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**Original Research Article**

# Genetic variability of TLR1 Gene and Its Relation with Different Traits in Pigs

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**A B S T R A C T**

TLR (toll-like receptor) gene is of prime importance in innate immunity response of different livestocks particularly pig. Genetic variations of the TLR1 gene were investigated by single strand conformationpolymorphism (SSCP) analysis. The present study was carried on blood samples of 96 individuals (5 ml each) of the pig population. The PCR-SSCP variants had significant effect on the litter size at birth and 7 days body weight while there were non-significant effect on piglet titer, dam titer, litter weight at birth, litter size at weaning, litter weight at weaning, body weights at birth, 14 days, 28 days, 42 days and 56days. The generated data will help for the detection of SNP markers related to disease resistance or susceptibility among pig population in India.

## Introduction

Swine production has become very important in India due to increased demand of pork and pork products among different section of our society especially in tribal population. The success of swine farming is greatly dependent on early body weight gain of the piglet, as it influences the survival and overall productivity. Morbidity and mortality are serious problem of piglets even after vaccination and optimum managmental condition. The extensive use of medicines for the control of diseases has led to development of resistance against the infectious agent and increased cost of production. Understanding the polymorphism pattern of genes responsible for immune responses are being carried out across the globe. Among the different genes involved in immune response of different livestock particularly pig, TLR (toll-like receptor) gene is of prime importance in innate immunity response. The growth has definite economic importance as well as genetic significance, because it reflects the overall interaction of genotype with all environmental factors under which it is expressed.

## Materials and Methods

Blood samples of 96 individuals (5 ml each) from the pig population were collected from

the ear vein in large pig and cephalic vein in piglets. These samples were collected in vaccutainers. An anticoagulant (EDTA) was mixed in blood. 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).A pair of synthetic oligonucleotide (primers) was required to prime DNA synthesis. The size of TLR1 gene was 2391 bp which was fragmented into five primers. (Shinkai *et al.*, 2006). Primer under study were synthesized by Xcelaris Lab.

Before running in PAGE, the 2 µl of PCR products were checked in 2% agarose for the amplification. Single strand conformational polymorphism is a tool for mutation detection at DNA level. It is a conformation based scanning method of polymorphism detection which is simple and widely used method.

In SSCP the target region is amplified by PCR and then amplified region is denatured to generate single stranded DNA and separated by electrophoresis on a non-denaturing polyacrylamide gel. The single stranded fragments adopt three dimensional conformations, which is dependent on the primary sequence. To explore genetic polymorphism in TLR1gene, amplified PCR products were subjected for SSCP through polyacralamide gel electrophoresis. Mixed Model Least Square and Maximum Likelihood MethodAs the data were non-orthogonal in nature with disproportionate subclass numbers, the same were subjected to Maximum Likelihood Method (Harvey, 1966 and 1990). Genotype based Growth and Reproductive traits: - The following mathematical model for reproductive traits was used:

$Y_{ij} = \mu + G_i + e_{ij}$.

Where,

$Y_{ij}$ = value of $j^{th}$ animal belonging to $i^{th}$ genotypic variants.

$\mu$ = population mean,

$G_i$ = Effect of $i^{th}$ genotypic variants, 1 to 3 and 1 to 3.

$e_{ij}$ = Random error associated with $Y_{ij}$ which is normally and independently distributed with mean zero and variance $\sigma^2_e$

## Results and Discussion

PCR-SSCP Polymorphism analysis of TLR1 Gene with the first primer having forward and reverse base sequence as CCCTCCAGGATCTATACCG and GGGTCTTCTCTTTCCCCGTA respectively, three different SSCP variants were found which were ABCD, ABCC and BBCC. The PCR-SSCP variants had significant effect on the litter size at birth and 7 days body weight while there were non-significant effect on piglet titer, dam titer, litter weight at birth, litter size at weaning, litter weight at weaning, body weights at birth, 14 days, 28 days, 42 days and 56days. ABCD genotype showed significantly ($P<0.05$) highest litter size at birth (08.97) followed by BBCC (08.25).

The genotype BBCC had the highest body weight (02.18) at 7 days. Bergman (2009) reported different polymorphic pattern of TLR1 gene. TLRs play an essential role in initiating the immune response against pathogens and can recognize a wide variety of pathogen associated molecular patterns from bacteria, viruses, and fungi. Though Liu *et al.*, (2011) reported that there were differences in anti-disease ability produced by different SSCP variants in a study of 14 chicken breed in China.

**Blood samples of 96 individuals (5 ml each) from the pig population**

| FRAGMENT NOS. | FORWARD PRIMER | REVERSE PRIMER | PRODUCT SIZE |
|---|---|---|---|
| 1 | CCCTCCAGGATCTATACCG | GGGTCTTCTCTTTCCCCGTA | 629 |

Contrary to that immune response developed against sheep RBC is non- specific that might be the reason for non-significant effect of SSCP variants of TLR1 gene on immune response like dam titer and piglet titer against the Sheep RBC in this study. The PCR-SSCP analysis of TLR -1 gene (first fragment) under study revealed the polymorphic pattern of genotypes in Swine. It may be stated that there was no significant association of TLR -1 gene polymorphism with the Humoral Immune response against Sheep RBC. Considering the association of revealed polymorphic variants of TLR -1 gene, will be an aide for the genetic improvement of the pig population.

## References

Bergman, I.M, Rossenberg, J.K, Edman, K and Edfors I. 2009. European wild boars and domestic pigs display different polymorphic patterns in the Toll like receptor (TLR) 1 TLR2 and TLR6 genes. *Immunogenetics* 62, 49-58

Buschmsnn, H.V.; Jung, H.; Krausslich; and Radzikowski, A. 1974. A study of immune response to sheep erythrocytes in several breeds of swine. *Med. Microbiol. Immunol*. 159-179.

Harvey W.R. 1990. Mixed Model Least Squares and Maximum Likelihood Computer Program. Users Guide for LSMLMW and MIXMDL.

Kramer, C. Y. 1957. Extension of multiple range test of group correlated adjusted means. *Biometrics*, 13: 13-18.

Sambrook, J.; Fritsch, E. F. and T. Maniatis, 1989. Molecular cloning: A Laboratory Manual 2nd ed. Cold spring Harbour; Cold Laboratory press; NY.

Shinkai, H; Tanaka M; Morozumi, T; Eguchi-Ogawa, T; Okumura, T; Muneta, Y; Takashi Awata. Hirohide Uenishi 2006. Biased distribution of single nucleotide polymorphisms (SNPs) in porcine Toll-like receptor 1 (TLR1), TLR2, TLR4, TLR5, and TLR6 genes, *Immunogenetics* (58): 324–330