Genome-wide association study and fine mapping of a QTL on SSC13 for Osteochondrosis in Duroc pigs

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Introduction

Osteochondrosis (OC) is a common joint disorder occurring in growing domestic animals. It is characterized by a disturbed endochondral ossification in the articular cartilage and the epiphyseal growth plates, and is most likely caused by failure of blood supply to the growing cartilage (reviewed by Ytrehus et al., 2007). OC is known as the most important cause of leg weakness in pigs and therefore an important trait both due to animal welfare concerns and economic reasons.

Risk factors causing OC include rapid growth, anatomic conformation, trauma, dietary imbalances, and genetic predisposition (Ytrehus et al., 2007). Both genetic and genomic studies have been conducted to elucidate the mechanisms causing OC. The heritability of OC in pigs varies from 0.08-0.42 in different breeds and studies (Aasmundstad et al., 2013, Jørgensen & Andersen, 2000, Kadarmideen et al., 2004). Genomic regions associated with OC have been detected in several studies. In a previous study we identified QTLs for OC on SSC2, 5, 6, 7, 8, 13 and 14 in the Norwegian Landrace breed (Grindflek et al. 2014). Moreover, QTL regions have been identified on SSC5, 13 and 15 in Large White x Wild Boar crosses (Andersson-Eklund et al. 2000) and on SSC2, 3, 6, 10 and 14 in Duroc x Pietrain crosses (Laenoi et al. 2011). Gene expression analysis has suggested roles for extracellular matrix genes, angiogenesis genes and immune response genes (Rangkasenee et al. 2013). The results of these studies indicate that OC is a complex genetic trait, and so far no causative mutations have been identified.

The aim of this study was to detect QTL regions contributing to OC in Duroc pigs and to use a fine mapping strategy to identify putative causative SNPs. For this purpose, boars were subjected to a computed tomography (CT) scan for measurement of OC on eight anatomical locations. All the phenotyped pigs were genotyped on the Illumina 60K SNP chip. A subset of 140 animals had Axiom 660K array genotypes available, making imputation feasible before a GWAS was conducted. Moreover, whole genome sequence data from 23 Duroc boars was used for subsequent SNP detection and fine mapping.

Material and methods

Animals and phenotypes

Data from 5975 purebred Duroc boars from Norsvin’s boar testing station (Hamar, Norway) was included in this study. The boars entered the testing station at approximately 30 kg live weight and were kept in groups of 12 pigs per pen. Ear samples for DNA extraction were...
collected when entering the test. At the end of the test period (approximately 120 kg live weight), all boars were subjected to a CT scan for measurement of OC as well as body composition traits. Prior to scanning, the boars were sedated using Azaperone (Stresnil Vet ®, Janssen-Cilag Ltd., Buckinghamshire, UK), which was injected intramuscularly. All animals were cared for according to laws, internationally recognized guidelines and regulations controlling experiments with live animals in Norway, according to the rules given by the Norwegian Animal Research Authority (The Animal Protection Act of December 20th, 1974, and the Animal Protection Ordinance Concerning Experiments with Animals of January 15th, 1996).

Osteochondrosis was scored from CT images as described in detail by Aasmundstad et al. (2013). For eight anatomical locations, a score between 0 and 5 was assigned and the sum of phenotypes from all the locations, the total score, obtained the highest heritability (Aasmundstad et al. 2013) and was therefore used for association analysis.

Genotyping and imputation

The genotyping was performed at CIGENE, University of Life Sciences, Norway. All the animals in this study were genotyped using the Illumina porcine 60K SNP chip (Illumina, San Diego, USA). Some of the boars (n=140) were also genotyped using the Axiom porcine 660K array from Affymetrix (Affymetrix Inc., Santa Clara, CA, USA). All genotypes were filtered based on call rate > 0.97 and minor allele frequency (MAF) > 0.01. Imputation of all animals to 660K was done using FImpute v.2.2 (Sargolzaei et al., 2014) using default settings and the complete pedigree as additional information. Altogether, 29,433 SNPs were shared between the arrays and checked for matching genotypes and allele frequencies before imputation.

