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Effects of g.10329C>T polymorphism in the *SCD* gene with milk production traits in Bangladeshi cattle: evidence from mutant protein function analysis

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ABSTRACT

This study aimed to pinpoint the single nucleotide polymorphisms (SNPs) within the stearoyl-CoA desaturase (*SCD*) gene that is notably linked to milk production traits and to evaluate the structural and functional consequences of mutant proteins. Through pooled DNA sequencing, five SNPs were identified within the *SCD* gene. Among these, only one exonic SNP (g.10329C>T) was non-synonymous, resulting in an amino acid change from alanine to valine in the protein. Structural and functional analysis of the g.10329C>T mutation revealed discernible differences between wild-type and mutant proteins, indicating consequential effects on the observed phenotype. Subsequently, this identified SNP was genotyped in 100 milking cows for association analysis. The SNP g.10329C>T demonstrated significant allele substitution effects on milk yield traits. These findings suggest that the identified polymorphism (g.10329C>T) influences milk yield traits within the studied population and could serve as a genetic marker for cattle selection processes aimed at enhancing productivity with proper validation.

Keywords: Single nucleotide polymorphism, association, *SCD*, mutant proteins

INTRODUCTION

In Bangladesh, in recent decades a large number of local cattle have been replaced by crossbred cattle to improve milk production for higher demand. The cross-breeding has been started in 1936. The pure Hariana bulls were imported to upgrade the local cattle through natural services from India. In 1958 by Directorate of Livestock Services (DLS) started cross-breeding with the Artificial Insemination (AI) program which was strengthened in 1975-76 and to date it is going on. In the 1960s, Sindhi, Sahiwal and Tharparkar bulls were imported from Pakistan and in 1973, Friesian and Jersey bulls were imported from Australia to cross with indigenous cattle for upgrading milk production. At present time, the cattle of Bangladesh are composed of pure local breeds and crossbreds (Hossain et al., 2002; Hamid et al., 2017). Local cattle in Bangladesh are small in size and horned, and they are used for ploughing all over the country. Even under ideal dietary, management, and environmental conditions, their production level is minimal (Hossain et al., 2002). The National Health Strategy recommends a daily milk ratio of 250 millilitres/person. The actual average daily consumption is 193 millilitres/person, less than the recommended intake (DLS, 2021). The low level of national production cannot meet the demand

of Bangladesh's growing population (DLS, 2021). In contrast to the world, the milk production growth rate in Bangladesh is very negligible due to a lack of proper selective breeding guidelines. Over the past five decades, Bangladesh has endeavoured to bridge the significant gap between milk production and demand (NLDP, 2007). The National Livestock Development Policy, under the Ministry of Fisheries and Livestock, has outlined a three-tiered approach to address this challenge: a) short-term objectives involve the insemination of the highest-performing crossbred Holstein-Friesian cows, which yield 10 kg or more of milk, under intensive management systems, utilizing imported semen from progeny-tested bulls; b) mid-term goals entail inseminating crossbred Holstein-Friesian cows that produce 6-10 kg of milk, reared within semi-intensive management systems, with semen sourced from 50% Holstein-Friesian bulls proven through progeny testing; c) long-term aspirations focus on inseminating native cows, managed under low-input production systems, with semen from superior progeny-tested or pedigreed bulls of local cattle breeds, aiming to enhance milk production. This strategic framework aims to gradually enhance milk production to meet the nation's growing demand, addressing both immediate and future needs within the dairy industry (NLDP, 2007).

Milk, rich in protein, lipids, lactose, and calcium, is a top dietary source of nutrition. It contains essential micronutrients and bioactive components like vitamins, minerals, immunoglobulins, and enzymes, which play an important role in the newborn's health and development (Medrano et al., 2000). Thus, milk and dairy products are vital for a balanced diet (Pereira, 2014). The fat content and composition of milk influence the quality of dairy products (Chilliard et al., 2003). Excess carbohydrates are converted into fatty acids through de novo lipogenesis in the liver, adipose tissue, and mammary glands, forming triglycerides stored as energy reserves in animals (Berg, 2019). According to published studies by Mele et al. (2007) and Moioli et al. (2007), the differences in mammary *SCD* activity, linked to either polymorphism of *SCD* and regulation of expression or differences in downstream factors that would affect interactions between enzymes and substrates, may be the vital reason for diet-independent variations in milk fat's conjugated linoleic acid (CLA) content (Peterson et al., 2002). The production of monounsaturated fatty acids (MUFA) can occur through several pathways; a key pathway involves the process of fatty acid synthesis, which takes place in the cytoplasm of animal cells and is regulated by the *SCD* enzyme. The key step is the desaturation process, where *SCD* introduces a double bond into the fatty acid chain at a specific position (between carbons 9 and 10). This converts a saturated fatty acid into a MUFA (Shimakata and Mihara, 1972; Strittmatter et al., 1974; Enoch et al., 1976). Because of biosynthesis function, numerous independent studies have already examined the associations between polymorphisms in a number of genes, including stearoyl-CoA desaturase (*SCD*) and milk traits (Hayes and Goddard, 2001; Grisart et al., 2002; Khatib et al., 2007; Khatib et al., 2008; Ozkan and Yakan, 2020; Ahsani et al., 2022; Azis and Anggraeni, 2023). Several cattle breeds have reported associations between a missense polymorphism in exon 5 (GenBank AY241932: g.10329CAla > TVal) and the concentration of certain individual fatty acid and fatty acid unsaturation indices in the milk fat (Mele et al., 2007; Moioli et al., 2007; Schennink et al., 2008; Alim et al., 2012; Houaga et al., 2018; Ma et al., 2021).

Additionally, research on the *SCD* gene in cattle has led to the discovery of 8 SNPs that together constitute 2 haplotypes (A and B) (Medrano et al., 2000). The animal *SCD* system consists of four hydrophobic transmembrane domains that are anchored in the endoplasmic reticulum membrane (Buist, 2004). This literature reported (Buist, 2004) that the *SCD* protein, a key enzyme in the production of fatty acids and a mitochondrial protein, has a transmembrane nature. Maryam et al. (2016) reported that p.K158I (Ala>Val) in *SCD* is localized close to the steroid-binding domain, a functionally active area involved in fatty acid synthesis. They also hypothesized that since this variant changes hydrophilicity to hydrophobicity, it may facilitate transport across the mitochondrial membrane and boost fatty acid production or that the presence of this substitution in the vicinity of the steroid-binding

domain may alter protein functionality. This hydrophilic to hydrophobic substitution may have contributed to the rise in milk fat content in cows (Maryam et al., 2016). The biosynthesis function and previous association effects on milk fat suggest that the *SCD* gene may also affect production traits like milk yield, fat percentage, and protein percentage, which are the main breeding goals in current selection schemes of dairy cattle. In this study, we chose the *SCD* gene as a potential candidate gene for milk production traits, considering both its biological role and genomic location. The objective was to investigate SNPs within the *SCD* gene, analyze the protein structure to understand the implications of these genetic variations and evaluate their correlation with milk production traits in Bangladeshi Local and Holstein Cross cows. This research aims to potentially utilize of identified SNPs as genetic markers in cattle selection processes.

MATERIALS AND METHODS

Experimental animals and phenotypic data collection

A total of one hundred crossbred (Bangladeshi Local X Holstein) F1 generation cows were selected from Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka those are reared in the same environment and management condition. Milk samples were collected from each lactating cow once in every lactation (within 90-100 days) period. After collection, milk samples were immediately transferred to the Animal Biotechnology Division of the National Institute of Biotechnology (NIB) to generate phenotypic data like protein and fat percentage using an auto milk analyzer (Lactoscan, Milk Analyzer, Bulgaria). Milk yield data from each milking cow (305 days' milk yield) was collected from CCBDF. The complete workflow employed in this study is shown in Figure 1.

