



Association of *MC4R*, *RYR1* and *PRKAG3* single nucleotide polymorphisms with body weight in crossbred piglets

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ABSTRACT

An attempt was made to study the association of *MC4R*, *RYR1* and *PRKAG3* SNPs with body weight from birth to 8 weeks in 238 crossbred pigs. The lower value of PIC, observed heterozygosity, expected heterozygosity and number of effective alleles for *RYR1* and *PRKAG3* SNP revealed that population under investigation was of low diversity maintaining a single allele. These values were intermediate for *MC4R* SNP representing that forces had been operated to maintain both alleles in the population. Chi square value was significant for *MC4R* showing significant departure from the Hardy–Weinberg equilibrium. Effect of all SNPs was non-significant on body weight at all ages. Though non-significant, GG genotype of *MC4R* SNP, NN genotype of *RYR1* SNP and QR genotype of *PRKAG3* SNP had better weight at 6 and 8 week as compared to their contemporary genotypes, i.e. AG and AA genotype of *MC4R* SNP, Nn genotype of *RYR1* SNP and RR genotype of *PRKAG3* SNP. Fourth parity piglets had higher body weight at all ages as compared to those born in other parities. Piglets born from March to June had a higher growth at most of the weeks as compared to rest of the seasons. Piglets born in 2016 had higher growth at most of the weeks. The effect of sex was non-significant on body weight at all ages.

Key words: Body weight, Crossbred pig, *MC4R*, *PRKAG3*, *RYR1*, SNP

Commercial pig farming for meat production is one of the best and profitable businesses in India. Pigs as compared to other livestock species contribute faster economic return to the farmers. Total pig population in the country was 10.3 million as per 19th Livestock Census (2012). Pigs are mainly concentrated in North-Eastern part of India (40% population). Pigs constitute 2.01% of the total livestock population (19th Livestock Census). Pork is rich in vitamins like thiamine, niacin and riboflavin and is highly preferred globally. The total meat production in the country is 7.4 MT wherein pig contributes 6.5% (BAHS 2017). Selection based on growth has been of great importance to the pig industry because of cost associated with feeding and consumer preference for lean meat. Studies in the field of Animal and Veterinary Sciences are presently geared towards the molecular approach of physiological mechanisms such as growth and metabolism. Growth is influenced by genetic and environmental factors along with their mutual interactions. Single nucleotide substitution in different genes has also been reported to be associated with growth traits in different swine breeds. Ryanodine receptor gene – *RYR1* (Halothane gene) had its influence on body

weight gain (Fisher and Mellett 1997, Krenkova *et al.* 1999) and feed conversion ratio (Larzul *et al.* 1997). The *MC4R* is involved in regulation of feed intake and growth related traits in various pig lines (Kim *et al.* 2000). *PRKAG3* gene had significant association with body weight, feed intake and feed conversion ratio in meat type chicken (Jin *et al.* 2016). The present investigation was therefore undertaken to determine effect of *MC4R*, *RYR1* and *PRKAG3* SNPs on body weight in crossbred pigs.

MATERIALS AND METHODS

A total of 238 crossbred (75% Landrace × 25% Bareilly local) piglets born at Swine Production Farm of the institute from 2013–16 were considered in the study. Genotype of each animal for 3 SNPs at porcine genes [Halothane/Stress/Porcine Stress Syndrome / Ryanodine Receptor 1(*HAL/PSS/RYR1*) gene; Melanocortin-4 Receptor (*MC4R*) gene; Protein Kinase, Amp-Activated, Gamma 3 (*PRKAG3*) gene], already attained by PCR-RFLP procedure and recorded in Farm Register was collected. Details of SNPs along with primer sequence, PCR program, restriction enzyme and amplicon size is given in Table 1. The body weight at birth and thereafter at weekly interval up to 8 week was collected from the Farm Records.

