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INVESTIGATION OF DNA METHYLATION ON PORCINE REFERENCE SEQUENCE GENES FOR BOAR TAINT TRAIT

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Abstract

Boar taint (BT) is an offensive flavor in non-castrated male pigs. However, studies of genome-wide DNA methylation profiles on all reference sequence genes to reveal epigenetic information associated with BT was scarce. Reduced representation bisulfite sequencing (RRBS) is an efficient technology to identify candidate epigenetic biomarkers associated with BT. Three different BT levels were analyzed using RRBS data to calculate differentially methylated genes (DMGs). In this study, we found 411 DMGs (Q-value < 0.01) out of 4566 reference genes. Hyper-methylation DMGs were revealed to be mainly enriched in five significant pathways (qvalue < 0.05). These results could contribute to the understanding of methylation levels on reference sequence genes in pigs and the usage of genomic selection for low BT in the breeding programs.

Keywords: boar taint, DNA methylation, reduced representation bisulfite sequencing, differentially methylated genes

INTRODUCTION

The offensive odor and/or flavor of boar taint (BT) in porcine meat is primarily caused by the accumulation of skatole (Patterson, 1968) and androstenone (Gower, 1972) in a percentage of non-castrated male pigs (boars). Surgical castration is an effective solution to avoid BT but results in reduced feed conversion efficiency, leaner carcass values and increased fecal and urinary nitrogen, in addition to animal welfare concerns (Bonneau, 1998; Claus *et al*., 1994). As levels of skatole and androstenone traits exhibit moderate to high heritability (Strathe *et al*., 2013a, 2013b), selection of low genetic merit of BT can be an effective approach to avoid BT and other disadvantages of surgical castration. Recent studies identified and exploited genetic variation of androstenone and skatole (Rowe *et al*., 2014) and predicted genomic breeding values for genomic selection (de Campos *et al*., 2015). In addition, gene expression and expression quantitative trait loci (eQTLs) profiles associated with BT in pigs were revealed by system genomics analyses (Drag *et al*., 2017, 2018).

As an important epigenetic modification, DNA methylation has been examined to be associated with growth (Jin *et al*., 2014), immune response (Wang *et al*., 2017) and reproduction traits (Bell *et al*., 2011) in pigs. Reduced representation bisulfite sequencing (RRBS) has been implemented to analyze patterns of DNA methylation by reducing the portion of the genome digestion (Meissner *et al*., 2005). The RRBS method primarily focuses on the enrichment of CpG-rich regions rather than the non-CpG regions (Meissner *et al*., 2005). In mammals, DNA methylation almost exclusively occurs at CG dinucleotides with 70-80% throughout the genome (Ehrlich *et al*., 1982; Law and Jacobsen, 2010). Currently, RRBS analysis of the pigs has been presented using intestinal tissue (Gao *et al*., 2014), ovaries (Yuan *et al*., 2016), neocortex, liver, muscle and spleen (Choi *et al*., 2015) and prepubertal (Chen *et al*., 2018) and adult (Wang and Kadarmideen, 2019) testis.

Gene information from Reference Sequence (RefSeq) database (Pruitt *et al*., 2007) is used as the reference standards for well-characterized genes. To our knowledge, no studies have investigated the genome-wide DNA methylation profiles using RRBS data on all reference sequence genes (RefSeqGenes) to reveal epigenetic information associated with BT. Therefore, the aim of this study is to analyze differentially methylated genes (DMGs) based on RefSeqGene in three different (high, medium and low) BT levels and then reveal candidate DMGs for further epigenetic application in pig breeding programs for low BT levels.

MATERIALS AND METHODS

The study was carried out using three groups of high (n = 3), medium (n = 3) and low (n = 3) BT levels based on the most extreme estimated breeding values (EBVs) within high and low groups and the closest mean values in the medium group. The EBVs of low, medium and high BT were calculated by summing the EBVs of skatole concentration and human nose score (HNS). Nine pigs were slaughtered at a

commercial slaughterhouse (Danish Crown, Herning, Denmark), and 150 mg testis tissue samples for each pig were retrieved by punch biopsy for RRBS sequencing.