Screening of polymorphisms and genotyping

Blood samples were collected from a chosen subset of cows (n = 100) for DNA extraction, which was performed using the TIANamp Blood DNA Kit following the manufacturer's guidelines. Primers designed according to Alim et al. (2012) and synthesized by Invitrogen (Invitrogen

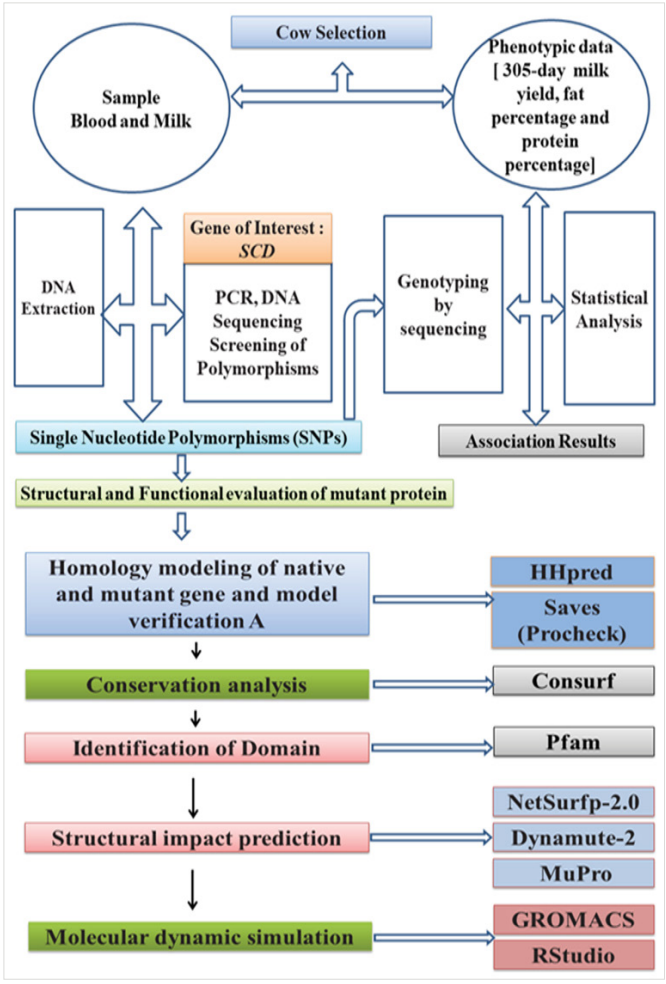


Figure 1. Workflow employed for the study

Life Technologies, China) were utilized. A DNA pool was created (50ng/µl/animal) and subjected to sequencing to identify potential SNPs. PCR amplification of the pooled samples was conducted using a programmable thermal cycler (Biometra GmbH, Germany) and Invitrogen's DreamTaq Green PCR Master Mix. The 25 µL reaction mixture contained 50 ng of genomic DNA, 1 µl specific *SCD* gene primers, 12.5µl of premix and 8.5 µl ddH_2O. The amplification process included 5 min at 94 °C for initial denaturing followed by 35 cycles at 94 °C for 30 s; annealing at 58 (°C) for 30 s, extension at 72 (°C) for 30 s and a final extension at 72 (°C) for 7 min. Gel electrophoresis of PCR products was carried out using 2% agarose gel and Azure c150 gel imaging workstations to confirm amplification. Subsequently, PCR products were sequenced at the Molecular Biotechnology Division, NIB, using the ABI3500 sequencer (Applied Biosystems, USA).

Sequence data analysis was performed using BioEdit Sequence Alignment Editor version 7.0.9.0 and ClustalW multiple sequence alignment programs to detect genetic polymorphisms. Finally, the identified polymorphism (g.10329C>T) was genotyped in all animals using PCR and sequencing techniques.

Structural and functional Impact Prediction of SCD mutant protein associated with milk traits

Non-synonymous polymorphisms in the *SCD* gene were analyzed for their impact on protein structure and function, affecting milk phenotype. HHpred, a web-based tool (https://toolkit.tuebingen.mpg.de/tools/hhpred), was used to detect remote protein homology and predict structures, while PROCHECK (https://servicesn.mbi.ucla.edu/PROCHECK/) evaluated structural integrity. SAVES web server and the Ramachandran plot confirmed structure quality. MUpro (https://www.ics.uci.edu/~baldig/mutation.html) predicted the effect of SNPs on protein stability using Support Vector Machines and Neural Networks. DynaMut2 analyzed protein motion, flexibility, and stability impact (Rodrigues et al., 2021). Conservation profile analysis was done with the Consurf server (Ashkenazy et al., 2016), and domain names were determined using the Pfam server (Apweiler et al., 2001). Molecular dynamics simulations of wild-type and mutant *SCD* proteins were performed using GROMACS for 100 ns (Abraham et al., 2015).

Statistical analysis

Pedigrees of the genotyped animals were traced back three generations, resulting in a total of 300 animals included in the analysis. The kinship matrix was calculated using MATLAB version 7.11.0.584. POPGENE software, version 1.32, was utilized to assess allelic and genotypic frequencies for the loci and to perform the Hardy-Weinberg equilibrium test. SAS 9.1.0 software (SAS Institute Inc. USA) was employed to estimate the genotypic effects on milk production traits. The analysis used the mixed procedure with an animal model (Lynch and Walsh, 1997):

$$Y = \mu + hys + L + G + \alpha + e,$$

where:

- Y is the phenotypic value;
- μ is the overall mean;
- hys is a herd-year-season effect;
- L is the fixed effect of lactation;
- G is a fixed effect corresponding to the genotype of polymorphisms;
- α is a random polygenic component for pedigree relationships;
- e is a random residual.

Bonferroni correction, adjusting the significance threshold to account for multiple comparisons, reducing the likelihood of false positives, was performed for multiple t-tests. The least squares mean was utilized for multiple comparisons to estimate the effects of *SCD* polymorphic genotypes on milk production traits. This likely involved comparing the means of different genotypic groups. Falconer and Mackay's equation was employed to compute the additive (a), dominance (d), and allele substitution (α) effects. These effects offer insights into how alleles at a specific locus influence the phenotype. Falconer and Mackay's (1996) equation was employed to compute the additive (a), dominance (d), and allele substitution (α) effects i.e.:

$$a = (AA - BB) / 2$$

$$d = AB - (AA + BB) / 2$$

$$\alpha = a + d\,(q - p)$$

where *AA* and *BB* are homozygous, *AB* is heterozygous genotype, and *p* and *q* are the allele frequencies.

RESULTS

Screening of single nucleotide polymorphisms and genotypes

In this study, we identified five SNPs within the *SCD* gene (GenBank: AY241932) through sequence analysis of pooled DNA samples. Among these SNPs, two (g.6926A>G and g.8646A>G) were situated within introns 3 and 4, while the remaining three (g.10153A>G, g.10213T>C, and g.10329C>T) were located in exon 5 (Figure 2).

