Chapter 1: Introduction

Bangladesh is a densely populated (1115.62 people per square kilometer) country ranked 10th globally (World population review, 2019). Agriculture and livestock play a crucial role in balancing the country's GDP and food safety by providing essential nutrients. Livestock contributes 40 % of the global value of the agricultural sector, as the livestock value chain is much simpler than any other product (FAO, 2021). According to the Department of Livestock Services (DLS), the Gross Domestic Products (GDP) growth rate of the livestock is 3.40%, regardless of the livestock sub-sector accounted for 1.54% of the total GDP in the fiscal year 2017-18, slightly decreasing year by year. Meat consumption in Bangladesh is 2.2 million tons, where beef supply is about 60%, with 40% of the national cattle herd slaughtered annually (UNIDO, 2019). Due to high demand and supply chain issues, rural farmers are now eager to adopt a genetic approach to investigate indigenous cattle with high carcass quality.

The most common cattle found in Bangladesh are Native and Exotic breed: Sahiwal, Sindhi, Holstein-Friesian, Jersey and Brahman. Most of the smallholder farmers in Bangladesh rear indigenous cows, which yield about 50% meat consistency for their weight. Nearly 85% of cattle in Bangladesh are indigenous in origin and the mature live weight is low (187-227 kg) compared to other exotic breeds and crosses (350-400kg) (Bhuiyan et al., 2007). The native cattle have striking features like prominent humps, deep shoulders, and well-developed hindquarters, but the head is slim with pointed, crescent-shaped horns. The meat acquired from this breed of cattle contains significantly higher intramuscular fat and is generally delicate and tender and the yields of the native cows are about 47-52 percent of their weight in meat (The Daily Star, 2018). Some indigenous types are distinct due to their unique characteristics, such as Red Chittagong cattle, Pabna cattle, and Munshiganj cattle.

Red Chittagong Cattle (RCC) are commonly found in the Chattogram district (Former Chittagong district). RCC is commonly found located in the plains of Chattogram, Noakhali and southern regions of Cumilla. This breed has a distinctive character with attractive red body color. Compared to other indigenous cattle, good milk and meat production make them top preferred in that region (Hossain et al., 2005).

RCC male and female cattle can be used for meat consumption and supply. It is indigenous and most potential type of domestic animal of cattle livestock in the world and economically higher profitable than other available cattle of Bangladesh (Khan *et al.* 2012). The meat of RCC has a high amount of intramuscular fat with juicy and fattening characteristics. The amount of meat yields roughly half of its actual weight (The Daily Star, 2018).

Beef demand has increased due to its worldwide consumption, which creates a huge shift in beef sector industries. Carcass traits such as tenderness (Maltin et al., 2003) and flavor (Tarrant, 1998) have been rated by consumers as the most critical elements for the beef sector. The percentage of carcass quality can be calculated based on the rib-eye length and its width to score of muscle as well as grade fat thickness at a certain point on the outer edge of the rib-eye. In contrast, marbling is a part or fragment of fat located in the rib eye muscle that is directly associated with yield quality. Therefore, the higher amount of marbling presents in yield, the better the quality. Apart from marbling, muscle consistency in beef plays an essential role in its texture and flavor. Tenderness, juiciness, and flavor are the crucial properties of meat. The factors associated with these properties such as weight, composition, conformation, and contamination can also change the carcass quality traits. Premium quality of meat tenderness is very famous among the consumers, and even they are very well aware of its importance towards carcass quality. As per the study, juiciness is closely associated with tenderness and red meat (Huffman et al., 1996).

The quality grade is calculated by the marbling score and further adjusted by alternative carcass traits such as meat color, fat color, texture and development when there is a particular defect in these traits (USDA, 1989). Higher quality grades have a heavier carcass weight with a higher marbling score, redder meat color, and whiter fat color (Moon et al., 2003). Understanding carcass data helps determine quality grade and yield grade; it also looks at carcass weight, fat thickness, and rib eye area. Carcass data opens new opportunities to fill the gap between consumer and provider chains, which increases the supply and gives the perspective to understand customer requirements and needs of the market. Carcass data can be obtained with the help of further analyses. The degree of analysis and data sensitivity depends on the approach used for data understanding, i.e. basis or deep understanding.

Primary understanding deals with breed type and its phenotypic evaluation. This approach allows a physical scan of the breed and looks for its physical condition. The advantage of using this approach is that it is cost-effective and has a lower time duration, but there is no validation on carcass quality. Under the advanced understanding approach, ultrasound scanning is used in this approach. This can predict the carcass quality of mature cattle or breed cattle. We can track crossbred information and take precautions based on the data information and its analyses; apart from this, it also provides information on the fat thickness and rib area. The advantage of using this approach is that no physical damage is required to obtain carcass data; even the monitoring of cattle breeds can be done systematically. The primary limitation of this approach is, it requires a certified trained person to predict the data first and later help with the analyses. Another limitation is that it is not cost-effective as ultrasound scanning machinery is very costly and requires maintenance.

The genetic approach by using modern life science technology that uses DNA and its principles to locate and identify genes associated with carcass traits, provides information on genes associated with carcass quality and controls genes associated with carcass traits. The advantages of using this approach are that we can track all genes and their associations with their functions even if we can use this information for effective crossbreeding. Limitations are required times to analyze along with the fact that a trained person is required to conduct a systematic protocol. Cattle genomic data has substantial predictive value since it allows for identifying animals with higher productive potential. In this regard, data collected from molecular markers provide vital information regarding selection programs and genetic improvement and is frequently referred to as molecular markers-assisted selection

Selection for carcass traits is difficult since they are expressed late in life. Beef quality and carcass traits are typically low to moderate heritability and are mostly reported after slaughter (Marshall, 1999; Gill et al., 2009). Therefore, through understanding the carcass data, we come to know various aspects that can be applied to improve the consistency of the carcass, and genetics can be the most famous successful approach. Gene variations such as Single Nucleotide Polymorphisms (SNPs) can be used as significant markers towards cattle selection for indirectly improving beef quality traits instead of direct measurements (Ge et al., 2001; Allan et al., 2007; Dekkers and Hospital, 2002; Shin and Chung, 2007).

So the genetic approach to identify genes and related markers related to carcass traits and beef quality is suitable to identify superior animal among populations. This study focuses on using a genetic approach, i.e. DNA markers, to investigate indigenous cattle of Bangladesh with good carcass quality. This approach is a forward step towards the agricultural farming system's balancing its existing challenges and helping avoid the wastage of meat in the beef sector. Therefore, the objective is to develop potential markers in *MYF5* and *CACNA2D*1 gene loci to analyze carcass traits to investigate indigenous cattle.

CACNA2D1 gene is located within the genomic region of seven QTLs, for carcass weight (Casas et al., 2001), average daily gain (Casas et al., 2001), stature (Ashwell et al., 2005), bone percentage (Gutierrez-Gil et al., 2009), meat-to bone ratio (Gutierrez-Gil et al., 2009), residual feed intake (Sherman et al., 2009). Four QTLs affect the longissimus muscle area (Takasuga et al., 2007; Mizoshita et al., 2004), marbling score (Yokouchi et al., 2009), feed conversion ratio (Sherman et al., 2009), and somatic cell count (Longeri et al., 2006) have also been found near the area (NAGRP, 2010). The role of *MYF5* is considered to be inherent for the innovation and growth of straight muscles and the sustainment of their physical appearance. Hence, they are believed to be candidate genes for growth and meat quality characters (Maak et al., 2006). A study on candidate genes identifies SNPs in genes that most likely cause mutation in a phenotypic trait grounded on physiological and endocrinological. Nevertheless, little research has been done on the relationship between the polymorphism of the *MYF5* gene and meat quality traits. *CACNA2D1* and *MYF*5 polymorphisms associated with carcass and meat quality traits in Indigenous cattle breeds of Bangladesh have not been analyzed. The objectives of the present study are to identify polymorphisms of the *CACNA2D1* and *MYF5* gene and to analyze associations between SNPs and carcass and meat traits.

The results could provide new data and evidence concerning the convenience of these markers for gene-assisted selection in cattle breeding programs. It might add to a superior conception of the hereditary control of carcass traits and will be helpful for additional practical genomic studies.

Specific objectives:

1. To identify genetic polymorphisms of the genes associated with carcass and meat quality traits in cattle.
2. To estimate changes on population of the identified polymorphisms in RCC and crossbred cattle of Bangladesh.
3. To predict the functional impact of the detected polymorphisms to prioritize of genetic markers for future selection programs to improve meat quality traits in indigenous cattle of Bangladesh.

Chapter 2: Review of Literature

Bangladesh is an agro-based developing country where agriculture is considered the jewel crown of the country, and livestock plays a crucial role in the national economy as an integral part of that. The contribution of livestock production is almost 3.31%, the second-highest among all other sub-sector of agriculture in Bangladesh (BER, 2017).Nearly 24.5 million heads of cattle are distributed throughout the country (Table 1). About 80% of cattle are indigenous type (Hamid et al., 2017). Depending upon the climate, soil type and availability of fodder in the country, the different types or varieties of cattle are available in different parts of the country, like RCC in Chattogram, Pabna type (in Pabna region), North Bengal Grey, Munshiganj type etc. (Khan et al., 2000).

Table 1: Livestock population of Bangladesh (BER, 2017-2018)

Livestock species	Livestock populations (In millions)
Cattle	24.09
Sheep	3.46
Goat	26.1
Buffalo	1.49
Poultry	338
Total livestock	393.14

2.1 Cattle genetic resources in Bangladesh

Indigenous cattle of Bangladesh are regarded as potential in some regions. They are recognized by their local names, like Pabna cattle, Red Chittagong cattle, Munshiganj cattle, North Bengal Gray cattle and other local native types (Table 2). After the artificial insemination program started, many crossbred cows have effectively been added to the different parts of the country. Thus, the cattle genetic resources of the country consist of Native cattle, Red Chittagong cattle, Pabna cattle, North Bengal Grey cattle, Munshiganj cattle, crossbred cattle and exotic breeds (Holstein and Friesian, Sahiwal, Sindhi, and Jersey).

The RCC is a valuable indigenous bovine genetic resource of Bangladesh with many attributes that are better than other available indigenous types. These cattle are readily distinguishable from others due to their distinct phenotypic features (Alam et al., 2007). The history of the development of this variety is not yet clear, and sometimes it is considered that this variety has evolved by inbreeding among them under the conditions of natural selection. These cattle are not yet recognized internationally as a breed but as a type or variety (Mason, 1998). The positive features of RCC lie in its ability to withstand extreme tropical climates and to survive on low-quality feed during periods of feed shortages. Furthermore, they are reputed to give birth every year, and lower calf mortality can give birth of 8-10 calves in a lifetime, attain sexual maturity earlier, are suitable for tillage operation, and traction, swift in their movement, hardworking in hot, humid, and rainfall conditions are considered unique RCC characteristics.

Growth is one of the essential characteristics of cattle and has been investigated for many years (Blasco and Gomes, 1993; Bathaei and Leroy, 1998). The coat color of RCC is red (deep as well as light), their muzzle, eyeball, eyebrow, tail switch and hoof are whitish/brick red in color and small-headed, its body size is comparatively small, but their physical condition is very strong and stout. Habib et al. (2003) stated that the height at withers, body length and heart girth of Red Chittagong cow are 107.71±0.93, 114.38±1.56 and 139.85±1.63 cm, and Red Chittagong bull is 125.00±0.87, 134.00±1.63 and 168.00±1.67 cm, respectively. Males are heavier than females (150-400 vs 150-200 kg) (FAO, 2004). It is found more or less everywhere in the Chattogram district and Chattogram Hill Tract region but is rare in other parts of the country. A relatively higher concentration of RCC is found in the Upazillas of Raozan, Anawara, Potiya, Chandonaish and Bashkhali of Bangladesh.

The Pabna and North Bengal Grey varieties have evolved from the crossbred foundation with mainly Hariana, Tharparker and Sahiwal bulls distributed in these in 1936 and an intensive visual selection during the last 1930-1950 localities (Hamid et al., 2017). They possess good body size and some draft features and are famous as a cart bullock in the market. However, in the Pabna region, continual infusion of Sahiwal, Australian Friesian Sahiwal (AFS), Holstein-Friesian and Jersey are in the process of hybridization.

On the other hand, there is no evidence of foreign blood in the Red Chittagong and Munshigonj varieties. These two varieties have been developed in the locality by natural selection and breeding among themselves for a long historic period (DLS, 2015).

Table 2: Available indigenous cattle of Bangladesh

Type	Geographic distribution	Agro-ecological zone
Non-descriptive deshi	Throughout country	All agro-ecological zone
Red Chittagong	Chattogram	Chattogram coastal plain
Pabna	Sirajganj, Pabna	Active Brahmaputra and Jamuna flood plain
North Bengal grey	Rajshahi, Northern part	Tista flood plain
Munshiganj	Munshiganj district	Young Brahmaputra and Jamuna flood plain

Source: ILRI, 2004

The non-descript indigenous cattle of Bangladesh are of the Zebu type, having developed hump. It is assumed that they have evolved in this area over the centuries for natural selection and farmers' interest in draft power to perform agricultural practices. Therefore, their production performances in meat and milk are far below that of specialized breeds as no selection pressure or attention was paid either to milk or meat yield from the very beginning of domestication.

In Bangladesh, a large number of non-descriptive cattle have been replaced by crossbred cattle during the last decade. It was started in 1958 by the Department of Livestock Services (DLS) with an artificial insemination (AI) program, which was strengthened in 1975-76. In the 1960s, the Savar dairy farm was established for the dairy development program of the government. The farm started to work with Sindhi, Sahiwal and Tharparkar breeds to cross with indigenous cattle.

2.2 Carcass and meat quality traits

In the beef industry, meat quality, like other commercially significant animal traits, is complicated, polygenic, and influenced by an unknown number of quantitative trait loci (QTL) (Grisart et al., 2002). The uniformity of carcasses is a desirable attribute. The key carcass characteristics of economic importance are quality grade, yield grade, and carcass weight, of which quality grade and carcass weight are the most readily handled carcass problems. Despite their relevance, as they are displayed late in life, selection for these characteristics has been difficult.

The efficiency of DNA-based carcass trait selection tools relies primarily on the marker characteristics of the loci used and the ability to collect large quantities of carcass data either directly from cattle slaughter, with ultrasound, or both. The economic characteristics of meat quality and carcass merit should benefit the most from the marker-assisted collection, including the relative difficulties in gathering performance data, the relative magnitude of heritability and phenotypic variance found in the characteristics, the currently available amount of performance details. Many candidate genes have been reported for carcass quality.

Several genes naturally control or regulate carcass traits related to beef quality, and single nucleotide polymorphisms (SNPs) in the genes can be important markers for enhanced meat production (Dekkers and Hospital, 2002; Shin and Chung, 2007). Beef quality and carcass traits typically have low to moderate heritability and are often reported post-slaughter (Marshall, 1999; Gill et al., 2009). Hence, SNPs can be used instead of direct measures as indicators for indirectly improving beef quality traits (Ge et al., 2001; Allan et al., 2007). To better describe the robustness of correlations of polymorphisms in candidate genes with economically relevant characteristics across beef cattle populations, studies with different populations are needed before this type of genetic knowledge can be used effectively in breeding and management decisions.

2.3 Improvement of carcass traits related to beef quality

This selection approach is most effectively implemented for highly heritable traits that are simply recorded before reproductive age. However, meat quality traits can usually only be measured post-slaughter and often have low heritability (Marshall et al., 1999).

Therefore, making progress using direct measurement is difficult for these traits. Marker-assisted selection can significantly increase the rate of genetic improvement in such traits reported by MacNeil et al. (2002), using markers linked to economically relevant traits, which can be used to predict the genetic merit of an animal. Impartial and objective confirmation experiments must be carried out in different breeds to decide if noticeable results are present in the breed/population under selection until such marker information can be included in breeding programs.

2.4 Genes responsible for carcass quality and meat traits

Carcass and meat quality traits, which are controlled by multiple genes, are economically important in the cattle industry. To identify, map and analyze quantitative trait loci (QTL) affecting production traits and to use genetic markers for marker-assisted selection to increase the frequency of favorable QTL alleles in target populations (Stone et al., 2005). Marker-assisted selection first requires the identification of a candidate gene or anonymous genetic markers associated with the traits of interest. The candidate gene may be selected based on a known relationship between physiological or biochemical processes and production traits and can be tested as a QTL.

Table 3: Genes related to carcass quality and meat traits

Gene responsible	Character included	Authors
LRGUK, TRIM24, SVOPL, TEX37, CA10, OXSR1	Hot carcass weight	Carvalho, 2019
CAPN1	Shear firmness, colour, marbling	Williams, 2008
CAST	Peak fore, total energy, hardness	Stone et al., 2005
DGAT1	Longissimus muscle	Fujii et al., 1991
SCD1, EMCN, LNX1, EIF5, SNORA28,DSC3	Marbling	Williams, 2008
OR2S2, 5S_rRNA,SNORA69; ITGA9, LOC100299372, LOC523083, LOC532403, LOC613441	Backfat thickness	Carvalho, 2019
TWIST2, SFXN1, CMYA5, CPQ, MRS2, C963T, UASMS1	Rib eye area	Carvalho, 2019; Da Silva, 2012
MYF5	Meat quality characters	Maak et al., 2006
CACNA2D1	Stature, average daily gain	Casas et al., 2001; Ashwell et al., 2005

2.5 Candidate genes

The study of associations of candidate genes is a step towards the knowledge of the genetic basis of productive traits. Compared to other genomic approaches, it can be more easily and efficiently implemented in breeding programs.

2.5.1 Strategies used in the candidate gene approach

2.5.1.1 Selecting a candidate gene

The first critical step in conducting candidate gene studies is the choice of a suitable candidate gene that may plausibly play a relevant role under investigation.

2.5.1.2 Choosing a DNA polymorphism

Once a candidate gene has been identified, researchers must determine which polymorphisms will be most beneficial for testing in an association study (Miles et al., 2008). To do this, they must first discover existing gene variations and then determine which variants result in proteins with changed activities that may impact the characteristic of interest. In many situations, researchers may be aware of a gene's nucleotide sequence but lack knowledge about functional variation in the gene. To do this, they must first discover existing gene variations and then determine which variants result in proteins with changed activities that may impact the characteristic of interest. In many situations, researchers may be aware of a gene's DNA sequence but lack of knowledge about its functional variation.

Detecting genetic variants is a laborious process that often involves sequencing, determining the sequence of DNA building blocks (i.e., nucleotides) for the entire gene in both affected and unaffected individuals to look for consistent differences. Alternatively, the researchers can employ screening to isolate small gene sections from many individuals and compare their mobility in a gelatinous material. Differences in mobility in these analyses may indicate nucleotide variations (Malhotra and Goldman, 1999). To confirm that a potential nucleotide variation exists and determine its exact location in the genome, investigators must conduct additional studies, typically based on the direct sequencing of the DNA section in question. This information also allows researchers to determine whether the nucleotide variation is likely to have functional significance, either because it results in amino acid changes in the resulting protein or because it occurs in DNA regions controlling a gene's activity.

Finally, to be useful for candidate gene studies, the variant should occur with sufficient frequency to allow detection of differences between individuals with and without the trait under investigation. However, not all genes have an easily identifiable common functional variant that can be exploited in association studies. In many cases, researchers have identified only changes in individual nucleotides (i.e., single nucleotide polymorphisms (SNPs) that have no known functional significance. Nevertheless, SNPs can be potentially valuable for narrowing a linkage region, because it would be prohibitively difficult to sequence all these genes. Publicly available SNP data is a great resource for candidate gene and association studies (Kwon et al., 2000).

