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A genome-wide scan reveals novel loci associated with liability to scrotal and inguinal hernia in Large White pigs

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ABSTRACT: A genome-wide study (GWAS) was performed to identify genomic regions associated with variation in hernia liability in pigs. Breeding values were estimated for a Large White and a Landrace line based on offspring phenotypes (1,359,765 purebred and crossbred male animals) using a binary trait animal model. The incidence of hernia in the evaluated population was on average 0.42% and the heritability estimate was 0.31±0.01. Estimated breeding values were deregressed for 2,750 Large White animals that were genotyped using the Porcine SNP60 Beadchip. Deregressed estimated breeding values (DEBV) were taken as the response variable in the GWAS. The GWAS discovered 10 SNPs associated with hernia in five QTL regions. The most significant SNPs per region explained between 1.22% and 1.60% of the total phenotypic variance. Four genes (*CYP19A1, RHOA, EGF, LEF1*) were proposed as candidate genes for this trait.
Keywords: hernia; genome wide association study; pigs

Introduction

Scrotal and inguinal hernias are the most frequent congenital defects in pigs. Incidences range from 0.39% to 1.09% (Thaller et al. (1996); Larzul et al. (2008); Mattsson (2011)). An inguinal hernia occurs when part of the small intestine passes through the internal inguinal ring and is present in the inguinal canal. A scrotal hernia occurs when part of the small intestine passes all the way through the inguinal canal and enters the scrotum (Grindflek et al., (2006); Zhao et al. (2009)). The distinction between inguinal and scrotal hernias cannot easily be made without clinical examination (Du et al., 2009), therefore these two traits were considered as a single trait, hereafter called 'hernia'. Identification of genomic regions controlling hernia is of great interest to breeding programs, both to improve animal welfare as well as for economic reasons.

The use of deregressed estimated breeding values (DEBV) obtained from a binary trait model is considered a powerful approach for difficult traits like hernia because of its low incidence and affected animals typically not being available for genotyping (Ostersen et al. (2011)).

The aim of this study was to identify SNPs with significant association with hernia using DEBV.

Materials and Methods

Phenotypes. Phenotypes included 1,359,765 records of purebred and crossbred male offspring from Large White (LW) and Landrace (LR) sow lines recorded on 52 different farms. Presence of hernia was recorded as either 1 (affected) or 0 (not-affected). In addition, the year and season of birth, number of littermates, and parity of the mother was recorded.

Genotypes. For the purpose of GWAS the genotyped animals were selected to be ancestors of the phenotyped animals. LW animals (608 males and 1,962 females) were genotyped using the Porcine SNP60 Beadchip. Quality filtering of the genotype data was done in two steps. In step one, SNPs with GenCall score <0.15, located on SSCY, with unknown position on the genome build10.2 reference (Groenen et al. (2012)), and animals with a missing genotype frequency ≥0.30 were removed. In step two SNPs with a call rate <0.05, minor allele frequency <0.01 and/or a strong deviation from Hardy Weinberg equilibrium ($\chi 2>600$) were removed. For males, SNPs on SSCX outside the pseudoautosomal regions were also removed if the frequency of heterozygous calls was >0.05. Animals with missing genotype frequency ≥0.05 were removed in step two.

Genotyped parent-offspring pairs were checked for Mendelian inconsistencies. Offspring with >1% Mendelian inconsistencies were excluded. Parents with >1% Mendelian inconsistencies with each of their offspring were excluded as well. The final genotype data consisted of 2,102 animals and 38,632 SNPs.

Pedigree. For phenotyped animals, pedigree was collected up to 20 generations. The pedigree data contained 1,434,713 animals.

Breeding Value Estimation. A binary single trait animal model was used to estimate the heritability and breeding values (EBV) by restricted maximum likelihood methodology implemented in the software ASReml (Gilmour et al. (2009)). Because a binary model was fitted, the effects evaluated in this analysis were calculated taking into account the underlying logistic scale using the Logit link function (Gilmour et al. (2009)). The following model was applied:

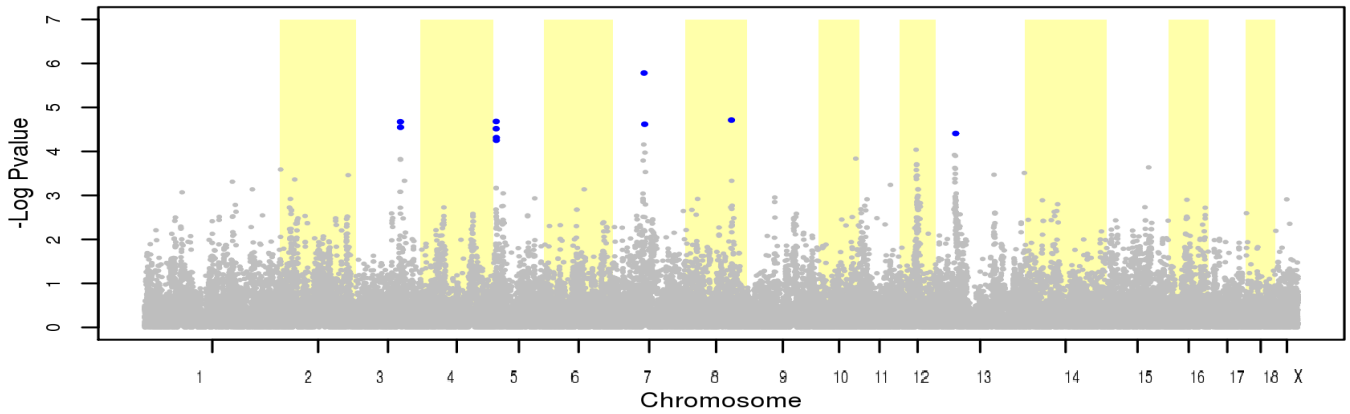

Figure 1. Associations between hernia and 38,632 genome-wide SNP. -log10 of P values (y-axis) are plotted against the genome position (x-axis) for each SNP. Blue dots represents SNPs that surpassed the FDR ≤ 0.20 threshold.

$Y_{ijklmn} = \mu + HYS_i + TNB_j + P_k + LL_l + c^2{}_m + a_n + e_{ijklmn}$ (1) where Y_{ijklmn} was the hernia status of male offspring n, HYS_i was the fixed effect of herd-year-season of birth (1194 classes), TNB_j was the fixed effect of number of littermates (30 classes), P_k was the fixed effect of the parity of the mother (7 classes) and LL_l was the fixed effect of the type of litter (4 classes, LW_{pure}, LW_{cross}, LR_{pure}, LR_{cross}). The random effects included the common litter effect ($c^2{}_m$) assumed to be normally distributed ~N(0, $I\sigma_c^2$), where I is a known identity matrix and σ_c^2 is the unknown variance between litters, and the additive genetic effect (a_n) assumed to be normally distributed, N(0, $A\sigma_a^2$), where A was a known matrix of additive genetic relationship among animals and σ_a^2 was the unknown genetic variance between animals. The residual error (e_{ijklmn}) was defined on the logistic scale, setting the residual variance to 1.

The EBV obtained with model 1 were deregressed according to Garrick et al. (2009), whereby parents average effects were removed as part of the deregression process. A weighting factor (w) was estimated based on the reliability of the calculated DEBV. A value of 0.5 was assumed for the scalar c, following the approach of Garrick et al. (2009).

Association analysis. A GWAS was performed in the software ASReml (Gilmour et al. (2009)) applying the following model:

$$DEBV_{ij}\, w = \mu + SNP_i + a_j + e_{ij} \qquad (2)$$

where $DEBV_{ij}$ is the observed DEBV for genotyped animal j, μ is the overall mean DEBV of the genotyped animals, SNP_i is the SNP genotype coded as 0, 1 or 2 copies of one of the alleles, a_j is the additive genetic effect and e_{ij} the residual error. The weighting factor w was used in the GWAS to account for the differences in the amount of offspring information available for the estimation of the DEBV (Garrick et al. (2009)).

Animals with a weighting factor higher than zero and a minimum reliability of 0.08 (1,361 LW animals) were included in the GWAS.

To account for multiple testing a false discovery rate (FDR) implemented in the R package 'qvalue' (Dabney et al. (2010)) was applied. A FDR ≤ 0.20 was set to define significant associations.

QTL regions. QTL regions were defined starting at the first significant SNP and continued until no significant SNP was found within the next 10 Mbp from the last significant SNP. Multiple QTL regions could be defined on the same chromosome when the distance between consecutive significant SNPs was more than 10 Mbp. The genetic variance explained by each QTL was calculated using the estimated allele substitution effect obtained from model 2 and the observed allele frequencies of the most significant SNP of each QTL region. The result was expressed as percentage of the phenotypic variance explained by the SNP ($\frac{\sigma^2_{SNP}}{\sigma^2_P} \times 100$).

