Indian Res. J. Genet. & Biotech 7(2) : 174 – 178 (2015)

Effect of TLR6-1 on different economic traits in swine

Nandani Kumari[1], Lal Babu Singh[2], Saroj Kumar Thakur[3] and Kiran Mishra[4]

[1]Deptt. of A.G.B., R.V.C., B.A.U. [2]Department of A.G.B., R.V.C., B.A.U. [3] A.I.O., F.S.B., Hotwar, Ranchi, Jharkhand Govt. [4]College of animal and veterinary science, Durg, Chhattisgarh

(Received: January 2015; Revised: March 2015; Accepted: April 2015)

Abstract

Effect of TLR6 gene polymorphism and its relationship with different economic traits has been analysed and documented in the current article. A total of forty-eight pigs were taken and the polymorphism of TLR6 gene by SSCP was seen. The set of forward and reverse primers (TLR6-1) was taken to amplify and see the polymorphism at the locus. The effect of haplotypes thus observed was seen on a total of five growth traits namely body weight at birth, body weight at seven days, body weight at fourteen days, body weight at twenty-eight days, body weight at forty-two days and four reproductive traits namely litter size at birth, litter weight at birth, litter size at weaning, litter weight at weaning. The population means were observed to be 09.030 ± 00.5850, 10.945 ± 00.8840 kg, 09.378 ± 00.7323 and 81.275 ± 6.7156, 01.178 ± 00.086, 01.689 ± 00.3480, 02.950 ± 00.5387, 04.057 ± 00.5821, 05.976 ± 00.5881 & 06.935, ± 00.9902 kg, for litter size at birth, litter weight at birth, litter size at weaning, litter weight at weaning, body weight at birth, body weight at 7-day, body weight at 14-day, body weight at 42-day and body weight at 56-day respectively. Significant effect was observed for body weight at birth. Effect on other traits although non-significant showed inter-haplotypes variations. Effect of TLR 6 gene could be more evident and informative if further results were taken and pooled over for all the available primers of TLR6 gene as effect would be significant at those loci for other traits as well.

Key words- SSCP, TLR6, Haplotypes, PCR, Growth traits, Reproductive traits.

Introduction

Swine production is getting momentum in Eastern India and N.E. regions including Jharkhand due to high demand of pork and pork products. A number of farmers and unemployed youths have started swine production programme for their livelihood and income generation. Swine breeders as well as farmers have exploited their positive aspects like better genotypes or breeds, short generation interval, high prolificacy and fast growth rate. But during an outbreak of fatal disease, they witness heavy loss due to high mortality and morbidity. Livestock diseases cost producers and consumers millions of dollars every year due to mortality and morbidity. It is also true that medication and vaccination are effective for controlling many diseases but some diseases still have a devastative effect when outbreaks occurs. Breeding for disease resistance is an alternative way to solve the problem of diseases in farms in long term perspective and to minimise the costs of disease control. Animal breeders and genetists are now focusing attention towards proper utilization of disease resistance aspect of livestocks. Toll like receptor are class of proteins that play a key role in the immune response system. It recognizes conserved molecules derived from microbes. Polymorphisms and/or differences in the production of immune molecules, such as TLRs, have a profound influence on responses to a wide range of pathogens and are associated with resistance and susceptibility to diseases Boger *et al* (2012). TLR1, TLR2, TLR4, TLR5, and TLR6 that recognize lipids or proteins, which are the cell wall components of extracellular pathogens, are located on the cell surface. On the other hand, TLR3, TLR7, TLR8 and TLR9 that recognize nucleic acids, such as RNA and DNA, derived from bacteria and viruses are expressed in the cytoplasm (Akira and Takeda 2004).

Materials and Method

A total of 48 pigs from three genetic groups namely Tamworth, Desi and T&D maintained at Pig farm

Corresponding authors- e-mail: drnandanikumari@gmail.com
Published by the Indian Society of Genetics, Biotechnology Research and Development
Biotech Bhawan 5 E Nikhil Estate, DPS Road, Shastripuram, Agra 282007
Online management by www.isgbrd.co.in

Ranchi veterinary College were utilized for this investigation. Observations of weight at birth (Birth weight) of all the 48 animal were taken under controlled managerial conditions.