2.5.2 *CACNA2D1* gene

The calcium channel, voltage-dependent, alpha-2/delta subunit 1 (*CACNA2D1*) gene encodes a protein linked with voltage-gated calcium channels. It increases the density of calcium channels triggered by a high voltage at the plasma membrane. The *CACNA2D1* gene in cattle has been located on BTA 4q18 (Buitkamp et al., 2003). It is in the genomic area of seven QTLs for carcass weight (Casas et al., 2001), average daily growth (Casas et al., 2001), height (Ashwell et al., 2005), bone % (Gutierrez-Gil et al., 2009), meat-to bone ratio (Gutierrez-Gil et al., 2009), residual feed consumption (Sherman et al., 2009) and somatic cell score (Zhang et al., 1998; Rupp and Boichard, 2003). Four QTLs affect the longissimus muscle area (Takasuga et al., 2007; Mizoshita et al., 2004), marbling score (Yokouchi et al., 2009), feed conversion ratio (Sherman et al., 2009), and somatic cell count (Longeri et al., 2006) have also been found near the area (NAGRP, 2010). Previous research has shown that mutations in calcium channel genes cause various genetic illnesses in humans and animals (Robinson et al., 2000). *CACNA2D1* and *RYR1* mutations, for example, induce human malignant hyperthermia syndrome (Loke and MacLennan, 1998; Robinson et al., 2000), central core illness (Robinson et al., 2002), and porcine stress syndrome (Fujii et al., 1991). Except for studies on porcine malignant hyperthermia induced by *RYR1* mutation, most recent calcium channel gene research has been performed in humans and model animals.

Few studies have been conducted on livestock such as cattle, pigs, and sheep, except for the studies on porcine malignant hyperthermia caused by *RYR1* mutation. (Li et al., 2005) reported that porcine *CACNA2D1* is located on porcine chromosome 9. Li et al.,

(2008) reported three QTLs regulating ovulation rate, shoulder weight, and body weight at ten weeks of age have been discovered in the domain, as has one QTL controlling pH up to 24 hours post mortem near the *CACNA2D1* gene. The *CACNA2D1* gene might be examined as a candidate gene impacting reproductive, carcass, and growth characteristics, as well as meat quality features in pigs with a single nucleotide substitution (C> A) in the 30 UTR discovered by PCR-SSCP (Li et al., 2007). There is no study of identifying polymorphisms of the *CACNA2D1* for carcass and meat traits in Bangladeshi cattle population.

2.5.3 *MYF5* gene

The myogenic determination (*MyoD*) gene family controls embryo development, maturation, and function (Te Pas et. al., 1999). MyoD is made up of four genes: *MYF3 (MyoD*) 1, *MYF4* or Myogenin (*MyoG*), *MYF5*, and *MYF6*. Myogenic factor 5 *(MYF5*) has been fine-mapped for QTLs for birth weight, preweaning average daily gain, and average daily gain on cattle chromosome 5 (Li , 2002) and three chromosomal regions (0 to 30 cM, 55 to 70 cM, and 70 to 80 cM) that are significantly associated with carcass and meat quality traits have been identified. *MYF5* and *MYF6* are thought to be essential for straight muscle innovation and growth and the maintenance of their outward look. As a result, they are thought to be candidate genes for growth and meat quality (Maak, 2006; Verner, 2007). SNPs in genes that are most likely to induce mutation in a phenotypic characteristic based on physiological and endocrinological data are identified in candidate gene studies (Shin, 2007). Polymorphisms in the *MYF5* gene have previously been linked to growth traits in Canadian cattle (Li, 2004) growth and average daily gain in Korean (Han woo) cattle (Chung, 2005), growth traits in Chinese (Qinchuan) cattle breed (Zhang, 2007), and growth and carcass traits in Korean (Han woo) cattle (Bhuiyan, 2007). Nonetheless, few studies have been conducted on the connection between *MYF5* gene polymorphism and meat quality characteristics in the Asiatic region. Polymorphism identification and association in *MYF5* gene and to analyze associations between SNPs and carcass and meat traits yet not conducted in Bangladesh. The results could provide new data and evidence concerning the convenience of these markers for gene-assisted selection in cattle breeding programs.

2.6 Marker-assisted selection

Cattle genomic data has substantial predictive value since it allows for the identification of animals with higher productive potential. In this regard, data collected from molecular markers stand out as providing vital information regarding selection programs and genetic improvement and are frequently referred to as molecular markers assisted selection (Teneva, 2010). By selecting parent stock that carries the gene, marker aided selection (MAS) may be used to enhance the frequency of favorable variants of a gene within a population. Selecting parents who are homozygotes for the desired allele guarantees that all gametes (sperm or egg cells) generated by that parent are homozygotes for the intended allele. To utilize MAS, markers for the phenotype and alleles of interest must be available. Furthermore, the quest for molecular markers encourages substantial progress in the genetic improvement of commercial species destined for animal production, particularly by increasing the accuracy of genetic value prediction for improved animal selection. Populational-genetic parameters (allele and genotype frequency estimates) allow for rate comparisons between populations and reveal variations in their genetic compositions, which contribute to phenotypic variance (Pereira et al., 2015).

2.6.1 Uses of marker-assisted selection in carcass related traits

The uses of marker-assisted selection (MAS) include low heritability traits (traits with observed or measured values that are poor). Traits that cannot be measured until later in life, potentially after the animal has reproduced (carcass or maternal traits), traits are difficult and/or expensive to measure, are not routinely measured or selected for currently (tenderness), and which are genetically correlated with another trait do not want to change. At the polygenic level, the traits may be positively correlated, but selection for a gene marker for marbling that is not associated with increased carcass fatness might be desirable. This would entail selection for marbling genes without pleiotropic effects on fatness (Van et al., 2007).

Depending on the kind of characteristic, the predicted advantages of altering allele frequencies and the consequent changes in phenotype differ. For a variety of reasons MAS is expected to enhance a variety of product characteristics, including carcass marbling and softness. Even though, these characteristics are tough to achieve. In reality, Warner-collection Bratzler's Shear force data is challenging to get because of

the high cost of obtaining large numbers of steak samples from sire identifiable animals and then obtaining shear force readings from certified labs (Weaber et al., 2007). Because the animals from which these observations are collected are incapable of reproducing, selection concentrates on the sires of animals with desirable characteristics. MAS also permits a selection among related individuals that do not show the trait (carcass trait in females) thus may be employed in introgression techniques to select both for and against introgressed and undesirable (Hillel et al., 1990). When half of the selected candidates were killed to assess phenotypic traits on carcasses, MAS provided an extra gain of 24%. When the non-selected halves were slaughtered, the marker phenotype data may be utilized to choose in the following generation, providing an extra gain of 64% (Goddard, 2007)

2.6.2 Limitations of marker-assisted selection for production and carcass traits

Selection based on marker information for a particular gene while ignoring other sources of genetic information such as expected progeny differences (EPD) will produce poor outcomes for overall trait improvement (Weaber et al., 2007). Ignoring information regarding genotype's positive merit via phenotypically derived genetic predictors such as EPD is discouraged. For example, marbling and softness are complicated characteristics regulated by numerous genes, with just a few genes containing useful markers. When both MAS and EPD are utilized, more responsiveness to selection is gained. When accessible, EPD should be the significant driver in selection decisions, with marker data serving as a backup for particular knowledge selection (Weaber et al., 2007).

The mode of inheritance is also significant like is the characteristic recessive or co-dominant. If the gene is recessive, it must be transferred aseptically into the population such that both the sire and dam populations are for calves to have a high chance of acquiring the recessive allele from both parents (Weaber et al., 2007). When the recessive trait is the desired phenotype, calves must be homozygous recessives before the beneficial impact is apparent. It is difficult and time-consuming to select for recessive characteristics. Consider picking a red coat colour from a herd of exclusively black (the dominant colour of red) animals.

To demonstrate the significance of gene frequency, data for a single gene locus was simulated. Consider a homozygous sire with two copies of the beneficial allele, which is mated to a herd of cows with a gene frequency of around 50%. Eight generations of selection are required before the calves have a gene with a 90% frequency. Even after 25 generations of selection, the gene frequency in calves is less than 50% because cows and bulls are scarce.

The argument is that using DNA markers for selection is not a quick remedy. Genetic testing and selection based on markers, particularly single gene markers, rather than panels that account for substantial amounts of variance, can be a major challenge. Researchers continue to probe the bovine genome for DNA markers linked to a wide range of characteristics. With only a few genes on the market, interpreting genotyping data is already somewhat tricky. The introduction of a vast number of DNA marker tests will soon create a massive quantity of data.

2.7 From biomarkers to molecular mechanisms of meat quality

The integration of biomarker data is a potential method for elucidating the molecular processes and biological networks that govern beef quality. So far, genomic studies have resulted in gene or protein catalogues. Many studies have found that the amount of gene expression in general, and more specifically the combination of individual gene expression, rather than a master gene, is responsible for phenotypic diversity (e.g., beef softness or marbling).

Computational biology is a branch of biology that uses computers to solve problems. Mining the information available from genetic studies with advanced bioinformatics aids in the depiction of processes by providing insight into physiological pathways and may aid in the search for novel candidate biomarkers. Indeed, computational biology has made it possible. Another way is using model animals to gain a better understanding of beef quality. Because of the incompleteness of the genome annotation, relevant information in beef is frequently absent. Alternative techniques include mining genome-wide data sets from worldwide databases (in silico approach) using online and interactive processes and databases (Kaspric et al., 2014) or using model species (*in vivo* and *in vitro* approaches).

This is important for identifying gene networks involved in building the quality phenotype, such as the development of muscle and adipose tissues, which define the lean-to-fat ratio (Kaspric et al., 2014). The knowledge obtained from research in non-ruminant and ruminant species can help us better comprehend the relationships.

2.8 Techniques used for gene isolation

2.8.1 PCR

The invention of the polymerase chain reaction (PCR) technology has had a dramatic impact on current molecular genetics, resulting in an explosion of systematic and population research (Schmidt et al., 2000). This technique offers a high-resolution capability. It also allows for highly comprehensive descriptions of genetic diversity in DNA and molecular components. This method employs two short single-strand DNA primers (usually 20 bp to 25 bp in length) to start DNA replication at a specified location on the DNA molecule. The DNA is then copied using a thermally stable DNA polymerase by expanding the primers and synthesizing complementary primers (strands of DNA). Denaturing the DNA repeatedly, re-annealing the primers, and duplicating the DNA will exponentially increase the target sequence between the primers. Following the amplification procedure, enough DNA is accessible for direct electrophoresis examination. The PCR-amplified DNA can be digested with a restriction enzyme and visualized by gel electrophoresis to determine if the PCR fragments have been cleaved, a process known as restriction fragment length polymorphism (RFLP), the first form of deoxyribonucleic acid (DNA) marker used to construct the first true genomic maps. There are a large number of restriction enzymes, each of which has a different specific recognition site.

2.8.2 Sanger sequencing

Sanger sequencing is a type of targeted sequencing that uses oligonucleotide primers to locate particular DNA sections. Denaturation of the double-stranded DNA is the first step in Sanger sequencing (Jamuar et al., 2016). The single-stranded DNA is then annealed to oligonucleotide primers and lengthened with a combination of deoxynucleotide triphosphates (dNTPs), that supply the arginine (A), cytosine (C), tyrosine (T), and guanine (G) nucleotides required to construct the new double-stranded structure.

A modest amount of chain-terminating dideoxynucleotide triphosphates (ddNTPs) for each nucleotide is also present. The sequence will keep extending with dNTPs until a ddNTP connects.Because the dNTPs and ddNTPs have an equal probability of connecting to the sequence, each one will end up at a different length. A fluorescent marker is included in each ddNTP (ddATP, ddGTP, ddCTP, and ddTTP). When a ddNTP is connected to the elongating sequence, the base fluoresces depend on the nucleotide associated with it. A is represented by green fluorescence, T by red, G by black, and C by blue. A laser in the automated system that reads the sequence detects a fluorescence intensity, which is converted into a "peak." When a heterozygous variation occurs inside a sequence, two fluorescent dyes of equal intensity will capture the locus. When a homozygous variant is present, the expected fluorescent color is replaced completely by the new base pair's color.

2.9 Single nucleotide polymorphisms

Single nucleotide substitutions/polymorphisms (SNPs) occur during DNA replication when one base or nucleotide is substituted for another. This phenomenon is commonly referred to as a substitution codon encodes for a different amino acid. Non-synonymous SNPs are further classified as missense or nonsense variants. A missense SNP occurs when the codon substitution alters the amino acid, and a nonsense SNP occurs when the codon substitution results in a stop codon that prematurely stops protein translation (Klug et al., 2013). This is important because SNP located within exons or regulatory regions can alter gene function/expression and, consequently, an animal's health or performance mutation, point mutation, or base substitution. There are two types of nucleotide substitutions: transitions and transversions. Transitions occur when a purine is substituted for a purine or when pyrimidine is substituted for another one-ring pyrimidine. Transversions occur when a one-ring purine is interchanged with a two-ring pyrimidine or vice versa (Vignal et al., 2002). Studies examining SNPs associated with human disease suggest that the most prevalent nucleotide substitutions are as follows: C substituted for T, T substituted for C, G substituted for A, and substituted to G. Thus, transitions are more common than transversions (Antonarakis and Cooper, 2013). Single nucleotide polymorphisms are found throughout the genome in both coding (i.e., exons) and non-coding regions (i.e., introns, intergenic regions, 5' or 3' untranslated regions, promoters, and transcription factor binding sites).

As Shen et al. (1999) discussed, “the frequency of SNPs varies between genomic regions and between coding and non-coding sequences.” Variants found within coding regions can be characterized as synonymous (codon encodes for the same amino acid) or non-synonymous (Ibeagha-Awemu et al., 2008).

It is important to note that although synonymous SNPs were previously deemed insignificant, recent studies suggest that synonymous SNPs may alter protein structure, function, and expression by operating in pre-mRNA splicing, as well as mRNA stability and structure. In addition, synonymous variants may influence protein translation and co-translational protein folding (Hunt et al., 2009). While SNP may be multiallelic (i.e., containing three or more nucleotides), most SNPs are bi-allelic containing only two nucleotides. This tendency to be bi-allelic can be attributed to the low frequency of single base-pair substitutions and the higher frequency of transitions compared to transversions (Vignal et al., 2002). Another key characteristic of SNPs is their high abundance in comparison to other genetic variants. In 2013, dbSNP listed 13,146,622 SNPs for the bovine genome and 66,994 of these were non synonymous SNPs (Adelson et al., 2014). Daetwyler et al. (2014) reported 26.7 million SNPs identified during the 1000 bull genomes project. In addition to genetic stability and the ease at which they can be analyzed with high-throughput technology, this high prevalence makes SNPs a valuable tool in genomic analyses.

2.10 Application of the data in breeding

To date, breed improvement has been accomplished by phenotypic selection focused on easily measurable characteristics, as mentioned above. Throughout the previous four decades, the techniques for selection have been improved. Methods for trait measurements have been conducted based on individuals have been substituted with estimated "breeding values" based on all relevant information about the individual's genetic quality, including information relatives, parents, off-spring and siblings. Nonetheless, many of the economically essential characteristics, particularly those related to meat quality variation, are challenging to quantify and "quantitative" in nature. By genotyping individuals and incorporating the information into the computation of the breeding value currently obtained through phenotypic measures, information on a few genes may be easily included in selection processes.

As previously mentioned, there are now numerous examples of genes regulating essential meat production characteristics that have been discovered. And a few experimental projects include this information in the selection process. However, the only information provided for most characteristics is a QTL location based on weakly connected markers rather than the gene or variation within the gene. Since different alleles at the marker loci can be associated with favorable or unfavorable alleles in different populations, the QTL information is specific to the study population; thus, the linkage phase between a marker and QTL had to be established for each family in which the markers are used. When compared to utilizing markers separately, the integration of surrounding marker information to build haplotypes containing the QTL enhances the confidence in accurately identifying genomic regions having beneficial alleles.

The rate of genetic advancement that can be achieved in selecting meat quality traits is determined by the quantity of genetic variation that can be handled. The amount of improvement that may be achieved using markers in the selection process is determined by the degree of variation indicated by the genes included in the selection criteria. Meat quality features contribute 10 to 30% of the diversity in beef and pork (Burrow et al., 2001), while individual genes may only explain a tiny part of this variation. Some traits, on the other hand, that have a well-defined biological basis and impact specific elements of meat quality, have a considerably more significant genetic contribution, such as the size and number of fibers in specific muscles, which affect lean muscle growth (Rehfeldt et al., 2000). Indeed, the myostatin gene, which has been linked to double muscling in some cattle breeds, has been demonstrated in mice to have a significant role in controlling muscle fiber size, type, and quantity (Rehfeldt et al., 2006). It is likely that even for a gene that is responsible for a large proportion of the genetic variation, the effect on the phenotype may be dependent on interactions with other genes (epistasis).

Chapter 3: Materials and Method

3.1 Study area

The field study was carried out at the Hathazari Upazilla, situated in the Chattogram district close to the Bay of Bengal and lying between latitudes 22º20'0" N to 22º32'30"N and between longitudes 91º47'30"E to 91º52'30"E which was taken by the widely used Garmin etrex GPS machine (Dutta et al., 2011) during February 2019 to December 2019. The molecular study was carried out at the laboratory (PRTC) of Chattogram Veterinary and Animal Sciences University (CVASU).

3.2 Selection of experimental animals

Red Chittagong and crossbred (RCC cross with local population) cattle were selected as target animals. A baseline survey was conducted in the Hathazari Upazilla by using a predesigned well-structured questionnaire to collect data on the present status of RCC, crossbred (a cross between RCC and local) based on their phenotypes. We examined the animals and selected healthy animals for sample collection. We randomly selected a total of 80 cattle, counting RCC 40 and crossbred 40. Cattle of different age groups, as reported by the owners and workers, were selected and identified. They feed their animal in traditional feeding system which were supplemented with standard concentrate mixture (wheat bran,rice bran, maize etc.), vitamins and mineral mixture and prepared for the next Eid-ul-Adha. Animal data were collected through a pre-prepared questionnaire.

3.3 Sample collection

For the molecular mechanisms, mainly blood was used as raw material to understand the underlying biological processes. In these consequences, blood samples were collected from RCC and crossbred cattle, and the detailed procedures of blood collection, DNA extraction, PCR, gene sequencing of PCR products were described as below: Blood samples were collected from eighty animals during the study period, and the individual animal was considered as a sampling unit. About 2-3 ml of peripheral blood was collected from the jugular vein in vials containing K2-EDTA (100 µg/ml Dipotassium Ethylene diamine tetraacetic acid). The vials were carried to the laboratory

PRTC of CVASU in a cool box and kept in the refrigerator at -20°C until further genomic DNA was extracted.

