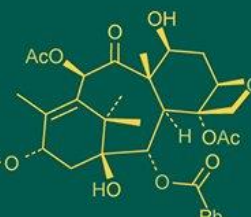
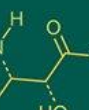
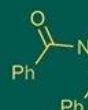


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Priyanka Pati
MVSC Scholar, Department of
Animal Genetics and Breeding,
NTR College of Veterinary
Science, Sri Venkateswara
Veterinary University,
Gannavaram, Andhra Pradesh,
India

Vinoo Regula
Professor, Sri Venkateswara
Veterinary University,
Tirupati, Andhra Pradesh,
India

Sudhakar Krovvidi
Associate Professor and Head,
Department of Animal
Genetics and Breeding, NTR
College of Veterinary Science,
Sri Venkateswara Veterinary
University, Gannavaram,
Andhra Pradesh, India

Kalyan Chakravarthy
Senior Scientist and Head,
ICAR-AICRP on Pigs, SVVU,
Tirupati, Andhra Pradesh,
India

Meghana Kondapu
Assistant Professor,
Department of Animal
Genetics and Breeding, NTR
College of Veterinary Science,
Sri Venkateswara Veterinary
University, Gannavaram,
Andhra Pradesh, India

Corresponding Author:
Meghana Kondapu
Assistant Professor,
Department of Animal
Genetics and Breeding, NTR
College of Veterinary Science,
Sri Venkateswara Veterinary
University, Gannavaram,
Andhra Pradesh, India

Evaluation of meat quality traits associated CAST and IFNA16 genes polymorphism in synthetic SVVU T-17 swine of Andhra Pradesh

Priyanka Pati, Vinoo Regula, Sudhakar Krovvidi, Kalyan Chakravarthy and Meghana Kondapu

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Abstract

The aim of the study was to characterize the polymorphism of the Calpastatin (*CAST*) and Interferon- α -16 (*IFNA16*) genes identified with restriction enzyme (*HinfI*) in LWY and SVVU-T17 population of Andhra Pradesh. Polymerase chain reaction-restriction-fragment-length-polymorphism (PCR-RFLP) was used to genotype the animals at SNP 76872 G>A for *CAST* gene and 413G>A SNP for *IFNA16* gene. The *CAST* gene (G76872A) genotypic and allelic frequencies of AA, GA, GG and A and G were found to be 0.39, 0.49, 0.12 and 0.63, 0.37 (LWY); 0.008, 0.47, 0.522 and 0.24, 0.76 (SVVU T-17) whereas, *IFNA16/HinfI* (rs701706389) genotyping revealed that two genetic groups were monomorphic with fixation of A allele. The Polymorphic Informative Content (PIC) value was medium and ranged from 0.29 to 0.36 for *CAST* gene. Diversity estimates (F_{IS}) value were negative for two genetic groups i.e., LWY (-0.27) and SVVU T-17 (-0.06). The Chi-Square test values revealed that LWY population was under HWE and SVVU-T17 population was deviated from HWE. Differences in genotypic and allelic frequencies at *CAST/HinfI* locus between LWY and SVVU T-17 were significant ($P < 0.01$). The results revealed the existence of polymorphism for *CAST* gene and it could be a marker to establish association with meat quality traits.

Keywords: Swine, IFNA16, CAST gene, polymorphism

Introduction

The consumers demand for meat is focused on quantitative as well as the qualitative characteristics of meat. Pork quality is determined by factors viz. pH45min after slaughter, final pH after slaughter, water-holding capacity, drip loss, tenderness, and sensory attributes (Sellier, 1998) [25]. Meat quality is a complicated polygenic trait that is influenced genetically both within and between breeds (Andersson, 2001) [1]. Over the past two decades, genomic techniques have been developed to identify genes associated with these traits, as improvement through conventional selection is difficult due to their low heritability, high cost of evaluation, and the fact that these traits can only be measured after slaughter. Genetic markers that are predictive of pork quality could be identified and used for genetic selection programs.

Calpastatin is an endogenous inhibitor protein specifically acting on Calpain, Ca^{2+} dependent cysteine proteinase and it plays a key role in meat tenderness by degrading myofibrillar proteins after slaughter. Previous studies suggest that Calpastatin (*CAST*) is an excellent candidate gene for studying variation in pork quality as it is highly related to the rate of meat tenderization and protein turnover after slaughter (Ernst *et al.*, 1998; Goll *et al.*, 1998; Parr *et al.*, 1999; Hao *et al.*, 2000) [6, 8, 21, 10]. *IFNA16* is a pleiotropic inflammatory cytokine with antiviral, antiproliferative, apoptotic, and immunoregulatory properties. The studies revealed that porcine *IFNA16* polymorphisms are related to intra muscular fat content and/or fatty acid composition (Mekchay *et al.*, 2022) [17]. Owing to the significance of these two genes the objective of this study was aimed to examine the polymorphism of the *CAST* and *IFNA16* genes.

