

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



A thesis
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*A thesis submitted to the
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**DEPARTMENT OF GENETICS AND ANIMAL BREEDING
FACULTY OF VETERINARY MEDICINE**

**CHATTOGRAM VETERINARY AND ANIMAL SCIENCES UNIVERSITY
CHATTOGRAM-4225**

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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JUNE, 2020

Authorization

I, DR. Arjuman Lima assure that I have performed all works furnished here in this report. The information has been collected from books, national and international journals, websites and other references. All references have been acknowledged accordingly.

I hereby declare that I am the sole author of the thesis “Nucleotide sequencing and detection of mutation in carcass associated candidate genes in indigenous cattle of Bangladesh”. I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of scholarly research. I further authorize the CVASU to reproduce the thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

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The Author

June, 2020

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List of Abbreviations

A	Adenine
ADG	Average daily gain
AFS	Australian friesian sahiwal
AI	Artificial insemination
Asp	Aspartic
BCAA	Branched-chain amino acid
BER	Bangladesh economic review
BLAST	Basic local alignment search tool
BONEP	Bone percentage
bp	Base pair
BTA	<i>Bos taurus</i> autosome
C	Cytosine
<i>CACNA2D1</i>	Calcium voltage-gated channel auxiliary subunit alpha2delta 1
Chr	Chromosome
cm	Centimeter
cM	Centromeric
CVASU	Chattogram Veterinary and Animal Sciences University
CWT	Carcass weight
Cys	Cysteine
dbSNP	The single nucleotide polymorphism database
ddATP	Dideoxyadenosine triphosphate
ddCTP	Dideoxycytidine triphosphate
ddGTP,	Dideoxyguanosine triphosphate
ddTTP	Dideoxythymidine triphosphate
DLS	Department of livestock services
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
dsDNA	Double stranded DNA
e.g.	Example
EDTA	Ethylene demine tetra acetic acid

EPD	Expected progeny differences
FABG	Favorprep Blood Genomic
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FCR	Feed conversion ratio
G	Guanosine
gDNA	Genomic DNA
GDP	Gross domestic product
Ile	Isoleucine
ILRI	International livestock research institute
kg	Kilogram
LMA	Longissimus muscle area
MAS	Marker assisted selection
ml	Milliliter
MoyD	Myogenic determination
mRNA	Messenger RNA
MTBR	Meat-to-bone ratio
<i>MYF</i>	Myogenic factor
MyoG	Myogenin
NAGRP	National animal genome research program
NCBI	National Center for Biotechnology information
ND	Non-descriptive
PCR	Polymerase chain reaction
PCR-SSCP	Polymerase chain reaction-single-strand conformation polymorphism
PIC	Polymorphism information content
PRTC	Poultry Research and Training Centre
QTL	Quantitative trait loci
RCC	Red Chittagong cattle
RFI	Residual feed intake
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid

SCS	Somatic cell score
SNP	Single nucleotide polymorphism
T	Thymine
TAE	Tris-acetate-EDTA
Trp	Tryptophan
Tyr	Tyrosine
UNIDO	UNIDO United Nations Industrial Development Organization
USA	United State of America
USDA	United States Department of Agriculture
UV	Ultraviolet
α -KIC	A-ketoisocaproate
%	Percentage
μ l	Micro liter

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Dedication

To my ever loving parents

Mr. Mahabubur Rahman

and

Mrs. Jarna Tara Begum

Abstract

Carcass related traits are very complex and difficult to measure phenotypically, they are not usually included in selection programs. Several studies have indicated associations between genes associated with carcass traits and the genetic polymorphisms in these genes. However, genetic research on indigenous cattle carcass traits in Bangladesh is scanty. Therefore, this study was carried out to screen polymorphisms of the *CACNA2D1* gene for carcass weight, dressing percentage, meat percentage, and backfat thickness and *MYF5* for live weight, loin eye height, loin eye area and water holding capacity. To identify and assess the association between polymorphisms and carcass traits a total of 80 animals were randomly selected. Genomic DNA was extracted from collected blood samples using a commercial DNA extraction kit for amplification of the fragments of exon 25 of *CACNA2D1* gene and exon 2 of *MYF5* gene using Polymerase Chain Reaction (PCR). PCR products were sequenced using DNA sequencing. Phylogenetic analysis was also performed to explore the genetic diversity of different cattle. This preliminary research on polymorphism of the *CACNA2D1* gene exon 25 in cattle revealed C1993A, T2058A, C2068A might be helpful as genetic markers in Red Chittagong Cattle (RCC) and Crossbred (RCC × Local) cattle. According to the score in Polyphen2, it can be confidently predicted to be deleterious. The SNP C1993A resulted in a missense mutation leading Leu (L) to 658 Ile (I) amino acid substitution, which may 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. The absence of this mutation in the RCC population may have a positive effect, and carcass quality may be better in RCC than crossbreed as those mutations may negatively impact the cattle population. In case of *MYF5* the mutation at the 1795C>T position, which is intronic, does not cause an amino acid substitution but several recent investigations revealed that SNPs in the intron region had significant associations with carcass and meat quality traits. Results from this study indicate that the *CACNA2D1* gene and *MYF5* have potential effects on carcass and meat quality traits, opening up possibilities for cattle breeding and improvement in gene-assisted selection and future research is necessary to evaluate other candidate genes. Moreover, the results for cattle carcass traits would be a scientific basis for the conservation and utilization of cattle genetic resources.

Keywords: Carcass trait, indigenous cattle, DNA sequencing, polymorphism, meat quality.