Polymorphic information content, observed and expected heterozygosity and number of effective allele were calculated using POPGENE32 software. Association of

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SNPs with body weights was determined using PROC GLM Module of SAS 9.3 software with following model:

$$y_{ijklmn} = \sum_{i=1}^3 G_i + P_j + Y_k + SN_l + SX_m + e_{ijklmn}$$

where y_{ijklmn} , observation on i^{th} genotype, j^{th} parity, k^{th} year of birth, l^{th} season of birth and m^{th} sex; μ , overall mean; $\sum_{i=1}^3 G_i$, effect of i^{th} genotype for *RYR1*, *MC4R* and *PRKAG3*; P_j , effect of j^{th} parity; Y_k , effect of k^{th} year of

birth; SN_l , effect of l^{th} season of birth; SX_m , effect of m^{th} sex; e_{ijklmn} , random error $\sim \text{NID}(0, \sigma^2 e)$.

RESULTS AND DISCUSSION

Least-squares analysis of variance and means of body weights are shown in Table 2. The PIC, observed heterozygosity, expected heterozygosity and number of effective alleles were low for *RYR1* (0.08, 0.09, 0.08 and

Table 1. SNPs along with primer sequence, PCR program, restriction enzyme and amplicon size

Gene	Location (Porcine chromosome no)	Primer sequence (5'-3')	Amplicon (bp)	PCR program	SNP position	Restriction enzyme	GenBank	Source
MC4R	1q22-q27	F:TACCTGACCA TCTTGATTG R:ATAGGAACAG ATGATCTCTTT	226	95°C 5 min (94°C 1 min, 52.5°C 45 sec, 72°C 1 min) 40 cycles, 72°C 5 min	C.1426A>G	<i>TaqI</i>	AF087937.1 (2000)	Kim <i>et al.</i>
RYR1	6 q1.1-q1.2	F:TCCAGTTTGCCA CAGGTCCCTACCA R:TTCACCGGAGT GGAGTCTCTGAGT	659	95°C 5 min (94°C 1 min, 60°C 45 sec, 72°C 1 min) 40 cycles, 72°C 5 min	1843C>T	<i>HhaI</i>	M91452.1	Fujii <i>et al.</i> (1991)
PRKAG3	15q2.4-q2.5	F:GGAGCAAATG TGCAGACAAG R:CCCACGAAGC TCTGCTTCTT	259	95°C 5 min (94°C 1 min, 9°C 45 sec, 72°C 1 min) 40 cycles, 72°C 5 min	c.599G>A	<i>BsrBI/MbiI</i>	AF214520.2	Milan <i>et al.</i> (2000)

Table 2. Least square means of body weights at different ages across the various effects