3.4 **DNA extraction**

Total genomic deoxyribonucleic acid (gDNA) was extracted from the whole blood samples by using the FavorPrepTM blood genomic DNA extraction mini kit. Briefly, the frozen blood samples were first thawed, and 200 µl whole blood samples were transferred to a micro-centrifuge tube with 20 µl proteinase-k and 200 µl FABG buffer and mixed thoroughly by pulse-vortexing. The sample was then incubated for 15 minutes at 60°C temperature while being vortexed every 3-5 minutes interval to lysate it. Then 200 µl of 100 % ethanol was added and mixed through by pulse vortexing for 10 seconds and briefly spined the tube to remove the drops from the inside of the tube. The mixture was then transferred to the FABG column with a collection tube and centrifuged at 6000×g for a minute. Spined the FABG mini-column after adding 400 µl W1 buffer at 18000×g (full speed) for 30 seconds and 750 µl wash buffer at 18000×g for 30 seconds, respectively, and discarded the flow-through in a consecutive manner. Centrifugation continued for an additional 3 minutes to dry the column and finally transferred the FABG mini-column to the elution tube where 120 µl elution buffers were added to the center of the column. The total DNA was collected and stored at -20°C after completing the final centrifugation for 1 minute at full speed.

3. 5 DNA quantification

Quantitation of nucleic acids was performed to determine the average concentrations of DNA or RNA present in a mixture and their purity. Here, DNA was quantified by the method of electrophoretic quantitation, then fluorometric quantitation. Initially, 4 ml of PCR product mixed with 1 ml of 6x gel loading dye (Composition: bromophenol blue and xylene cyanol FF, Thermo Fisher Scientific) from each tube along with 1 kb plus Gene Ruler DNA ladder (Thermo Scientific) on a 1.5 percent agarose gel at a steady voltage of 80-90 V for 40 minutes at 1x TAE buffer. For validation of PCR amplification, a horizontal agarose gel electrophoresis was performed (Agaro-power gel electrophoresis system-Bioneer, Korea). According to the manufacturer's instructions, the Qubit 2.0 fluorometer (Invitrogen, Life Technologies, Carlsbad, CA, USA) for quantification was used. The Qubit fluorometer calculates concentration based on the fluorescence of a dye that binds to double-stranded DNA (dsDNA).

The Qubit fluorometer picks up this fluorescence signal and converts it into a DNA concentration measurement by using DNA standards of known concentration.
Qubit dsDNA BR Assay Kit was used for DNA quantification. Based on DNA concentration derived from the Qubit measurements and the volume of the DNA extract, total DNA yield was calculated with a simple multiplication.

3.6 Primers used

To amplify the DNA sequence of the bovine *CACNA2D1* gene into a 249-bp product, one pair of primers (forward and reverse) was used from the paper of Yuan et al. (2011). The primer recorded by Ujan et al. (2011) was used to amplify the *MYF5* gene. To dilute the primers and give a final concentration of 20pmol/µl at first, they were dissolved in nuclease-free water. The PCR conditions for amplification were optimized and standardized.
The primer sequence, amplified region and product size of the amplicons are listed in Table 4.

Table 4: Primers of the bovine *CACNA2D1 and MYF5* gene designed for PCR

Primer name	Nucleotide sequence	Length	Chromosome no	Exon no	Amplicon size (bp)	Tm (ºC)
CACNA2D1	F: 5'-GTTTCCACTACCTATGATTGC-3'	21	9	Exon 25	249	61.9
	R: 5'-ACTGAACCAAGATTTGACCAC-3'	21				
MYF5	F: 5′-ACGCTTTGCTCTTGTTCC-3′	16	5	Exon 2	283	58
	R: 5′-AATACTGCCTGCTTGACGA-3′	17				

Reference: (Yuan et al., 2011, Ujan et al., 2011)

3.7 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a powerful method for amplifying particular segments of DNA. PCR makes billions of copies of a specific DNA fragment or gene, which allows detection and identification of gene sequences using visual techniques based on size and charge. This technique is currently widely used by clinicians and researchers to diagnose diseases, clone and sequence genes, and carry out sophisticated quantitative and genomic studies in a rapid and very sensitive manner.

The PCR reaction was performed in a thermocycler (2720 Applied Biosystem, Thermo Fisher Scientific, Singapore) in 25µL volume containing 0.5 µL of each primer (forward and reverse), 12.5µL 2x DreamTaq Green PCR Master Mix (Thermo Scientific) containing DreamTaq™ DNA polymerase, optimized DreamTaq Green buffer, $MgCl_2$ and dNTPs. About 2µL of genomic DNA was used as a template. The PCR conditions for two primers are summarized in Table 5. The annealing temperature was set by several trials on the basis of clear band. The PCR products were visualized following electrophoresis through a 1.8% ethidium bromide stained agarose gel, and the fragments were photographed under the gel documentation unit.

Table 5: Steps and conditions of thermal cycling for *CACNA2D1* and *MYF5* primers in PCR

Sl. No.	Step	For the *CACNA2D1* gene	For the *MYF5* gene
1.	Initial denaturation	95°C for 5 minutes	95°C for 5 minutes
2.	Final denaturation- 32 cycle	94°C for 30 seconds	94°C for 30 seconds
3.	Annealing	61.9°C for 30 sec	58°C for 30 sec
4.	Initial extension	72°C for 30 seconds	72°C for 30 seconds
5.	Final extension	72°C for 10 minutes	72°C for 10 minutes
6.	Final holding	4°C	4°C

3.8 Agarose gel electrophoresis

Initially, a sufficient electrophoresis buffer (1x TAE) was prepared to fill the electrophoresis tank and to cast the gel. To make the gel, dissolve 1x TAE buffer solution (50x TAE Electrophoresis Buffer, Thermo Scientific, Lithuania) in agarose powder (Agarose molecular biology grade, France). The solution is then heated in a microwave oven at 100°C and cooled to roughly 55°C to create a 1.5 percent agarose gel solution, which is combined with 2-3 drops of ethidium bromide. The solution was then put into a casting tray, which served as a mold. When the gel solution hardens, a well-former template (also known as a comb) is put across the end of the casting tray to produce wells. After that, the solution was poured into the mold with an appropriate comb to form the sample slots in the gel when the gel solution solidified. After that, the gel set was placed in a buffer-filled electrophoresis chamber with positive and negative electrodes at both ends. A micropipette or transfer pipette is used to transport around 5L samples to the sample wells. To compare the amplicon size of a gene product, the first hole was filled with 1 kb plus Gene Ruller DNA ladder (Thermo Scientific). A negative control containing all the reaction components except the template DNA was also made to check for any contamination of the foreign DNA in the reaction components.

The electrophoresis equipment was powered by a direct current (D.C.) power source, and an electrical current was applied. For around 40 minutes, electrophoresis was performed at 80-90V. Charged molecules in the sample enter the gel via the good wall. Molecules with a net negative charge migrate to the positive electrode (anode), while molecules with a net positive charge move to the negative electrode (cathode).The buffer acts as an electrical conductor and controls the pH, which is crucial for the charge and stability of biological molecules. Since DNA has a high negative charge at neutral pH, it migrates across the gel towards the positive electrode during electrophoresis.

3.9 Gene sequencing of PCR product

3.9.1 PCR product purification

The PCR substance was purified using the procedure of centrifugation. To complete the PCR combination, a 1:1 volume of Binding Buffer was applied first and thoroughly combined. The colour of the solution has been checked.

For DNA binding, the yellow colour suggests an ideal pH (If the solution colour is orange or violet, 10 μL of 3 M sodium acetate was added, pH 5.2 solution, and blended. The colour of the mixture would turn yellow). Up to 800 μL of the solution was then moved to the purification column of GeneJET, centrifuged for 30-60 seconds, and the flow-through was discarded. (Notes: Once the cumulative amount reaches 800 μL, the solution should be applied to the column in phases. After the addition of 800 μL of the solution, centrifuge the column for 30-60 s and discard the flow through. Repeat until the column membrane has been applied to the whole solution. After each application, securely close the bag with GeneJET Purification Columns.

After that, 700 μL of wash buffer was applied to the purification column of GeneJET and centrifuged again for 30-60 seconds after the flow-through was discarded and the purification column was inserted back into the collection tube. The empty GeneJET purification column was centrifuged for an additional 1 minute to entirely eliminate all remaining wash buffer (Note: This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions). Finally, a clean 1.5 mL microcentrifuge tube was moved to the GeneJET purification column, and 50 μL of Elution Buffer was applied to the middle of the well of the GeneJET purification column membrane and centrifuged for 1 minute. Finally, the purification column of GeneJET was discarded, and the filtered DNA was deposited at -20 °C before sequencing was completed.

3.9.2 Sequencing

Purified PCR products have been submitted to Sanger sequencing methods by commercial suppliers (Bioneer Corp, South Korea) for sequencing. Sanger-sequenced with a Big dye terminator v3.1 sequencing kit and a 3730xl automated sequencer were the filtered PCR products (Applied Biosystems, Foster City, CA). Nucleotide sequences were then calculated in the Macrogen on both strands of PCR amplification materials.

3.9.3 The analytical method of sequencing

The processing and analysis with Bioedit (www.mbio.ncsu.edu/BioEdit/) of nucleotide sequences and deduced amino acid sequences was carried out. Using BLAST (Altschul et al., 1990), the reference sequences for the respective genes from the National Center for Biotechnology Knowledge (NCBI) database were compared with the reference

sequences for the respective genes and several alignments were achieved using ClustalW software (https://www.genome.jp/tools-bin/clustalw).For allele and genotype frequencies the GenePop software (https://genepop.curtin.edu.au/) and the GeneScreen program (https://bio.tools/genescreen) have been used to evaluate genotypic polymorphism results. Heterozygosity and polymorphism information quality (PIC) values for each locus were computed by using p,q CHWE: PolyPICker (https://www.genecalculators.net/pq-chwe-polypicker.html). The nucleotide sequences were analyzed to compare the nucleotide sequences, evolutionary divergence, evolutionary relationships, and maximum composite likelihood estimation using NCBI BLAST (Johnson et al., 2004; Madden, 2013) and MEGA6 software (Tamura et al., 2013).

3.10 Phylogenetic analyses

The multiple alignment analysis was performed using the Clustal W program (Thompson et al., 1994), while the phylogenetic analysis was performed by the Neighbour-joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) using the MEGA software, version-5 (Tamura et al., 2011). The tree stability was estimated by a bootstrap analysis for 1,000 replications (Felsenstein, 1985).

3.11 Impact of the mutation on protein

The identified polymorphism impact on the coding region of the peptide has been identified by Polyphen software (http://genetics.bwh.harvard.edu/pph2/). The score from this software was used to predict any possible impact on the structure and function of a functional protein (Adzhubei et al., 2010).

Chapter 4: Results

Investigation of the promoter and the exon sequences of pooled DNA revealed to identify polymorphisms (if present) in the *CACNA2D1* gene and the *MYF*5 gene in Red Chittagong Cattle (RCC) and crossbred cattle of Bangladesh. The fragments of exon 2 of the *MYF*5 gene and exon 25 of *CACNA2D1* were amplified in both genotype using genomic DNA and sequences subjected to DNA analysis. The impact of mutations on the structure and function of related proteins was also detected. Finally, association analysis was also done to identify the relationship between identified polymorphic loci in the studied cattle breeds.

4.1 *CACNA2D1* gene

4.1.1 PCR amplification of *CACNA2D1* gene

DNA from test animals were used in PCR to amplify a 249-bp fragment of exon 25 of the *CACNA2D1* gene. PCR products were separated by agarose gel electrophoresis (Figure 1). We observed clear bands at expected fragment length, and the absence of any non-specific band indicates a reasonable specificity.

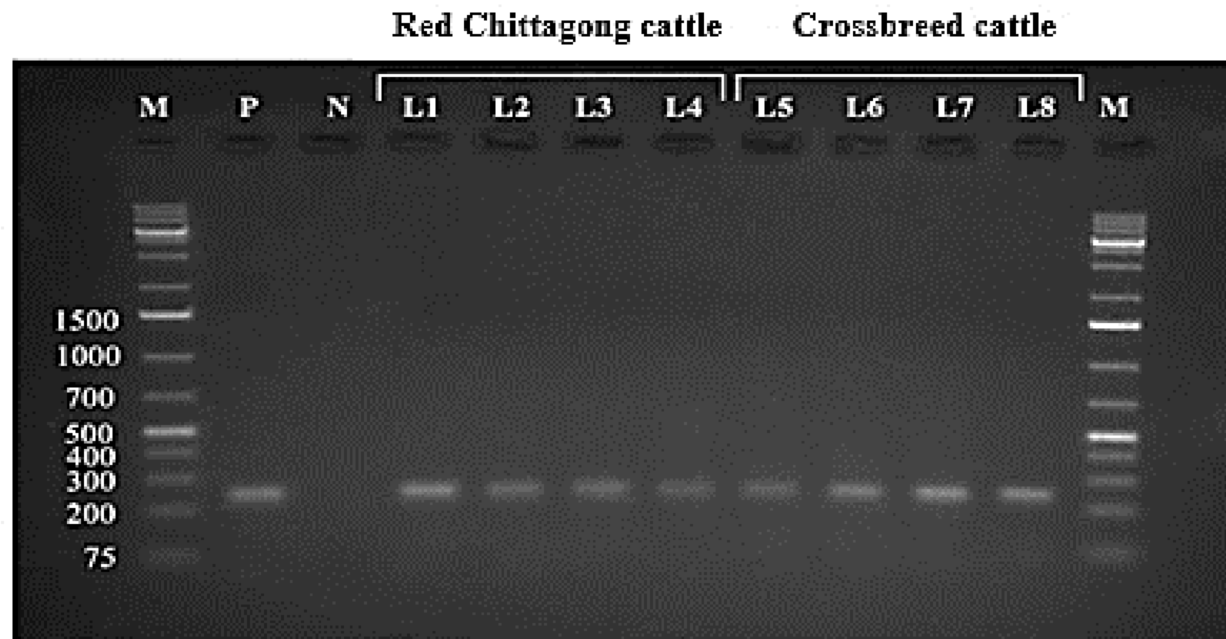


Figure 1: Gel electrophoresis image for amplicon of *CACNA2D1* exon 25.

Lane (M) is a DNA marker/ladder; Lane P is positive sample and Lane N is negative sample; Lane L1-L4 and L5-L8 represents the PCR product of the *CACNA2D1* gene (249 bp) of two indigenous cattle

4.1.2 Nucleotide sequences of *CACNA2D1* gene

Initial processing and analysis of nucleotide sequences were performed using BioEdit version 7.0.5.3. The sequence quality was good with less contamination. The sequence chromatogram showed evenly spaced peaks, each with only one color and crisp, clean bands, well separated and with no ambiguity, indicating proper base calls. Peak heights vary within a 3-fold "Noise" (baseline) peak may be present, but the number is relatively minimal (Figure 2).

Curated sequences were compared with the reference sequences (Figure 3) for respective genes from the National Center for Biotechnology Information (NCBI) database using BLAST (Altschul et al., 1990). Blast results showed that the sequenced *CACNA2D1* gene fragment in this study fragment showed 98% homology with the sequence of *Bos indicus* (NCBI accession no: NC_032653.1).

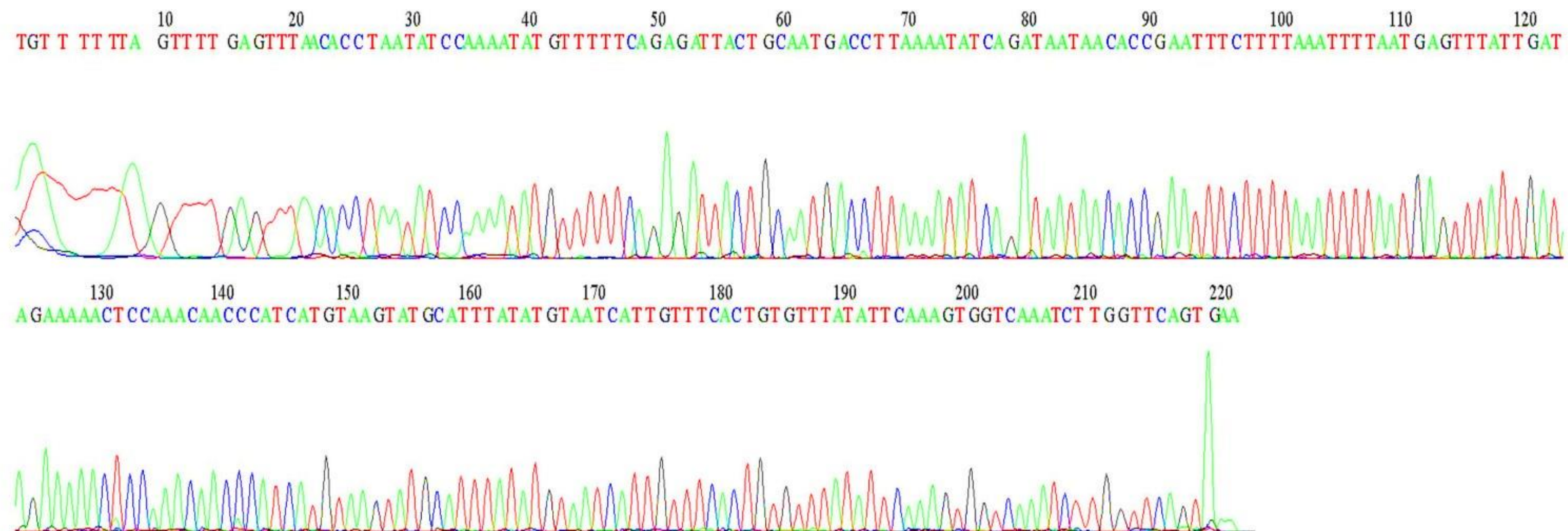


Figure 2: Nucleotides sequence of *CACNA2D1* gene

NC_032653.1:39451858-40012939 Bos indicus isolate QUIL7308 breed Nelore chromosome 4, Bos_indicus_1.0, whole genome shotgun sequence

Sequence ID: **Query_11421** Length: **561082** Number of Matches: **1**

Range 1: 526766 to 526983

Score	Expect	Identities	Gaps	Strand
383 bits(207)	4e-108	214/218(98%)	1/218(0%)	Plus/Plus

```
Query  3       TTTTT-TAGTTTTGAGTTTAACACCTAATATCCAAAATATGTTTTTCAGA
               |||||  ||||||||||||||||||||||||||||||||||||||  |||
Sbjct  526766  TTTTTAAAGTTTTGAGTTTAACACCTAATATCCAAAATATGTTTTYYAGA

Query  62      ATGACCTTAAAATATCAGATAATAACACCGAATTTCTTTTAAATTTTAAT
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526826  ATGACCTTAAAATATCAGATAATAACACCGAATTTCTTTTAAATTTTAAT

Query  122     ATAGAAAAACTCCAAACAACCCATCATGTAAGTATGCATTTATATGTAAT
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526886  ATAGAAAAACTCCAAACAACCCATCATGTAAGTATGCATTTATATGTAAT

Query  182     CTGTGTTTATATTCAAAGTGGTCAAATCTTGGTTCAGT  219
               ||||||||||||||||||||||||||||||||||||||
Sbjct  526946  CTGTGTTTATATTCAAAGTGGTCAAATCTTGGTTCAGT  526983
```

Figure 3: BLAST result of the *CACNA2D1* gene with the sequence of *Bos indicus* (NCBI accession no: NC_032653.1)

4.1.3 Multiple sequence alignment of *CACNA2D1* gene

The color key comparison of nucleotide sequences by NCBI blast revealed similarity between all tested samples when sequences of the studied cattle population aligned with related *Bos taurus* sequences. The visual depictions of the alignment as deletion mutations (indels or gaps) that appear as hyphens in all studied population sequences in the alignment (Figure 4).