Five ml of blood was collected along with anticoagulant from each of the experimental animal. Genomic DNA was isolated and purified from white blood cells using proteinase-K digestion and standard phenol-chloroform extraction as per the standard protocol described by Sambrook, *et. al.,*(1989). The base sequence of synthetic oligonucleotide /primers (One forward and another backward) taken to prime DNA amplification to see the polymorphism named as TLR6-1 in TLR6 was as follows-

Primer name
Sequence of Forward primer
Sequence of Reverse primer
Fragment size
TLR6- 1
TTTGGATGCCTAGCAAGATA
GGGATGGCACTTTTCCAGAT
425

To explore genetic polymorphism in TLR-6 gene, amplified PCR products were subjected for SSCP through polyacrylamide gel electrophoresis. Silver staining method was described by Bassam,*et.al.,*(1991). The data was statistically analyzed with available computer software SPAB and Least Square Analysis Harvey's model(1990).

The following economic traits were considered to see the effect of TLR6 gene on them –

I. Growth traits-
- I.1. Body weight at birth
- I.2.Body weight at seven days
- I.3.Body weight at fourteen days
- I.4.Body weight at twenty-eight days
- I.5.Body weight at forty-two days

II. Reproductive traits-
- II.1.Litter size at birth
- II.2Litter weight at birth
- III.4.Litter size at weaning
- III.5.Litter weight at weaning

Results and Discussion

The PCR-SSCP association with different traits for TLR6-1 gene was analyzed and shown in table 4.2. & 4.3. A total of six haplotypes were observed in case of 1st primer namely A, B, C, D, E and F for the four SSCP gels obtained (Figure 1). The haplotypes had non-significant effect on all other traits under study (litter size at birth, litter weight at birth, litter size at weaning, litter weight at weaning, body weight at seven days, body weight at fourteen days, forty two days and fifty six days.) except body weight at birth.

The population means were observed to be 09.030 ± 00.5850, 10.945 ± 00.8840 kg, 09.378 ± 00.7323 and 81.275 ± 6.7156, 01.178 ± 00.086, 01.689 ± 00.3480, 02.950 ± 00.5387, 04.057 ± 00.5821, 05.976 ± 00.5881 & 06.935, ± 00.9902 kg, for litter size at birth, litter weight at birth, litter size at weaning, litter weight at weaning, body weight at birth, body weight at 7-day, body weight at 14-day, body weight at 42-day and body weight at 56-day respectively (Tables 1 and 2) .

Significant effect was reported for body weight at birth ($F<0.01$). Haplotype A, differed significantly from all except for haplotypes B and F. Haplotypes C&D did not differ significantly from each other and D had significantly highest body weight (1.365 ± 0.1960 kg) at birth than other haplotypes while F had significantly lowest body weight at birth among all with a mean value of 1.028 ± 00.1492 kg.

The maximum body weight at 7-day was 02.026 ± 00.7916 kg for haplotype D and it was lowest (1.429 ± 00.7473 kg) for haplotype E .

The trait, 14-day body weight showed a highest value of 03.712 ± 01.2254 kg for haplotype D and a lowest value of 02.275 ± 00.9331 kg. for haplotype F.

With respect to body weight at 42-day, the haplotypes had non-significant effect on F-value. Among all the haplotypes, A had maximum value of 06.675 ± 00.6618 kg and the lowest value of 05.431 ± 01.3376 kg was found for haplotype D.

In case of 56-day body weight, the haplotype F had the highest value of 08.909 ± 017150 kg and D had the lowest value of 05.377 ± 02.2523 kg.

Maximum value of mean litter size at birth was 10.249 ± 01.5726 in case of hyplotype E whereas minimum value was reported (07.737 ± 01.2685) for haplotype F. The highest litter weight at birth was observed to be 11.884 ± 02.0109 kg for haplotype D followed by haplotype C (11.8781 ± 01.331 kg). The lowest value of mean litter weight at birth was observed with haplotype F (08.336 ± 01.5313).