Materials and methods

Sampled population

A total of 218 blood samples were collected from unrelated individuals in swine populations of Large White Yorkshire (LWY) (n = 101), and synthetic SVVU-T17 (n = 117) from different parts of Andhra Pradesh for isolation of genomic DNA. The present study was conducted at the Department of Animal Genetics and Breeding, NTR College of Veterinary Science Gannavaram and was approved by the Institutional Ethics Committee (IAEC No: 11/IAEC/NTRCVSc/2023). Genomic DNA was isolated by high salt method (Miller *et al.*, 1988; Aravindakshan *et al.*, 1997) [18, 21] with minor modifications. The quality and purity of DNA was checked by agarose gel electrophoresis and quantification by Nano spectrophotometer.

Amplification of gene regions and genotyping

The oligonucleotide primers (Table1) reported by Rodriguez *et al.* (2022) [22], were used for amplifying DNA region of intron 6 (76872G>A) in *CAST* gene whereas, primers were custom designed using Primer Quest Tool (IDT) online version for amplifying the genomic region spanning SNP 413G>A (rs701706389) in the *IFNA16* gene. The polymerase chain reaction (PCR) was performed in a 15 µl reaction volume consisting 7.5 µl 2X PCR master mix, 5 pmoles each of forward and reverse primers, and one µl genomic DNA (100 ng). Cyclic condition of PCR included one cycle of initial denaturation (94 °C/5 min) followed by thirty eight cycles of denaturation (94 °C/30 seconds), annealing (50 °C/30 seconds), extension (72 °C/30 seconds) and a cycle of final extension (72 °C/7 min). The PCR products were separated by 1.5% agarose gel electrophoresis. The amplified fragment of *CAST* and *IFNA16* gene was digested with *HinfI* enzyme. Digestion was conducted at 37 °C for 12 h and in a 10 µl reaction solution that includes 3 µl of PCR amplicon 1.5 µl buffer, 0.5 µl (5 units) of restriction endonucleases (*HinfI*), and 5µl of Nuclease free water,. The resulting digestion products were electrophoretically analysed on 2% agarose gel using 1X TAE buffer at constant voltage of 80V for 45 minutes for genotyping. The fragment size of PCR-RFLP products was determined with 50bp and 100bp DNA ladder (O'Gene Ruler DNA Ladder, ThermoFisher). The amplified product was visualized as a single compact fluorescent band of expected size under Omega Fluor™ Plus Documentation Systems, BioExpress, USA. Genetic group wise the number of restriction sites and restriction fragment sizes were noted to genotype the individuals.

Population genetic analysis

Population genetic indicators were estimated using POPGENE version 1.32 (Yeh *et al.*, 1999) [26]. Polymorphic Information Content (PIC) values were also calculated to evaluate the degree of genetic variation. Population differentiation was assessed using FIS (inbreeding coefficient) values estimated through the online tool Wright_Fst Calculator.

Results and Discussion

The polymorphism in intron 6 of the *CAST* (G76872A) and *IFNA16* (c.413G>A) (rs701706389) genes was evaluated using the PCR-RFLP technique. The electrophoretic PCR-RFLP patterns are presented in Figure 1 depicting the digestion of the 376 bp *CAST* PCR product with the *HinfI*

restriction enzyme revealing three distinct genotypes: AA (376 bp), GA (376, 247, and 129 bp), and GG (247 and 129 bp). Genotype and allele frequencies for the *CAST* locus are presented in Table 2. In the LWY population, the GA genotype showed the highest frequency (0.49), followed by AA (0.39) and GG (0.12). In contrast, the SVVU T-17 population exhibited the highest frequency for the GG genotype (0.52), followed by GA (0.47), while only one animal was identified with the AA genotype (0.008). Correspondingly, the frequency of allele A was higher in LWY (0.63), whereas allele G predominated in SVVU T-17 (0.76).

