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A genome-wide association study reveals a novel candidate gene for sperm motility in pigs

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ABSTRACT

Sperm motility is one of the most widely used parameters in order to evaluate boar semen quality. However, this trait can only be measured after puberty. Thus, the use of genomic information appears as an appealing alternative to evaluate and improve selection for boar fertility traits earlier in life. With this study we aimed to identify SNPs with significant association with sperm motility in two different commercial pig populations and to identify possible candidate genes within the identified QTL regions. We performed a single-SNP genome-wide association study using genotyped animals from a Landrace-based (L1) and a Large White-based (L2) pig populations. For L1, a total of 602 animals genotyped for 42,551 SNPs were used in the association analysis. For L2, a total of 525 animals genotyped for 40,890 SNPs were available. After the association analysis, a false discovery rate q-value $\leq$0.05 was used as the threshold for significant association. No SNPs were significantly associated with sperm motility in L1, while six SNPs on *Sus scrofa* chromosome 1 (position 117.26–119.56 Mb) were significant in L2. The mitochondrial methionyl-tRNA formyltransferase (*MTFMT*) gene, which affects translation efficiency of proteins in sperm cells, was identified as a putative candidate gene. The significant markers identified in this study may be useful to enhance the genetic improvement of sperm motility by selection of boars at an earlier age under a marker assisted selection strategy.

1. Introduction

Sperm motility is defined as the proportion of moving sperm cells in an ejaculate and it has been described as a useful indicator of sperm fertilizing ability (Gadea, 2005; Broekhuijse et al., 2012). Such a relationship with fertilizing capacity has made this trait one of the most widely used parameters in sperm quality evaluations.

Traditional sire pig breeding programs have based their selection mainly on production traits, neglecting the potential benefits of selection for improved boar semen traits on fertility. According to Robinson and Buhr (2005), commercially important traits, such as growth rate, feed efficiency

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and lean yield, should not be the unique criteria for selection, since this approach can result in reduced semen production due to the negative correlations between some production and semen traits (Oh et al., 2006).

One of the major limitations to include fertility and sperm quality traits in breeding programs is their measurement after puberty, which prolongs the generation interval. Thus, molecular information can be used to evaluate and improve selection for boar fertility traits earlier in life (Kaewmala et al., 2011). In this context, quantitative trait loci (QTL) linkage mapping based on microsatellite markers has already been applied to study sperm motility in pigs (Xing et al., 2009). However, the large identified QTL regions and the mapping dependency on the considered resource families (Rothschild et al., 2007) limited the expected success. Moreover, candidate genes have been proposed (Lin et al., 2006; Kaewmala et al., 2011; Gunawan et al., 2011, 2012) based on prior knowledge of their physiological functions in order to elucidate questions about the genetic control of sperm motility. Nevertheless, because of the complexity of this trait, with several biological mechanisms involved in its expression, some genes can be chosen under a high level of subjectivity.

Genome-wide association studies (GWAS) can overcome these limitations using high-throughput genotyping technologies to assay the Single Nucleotide Polymorphisms (SNPs) and relate them to traits of interest (Pearson and Manolio, 2008). The density of the Illumina PorcineSNP60 BeadChip (Ramos et al., 2009) is sufficient to find SNPs in linkage disequilibrium (LD) with causative mutations at population level (Zhang et al., 2012), resulting in QTL detection at high resolution (Duijvesteijn et al., 2014). Additionally, posterior studies can be done by exploiting the QTL regions through online database tools (for example NCBI, Emsembl, Pig QTL database, and others) in order to find candidate genes inside them, thus avoiding subjective gene selection.

GWAS have been performed in pigs for many traits, such as boar taint (Duijvesteijn et al., 2010), body composition (Fan et al., 2011), sow reproductive traits (Onteru et al., 2012) and feeding behavior (Do et al., 2013). However, to date, GWAS applied to boar sperm motility has not been reported yet, and its implementation is essential to better understand the genetic architecture of this trait that is relevant for both pig breeding and reproduction. Toward this orientation, we aimed to identify SNPs with significant association with sperm motility in two different commercial pig populations and to identify possible candidate genes within the QTL regions.

2. Materials and methods

2.1. Ethics statement

The data used for this study were obtained as part of routine data recording in a commercial breeding program. Samples collected for DNA extraction were only used for routine diagnostic purposes of the breeding program. Data recording and sample collection were conducted strictly in line with the Dutch law on the protection of animals (Gezondheids-en welzijnswet voor dieren).

Table 1
Descriptive statistics of sperm motility.

Line	Number of observations	Number of animals	Mean (%)	SD	Min	Max
L1	32,884	760	86.00	7.36	10.00	99.00
L2	32,576	645	85.85	7.50	10.00	99.00

Number of observations, number of animals, mean, standard deviation (SD), minimum (min) and maximum (max) values of sperm motility in the two populations evaluated (L1 = Landrace-based and L2 = Large White-based).

2.2. Animals and phenotypes

The phenotypic data consisted of repeated observations of fresh sperm motility of boars from two commercial lines: L1 (Landrace, $n = 760$) and L2 (Large White, $n = 645$). The ejaculates were collected from October of 2006 to December of 2012 and evaluated using the CASA method – Computer Assisted Sperm Analysis (Amann and Katz, 2004). The CASA equipment calculates the estimated percentage of motile cells in a continuous range of results, in other words, it provides the exact number of sperm cells that were considered to be motile or immotile (Broekhuijse et al., 2011) and measures the proportion of these cells in the ejaculate, which ranges from 0% to 100%. The motility is calculated according to some specific parameters, as velocity of movement, the width of the sperm head's trajectory and frequency of the change in direction of the sperm head (Mortimer, 2000; Broekhuijse et al., 2012).

The phenotypic data consisted of 32,884 ejaculates for L1 and 32,576 ejaculates for L2 (Table 1). The number of ejaculates per boar was on average 43.27 ± 36.81 for L1 and 50.51 ± 39.15 for L2. In both lines the mean sperm motility was around 86%, ranging from 10% until 99% – only observations of sperm motility $\geq$10% were included in the dataset because observations <10% could be caused by evaluation errors (Table 1). The complete pedigree file (up to 10 generations) contained a total of 38,425 animals for L1 and 38,994 animals for L2.

2.3. Assessment of pseudo-phenotypes (deregressed EBVs)

The estimated breeding value (EBV) of each animal was obtained using a repeatability animal model in ASReml 3.0 (Gilmour et al., 2009). The analysis were performed within line applying the following model:

$$y_{ijkl} = \mu + AI^{*}YW_i + \mathrm{lab}_j + b_1 * \mathrm{conc}_{ijkl} + b_2 * \mathrm{int}_{ijkl} + b_3 * \mathrm{age}_{ijkl} + p_k + a_k + e_{ijkl}, \quad (1)$$

where y_{ijkl} is the phenotypic measurement (fresh sperm motility); μ is the overall population mean; $AI^{*}YW_i$ is the combined effects of AI station, year and week of semen collection; lab_j is the effect of the laboratory where the samples were analyzed; conc_{ijkl} is a covariate of the semen concentration; int_{ijkl} is a covariate of the effect of the interval between collections; age_{ijkl} (months) is a covariate of