Species/Abbrv	* * * * * * * * * * * * * * * * * * * * * *
1. Bos_Taurus_CACNA2D1	A G T T T T G A G T T T A A C A C C T A A T A
2. CACNA2D1_RCC22	A G T T T T G A G T T T A - C A C C T A A T A
3. CACNA2D1_RCC23	A G T T T T G A G T T T A - C A C C T A A T A
4. CACNA2D1_RCC29	A G T T T T G A G T T T A - C A C C T A A T A
5. CACNA2D1_RCC32	A G T T T T G A G T T T A - C A C C T A A T A
6. CACNA2D1_RCC38	A G T T T T G A G T T T A - C A C C T A A T A
7. CACNA2D1_Crossbred1	A G T T T T G A G T T T A - C A C C T A A T A
8. CACNA2D1_Crossbred11	A G T T T T G A G T T T A - C A C C T A A T A
9. CACNA2D1_Crossbred14	A G T T T T G A G T T T A - C A C C T A A T A
10. CACNA2D1_Crossbred3	A G T T T T G A G T T T A - C A C C T A A T A
11. CACNA2D1_Crossbred15	A G T T T T G A G T T T A - C A C C T A A T A

Figure 4: Comparison of *CACNA2D1* gene sequences of *Bos taurus* with sequences of gene in studied cattle population showing a deletion at 219 bp position.

4.1.4 Phylogenetic analysis of *CACNA2D1* gene

The nucleotide sequences of the *CACNA2D1* gene sequenced in the present study (for crossbred cattle and RCC) and five published sequences for different breeds were used to construct a phylogenetic tree (Figure 5). The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were calculated using the Maximum Composite Likelihood technique and are expressed in terms of the number of base substitutions per site. Evolutionary comparisons took into account variations in composition bias among sequences. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA7. Phylogenetic analysis revealed that the sequence of crossbred belongs to a distant tree branch compared to RCC.

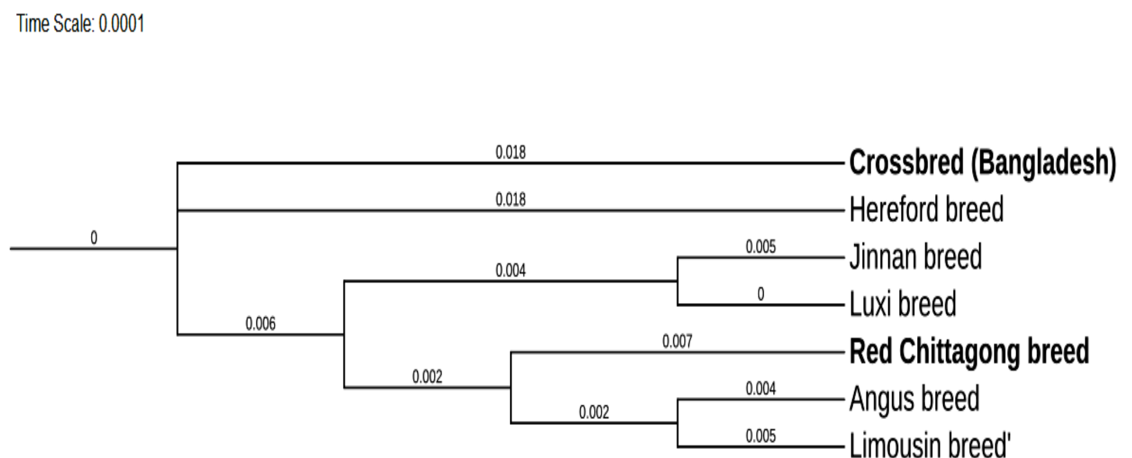


Figure 5: Phylogenetic tree for *CACNA2D1* gene in different breeds of cattle

4.1.5 Mutations detected in the *CACNA2D1* gene

In this study, three SNPs were observed in exon 25 of the *CACNA2D1* gene. The SNPs were c.1993C>A, c.2058T>A, and c.2068C>A (Table 6). Two SNPs, viz. c.1993C>A and c.2068C>A were missense mutations. The c.1993C>A is a transversion mutation that results in a pyrimidine to purine base substitution. It causes an amino acid change (leucine to isoleucine) at position 658 (p.Leu658Ile) of the *CACNA2D1* protein. The c.2068C>A substitution causes phenylalanine to threonine substitution at position 683 (p.Phe683Thr). It is a transversion mutation. The c.2058T>A was the same sense mutation, meaning that this substitution does not change the amino acid sequences in the resulting protein. Sequencing results for identified polymorphisms in the *CACNA2D1* gene are presented in Figures 6, 7 and 8.

Table 6: Polymorphic sequence variations detected in the *CACNA2D1* gene in Bangladeshi cattle

Region	Coding base (bp)	Base change	RNA codon change	Amino acid change	Types of mutation
Exon 25	1993	C-A	CUU-AUU	Leu(L) 658 Ile(I)	Missense
	2058	T-A	ACU-ACA	-	Same-sense
	2068	C-A	CCA-ACA	Phe(P) 683 Thr(T)	Missense

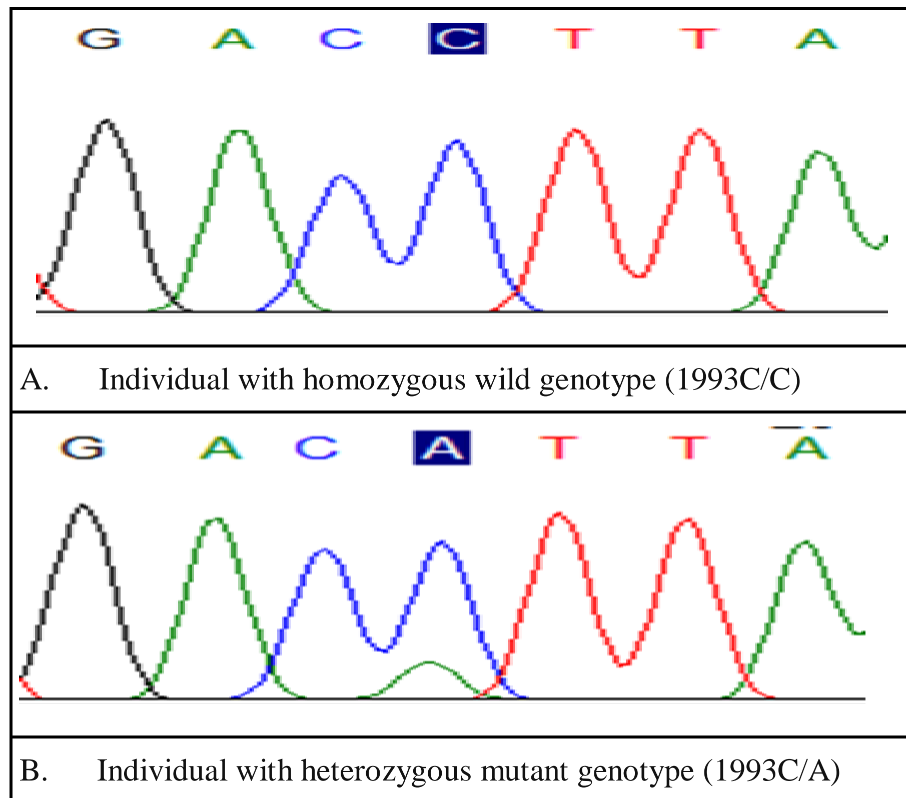


Figure 6: Sequencing results of the C1993A mutation in the *CACNA2D1* gene in Bangladeshi cattle.

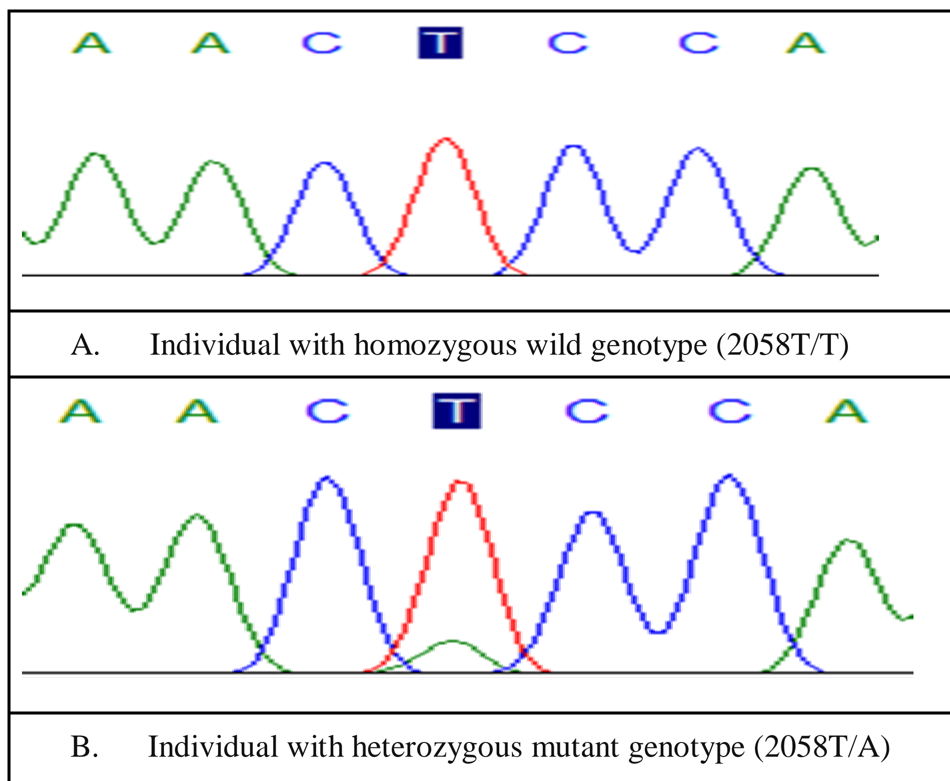
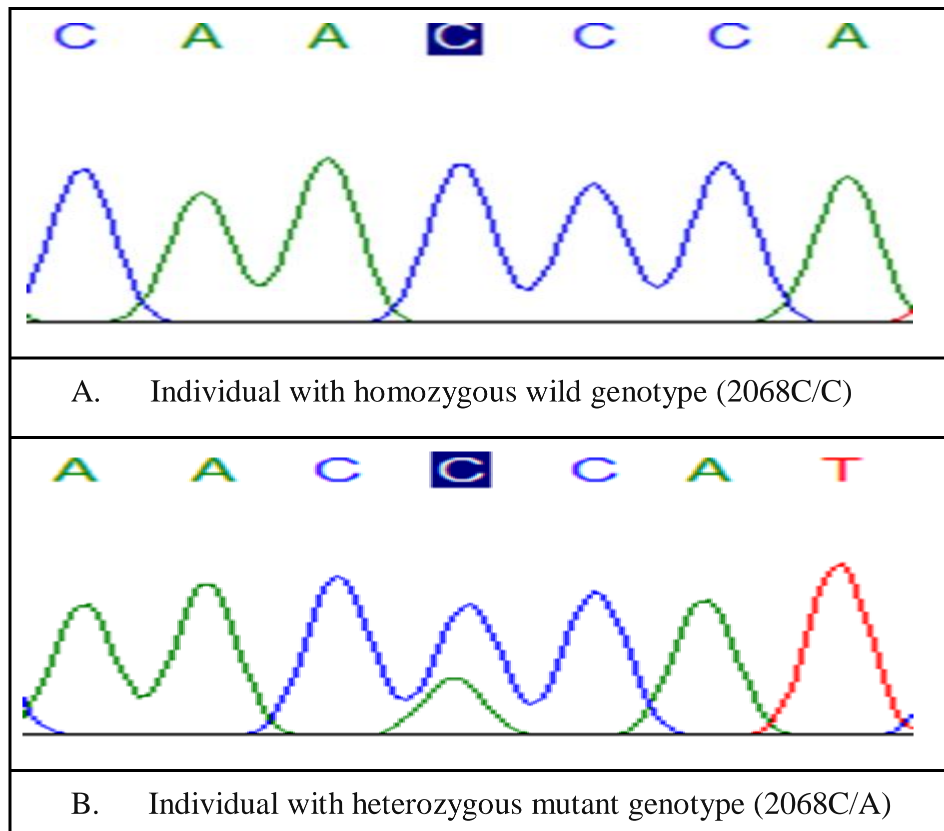


Figure 7: Sequencing results of the T2058A mutation in the *CACNA2D1* gene in Bangladeshi cattle.



A. Individual with homozygous wild genotype (2068C/C)

B. Individual with heterozygous mutant genotype (2068C/A)

Figure 8: Sequencing results of the C2068A mutation in the *CACNA2D1* gene in Bangladeshi cattle

4.1.6 Overall genotypic and allelic frequencies of different polymorphhisms detected in *CACNA2D1* gene

4.1.6.1 C1993A mutation in *CACNA2D1*

For C1993A mutation, only crossbred cattle were polymorphic, whereas RCC was monomorphic for the locus. The allelic frequencies in crossbred cattle were 0.70 for wild type allele and 0.30 for mutation allele. Two possible genotypes CC and CA, out of three possible genotypes, were observed. The frequencies for CC and CA genotypes in crossbred were 0.40 and 0.60, respectively (Table 7).

According to the classification of PIC (low polymorphism if PIC value <0.25, moderate polymorphism if PIC value 0.25-0.50, and high polymorphism if PIC value >0.50), C1993A locus was found to be moderately polymorphic (PIC value 0.33) in crossbred cattle.

Table 7: Overall genotypic and allelic frequencies of C1993A mutation detected in *CACNA2D1* gene

Genotype	Genotypic frequency			Allelic frequency		He	PIC
	CC	CA	AA	C	A		
Red Chittagong	1.0	-	-	1.0	-	-	-
Crossbred	0.40	0.60	0.00	0.70	0.30	0.42	0.33
Overall	0.70	0.30	0.00	0.85	0.15	0.25	0.22

4.1.6.2 T2058A mutation in *CACNA2D1* gene

Table 8 shows the genotypic and allelic frequencies of 2058T>A in RCC and crossbreds. For this SNP, a higher frequency of T (85%) than that of the A allele was recorded. RCC was monomorphic, while crossbred cattle had two genotypes in the locus. The frequency of heterozygous mutant genotypes was 60% in crossbred cattle. PIC value for T2058A indicates it was moderately polymorphic in crossbred cattle.

Table 8: Overall genotypic and allelic frequencies of T2058A mutation detected in *CACNA2D1* gene

Genotype	Genotypic frequency			Allelic frequency		He	PIC
	TT	TA	AA	T	A		
Red Chittagong	1.0	-	-	1.0	-	-	-
Crossbred	0.40	0.60	0.00	0.70	0.30	0.42	0.33
Overall	0.70	0.30	0.00	0.85	0.15	0.25	0.22

4.1.6.3 C2068A mutation in *CACNA2D1* gene

Genotypic and allelic frequencies of c.2068C>A are displayed in Table 9. Homozygous wild CC and heterozygous mutant CA genotypes were identified for the C2068A polymorphic site in crossbred cattle. This SNP showed a higher frequency of the C than the A allele (85% frequency in the whole population).

Table 9: Overall genotypic and allelic frequencies of C2068A mutation detected in *CACNA2D1* gene

Genotype	Genotypic frequency			Allelic frequency		He	PIC
	CC	CA	AA	C	A		
Red Chittagong	1.0	-	-	1.0	-	-	-
Crossbred	0.40	0.60	0.00	0.70	0.30	0.42	0.33
Overall	0.70	0.30	0.00	0.85	0.15	0.25	0.22

4.1.7 Impact of detected mutations on CACNA2D1 protein

This SNP resulted in a missense mutation in the CACNA2D1 protein, which led to Leu (L) 658 Ile (I) amino acid substitution. The identified polymorphism has a significant impact on the 1993 bp coding region of the CACNA2D1 peptide. According to the score in Polyphen2 (0.00 to 1.00), the larger the score, the more confidently predicted to be deleterious (http://genetics.bwh.harvard.edu/pph2/). Therefore, this mutation is predicted to cause damage (with a score of 0.875) to the structure and function of the functional CACNA2D1 protein. Other amino acid substitutions in *CACNA2D1* are caused by missense mutations at the 683 bp position, indicating a benign phenotypic effect (with a score of 0.013) since the value is smaller (close to 0.00). The g. 2068C>A mutation was found to cause a moderate functional change in the CACNA2D1 protein, resulting in the conversion of phenylalanine to threonine (Figures 9 and 10).

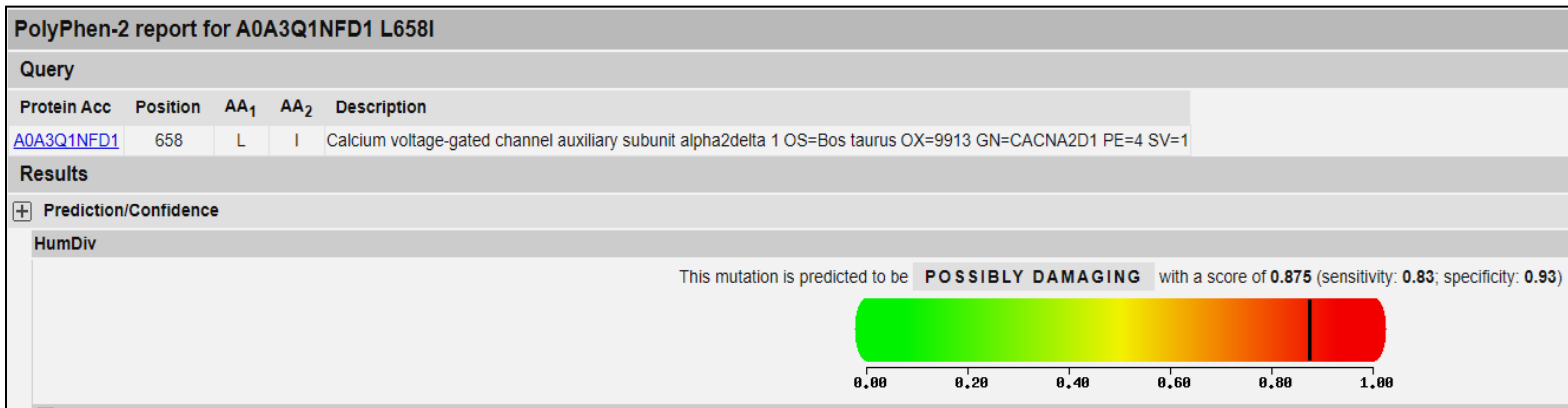


Figure 9: Impact of C1993A substitution *CACNA2D1* gene on the structure and function of CACNA2D1 protein.

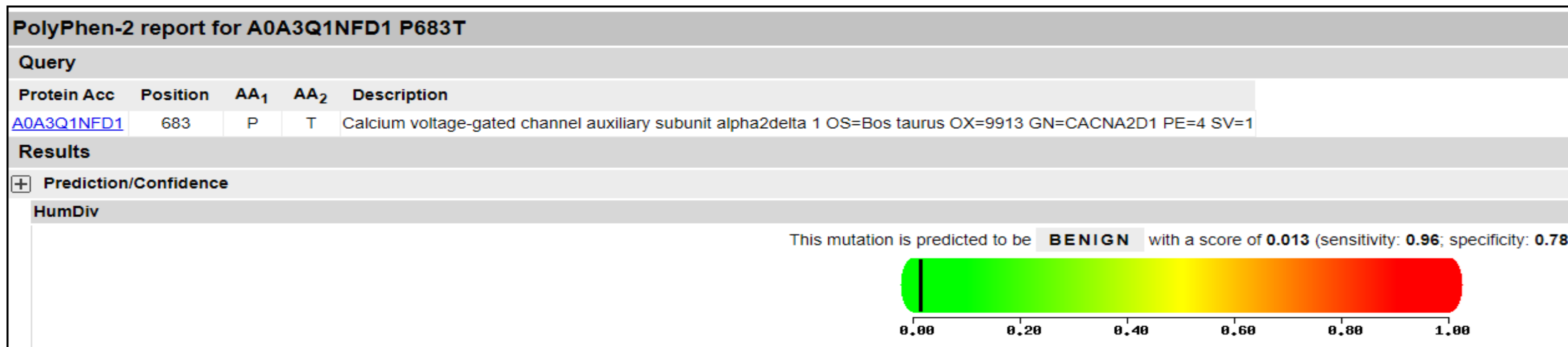


Figure 10: Impact of C2068A substitution in *CACNA2D1* gene on the structure and function of CACNA2D1 protein.

