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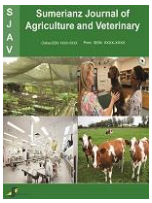

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Genetic Variation in Protein Structure and Phylogenetic Relationships of West African Dwarf Sheep: An Investigative Analysis

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Abstract

This research aimed to ascertain the secondary and tertiary protein structure variations in the CD14 gene of the West African Dwarf sheep (*Ovis aries*) from Cross River and Akwa Ibom States, Nigeria. The research used forty samples (20 from each state) of West African Dwarf sheep (WAD) from farms and markets in the two States. One ml of blood was collected from each sheep through jugular venipuncture for DNA extraction. The region of ***CD14*** of African Dwarf sheep was amplified by the Polymerase Chain Reaction (PCR) technique using specific sets of forward and reverse primers. The percentage of alpha helix in the secondary protein structure of WAD from AKS was higher than that detected in CRS. For the extended strand, the highest percentage was recorded from AKS17 (15.85%), while the lowest was from CRS14 (14.44%). Random coils had the highest percentage in the secondary protein structure and were generally similar among the selected WAD. The folding of the tertiary protein structure was generally similar among the WAD showing a close relationship in the activities of *CD14* among the sheep. There was a close relationship among the WAD from Akwa Ibom and Cross River States based on *CD14* gene variation.

Keywords: CD14 molecule; Protein structure; Sheep; Variation and phylogenetics.

1. Introduction

The West African Dwarf (WAD) sheep are relatively small ruminants, usually with a crimped hair called wool and often with horns forming a lateral spiral. These sheep are highly valued for their prolificacy, sexual precocity, and good fertility, making them important assets for agricultural communities in West Africa. Their ability to reproduce efficiently contributes significantly to efforts aimed at enhancing food security in the region. They are primarily reared for meat production [1] and are also a great support system for boosting food security. They are also occasionally raised for pelts, as dairy animals, or as model organisms for science [2] as well as cultural practices [3]. In Nigeria, sheep and other small ruminant represent about 63.70% of the total grazing domestic animals [4]. As reported by Adjibode, *et al.* [5] variation in carcass yielded 43.6% to 55.8% of the live weight of the animal. By providing a sustainable source of meat, milk, and other products, West African Dwarf sheep play a crucial role in addressing nutritional needs and alleviating poverty among local populations.

The WADs differ from their wild relatives and ancestors in several respects, having become uniquely neotenic as a result of selective breeding by humans [6]. A few primitive breeds of the sheep retain some of the characteristics of their wild relatives, such as short tails. Wild sheep show greater variations in terms of response to environmental changes and disease resistance [7]. Over time of selective breeding, WAD sheep is now reported as being hardy and possesses adaptability traits which include the ability to survive seasonal fluctuations, drought resistance and tolerance to diseases prevalent in the areas where they live [8]. The sheep also display variation in their colour pattern. As an African indigenous breed, WAD is not exempted from the harsh climatic condition that characterizes the tropics. Over the years, this environment has conferred great resilience on the WAD when compared to exotic breeds. These attributes of the dwarf sheep contribute to their resistance to disease partly initiated by the clusters of differentiated (CD14) gene.

Manipulation of the host immune response is the most precise and effective tool to lower disease incidences and to nullify the limitations associated with antibiotic treatment or vaccination [9]. Clusters of differentiation (CD14) is an important molecule for innate immunity. The cluster of differentiation (CD) is a multifaceted molecule with a variable range from CD1 to CD166, each exhibiting differential structures and functions. The CD14 is the most important part of this complex known to play a vital role against several enterotoxigenic bacteria. Its pattern recognition receptor binds mainly with LPS (lipopolysaccharide), lipoteichoic acid, and arachidonic acid and thus releases various cytokines that act as body defense [10].

There exists an imperative need to bolster disease-resistance traits within this specific breed. Regrettably, scant documentation is available regarding the CD14 gene in sheep, notably concerning protein structure variations or molecular characterization, as underscored by Singh, *et al.* [7]. Limited information exists regarding the CD14 gene in sheep, particularly regarding protein structure variations or molecular characterization. Singh, *et al.* [7] noted the scarcity of reports in this regard. Considering these gaps, our current investigation aimed to isolate and sequence the CD14 gene from indigenous dwarf sheep. Additionally, we also utilize bioinformatics tools to determine the secondary and tertiary protein structure characteristics of the CD14 gene.

2. Materials and Methods

2.1. Location and Sampling

Forty West African Dwarf sheep were sampled from farms and markets in Cross River and Akwa Ibom States, Nigeria. The samples constituted twenty each from the two States.

2.2. Blood Collection

One ml of blood was collected from each sheep through jugular venipuncture into EDTA bottles for DNA extraction.

