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Potential Application of Genomics to Reduce Boar Taint Levels in Three Canadian Swine Breeds

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ABSTRACT: The objective of this study was to investigate the possibility of reducing the amount of androstenone and skatole in fat tissues of intact males using genetic markers. A total of 3,474 pigs were genotyped for 97 SNP markers from which 61, 80 and 83 of genotyped SNPs were polymorphic (MAF>0.05) in Duroc, Landrace and Yorkshire pigs, respectively. Approximately 51% and 5% of Duroc, 27% and 15% of Landrace, and 24% and 11% of Yorkshire pigs had androstenone and skatole levels that were above consumer acceptance thresholds, respectively. A two-step validation analysis was performed to examine the association of SNPs with androstenone and skatole levels. A number of SNPs had significant association with androstenone and skatole levels in each of the breeds. Using genetic markers for selection of breeding animals against high levels of boar taint is promising.
Keywords: pig; boar taint; genomics

Introduction

Boar taint is in an unpleasant odour and flavour emanating from meat originating from intact male pigs. Castration of young piglets is common practice in Canada and is effective in preventing boar taint. However, there is increasing interest in raising pigs without castrating males. Boar taint is caused by the accumulation of androstenone and skatole in fat tissues. Androstenone is a steroid produced in the testes as the boar nears puberty and it acts as a sex pheromone to regulate reproductive development in gilts and induce a mating stance in sows. Skatole results from the bacterial breakdown of tryptophan in the gut (reviewed in Zamaratskaia and Squires, 2009). Genetic selection aiming to reduce boar taint to an acceptable level for consumers offers a potential solution to this emerging issue. Previous studies have shown that these two boar taint compounds are moderately to highly heritable, suggesting that genetic selection to reduce boar taint in intact males is possible. At the University of Guelph, researchers previously identified genetic markers for boar taint in candidate genes that code for enzymes involved in the synthesis and degradation of boar taint compounds - androstenone and skatole. The objective of this study was to investigate the possibility of reducing androstenone and skatole levels in fat tissues of swine using genetic markers.

Materials and Methods

Animal sampling and genotyping. A total of 3,297 Canadian Duroc (n=976), Landrace (n=1128) and Yorkshire (n=1193) boars were sampled and genotyped for 97 SNPs located in 40 candidate genes. A fat sample was collected from animals at the slaughter plant or *via* biopsies (Baes et al, 2013) and sent to the laboratory for DNA extraction and to measure levels of boar taint compounds. The weight of the animal at sampling was recorded if available or it was estimated from the weight recorded at scanning.

Boar taint measurements: Fat samples were processed to measure 5α-androstenone and skatole levels using an Enzyme Linked Immunoassay (ELISA) (Squires and Lundström (1997)) and high performance liquid chromatography (HPLC) with fluorescence detection (Lanthier et al. (2007)), respectively. The number of animals for which a fat sample was processed included 644 Duroc, 837 Landrace, and 871 Yorkshire.

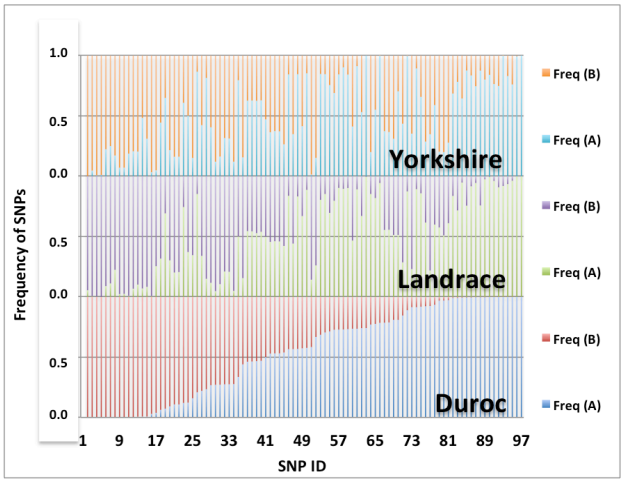
Analysis. A two-step analysis was performed using the SAS GLM and REG procedures (2002-2008), respectively. Since distributions of both compounds were highly skewed, the natural logarithms of androstenone and skatole were calculated and used as phenotypes. SNPs were coded as 0, 1 and 2 for AA, AB and BB coded genotypes, respectively. Data and SNP filtering were also performed prior to data analysis. SNPs with minor allele frequencies (MAF) less than 0.05 within breed were excluded. Animals weighing less than 90 kg were excluded since they may not have reached sexual maturity. Animals heavier than 150 kg and older than 300 days were also excluded due to their age and the potential reduction of boar taint compounds over time in older animals. The SAS GLM procedure was used to adjust phenotypes for season, as well as for boar's age and weight at time of sampling. Residuals of the GLM procedure were then used in the second phase of analysis where the SAS REG procedure was used to identify the best fitting model. SNPs were included in the analysis of androstenone and skatole as covariates using backward elimination model selection. SNPs with p>0.10 were eliminated at every step of the backward elimination.

