Indian Journal of Animal Sciences **96** (3): 243–246, March 2026/Article
https://doi.org/10.56093/ijans.v96i3.143165

Differential muscle methylome analysis in Mali and Hampshire pig breeds

PRAJWALITA PATHAK[1A], N H MOHAN[2,A✉], JURI DEKA[1], JAYA BHARATI[1], A K DAS[1], R THOMAS[1], R SINGH[3], V K GUPTA[1] and B C DAS[1]

ICAR-National Research Centre on Pig, Rani, Guwahati 781 131 Assam India

Received: 12 June 2025; Accepted:19 June 2026

ABSTRACT

Muscle development and carcass yield of meat indigenous and exotic pigs vary significantly. DNA methylation, being a major epigenetic factor, could regulate myogenic process through regulation of gene expression. The objective of the present study was to compare genome-wide methylation in indigenous (Mali) and exotic (Hampshire) breeds of pigs. A total 1549 differentially methylated regions (P<0.1) in the genome with hypermethylation around the promoter-transcription start site of *MYMK* gene were identified in Mali pigs. No differences in the DNA methylation was observed with respect to promoter region of other myogenic genes. Ontology of genes whose bodies were differentially methylated, were related to gaseous exchange, lipid metabolism, opioid, nephrin and DARPP-32 signalling. Based on the study, it may be concluded that differences exist in the muscle tissue DNA methylation between Mali and Hampshire pigs. Further studies are required to understand the implication of the differential methylation in myogenic process in pigs.

Keywords: DNA methylation, Muscle, Myogenesis, Pig

Muscle development is an important economic traits in pig as it is directly related to meat production. Therefore, efforts for improving carcass yield have always received highest priority among breeders. Muscularity of pig is influenced by different factors such as breed, age, feeding and management that determine the meat yield (Sharples *et al.* 2016). Recently, difference between muscle transcriptome between indigenous, Mali and exotic, Hampshire breeds have been reported (Mohan *et al.* 2023). DNA methylation is one of the major epigenetic modifications that has been shown to regulate gene expression during several physiological and pathological conditions (Carrio and Suelves 2015). DNA methylation is an important epigenetic factor for regulating muscle development (Tsumagari *et al.* 2013, Jin *et al.* 2014, Zhang *et al.* 2019, Li *et al.* 2021, Yang *et al.* 2021). Differences in genome-wide methylation have been shown in muscles with diverse metabolic characteristics (Shen *et al.* 2016, Chen *et al.* 2019). DNA methylation can influence myogenic lineage development (Carrio *et al.* 2015) and modulate muscle gene expression (Yang *et al.* 2017). Understanding differences in DNA methylation in muscular tissue can provide insights to regulation of myogenesis. The objective of the present study was to compare genome-wide methylation in the muscle of an indigenous (Mali) and exotic (Hampshire) breeds of pigs which differ in muscularity to understand the regulation of myogenic gene expression.

Present address: [1]Krishi Vigyan Kendra, Dhemaji, Simen Chapori 787 061, Assam, India. [2]ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001, India. [3]ICAR-Indian Veterinary Research Institute, Bareilly, 243 122. Uttar Pradesh. India. [A]Both the authors have equally contributed to the study. ✉Corresponding author email: mohannh.icar@nic.in

MATERIALS AND METHODS

Semimembranosus muscle samples were obtained using biopsy under local anaesthesia from two different breeds of pigs (Hampshire, and Mali), aged 35-40 days, maintained at ICAR-National Research Centre on Pig, Guwahati, Assam. The study was approved by institutional animal ethics committee. The muscle tissues were cleaned in normal saline and processed for methylated DNA immunoprecipitation (MeDIP) followed by next generation sequencing**.** From each breed, one pig was selected for constructing MeDIP DNA libraries. Briefly, approximately 8µg of DNA extracted was sonicated to 100–500-bp fragments. The overhangs after fragmentation were converted to blunt ends by end repair followed by 3' end adenylation and multiple indexing adapter ligation. The fragments ligated with adaptors were immunoprecipitated with a monoclonal anti-methyl cytosine antibody. The enriched fragments with methylation were purified and amplified through adaptor-mediated PCR. Later, 8.5pmol of the MeDIP library was subjected to paired-end (2 x 100) sequencing using an Illumina HiSeq 2500 in a commercial facility. The quality of the raw sequences generated from MeDIP sequencing was assessed with FastQC (version 0.11.9) accessible at https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Low quality bases with Phred score

