



Genetic Analysis of An Giang Ongole Cattle Based on Mitochondrial DNA D-Loop, Y Chromosomal Haplotypes and Polymorphism of Genes Associated with Economic Traits

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Abstract | The Ongole cattle are popularly raised in the Seven Mountains region of An Giang Province, Vietnam. This breed is a main source of animals that often participate in the famous bull-racing festival. A small number of cattle in the Phum squirrels have a pure Ongole phenotype. However, due to the practice of castration of male calves and Sindization, purebred Ongole are in danger of extinction, whereas genetic features have not been investigated. This study aimed to examine the genetic features of Ongole cattle by utilizing molecular markers. We examined the nucleotide sequences of mitochondrial DNA and the *SRY* gene on the Y chromosome, as well as conducted genotyping of the *NCAPG*, *DGAT1*, and *RNF212* genes in the Ongole cattle population. The sequence investigation of the mitochondrial DNA revealed that the cattle possess the *Bos indicus* type I1 haplotype, suggesting relatively high genetic diversity in the maternal lineage. The sequence analysis of the *SRY* gene suggests that all the males possess a zebu-type haplotype, which may be derived from a cross between *Bos javanicus* and zebu. The results of the genotyping of the functional genes indicated that the *NCAPG* and *DGAT1* genes are polymorphic, whereas the *RNF212* gene is monomorphic. Particularly, the presence of the desirable alleles of the *NCAPG* and *DGAT1* genes will be important for the selection of animals based on their potential performances in meat and milk productivity. The present findings could be informative for future conservation and breeding of this bull-racing cattle breed.

Keywords | Ongole cattle, Genetic diversity, mtDNA, Polymorphisms, SNPs, Y chromosome

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INTRODUCTION

An Giang Province has many hills, vast plains, and a very diverse climate. Therefore, cattle raising is very suitable and has become a long-standing profession closely associated with the economic, cultural, and religious tra-

ditions of the Khmer ethnic minority. There are over six unique native cattle breeds and fourteen imported cattle breeds in Vietnam (Pham *et al.*, 2013; Hoang Kim Giao, 2009), respectively. Cattle plow the fields, pull carts, and transport crops and rice, which are the main livelihoods of the Khmer people, especially at the Seven Mountains Bull

Racing Festival every year. The goal of bull-racing is to preserve and promote historical and intangible cultural values, pass on the unique traditional art form of the region, and combine conservation with economic development and tourism in the Seven Mountains region. Therefore, in 2020, the Project to Preserve and Promote the Seven Mountains Bull Racing Festival was approved by the People's Committee of An Giang Province. The province is preparing a roadmap to upgrade it to the Bull Racing Festival of the Khmer people in the Mekong Delta, moving towards the International Bull Racing Festival in An Giang. However, genetic characteristics and strategies for improving the quality of breeding stock have not received much attention.

Accordingly, Ongole cattle are still chosen by local people as a traditional racing bull breed because they are healthy, have long legs, and run faster than other bull breeds. In some Khmer villages, cattle still have the purebred Ongole phenotype. However, the racing bull herd is facing the risk of extinction because of crossbreeding, the practice of castrating male calves, and the cattle Sindization program. The number of breeding animals is increasingly scarce (Hoang Kim Giao, 2009), breeders import breeding animals from Cambodia through trails and open paths; and they select and train racing bulls based on folk experience. Consequently, this pure population has rapidly decreased. For that reason, assessing the genetic characteristics of An Giang Ongole cattle is important to designing conservation programs for the sustainable development of the agricultural sector and the livelihood of poor ethnic minority farmers in An Giang, Vietnam.

