Genome-wide association study of birth weight and pre-weaning body weight of crossbred pigs

KARTHIKEYAN A1, AMIT KUMAR2, RAJNI CHAUDHARY3, AAMIR BASHIR WARA4, AKANSHA SINGH5, N R SAHOO6, MOHD BAQIR7 and B P MISHRA8

Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh 243 122 India

Received: 04 September 2019; Accepted: 23 September 2019

ABSTRACT

In piggery, birth weight and body weight remains most vital economic trait as they directly influence on the production performance of the farm. Implementing the genomic selection would pay way for rapid genetic gain along with increased accuracy than conventional breeding. Prior to genomic selection, genome wide association study (GWAS) has to be conducted in order to find informative SNPs associated with the traits of interest in a given population. Under this study 96 crossbred pigs were genotyped using double digest genotype by sequencing (GBS) technique using Hiseq platform. Raw FASTQ data were processed using dDOCENT Pipeline on Reference based method and variants were called using Free Bayes (version 1.1.0-3). Using Plink (v1.09b), variants having MAF>0.01, HWE<0.001 and genotyping rate >80% were filtered out and 20,467 SNPs were retained after quality control, for ascertaining GWAS in 96 pigs. Before conducting association studies, the data were adjusted for significant non-genetic factors affecting the traits of interest. GWAS was performed using Plink software (v1.9b) identified 9, 11, 12, 23, 28, 30, 33 and 42 SNPs significantly (adjusted P<0.001) associated with birth weight, body weight at weekly interval from 1st week to 8th week, respectively. A large proportion of significant (adjusted P<0.001) SNPs were located on SSC10, SSC6, SSC13, SSC8 and SSC1. One genome wide significant SNP and four genome wide suggestive SNPs were identified. Two common SNPs affecting all body weight at different weeks were located on SSC5:40197442 and SSC13:140562 base pair position. This study helps to identify the genome wide scattered significant SNPs associated with traits of interest which could be used for genomic selection, but further validation studies of these loci in larger population are recommended.

Key words: Birth weight, Crossbred pigs, GBS, GWAS, Pre-weaning weight

As compared to other livestock farming, piggery has better feed conversion efficiency, higher fecundity and better utilization of non-conventional feed resources into valuable nutritious meat. Therefore, per animal productivity improvised through crossbreeding programme with exotic inheritance might be a better strategy to address the required demand for animal protein. In Indian scenario, commercial pig production involves rearing of crossbred pigs under intensive farming system. The advent of sequencing technology driven by the human genome project has led to generate large numbers of polymorphic genetic markers, i.e. Single Nucleotide Polymorphism (SNPs) across the livestock species. SNPs appear to be a marker of choice because of its abundant widespread coverage of the genome and ease of genotyping (Visscher et al. 2000). GWAS technique employed in the field of animal breeding for the identification of causal mutation/genes present wide across the genome to study the genetic mechanism behind the complex nature of the production traits in livestock (Johnson and O’Donnell 2009). As compared to traditional QTL mapping strategies, GWAS covers the major advantages both in the power to detect causal variants with modest effects and indicating the narrower genomic regions that harbour causal variants (Greely 2007). Till date, Genome-wide association studies have been used to identify important genomic regions influencing complex traits (Hayes and Goddard 2010), to identify markers that would increase estimated breeding values (EBVs) accuracy.

Several literatures were available on GWAS in Pigs for production, welfare and disease resistance traits. Yet those research findings cannot be implemented in native population until unless those findings validated under native population or been investigated across wider genetic base and also accommodating population from several generations. However, in Indian scenario GWAS studies on pigs have not yet been reported till date and also

Present address: 1,3,5PhD scholar (karthikeyan0318@gmail.com, rajnichaudhary79@gmail.com, vetakki10@gmail.com), 2,8Senior Scientist (vetamitchandan07@gmail.com, vet.nihar@gmail.com), Animal Genetics; 3Joint Director (R) (bpmishra_1@hotmail.com), IVRI Izatnagar. 4Veterinary Assistant Surgeon (amirwar10@gmail.com), Jammu and Kashmir. 5Veterinary Assistant Surgeon (drbaqirvet@gmail.com), Ladakh.
conducting such studies would aid in formation of reference population for future genomic selection in pigs. Hence, present study aims to find Genome Wide Association of informative SNPs with growth performance in crossbred pigs.