RRBS adapters and reads less than 20 bases long were trimmed by *Trimmomatic* software (Bolger *et al*., 2014). Then, *Bismark* software (Krueger and Andrews, 2011) was applied to map clean reads to the pig reference genome (Sscrofa11.1/susScr11), and then determine the methylation levels for each cytosine site.

The DMGs were defined for significant genes after the comparisons against methylation levels on RefSeqGenes using R package *GeneDMRs* (https://github.com/xiaowangCN/GeneDMRs). To account for multiple hypothesis testing, P-values can be adjusted to Q-values by false discovery rate (FDR) method (Hochberg, 1995). RefSeqGene database (Sscrofa11.1/susScr11) used in this study was downloaded from the University of California Santa Cruz (UCSC) website (http://genome.ucsc.edu/cgi-bin/hgTables).

The pathway terms were analyzed in hypo-methylated and hyper-methylated categories based on R package *clusterprofiler* (Yu *et al*., 2012) and *KEGG.db* (version 3.2.3) of *Sus scrofa* organism. According to R package *GeneDMRs*, the hypo-methylated and hyper-methylated DMG was defined when the methylation difference between low and high BT levels was positive and negative, respectively.

RESULTS AND DISCUSSION

In this study, a total of 4566 RefSeqGenes were used for analysis. Finally, 2772 genes of them with annotated genomic position and cytosine sites were used for DMG detection. After statistical analysis, 411 genes were defined as DMGs with Q-values less than (<) 0.01. Compared to the distribution of 2772 genes on porcine genome, the percentages of 411 DMGs on chromosome 3 and chromosome 17 showed the larger ratios than before, but DMGs on chromosome X were not detected (Figure 1A and Figure 1B). In addition, hypo-DMGs were mostly located on chromosome 17, while hyper-DMGs were mostly located on chromosome 6 (Figure 1C and Figure 1D).