The *CAST*-derived genotypes designated in the present study as AA, GA, and GG correspond to the AA, AB, and BB nomenclature reported in earlier literature (Kocwin-Podsiadla *et al.*, 2003; Kušec *et al.*, 2015) [12,14], since the SNP position and *HinfI* restriction site remain identical. The higher heterozygote frequency observed in LWY was in agreement with reports in crosses involving LWY and other pure breeds (Đurkin *et al.*, 2009; Kušec *et al.*, 2015) [4,14]. Dearth of literature on polymorphism of *CAST* G76872A in Large White Yorkshire populations limited the comprehension on the genetic structure with respect to this locus. However, contrary observations with higher frequencies of the BB genotype were reported by Kocwin-Podsiadla *et al.* (2003), Rybarczyk *et al.* (2010), and Kenchawong *et al.* (2020) [12, 24, 11], in populations involving LWY crosses. Similarly, except for Đurkin *et al.* (2009) [4], who reported a higher frequency of the A allele comparable to the present findings, most studies documented the B allele as predominant, with frequencies ranging from 0.45 to 0.96 across various Yorkshire-derived crossbred populations (Kocwin-Podsiadla *et al.*, 2003; Kurył *et al.*, 2003; Rybarczyk *et al.*, 2010; Gandolfi *et al.*, 2011; Ropka-Molik *et al.*, 2014; Kenchawong *et al.*, 2020) [12, 13, 24, 7, 23, 11]. The disparity of present findings with the literature reviewed against could be attributed to the difference in the genetic makeup of the populations and the selection criteria followed owing to the purpose for which they are reared. Most of the reviewed populations were either commercial stocks or triple crosses.

The genotypic frequency at *CAST/HinfI* locus in synthetic SVVU T-17 pigs could not be compared either with similar genetic groups or desi (indigenous) pigs, the present study being first of its kind on this population. In SVVU T-17 pigs, despite the breed having been developed from crosses involving LWY and indigenous pigs with an intended genetic composition of 75% LWY and 25% desi inheritance, the genotype and allele frequencies differed markedly from those observed in LWY. In SVVU T-17, allele A became the minor allele (0.24), while allele G predominated. The higher frequency of the G allele observed in the present study is consistent with previous reports in synthetic hybrids (Kocwin-Podsiadla *et al.*, 2003) [12], Zlotnicka spotted indigenous pigs of Poland (Kurył *et al.*, 2003) [13], Mongcai pigs native to Vietnam (Ngu *et al.*, 2012) [19], and Creole pigs of Argentina (Rodriguez *et al.*, 2022) [22]. Near fixation of the G allele has also been reported in Pietrain pigs of Belgian origin (Rybarczyk *et al.*, 2010) [24] and Duroc-derived hybrid populations, possibly due to selection pressure (Kenchawong *et al.*, 2020) [11].

For the *IFNA16* gene, amplification of the 237 bp fragment was successful; however, digestion with the *HinfI* restriction enzyme did not reveal any polymorphism. All LWY and

SVVU T-17 samples displayed an undigested 237 bp fragment corresponding to the AA genotype (Figure 2). This indicated fixation of the 'A' allele and absence of the 'G' allele in both populations. Earlier studies demonstrated that IFN- α subtypes are involved in adipogenesis, lipogenesis, and lipid metabolism (Gunfred *et al.*, 1991; Lee *et al.*, 2016) [9, 15]. Furthermore, due to the proximity of the *IFNA16* gene to QTL regions associated with intramuscular fat content and fatty acid composition in swine (Ma *et al.*, 2013; Park *et al.*, 2017) [16, 20], the gene has been hypothesized to influence lipid accumulation and meat quality traits. Nevertheless, both populations evaluated in the present study were monomorphic at the *IFNA16*/*HinfI* locus.

Population genetic analysis revealed that genotype distribution at the *CAST* locus in LWY confirmed to Hardy-Weinberg equilibrium ($P > 0.05$), whereas the SVVU T-17 population deviated significantly from equilibrium ($P < 0.05$). The observed genotype frequencies in LWY (AA: 0.39, GA: 0.49, GG: 0.12) did not differ significantly from expected frequencies. Significant differences in genotype and allele frequencies were detected between LWY and SVVU T-17 populations ($P < 0.001$), indicating that the SVVU T-17 population has evolved and stabilized distinctly from the LWY population despite its genetic contribution from LWY ancestry. The GA and GG genotypes were almost equal in