MATERIALS AND METHODS

**Data collection:** Phenotype data of ninety-six crossbred piglets (75% landrace × 25% Ghurrah) maintained at Swine Production Farm, Indian Veterinary Research Institute (IVRI), Iztanagar were collected. The records on birth weight (W0), body weight at weekly interval from 1st week (W1), body weight at 2nd week (W2), body weight at 3rd week (W3), body weight at 4th week (W4), body weight at 5th week (W5), body weight at 6th week (W6), body weight at 7th week (W7) and body weight at 8th week (W8) weaning weight, respectively were collected. For genotyping, blood samples were collected in optimum aseptic condition and DNA was isolated from the collected blood samples. The use and care of animal for this purpose were in accordance with ethical standards laid by Institutional Animal Ethics Committee, IVRI.

**Adjustment for non-genetic factors:** The phenotypic data distributed over different seasons, year, sex and litter size group were subsequently analysed for their influence of the traits under study. The influence of various non-genetic factors (season of birth, year of birth, litter size and sex of the individual) on birth weight and body weight at different weeks of age was studied by least squares analysis method as described by Harvey (1990).

**Genotyping:** Genotyping was done using double digestion genotyping by sequencing (ddGBS) technique, a highly multiplexed technique based on an Illumina HiSeq sequencing platform. Two restriction enzymes, i.e. ApeKI and PstI were used to digest the genome into several smaller fragments and subsequently these fragments were ligated to universal sequencing adapters and barcode for respective samples after fragment size selection. It was followed by sequencing in parallel high throughput Illumina HiSeq sequencing technologies. The SNP calling pipeline was followed after Quality Check of all individual samples which included initial quality filtering followed by mapping to *Sus scrofa* version 11.1 reference genome using Burrows-Wheeler Aligner (BWA) algorithm. Raw FASTQ files were subjected to dDOCENT pipeline in order to call SNPs based on reference sequence with the default settings for reads trimming and mapping MEM algorithm of BWA.

**Quality Control and Statistical Analysis:** SNPs were called from read mapped using FreeBayes (version v0.9.10). Total of 10,029,009 biallelic variants were called with genotyping rate of 0.344 from 96 animals sequenced. After stringent quality control done both at the level of individual samples as well as that of the individual SNPs (MAF >0.01, genotyping rate >0.8, HWE<0.001) 20,467 SNPs were left out with 0.86 genotyping call rate. Out of which only 14,518 SNPs were utilized for ascertaining GWAS with birth weight and pre-weaning body weight at weekly intervals, where rest belonging to unmapped scaffolds and allsomes were discarded. The statistical analysis of each SNP for association with the traits of interest were accomplished using linear model in which the trait score is the y-variable and genotype is one of the explanatory variables.

\[ y = \beta_1 x + \beta_0 + e \]

where \( y \), a continuous valued phenotype; \( x \), SNP genotype at a given locus; \( \beta_1 \), regression coefficient or the parameter that represents the strength of association between the SNP \( x \) and the phenotype \( y \); \( \beta_0 \), intercept term; and \( e \), noise or the part of \( y \) that is not explained by the SNP \( x \) (e.g. environmental effect).

**Genome wide association study:** The basic association analysis was accomplished using PLINK v1.9 for all the traits of interest. The genomic-control corrected P-values were used for checking the possible association of SNPs with all the selected traits. Based on the Bonferroni method, the significance level for significant SNPs was given by 0.05/N and for suggestive SNPs was given by 1/N, where \( N \) is the number of informative SNPs. Therefore under present study, the significance threshold of \( P <3.44 \times 10^{-6} \) (0.05/14518 = 3.44 \times 10^{-6}) for Genome wide significant SNPs and \( P <6.88 \times 10^{-5} \) (1/14518 = 6.88 \times 10^{-5}) for genome wide suggestive SNPs as determined for traits of interest. The Manhattan were generated for each association reports through package “GWAS Tools” in R environment. The top ten significant (adjusted \( P<0.001 \)) SNPs associated with each trait were listed out and a region flanking around the variation spanning 1 Mb was screened using UCSC genome browser for presence of any coding genes.