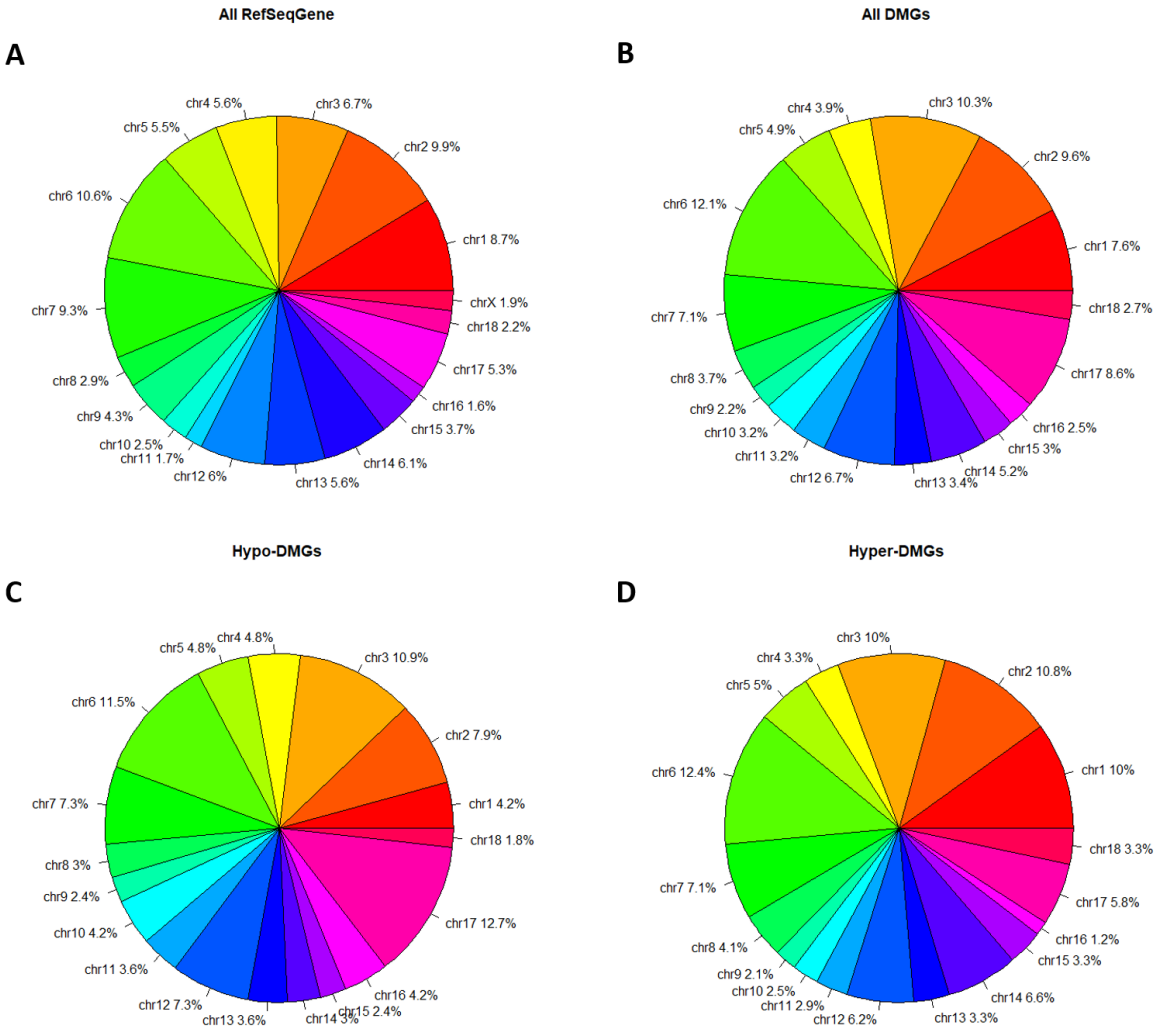

Figure 1. Percentages of all (**A**) RefSeqGene, (**B**) DMGs (Q-value < 0.01), (**C**) hypo-DMGs (Q-value < 0.01) and (**D**) hyper-DMGs (Q-value < 0.01) in different chromosomes.

Our results revealed that most of the DMGs were enriched in hyper-methylated category and hypo-methylated DMGs were seldom enriched (Figure 2). Five pathways of them were significant (qvalue < 0.05) including cellular senescence, Th17 cell differentiation, AMPK signaling pathway, FOXO signaling pathway and chronic myeloid leukemia (Table 1). In a period of cellular proliferation or a rapid manner in response to acute stress, senescence usually occurs. The cellular senescence is activated by normal cells in response to various types of stress including telomere uncapping, DNA damage, oxidative stress and oncogene activity (Ben-Porath and Weinberg, 2005). In our study, 11 DMGs (NM_001038639, NM_001113452, NM_001123080, NM_001135959, NM_001145750, NM_001244550, NM_001285967, NM_001287846, NM_214015, NM_214124 and NM_214128) were involved in the cellular senescence pathway (qvalue = 0.0026). Additionally, T helper 17 cells (Th17), a subset of pro-inflammatory T helper cells, can produce interleukin 17 (IL-17). Th17

are related to T regulatory cells because the signals that cause Th17s to differentiate actually inhibit T regulatory cells differentiation (Hartigan-O'Connor *et al*., 2011). AMP-activated protein kinase (AMPK) is one of the central regulators of cellular and organismal metabolism. It has critical roles in regulating growth, reprogramming metabolism and cellular processes (Mihaylova and Shaw, 2011). Forkhead box O (FOXO), one subfamily of the fork head transcription factor family, plays important roles in cell fate decisions and in a wide range of cancers as a tumor suppressor (Farhan *et al*., 2017). Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder characterized by the presence of BCR-ABL, while BCR-ABL is an oncogene created by the fusion of *BCR* and *ABL* genes (Rowley, 1973). The juxtaposition of these genes encodes a novel fusion gene that translates into a protein with constitutive TK activity when they are in response to genetic mutation (Sinclair *et al*., 2013).