The D-loop area of mitochondrial DNA (mtDNA) and *SRY* gen at the Y chromosome is normally implemented in genetic characterization and phylogenetic analyses of populations of the nearby animals, in view that they're transmitted to subsequent technology via means of the best maternal and paternal lineages, respectively. They were also used to estimate the genetic diversity and powerful size of the population (Anderson *et al.*, 1982; Mannen *et al.*, 2017). In this study, we investigated the nucleotide sequences of hypervariable D-loop location of mtDNA and male-particular *SRY* gene on Y-chromosomal of the Ongole livestock, aiming to make clear the phylogenetic relation and genetic variety of the Ongole livestock populace within the An Giang Seven Mountains area, Vietnam. Moreover, because of the latest development of marker-assisted choice in local animals, numerous polymorphisms of the genes associated with economically vital developments of livestock, such as milk and meat productivities, in addition to reproductive performance, have been offered in livestock (Hoashi *et al.*, 2007; Yamada *et al.*, 2008). However, the genetic information on these important economic traits of the majority of Vietnamese native cattle breeds has not been studied due to funding limitations and breed improvement

policies. In this research, therefore, we genotyped the polymorphisms of non-SMC condensin I complicated subunit G (NCAPG), the allele A > G related to carcass weight (Eberlein *et al.*, 2009), diacylglycerol o- acyltransferase 1 (DGAT1) related to milk yield and milk fat proportion (Grisart *et al.*, 2002), and the mutation C > T within the ring finger protein 212 (RNF212), which performs a vital function in regulating crossover all through meiotic recombination and is associated with excessive fertility (Sandor *et al.*, 2012). The genotype information of those genes within the Ongole livestock could be informative about the expected genetic performance of the population of this livestock breed.

Through the genetic exploration of An Giang Ongole livestock, the usage of mtDNA, *SRY* haplotype diversity, and the genotypes of genes associated with economically important traits, we disclosed the genetic characteristics of An Giang Ongole cattle, which could be informative for future conservation and breeding of this bull racing cattle breed.

MATERIALS AND METHODS

SAMPLE COLLECTION

Because of budget constraints, there were a total of thirty blood samples from 2 to 5-year-old healthy and unrelated cattle (fifteen females and fifteen males) collected from a household raising Ongole cattle in the Khmer Phum squirrels of the Seven Mountains region, An Giang Province. Following the ethical use of animals, about 5 mL of blood was taken from the jugular vein, and the blood tube was stored at 4°C. Genomic DNA was extracted using the basic steps of collecting white blood cells by centrifugation, protein digestion with Proteinase K, DNA extraction with a mixture of phenol chloroform and isoamyl alcohol, and DNA precipitation with ethanol (Barker, 1998). The concentration of total DNA products was measured using a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific, USA).

ANALYSIS OF mtDNA AND SRY GENE HAPLOTYPE DIVERSITY

To analyze the mtDNA gene haplotype diversity of Ongole cattle, a 650-bp mtDNA fragment was amplified using primer pairs (Table 1) according to the forward primer of Loftus *et al.* (1994), and the reverse primer of Le *et al.* (2018), and the amplified fragment was directly sequenced using these primers. PCR reactions have been completed in 10µL DNA, 0.2µM primers, 0.2µmol/L dNTP, 2×PCR buffer, and 1U Kod FX Taq DNA polymerase (Toyobo, Osaka, Japan), for 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 90 s. After PCR amplification, the PCR productions were

Table 1: Primer sequences, lengths and annealing temperatures of functional genes.

Gene	Primer sequences (5'- 3')	Lengths (bp)	Annealing temperatures (°C)	Resources
mtDNA	F: CTGCAGTCTCACCATCAACC R: CCTTTGACGGCCATAGCTGA	650	58	Loftus <i>et al.</i> (1994) Thu <i>et al.</i> (2018)
SRY	F: CCGGGCTATAAATATCGACCT R: GATGAAACCTTGGGTCTCACAG	1062	58	Nijman <i>et al.</i> (2008)
NCAPG	F: ATTTAGGAAACGACTACTGG R: ATTTGTATCTCTTATTATCATC	129	51	Eberlein <i>et al.</i> (2009)
DGAT1	F: GCACCATCCTCTTCCTCAAG R: GGAAGCGCTTTCGGATG	411	66	Grisart <i>et al.</i> (2002)
RNF212	F: GGGTCACCACAGTCCAGAGT R: GCTGCCTGTAAGGAGGTTCT	567	58	Sandor <i>et al.</i> (2012)