4.2 *MYF5* gene

4.2.1 PCR amplification of *MYF5* gene

All of the eighty animals tested had a 283-bp amplified product amplification. The PCR products were separated on 1.5% agarose gels. Figure 11 shows the gel electrophoresis image of the *MYF5* PCR product. The clarity of the image indicates a reasonable specificity of the PCR reaction.

4.2.2 Nucleotide sequences of *MYF5* gene

Analysis of nucleotide sequences resulted in good quality with limited or no contamination. Peaks are evenly-spaced, and the heights vary no more than 3-fold. Peak with only one colour and crisp, clean bands, well separated and with no ambiguity about the proper base call. “Noise” (baseline) peaks may be present, but the number is negligible (Figure 12).

The studied sequences were compared with the reference sequences for the *MYF5* gene from the NCBI database using BLAST (Altschul et al., 1990) (Figure 13). Alignment analysis revealed 99% homology with the sequence of *Bos indicus* (NCBI accession no: EF197851.1).

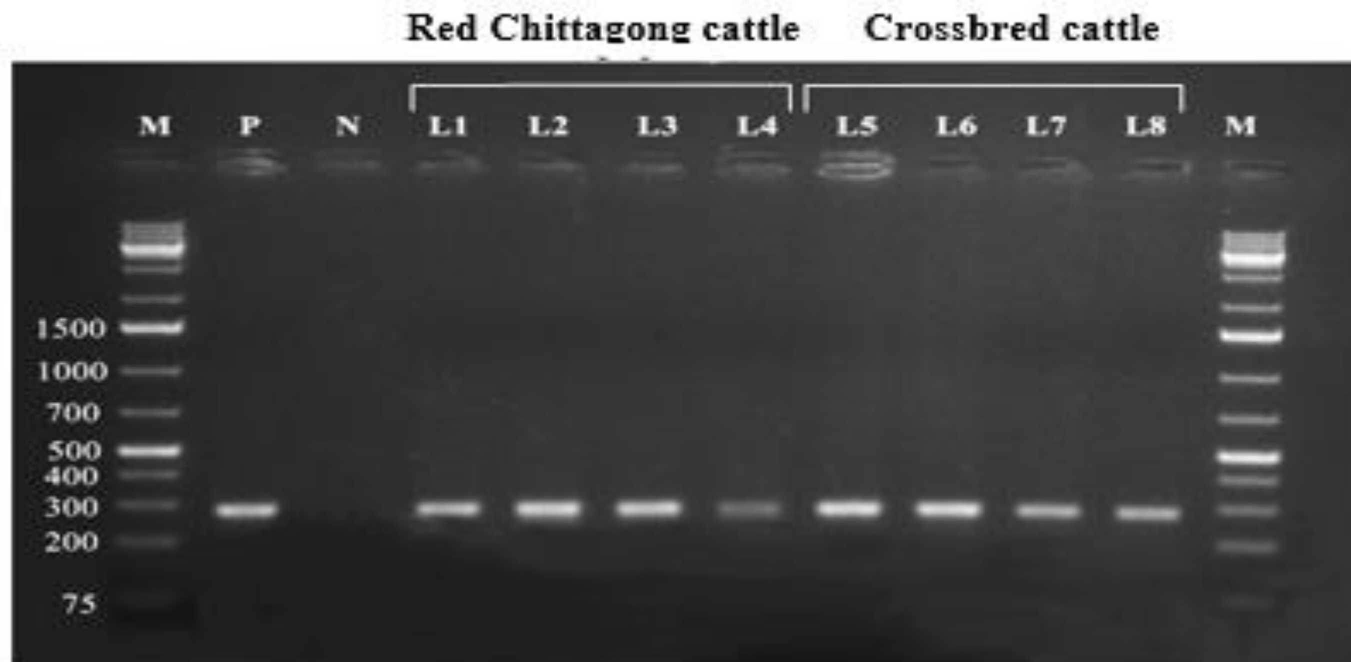


Figure 11: Gel electrophoresis image for amplicon of *MYF5* gene.

Lane (M) is a DNA marker/ladder; Lane P is positive sample and Lane N is negative sample; Lane L1-L4 and L5**-** L8 represents the PCR product of the *MYF5* gene (283bp) of two indigenous cattle

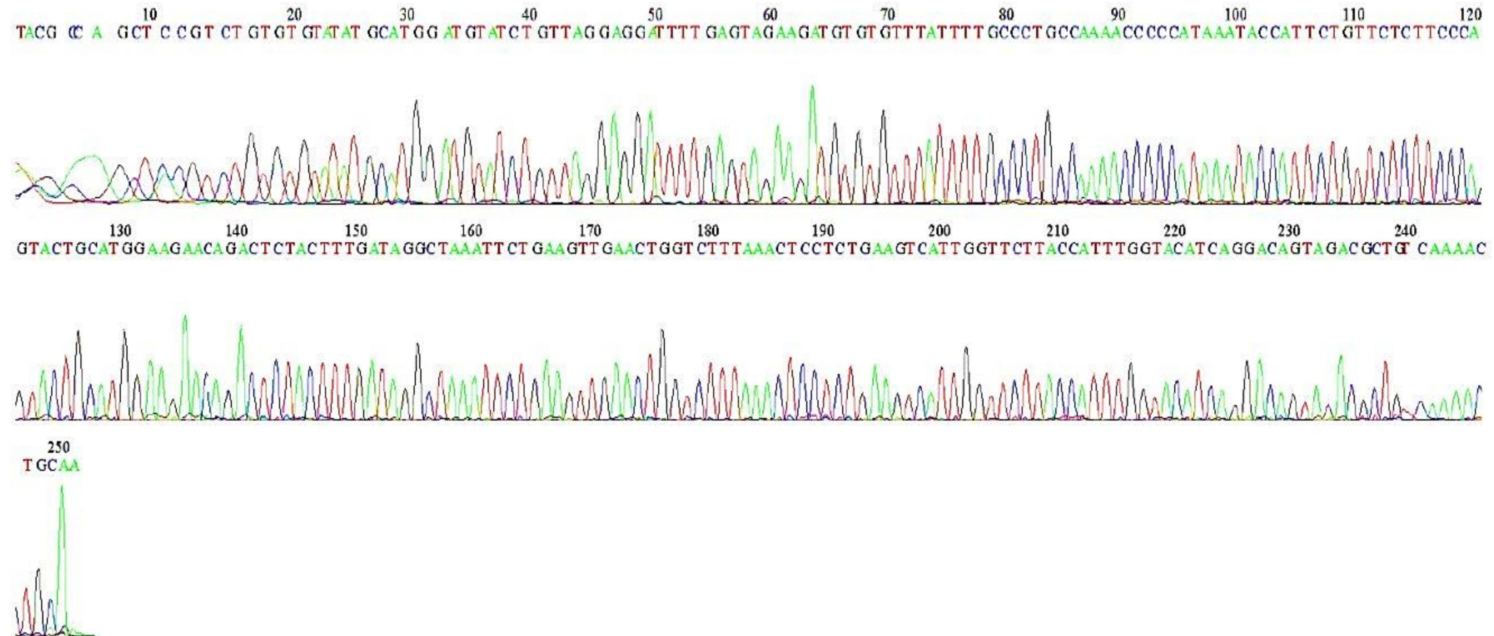


Figure 12: Nucleotides sequence of *MYF5* gene.

Bos indicus myogenic factor 5 (Myf-5) gene, complete cds

Sequence ID: EF197851.1 Length: 3328 Number of Matches: 1

Range 1: 1588 to 1832 GenBank

Score	Expect	Identities	Gaps	Strand
444 bits(231)	5e-120	245/247(99%)	2/247(0%)	Plus/Minus

```
Query  10    AAGCTCACGTCTGTGTGTATATGCATGGATGTATCTAGTTAGGAGGATTTTGAGTAGAAG  69
             |||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||
Sbjct  1832  AAGCTCACGTCTGTGTGTATATGCATGGATGTATCT-GTTAGGAGGATTTTGAGTAGAAG  1774

Query  70    ATGTGTGTTTATTTTGCCCTGCCAAAACCCCCATAAATACCATTCTGTTCTCTTCCCAGT  129
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1773  ATGTGTGTTTATTTTGCCCTGCCAAAACCCCCATAAATACCATTCTGTTCTCTTCCCAGT  1714

Query  130   ACTGCATGGAAGAACAGACTCTACTTTGATAGGCTAAATTCTGAAGTTGAACTGGTCTTT  189
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  1713  ACTGCATGGAAGAACAGACTCTACTTTGATAGGCTAAATTCTGAAGTTGAACTGGTC-TT  1655

Query  190   AAACTCCTCTGAAGTCATTGGTTCTTACCATTTGGTACATCAGGACAGTAGACGCTGTCA  249
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1654  AAACTCCTCTGAAGTCATTGGTTCTTACCATTTGGTACATCAGGACAGTAGACGCTGTCA  1595

Query  250   AAACTGC  256
             |||||||
Sbjct  1594  AAACTGC  1588
```

Figure 13: BLAST result of the *MYF5* gene with the sequence of *Bos indicus.* (NCBI accession no: EF197851)

4.2.3 Multiple sequence alignment of *MYF5* gene sequences

Sequences of the ten studied population of RCC and crossbred cattle are aligned with related *Bos taurus* sequences for *CACNA2D1* gene, and there are no visual changes present (Figure 14). The *Bos indicus MYF5* gene showed a nucleotide deletion compared to five studied animals of crossbred and the five studied RCC with *Bos taurus* reference sequence.

Species/Abbrv	* * * * * * * * * * * * * * * * * * * *
1. MYF5_Bos_taurus	A G G A G T T T A A A G A C C A G T T C A
2. MYF5_RCC38	A G G A G T T T A A A G A C C A G T T C A
3. MYF5_RCC32	A G G A G T T T A A A G A C C A G T T C A
4. MYF5_RCC29	A G G A G T T T A A A G A C C A G T T C A
5. MYF5_RCC03	A G G A G T T T A A A G A C C A G T T C A
6. MYF5_RCC04	A G G A G T T T A A A G A C C A G T T C A
7. MYF5_Bos_indicus	A G G A G T T T A A - G A C C A G T T C A
8. MYF5_Cross01	A G G A G T T T A A A G A C C A G T T C A
9. MYF5_Cross02	A G G A G T T T A A A G A C C A G T T C A
10. MYF5_Cross03	A G G A G T T T A A A G A C C A G T T C A
11. MYF5_Cross18	A G G A G T T T A A A G A C C A G T T C A
12. MYF5_Cross20	A G G A G T T T A A A G A C C A G T T C A

Figure 14: Comparison of *MYF5* gene sequences of *Bos taurus* and *Bos indicus* with sequences of *MYF5* gene in Bangladeshi cattle.

4.2.4 Phylogenetic analysis of *MYF5* gene

The nucleotide sequences of the *MYF5* gene of crossbred cattle and RCC from this study and four published sequences of cattle breeds, one buffalo breed and one sheep breed *MYF5* gene from the NCBI database was utilized to construct the phylogenetic tree (Figure 15). The present result showed *MYF5* gene was not phylogenetically diverse in crossbred cattle and the RCC. Both breeds in the present study clustered into a common cluster with *Bos indicus*. Moreover, the *MYF5* gene was shown to be phylogenetically diverse among the other species studied in this study.

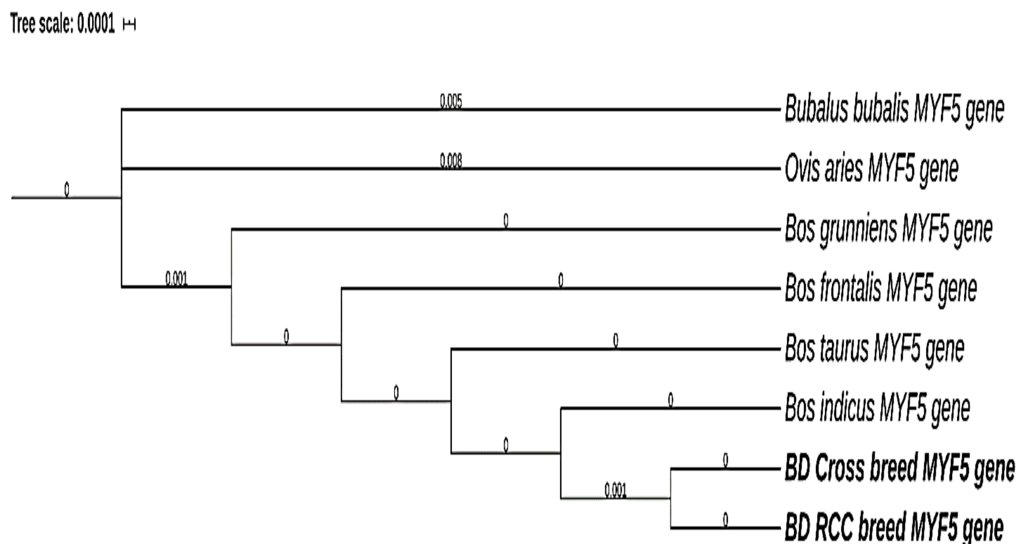
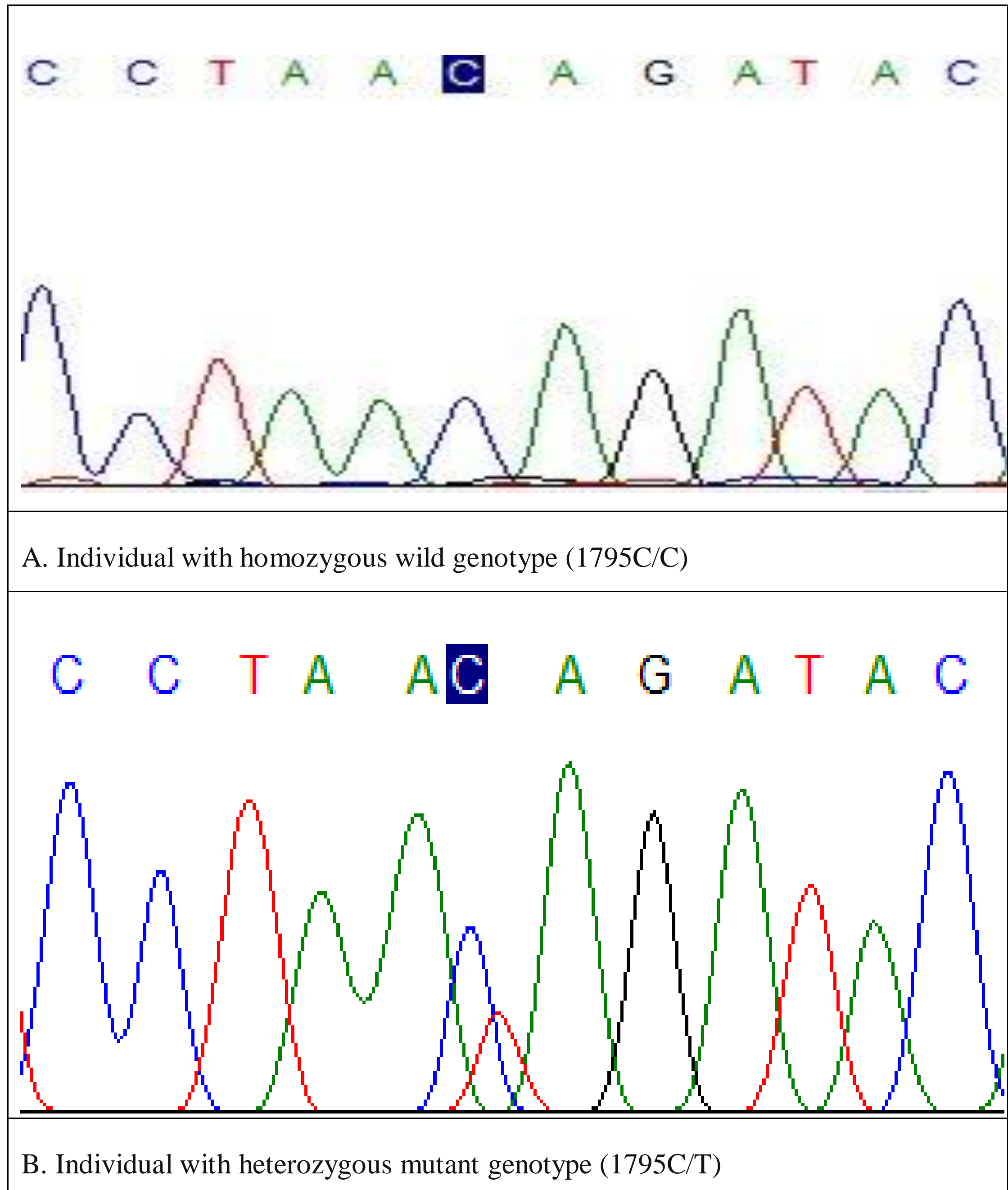


Figure 15: Phylogenetic tree of *MYF5* gene. BD Cross indicates Bangladeshi crossbred cattle (in the current study); BD RCC indicates Red Chittagong cattle of Bangladesh (in the current study).

4.2.5 Mutations detected in the *MYF5* gene

The present study sequenced *MYF5* gene sequences that cover 283 bp. Compared with reference *MYF5* (Sequence ID: EF197851.1), one SNP was identified in the non-coding part of *MYF5* gene c.1795C>T. The c.1795C>T is an intronic mutation hence does not cause an amino acid substitution. Sequencing chromatograms for this polymorphism in the *MYF5* gene are illustrated in Figure 16.



A. Individual with homozygous wild genotype (1795C/C)

B. Individual with heterozygous mutant genotype (1795C/T)

Figure 16: Sequencing results of the C1795T mutation in the *MYF5* gene in Bangladeshi cattle.

4.2.6 Overall allelic and genotypic frequencies of different polymorphisms in *MYF5* gene

4.2.6.1 C1795T in *MYF5* gene

Genotype and allele frequencies of c.1795C>T for RCC and crossbred are presented in Table 10. In the studied cattle population, two genotypes were observed (homozygous wild CC and heterozygous mutant CT) for the 1795 locus. There is no homozygous mutant TT genotype observed. This SNP displayed a higher frequency of C (90% frequency in the whole population) than the T allele, and the homozygous wild genotype CC was highly predominated in the studied population (average 0.80). According to the PIC classification, the C1795T locus was low polymorphic (PIC value of 0.16) in both breeds.

Table 10: Overall genotypic and allelic frequencies of C1795T mutation detected in *MYF5* gene.

Genotype	Genotypic frequency			Allelic frequency		He	PIC
	CC	CT	TT	C	T		
Red Chittagong	0.80	0.20	0.00	0.9	0.10	0.32	0.16
Crossbred	0.80	0.20	0.00	0.9	0.10	0.32	0.16
Overall	0.80	0.20	0.00	0.90	0.10	0.32	0.16

Chapter 5: Discussion

In beef cattle breeding, a major consideration is being paid to a faster growth rate. QTL mapping and identification of causative genes that affect growth traits will greatly enhance the progress towards this goal (Li et al., 2004). Polymorphisms in candidate genes and their association with economic traits have been performed to ascertain the genetic basis of production traits and to develop marker-assisted selection. This study investigated two candidate genes, *CACNA2D1* and *MYF5,* associated with carcass traits. The exons were amplified and sequenced to study genetic diversity and to detect whether any functional mutation is segregating in the Bangladeshi cattle population.