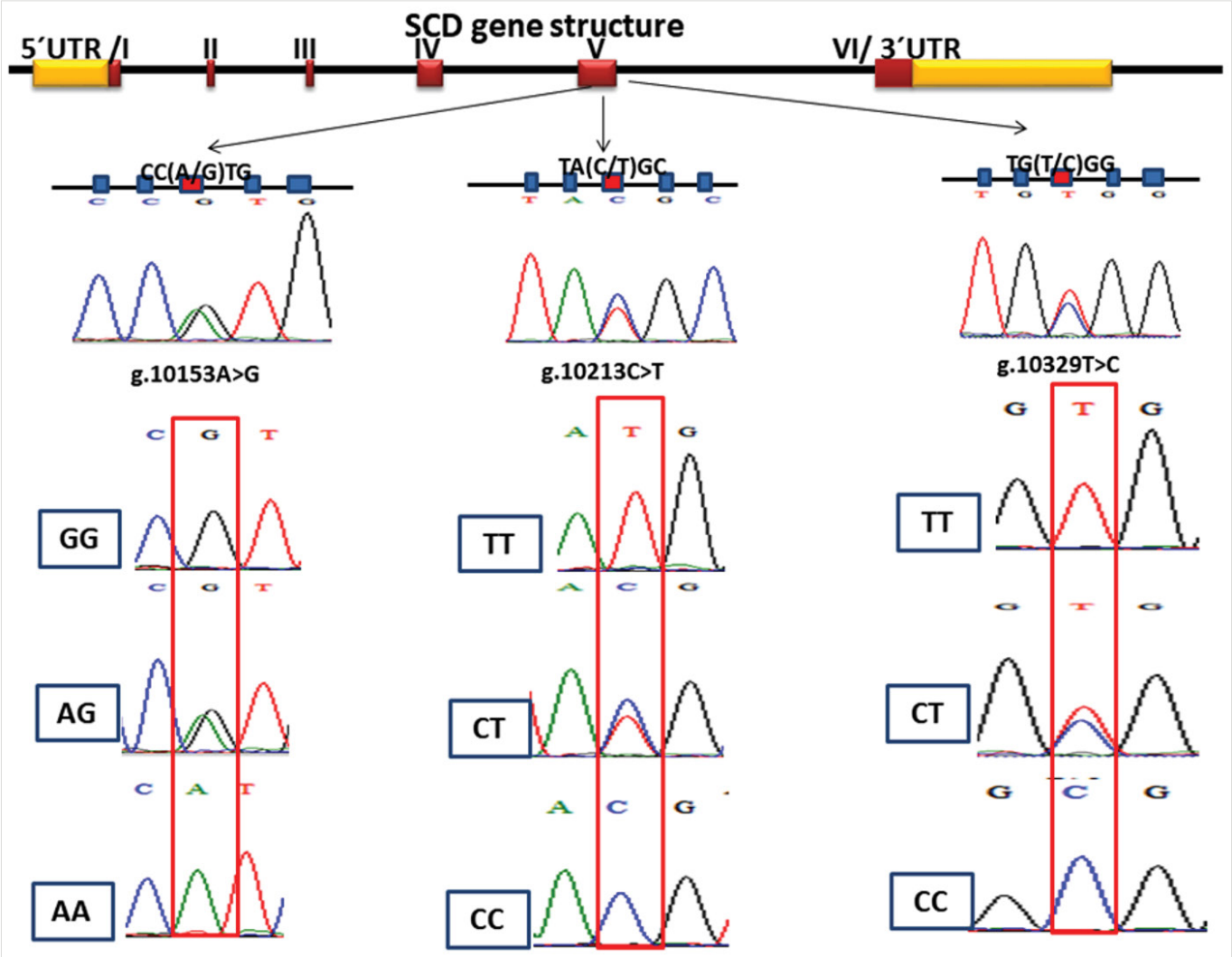


Figure 2. *SCD* gene structure and genotyping of individual polymorphisms by chromatograph differences in studied animals

Specifically, only the g.10329C>T SNP was anticipated to induce an amino acid substitution, resulting in a change from alanine to valine at position 293 within the *SCD* protein. Table 1 illustrates the genotypic and allelic frequencies, along with the results of the Hardy-Weinberg equilibrium test (χ^2). The chi-square test demonstrated that the genotypic frequencies of the g.10329C>T locus within the population were consistent with Hardy-Weinberg equilibrium (P>0.05) (Table 2), suggesting less selection pressure at this particular site.

Table 1. PCR primers used for sequencing the *SCD* gene

Exons	Sequence	Size of PCR product
Exon 1	L- GTTGGCAACGAATAAAAGAGG R- CGCGGTGATCTCAACTCTTC	384 bp
Exon 2	L- GGACCGGGTCTATGCCTATC R- CCATCCAGCCTCTCAGGAC	552 bp
Exon 3	L- GTTCCCTGGGACTCCTAAGC R- CCGGAACTTAACCACAAGGA	499 bp
Exon 4	L- GGCAACTCCATGACTTCTCC R- CATGACCGTCCTAGGTCAAC	594 bp
Exon 5	L- CCCATTCGCTCTTGTTCTGT R- CGTGGTCTTGCTGTGGACT	400 bp
Exon 6	L- GCCTCTGAGGGGATCTATTTG R- AGGCAGAGTTGTTGGCTTTC	543 bp

Source: Alim et al. (2012)

Table 2. Genotypic and allelic frequencies and Hardy–Weinberg equilibrium χ^2 test of *SCD* genotypes

Polymorphisms	Genotypic frequency			Allelic frequency		Hardy–Weinberg equilibrium χ^2 test
g.10329C>T	CC	CT	TT	C	T	$P > 0.05$
	0.23	0.65	0.12	0.55	0.45	

Structural and functional Impact Prediction of SCD mutant protein associated with milk traits

The three-dimensional model of the protein was generated (Figure 3) in PDB format using the HHPred server. The quality of the model was validated by inspecting the Ramachandran plot generated by the PROCHECK web server. The findings indicated that the majority of the residues of the three-dimensional models belonged to the most favored regions for both the wild and mutant versions (Figure 4).

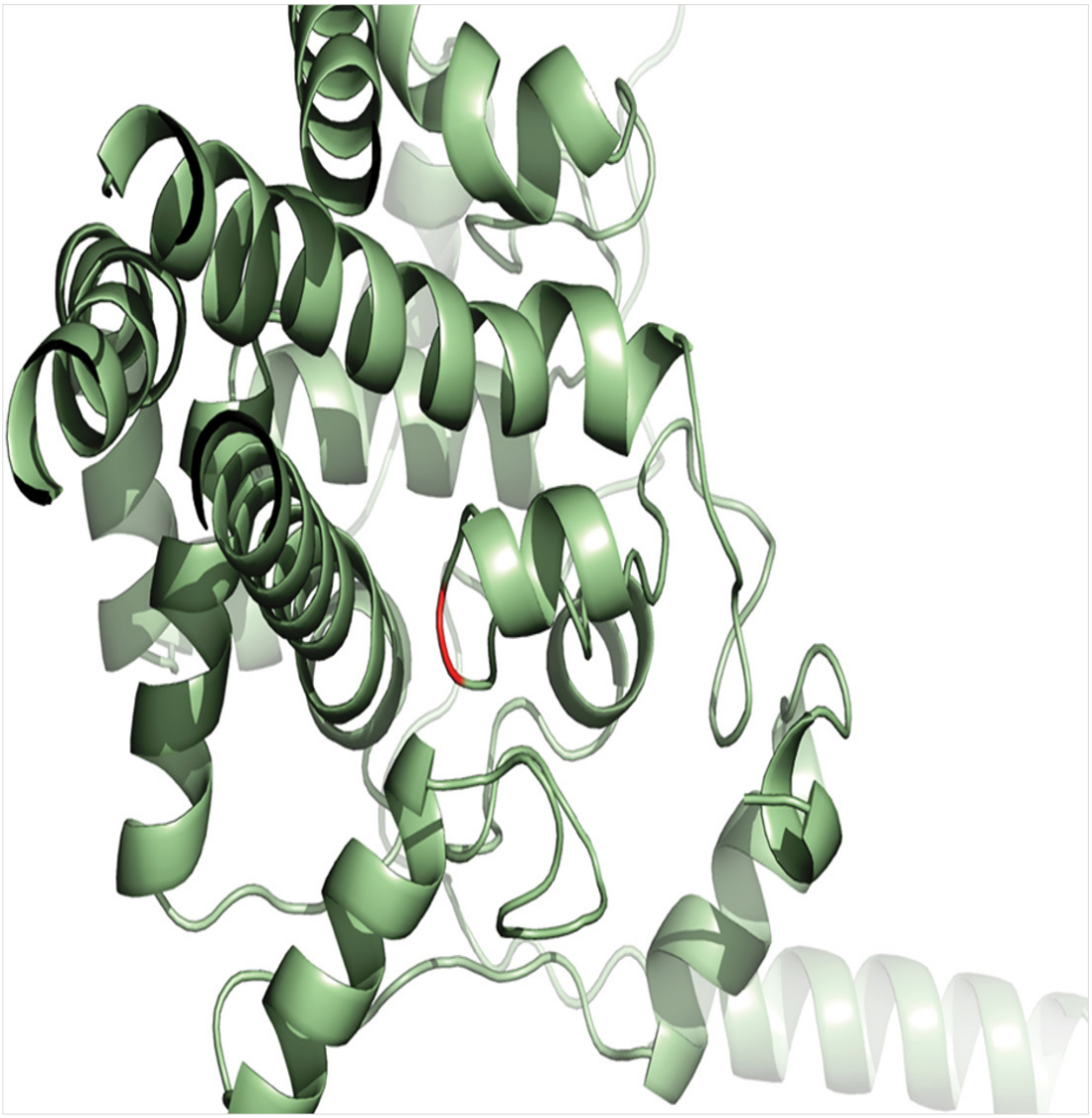


Figure 3. Three-dimensional model of *SCD* mutant g.10329 C>T (V293A) (the position of the mutated amino acid is marked in red)