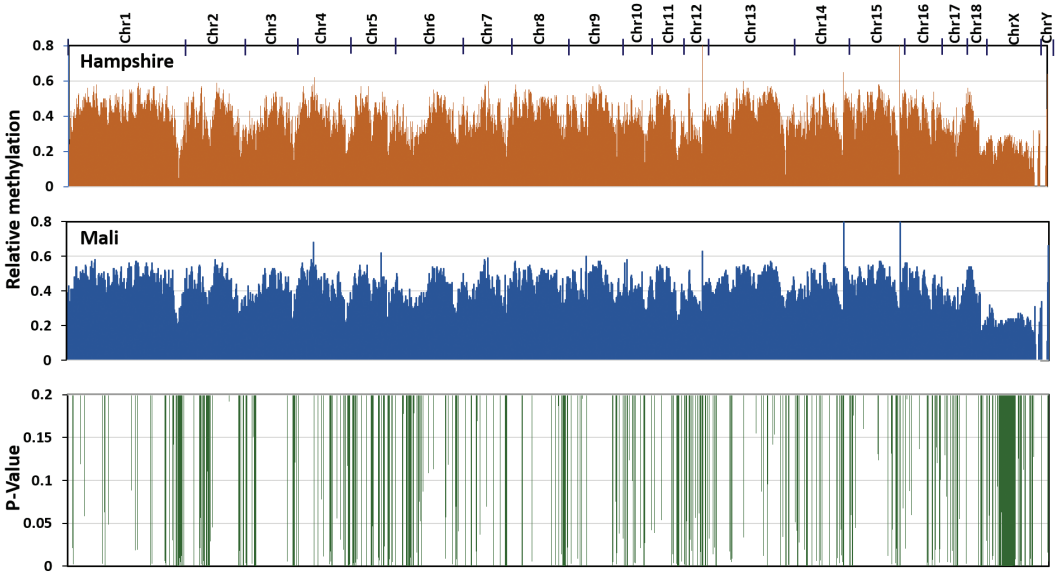

Fig. 1. Chromosome wise methylation in Hampshire and Mali breed of pigs

< 30 were removed using custom made perl script and the adaptor sequences were removed using cutadapt (version 3.3) (Kechin *et al.* 2017). The pre-processed reads were mapped to the *Sus scrofa* genome v11.1 downloaded from UCSC (http://genome.ucsc.edu/) with Burrows–Wheeler alignment (BWA) (version 0.7.17) (Li and Durbin 2009) using default parameters. The resulting SAM files were converted to BAM format and sorted using samtools (version 1.11) (Li *et al.* 2009). Peaks of each MeDIP sample were called against input by MACS (version 2.2.7.1) (Zhang *et al.* 2008)with p-value <10−5 and other parameters set at default values. Uniquely mapped reads with mapping quality score of ≥10 were analyzed using the MEDIPS software package (version 1.44) (Lienhard *et al.* 2014) to estimate methylation levels. Differential methylation analysis was carried out using MEDIPS for all consecutive 100bp windows across the genome and the CpG enrichment values were calculated. The annotation files were extracted from *Sus scrofa* file downloaded from UCSC website (http://genome.ucsc.edu/). The genomic regions 5 kb upstream and downstream of the TSS were considered proximal promoter regions. Methylation 1kb before Transcription Start Site (TSS) to Transcription End Site (TES) plus 1kb of myogenesis related genes (*MYOD1, MYOG, MYMX, MYMK, MYF5, PITX1, PITX3, TWIST2*) were examined. The wiggle files were generated using "exportWIG" function in MEDIPS to export genome wide coverage profiles for visualization in the UCSC genome browser. The methylation pattern of -1KB Methylated DNA immunoprecipitation sequencing data have been deposited in the NCBI Gene Expression Omnibus under the GEO accession number GSE184969 (GSM5602642, GSM5602643).

RESULTS AND DISCUSSION

The present study reports genome level methylation in the muscle of Mali and Hampshire breeds of pigs for first time. During the MEDIP sequencing 60.99 and 40.30 million raw reads were generated in Hampshire and Mali muscle, respectively. Clean reads were aligned to the *Sus scrofa* reference genome 11.1, with mapping rates were 97.87 to 99.02% in Hampshire and Mali samples corresponding to 59.69 and 39.10 million reads, respectively.