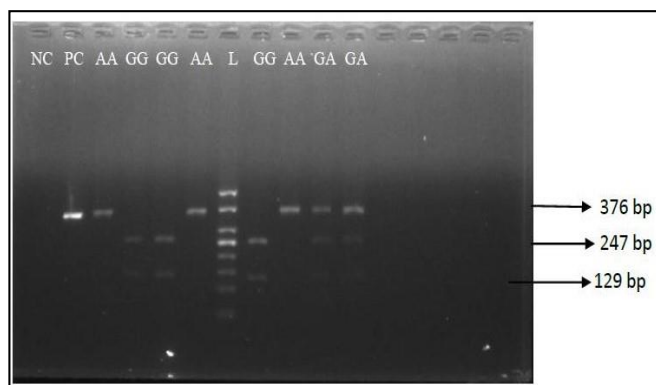
the synthetic population. The perusal of literature indicates that the higher genotypic frequency of 'GG' are associated with increase in ham, highest pH24 (Kowin-Podsiada *et al.*, 2004; Ngu *et al.*, 2012) [12, 19], and low level of intramuscular fat (Ropka-Molik *et al.*, 2014) [23], whereas, 'GA' is associated with preferable values of carcass length, ham length, muscle thickness, loin eye area and fat thickness (Kusec *et al.*, 2005) [14]. The polymorphism information content (PIC) values of LWY and SVVU T-17 were 0.36 and 0.298 respectively suggesting moderate level of polymorphism in these genetic groups and could be considered as reliable source of genetic variability. The PIC values restricted to less than 0.5 in the present studied population was due to the bi-allelic nature of the SNP'S (Eltaher *et al.*, 2018) [5], and could also be due to low mutation rates in SNP'S (Coates *et al.*, 2009; Eltaher *et al.*, 2018) [3, 5]. The expected heterozygosity (H_e) in the LWY population was slightly higher than SVVU T-17, revealing that the LWY population was more diverse since H_e depends on both the number of alleles (richness) and the evenness of the alleles in the population. Negative F_{IS} values observed in both populations is indicative of heterozygote excess (outbreeding) and is more accentuated in SVVU T-17 revealing a moderate effect of non-random mating within the population.

Table 1: Details of oligonucleotide primers, amplicon size and restriction enzyme digestion pattern

Gene/SNP	Primer sequence(5-3)	Amplicon (bp)	RE	PCR-RFLP Pattern(bp)
CAST 76872G>A	F: TTCCCATAGCCCACAAGAAG R: AATGAGCAGCCAACATCAGA	376	<i>HinfI</i>	A: 376 G: 247 + 129
IFNA16 413G>A	F: CATGGCTCTGGTGCATGA R: TGCAGATAGAGGGTGAGTCT	237	<i>HinfI</i>	A: 185 + 34 + 18 G: 219 + 18

Table 2: Distribution of genotypes and allele frequencies at *CAST*/*HinfI* locus in swine

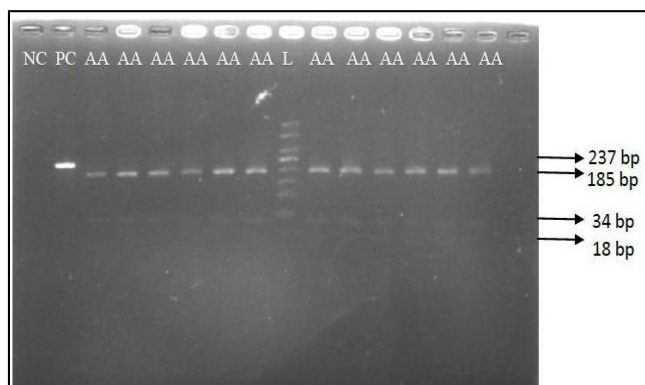
Breed/Group	Total no. of animals (n)	genotypic frequency			Allele frequency		χ^2 value	P value
		AA	GA	GG	A	G		
LWY	101	0.39 (39)	0.49 (50)	0.12 (12)	0.63	0.37	0.38	0.53
SVVU-T17	117	0.008 (1)	0.470 (55)	0.522 (61)	0.24	0.76	8.66	0.003



Genotypes are shown at the top of the lane.

L: 50bp ladder, PC: Positive Control, NC: Negative Control

Fig 1: RFLP pattern of *CAST*/*HinfI* locus



Genotypes are shown at the top of the lane.

L: 50bp ladder, PC: Positive Control, NC: Negative Control

Fig 2: RFLP pattern of *IFNA16*/*HinfI* locus

Conclusion

Significant differences in the allele frequencies between the genetic groups in the present study demonstrated that evolution of SVVU T17 was achieved by traditional selection utilizing the merits of LWY on desi pigs. As pigs are primarily for meat, considering the association of CAST polymorphs with certain traits in selection criteria would improve the genetic progress for meat traits making SVVU17 more acceptable. Hence, investigation for polymorphism at other putative SNPs in CAST and IFNA16 genes and determining their association with meat quality traits in large population would be a possible way in concluding these genes in MAS.

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Conflict of Interest

No potential conflict of interest was reported by the authors.

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