Sequence data analyses and imputation

Whole genome sequence data was available from 23 Duroc boars at a 9-17X coverage. All of the sequenced animals were 60K genotyped. Moreover, 11 of the sequenced animals were available with OC phenotypes and six of them were among the 660K genotyped animals. The reads were 2 x 100 bp Illumina paired-ends and were aligned to Sscrofa build 11.1 using BWA-aln v.0.7.5 (Li & Durbin, 2009). Duplicates were marked and files sorted with SAMtools v.0.1.19 (Li et al. 2009) and SNPs were detected in the most significant QTL region using FreeBayes v.1.0.2 (Garrison & Marth, 2012). Reads were filtered using VCFtools v.0.1.14 (Danecek et al. 2011) and SAMtools bcftools v.1.3 (Li et al. 2009). Beagle v.4.1 (Browning & Browning, 2016) was used to phase the SNPs detected in the sequence data. Prior to imputation, the 660K data was compared to the sequence data using conform-gt (Browning & Browning, 2016) to exclude variants that were not present in the sequence data and to adjust records to match chromosome strand and allele order. After imputation, SNPs with a MAF < 0.01 were removed before further analyses.

Genome-wide and regional association analyses

GWAS was performed using the R v.3.2.4 package GenABEL v.1.8-0 (Aulchenko et al. 2007). The total OC score was pre-corrected for herd*birthyear, mother’s litter number, OC referee, pen and animal ID. Association testing was performed using a kinship matrix of relatedness estimated from genotyped SNPs using identity-by-state and the p-values were
corrected for genomic control by dividing the observed test statistics by a calculated genomic inflation factor.

Regional association analysis was conducted for the imputed sequence variants in the QTL region. The analysis was run in ASReml v.3.0 (Gilmour et al. 2009) by fitting each SNP as a fixed effect in the model. The phenotypes were the same as for the GWAS analysis and a pedigree based relationship matrix was fitted. The \( p \)-values were corrected for multiple testing using the Bonferroni correction method. The Ensembl variant effect predictor (VEP) was used to predict the effect of the detected SNPs (McLaren et al. 2010).

**Results and discussion**

In this study, we identified a QTL region for OC in Duroc pigs on SSC13 (Figure 1). Eight SNPs at \(~44\) Mb had a \( p \)-value \( < 1.0^{-4} \) and were considered significant. QTLs on SSC13 have been reported previously for OC in Large White x Wild boar pigs (Andersson-Eklund et al. 2000) and for leg score in Large White x Meishan pigs (Lee et al. 2003). These studies used microsatellite markers, however, making it difficult to predict overlap with our QTL region. The most significant SNP in our study was rs325689994 at 43.89 Mb \( (p=9.7e-05) \). This is in an intergenic region between \( PTPRG \) (protein tyrosine phosphatase, receptor type G) and \( C3orf14 \). Intergenic SNPs may have regulatory functions, however, it is difficult to assign putative functional SNPs in such regions. We therefore proceeded with fine mapping to get closer to the causative variant.

Whole genome sequences of 23 Duroc pigs were used for SNP detection, and variants were detected in a 10 Mb region \( (36-46\) Mb) surrounding the QTL for OC on SSC13. After filtering, 39,027 SNPs were available for imputation and analyses.

The fine mapping analysis confirmed a previously detected QTL in this region (Grindflek et al. 2014), but changed the position of the most significant SNP to 44.03 Mb (Figure 2). This is 0.14 Mb away from the most significant 660K SNP. The most significant SNP from the fine mapping analysis (rs342618352) was characterised by VEP as an intronic variant of \( CADPS \) (calcium dependent secretion activator). CADPS is responsible for Ca2+-regulated exocytosis of secretory vesicles. It is mainly expressed in neuroendocrine cells and a role in osteochondrosis is not obvious. Whether the causal variant underlying this QTL is regulatory or whether the most significant SNP is in LD with the functional variant is still unclear.
Figure 1. Manhattan plot showing genome-wide association results for Osteochondrosis in Duroc.
Figure 2. Association analysis using sequence variants within the QTL region on SSC13.

List of References