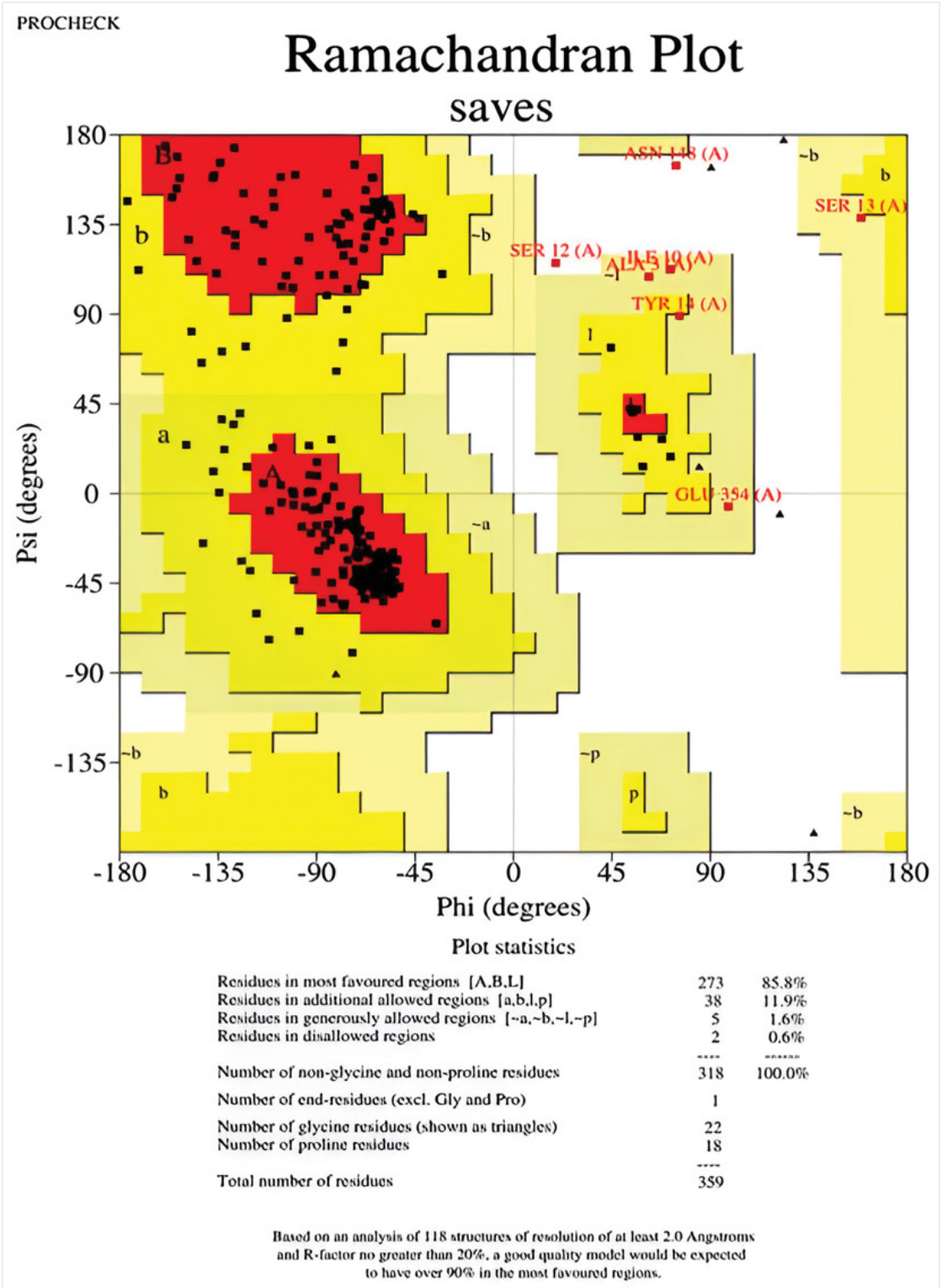


Figure 4. Ramachandran plot of *SCD* mutant g.10329 C>T (V293A) generated by PROCHECK

The conservation profile findings were displayed in the form of a structural representation of the protein sequence, with the putative structural and functional residues highlighted. Here, the V293A position in *SCD* belonged to the average conservation profile (Figure 5).