2.3. DNA Extraction

Genomic DNA extraction was carried out using quick - DNA™ miniprep plus kit protocol. Four volumes of genomic lysis buffer were added to the sample after vortexing for 4-6 seconds, then let to stand at room temperature for 5-10 minutes and thoroughly centrifuged to remove particulate debris. The mixtures were then transferred to a Zymo-Spin™ column in a collection tube and centrifuged at 10000 g for one minute. The collection tubes were discarded with the flow through. The Zymo spin column was transferred to a new collection tube. 200µl of DNA pre-wash buffer was added to the spin column, followed by one-minute centrifugation at 10000 g. 500 µl of g-DNA wash buffer was added to the spin column followed by centrifugation for one minute at 10000 g. After this, the spin column was then transferred to a micro centrifuge tube. 50µl of DNA elution buffer was added to the spin column and incubated for 30 seconds at room temperature. The last step was repeated to elute the DNA. The eluted DNA in the microcentrifuge tube was stored at less than 20˚C pending the amplification.

2.4. Sequencing of *CD14* gene

Sequencing reaction was performed in International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria with AB13730×L sequencer using 20 µl reaction comprising approximately 20 ng of purified PCR product as template DNA, 8 µl of Big Dye Terminator Reaction Mix (dNTPs, ddNTPs, buffer, enzyme and $MgCl_2$), 8 µl of deionized water, 2 µl of primer programmed as 25 cycles at 96 ^oC for 10 seconds, 60 ^oC for five seconds and 60 ^oC for four minutes.

2.5. Statistical Analysis

Bioedit software version 7.2.5 [11] was employed to view and edit the sequences. MEGA 7.0 was used for multiple sequence alignment of all the samples excluding all the gaps [12]. The phylogenetic relationship among the WAD sheep was constructed using aligned CD14 gene sequences on MEGA software. The secondary protein structure was predicted using online Gor4 software from https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html while the tertiary structure was predicted using phyre2 from http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index.

3. Results

3.1. Secondary and Tertiary Protein Structure

Table 1 shows the percentage subunits of alpha, extended strand and random coil found in the secondary protein structure of the ***CD14*** gene of *Ovis aries* from AKS and CRS. Five individuals each from AKS and CRS were selected for secondary protein structure evaluations. The percentage of alpha helix in the secondary protein structure of WAD from AKS was higher than that detected in CRS. For the extended strand, the highest percentage was recorded from AKS17(15.85%) while the lowest was from CRS14 (14.44%). Random coils had the highest percentage in the secondary protein structure and were generally similar among the selected WAD sheep. Figure 1, 2, 3, 4, 5 shows the tertiary protein structure of WAD from AKS and CRS. The folding of the tertiary protein structure was generally similar among the WAD sheep.

3.2. Phylogenetic Relationship

The phylogenetic analysis results, depicted in Figure 6, showed the genetic relationships among West African Dwarf (WAD) sheep. These results revealed the clustering of sheep into distinct subgroups, delineated by two major clusters. The first major cluster comprised three samples of WAD sheep exclusively from Akwa Ibom State denoted as AKS1, AKS2, and AKS3. Conversely, the second major cluster included samples from both Akwa Ibom and Cross River States. The results of the phylogenetics revealed a close relationship among the sheep.

3.3. Discussion

The molecule CD14 stands out as one of the most significant among the diverse array of CD molecules, ranging from 1 to 166, as documented by Chessa, *et al.* [10]. During our investigation, distinct subunits were identified in the secondary protein structure of the CD14 gene of *Ovis aries* originating from Akwa Ibom State (AKS) and Cross River State (CRS). Notably, variations were observed in the secondary protein structure, including alpha helix, extended strand, and random coil, across the five individuals selected from each region. Specifically, individuals from AKS exhibited a higher percentage of alpha helix compared to those from CRS, suggesting regional differences. Additionally, variability within regions was evident, with AKS displaying the highest percentage of extended strands among the samples analyzed. Furthermore, the random coil exhibited both the highest percentage and highest similarity in the secondary protein structure among the selected WAD sheep. These differences underscore the genetic diversity within these populations, providing breeders with valuable insights for strategic breeding programmes. This diversity facilitates the inheritance of a broad range of traits, enabling breeders to tailor programs to specific goals, such as disease resistance or wool quality enhancement, as emphasized by Pleurdeau, *et al.* [2].

These findings align with the notion proposed by Pleurdeau, *et al.* [2] that WAD sheep may serve as model organisms due to their genetic proximity compared to other small ruminants. Analysis of the tertiary protein structure further revealed minimal variation when combining genes from AKS and CRS sheep, suggesting a close relationship in CD14 activities among these populations. The close relationship observed among the sheep populations, as revealed also by the phylogenetic analysis, underscores the genetic coherence and shared ancestry among WAD sheep breeds. This finding corroborates previous research highlighting the genetic similarity and relatedness among WAD sheep populations across different regions [10]. This similarity could imply consistency in traits related to CD14 function and may aid breeders in managing their flocks effectively. Moreover, the observed genetic stability in CD14 among WAD sheep holds implications for selective breeding strategies. By leveraging this stability, breeders can enhance desirable traits, such as disease resistance, over successive generations. The genetic relationships among WAD sheep populations are essential for conservation efforts, breeding programs, and the sustainable management of genetic resources. This targeted approach may lead to improved overall flock health and performance.