**RESULTS AND DISCUSSION**

The Least square means of overall population mean±SE (kg) for W0, W1, W2, W3, W4, W5, W6, W7 and W8 were0.97±0.03, 2.30±0.07, 3.69±0.11, 5.07±0.17, 6.54±0.25, 7.92±0.31, 9.35±0.36, 10.85±0.40 and 12.35±0.5 kg, respectively (Table 1).

**Effect of non-genetic factors on traits under study:** The effect of season of farrowing (summer and winter) was significant on W2, W3, W4, W5, W6 W7, and W8, hence these records were adjusted using Least Squares constants before ascertaining GWAS. Whereas, its effect was non-significant on W0 and W1. Body weights of piglets born in winter seasons were recorded significantly higher body weights than piglets born in summer season, these findings were in accordance with similar studies (Panduranga reddy et al. 2013, Naha et al. 2017). Similar results were noticed in other studies also. The effect of year of farrowing, sex and litter size group were also found non-significant for all the traits selected for association study. The group wise least square means along with error values for different subclass under year of farrowing, sex, litter size groups for all traits under study were given in detail under the Table 1. The genetic evaluation of same population had been accomplished using random regression models and animal models (Chaudhary et al. 2019, Chaudhary et al. 2020)
Total of 20,467 SNPs were left out with 0.86 genotyping call rate from 96 animals were genotyped by GBS technology. Similarly, had detected 41,108 autosomal SNPs was detected by GBS technique from 2,936 Duroc boars (Tan et al. 2017).

Association of informative SNPs for birth weight: GWAS is a powerful method to identify the mutations or genes under lying complex traits in domestic animals. One genome wide significant SNP was found, i.e. SSC7:24603150 and four genome wide suggestive SNPs, i.e. SSC6:38758955, SSC6:31135409, SSC7:21610704 and SSC6:61588085 were found under present studies. Majority of the significant SNPs (adjusted P<0.001) were found to be located on SSC10, SSC6, SSC13, SSC8 and SSC1.

A total of 10 significant SNPs (adjusted P<0.001) were found with three of them located on chromosome 6 were associated with W0. SNP SSC6:160752820 was found located at intron 15 of the EPS15 gene on chromosome 6. EPS15, a known imprinted gene reported to be expressed in placenta, impacts birth weight (Kappil et al. 2015). Other genes in nearby vicinity of top 10 significant SNPs (Table 2) that are flanking 1 Mb region were CYB5R1, BTN1A1, SHCBP1, NFATC2, ZNF300, SLCA57, CCDC154 and KCNV1. Similar studies conducted in 532 pigs for litter weight born alive (LWB) genotyped using GBS yield 1,67,355 SNPs. GWAS study showed 20 significant SNPs were associated with LWB (Wu et al. 2018). Manhattan Plot for SNPs associated birth weight was projected in Fig. 1.

Association of informative SNPs for pre-weaning weekly body weight: At adjusted P<0.001 value of genomic-control corrected P-value, 11 significant SNPs were found to be associated with W1. The genome scan for genes within 1 Mb of top ten Significant SNPs revealed presence of NFATC2, CD83, SLCA57, ANK2 and CPNE4 in the intergenic region. Whereas some SNPs were found within genic regions of ISL1 (Exon 3), DAAM2 (Intron 2), KDM4B (Intron 18), RAB3C (Intron 3) and MATE2 (Intron 2). NFATC2 found to play a role in skeletal muscle growth mediated through the PFG2 receptor (Horsley and Pavlath 2003), while CD83 found to be up regulated in highest weight gain group among landrace crossbreds indicating...
its role in weight gain (Lessard et al. 2018). Similarly, DAAM2 was associated with back fat thickness and carcass length in a GWAS study conducted in F2 generation of Landrace crosses (Falkner-Gieske et al. 2019).

For W2, 12 SNPs were associated at genomic-control corrected P-value P<0.001. The genome scan for genes within 1 Mb of top 10 significant SNPs revealed presence of CPNE4, POPDC3, MROH9, SUSD4, ALG10, OR4C11, SHCBP1 present in the intergenic region and two SNPs found within the genic region of NPG3 (Intron 2) and ISL1 (Exon 3). POPDC3 gene encodes protein which has been abundantly expressed in cardiac and skeletal muscle and its copy number variation in white leghorn associated with production traits (Yi et al. 2014).