To test the effectiveness of using genetic markers for selection against boar taint, 80% of the older boars with boar taint measures (training group) were used to determine which markers associated with the traits, and the remaining 20% of the younger boars with boar taint measures were used for validation. The numbers of animals in training and validation groups were 480 and 110 in Duroc, 564 and 134 in Landrace, and 550 and 141 in Yorkshire breed, respectively. Within each training group, the backward elimination regression model was used to find the best model as previously explained. Given the high observed levels of androstenone in Duroc and high levels of skatole in Landrace and Yorkshire breeds, the association of SNPs with androstenone in Duroc and with skatole in Landrace and Yorkshire breeds were tested. The number of unfavorable alleles in the significant SNPs were counted for each of the animals in the validation group and then correlated to their boar taint compound levels.

Results and Discussion

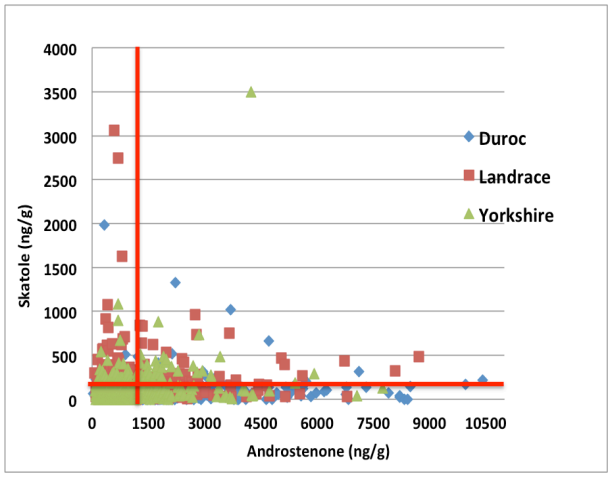
Data editing. To select for boar taint, polymorphic markers that are associated with boar taint compounds are needed. From an initial set of 124 SNPs, 97 SNPs were chosen for further analyses (Figure 1). Following SNP filtration for MAF<0.05, the number of markers remaining was 61, 80 and 83 for Duroc, Landrace and Yorkshire pigs, respectively.

Figure 1: Frequency of 97 markers tested within each breed



Boar taint compounds. The distribution of androstenone and skatole levels is plotted in Figure 2. Consumer acceptance levels of 1000 ng/g androstenone and 200 ng/g skatole in fat samples were based on Walstra et al. (1999). About half of the Duroc pigs and one quarter of the Landrace and Yorkshire pigs had androstenone levels exceeding the consumer acceptance level. Approximately 95% of the Duroc pigs had skatole levels below the consumer acceptance level. In comparison to Duroc, levels of skatole were higher in Landrace and Yorkshire breeds (Table 1). Across all the three breeds, about 5% of boars were above the consumer acceptance levels for both measured compounds, 28% were too high for androstenone, but were acceptable for skatole, 6% were too high for skatole, but were acceptable for androstenone and 61% were acceptable for both compounds.

Figure 2: Distribution of androstenone and skatole in sampled pigs



The Horizontal and vertical red lines specify the consumer acceptable level of 200 ng/g and 1000 ng/g for skatole and androstenone, respectively.

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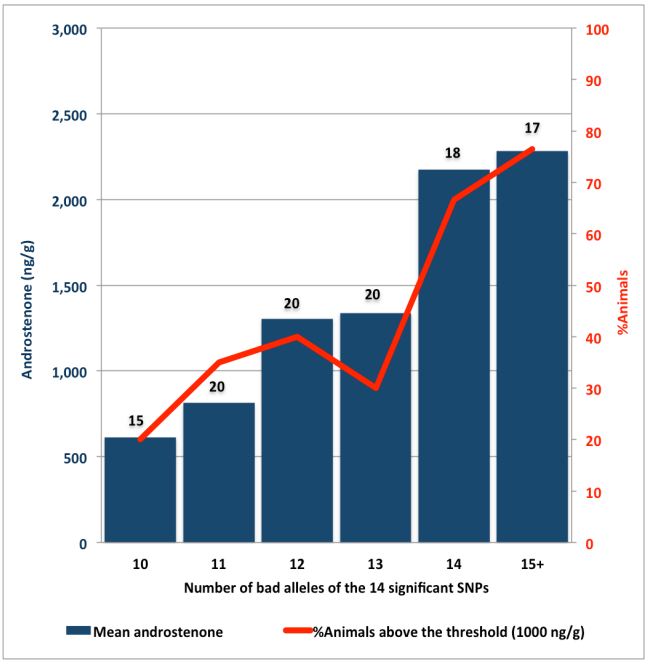
Effectiveness of the SNP markers. The best models included 14, 23 and 12 SNPs for the Duroc (androstenone), Landrace (skatole) and Yorkshire (skatole) breeds, with an R-square of 22%, 14% and 14%, respectively. The number of unfavorable SNP alleles which had a significant effect on increasing boar taint was counted and the boars were grouped based on this number. Levels of each boar taint compound were plotted against the number of unfavorable SNP alleles (Figure 3). In Duroc pigs, the number of unfavorable alleles were significantly correlated (r= 0.33, p<0.001) with androstenone levels in fat. The number of unfavorable alleles was significantly correlated (r= 0.23, p<0.01) with skatole levels in Landrace pigs (Figure 4). No