electrophoresed in an agarose gel in TAE buffer, stained with 6X GelRed® dye (Biotum, USA), and separated by electrophoresis on a 2% agarose gel in 0.5X TAE buffer at 100V for 25 minutes. HaeIII was used as a standard-size ladder. The electrophoresis results were examined on the Gel DocTM XR+ machine (Bio Rad, USA). The electrophoresis results after purification with Exo sap enzymes were sequenced by the Sanger method. The earned sequences were aligned using MEGA11 software (Tamura *et al.*, 2021), and finally generated a 240 bp documentation, from 16,023 to 16,262 of the mtDNA gene sequence, referenced with sequences and accession codes on the NCBI as V00654 (*Bos taurus*) and L27733 (*Bos indicus* I1) (Troy *et al.*, 2001; Chen *et al.*, 2010).

To investigate the Y-chromosomal haplotypes of fifteen male Ongole cattle, a 1,062bp piece of the SRY gene within the male-specific region of the cattle Y chromosome has been amplified using the primer pair indexed in Table 1 (Nijman *et al.*, 2008), and the amplified fragments have been sequenced at once sequenced with the use of those primers. PCR reactions have been done in 10 µL reaction combos containing 10 ng of genomic DNA, 0.2 µM primers, 0.25 µmol/L dNTP, 2 × PCR buffer, and 1 U Kod FX Taq polymerase (Toyobo, Osaka, Japan), for 35 cycles of denaturation at 94°C for fifteen seconds, annealing at 58°C for thirty seconds, and extension at 72°C for forty-five seconds (Verkaar *et al.*, 2004). The earned sequences were aligned by Clustal W and then constructed into a phylogenetic tree by the Neighbor-Joining tree in MEGA11 software (Tamura *et al.*, 2021).

INVESTIGATION OF GENETIC RELATIONSHIPS BETWEEN CATTLE POPULATIONS

Analysis of genetic relationships between populations through evaluation of mitochondrial D-loop region sequence polymorphisms of domestic and exotic cattle groups calculated according to the method of Tamura and Nei. (1993). The phylogenetic tree of An Giang Ongole cattle was constructed based on 240 bp mtDNA of 127 se-

quences, including 30 sequences of An Giang Ongole cattle and 97 sequences of native cattle in South Asian countries, China, Southeast Asia, and India, such as China (Lei *et al.*, 2006), Bhutan (Chen *et al.*, 2010; Lin *et al.*, 2007), Nepal (Takeda *et al.*, 2004; Fujise *et al.*, 2003), Bangladesh (Bhuiyan *et al.*, 2007), India (Chen *et al.*, 2010), Pakistan (Chen *et al.*, 2010), Myanmar (Chen *et al.*, 2010), Cambodia (Chen *et al.*, 2010), Laos (Chen *et al.*, 2010), and Vietnam (Chen *et al.*, 2010), using the Neighbor-Joining (NJ) method by MEGA11 software (Tamura *et al.*, 2021).

To identify genetic relationships between populations via the assessment of SRY gene sequence polymorphisms of An Giang Ongole cattle and exotic cattle groups through reference to sequences with codes in the gene bank DQ336526 (*Bos taurus*), DQ336527 (*Bos indicus*), and DQ336528 (*Bos javanicus*), Banteng cattle, or Southeast Asian wild cattle (Namikawa *et al.*, 1983).

DETERMINATION OF FUNCTIONAL GENOTYPES

For genotyping of the functional genes, *NCAPG*, *DGAT1*, and *RNF212* genes were amplified using the primer pairs listed in Table 1 and genotyped by PCR-RFLP using *Ts-p509I*, *EaeI*, and *AlwI* restriction enzymes, respectively, according to Eberlein *et al.* (2009); Grisart *et al.* (2002); Sandor *et al.* (2012), respectively (Table 2). PCR reactions were carried out in 10 µl reaction mixtures containing 10 ng of genomic DNA, 0.2 µM primers, 0.25 µmol/L dNTP, 2 × PCR buffer, and 1 U Kod FX Taq DNA polymerase (Toyobo, Osaka, Japan) for 35 to 40 cycles of denaturation at 94°C for 30–120 s, annealing at the temperatures indicated in Table 1 for 30–60 s, and extension at 72°C for 30–120 s.