Factor	Least square means of body weights								
	Birth	1 week	2 week	3 week	4 week	5 week	6 week	7 week	8 week
μ	1.03±0.21	2.36±0.51	3.73±0.82	5.18±1.22	6.58±1.62	8.01±1.92	9.53±2.28	11±2.62	12.87±2.77
Parity				*	*	**	*	*	**
1	1.02±0.04	2.11±0.11	3.53±0.17	4.70±0.25	5.90±0.33	7.53±0.40	8.90±0.47	10.41±0.54	1.02±0.04
2	1.02±0.04	2.19±0.11	3.66±0.18	4.91±0.26	6.15±0.35	7.74±0.42	9.17±0.49	10.54±0.57	1.02±0.04
3	0.98±0.06	2.17±0.14	3.21±0.23	4.10±0.34	5.00±0.46	6.27±0.54	7.85±0.64	8.91±0.74	0.98±0.06
4	0.91±0.10	1.92±0.25	3.39±0.40	5.04±0.60	6.50±0.79	8.43±0.94	10.70±1.12	11.58±1.29	0.91±0.10
Season			*	**	**	**	**	**	**
November–February	0.96±0.05	2.05±0.14	3.19±0.22	4.14±0.33	4.92±0.44	6.35±0.52	7.84±0.62	9.20±0.71	10.38±0.76
March–June	1.01±0.05	2.14±0.12	3.55±0.20	5.03±0.30	6.47±0.40	8.14±0.47	10.00±0.56	11.05±0.64	12.43±0.68
July–October	0.97±0.05	2.10±0.12	3.60±0.19	4.89±0.29	6.27±0.38	7.98±0.46	9.62±0.54	10.83±0.62	11.84±0.66
Year		**							**
2013	1.01±0.06	2.09±0.15	3.52±0.25	4.68±0.37	5.75±0.49	7.22±0.59	8.82±0.70	10.05±0.80	11.19±0.86
2014	0.96±0.08	1.87±0.23	3.12±0.37	3.96±0.55	4.96±0.73	6.86±0.86	8.42±1.02	9.20±1.18	9.59±1.25
2015	0.93±0.05	2.05±0.12	3.49±0.20	4.92±0.29	6.20±0.39	7.80±0.46	9.45±0.55	10.73±0.63	12.04±0.68
2016	1.03±0.04	2.38±0.09	3.65±0.14	5.19±0.21	6.63±0.28	8.08±0.33	9.93±0.39	11.45±0.45	13.37±0.48
Sex									
M	1.01±0.05	2.12±0.12	3.47±0.20	4.71±0.29	5.92±0.38	7.47±0.46	9.07±0.54	10.25±0.62	11.32±0.66
F	0.96±0.04	2.08±0.11	3.43±0.18	4.66±0.27	5.86±0.36	7.52±0.43	9.24±0.51	10.46±0.59	11.78±0.62
SNP <i>MC4R</i>									
AA	0.98±0.05	2.00±0.13	3.35±0.21	4.55±0.32	5.81±0.42	7.56±0.50	9.29±0.59	10.57±0.68	11.67±0.72
AG	0.96±0.05	2.11±0.11	3.39±0.19	4.67±0.28	5.82±0.37	7.28±0.43	8.86±0.51	10.04±0.59	11.28±0.63
GG	1.01±0.05	2.19±0.13	3.61±0.20	4.84±0.30	6.04±0.40	7.64±0.48	9.31±0.57	10.47±0.65	11.70±0.69
SNP <i>RYR1</i>									
NN	1.00±0.04	2.17±0.10	3.57±0.16	4.85±0.23	6.10±0.31	7.65±0.37	9.33±0.43	10.42±0.50	11.65±0.53
Nn	0.97±0.06	2.03±0.15	3.32±0.25	4.52±0.37	5.67±0.48	7.34±0.58	8.98±0.68	10.30±0.78	11.45±0.83
SNP <i>PRKAG3</i>									
QR	0.99±0.04	2.09±0.11	3.39±0.17	4.67±0.26	5.93±0.34	7.61±0.41	9.29±0.48	10.44±0.56	11.68±0.59
RR	0.98±0.05	2.11±0.13	3.50±0.22	4.71±0.32	5.84±0.43	7.37±0.51	9.02±0.60	10.28±0.69	11.42±0.73

*Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$.

Table 3. Allele frequency, genotype frequency, polymorphic information content, heterozygosity and number of effective allele for different SNPs with test for Hardy-Weinberg equilibrium in crossbred pigs

Locus	No. of individual(s)	No. of allele	Allele frequency		Genotype frequency			No of effective allele (N_e)	PIC	Heterozygosity		Test for HWE		
			A	G	AA	AG	GG			Observed (H_o)	Expected (H_e)	χ^2	DF	Pr> χ^2
SNP <i>MC4R</i>	238	2	A (0.47)	G (0.53)	AA (0.185)	AG (0.567)	GG (0.248)	1.99	0.37	0.57	0.5	4.5969	1	0.032
SNP <i>RYR1</i>	238	2	N (0.95)	n (0.05)	NN (0.91)	Nn (0.09)	Nn (0.09)	1.09	0.08	0.09	0.08	0.507	1	0.4764
SNP <i>PRKAG3</i>	238	2	R (0.91)	Q (0.08)	QR (0.185)	RR (0.815)	RR (0.815)	1.2	0.15	0.18	0.17	2.469	1	0.1161