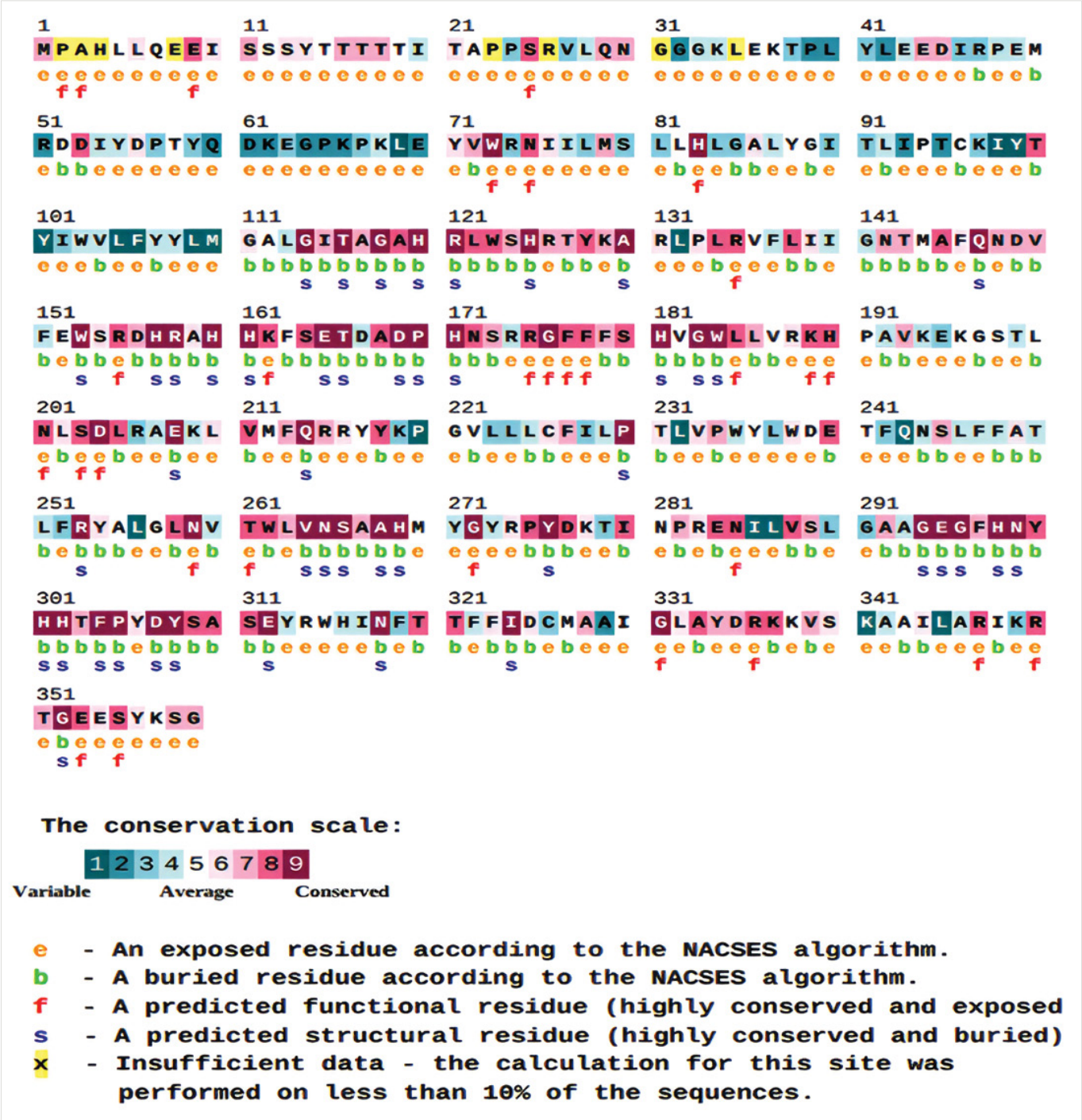


Figure 5. Conservation profile analysis of *SCD* protein

SCD contained only one domain namely Acyl-CoA desaturase and the *SCD* mutant V293A belonged to that domain (Figure 6). The Acyl-CoA desaturase domain is an essential component of desaturase enzymes, playing a fundamental role in lipid homeostasis and cell function. This domain catalyzes the introduction of double bonds into fatty acyl chains of lipids, leading to the formation of unsaturated fatty acids.

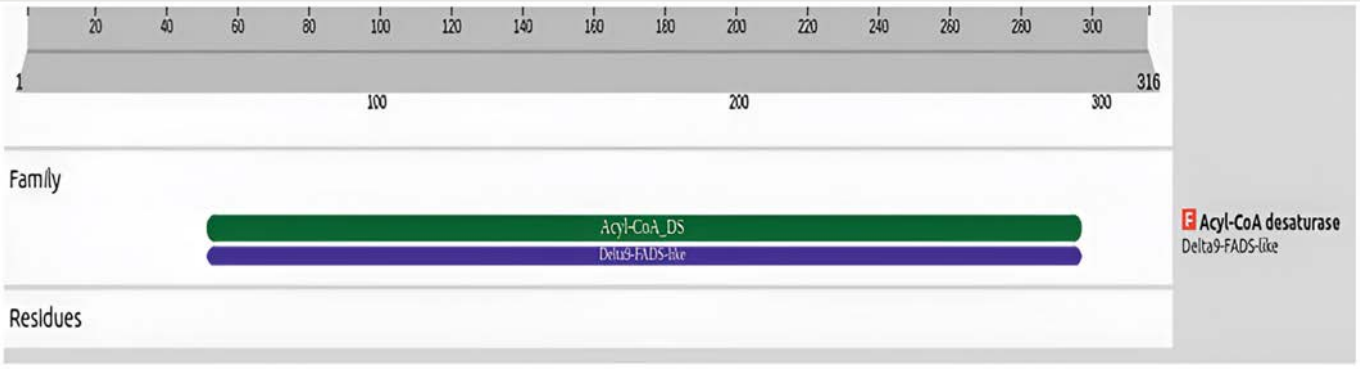
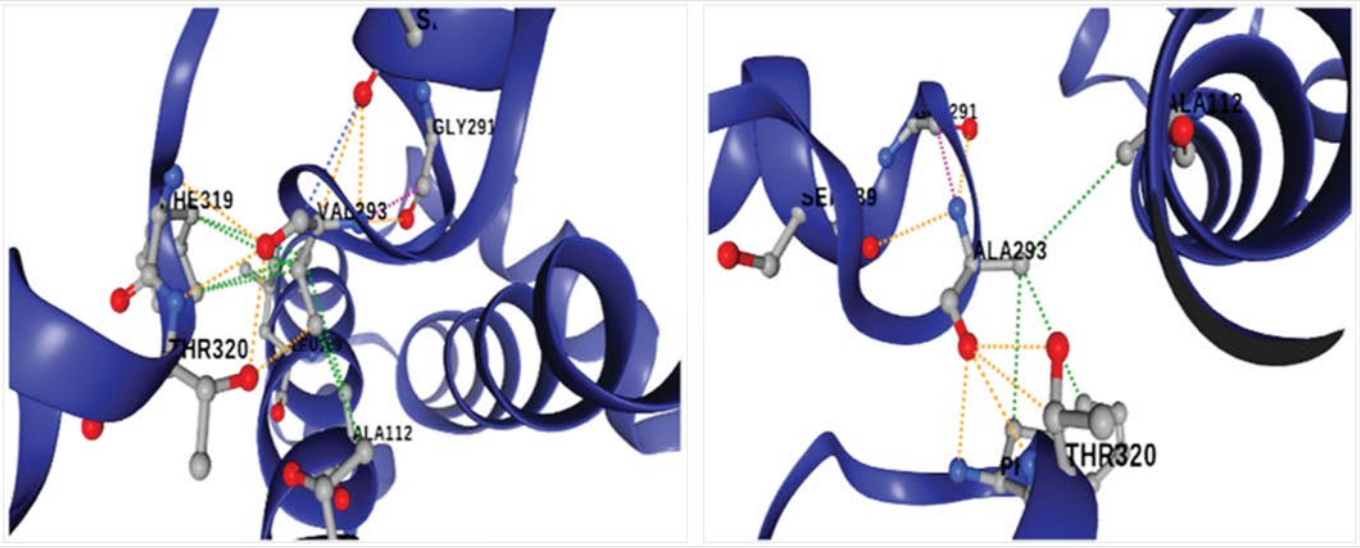


Figure 6. *SCD* domains

Interatomic interactions prediction with Mupro analysis revealed that the mutation V293A in *SCD* decreased the stability of the protein. The delta G for mutations in V293A in *SCD* was -1.7005351 kcal/mol. Dynamut2 predicted stability change (ΔΔGStability) for mutations in *SCD* was -1.74 kcal/mol. A negative value indicates a destabilizing effect of protein. The mutation was more unstable for the protein if the ΔΔG value was negative (Figure 7).

We calculated the RMSD to check the reliability of the systems. Variation in the RMSD value indicates a change in the protein. Throughout the simulation, the RMSD value between wild-type *SCD* and the mutant changed (Figure 8).

The regional flexibility of the protein was evaluated using the RMSF method which showed that the mutant protein was more flexible (Figure 9).



a) *SCD* Wild type b) *SCD* V293A Mutant

Figure 7. Interatomic interactions of *SCD* wild type and V293A mutant revealed by dyanmute2 analysis

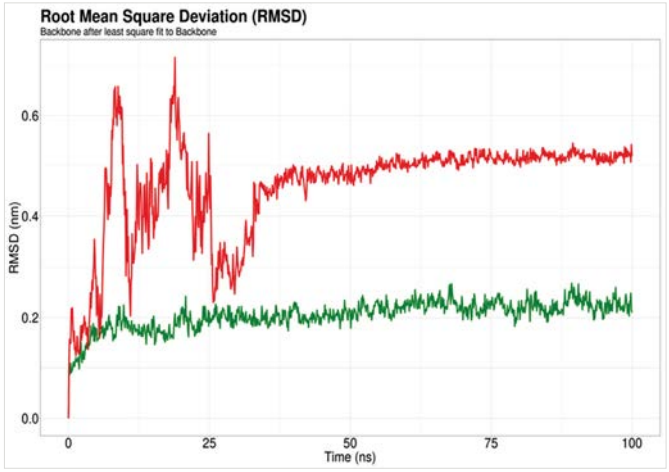


Figure 8. RMSD of the wild type (green) *SCD* and the V293A mutant (red) (X-axis represents the time (ns) while the Y-axis represents the RMSD value (nm))