In this study, a more invasive search was performed for the coding exons of two genes, *CACNA2D1* and *MYF5*, respectively. The research after sequencing was executed in two phases. In the first phase, the genetic diversity of those genes in Bangladeshi cattle was carried out through phylogenetic analysis to improve animal genetics by using genomic research. In the second phase, polymorphism was identified and association with carcass trait was analyzed as a way to use genetic markers for marker-assisted selection to increase the frequency of favorable QTL alleles in target populations.

5.1 *CACNA2D1* gene

5.1.1 Nucleotide sequencing

The sequence of the same region was subjected to close similarity with the genome sequence and assembly of *Bos indicus*. The designated sequences revealed 99% homology with the sequence of *Bos indicus* (NCBI accession no: NC_032653.1) reported by Lowe et al. (1997), which indicates the sequencing procedure and quality is good. So, the indigenous cattle in Bangladesh, RCC and crossbred can assess by these molecular markers associated with meat quality and carcass merit. And this sequence result is perfect for further analysis to identify genetic polymorphisms of the gene associated and to analyze associations between SNPs and carcass and meat quality traits in cattle.

5.1.2 Genetic divergence based on Phylogenetic analysis

The phylogenetic analysis of the *CACNA2D1* gene sequence in indigenous Bangladeshi cattle with the other cattle breeds was based on the seven nucleotide sequences used to construct the phylogenetic tree. The phylogenetic tree indicated that the crossbred of the present study was out-grouped from the other cattle breeds, including the RCC breed. The Angus breed and the Limousin breed were clustered together in a single sub-clade, and both of them showed the same amount of divergence from the common ancestor followed by the Red Chittagong cattle (this study). No phylogenetic analysis was found in different cattle breeds for this gene. One phylogeny was constructed by (Magotra et al., 2017) when a phylogenetic tree was constructed for predicting the evolutionary relationship between different species based on the nucleotide sequence of *CACNA2D1* gene. Both are beef cattle, which indicates we can select RCC as a good beef cattle ancestor.

5.1.3 Alignment analysis

Indels, a collective term that does not distinguish between insertions and deletions, occur during the evolution of a given taxon and therefore carry the evolutionary signal. This study showed a complete deletion where the entire column of nucleotide sequence was deleted when comparison was done with *Bos taurus* and other portions were the same as the reference sequence. This might be because some parts of the gene are highly conserved, which means that relatively few mutations occur within these areas over time. Other parts, known as hyper-variables, are more sensitive to insertion, deletion, and substitution changes.

5.1.4 Polymorphisms in *CACNA2D1* gene

Identification of the candidate genes responsible for variation in quantitative traits has been a challenge in modern genetics. These studies reported an association between variants in two genes and carcass-related traits, which implicates their essential role in the beef industry of Bangladesh.

In the current study, exon 25 of the *CACNA2D1* gene was screened in Bangladesh's indigenous cattle (RCC and crossbred). The *CACNA2D1* gene was polymorphic in this study. Three mutations were detected by PCR and DNA sequencing.

The detected mutations can be beneficial or harmful based on their impacts on the resulting polypeptide (Figures 9 and 10). The cattle *CACNA2D1* gene has been mapped to BTA 4q18 (Buitkamp et al., 2003). It is located within the genomic region of several QTLs, and it is a polymorphic gene whose polymorphism has been proven to be significantly associated with carcass weight, dressing percentage, meat percentage, and backfat thickness (Hou et al., 2010). Therefore, the *CACNA2D1* gene is considered to be one of the potential candidate genes influencing carcass and meat quality traits. The QTLs recorded in several studies were average daily gain (ADG) and carcass weight (CWT) (Casas et al., 2001), meat-to-bone ratio (MTBR) and bone percentage (BONEP) (Gutierrez-Gil et al., 2009), residual feed intake (RFI) (Sherman et al., 2009), somatic cell score (SCS) (Zhang et al., 2007).

Also, four QTLs have been found near the area of the *CACNA2D1* gene (http://www.animalgenome.org/cgibin/QTLdb/BT/draw_chromap?optqtl=&chromos=4&orderqtl=QTL_symbol&scale=4&density=10&submit=GO). Those QTLs are for somatic cell count (SCC) (Longeri et. al., 2006), longissimus muscle area (LMA) (Takasuga et al., 2007), marbling score (MARBL) (Yokouchi, et al., 2009), feed conversion ratio (FCR) (Sherman et al., 2009).

Compared with reference coding sequence data of the *CACNA2D1* gene, two SNPs were identified in exon 25 of the *CACNA2D1* gene, viz. c.1993C>A, c.2058T>A, and c.2068C>A predicted to cause an amino acid change. Two SNPs, viz. c.1993C>A and c.2068C>A were missense mutations. The c.1993C>A substitution results in an amino acid change (leucine to isoleucine) at position 658 (p.Leu658Ile). The c.2068C>A causes phenylalanine to threonine substitution at position 683 (p. Phe683Thr). These two mutations were not reported earlier. These novel polymorphisms can help identify the region of the indigenous cattle breeds of Bangladesh. There are many novel mutations found in the *CACNA2D1* gene. A526745G mutation was reported on Hou et al., (2010). Non-synonymous mutation of SNP C526740G, replace Cysteine (Cys) to Tryptophan (Trp) and SNP G537917T ensuing Aspartic (Asp) to Tyrosine (Tyr) amino acid reported by Yuan et al., (2011).

Previous research studies on the cattle *CACNA2D1* gene indicated this could be considered as a potential candidate gene influencing carcass, meat quality, growth, and developmental traits. In the reference paper (Yuan et al., 2011) from which we selected

the primer, the A526745G mutation was significantly associated with carcass weight, dressing percentage, meat percentage, and backfat thickness. In this preliminary research on polymorphism of the *CACNA2D1* gene exon 25 in cattle revealed three new SNPs that might be useful as genetic markers. Further association studies are needed for association with carcass data.

Results from this study indicate that the *CACNA2D1* gene has potential effects on carcass and meat quality traits, opening up possibilities for cattle breeding and improvement in gene-assisted selection. Conserved domains and protein structures provide useful information for analyzing the evolution and species relationships.

5.1.5 Impact of mutations in *CACNA2D1* gene

According to the central dogma theory, DNA makes RNA and RNA make protein. DNA mutations can cause changes in the structure of an encoded protein and a reduction or complete loss of its expression. Because a change in the DNA sequence affects all copies of the encoded protein, it might change the protein's three-dimensional structure. A protein's function in an organic system is primarily determined by its structure. Base-pair substitutions may lead to an incorrect amino acid in the corresponding position in the encoded protein, and of these, a large proportion results in altered protein function.

Therefore, the physiological role of leucine is to work with insulin to activate the switch that stimulates muscle protein synthesis when amino acids and energy from food become available. (Garlick, 2005) report that leucine level must be sufficiently high to play the signaling and metabolic roles. Leucine activates the signaling factor of the mammalian target of rapamycin (mTOR) signaling to promote protein synthesis in skeletal muscle and adipose tissue (Li et al., 2011). Besides, leucine regulates blood glucose levels by promoting gluconeogenesis and aids in the retention of lean mass in a hypocaloric state. It is beneficial to animal nutrition and clinical application (Li et al., 2011). The remainder portion of leucine after protein synthesis is converted to α-ketoisocaproate (α-KIC) and β-hydroxy-β-methyl butyrate (HMB) in skeletal muscle, which also inhibits protein degradation and regulates energy homeostasis (Duan, 2016).

Isoleucine (I), as a branched-chain amino acid (BCAA), has a vital role in regulating body weight and muscle protein synthesis (Liu et al., 2021). Isoleucine (I), as a branched-chain amino acid (BCAA), has a vital role in regulating body weight and

muscle protein synthesis. Some previous studies on mice show that isoleucine significantly increases muscle and fat mass, causing insulin resistance and upregulating the essential adipogenic and myogenic proteins (Liu et al., 2021). The primary proven function from the previous study is to develop the immune system, including immune organs, cells and reactive substances (Gu et al., 2019).

The SNP C1993A resulted in a missense mutation leading to a Leu (L) 658 Ile (I) amino acid substitution. This mutation has a significant impact on the 1993 bp coding region of the CACNA2D1 peptide. It is predicted to cause possible damage due to the less significant effect of isoleucine in protein synthesis. This detrimental effect may cause a reduction in muscle mass along with body weight. As a result, the absence of this mutation in the RCC population has a positive, and carcass quality in the RCC may be higher than in crossbred.

The amino acid substitution in *CACNA2D1* at the 683 position was predicted to have a mild phenotypic effect (with a score of 0.013) in the Polyphen2 analysis. The g.2068C>A mutation was deduced to cause a moderate functional change in the CACNA2D1 protein and resulted in phenylalanine to threonine. Phenylalanine may promote intestinal development and increase intestinal digestive enzyme activity in calves, thereby promoting the growth and development of calves (Cao et al., 2019). On the other hand, threonine imbalance reduces skeletal muscle growth in young animals, but the underlying mechanism is unclear (Wang et al., 2007). Therefore, the mutation may not cause any significant change in carcass-related traits in cattle.

5.2 *MYF5* gene

5.2.1 Nucleotide sequencing

The sequence of the *MYF5* gene was subjected to BLAST analysis using *Bos indicus* sequences as the reference. The designated sequences revealed 99% identical bases (245/247) with the sequence of *MYF5* myogenic factor 5 *Bos indicus* (zebu cattle), Gene ID: 10955894, NCBI accession no: EF197851.1) reported by Zhong et. al., (2006). This finding indicates that the sequence procedure and quality are suitable (2 gaps only). So assessment of the indigenous breed of cattle in Bangladesh, the RCC and crossbred for this trait of meat quality and carcass merit is possible.

Moreover, this sequence result is perfect for further analysis to identify genetic polymorphisms in this gene and analyze associations between SNPs and carcass and meat quality traits in cattle.

5.2.2 Genetic divergence based on Phylogenetic analysis

Nucleotide sequences of the *MYF5* gene of crossbred cattle and RCC were used in this study, and four published sequences of cattle breed, one buffalo breed and one sheep breed *MYF5* gene from the NCBI database were used to construct the phylogenetic tree (Figure 15). The present result showed the *MYF5* gene was not phylogenetically diverse in crossbred cattle and the RCC. Both genotypes in the present study clustered into a common cluster with *Bos indicus*. Moreover, the *MYF5* gene was shown to be phylogenetically diverse among the other species. Similar pattern was observed by (Zhao et al., 2020) where *Bubalus bubalis* and *Ovis aries* were also more closely related and away from the bovine species.

5.2.3 Alignment analysis

Alignment analysis did not show any insertions or deletions. This might be because some parts of the gene are highly conserved, which means that relatively few mutations occur within these areas over a period. One deletion was found in the *Bos indicus* reference sequence. All samples of RCC and crossbred along with the *Bos taurus* reference sequence show similarity, indicating strong preservation of genetic material with no recombination in the target region of the *MYF5* gene.

5.2.4 Polymorphism in *MYF5* gene

One SNP was identified in the non-coding part of the *MYF5* gene c.1795C>T, an intronic mutation that does not cause an amino acid substitution. The sequence of DNA in between exons is initially copied into RNA but which part is cut out of the final RNA transcript, therefore, does not change the amino acid code. Some intronic sequences are known to affect gene expression. At present, the function of introns is less well-understood than that of exons. On average, introns are much larger than exons. They may harbour promoters or other regulatory modules that modify gene expression and might influence the penetrance and expressivity of phenotypes. This may play a significant role in modifying gene expression patterns (Pagani et al., 2004). Thus, it may be that, in some cases, intron variations contribute to phenotypic heterogeneity.

The association results between c.1795C>T SNP genotypes of the *MYF5* gene and growth traits are not consistent with previous investigations. Several studies reveal that g.1911A>G SNP polymorphism had a significant association with the average daily gain (ADG) in Canadian beef cattle (Li et al., 2004).Average daily gain (ADG) and yearling weight in Hanwoo (Chung and Kim, 2005) and growth related traits in Chinese cattle (Zhang et al., 2007). Based on the QTL studies and functions, the *MYF5* gene was considered as a positional candidate gene in the chromosomal region at 0 to 30 cm. More preciously it has been mapped at 19 cM on BTA5 (Grosse et al., 1999).

5.2.5 Impact of mutations in *MYF5* gene

As discussed in review section, member of the *MYOD* gene family have potential roles in muscle development in cattle. The significantly associated SNPs c.1795C>T of the *MYF5* gene located in the intron regions suggest that they are not causative mutations and can be closely linked to other mutations in the coding or regulatory regions of the genes. Recent investigations revealed that SNPs in the intron region had significant associations with carcass and meat quality traits (Di et al., 2005; Sherman et al., 2008) in cattle. Introns have also been shown to affect mRNA stability or transcriptional efficiency and thus exert specific biological effects on genes (Le et al., 2003).

Basically, *MYF5* gene is involved in muscle development from commitment and proliferation through muscle fiber formation during embryonic development to their postnatal maturation and function (Te et al., 1999). These genes are considered as candidate genes for meat production traits due to their potential roles in muscle fiber development (Wyszynska-Koko et al., 2006; Verner et al., 2007).

Overall, By using previously studied candidate genes and genetic markers *CACNA2D1* and *MYF5* which are linked with the trait of interest carcass quality, it is possible to perform selection of young animals at an early stage of breeding and choose the best animals for crossing, allowing for much faster improvement of the selected trait than standard methods. By speeding breeding development, such a strategy can increase the financial benefits of animal production. Access to candidate genes may provide a more comprehensive knowledge of the genetic basis for expressing quantitative differences across people (Noguera et al., 2003).

The main objective of this study was to identify polymorphisms of the *CACNA2D1* and *MYF5* gene associated with carcass weight, dressing percentage, meat percentage, and backfat thickness (Hou, 2010) in cattle population and make association further and here detected three new polymorphisms which may have a detrimental effect on this cattle population which was discussed earlier. This preliminary research on polymorphism of the *CACNA2D1*gene exon 25 in cattle revealed C1993A, T2058A, C2068A might be helpful as a genetic marker in Crossbred and RCC cattle. The absence of this mutation in the RCC population may have a positive effect, and carcass quality may be better in RCC than crossbred as those mutations may negatively impact the cattle population.

In the case of the *MYF5* gene, the target was to check the presence of a previously identified polymorphism at position 1553, which was transverse mutation A to T in exon 2 ANS significantly associated with live weight, loin eye height, loin eye area and water holding capacity (Ujan et al., 2011). However, we have found the mutation at the 1795C>T position, which is intronic, does not cause an amino acid substitution, but further association studies are needed to determine the effect. Further association of results with carcass data along with another suitable polymorphism detection is necessary. Results from this study indicate that the *CACNA2D1* gene has potential effects on carcass and meat quality traits, opening up possibilities for cattle breeding and improvement in gene-assisted selection.

Conclusion

This study is one of the first works to create a foundation for future genetic studies to assess carcasses in indigenous cattle of Bangladesh. This study preliminarily suggested that the *CACNA2D1 and MYF5* gene sequences in RCC and crossbred cattle are polymorphic. This study explored three SNPs in exon 25 of *CACNA2D1*, exon 2 of *MYF5*. The association of these SNPs of *CACNA2*D1 and *MYF5* genes was also investigated and the impact on the protein was detected for significant change due to these mutations. However, the effect of SNPs of *CACNA2*D1 was significant (polyphen2 score nearer one) on carcass traits in these indigenous cattle. Heterozygous mutant (CA) at C1993A loci and C2068A loci in the *CACNA2D1* gene recorded with a significantly related trait in this candidate gene.

On the other hand, T2058A**,** a homozygous type of mutation, may have a less significant impact. Heterozygous mutant CA for the 1795 locus in the *MYF5* gene of the studied cattle population was detected as an intronic type of mutation, which may have a deadly impact. The association between polymorphisms of this study identified in *CACNA2D1* exon 25, *MYF5* exon 2 and carcass traits in indigenous Bangladeshi cattle indicates its perspective in future molecular breeding. However, the present study investigated only a small number of animals, and further investigation is required to confirm the link with carcass associated traits in different cattle breeds of Bangladesh. The genomic selection was developed to improve artificial selection programs by incorporating single nucleotide polymorphism (SNP) data. This genomic selection may improve the rate of genetic gain for traits that are difficult to measure at the phenotypic level. Phenotypic observations do not have to be collected for each animal. Estimated breeding values (EBV) can be calculated for traits recorded in a reference population.

The candidate gene method that used in this study has been demonstrated to be highly powerful for analyzing the genomic structure of quantitative traits, making it a considerably more successful and cost-efficient strategy for direct gene identification. Advances in molecular genetics have enabled the advancement of breeding animal genome mapping.

The likelihood of success in identifying novel genes and markers indicates that their application in selection programs will undoubtedly be amplified and efficient in improving breeding animal quality. In the face of molecular marker developments and applications, we can detect evidence in the breadth of livestock production, product quality via animal selection by genotypic profile, and the sustainability of the animal improvement system. As a result, these markers can anticipate innovations and decisions in animal reproductive programs.

Several novel mutations were identified in the *CACNA2D1* and *MYF5* genes for indigenous cattle of Bangladesh in this study. Substantial differences in allele frequencies were observed among different cattle breeds. The results presented here show significant associations between the *CACNA2D1* and *MYF5* SNPs with growth and carcass traits. These identified SNPs can be used as markers for the selection of animals and can potentially be used for cattle breeding using modern methods such as marker-assisted selection or marker-assisted introduction. In this context, it can conclude that the use of molecular markers in animal production assists the selection effectiveness in breeding programs by predicting and selecting superior animals and consequently contributing to quality in animal production.

Due to time and resource limitations, we conducted the study on a small scale. In the future, the study can be conducted involving a larger sample size. Moreover, there are many genes related to the carcass traits in cattle. To improve carcass traits through marker-assisted selection, it is better to search for more candidate genes in Bangladeshi cattle. Accumulation of database by doing such work can lead to set genomic library for indigenous cattle of Bangladesh

References

Adelson, D. L., Qu, Z., Raison, J.M. and Lim. S.L., 2014. Bovine genomics. In: D. Garrick and A. Ruvinsky, editors, *The genetics of cattle*. CABI, Boston, MA, 130-152.

Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., Kondrashov, A. S. and Sunyaev, S.R., 2010. A method and server for predicting damaging missense mutations. *Nature Methods*, *7*(4), 248-249.

Alam, M.M., Sarder, M.J.U., Ferdousi, Z. and Rahman, M.M., 2008. Productive and reproductive performance of dairy cattle in Char areas of Bangladesh. *The Bangladesh Veterinarian*, 25(2), 68-74.

Allan, M.F., Thallman, R.M., Cushman, R.A., Echternkamp, S.E., White, S.N., Kuehn, L.A., Casas, E. and Smith, T.P.L., 2007. Association of a single nucleotide polymorphism in SPP1 with growth traits and twinning in a cattle population selected for twinning rate. *Journal of Animal Science*, 85(2), 341-347.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J., 1990. Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403-410.

Andersson, L. and Georges, M., 2004. Domestic-animal genomics: deciphering the genetics of complex traits. *Nature Reviews Genetics*, 5(3), 202-212.