1.09) and *PRKAG3* (0.15, 0.18, 0.17 and 1.20) SNP locus revealing that population under investigation was of low diversity maintaining a single allele (Table 3). The corresponding values for *MC4R* (0.37, 0.57, 0.5 and 1.99) SNP locus were intermediate representing that forces had been operated to maintain both alleles in the population. Chi squares value was significant for *MC4R* ($\chi^2=4.6$) showing significant departure from the Hardy-Weinberg equilibrium. Body weight showed a continuous increase over age from birth to 8 week. *RYR1* (1843C>T) had two genotypes, i.e. dominant homozygote (NN) and heterozygote (Nn). Genotypic frequencies were 0.91 and 0.09 for NN and Nn, respectively; while, the allelic frequencies were 0.95 and 0.05 for N and n, respectively. At *MC4R* (C.1426A>G) SNP site, two alleles (i.e. A and G) and three genotypes (i.e. AA, AG and GG) were observed in present population. Out of 238 animals, 135 were AG with genotypic frequency of 0.567, 44 were AA with genotypic frequency of 0.185 and 59 were GG with 0.248 genotypic frequency. The frequency of A and G allele was 0.47 and 0.53 in crossbred pigs. Two genotypes were observed at *PRKAG3* (c.599 G>A) SNP locus, i.e. heterozygote QR and homozygote RR. RR with genotypic frequency of 0.815 and QR with genotypic frequency of 0.185 were observed in present population and the allelic frequencies for R and Q were 0.91 and 0.09, respectively.

Effect of all 3 SNPs was non-significant on body weight at all the ages (Table 2). The present results were however contrary to the findings of previous workers. Fisher and Mellett (1997), Larzul *et al.* (1997), Razmaitė (2006) and Pietruszka *et al.* (2008) observed significant effect of *RYR1* (1843C>T) SNP on body weight in different breeds of pigs at different ages. Significant effect of *MC4R* (C.1426A>G) on body weight (at 10 to 20 week) was reported by Kim *et al.* (2000), Houston *et al.* (2004) and Switonski *et al.* (2010) in pigs. This difference in results may be attributed to the variation in genetic makeup of the population / breed and sample size under investigation. Hernandez-Sanchez *et al.* (2003) and Houston *et al.* (2004) also reported that the effect of *MC4R* (C.1426A>G) SNP on growth is breed or line specific.

Though non-significant, GG genotype of *MC4R* (C.1426A>G) SNP, NN genotype of *RYR1* (1843C>T) SNP and QR genotype of *PRKAG3* (c.599 G>A) SNP had better growth at 6 and 8 week as compared to their contemporary genotypes, i.e. AG and AA genotype of *MC4R* (C.1426A>G) SNP, Nn genotype of *RYR1* (1843C>T) SNP and RR genotype of *PRKAG3* (c.599 G>A) SNP. The better growth in NN genotype of *RYR1*(1843C>T) SNP was in agreement with findings of Pietruszka *et al.* (2008) in Polish Synthetic pigs. Larzul *et al.* (1997) and Razmaitė (2006) however observed better growth in nn genotype of *RYR1* (1843C>T) SNP as compared to their contemporary genotypes in Large White crossbred pigs. Kim *et al.* (2000) observed better growth in GG genotype of *MC4R* (C.1426A>G) SNP. Their results were similar to our investigation. Hernandez-Sanchez *et al.* (2003) and Houston

et al. (2004) however observed better growth in AA genotype of *MC4R* (C.1426A>G) SNP as compared to their contemporary genotypes in Landrace and Large White crossbred pigs.

Effect of parity was significant on most of the body weights, except for weight at birth, 1 and 2 week. Fourth parity piglets had higher body weight as compared to those born in other parities. Similar observations were also reported by Deka (2002) and Chhabra *et al.* (2005) in Landrace and Large White Yorkshire crossbred pigs. In the present investigation, effect of season of birth was significant on body weight from 2–8 weeks and non-significant at birth and 1 week. Piglets born from March to June had a higher growth at most of the weeks as compared to rest of the seasons. However, Mukhopadhyay *et al.* (1992) and Chhabra *et al.* (2005) reported higher body weight at winter season in Landrace crossbred pigs. The effect of year of birth was non-significant on body weight at most of the ages, except at 1st and 8th week. Similar results were also observed by Nath *et al.* (2002) and Chhabra *et al.* (2005) in Landrace and Large White Yorkshire crossbred pigs. Piglets born in 2016 had higher growth at most of the weeks. The effect of sex was non-significant on body weight at all of the ages. However, males were higher in body weight as compared to females.

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