# 783. Genomic regions associated with backfat thickness show pleiotropic effect on osteochondrosis in pig

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## Abstract

The aim of this study was to perform genome-wide association analyses for backfat thickness and osteochondrosis in Landrace pigs and to fine map pleiotropic genomic regions. In order to characterise genomic regions, phenotypic information of 5,000 animals with osteochondrosis scored from CT images and 40,000 animals with backfat thickness scored from CT or ultrasound images were analysed. All animals were genotyped with a medium density SNP chip and a subset of them were genotyped with a high-density SNP chip as well, allowing for imputation. Two genomic loci were found in common for osteochondrosis and backfat thickness, one on chromosome 5 and one on chromosome 14. For both regions, an antagonistic relationship was found. Fine mapping using an impact score approach identified the *CCND2* gene as the most likely causal gene on chromosome 5, whereas a mutation in *CRTAC1* had the highest impact score in the chromosome 14 region.

## Introduction

Osteochondrosis (OC) is a developmental disease usually occurring in joints in an early stage of life in pigs as well as other domestic animals. It is characterised 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 (e.g. Ytrehus *et al.*, 2007). OC is the most important cause of leg weakness in pigs and is therefore an important trait due to both animal welfare concerns and economic reasons. In Norwegian Landrace pigs, the heritability of OC was shown to vary from 0.06-0.21 depending on anatomical location, and the total OC score had a heritability of 0.31 (Aasmundstad *et al.*, 2013). Genomic studies have been conducted to elucidate the mechanisms causing OC. Quantitative trait loci (QTL) regions associated with OC have been identified on *Sus scrofa* chromosome (SSC) 2, 3, 5, 13 and 15 (Andersson-Eklund *et al.*, 2000; Laenoi *et al.*, 2011).

Backfat (BF) is an important carcass composition trait in pig production. Lean meat is the most valuable product so breeding programs have aimed for more growth of lean meat and reduced levels of BF (Lonergan *et al.*, 2001). Heritability of BF in Landrace pigs is 0.42 (unpublished data, H. C. Oliveira). Genomic studies have showed numerous candidate genes influencing backfat thickness and QTLs identified over the years can be found in the animal QTL database (https://www.animalgenome.org/QTLdb).

The aim of this study was to detect genomics regions contributing to BF in Landrace pigs and to fine map regions showing pleiotropy with OC.

## Materials & methods

**Animals.** The animals included in this study were purebred Landrace boars and sows born between 2013 and 2020 in Norway. Animals used in the OC analysis were boars from the nucleus boar testing station in Hamar, Norway, that entered the testing station at approximately 30 kg live weight and lived in groups of 12 pigs per pen. At the end of the test period (at approximately 120 kg live weight), boars were subjected

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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. OC was scored from CT images as described in detail by Aasmundstad *et al.* (2013). For eight anatomical locations (distal and lateral humerus and femur, right and left side), a score for OC between 0 and 5 was assigned and the sum of phenotypes from all the locations, the total score, was used as the OC phenotype. A total of 5,000 animals were included for the OC association analysis.

Animals used in the BF analysis were boars from the boar testing station as well as boars and sows from Norwegian nucleus farms. BF measurements were done using CT data on boars from the boar testing station and ultrasound on the other animals. Backfat was measured in millimeter and 40,000 animals were included in the BF association analysis.

**Genotyping.** Ear samples for DNA extraction were collected and stored at -20 °C until used (Caisley ear sampling protocol). DNA for genotyping was extracted from the ear samples using BioSprint DNA Kit (Qiagen, Hilden, Germany) by BioBank (Hamar, Norway). DNA concentration and quality was measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, DE, USA). The genotyping was performed at CIGENE, University of Life Sciences, Norway and at NEOGEN, Scotland, UK. All the animals in this study were genotyped using Illumina porcine medium-density SNP chips (50K, 80K) (Illumina, San Diego, USA) or a custom made 25K SNP chip (Illumina and Neogen). Some of the boars 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 performed using FImpute v3 (Sargolzaei *et al.*, 2014) with default settings and the complete pedigree as additional information. SNPs that were shared between the SNP chips were checked for matching genotypes and allele frequencies before imputation.

**Genome wide association study and fine mapping.** Genome-wide association study (GWAS) was performed using the GCTA software (Yang *et al.*, 2011). The total OC score and the backfat measurements were included as phenotypes using a linear animal model.

Fine mapping was conducted using the porcine combined annotation dependent depletion (pCADD) method (Derks *et al.*, 2021). The pCADD pipeline takes the top SNP as input and identifies SNPs from whole genome sequence (WGS) data that are in high linkage disequilibrium (LD; >0.7) with the top SNP. SNPs from WGS data are then ranked based on the pCADD scores, chromatin data information (liver) and gene expression data. The output from the pipeline is a list of likely causal variants. WGS and gene expression data from the Landrace population were available from previous studies (e.g. Van Son *et al.*, 2017a,b).

## Results

GWAS were conducted using 660K imputed genotypes to identify genomic regions associated with OC and BF in Landrace boars. The prevalence of OC for the animals included in this study ranged between a total score of 0-19 with mean=2.81 (SD=2.54). BF ranged from 2 to 18 mm with mean=7.44 (SD=1.82). The GWAS detected significant loci for both OC (Figure 1) and BF (Figure 2) (corrected *P*-value<$1.0^{-10}$) on SSC5 at 66 Mb and on SSC14 at 109 Mb. For BF, additional significant loci were detected on SSC2 44 Mb (highly significant), SSC4 1 Mb, SSC5 15 Mb and SSC18 11 Mb.