In case of litter size at weaning, haplotype C had the highest mean of 09.804 ± 00.8808 followed by E whose mean value was 09.736 ± 01.2563. The lowest value of litter size at weaning was found with haplotype F (07.389 ± 01.0134). In case of litter weight at weaning the haplotype E had highest value of 94.913 ± 14.4206 kg while it was lowest for haplotype F (69.244 ± 11.6323 kg).

Figure 1. Haplotyping pattern under TLR6-1 (Gel-3)

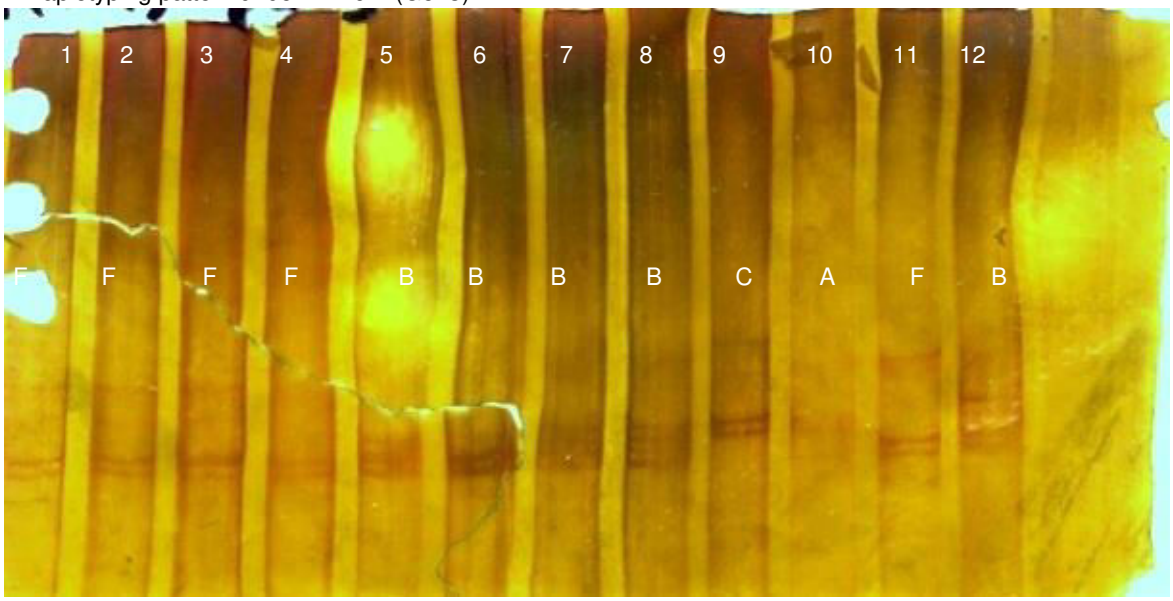

Table.4.2.Association of TLR6-1 PCR-SSCP Variants/SNPs with growth traits

Traits → / Haplotypes ↓	Birth Wt(Kg)	7day Wt(Kg)	14day Wt(Kg)	28day Wt(Kg)	42day Wt(Kg)	56day Wt(Kg)
μ	01.177± 00.0862	01.689± 00.3480	02.950± 00.5387	04.097± 00.5821	05.976± 00.5881	06.935± 00.9902
A	01.085± 00.0970[a]	01.795± 00.3917	02.972± 00.6063	04.528± 00.6552	06.675± 00.6618	07.915± 01.1143
B	01.098± 00.1117[ab]	01.613± 00.4512	02.774± 00.6984	03.824± 00.7547	05.744± 00.7624	06.325± 01.2837
C	01.329± 00.1297 [b]	01.534± 00.5239	02.624± 00.8111	03.896± 00.8764	05.544± 00.8853	06.475± 01.4907
D	01.365± 00.1960[c]	02.026± 00.7916	03.712± 01.2254	04.499± 01.3242	05.432± 01.3376	05.377± 2.2523
E	01.160± 00.1850[d]	01.429± 00.7473	02.275± 00.9331	03.049± 01.2501	05.958± 01.2627	06.612± 02.1262
F	01.028± 00.1492[d]	01.737± 00.6028	03.343± 01.1568	04.786± 01.0084	06.503± 01.0186	08.909± 01.715
F value	03.582*	00.506	01.188	01.509	00.721	01.114