After PCR amplification and restriction enzyme digestion, the PCR fragments were electrophoresed in an agarose gel in TAE buffer and stained with Gelred. HaeIII was used as the standard size scale: 2,000, 1,500, 1,000, 600, 500, 400, 300, 200, and 100 bp, and visualized using a UV transilluminator. Allele and genotype frequencies were calculated according

Table 2: Restriction enzymes and genotyping.

Gene	Cut positions	Restriction enzymes	Temperature (°C) / time	Genotype	Allele size (bp)
NCAPG (A>G)	5'...AA/TT..3'	<i>Tsp509I</i>	65/45 minutes	GG GT TT	129 129; 66; 63 66; 63
<i>DGAT1</i> (K232A)	5'...AA/GC...3'	<i>EaeI</i>	37/24 hours	KK KA AA	411 411; 203; 208 203; 208
RNF212 (C>T)	GGATC(N)4 CCATG(N)5	<i>AlwI</i>	37/24 hours	CC CT TT	386; 168; 13 386; 203; 168; 183; 13 203; 183; 168

to the Hardy-Weinberg equilibrium (HWE) principle, based on the difference between predicted and detected values ($p = P + H/2$, $q = Q + H/2$), wherein p and q are allele frequencies (Mader, 2001; Dino *et al.*, 2018; Le *et al.*, 2018).

The research question in this study is to determine; What is the genetic diversity and phylogenetic relationship of the Ongole cattle population in An Giang? How are gene polymorphisms related to important economic traits such as meat yield, milk quality, and fertility?

RESULTS AND DISCUSSION

mtDNA HAPLOTYPIC DIVERSITY IN AN GIANG ONGOLE CATTLE

To estimate the genetic diversity of mtDNA haplotypes, thirty An Giang Ongole cattle samples from households in An Giang Province were sequenced for the nucleotides of the D-loop region. Analysis of 240 bp mtDNA from position 16023-16262 indicated that these cattle possess 10 nucleotide polymorphisms and 8 haplotypes that all belong to the zebu-type haplogroups of the bovine mtDNA (Table 3 and Figure 1), including I1, I2, and 1 new haplotype belonging to I1. This new haplotype was submitted to GenBank with accession number OR449750. We tentatively designated this new haplotype as I1.ONH. When compared with the standard reference sequence V00654 (*Bos taurus*), the D-loop region of mtDNA in Ongole cattle had nine substitution polymorphisms (C<->T; G<->A). Comparing with the standard reference sequence L27733 (*Bos indicus* I1), the D-loop region of mtDNA has 10 substitution polymorphisms (including one deletion position 16144), and 20 Ongole cattle individuals had the same genotype as the reference sequence of a single haplotype I1. This haplotype is the common genotype in the zebu cattle group; 3 individuals have genetic characteristics similar to the I1 49 genotype that was found in native cattle in Laos, Cambodia, and Vietnam (Chen *et al.*, 2010); and 1 individual has the I1a genotype, which is the genotype commonly found in native cattle in South Asian countries, known as the cradle of domesticated zebu cattle (Chen *et al.*, 2010).

This result suggests that Ongole cattle in An Giang belong to the *Bos indicus* I1 group.

Table 3: mtDNA haplotype diversity of Vang cattle.

		Nucleotide position									
		1	1	1	1	1	1	1	1	1	1
		6	6	6	6	6	6	6	6	6	6
Cattle	Haplotype	0	0	0	0	0	1	1	1	1	2
		5	5	7	8	8	2	2	4	4	3
		1	2	5	5	6	5	8	2	4	4
L27733 ^a	I1	C	C	C	T	T	T	C	T	-	C
V00654 ^b	T3	C	C	T	C	T	T	C	T	A	C
Ongole (n=20)	I1	.	.	.	.	.	.	.	.	-	.
Ongole (n=3)	I1 49	.	.	.	.	.	.	.	.	-	T
Ongole (n=1)	I1.ONH ^c	.	.	C	T	.	.	.	.	-	.
Ongole (n=1)	I1 55	.	.	.	.	.	.	A	.	-	.
Ongole (n=1)	I1 13	.	.	T	T	.	C	.	.	-	.
Ongole (n=1)	I1 11	T	.	T	T	.	.	.	.	-	.
Ongole (n=1)	I1 1a	.	.	T	T	.	.	.	.	-	.
Ongole (n=2)	I2 28	.	.	C	C	C	.	.	C	-	T