Candidate genes. Candidate genes were searched for in the QTL regions and the neighboring upstream and downstream 2 Mpb regions based on the NCBI *Sus scrofa* build 3.1 (NCBI (2014)). The gene functions were examined by reviewing the literature.

Results and Discussion

Quantitative analysis. The incidence of hernia was on average 0.42% and the heritability was estimated to be 0.31±0.01. Estimating the heritability of binary traits is not straight forward and estimates reported for hernia vary from 0.03 to 0.27, in different populations (Thaller et al. (1996); Ranberg (2007); Larzul et al. (2008)). Contradictory results on the heritability of other binary traits have been reported, although the factors influencing these differences remain unclear (Villemereuil et al. (2012)). The proportion of variance due to common litter effects, c^2, was estimated

to 0.53. A significant c^2 has been reported before and was shown to remarkably improve the fit of a binary model for congenital defects in pigs (Thaller et al. (1996)). The large c^2 suggests that the intrauterine, and possible postnatal, environments substantially influenced this type of disorder (Thaller et al. (1996)).

QTL regions and candidate genes. Distributed over 5 QTL regions, 10 significant SNPs were found to be associated with hernia (Figure 1). The five QTL regions were located on SSC3, SSC5, SSC7, SSC8 and SSC13 (Table 1). The most significant SNPs of each QTL region identified in this study explained between 1.22% and 1.60% of the total variance of hernia incidence (Table 1). In general, the proportion of total variance explained by significantly associated SNPs was low.

Table 1. Regions associated with hernia (FDR ≤0.20). Genome position, number of SNPs and the proportion of the phenotypic variance explained by the most significant SNP per region.

Chr	Position (Mbp)	SNP per region	Proportion (%)
SSC3	104.46 - 104.54	2	1.29
SSC5	5.04 – 5.36	4	1.22
SSC7	53.76 – 54.48	2	1.60
SSC8	119.72	1	1.31
SSC13	34.53	1	1.23

The three regions identified on SSC3, SSC5 and SSC8 do not overlap with previous results, although other regions on these chromosomes have previously been reported to harbor QTL for hernia (Ding et al. (2009); Grindflek et al. (2006); Knorr et al. (2006); Stinckens et al. (2011)). In the QTL region on SSC8 the candidate genes pro-epidermal growth factor precursor (*EGF*) and lymphoid enhacer-binding factor 1 (*LEF1*) are located. Mutations in *EGF* have been related to connective-tissue problems, such as inguinal hernia (Schrijver et al., 1999). *LEF1* is associated with β-catenin which mediate anti-mullerian hormone (*AMH*) (Allard et al. (2000)). *AMH* is involved in the swelling reaction of gubernaculum occurring during the first phase of testicular descent (Grindflek et al. (2006); Zhao et al. (2010)).

The region on SSC7 has previously been reported to be associated with hernia. The SSC7 QTL overlaps with the region previously identified by Grindflek et al. (2006) and Knorr et al. (2006). The gene cytochrome P450 family 19A1 (*CYP19A1*) was a candidate gene for this QTL, suggested by Grindflek et al. (2006) based on its location in the homologous region in human. However, based on the current pig genome build 10.2 (Groenen et al. (2012)) this gene is located on SSC1. No other genes in this region are proposed as candidates for this QTL.

The QTL region identified on SSC13 has been previously reported by Grindflek et al. (2006) and Ding et al. (2009). In this region the gene ras homolog family member A (RHOA) is located, that regulates the contraction and shortening of smooth muscle tissues (Zhang et al., 2012). Development of inguinal hernia is readily explained by the persistence of smooth muscle component around the processus vaginalis after the descent of the testis into the scrotum (Hosgor, M., et al. (2004)). The most significant SNP located in this region accounted for a modest proportion of the total variance (1.23%).

Differences in power of the current study and studies from Grindflek et al. (2006) and Ding et al. (2009) may contribute to finding different regions on SSC3, SSC5, SSC8. Stinckens et al. (2011) was the only previous study employing the Porcine SNP60 Beadchip to analyze hernia in a different Large White population. A QTL for hernia was reported on SSC5, without specifying its location. They also reported associations on SSC6 and SSCX, which were not confirmed in our study.

Conclusion

The use of DEBVs in combination with a binary trait model appears to be a powerful approach for difficult traits like hernia that have low incidence and where affected animals are typically not available for genotyping. Novel QTL regions were detected on SSC3, SSC5, and SSC8, while previously know QTL regions were narrowed considerably.

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