation of bone formation and growth are calcitriol, growth hormone, thyroxine, sex hormones, parathyroid hormone and calcitonin (Martini, 1998). Thus it is obvious that bone growth and osteochondrosis have a very complex genetic background which is partly the same as that of general body growth. Several candidate genes of growth have been mapped in pigs, including those encoding growth hormone, leptin, insulin-like growth factors, calcitonin, parathyroid hormone, thyroid stimulating hormone and/or their receptors (PGBase at www.ri.bbsrc.ac.uk/cgi-bin/, May 1999).

By using information on homologous regions of the human gene map, the number of candidate genes can
be further increased in the regions of the located QTLs (e.g. www.ncbi.nlm.nih.gov/Omim/, May 1999).

In the present study, two of the suggestive QTLs for bone dimension and one for osteochondrosis were located on chromosomes where QTLs for growth and carcass composition have been found in the same material, i.e. on chromosomes 2, 4 and 13. A paternally expressed QTL with large effects on body composition traits such as meat percentage, meat + bone percentage and muscle area has recently been mapped to the IGF2 locus on chromosome 2 (Jeon et al., 1999; Nezer et al., 1999). However, the QTL for the bone dimension trait was located approximately 75 cM from the IGF2 locus (Jeon et al., 1999). The location was close to a region where a suggestive QTL for growth rate at test has been reported (Knott et al., 1998) and the insulin receptor locus is located (Marklund et al., 1996). This region is homologous to either the human chromosome region 19p or 11q. Human 19p13 harbours potential candidate genes for bone dimensions including tyrosine kinase-2 (TYK2) and cartilage oligomeric matrix protein (COMP), whereas the osteopetrosis (OPTB1) and osteoporosis-pseudoglioma (OPS) genes are found on human chromosome 11q12–q13. In addition, a QTL affecting bone mineral density in humans was recently mapped to the latter region (Koller et al., 1998).

The region on chromosome 4 where a QTL for bone dimension was suggested is not very well defined, but coincides with the region where QTLs affecting carcass composition and growth rate have been identified (e.g. Andersson et al., 1994; Andersson-Eklund et al., 1998a; Knott et al., 1998; Rohrer & Keele, 1998; Walling et al., 1998). The F13B, gene which is located within the peak region for the bone dimension QTL, has its human homologue at chromosome 1q31. This is the location of a possible candidate gene, osteocalcin (BGLAP), which is associated with bone mineralization. However, the same region of pig chromosome 4 also shares homology with human chromosome 8 (Rettenberger et al., 1995), but the pig comparative map of chromosome 4 is not informative enough to enable an efficient search for candidate genes.

QTLs on chromosome 13 affecting early growth in pigs have been reported in studies of wild boar crosses (Andersson et al., 1994; Knott et al., 1998) and in pure-bred Large White pigs (Nyström et al., 1997). Chromosome 13 harbours the PIT1 locus, which codes for a transcriptional factor of growth hormone and has been reported to influence early growth in a cross between Chinese and European pigs (Yu et al., 1995). In the present study we identified a QTL with negative additive effect of the wild boar allele on the prevalence/severity of osteochondrosis in the region homologous to human 3p, where both PIT1 and the parathyroid hormone receptor (PTHr) genes are located.

At pig chromosome 5 we located a QTL affecting osteochondrosis to a position between the interferon-γ (IFNG) and the insulin-like growth factor-1 (IGF1) genes. This region is homologous to human chromosome 12q14–q24 where a potential candidate gene, cartilage homeoprotein 1 (CART1), is located. The region on pig chromosome 17 harbouring a QTL for bone dimensions shares homology with human chromosome 20p where at least two potential candidate genes have been located: bone morphogenetic protein-2 (BMP2) which induces cartilage and bone formation, and the gonadotropin-releasing hormone-2 (GNRH2).

We found two QTLs per trait at the suggestive significance level, which is twice as many as expected by chance. Two different QTLs were significant at the 5% genome-wide level in testing five independent traits, which also is more than expected by chance. However, the power of the present experiment was low, especially for low-heritability traits such as osteochondrosis. Beavis (1996) showed in simulation studies, where 10 QTLs explained 30% of the phenotypic variation in an F2 population, that the power was increased from 12% to 57% when the number of progeny increased from 100 to 500. The power for a trait with high heritability (63%) was 33% and 86% in the corresponding situations. A low heritability could partly explain why we identified fewer QTLs for the present traits than has been described for e.g. fat deposition traits (Knott et al., 1998). Furthermore, we do not know the osteochondrosis status in the wild boar population or the actual bone dimensions in any of the founder populations, although the wild boar has relatively longer legs than the domestic pig. If the founder populations were not fixed for alternative QTL alleles for the studied traits the power of the analysis would be reduced. Beavis (1996) concluded that QTL studies using small numbers of progeny are not very powerful if there are many small-effect QTLs in the genome. Under these circumstances only a small proportion of the QTLs are identified and their effects are overestimated. However, the QTLs that are identified are not expected to be false as long as appropriate significance thresholds for the test statistics are used. Our conclusion from the present study is that we have identified some of the chromosome regions that have an impact on the variation of bone dimension and osteochondrosis in a cross between wild boar and Large White pigs. The substantial effect of the chromosome 5 QTL calls for further studies within commercial populations to find out whether the QTL segregates, and to evaluate whether marker-assisted selection could be used to reduce the prevalence of osteochondrosis.
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References