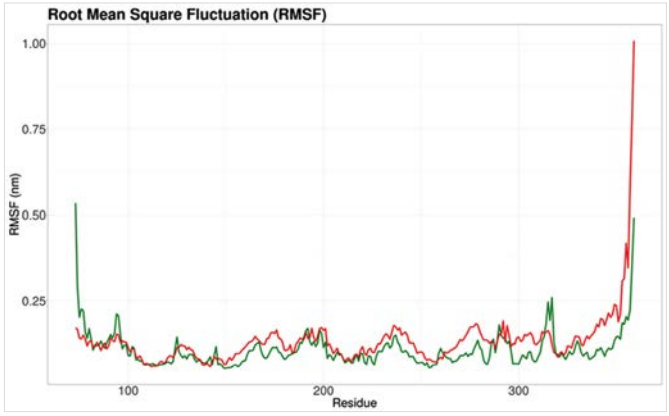


Figure 9. RMSF of the wild-type (green) *SCD* and the V293A (red) mutant (X-axis represents the amino acid residues while the Y-axis represents the RMSF value (nm))

The degree of compactness is measured using the radius of gyration. After 20 ns, the mutant protein had a higher radius of gyration compared to the wild type (Figure 10).

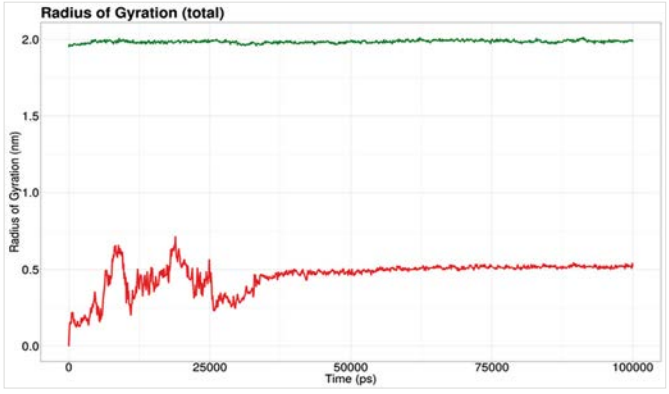


Figure 10. The radius of gyration of the wild-type *SCD* (green) and the V293A mutant (red) (the X-axis represents the time (ps) while the Y-axis represents the area (nm^2))

The stability of proteins' hydrophobic cores was predicted using solvent-accessible surface area (SASA) in molecular dynamic simulations. Here, the SASA values declined gradually for both the wild type and the mutant. However, for the mutant, the SASA values remained higher than the wild type throughout the simulation period indicating a higher probability of disruption by solvents (Figure 11).

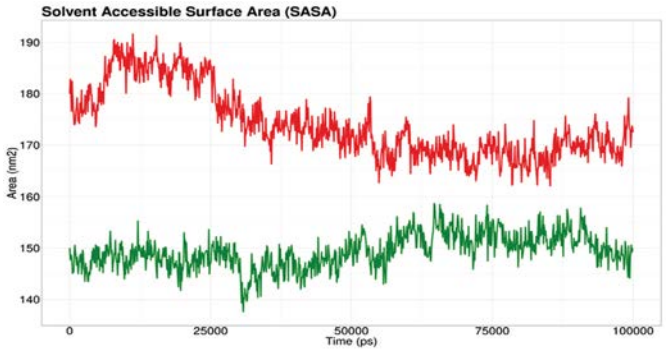


Figure 11. SASA of the wild-type *SCD* (green) and the V293A (red) mutant (X-axis represents the time (ps) while the Y-axis represents the SASA value (nm))

Association and effects of SNPs

The SNP (g.10329C>T) was initially identified as associated ($P \leq 0.0001$) with milk traits, with raw P values (<0.05). However, subsequent Bonferroni correction for multiple t-tests did not render these associations significant. Nevertheless, significant additive and allele substitution effects were observed for milk yield traits at the g.10329C>T loci ($P < 0.01$) (Table 3). The T allele demonstrated the potential to increase milk production by 198.46 kg over a full lactation period compared to the C allele at the g.10329C>T position (Table 3).

Table 3. Effects of g.10329 C>T polymorphism of different *SCD* genotypes on Bangladeshi local cattle (LSM ± SE)

Locus	Genotype (n)	Milk yield (kg)	Fat (%)	Protein (%)	Additive (a), Dominant (d) and Allele substitution (α) effects		
					Milk yield (kg)	Fat (%)	Protein (%)
g.10329 C>T	CC (23)	1946.37 ± 155.919	3.79 ± 0.097	3.07 ± 0.058	188.931 (a)*	0.029 (a)	-0.023 (a)
	CT (65)	1622.13 ± 90.020	3.59 ± 0.056	3.11 ± 0.027	-95.309 (d)	-0.078 (d)	0.018(d)
	TT (12)	1568.51 ± 209.188	3.64 ± 0.013	3.12 ± 0.020	198.462 (α)*	0.037 (α)	-0.025 (α)
P-value		<0.0001 (0.36-0.46 corrected)	<0.0001 (0.90 corrected)	<0.0001 (0.80 corrected)			
Maximum yield		3003.781	4.592	3.320		-	
Minimum yield		939.400	2.867	2.895		-	

Notes: * Significant effect at $P < 0.05$ level; n = number of animals

DISCUSSION

Stearoyl-coenzyme A desaturase (*SCD*), a multifunctional complex enzyme for cellular biosynthesis of MUFAs by catalyzing the introduction of a double bond into saturated fatty acyl-CoAs, has been studied for its association with milk production traits and milk fat-related traits in dairy cattle (Taniguchi et al., 2004; Conte et al., 2006; Mele et al., 2007; Macciotta et al., 2008; Schennink et al., 2008; Kgwatalala et al., 2009; Alim et al., 2012; Houaga et al., 2018; Ma et al., 2021). The primary mechanisms involved in milk fat synthesis include de novo synthesis of short-chain fatty acids (*FASN*, *ACACA*, and *SCD*), the uptake, transport, and activation of long-chain fatty acids (*LPL*, *VLDLR*, *ACSL*, *FABP3*, and *SCD*), triglyceride synthesis (GPAM, LPIN1, DGAT, and AGPAT6), signalling pathways, and the production of transcription factors (*mTOR*, *SREBP*, *PPARG*, and *AMPK*) (Guo et al., 2024). Macciotta et al.(2008) reported that Italian Holsteins cows with CC homozygous genotype at the *SCD* locus (g.10329C/T) produced higher milk and protein compared to homozygous TT cows; heterozygous CT cows were in an intermediate position. Similar to Macciotta et al.(2008), Taniguchi et al.(2004) also reported that cows with (A293V) AA genotypes producing higher milk fat compared to VV genotypes in Japanese Black cows. Interestingly Alim et al. (2012) reported the association of heterozygous genotypes with different milk production traits in Chinese Holsteins and heterozygous CT cows produced more milk than the cows of homozygous CC or TT genotypes. Similar to Alim et al. (2012), Maryam et. al. (2016) reported that homozygous genotypes produced less fat than heterozygous genotypes in buffalo for the same SNP. Recently, it has been reported that in Polish Holstein-Friesian cows, the A293V (c.878C/T) mutation in *SCD* has been associated with milk fatty acids and altered the amino acid alanine to valine (Kesek-Wozniak et al., 2020). Constantly, Bouwman et al. (2011) documented that the A allele of SNP (A293V; c.878C/T) in *SCD* was linked to greater milk fatty acid levels, while other research indicated that the V allele had a less significant impact on milk fats in the White Fulani and Borgou cow breeds (Duchemin et al., 2013; Houaga et al., 2018). Very recent reports demonstrated that the *SCD* could be one of the candidates and g.10153A>G SNP (AG genotype) as a genetic marker to assist selection in reducing saturated fatty acid and increasing MUFA in the milk content of Holstein Friesian cows in Indonesia (Azis and Anggraeni, 2023). Our findings showed significant substitution effects and association at g.10329 C>T locus for milk yield trait. At g.10329 C>T locus, homozygous CC genotypes produced more milk compared to homozygous

TT genotypes and heterozygous AG genotypes were in an intermediate position but the result is inconsistent with Bayraktar and Ozcan (2023), they reported that heterozygous CT genotypes significantly associated with total milk yield similar to Alim et al. (2012).