Table 1. Descriptive statistics on androstenone and skatole levels in Canadian boars*

Boar taint compound	Breed		
	Duroc	Landrace	Yorkshire
Androstenone (ng/g)			
Number of animals	627	768	805
Mean	1,650	954	840
Range	35-18,820	65-13,748	75-7,470
%Unacceptable	51	27	24
Skatole (ng/g)			
Number of animals	606	725	755
Mean	70	124	94
Range	0-1,986	0-3,062	0-3,502
%Unacceptable	5	15	11

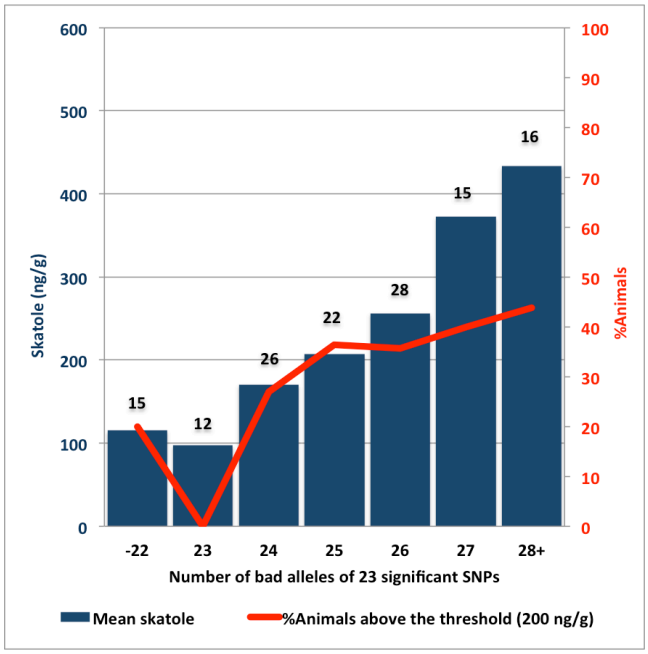
*Statistics based on all the sampled boars before excluding light weight and very old or heavy individuals.

Figure 3. Relationship between number of unfavorable alleles of selected SNPs and levels of androstenone in the Duroc validation group



Number of animals in each group is shown on top of each bar. The 15+ group includes 9, 6 and 2 animals that were found to have 15, 16 and 17 unfavourable alleles, respectively

Figure 4. Relationship between number of unfavorable alleles of selected SNPs and levels of skatole in the Landrace validation group



Number of animals in each group is shown on top of each bar. The -22 group includes 4 and 11 animals with 21 and 22 unfavorable alleles, respectively. The 28+ group includes 10, 4 and 2 animals that had 28, 29 and 30 unfavorable alleles, respectively.

significant correlation was found between the number of unfavorable alleles and skatole levels in Yorkshire pigs. This might be due to relatively small proportion of Yorkshire pigs (11%) with high levels of skatole.

The selected subset of the genetic markers for each boar taint compound could be used together with national genetic values to calculate markers assisted EBVs (MEBV), which could be used in a selection index to decrease the androstenone and skatole levels in purebred animals. The current results offer a promising application with regards to using SNP markers to reduce boar taint in Canadian pigs.

Conclusion

Results suggest that using genetic markers for selection of the breeding animals against the high levels of boar taint is possible. With recent advances in genomic technology, it is becoming more affordable to genotype animals for SNP markers associated with boar taint levels. More phenotypes and genotypes on animals would increase the accuracy of predictions. The efficiency of using markers to decrease boar taint in purebreds must be validated in commercial crossbred pigs and possible negative impacts of markers on production traits should be considered.

Acknowledgements

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