The top SNP of the SSC5 QTL is AX-116685771 for both traits, showing opposing effects where the A allele increases BF and decreases OC. The SNP explains 0.5% of the genetic variance for OC and 0.1% for BF. Fine mapping of this region returned 47 variants in LD with the top SNP. The variant with the highest impact

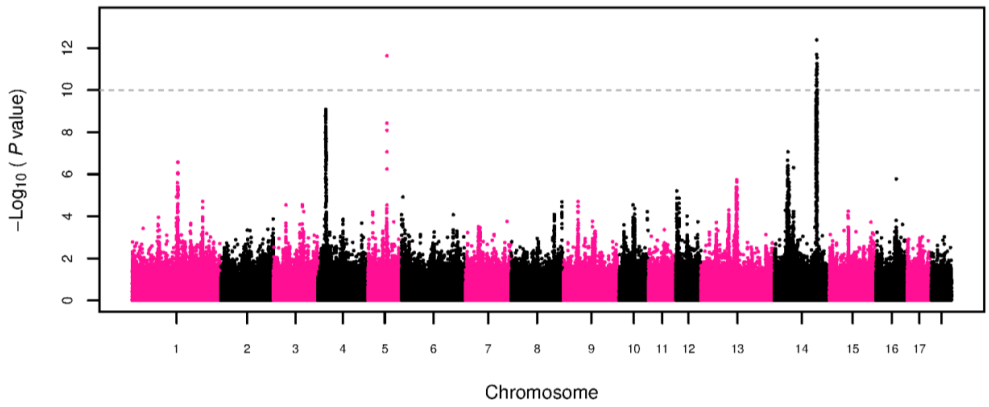


**Figure 1.** GWAS results of OC in Landrace pigs.

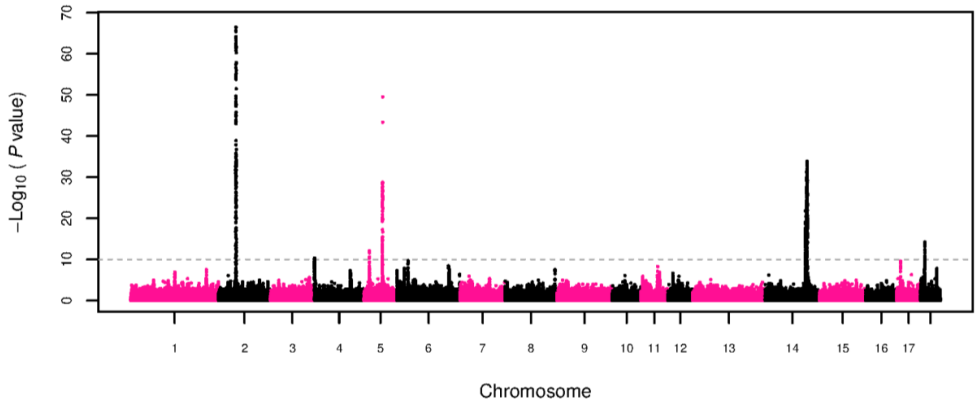


**Figure 2.** GWAS results of BF in Landrace pigs.

score was an intronic variant in the cyclin D2 gene (*CCND2*) with a pCADD score of 13.5. All 47 variants from this region were located in *CCND2* or downstream of this gene.

For the SSC14 QTL, AX-116521996 is the top SNP for OC whereas AX-116659727 is the top SNP for BF (LD between these two SNPs is 0.9). AX-116659727 is also highly significant for OC and the SNP shows opposing effects for the two traits in the same manner as on SSC5. The top SNPs explains 0.1% of the genetic variance for both OC and BF. Using AX-116659727 as top SNP, fine mapping of this region returned 319 variants in LD. The variant with the highest impact score was an intronic variant in cartilage acidic protein 1 (*CRTAC1*; pCADD=17.8).

## Discussion

Breeding for increased growth rate and more lean meat has reduced the level of BF. However, pigs that grow faster are also more susceptible to OC and by including phenotypes on OC in the breeding goal, it is possible to balance the selection. Genomic information can be useful to identify genes showing pleiotropic effects on different traits and this study identified two QTL regions in common for OC and BF; on SSC5 and 14.

A previous study also identified the region on SSC5 for BF in four different breeds (Gozalo-Marcilla *et al.*, 2021). They suggested a causal role for the fibroblast growth factor 23 (*FGF23*) gene as its expression in osteocytes is linked to adipose tissue secretion of proteins. In this study, fine mapping identified *CCND2* as the most likely causal gene on SSC5. It is involved in the control of the cell cycle (Chiles, 2004) and knock-out studies in mice show numerous consequences in development of organs and functions. The two genes *FGF23* and *CCND2* are located just 50 kb apart and both genes are good biological candidates for both traits. It is also possible that the two genes are somehow interacting or that one gene is affecting BF and the other OC.

On SSC14, a mutation in *CRTAC1* showed the highest impact score. This gene is an extracellular matrix protein of articular cartilage (Steck *et al.*, 2007) and a promising candidate gene for OC, however, a function related to BF is not clear. It is also possible that two closely linked genes are causing the common QTL region on SSC14. The fine mapping of this region also listed several other potential candidate genes with high impact score, but further studies are needed to pinpoint the causal gene.

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