The results of the mutational impact analysis conducted by Mupro suggest that mutations in *SCD* are associated with a destabilizing effect on the protein structure. This implies that these mutations could potentially disrupt the functionality of the protein to varying extents. The analysis of interatomic interactions further supports this idea, revealing discrepancies between the mutants and the wild type in terms of their interactions with neighbouring residues. Such differences indicate that the function of the proteins may be affected by these mutations. A protein's activity, regulation, and function rely heavily on the stability of its structure, any reduction in stability can lead to protein degradation, misfolding, and eventual dysfunction. This highlights the importance of comprehending the structural consequences of mutations in proteins like *SCD* (Rozario et al., 2021). Sequence conservation analysis unveiled that the mutation is located in average conserved locations suggesting that they might have important impacts on the functioning of the protein. The analysis of sequence conservation indicates that the mutation sites are situated in moderately conserved regions. This suggests that these mutations may exert significant effects on the protein's functioning. Conserved regions are typically crucial for protein structure and function, making mutations occurring in these regions more likely to have functional repercussions. Our result showed that *SCD* contained only the Acyl-CoA desaturase domain and the *SCD* mutant V293A was in the domain. The Acyl-CoA desaturase domain, also known as the fatty acid desaturase domain, is a conserved structural motif found in enzymes known as desaturases. These enzymes play a crucial role in lipid metabolism by introducing double bonds into fatty acyl chains. These double bonds are essential for the formation of unsaturated fatty acids, which are important components of various cellular structures, energy storage molecules, and signalling molecules. A molecular dynamics simulation complex lasting 100 nanoseconds highlighted distinct variances between the wild-type *SCD* and the V293A mutant in terms of RMSD, RMSF, Radius of Gyration, and SASA profiles. This indicates that the mutation in the *SCD* protein is likely to disrupt its normal function. Consequently, it is plausible to predict that the mutation could affect both the structure and function of the protein, potentially influencing the observed phenotype. However, additional in vitro and in vivo experiments involving a larger number of animals and a more extensive dataset are imperative to validate our findings further.

CONCLUSIONS

The present research has unveiled significant effects of allele substitution related to milk yield traits at the g.10329C>T locus. Throughout the entire lactation period, the presence of the C allele correlated with a noteworthy increase of 198.46 kg in milk production compared to the T allele at the g.10329C>T location. Furthermore, we did a series of structural and functional analyses like structural prediction, interatomic interactions, conservation profile analysis, domain identification, and molecular dynamic simulation of mutant protein to find out the allele substitution effects. There have not been any previous reports with structural and functional analysis of mutant protein to support these findings. The association results along with structural and functional evaluation of the *SCD* mutant and wild protein, and useful information provided by this study could be used with proper validation in selective breeding initiatives aimed at enhancing the milk production performance of dairy cattle.

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REFERENCES

Abraham, M.J., Murtola, T., Schulz, R., Pall, S.R., Smith, J.C., Hess, B., Lindahl, E. (2015) GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX, 1, 19-25. DOI: https://doi.org/10.1016/j.softx.2015.06.001

Ahsani, M.R., Mohammadabadi, M., Buchkovska, V., Levstafiieva, Y.M., Kucher, D., Kochuk-Yashchenko, O., Babenko, O., Stavetska, R., Oleshko, V., Kalashnyk, O. (2022) Association of Stearoyl CoA Desaturase Expression with Cattle Milk Characteristics. Iranian Journal of Applied Animal Science, 12, 271-279.

Alim, M.A., Fan, Y.P., Wu, X.P., Xie, Y., Zhang, Y., Zhang, S.L., Sun, D.X., Zhang, Q., Liu, L. (2012) Genetic effects of stearoyl-coenzyme A desaturase (*SCD*) polymorphism on milk production traits in the Chinese dairy population. Molecular Biology Reports, 39, 8733-40. DOI: https://doi.org/10.1007/s11033-012-1733-6

Apweiler, R., Attwood, T.K., Bairoch, A., Bateman, A., Birney, E., Biswas, M., Bucher, P., Cerutti, L., Corpet, F., Croning, M.D.R. (2001) The InterPro database, an integrated documentation resource for protein families, domains and functional sites. Nucleic acids research, 29, 37-40. DOI: https://doi.org/10.1093/nar/29.1.37

Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., Ben-Tal, N. (2016) ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. Nucleic acids research, 44, W344-W50. DOI: https://doi.org/10.1093/nar/gkw408

Azis, R., Anggraeni, A. (2023) Association of Stearoyl-CoA Desaturase Gene Polymorphisms on Milk Fatty Acid Composition of Holstein Friesian Cows in Indonesia. Tropical Animal Science Journal, 46, 27-35. DOI: https://doi.org/10.5398/tasj.2023.46.1.27

Berg, J S.L., Tymoczko, J, Gatto, G. (2019) Biochemistry. 9th ed. New York (NY): W.H. Freeman and Company.

Bayraktar, M., Ozcan, B.D. (2023) Association between Stearoyl CoA Desaturase (*SCD*) Gene Polymorphisms and Milk Production in Holstein Cattle Breed. Turkish Journal of Agriculture-Food Science and Technology, 11, 799-802. DOI: https://doi.org/10.24925/turjaf.v11i4.799-802.5969

Bouwman, A.C., Bovenhuis, H., Visker, M.H.P.W., van Arendonk, J.A.M. (2011) Genome-wide association of milk fatty acids in Dutch dairy cattle. BMC genetics,12, 1-12. DOI: https://doi.org/10.1186/1471-2156-12-43

Buist, P.H. (2004) Fatty acid desaturases: selecting the dehydrogenation channel. Natural product reports, 21, 249-62. DOI: https://doi.org/10.1039/B302094K

Chilliard, Y., Ferlay, A., Rouel, J., Lamberet, G. (2003) A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. Journal of Dairy Science, 86, 1751-70. DOI: https://doi.org/10.3168/jds.S0022-0302(03)73761-8

Conte, G., Mele, M., Castiglioni, B., Serra, A., Viva, M., Chessa, S., Pagnacco, G., Secchiari, P. (2006) Relationship between bovine *SCD* polymorphism locus and mammary gland desaturation activity. In: 8th World Congress on Genetics Applied to Livestock Production, pp. 22-37. Instituto Prociancia, Belo Horizonte, Minas Gerais, Brazil.

DLS (2021) Available at: https://www.dls.gov.bd/site/page/22b1143b-9323-44f8-bfd8-647087828c9b/Livestock-Economy

Duchemin, S., Bovenhuis, H., Stoop W.M., Bouwman, A.C., Van Arendonk, J.A.M., Visker, M. (2013) Genetic correlation between composition of bovine milk fat in winter and summer, and DGAT1 and *SCD*1 by season interactions. Journal of Dairy Science, 96, 592-604. DOI: https://doi.org/10.3168/jds.2012-5454

Enoch, H.G., Catala, A., Strittmatter, P. (1976) Mechanism of rat liver microsomal stearyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. Journal of Biological Chemistry, 251, 5095. DOI: https://doi.org/10.1016/S0021-9258(17)33223-4

Falconer, D.S., Mackay, T.F.C. (1996) Introduction to Quantitative Genetics. 4th edn. Longman Scientific and Technical, New York.