^a*Bos indicus* I1; ^b*Bos taurus*; ^cNew Haplotype.

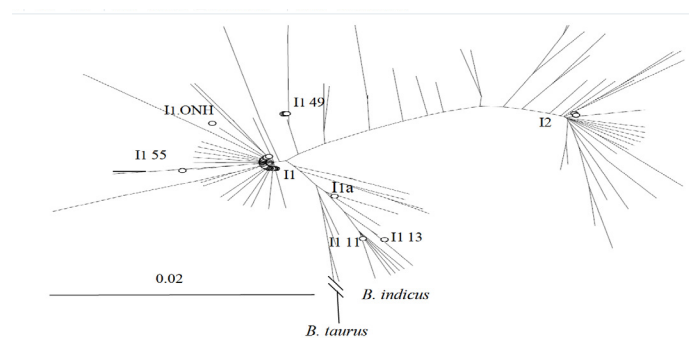


Figure 1: Phylogenetic tree of Ongole cattle* (*Constructed based on 240 bp mtDNA of 127 sequences, including 30 An Giang Ongole cattle and 97 sequences of indigenous cattle in South Asia, China, Southeast Asia, and Europe, using the Neighbor-Joining (NJ) method by MEGA11 software. Eight haplotypes observed in An Giang Ongole cattle are represented by circles).

The ammalian mitochondrial DNA is a small genome (about 16.3 kb in size), circular in structure, showing maternal inheritance, no recombination, and a rapid mutation rate compared to the nuclear genome (Magee *et al.*, 2014). Analysis of the cattle phylogenetic tree from the D-loop sequence of mtDNA in wild bison shows many branches and complex branch structures. In contrast, analysis of the D-loop region of mtDNA in modern domestic cattle results in two distinct main branches representing two groups of *Bos taurus* and *Bos indicus* cattle. Of these, *Bos taurus* is divided into 5 genotype groups (T, T1, T2, T3, and T4) and 2 genotype groups in *Bos indicus* (I1 and I2) (Achilli *et al.*, 2009; Chen *et al.*, 2010). *Bos taurus* cattle populations are mainly distributed in Northern and Western Africa and almost the entire Eurasia region, from Northwestern Europe to Japan (Magee *et al.*, 2014), while zebu cattle were domesticated from the Indus Valley civilization about 8000 years ago (Loftus *et al.*, 1994). Currently, *Bos taurus* is also mainly distributed in South Asia, East Asia, Southeast Asia, and eastern and southern Africa (Magee *et al.*, 2014; Utsunomia *et al.*, 2019). In Vietnam, Berthouly *et al.* (2010) reported that there were over six unique native cattle breeds, and the indigenous cattle population in Ha Giang (Hmong cattle) belongs to the Taurine-zebu cattle group. Okuda *et al.* (2017) analyzed 27 mtDNA samples from Laotian native cattle, and the results also found only 3 haplogroups belonging to I1, no new haplotypes, and no haplotypes belonging to the *Bos taurus* group. Moreover, analyzing 28 mtDNA samples from Pesisir native cattle in Indonesia, Dino *et al.* (2018) presented that there was 1 haplotype I2, and 1 out of 5 haplotypes were new, all belonging to group I1 of *Bos indicus*; no haplotypes belonging to the *Bos taurus* group were detected. In addition, Chen *et al.* (2010) suggested that the near absence of the I2 group in Southeast Asia may be due to the later introduction of this group into the domestic cattle group in the country and possibly because the culture at that time prevented the spread of the I2 group. The later scattered appearance of I2 in the region may be explained by trade exchange.