The overall relative methylation score of Hampshire genome was slightly higher (0.08%) than that in Mali pigs. Increased methylation is reported to be associated with muscle lineage formation (Tsumagari *et al.* 2013). Chromosome-wise methylation mapping and differentially methylated regions (DMRs) is shown in Fig 1 and 2. (Detailed data in supplementary file 1 and 2). A total of 1549 and 25 DMRs (P<0.1) were identified across the pig chromosomes and mitochondrial DNA, respectively. Highest number of DMRs were observed in chromosome 13 (60.23%). No DMRs could be observed in chromosomes 17 and 18. The DMRs were traced to intergenic (92.12%; 1023 upregulated; 404 downregulated), intragenic (6.91%; 4 upregulated; 103 downregulated) and promoter regions (0.97%; 3 upregulated; 12 down regulated) of genome. The distribution of CpGs were similar between two breeds of pigs with 2.16 and 1.72% differences in relative frequency of CpGs and ratio of observed/expected ration of CpGs within the regions as compared to reference genome. The CpGs were less enriched across the genome of both the breeds as compared to the reference genome, except for chromosome 12, which was 1.2 times more enriched in Mali genome. The methylation in both Mali and Hampshire was similar to the methylation of *Sus scrofa* reference genome (Fig 2; circles E and F). Methylation across the mitochondrial DNA is shown in supplementary file 5.

No significant differences could be observed in the methylation pattern -1kb before Transcription Start Site (TSS) to Transcription End Site (TES) plus 1kb in most

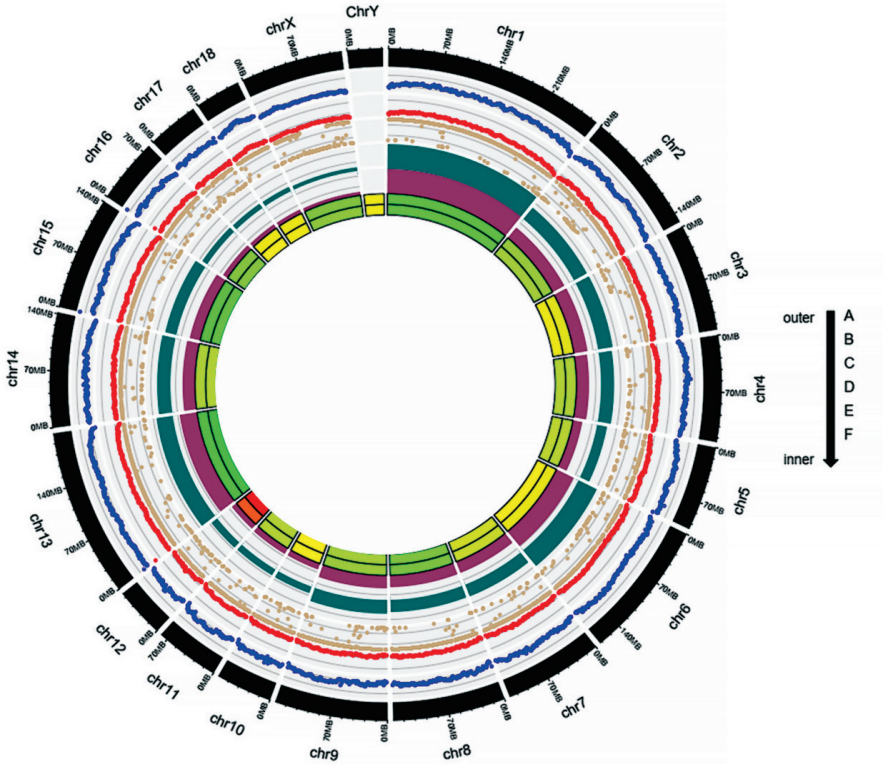

Fig. 2. Circos plot showing genome wide methylation in Mali and Hampshire breed of pigs.

A-Chromosome-wise methylation in Mali; B-Chromosome-wise methylation in Hampshire; C-p-value distribution of methylation across chromosomes. The inner dots indicate lowest p-values; D-Distribution of CpG methylations in Mali; E-Distribution of CpG methylations in Hampshire; F: Relative enrichment of CpGs as compared to reference genome (Sus scrofa build 11.1)- green-yellow-red indicating low-medium- high values;

of the myogenic (*MYOD1, MYOG, MYMX, MYF5, PITX1, TWIST2)* and related genes (*WNTA5, WNT9A, TGFB1, FGFR2*) between two breeds (figures in supplementary file 3). However, DNA hypermethylation around the promoter-*TSS* of Myomaker (*MYMK*) gene in Mali as compared to Hampshire (Fig. 3). Myomaker gene plays a critical role in fusion of myoblasts, which is a major step in formation of multinucleated muscle fibers (Zhang and Roy 2017, Chen *et al.* 2020). The hypermethylation of -1KB region of *MYMK* gene is suggestive of epigenetic modification in the Mali as compared to Hampshire, which needs further studies. A reduced promoter methylation of desmin gene (Chr15:

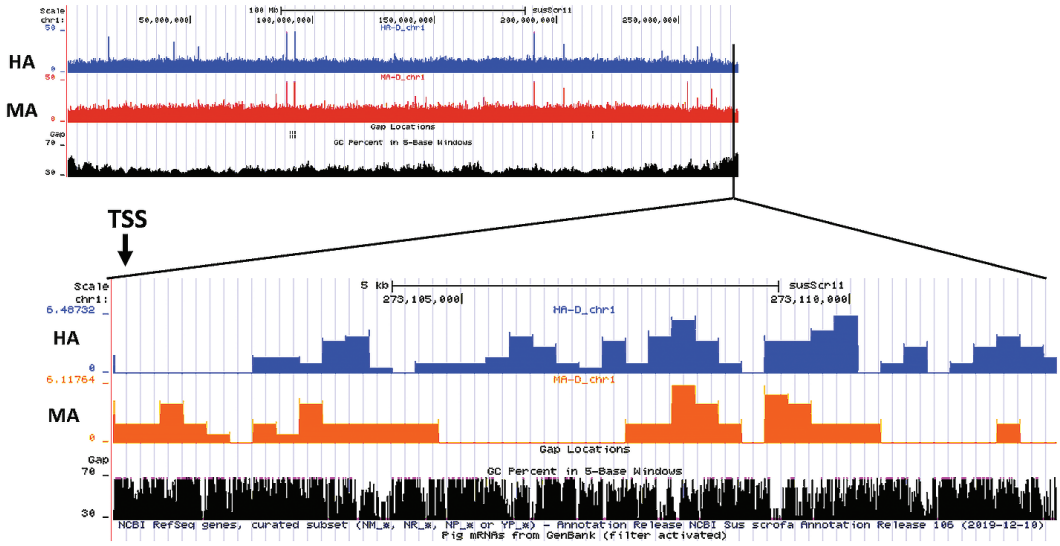

Fig. 3. Methylation in the promoter region of *MYMK* gene in Mali as compared to Hampshire breed of pig.

121428401-121428600) was observed in Hampshire as compared to Mali pigs. Demethylation of desmin CpGs has been reported (Carrio and Suelves 2015) as essential for myogenesis.

The ontology of genes whose bodies were differentially methylated were also examined, which were related to O_2 and CO_2 gaseous exchange, lipid metabolism, opioid, nephrin and DARPP-32 signalling (supplementary file 4). Differentially methylated promoter regions were traced to uncharacterised genes (ENSSSCG00000045321, ENSSSCG00000048719, ENSSSCG00000047552 and ENSSSCG00000049256). Gene ontology analysis of *DMR* corresponds to pathways related to nephrin, a protein associated with myoblast fusion in drosophila (Sohn *et al.* 2009) and DARPP-32, a tightly regulated hub molecule in neurons (Yger and Girault 2011), whose role in porcine muscle is presently unknown.

A limitation of the methylation study is the limited number of samples, and hence the results reported are influenced by the sample size, physiological factors, besides the genetics of the animals and dynamic nature of methylation. Since, the animals were maintained at same management and ambient environment, the observed variations in muscle methylome might have been resulted from underlying genetic differences. The results of the present study indicated differences in genome-wide methylation in the muscle of indigenous (Mali) and exotic (Hampshire) breeds of pigs. Further, large sample studies are required to understand methylation in the regulation of myogenesis, hence the muscularity of the animal and its application in selection process.

ACKNOWLEDGEMENTS

The authors are thankful to Indian Council of Agricultural Research and for providing resources and facilities for the study.

REFERENCES

Carrio E, Diez-Villanueva A, Lois S, Mallona I, Cases I, Forn M, Peinado M A and Suelves M. 2015. Deconstruction of DNA methylation patterns during myogenesis reveals specific epigenetic events in the establishment of the skeletal muscle lineage. *Stem Cells* **33**(6): 2025–2036.

Carrio E and Suelves M. 2015. DNA methylation dynamics in muscle development and disease. *Front Aging Neurosci* **7**: 19.

Chen B, You W, Wang Y and Shan T. 2020. The regulatory role of Myomaker and Myomixer-Myomerger-Minion in muscle development and regeneration. *Cell Molecular Life Science* **77**(8): 1551-1569.