Andersson, L., Haley, C.S., Ellegren, H., Knott, S.A., Johansson, M., Andersson, K., Andersson-Eklund, L., Edfors-Lilja, I., Fredholm, M. and Hansson, I., 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science*, 263(5154), 1771-1774.

Antonarakis, S.E. and Cooper, D.N., 2013. Human gene mutation in inherited disease: Molecular mechanisms and clinical consequences.

Ashwell, M.S., Heyen, D.W., Weller, J.I., Ron, M., Sonstegard, T.S., Van Tassell, C.P. and Lewin, H.A., 2005. Detection of quantitative trait loci influencing conformation traits and calving ease in Holstein-Friesian cattle. *Journal of Dairy Science*, 88(11), 4111-4119.

Asian Development Bank, Poverty in Bangladesh. (https://www.adb.org/countries/bangladesh/poverty)

Bathaei, S.S. and Leroy, P.L., 1998. Genetic and phenotypic aspects of the growth curve characteristics in Mehraban Iranian fat-tailed sheep. *Small Ruminant Research*, 29(3), 261-269.

Benyshek, L.L., 1981. Heritabilities for growth and carcass traits estimated from data on Herefords under commercial conditions. *Journal of Animal Science*, 53(1), 49-56.

BER, 2017. Bangladesh Economic Review, Financial system management unit (FSMU), finances division, ministry of finance, government of the people's republic of Bangladesh, 2018. (https://mof.gov.bd)

Bertrand, J.K., Green, R.D., Herring, W.O. and Moser, D.W., 2001. Genetic evaluation for beef carcass traits. *Journal of Animal Science*, 79, E190-E200.

Bhuiyan, A.K.F.H., Hossain, M.M. and Deb, G.K., 2007. Indigenous cattle genetic resources of Bangladesh and a way forward to their development. *Bangladesh Journal of Progressive Science and Technology,* 5(1), 105-112.

Bhuiyan, M.S.A., Kim, N.K., Cho, Y.M., Yoon, D., Kim, K.S., Jeon, J.T. and Lee, J.H., 2009. Identification of SNPs in *MYOD* gene family and their associations with carcass traits in cattle. *Livestock Science*, 126(1-3), 292-297.

Blasco, A. and Gomes, E., 1993. A note on growth curves of rabbit lines selected on growth rate or litter size. *Animal Production*, 57(2), 332-334.

Buitkamp, J., Ewald, D., Masabanda, J., Bishop, M.D. and Fries, R., 2003. FISH and RH mapping of the bovine alpha (2)/delta calcium channel subunit gene (CACNA2D1). *Animal Genetics*, 34(4), 309-310.

Burrow, H.M., Moore, S.S., Johnston, D.J., Barendse, W. and Bindon, B.M., 2001. Quantitative and molecular genetic influences on properties of beef: a review. *Australian Journal of Experimental Agriculture*, 41(7), 893-919.

Cafe, L.M., Robinson, D.L., Ferguson, D.M., McIntyre, B.L., Geesink, G.H. and Greenwood, P.L., 2011. Cattle temperament: persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. *Journal of Animal Science*, 89(5), 1452-1465.

Cao, Y., Liu, S., Yang, X., Guo, L., Cai, C. and Yao, J., 2019. Effects of dietary leucine and phenylalanine on gastrointestinal development and small intestinal enzyme activities in milk-fed holstein dairy calves. *Bioscience Reports*, 39(1), BSR20181733.

Carvalho, M.E., Baldi, F.S., Alexandre, P.A., Santana, M.H.D.A., Ventura, R.V., Bueno, R.S., Bonin, M.N., Rezende, F.M., Coutinho, L.L., Eler, J.P. and Ferraz, J.B.S., 2019. Genomic regions and genes associated with carcass quality in Nelore cattle. *Genetics and Molecular Research*, 18(1), 1-15.

Casas, E., Stone, R.T., Keele, J.W., Shackelford, S.D., Kappes, S.M. and Koohmaraie, M., 2001. A comprehensive search for quantitative trait loci affecting growth and carcass composition of cattle segregating alternative forms of the myostatin gene. *Journal of Animal Science*, 79(4), 854-860.

Chung, E.R. and Kim, W.T., 2005. Association of SNP marker in IGF-I and *MYF5* candidate genes with growth traits in Korean cattle. *Asian-Australasian Journal of Animal Sciences*, 18(8), 1061-1065.

Da Silva, R.C.G., Ferraz, J.B.S., Meirelles, F.V., Eler, J.P., Balieiro, J.C.C., Cucco, D.C., Mattos, E.C., Rezende, F.M. and Silva, S.L., 2012. Association of single nucleotide polymorphisms in the bovine leptin and leptin receptor genes with growth and ultrasound carcass traits in Nellore cattle. *Genetics and Molecular Research*, 11(4), 3721-3728.

Daetwyler, H.D., Capitan, A., Pausch, H., Stothard, P., Van Binsbergen, R., Brøndum, R.F., Liao, X., Djari, A., Rodriguez, S.C., Grohs, C. and Esquerre, D., 2014. Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. *Nature Genetics*, 46(8), 858-865.

Dekkers, J.C., Hospital F., 2002. Utilization of molecular genetics in genetic improvement of plants and animals. *Nature Reviews: Genetics*, 3, 22-32.

Di Stasio, L., Destefanis, G., Brugiapaglia, A., Albera, A. and Rolando, A., 2005. Polymorphism of the GHR gene in cattle and relationships with meat production and quality. *Animal Genetics*, 36(2), 138-140.

DLS, 2015. Annual report on livestock. Division of Livestock Statistics, Ministry of Fisheries and Livestock, Farmgate, Dhaka, Bangladesh.

DLS. (2017-18). Department of livestock services. Livestock Economy at a Glance. Dhaka, Bangladesh.

Duan, Y., Li, F., Li, Y., Tang, Y., Kong, X., Feng, Z., Anthony, T.G., Watford, M., Hou, Y., Wu, G. and Yin, Y., 2016. The role of leucine and its metabolites in protein and energy metabolism. *Amino Acids*, 48(1), 41-51.

Dutta, A.K., Pradhan, P., Roy, A. and Acharya, K., 2011. A subtropical agaric new to India. *Kavaka*, *39, 37*-39.

FAO | Animal production | Food and Agriculture Organization, 2021. (http://www.fao.org/animal-production/en/)

FAO, 2004. State of the World (SoW) animal genetic resources. Food and Agriculture Organization (FAO), Ministry of Fisheries and Livestock, Government of the Peoples Republic of Bangladesh, 65.

Felsenstein, J., 1985. Phylogenies and the comparative method. *The American Naturalist*, 125(1), 1-15.

Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V.K., Weiler, J.E., O'Brien, P.J. and MacLennan, D.H., 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science*, 253(5018), 448-451.

Garlick, P.J., 2005. The role of leucine in the regulation of protein metabolism. *The Journal of Nutrition*, 135(6), 1553S-1556S.

Ge, W., Davis, M.E., Hines, H.C., Irvin, K.M. and Simmen, R.C., 2001. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. *Journal of Animal Science*, 79(7), 1757-1762.

Gene Testing for Production and Carcass Traits: | UNL Beef (https://beef.unl.edu/beefreports/symp-2007-19-xx)

Gill, J.L., Bishop, S.C., McCorquodale, C., Williams, J.L. and Wiener, P., 2009. Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle. *Genetics Selection Evolution*, 41(1), 1-12.

Goddard, M.E., 1996. The use of marker haplotypes in animal breeding schemes. *Genetics Selection Evolution,* 28(2), 161-176.

Goddard, M. E., and Hayes, B. J. 2007. Genomic selection. *Journal of Animal Breeding and Genetics*, 124, 323-330.

Grisart, B., Coppieters, W., Farnir, F., Karim, L., Ford, C., Berzi, P., Cambisano, N., Mni, M., Reid, S., Simon, P. and Spelman, R., 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Research*, 12(2), 222-231.

Grosse, W.M., Kappes, S.M., Laegreid, W.W., Keele, J.W., Chitko-McKown, C.G. and Heaton, M.P., 1999. Single nucleotide polymorphism (SNP) discovery and linkage mapping of bovine cytokine genes. *Mammalian Genome*, 10(11), 1062-1069.

Gu, C., Mao, X., Chen, D., Yu, B. and Yang, Q., 2019. Isoleucine plays an important role for maintaining immune function. *Current Protein and Peptide Science,* 20(7), 644-651.

Gutiérrez-Gil, B., Williams, J.L., Homer, D., Burton, D., Haley, C.S. and Wiener, P., 2009. Search for quantitative trait loci affecting growth and carcass traits in a cross population of beef and dairy cattle. *Journal of Animal Science*, 87(1), 24-36.

Habib, M.A., Bhuiyan, A.K.F.H., Bhuiyan, M.S.A. and Khan, A.A., 2003. The red chittagong cattle: An animal genetic resource of Bangladesh. *Bangladesh Journal of Animal Science*, 32, 101-108.

Hamid, M., Rahman, A., Zaman, M.A. and Hossain, K.M., 2017. Cattle genetic resources and their conservation in Bangladesh. *Asian Journal of Animal Sciences*, 11, 54-64.

Hasnath, M.A., 1974. Studies on crossbred cattle. *Research Bulletin*, 1, 1-24.

Hillel, J., Schaap, T., Haberfeld, A., Jeffreys, A.J., Plotzky, Y., Cahaner, A. and Lavi, U., 1990. DNA fingerprints applied to gene introgression in breeding programs. *Genetics*, 124(3), 783-789.

Hossain, M.M., Bhuiyan, M.S.A., Bhuiyan, A.K.F.H. and Hanotte, O., 2005, November. Red Chittagong-indigenous cattle genetic resource with promise in Bangladesh. *Proceedings of the AHAT/BSAS International Conference*, 2, 82-83.

Hou, G.Y., Yuan, Z.R., Gao, X., Li, J.Y., Gao, H.J., Chen, J.B. and Xu, S.Z., 2010. Genetic polymorphisms of the *CACNA2D1* gene and their association with carcass and meat quality traits in cattle. *Biochemical Genetics*, *48*(9-10), 751-759.

Huffman, K.L., Miller, M.F., Hoover, L.C., Wu, C.K., Brittin, H.C. and Ramsey, C.B., 1996. Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. *Journal of Animal Science*, 74(1), 91-97.

Hunt, M.G., Moshier, S. and Milonova, M., 2009. Brief cognitive-behavioral internet therapy for irritable bowel syndrome. *Behaviour Research and Therapy*, 47(9), 797-802.

Ibeagha-Awemu, E.M., Lee, J.W., Ibeagha, A.E., Bannerman, D.D., Paape, M.J. and Zhao, X., 2008. Bacterial lipopolysaccharide induces increased expression of

toll-like receptor (TLR) 4 and downstream TLR signaling molecules in bovine mammary epithelial cells. *Veterinary Research*, 39(2), 1-12.

ILRI, 2004. Status, Trends, Utilization and Performance of FAnGR of Bangladesh, Consultancy Report of GEF-UNEP-ILRI FAnGR Asia Project, Kenya, 49.

Jamuar, S.S., Kuan, J.L., Brett, M., Tiang, Z., Tan, W.L.W., Lim, J.Y., Liew, W.K.M., Javed, A., Liew, W.K., Law, H.Y. and Tan, E.S., 2016. Incidentalome from genomic sequencing: a barrier to personalized medicine. *EBioMedicine*, 5, 211-216.

Johnson, K.J., Zecevic, A. and Kwon, E.J., 2004. Protocadherin α3 acts at sites distinct from classic cadherins in rat testis and sperm. *Biology of Reproduction*, 70(2), 303-312.

Júnior, G.F., Costa, R.B., De Camargo, G.M.F., Carvalheiro, R., Rosa, G.J.M., Baldi, F., Garcia, D.A., Gordo, D.G.M., Espigolan, R., Takada, L. and Magalhães, A.F.B., 2016. Genome scan for postmortem carcass traits in Nellore cattle. *Journal of Animal Science*, 94(10), 4087-4095.

Kaspric, N., Picard, B., Reichstadt, M., Tournayre, J. and Bonnet, M., 2014, April. Protein function easily investigated by genomics data mining using the ProteINSIDE web service. In 2. *International Work-Conference on Bioinformatics and Biomedical Engineering (IWBBIO)*.

Khan, M.K.I., Huque, K.S., Miah, A.G. and Khatun, M.J., 2000. Study on the performance of Red Chittagong cow under different production system. *Pakistan Journal of Biological Science*, 3(2), 318-319.

Khan M K I, Miah G, Khatun M J, Das A. 2012. Economic and genetic evaluations of different dairy cattle breed under rural conditions of Bangladesh. *Livestock Research for Rural Development*, 24 (1), 1-8.

Klug, F., Prakash, H., Huber, P.E., Seibel, T., Bender, N., Halama, N., Pfirschke, C., Voss, R.H., Timke, C., Umansky, L. and Klapproth, K., 2013. Low-dose irradiation programs macrophage differentiation to an iNOS+/M1 phenotype that orchestrates effective T cell immunotherapy. *Cancer Cell*, 24(5), 589-602.

Kwon, J.M. and Goate, A.M., 2000. The candidate gene approach. *Alcohol Research and Health*, 24(3), 164.

Le H., Nott, A. and Moore, M.J., 2003. How introns influence and enhance eukaryotic gene expression. *Trends in Biochemical Sciences*, 28(4), 215-220.

Li, C., Basarab, J., Snelling, W.M., Benkel, B., Murdoch, B. and Moore, S.S., 2002. The identification of common haplotypes on bovine chromosome 5 within commercial lines of Bos taurus and their associations with growth traits. *Journal of Animal Science*, 80(5), 1187-1194.

Li, C., Basarab, J., Snelling, W.M., Benkel, B., Murdoch, B., Hansen, C. and Moore, S.S., 2004. Assessment of positional candidate genes *MYF5* and *IGF1* for growth on bovine chromosome 5 in commercial lines of Bos taurus. *Journal of Animal Science*, 82(1), 1-7.

Li, C., Basarab, J., Snelling, W.M., Benkel, B., Murdoch, B., Kneeland, J., Hansen, C. and Moore, S.S., 2002. Identical by descent haplotype sharing analysis: application in fine mapping of QTLs for birth weight in commercial lines of *Bos taurus*. In Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France, 29, 481-484.

Li, F., Yin, Y., Tan, B., Kong, X. and Wu, G., 2011. Leucine nutrition in animals and humans: mTOR signaling and beyond. *Amino acids*, 41(5), 1185-1193.

Li, J.H., Chu, X.H., Guo, X.L. and Xu, N.Y., 2005. Radiation hybrid mapping of skeletal muscle calcium channel genes *CACNB1* and *CACNG1* to porcine chromosome 12 and *CACNA2D1* to porcine chromosome 9. *Animal Genetics,* 36(4), 358-359.

Li, J.H., Yang, M.S. and Xu, N.Y., 2007. Single nucleotide polymorphism in 30 UTR of porcine *CACNA2D1* gene and its genetic effect on meat quality trait. *Acta Agric Boreali Sin*, 22, 76-79.

Li, J.H., Yang, M.S. and Xu, N.Y., 2008. Mapping of porcine *CACNA2D1* gene and the integrative analysis of correlative genetic maps. *Journal of Northwest University (Natural Science Edition)*, 36, 49–53

Liu, S., Sun, Y., Zhao, R., Wang, Y., Zhang, W. and Pang, W., 2021. Isoleucine increases muscle mass through promoting myogenesis and intramyocellular fat deposition. *Food & Function*, 12(1), 144-153.

Loke, J. and MacLennan, D.H., 1998. Malignant Hyperthermia and Central Core Disease: Disorders of Ca2 Release Channels1. *The American Journal of Medicine*, 104(5), 470-486.

Longeri, M., Polli, M., Strillacci, M.G., Samore, A.B. and Zanotti, M., 2006. Quantitative trait loci affecting the somatic cell score on chromosomes 4 and 26 in Italian Holstein cattle. *Journal of Dairy Science*, 89(8), 3175-3177.

Lowe, T.M. and Eddy, S.R., 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research*, 25(5), 955-964.

Maak, S., Neumann, K. and Swalve, H.H., 2006. Identification and analysis of putative regulatory sequences for the *MYF5/MYF6* locus in different vertebrate species. *Gene*, 379, 141-147.

MacNeil, M.D. and Grosz, M.D., 2002. Genome-wide scans for QTL affecting carcass traits in Hereford× composite double backcross populations. *Journal of Animal Science*, 80(9), 2316-2324.

Madden, T., 2013. The BLAST sequence analysis tool. *The NCBI Handbook*, 2, 425-436.

Magotra, A., Gupta, I.D., Verma, A., Chaudhari, M.V., Arya, A., Vineeth, M.R., Kumar, R. and Selvan, A.S., 2017. Characterization and validation of point mutation in exon 19 of *CACNA2D1* gene in Karan Fries (*Bos taurus* x *Bos indicus*) cattle. *Indian Journal of Animal Research*, 51(2), 227-230.

Malhotra, A.K. and Goldman, D., 1999. Benefits and pitfalls encountered in psychiatric genetic association studies. *Biological Psychiatry*, 45(5), 544-550.

Malhotra, A.K. and Goldman, D., 1999. Benefits and pitfalls encountered in psychiatric genetic association studies. *Biological Psychiatry*, 45(5), 544-550.

Maltin, C., Balcerzak, D., Tilley, R. and Delday, M., 2003. Determinants of meat quality: tenderness. *Proceedings of the Nutrition Society*, 62(2), 337-347.

Marshall, D.M., 1999. Genetics of meat quality. *The Genetics of Cattle,* 605-636.

Mason, I. L. 1998. A world dictionary of livestock breeds, types and varieties. Third edition, CAB, Wallingford, 245-251.

Miles, C. and Wayne, M., 2008. Quantitative trait locus (QTL) analysis. *Nature Education* 1 (1), 208.

Mizoshita, K., Watanabe, T., Hayashi, H., Kubota, C., Yamakuchi, H., Todoroki, J. and Sugimoto, Y., 2004. Quantitative trait loci analysis for growth and carcass traits in a half-sib family of purebred Japanese Black (Wagyu) cattle. *Journal of Animal Science*, 82(12), 3415-3420.

Moon, S.S., Hwang, I.H., Jin, S.K., Lee, J.G., Joo, S.T. and Park, G.B., 2003. Carcass traits determining quality and yield grades of Hanwoo steers. *Asian-Australasian Journal of Animal Sciences*, 16(7), 1049-1054.

Mullenbach, R., 1989. An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends Genetics*, *5*, 391.

NAGRP, 2010. A snapshot of QTL on cattle chromosome 4. Cattle QTLdb, NAGRP, *Bioinformatics Coordination Program*. (http://www.animalgenome.org/cgi-bin/QTLdb)

Nei, M. and Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press.

Noguera, J.L., Varona, L., Gomez-Raya, L., Sanchez, A., Babot, D., Estany, J., Messer, L.A., Rothschild, M. and Perez-Enciso, M., 2003. Estrogen receptor polymorphism in Landrace pigs and its association with litter size performance. *Livestock Production Science*, 82(1), 53-59.

Pagani, F. and Baralle, F.E., 2004. Genomic variants in exons and introns: identifying the splicing spoilers. *Nature Reviews Genetics*, 5(5), 389-396.

Pereira, G.L., Regatieri, I.C., Ferraz, G.D.C., Neto, A.D.Q. and Curi, R.A., 2015. Perspectivas do uso de marcadores moleculares no melhoramento genético de equinos de corrida da raça Quarto de Milha. *Veterinária e Zootecnia*, 22(3), 347-369.