Thus, the analysis of An Giang Ongole cattle in this observation resulted in 8 haplogroups belonging to groups of I1, I2, I1 49, and 1 new haplotype, and their relative frequencies are higher than those results when compared with the previous reports in Lao, Japan, Indonesia, and Vietnam (Mannen *et al.*, 2000; Mannen *et al.*, 2017; Dino *et al.*, 2018; Le *et al.*, 2018). This finding initially suggests that the An Giang Ongole cattle population has relatively high genetic diversity in the maternal line.

STRY HAPLOTYPE DIVERSITY IN AN GIANG ONGOLE CATTLE

We also identified the Y chromosomal haplotype of the An Giang Ongole cattle by sequencing the 1,062 bp piece of the STRY gene on the Y chromosome. In the exploration of

the Y-chromosomal haplotype of cattle, Nijman *et al.* (2008) classified the Y-chromosomal haplotype of cattle into *Bos taurus*, *Bos indicus*, and Southeast Asian Banteng-*Bos javanicus* types by 5 SNPs in this segment. As shown in Table 4, all fifteen male cattle possess a unique haplotype with 10 polymorphic positions, in which 7 nucleotide positions were homologous to the haplotype nucleotide sequences in *Bos indicus*, and 7 nucleotide positions were homologous to the haplotype nucleotide sequences in *Bos javanicus* (Banteng), without the appearance of the haplotype *Bos taurus*. This Ongole haplotype was tentatively named OAGY.

Table 4: STRY haplotype diversity of Ongole cattle.

Haplotype	Nucleotide position									
	1	1	2	2	2	2	2	2	2	2
	7	7	0	0	1	1	2	2	3	3
	0	9	1	5	0	1	2	3	0	1
	7	0	8	9	3	6	9	8	7	4
AB039748 ¹	G	T	A	C	T	A	A	C	C	C
AY079145 ²	T	C	A	T	T	G	G	T	T	T
AY079146 ³	T	T	G	C	C	A	A	T	T	T
(OAGY ⁴ , (n=15)	T	T	A	T	T	A	A	T	T	T

¹*Bos taurus*; ²*Bos indicus*; ³*Bos javanicus*; ⁴An Giang Ongole cattle.

Genetic analysis of undomesticated local bulls in Indonesia showed that they carried the gene line of Banteng, Southeast Asian wild cattle (Namikawa *et al.*, 1983; Nijman *et al.*, 2003; Tanaka *et al.*, 2011). Besides Bali cattle, which are known as domesticated cattle originating from Banteng cattle, Madura cattle originated from a cross between Banteng and zebu cattle (Otsuka, 1983). Thus, Ongole An Giang bulls carry 7/10 nucleotide homology positions on the STRY gene similar to the nucleotide sequence of Banteng and zebu cattle, indicating that they may be hybrids between Banteng and zebu cattle. Genetic studies conducted on local breeds of Indonesian native cattle have indicated a gene flow of Banteng into several of these breeds (Namikawa *et al.*, 1983; Nijman *et al.*, 2003; Tanaka *et al.*, 2011; Mohamad *et al.*, 2009). In contrast, other research has shown that male cattle in Indonesia and Vietnam exhibit zebu characteristics (Dino *et al.*, 2018; Le *et al.*, 2018), while a limited presence of the *Bos taurus* type was also identified in Vietnam (Le *et al.*, 2018). These results imply a potential paternal gene flow from Banteng into Ongole cattle.

As a result, analysis of mtDNA gene nucleotypes and Y chromosome STRY gene polymorphisms showed that An Giang Ongole cattle originated from zebu cattle in both maternal and paternal lines, in which the paternal line may be a hybrid between Southeast Asian wild cattle and zebu cattle, and could be informative for considering the origin and history of An Giang Ongole cattle in Vietnam.