Grisart, B., Coppieters, W., Farnir, F., Karim, L., Ford, C., Berzi, P., Cambisano, N., Mni, M., Reid, S., Simon, P. (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, 222-31. DOI: http://www.genome.org/cgi/doi/10.1101/gr.224202

Guo, Y., Wei, Z., Zhang, Y., Cao, J. (2024) Research Progress on the Mechanism of Milk Fat Synthesis in Cows and the Effect of Conjugated Linoleic Acid on Milk Fat Metabolism and Its Underlying Mechanism: A Review. Animals, 14, 204. DOI: https://doi.org/10.3390/ani14020204

Hamid, M., Rahman, A., Zaman, M.A., Hossain, K.M. (2017) Cattle genetic resources and their conservation in Bangladesh. Asian Journal of Animal Sciences, 11 (2), 54-64.

Hayes, B., Goddard, M.E. (2001) The distribution of the effects of genes affecting quantitative traits in livestock. Genetics Selection Evolution, 33, 209-30. DOI: https://doi.org/10.1186/1297-9686-33-3-209

Hossain, K.B., Takayanagi, S., Miyake, T., Moriya, K., Bhuiyan, A., Sasaki, Y. (2002) Statistical genetic studies on cattle breeding for dairy productivity in Bangladesh: I. Genetic improvement for milk performance of local cattle populations. Asian-Australasian Journal of Animal Sciences, 15, 627-32. DOI: https://doi.org/10.5713/ajas.2002.627

Houaga, I., Muigai, A.W.T., Nganga, F.M., Ibeagha-Awemu, E.M., Kyallo, M., Youssao, I.A.K., Stomeo, F. (2018) Milk fatty acid variability and association with polymorphisms in *SCD*1 and *DGAT1* genes in White Fulani and Borgou cattle breeds. Molecular Biology Reports, 45, 1849-62. DOI: https://doi.org/10.1007/s11033-018-4331-4

Kesek-Wozniak, M.M., Wojtas, E., Zielak-Steciwko, A.E. (2020) Impact of SNPs in *ACACA*, *SCD*1, and *DGAT1* genes on fatty acid profile in bovine milk with regard to lactation phases. Animals, 10, 997. DOI: https://doi.org/10.3390/ani10060997

Kgwatalala, P.M., Ibeagha-Awemu, E.M., Mustafa, A.F., Zhao, X. (2009) Influence of stearoyl-coenzyme A desaturase 1 genotype and stage of lactation on fatty acid composition of Canadian Jersey cows. Journal of Dairy Science, 92, 1220-8. DOI: https://doi.org/10.3168/jds.2008-1471

Khatib, H., Monson, R.L., Schutzkus, V., Kohl, D.M., Rosa, G.J.M., Rutledge, J.J. (2008) Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle. Journal of Dairy Science, 91, 784-93. DOI: https://doi.org/10.3168/jds.2007-0669

Khatib, H., Zaitoun, I., Wiebelhaus-Finger, J., Chang, Y.M., Rosa, G.J.M. (2007) The association of bovine *PPARGC1A* and OPN genes with milk composition in two independent Holstein cattle populations. Journal of Dairy Science, 90, 2966-70. DOI: https://doi.org/10.3168/jds.2006-812

Lynch, M., Walsh, B. (1997) Genetics and Analysis of Quantitative Traits. Sinauer Associates, Inc., Sunderland, MA, USA.

Ma, Y., Khan, M.Z., Xiao, J., Alugongo, G.M., Chen, X., Chen, T., Liu, S., He, Z., Wang, J., Shah, M.K. (2021) Genetic markers associated with milk production traits in dairy cattle. Agriculture, 11, 1018. DOI: https://doi.org/10.3390/agriculture11101018

Macciotta, N.P., Mele, M., Conte, G., Serra, A., Cassandro, M., Dal Zotto, R., Borlino, A.C., Pagnacco, G., Secchiari, P. (2008) Association between a polymorphism at the stearoyl CoA desaturase locus and milk production traits in Italian Holsteins. Journal of Dairy Science, 91, 3184-9. DOI: https://doi.org/10.3168/jds.2007-0947

Maryam, J., Babar, M.E., Bao, Z., Nadeem, A. (2016) A novel selection signature in stearoyl-coenzyme A desaturase (*SCD*) gene for enhanced milk fat content in Bubalus bubalis. Tropical Animal Health and Production, 48, 1343-9. DOI: https://doi.org/10.1007/s11250-016-1092-8

Medrano, J.F., Johnson, A., DePeters, E.J., Islas, A. (2000) Genetic modification of the composition of milk fat: Identification of polymorphisms within the bovine stearoyl-CoA-desaturase gene. Pflugers Archiv, 439, R24-R.

Mele, M., Conte, G., Castiglioni, B., Chessa, S., Macciotta, N.P., Serra, A., Buccioni, A., Pagnacco, G., Secchiari, P. (2007) Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. Journal of Dairy Science, 90, 4458-65. DOI: https://doi.org/10.3168/jds.2006-617

Moioli, B., Contarini, G., Avalli, A., Catillo, G., Orru, L., De Matteis, G., Masoero, G., Napolitano, F. (2007) Short communication: Effect of stearoyl-coenzyme A desaturase polymorphism on fatty acid composition of milk. Journal of Dairy Science, 90, 3553-8. DOI: https://doi.org/10.3168/jds.2006-855

NLDP. (2007) National Livestock Development Policy, Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh.

Ozkan, H., Yakan, A. (2020) The relationship between milk fatty acid profile and expression levels of *SCD*, *FASN* and *SREBPF1* genes in damascus dairy goats. Kocatepe Veterinary Journal, 13, 294-303. DOI: https://doi.org/10.30607/kvj.728554

Pereira, P.C. (2014) Milk nutritional composition and its role in human health. Nutrition, 30, 619-27. DOI: https://doi.org/10.1016/j.nut.2013.10.011

Peterson, D.G., Kelsey, J.A., Bauman, D.E. (2002) Analysis of variation in cis-9, trans-11 conjugated linoleic acid (CLA) in milk fat of dairy cows. Journal of Dairy Science, 85, 2164-72. DOI: https://doi.org/10.3168/jds.S0022-0302(02)74295-1

Rodrigues, C.H.M., Pires, D.E.V., Ascher, D.B. (2021) DynaMut2: Assessing changes in stability and flexibility upon single and multiple point missense mutations. Protein Science, 30, 60-9. DOI: https://doi.org/10.1002/pro.3942

Rozario, L.T., Sharker, T., Nila, T.A. (2021) In silico analysis of deleterious SNPs of human MTUS1 gene and their impacts on subsequent protein structure and function. PloS one, 16, e0252932. DOI: https://doi.org/10.1371/journal.pone.0252932

Schennink, A., Heck, J.M., Bovenhuis, H., Visker, M.H., van Valenberg, H.J., van Arendonk, J.A. (2008) Milk fatty acid unsaturation: genetic parameters and effects of stearoyl-CoA desaturase (*SCD1*) and acyl CoA: diacylglycerol acyltransferase 1 (*DGAT1*). Journal of Dairy Science, 91, 2135-43. DOI: https://doi.org/10.3168/jds.2007-0825

Shimakata, T., Mihara, K. (1972) Reconstitution of hepatic microsomal stearoyl-coenzyme A desaturase system from solubilized components. Journal of Biochemistry, 72, 1163-74. DOI: https://doi.org/10.1093/oxfordjournals.jbchem.a130004

Strittmatter, P., Spatz, L., Corcoran, D., Rogers, M.J., Setlow, B., Redline, R. (1974) Purification and properties of rat liver microsomal stearyl coenzyme A desaturase. Proceedings of the National Academy of Sciences, 71, 4565. DOI: https://doi.org/10.1073/pnas.71.11.456

Taniguchi, M., Utsugi, T., Oyama, K., Mannen, H., Kobayashi, M., Tanabe, Y., Ogino, A., Tsuji, S. (2004) Genotype of stearoyl-coA desaturase is associated with fatty acid composition in Japanese Black cattle. Mammalian Genome, 15, 142-8. DOI: https://doi.org/10.1007/s00335-003-2286-8