Chen Z, Chu S, Xu X, Jiang J, Wang W, Shen H, Li M, Zhang H, Mao Y and Yang Z. 2019. Analysis of longissimus muscle quality characteristics and associations with DNA methylation status in cattle. *Genes Genomics* **41**(10): 1147–1163.

Jin L, Jiang Z, Xia Y, Lou P, Chen L, Wang H, Bai L, Xie Y, Liu Y, Li W, *et al.* 2014. Genome-wide DNA methylation changes in skeletal muscle between young and middle-aged pigs. *BMC Genomics* **15**: 653.

Kechin A, Boyarskikh U, Kel A and Filipenko M. 2017. cutPrimers: A new tool for accurate cutting of primers from reads of targeted next generation sequencing. *Journal of Comput Ational Biology* **24**(11): 1138-1143.

Li H and Durbin R. 2009. st and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**(14): 1754-1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R and Genome Project Data Processing S. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**(16): 2078-2079.

Li X J, Liu L Q, Dong H, Yang J J, Wang W W, Zhang Q, Wang C L, Zhou J and Chen H Q. 2021. Comparative genome-wide methylation analysis of longissimus dorsi muscles in Yorkshire and Wannanhua pigs. *Animal Genetics* **52**(1): 78–89.

Lienhard M, Grimm C, Morkel M, Herwig R and Chavez L. 2014. MEDIPS: genome-wide differential coverage analysis of sequencing data derived from DNA enrichment experiments. *Bioinformatics* **30**(2): 284–286.

Mohan N H, Pathak P, Buragohain L, Deka J, Bharati J, Das A K, Thomas R, Singh R, Sarma D K, Gupta V K and Das B C. 2023. Comparative muscle transcriptome of Mali and Hampshire breeds of pigs: a preliminary study. *Animal Biotechnology* **34**(8): 3946–3961.

Sharples A P, Stewart C E and Seaborne R A. 2016. Does skeletal muscle have an 'epi'-memory? The role of epigenetics in nutritional programming, metabolic disease, aging and exercise. *Aging Cell* **15**(4): 603–616.

Shen L, Du J, Xia Y, Tan Z, Fu Y, Yang Q, Li X, Tang G, Jiang Y, Wang J, *et al.* 2016. Genome-wide landscape of DNA methylomes and their relationship with mRNA and miRNA transcriptomes in oxidative and glycolytic skeletal muscles. *Scintific Reports* **6**: 32186.

Sohn R L, Huang P, Kawahara G, Mitchell M, Guyon J, Kalluri R, Kunkel L M and Gussoni E. 2009. A role for nephrin, a renal protein, in vertebrate skeletal muscle cell fusion. *Proceedings of the National Academy of Sciences of the United States of America* **106**(23): 9274–9279.

Tsumagari K, Baribault C, Terragni J, Varley K E, Gertz J, Pradhan S, Badoo M, Crain C M, Song L, Crawford G E, *et al.* 2013. Early de novo DNA methylation and prolonged demethylation in the muscle lineage. *Epigenetics* **8**(3): 317–32.

Yang Y, Fan X, Yan J, Chen M, Zhu M, Tang Y, Liu S and Tang Z. 2021. A comprehensive epigenome atlas reveals DNA methylation regulating skeletal muscle development. *Nucleic Acids Research* **49**(3): 1313–1329.

Yang Y, Liang G, Niu G, Zhang Y, Zhou R, Wang Y, Mu Y, Tang Z and Li K. 2017. Comparative analysis of DNA methylome and transcriptome of skeletal muscle in lean-, obese-, and mini-type pigs. *Scientific Report* **7**: 39883.

Yger M and Girault J A. 2011. DARPP-32, Jack of All Trades... Master of Which? *Front Behav Neurosci* **5**: 56.

Zhang W and Roy S. 2017. Myomaker is required for the fusion of fast-twitch myocytes in the zebrafish embryo. *Developmental Biology* **423**(1): 24–33.

Zhang W, Zhang S, Xu Y, Ma Y, Zhang D, Li X and Zhao S. 2019. The DNA methylation status of wnt and tgfbeta signals is a key factor on functional regulation of skeletal muscle satellite cell development. *Frontiers in Genetics* **10**: 220.

Zhang Y, Liu T, Meyer C A, Eeckhoute J, Johnson D S, Bernstein B E, Nusbaum C, Myers R M, Brown M, Li W and Liu X S. 2008. Model-based analysis of ChIP-Seq (MACS). *Genome Biology* **9**(9): R137.