Polymorphism of Genes Associated with Economic Traits

Next, we explored the polymorphisms of the genes associated with particular economic traits of cattle, including the *NCAPG* gene related to carcass weight; the *G* allele of this gene improves carcass weight (Eberlein *et al.*, 2009); the mutation that replaces *T* with *G* at position g.38245413 (exon 9), and changes the amino acid isoleucine to methionine at position 442 of the *NCAPG* protein (Setoguchi *et al.*, 2011); the *DGAT1* gene related to milk fatty acid composition; the *K* allele of this gene increases fat content (Grisart *et al.*, 2002); and the *RNF212* gene, which plays an important role in regulating crossing over during meiotic recombination; the *T* allele of this gene is known to increase the rate of meiotic recombination in cattle (Reynolds *et al.*, 2013; Qiao *et al.*, 2014).

Table 5: Genotype and allele frequencies in relation to economic traits.

Gene	n	Genotype			Allele frequency		Chi-square values
NCAPG	30	T/T	G/T	G/G	T	G	0.85
		28	2	0	0.97	0.03	
DGAT1	30	K/K	K/A	A/A	K	A	0.85
		28	2	0	0.97	0.03	
RNF212	30	CC	CT	TT	C	T	NA
		30	0	0	1	0	

NA: Not applicable; It does not follow Hardy Weinberg equilibrium.

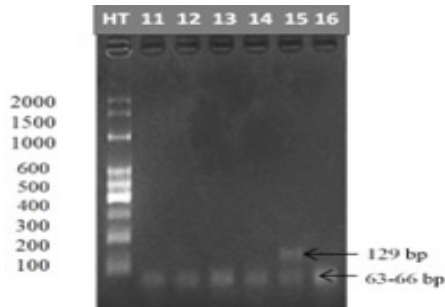


Figure 2: The *NCAPG* gene was cut by the *Tsp509I* enzyme in samples 11-16. Well HT: Standard DNA ladder (2,000, 1,500, 1,000, 600, 500, 400, 300, 200, and 100 bp).

The results of this study in Table 5 and Figure 2 showed that there were two genotypes, *TT* and *TG*, indicating that the *NCAPG* I442M gene showed polymorphism in this cattle population. It means that this population has detected the *T* > *G* substitution mutation related to carcass weight at a low allele frequency (*G* = 0.03). This result is different from the result of Okuda *et al.* (2017), who studied 27 local Lao cattle and suggested that 100% of the research samples had the *TT* genotype. Thus, there is the presence of the *G* allele of the *NCAPG* gene in the An Giang Ongole cattle population, meaning that there is a polymorphism of this gene. Moreover, the distribution of genotypes *KK* and *KA* in the

DGAT1 gene is 28 and 2 individuals, respectively (Figure 3), demonstrating that both alleles *K* and *A* of this gene are present in the An Giang Ongole cattle population. While analyzing 27 local cattle samples in Laos and 30 samples of Central Vietnam Yellow cattle, this result revealed that 100% of cattle in these two countries had only one genotype, *KK* (Okuda *et al.*, 2017; Le *et al.*, 2018). Thus, there is the presence of allele *A* of the *DGAT1* gene in the An Giang Ongole cattle population, meaning that there is a polymorphism of this gene at a low frequency (*A* = 0.03).

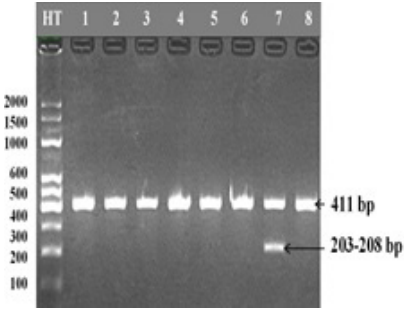


Figure 3: Cutting the *DGAT1* gene with the *EaeI* enzyme in samples 1-8. Well HT: Standard DNA ladder (2,000, 1,500, 1,000, 600, 500, 400, 300, 200, and 100 bp).