For W3, 23 SNPs were associated at genomic-control corrected P-value P<0.001. One genome wide suggestive SNP SSC6:38758955 was found associated with W3 with adjusted P value of 3.58E-05, it was found to be in near vicinity of downstream of SHCBP1. The genome scan for genes within 1 Mb of top 10 significant SNPs revealed presence of OR4C11, METTL6, FOXB2, SUSD4, CYB5R1, SUCLG2 and CPNE4 in the intergenic region and two SNPs found within the genic region of ERICH4 (Intron 2) and GASK1A (Exon 3). SUCLG2 gene involved in the tricarboxylic acid (TCA) pathway of energy metabolism and ATP production, reported to be expression higher level may be associated with improvements in meat quality traits by its role in regulating ATP production and postmortem pH decline (Velez-Irizarry et al. 2019).

Similarly, for W4, total of 28 significant SNPs (adjusted P<0.001) were found with to be associated. One genome wide suggestive SNP SSC6:38758955 was found to be associated with W4 with adjusted P-value of 1.99E-05, which was in near vicinity of downstream of SHCBP1. SHCBP1 mRNA and protein expression are restricted to actively dividing cells and proliferating cells and also its expression was regulated by growth factor indicating its association with the Shc adaptor molecule suggesting the role for this protein in signalling pathways governing cell cycle progression (Schmandt et al. 1999). The genome scan for genes within 1 Mb of top 10 significant SNPs revealed presence of METTL6, GABRG1, MROH9, CPNE4, FOXB2 present in the intergenic region and four SNPs were located within the genes of HIST4H4 (Intron 1), GASK1A (Exon 2), ITGB7 (Intron 14) and TMEM74 (Exon 1).

The SNPs (adjusted P<0.001) associated with W5 were 24 with involving one genome wide suggestive SNP SSC7:21607074 found within the HIST4H4 gene in the first intron with the adjusted P value of 2.90E-05. The genome scan for genes within 1 Mb of top 10 significant SNPs revealed presence of SHCBP1, FOXB2, KCNJ12, METTL6, MROH9, OR4C11, CPNE4 present in the intergenic region and two SNPs were located within the genes of GASK1A (Exon 2) and ITGB7 (Intron 14).

Similarly, for W6, total of 30 significant SNPs (adjusted P<0.001) were found with to be associated. With two genome wide suggestive SNP SSC7:21607074 with adjusted P-value of 1.52E-05 and SSC12:61588085 with adjusted P-value of 2.07E-05, which were in near vicinity of HIST4H4 and KCNJ12. RNA-seq analysis reported that bovine KCNJ12 gene expression was significantly up-regulated in adult stage of longissimus muscle than from fetal stage, pointing out its role in potential roles in bovine myocyte differentiation and muscle development (He and Liu 2013). Also studies have reported KCNJ12 gene missense mutation as a marker in cattle for beef breeding programs (Cheng et al. 2019). With one of the CNV significantly associated with seven growth traits in Nellore cattle was in overlap with KCNJ12 gene which is involved in affecting in growth traits (Zhou et al. 2016). The genome scan for genes within 1 Mb of top 10 Significant SNPs revealed presence of BTN1A1, SUSD4, CPNE4, FOXB2, PCMTD2, OR4C11 present in the intergenic region and one SNPs were located within the genes of TTL7 (Intron 14).

For W7, 33 SNPs were associated at genomic-control corrected P-value P<0.001. One genome wide suggestive SNP SSC7:21607074 was found associated with W7 with adjusted p value of 2.32E-05, it was found to be in near vicinity of HIST4H4 at intron 1. The genome scan for genes within 1 Mb of top 10 significant SNPs revealed presence of FTO, BTN1A1, KCNJ12 and CPNE4 present in the intergenic region and three SNPs found within the genic
region of TTLL7 (Intron 14), DCC (Intron 14) and ZBTB7C (Intron 2). ZBTB7C are known to play a role in regulation of fat cell differentiation, reported to be associated with QTL for back fat thickness in pigs (Hérault et al. 2018). Studies in pigs have provided evidence that FTO was associated with intramuscular fat deposition and average daily gain (Fan et al. 2009).