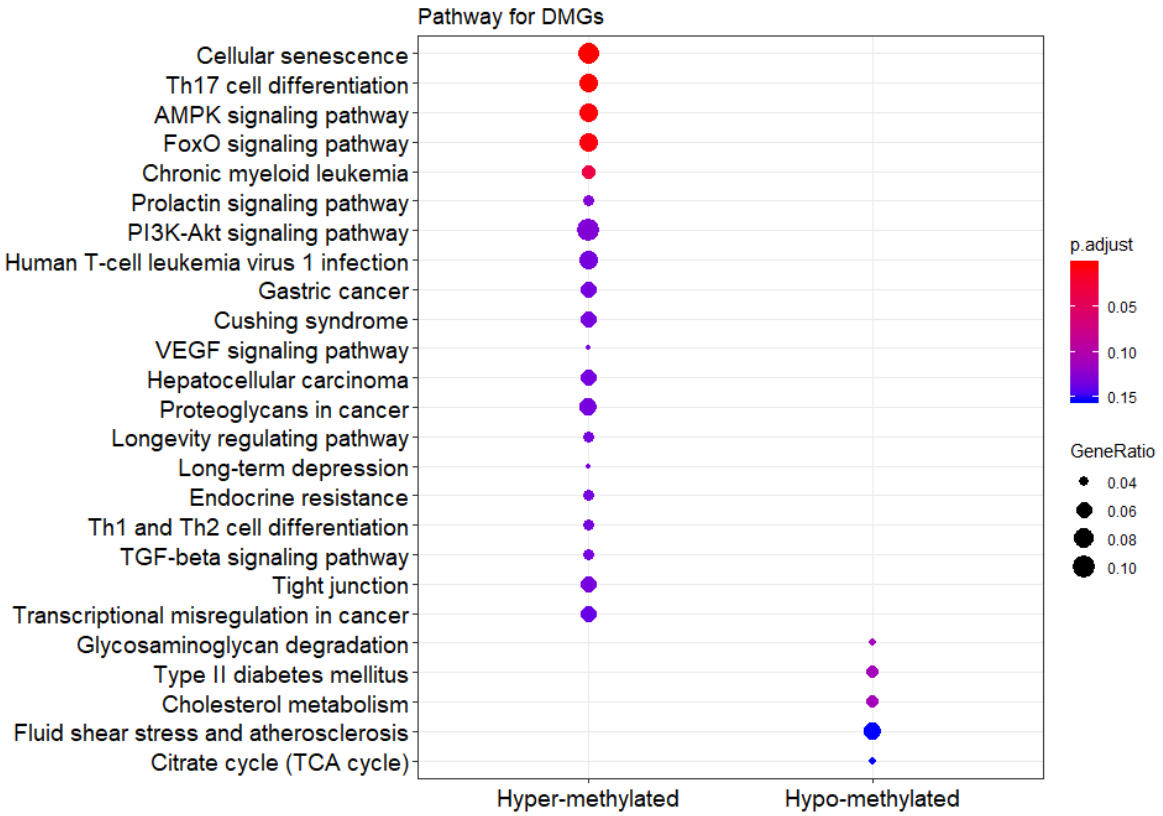

Figure 2. Pathways of the DMGs in the hypo-methylated and hyper-methylated categories.

Table 1. Significant pathways (qvalue < 0.05) for DMGs.

Pathway	Description	pvalue	p.adjust	qvalue	GeneRatio	Genes
ssc04218	Cellular senescence	1.84e-05	0.0031	0.0026	11/119	NM_001038639/NM_001113452/NM_001123080/NM_001135959/NM_001145750/NM_001244550/

						NM_001285967/NM_001287846/NM_214015/ NM_214124/NM_214128
ssc04659	Th17 cell differentiation	3.02e-05	0.0031	0.0026	9/119	NM_001038639/NM_001044567/NM_001113452/ NM_001246252/NM_001253352/NM_001315722/ NM_214015/NM_214128/NM_214290
ssc04152	AMPK signaling pathway	7.99e-05	0.0055	0.0047	9/119	NM_001135959/NM_001145750/NM_001243060/ NM_001243802/NM_001244062/NM_001287846/ NM_214025/NM_214157/NM_214172
ssc04068	FOXO signaling pathway	1.39e-04	0.0072	0.0061	9/119	NM_001038639/NM_001044588/NM_001123080/ NM_001135959/NM_001145750/NM_001244550/ NM_214015/NM_214124/NM_214172
ssc05220	Chronic myeloid leukemia	8.20e-04	0.034	0.029	6/119	NM_001038639/NM_001244550/NM_001246252/ NM_001285967/NM_214015/NM_214290

Conclusion

After the investigation of methylation levels on reference genes in pigs, 411 DMGs (Q-value < 0.01) were identified from the 4566 reference genes. We also found that five significant pathways (qvalue < 0.05) were mainly from the hyper-methylation DMGs. In this study, these results could be used in the genomic selection for low BT in the breeding programs.

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