Rehfeldt, C. and Kuhn, G., 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. *Journal of Animal Science*, *84*(suppl_13), E113-E123.

Rehfeldt, C., Fiedler, I., Dietl, G. and Ender, K., 2000. Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. *Livestock Production Science*, 66(2), 177-188.

Robinson, R.L., Curran, J.L., Ellis, F.R., Halsall, P.J., Hall, W.J., Hopkins, P.M., Iles, D.E., West, S.P. and Shaw, M.A., 2000. Multiple interacting gene products may influence susceptibility to malignant hyperthermia. *Annals of Human Genetics*, 64(4), 307-320.

Rupp, R. and Boichard, D., 2003. Genetics of resistance to mastitis in dairy cattle. *Veterinary Research*, 34(5), 671-688.

Saitou, N. and Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4), 406-425.

Sargolzaei, M., Schenkel, F.S., Jansen, G.B. and Schaeffer, L.R., 2008. Extent of linkage disequilibrium in Holstein cattle in North America. *Journal of Dairy Science*, 91(5), 2106-2117.

Schmidt, H. and Westheide, W., 2000. Are the meiofaunal polychaetes Hesionides arenaria and Stygocapitella subterranea true cosmopolitan species? Results of RAPD-PCR investigations. *Zoologica Scripta*, 29(1), 17-27.

Shen, W., Nada, K. and Tachibana, S., 1999. Effect of cold treatment on enzymic and nonenzymic antioxidant activities in leaves of chilling-tolerant and chilling-sensitive cucumber (*Cucumis sativus* L.) cultivars. *Journal of the Japanese Society for Horticultural Science*, 68(5), 967-973.

Sherman, E.L., Nkrumah, J.D., Li, C., Bartusiak, R., Murdoch, B. and Moore, S.S., 2009. Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. *Journal of Animal Science*, 87(1), 37-45.

Sherman, E.L., Nkrumah, J.D., Murdoch, B.M., Li, C., Wang, Z., Fu, A. and Moore, S.S., 2008. Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. *Journal of Animal Science*, 86(1), 1-16.

Shin, S.C. and Chung, E.R., 2007. Association of SNP marker in the leptin gene with carcass and meat quality traits in Korean cattle. *Asian-australasian Journal of Animal Sciences*, 20(1), 1-6.

Silva, M.V., Lopes, P.S., Guimarães, S.E. and Torres, R.A., 2003. Utilização de marcadores genéticos em suínos. II. Características de desempenho e de qualidade da carne. *Archivos Lationamericanos de Produccion Animal*, 11(1), 11-20.

Stone, R.T., Casas, E., Smith, T.P.L., Keele, J.W., Harhay, G., Bennett, G.L., Koohmaraie, M., Wheeler, T.L., Shackelford, S.D. and Snelling, W.M., 2005. Identification of genetic markers for fat deposition and meat tenderness on bovine chromosome 5: development of a low-density single nucleotide polymorphism map. *Journal of Animal Science*, 83(10), 2280-2288.

Takasuga, A., Watanabe, T., Mizoguchi, Y., Hirano, T., Ihara, N., Takano, A., Yokouchi, K., Fujikawa, A., Chiba, K., Kobayashi, N. and Tatsuda, K., 2007. Identification of bovine QTL for growth and carcass traits in Japanese Black cattle by replication and identical-by-descent mapping. *Mammalian Genome*, 18(2), 125-136.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28(10), 2731-2739.

Tarrant, P.V., 1998. Some recent advances and future priorities in research for the meat industry. *Meat Science*, 49, S1-S16.

Te P.M.F.W., Soumillion, A., Harders, F.L., Verburg, F.J., Van den Bosch, T.J., Galesloot, P. and Meuwissen, T.H.E., 1999. Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs. *Journal of Animal Science*, 77(9), 2352-2356.

Teneva, A. and Petrović, M.P., 2010. Application of molecular markers in livestock improvement. *Biotechnology in Animal Husbandry*, 26(3-4), 135-154.

The Daily Star, Aug 14, 2018
(https://www.thedailystar.net/news/lifestyle/spotlight/cattle-tales-1620187)

Thompson, J.D., Higgins, D.G. and Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research*, 22(22), 4673-4680.

Ujan, J.A., Zan, L.S., Ujan, S.A. and Wang, H.B., 2011. Association between polymorphism of *MYF5* gene with meat quality traits in indigenous Chinese cattle breeds. In International Conference on Asia Agriculture and Animal, IPCBEE, 13, 50-55.

UNIDO-The dairy and beef value chain in Bangladesh, 2019.
(https://www.unido.org/sites/default/files/files/2019-05/Bangladesh%20dairy%20and%20beef%20vc%20report%20%28Wei%27s%20final%20version%29%20.pdf)

USDA-Beef Quality and Yield Grades, 1989.
(https://meat.tamu.edu/beefgrading/)

Van Eenennaam, A.L., Weaber, R.L., Drake, D.J., Penedo, M.C.T., Quaas, R.L., Garrick, D.J. and Pollak, E.J., 2007. DNA-based paternity analysis and genetic evaluation in a large, commercial cattle ranch setting. *Journal of Animal Science*, *85*(12), 3159-3169.

Verner, J., Humpolíček, P. and Knoll, A., 2007. Impact of *MYOD* family genes on pork traits in Large White and Landrace pigs. *Journal of Animal Breeding and Genetics*, *124*(2), 81-85.

Vignal, A., Milan, D., SanCristobal, M. and Eggen, A., 2002. A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution*, 34(3), 275-305.

Wang, X.U., Qiao, S., Yin, Y., Yue, L., Wang, Z. and Wu, G., 2007. A deficiency or excess of dietary threonine reduces protein synthesis in jejunum and skeletal muscle of young pigs. *The Journal of Nutrition*, 137(6), 1442-1446.

Weaber, R.L.B., 2007, December. Gene-Testing for Production and Carcass Traits: What Does it Mean to a Rancher? *Range Beef Cow Symposium*, 19.

Williams, J.L., 2008. Genetic control of meat quality traits. *Meat Biotechnology*, 21-60.

World Population Review (2019), Bangladesh Population 2019. (http://worldpopulationreview.com/countries/bangladeshpopulation/)

Wyszyńska-Koko, J., Pierzchała, M., Flisikowski, K., Kamyczek, M., Różycki, M. and Kurył, J., 2006. Polymorphisms in coding and regulatory regions of the porcine *MYF6* and *MYOG* genes and expression of the *MYF6* gene in m. longissimus dorsi versus productive traits in pigs. *Journal of Applied Genetics*, 47(2), 131-138.

Yokouchi, K., Mizoguchi, Y., Watanabe, T., Iwamoto, E., Sugimoto, Y. and Takasuga, A., 2009. Identification of a 3.7-Mb region for a marbling QTL on bovine chromosome 4 by identical-by-descent and association analysis. *Animal Genetics*, 40(6), 945-951.

Yuan, Z.R. and Xu, S.Z., 2011. Novel SNPs of the bovine *CACNA2D1* gene and their association with carcass and meat quality traits. *Molecular Biology Reports*, 38(1), 365-370.

Zhang, Q., Boichard, D., Hoeschele, I., Ernst, C., Eggen, A., Murkve, B., Pfister-Genskow, M., Witte, L.A., Grignola, F.E., Uimari, P. and Thaller, G., 1998. Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. *Genetics*, 149(4), 1959-1973.

Zhang, R.F., Chen, H., Lei, C.Z., Zhang, C.L., Lan, X.Y., Zhang, Y.D., Zhang, H.J., Bao, B., Niu, H. and Wang, X.Z., 2007. Association between polymorphisms of *MSTN* and *MYF5* genes and growth traits in three Chinese cattle breeds. *Asian-Australasian Journal of Animal Sciences*, 20(12), 1798-1804.

Zhao, C., Raza, S.H.A., Khan, R., Sabek, A., Khan, S., Ullah, I., Memon, S., Abd El-Aziz, A.H., Shah, M.A., Shijun, L. and Wang, L., 2020. Genetic variants in MYF5 affected growth traits and beef quality traits in Chinese Qinchuan cattle. *Genomics*, 112(4), 2804-2812.

Zhong, J., Chen, Z., Zhao, S. and Xiao, Y., 2006. Classification of ecological types of the Chinese yak. *Acta Ecologica Sinica*, 26(7), 2068-2072.

Appendix-I

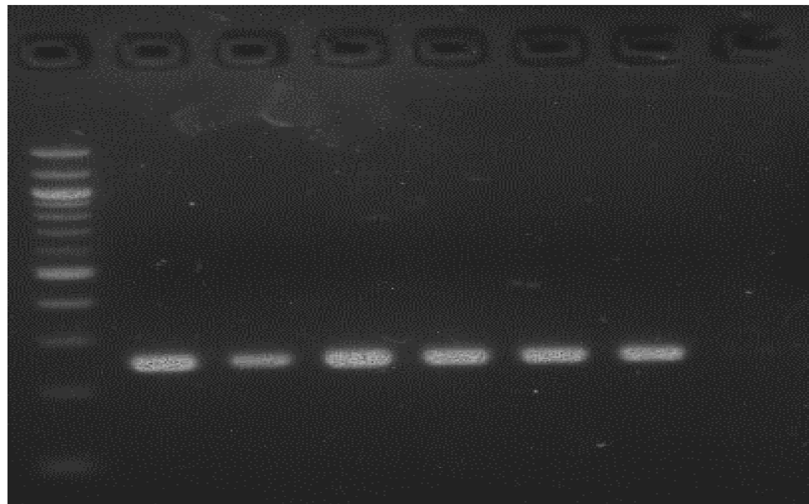


Figure: Gel electrophoresis image of *CACNA2D1* gene (249bp)

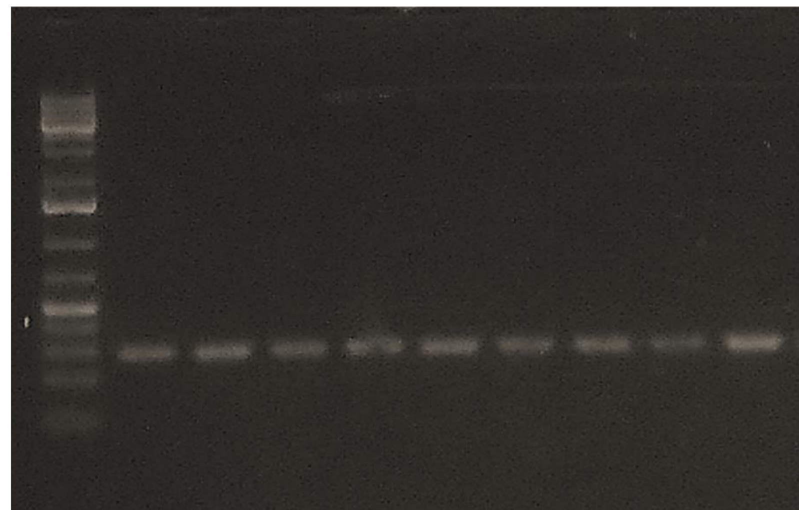


Figure: Gel electrophoresis image of *MYF5* gene (283bp)

Appendix-II

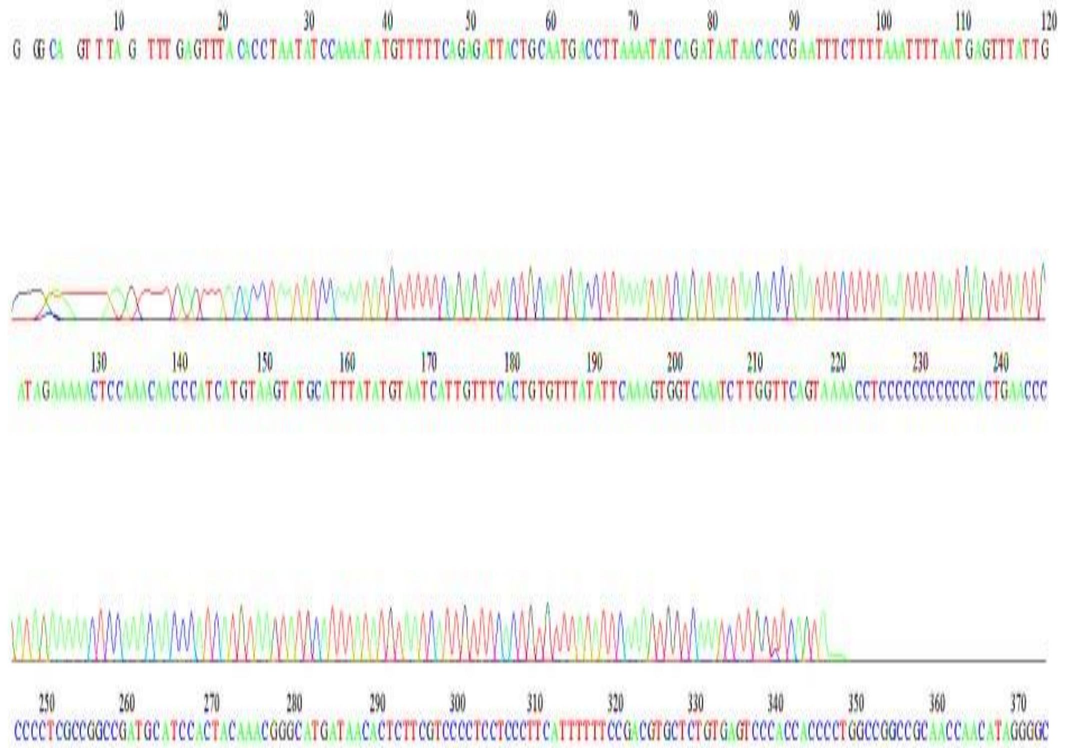


Figure: Sequencing data of *CACNA2D1* gene

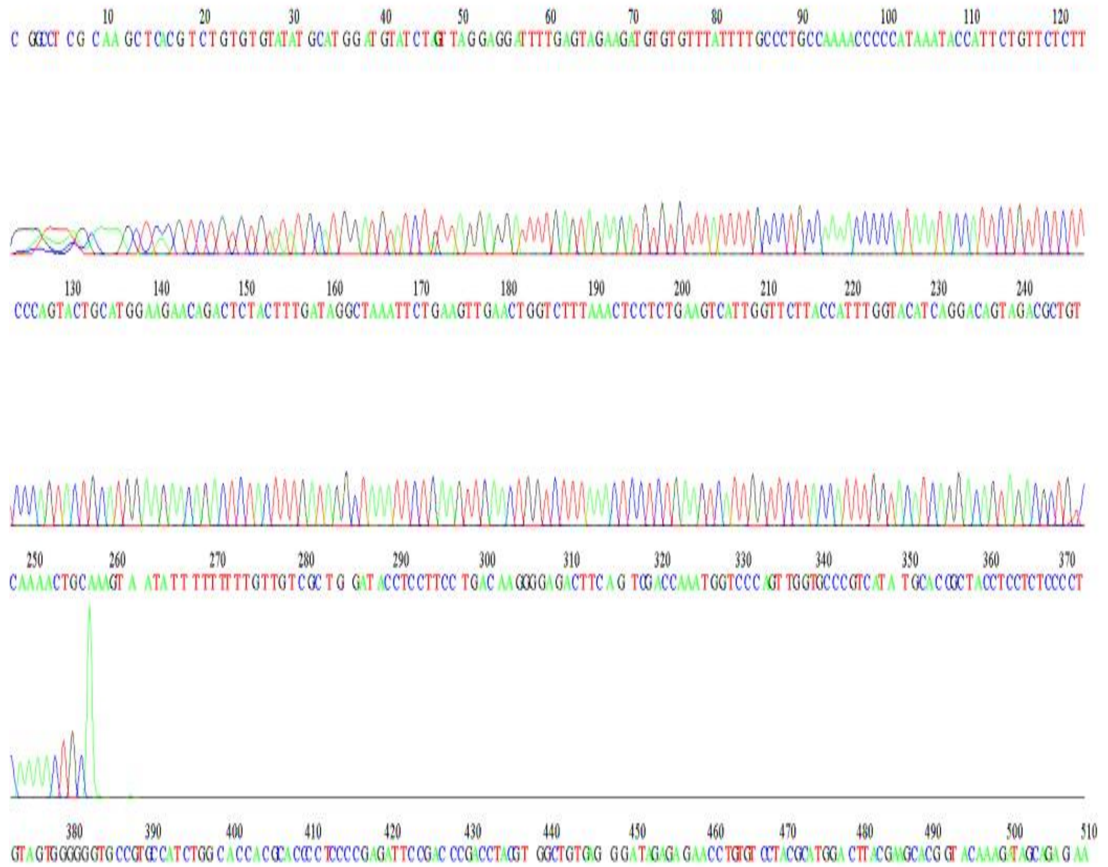


Figure: Sequencing data of *MYF5* gene

Appendix-III

NUCLEOTIDE SEQUENCING AND DETECTION OF MUTATION IN CARCASS ASSOCIATED CANDIDATE GENES IN INDIGENOUS CATTLE OF BANGLADESH

Department of Genetics & Animal Breeding

Chattogram Veterinary and Animal Sciences University

ID NO: **Date:**

1. Owner name: Contact no:
   Address
   Village: Upazilla:
   District:
2. Education status of the owner: : ◯ Low educated (1-5 class) ◯ Medium (6-9 class) ◯ Higher (10-11 class) ◯ Educated (Graduate)
3. Are they maintain record sheet: ◯ Yes ◯ No
4. Current population of Cattle at farm……………………..
5. Breed : ◯ RCC ◯ Cross ◯ Other
6. Age : ◯ Less than 1 year ◯ 1 year ◯ 1 year-2 year ◯ More than 2 year
7. Sex : ◯ M ◯ F
8. Source of purchase: ◯ own production ◯from other household
   ◯from market ◯others
9. Muscle at shoulder area, loin area, back fat
   ◯Under developed ◯Developed ◯Well developed
10. Body weight measurement :
    Body length: Heart Girth:
11. Body weight :…………………kg
12. Prevention and Control measures of Livestock:
    a. Do you face any disease of your animals? ◯Yes ◯No
    If yes, what kinds of diseases attack here…………………….
    b. If there was epidemic last year ◯ Yes ◯No
    if yes, the name of the diseases
13. How much blood do you collect? ◯ 2 ml ◯ 3 ml ◯ 5 ml
14. Do you use any antiseptic before collection of blood? ◯ Yes ◯ No
15. Do you use any anticoagulant in the test tube? ◯ Yes ◯ No
16. How do you store the test tubes immediately after collection?
    ◯In the test tube stand ◯ By other means
17. Others Information:

Biography

This is DR. Arjuman Lima, daughter of Mr. Mahabubur Rahman and Mrs. Jarna Tara Begum, from Anwara Upazilla under Chattogram district of Bangladesh. She completed the Secondary School Certificate (SSC) Examination in 2008 from Kapashgola City Corporation School, Chattogram, followed by Higher Secondary Certificate (HSC) Examination in 2010 from Govt. Women College, Chattogram. She obtained her Doctor of Veterinary Medicine (DVM) degree in 2017 from Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh with CGPA 3.81 out of 4.00. Now, she is a candidate for the degree of Masters of Science in Animal Breeding and Genetics at the Department of Genetics and Animal Breeding, Faculty of Veterinary Medicine, CVASU, Chattogram, Bangladesh. She is working as a researcher on few projects in this department. Her professional goal is to involve herself as an academician and researcher since her passion has always been to serve her country and to improve quality of life of the people.