For W8 (weaning weight), 42 SNPs were associated at genomic-control corrected P-value P<0.001. Manhattan Plot for SNPs associated weaning weight (W8) was depicted in Fig. 2. One genome wide significant SNP SSC7:24603150 was found associated with W8 with adjusted P-value of 3.42E-06, it was found to be in near vicinity of BTN1A1. BTN1A1 reported to be one of the candidate gene for several traits like milk fat yield, total solid, solid-nonfat and first milk yield in dairy goats (Qu et al. 2011). One genome wide suggestive SNP SSC6:31135409 was found associated with W8 with adjusted p value of 1.46E-05, it was found to be in near vicinity of FTO. Association studies in crossbred pig showed contribution in genetic variance from the polymorphism in the FTO gene was highest for back fat depth, meat area on the musculus longissimus, lumbrorum and thoracic tissues (Dvořáková et al. 2012). The genome scan for genes within 1 Mb of top 10 significant SNPs (Table. 2) revealed presence of TMEM132D, EHBPI and IGLON5 present in the intergenic region and five SNPs found within the genomic region of ZBTB7C (Intron 2), EHBPI (3‘UTR), SERINC2 (Exon 2), FRY (Intron 1) and MROH9 (5’UTR).

This study helps to identify the genome wide scattered significant SNPs associated with traits of interest which could be used for genomic selection, but further validation studies of these loci in larger population are recommended. In summary most of the candidate genes we identified function in growth related pathways, directly or indirectly, which were then further evaluated through literature mining to assess their biological functions. Further experimentation will be required to confirm the functions of these genes and elucidate the molecular mechanisms underlying growth traits. The gains from the incorporation of genome wide significant novel SNPs for estimation of genomic breeding value will increase the accuracy of selection in crossbreds at the early age in piggery industry to achieve faster genetic gain in our crossbred population.

ACKNOWLEDGEMENTS

The authors are thankful to ICAR-IVRI with the necessary facilities to carry out this work.

REFERENCES

Chaudhary R, Prakash V, Sailo L, Singh A, Karthikeyan A, Mehrotra A, Mondal S K, Sahoo N R, Kumar A. 2019. Estimation of genetic parameters and breeding values for growth traits using random regression model in Landrace × Desi (indigenous) crossbred pigs. Indian Journal of Animal Sciences 89(10).

Chaudhary R, Sailo L, Singh A, Karthikeyan A, Mehrotra A, Mondal S K, Sahoo N R and Kumar A. 2020. Genetic parameter estimates for individual growth performance of crossbred piglets in sub-temporeral conditions. Indian Journal of Animal Sciences 90(01): In Press.

Cheng J, Peng W, Cao X, Huang Y, Lan X, Lei C and Chen H. 2019. Differential Expression of KCNJ12 Gene and Association Analysis of Its Missense Mutation with Growth Traits in Chinese Cattle. Animals 9(5): 273.

Dvořáková V, Bartenschlager H, Stratil A, Horák P, Stupka R, Eitek J, Špryl M, Hrdlicková A and Geldermann H. 2012. Association between polymorphism in the FTO gene and growth and carcass traits in pigs crossses. Genetics Selection Evolution 44(1): 13.

Falkner-Gieske C, Blaj I, Preuß S, Bennennitz J, Thaller G and Tetens J. 2019. GWAS for meat and carcass traits using imputed sequence level genotypes in pooled F2-designs in pigs. G3: Genes, Genomes, Genetics 3: 404452.

Fan B, Du Z Q and Rothschild M F. 2009. The fat mass and obesity-associated (FTO) gene is associated with intramuscular fat content and growth rate in the pig. Animal Biotechnology 20(2): 58–70.

Greely H T. 2007. The uneasy ethical and legal uppinings of large scale genomic biobanks. Annual Review of Genomics and Human Genetics 8: 343–64.

Harvey W R. 1990. User’s guide for LSMLMW mixed model least square and maximum likelihood computer program (PC-2 version). Ohio State University Pres. Columbus 91.

Hayes B and Goddard M. 2010. Genome-wide association and genomic selection in animal breeding. Exploiting Genome-wide Association in Oilseed Brassicas: a model for genetic improvement of major OECD crops for sustainable farming. Genome 53(11): 876–83.

