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

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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, 24, 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

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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), Izatnagar were collected. The records on birth weight (W0), body weight at weekly interval from 1st week (W1), body weight at 2st week (W2), body weight at 3rd week (W3), body weight at 4th week (W4), body weight at 5st 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 allosome 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.

$$y = x\beta_1 + \beta_0 + \varepsilon$$

y, a continuous valued phenotype; x, SNP genotype at a given locus; β_1, regression coefficient or the parameter that represents the strength of association between the SNP x and the phenotype y; β_0, intercept term; and ε, 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)

Table 1. Least Square means and standard error of W0, W1, W2, W3, W4, W5, W6, W7 and W8 for different non-genetic effects in crossbred pigs

Effect	Classification	N	Body weight at 8th week of age (Weaning weight)							
			W0	W1	W2	W3	W4	W5	W6	W7	W8
Season	Summer(Apr–Sep)	73	0.90±0.04	2.13±0.11	3.24±0.15^{b}	4.36±0.25^{b}	5.47±0.36^{b}	6.30±0.44^{b}	7.61±0.52^{b}	8.92±0.57^{b}	10.32±0.71^{b}
	Winter(Oct–Mar)	23	1.04±0.05	2.47±0.13	4.15±0.19^{a}	5.79±0.31^{a}	7.61±0.44^{a}	9.55±0.53^{a}	11.09±0.63^{a}	12.78±0.70^{a}	14.38±0.86^{a}
Year	2015	23	0.89±0.05	2.15±0.14	3.70±0.19	5.16±0.32	6.66±0.46	7.77±0.56	9.12±0.66	10.51±0.73	11.59±0.90
	2016	73	1.05±0.04	2.44±0.10	3.69±0.15	4.98±0.24	6.42±0.35	8.08±0.42	9.58±0.50	11.19±0.55	13.11±0.68
Sex	Male	69	1.02±0.02	2.35±0.06	3.69±0.09	5.02±0.15	6.35±0.22	7.74±0.27	9.13±0.32	10.52±0.35	12.26±0.43
	Female	27	0.93±0.04	2.24±0.12	3.70±0.17	5.13±0.28	6.73±0.40	8.11±0.49	9.57±0.58	11.17±0.63	12.44±0.79
LSG	5 to 8	37	0.95±0.04	2.28±0.11	3.79±0.15	5.16±0.25	6.72±0.36	8.09±0.43	9.48±0.51	10.98±0.57	12.15±0.70
	>8	59	0.99±0.03	2.32±0.07	3.6±0.11	4.99±0.18	6.36±0.25	7.76±0.31	9.22±0.37	10.71±0.40	12.55±0.50
	Overall Mean	96	0.97±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	12.35±0.5

Means with different superscripts in the same column across each effect indicates statistically significant (P<0.05).

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, SSC7:21607074 and SSC: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*, *SLC5A7*, *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, SLC5A7, 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 PGF2 receptor (Horsley and Pavlath 2003), while *CD83* found to be up regulated in highest weight gain group among landrace crossbreds indicating

Fig. 1. Manhattan plot showing associated informative SNPs with birth weight.

Table 2. Top ten significant SNPs associated with Birth weight and Body weight at 8th week of age (Weaning weight)

Chr	SNP bp position	Adjusted P value	Genic region	Flanking Gene Symbol within 1Mb	Chr	SNP bp position	Adjusted p value	Genic region	Flanking Gene Symbol within 1Mb
SSC10	2.5E+07	0.00014	Intergenic	*CYB5R1*	SSC7	2.5E+07	3.42E-06	Intergenic	*BTN1A1L*
SSC7	2.5E+07	0.00014	Intergenic	*BTN1A1L*	SSC6	3.1E+07	1.46E-05	Intergenic	*FTO*
SSC6	3.9E+07	0.00016	Intergenic	*SHCBP1*	SSC1	9.8E+07	6.69E-05	Intron 2	*ZBTB7C*
SSC17	5.3E+07	0.00024	Intergenic	*NFATC2*	SSC3	7.9E+07	9.44E-05	3' UTR	*EHBP1*
SSC2	1.5E+08	0.00044	Intergenic	*ZNF300*	SSC6	5.9E+07	0.00011	Intergenic	*IGLON5*
SSC3	4.8E+07	0.00044	Intergenic	*SLC5A7*	SSC6	8.8E+07	0.00011	Exon 9	*SERINC2*
SSC6	1.6E+08	0.00046	Intron 15	*EPS15*	SSC11	8529788	0.00012	Intron 1	*FRY*
SSC3	4E+07	0.00047	Intergenic	*CCDC154*	SSC14	2.5E+07	0.00014	Intergenic	*TMEM132D*
SSC4	2.7E+07	0.00082	Intergenic	*KCNV1*	SSC3	7.9E+07	0.00014	Intergenic	*EHBP1*
SSC6	3.9E+07	0.00121	Intergenic	*SHCBP1*	SSC9	6.3E+07	0.00014	5' UTR	*MROH9*

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 crossbreds (Falker-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 ten 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. Onegenome 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 *p*H 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 $1.19E^{-05}$, 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 *TTLL7* (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, lumborum and thoracic tissues (Dvoøáková *et al*. 2012). The genome scan for genes within 1 Mb of top 10 significant SNPs (Table. 2) revealed presence of *TMEM132D, EHBP1* and *IGLON5* present in the intergenic region and five SNPs found within the genic region of *ZBTB7C* (Intron 2), *EHBP1* (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

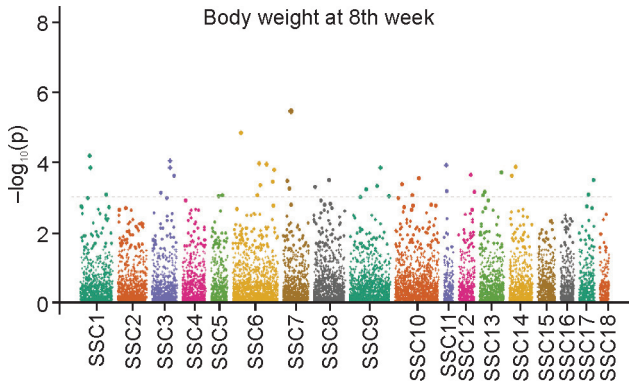

Fig. 2. Manhattan plot displaying associated informative SNPs with Body Weight at 8th week (weaning weight).

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.

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