In addition, the *RNF212* P259S gene is a *C* > *T* substitution mutation at position g.118327636 (NC_032655.1 Chromosome 6) that has changed the amino acid in proline to serine at position 259 of the *RNF212* protein. This substitution mutation increases the meiotic recombination rate in cattle. The meiotic recombination rate is positively correlated with reproductive efficiency, so this mutation may be an important marker related to high fertility in cattle (Sandor *et al.*, 2012; Kadri *et al.*, 2016). Therefore, to determine whether the analyzed samples have the *C* > *T* mutation at position g.118327636 (exon 12), we selected the restriction enzyme *AlwI* that can recognize and cleave at the mutation site based on the results from the screening filtering suitable restriction enzymes for the *RNF212* P259S mutation using the NEBcutter V2.0 program (New England Biolabs; <http://nc2.neb.com/NEBcutter2/>). The results of *RNF212* genotype analysis by PCR-RFLP showed that there was only one *CC* genotype, demonstrating that the *RNF212* gene did not exhibit polymorphism in the cattle population (Figure 4). Thus, we have not detected the *C* > *T* substitution mutation associated with high fertility in An Giang Ongole cattle.

The results of genotype analysis of 30 An Giang Ongole cattle showed that they carried carcass weight and milk fat ratio traits related to the polymorphism of the *NCAPG* and *DGAT1* genes. The polymorphisms of these genes analyzed in this study have been identified as genes closely related to specific traits of beef and dairy cattle (*B. taurine*). However, the distribution of these gene polymorphisms in the zebu group has not been clearly published. Significant dif-

ferences in allele frequencies of genes between *Bos taurine* and zebu cattle populations have been published (Kaneda *et al.*, 2011; Yonesaka *et al.*, 2016; Okuda *et al.*, 2017). In this study, we found that the polymorphism of the *NCAPG* and *DGAT1* genes published in *taurine* cattle was also observed in the An Giang Ongole cattle population.

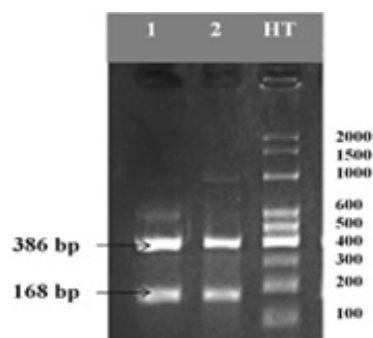


Figure 4: The *RNF212* gene has been cut by the *AlwI* enzyme in samples 1 and 2. **Well HT:** Standard DNA ladder (2,000, 1,500, 1,000, 600, 500, 400, 300, 200, and 100 bp).

At present, publications have shown very little polymorphism of the *NCAPG* and *DGAT1* genes in the zebu cattle group. In this study, the presence of the *G* and *A* alleles of these genes in the An Giang Ongole cattle population was detected. Thus, although An Giang Ongole cattle show polymorphism of genes related to carcass weight and milk fat quality traits, they do not show polymorphism of genes related to the trait regulating crossover during meiotic recombination.

CONCLUSIONS AND RECOMMENDATIONS

For the conservation and breeding of bull racing animals, it is necessary to accurately estimate their genetic characteristics, including genetic diversities, relations with other breeds, and potential genetic performances.

The investigation of mtDNA, *SRY* haplotype diversity, and genotypes of functional genes associated with economically traits explored that An Giang Ongole cattle has relatively high genetic diversity originated from zebu cattle in both maternal and paternal lines, in which the paternal line may be a hybrid between *Bos javanicus* and zebu cattle, and the presence of desirable alleles of some economically important traits.

This is the first study for a comprehensive genetic characterization of An Giang Ongole cattle using molecular markers, which could be informative for future conservation and breeding of this bull racing breed. However, the sample size representing the Ongole cattle population in this study was relatively small, which may have had certain limitations on the study results.

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NOVELTY STATEMENT

It is possible to evaluate population genetic diversity and select meat and milk traits in the An Giang Ongole cattle herd based on these molecular markers.

AUTHOR'S CONTRIBUTIONS

Nguyen Ba Trung: Conceived, designed, performed the experiments, and analyzed the data.

Nguyen Ba Trung and Pham Thi Kim Phuong: Wrote the paper, collected the samples, reviewed, and approved the final manuscript.

CONFLICT OF INTEREST

We certify that there is no conflict of interest

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