He H and Liu X. 2013. Characterization of transcriptional complexity during longisssimus muscle development in bovines using high-throughput sequencing. PloS One 8(6): 64356.

Hérault F, Damon M, Cherel P and Le Roy P. 2018. Combined GWAS and LDLA approaches to improve genome-wide quantitative trait loci detection affecting carcass and meat quality traits in pig. Meat science 135: 148–58.

Horsley V and Pavlath G K. 2003. Prostaglandin F2αt stimulates growth of skeletal muscle cells via an NFATC2-dependent pathway. The Journal of Cell Biology 161(1): 111–18.

Johnson A D and O’Donnell C J. 2009. An open access database of genome-wide association results. BMC Medical Genetics 10: 6.

Kappil M A, Green B B, Armstrong D A, Sharp A J, Lambertiini L, Marsit C J and Chen J. 2015. Placental expression profile

---

**Fig. 2.** Manhattan plot displaying associated informative SNPs with Body Weight at 8th week (weaning weight).
of imprinted genes impacts birth weight. *Epigenetics* **10**(9): 842–49.

Lessard M, Blais M, Beaudoin F, Deschene K, Verso L L, Bissonnette N, Lauzon K and Guay F. 2018. Piglet weight gain during the first two weeks of lactation influences the immune system development. *Veterinary Immunology and Immunopathology* **206**: 25–34.

Naha B C, Gaur G K, Patel B H M and Sahoo N R. 2017. Growth and litter traits in crossbred pigs across the non-genetic factors. *Indian Journal of Animal Research* **51**(4): 798–800.

Pandurangareddy P, Prakash M G, Kumari B P, Suresh J and Bharthi A. 2013. Genetic analysis of preweaning body weights in crossbred pigs. *Indian Journal of Animal Research* **47**(1): 70–74.

Qu Y, Liu Y, Ma L, Sweeney S, Lan X, Chen Z, Li Z, Lei C and Chen H. 2011. Novel SNPs of butyrophilin (BTN1A1) and milk fat globule epidermal growth factor (EGF) 8 (MFG-E8) are associated with milk traits in dairy goat. *Molecular Biology Reports* **38**(1): 371–77.

Schmandt R, Liu S K and McGlade C J. 1999. Cloning and characterization of mPAL, a novel Shc SH2 domain-binding protein expressed in proliferating cells. *Oncogene* **18**(10): 1867.

Tan C, Wu Z, Ren J, Huang Z, Liu D, He X, Prakapenka D, Zhang R, Li N, Da Y and Hu X. 2017. Genome-wide association study and accuracy of genomic prediction for teat number in Duroc pigs using genotyping-by-sequencing. *Genetics Selection Evolution* **49**(1): 35.

Velez-Irizarry D, Casiro S, Daza K R, Bates R O, Raney N E, Steibel J P and Ernst C W. 2019. Genetic control of longissimus dorsi muscle gene expression variation and joint analysis with phenotypic quantitative trait loci in pigs. *BMC Genomics* **20**(1): 3.

Visscher PM, Pong-Wong R, Whittemore C and Haley C S. 2000. Impact of biotechnology on (cross) breeding programmes in pigs. *Livestock Production Science* **65**(1): 57–70.

Wu P, Yang Q, Wang K, Zhou J, Ma J, Tang Q, Jin L, Xiao W, Jiang A, Jiang Y and Zhu L. 2018. Single step genome-wide association studies based on genotyping by sequence data reveals novel loci for the litter traits of domestic pigs. *Genomics* **110**(3): 171–79.

Yi G, Qu L, Liu J, Yan Y, Xu G and Yang N. 2014. Genome-wide patterns of copy number variation in the diversified chicken genomes using next-generation sequencing. *BMC Genomics* **15**(1): 962.

Zhou Y, Utsunomiya Y T, Xu L, Bickhart D M, Alexandre P A, Rosen B D, Schroeder S G, Carvalheiro R, de Rezende Neves H H, Sonstegard T S and Van Tassell C P. 2016. Genome-wide CNV analysis reveals variants associated with growth traits in *Bos indicus*. *BMC Genomics* **17**(1): 419.