Table 1. Association of TLR6-1 PCR-SSCP Variants/SNPs with reproductive traits

Traits → / Haplotypes ↓	LszB	LwtB(kg)	LszW	LwtW(Kg)
μ	09.378±00.7323	10.975±0.8840	09.031±00.5850	81.275± 06.7156
A	09.789± 00.8242	11.264± 00.9949	09.157± 00.6584	75.669± 07.5578
B	09.434± 00.9495	11.122±01.1461	09.225±00.7585	88.669± 08.7066
C	10.001± 01.1025	11.878± 01.3309	09.804± 00.8808	76.873±10.1105
D	09.056± 01.6658	11.884± 02.0109	08.874±01.3308	82.281± 15.2756
E	10.249±01.5726	11.367±01.8983	09.736±01.2563	94.913±14.4206
F	07.737±01.2685	08.334±01.5313	07.389±01.0134	69.244± 11.6323
F value	01.1254	01.3695	02.0913	01.7178

Russel et al.(2012) analysed selected bo*TLR1* SNPs within a Holstein Friesian herd. Significant associations were found for the tagging SNP -79 T > G and the 3'UTR SNP +2463 C > T. They observed favourable linkage of reduced CM with increased milk fat and protein, indicating selection for these markers would not be detrimental to milk quality. Furthermore, it was found that some of these bo*TLR1* SNPs underpin functional variation in bovine TLR1. Animals with the GG genotype (from the tag SNP -79 T > G) had significantly lower bo*TLR1* expression in milk somatic cells when compared with TT or TG animals. In addition, stimulation of leucocytes from GG animals with the TLR1-ligand Pam3csk4 resulted in significantly lower levels of CXCL8 mRNA and protein. SNPs in bo*TLR1* were significantly associated with CM.

Lim et al.(2013) found that iNOS (Inducible nitric oxide synthase) and TLR-4 (Toll-like Receptor-4) play crucial roles in innate immunity of poultry. This study was aimed to augment previous findings, which show the association of iNOS (C14513T) and TLR-4 (G4409T) polymorphisms with economic traits in Korean Native Black (KNB), Rhode Island Red (RIR) and Cornish chickens. Investigation in the effect of SNPs on economic traits (layday, layw, layno, bw150, bw270, layw270) was conducted. iNOS (C14513T) had a significant effect on the average body weight at 270 days of age (p0.05). The results obtained from using the candidate genes can be useful for the genetic improvement of body weight in both KNB and RIR breeds.

According to yang et al. (2013) SNPs of TLR6 (rs1039559, rs3821985, and rs3775073) may be affect the disease characteristics of stroke, such as NIHSS and MBI. Recent studies showed association between diseases and TLR6 polymorphisms. Two SNPs (rs3821985 and rs3775073) of the TLR6 gene were associated with the NHISS in ischemic stroke patients ($P< 0.05$). Also, three SNPs (rs1039559, rs3821985, and rs3775073) showed association with MBI in ischemic stroke patients ($P< 0.05$).

Conclusion

The PCR-SSCP analysis of TLR6-1 gene revealed the polymorphic pattern of haplotypes in Swine. There was significant association between many traits and TLR6 gene. Many traits showed non-significant association of traits with TLR6 gene but variation among haplotypes was found for the traits. Effect of TLR 6 gene could be more evident and informative if further results were taken and pooled over for all the available primers of TLR6 gene because the non-significant effect of SNPs/Haplotypes/polymorphism on different traits at one locus could be significant at other. Considering the association of revealed polymorphic variants of TLR6-1 gene, t can act as an aid in developing marker assisted selection.

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