However, it is crucial to address the challenge posed by the poor adaptability and disease resistance of WAD sheep, as highlighted by Singh, *et al.* [7]. Enhanced disease resistance associated with CD14 is integral to the immune system, presenting an opportunity for selective breeding to bolster overall flock health. Understanding individual differences and shared traits could allow breeders to implement tailored practices, including nutrition, health protocols, and environmental management, to optimize flock management. Additionally, identifying variations in protein structures aids in assessing vulnerabilities or strengths within the flock, informing breeding and management decisions. The analysis of variation in CD14 protein structures in the present study provides valuable insights for breeders to improve the health, resilience, and performance of West African Dwarf sheep populations.

4. Conclusion

In conclusion, our study sheds light on the genetic and protein structure diversity of the CD14 gene in West African Dwarf (WAD) sheep from Akwa Ibom State (AKS) and Cross River State (CRS). We found regional variations in CD14 protein structure, suggesting genetic diversity within these populations. Despite differences, AKS and CRS WAD sheep exhibited a close relationship in CD14 function, indicating consistent traits similarities. This stability offers opportunities for selective breeding to enhance disease resistance and flock health. Addressing challenges like poor adaptability and disease resistance remains critical. Overall, our findings provide insights for improving the resilience and productivity of WAD sheep populations through informed breeding strategies.

4.1. Conflict of Interest

The authors declare that there is no existing conflict of interest

Acknowledgment

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Table-1. Secondary protein structure subunits in CD14 of *Ovis aries*

Subunits	AKS15	AKS17	AKS18	AKS20	AKS11	CRS12	CRS14	CRS18	CRS19	CRS15
Alpha helix (%)	18.73	18.66	18.73	18.73	18.73	17. 96	17.96	17.89	17.96	18.02
Extended strand (%)	15.19	15.85	14.49	14.49	14.13	14.79	14.44	15.79	15.14	14.84
Random coil (%)	66.08	65.49	66.78	66.78	67.14	67.52	67.61	66.32	66.90	67.14

AKS= Akwa Ibom State; CRS= Cross River

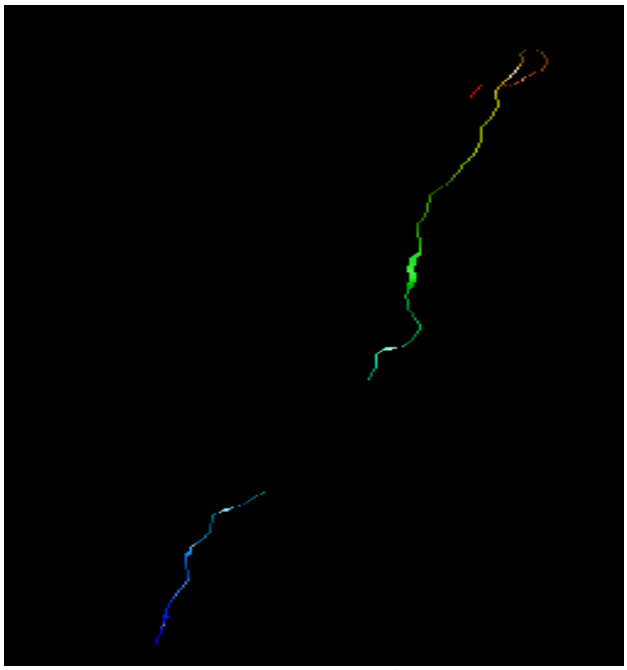

Figure-1a. Tertiary protein structure of AKS15 *CD14* gene

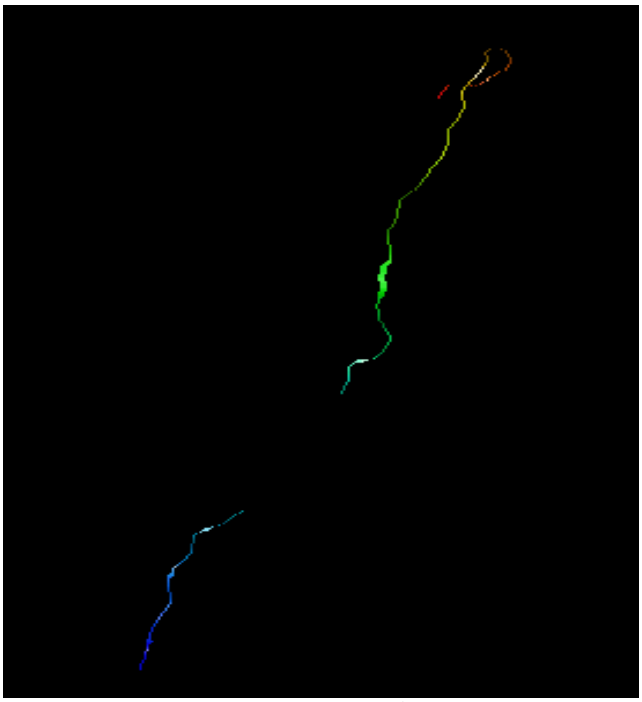

Figure-1b. Tertiary protein structure of AKS17 *CD14* gene

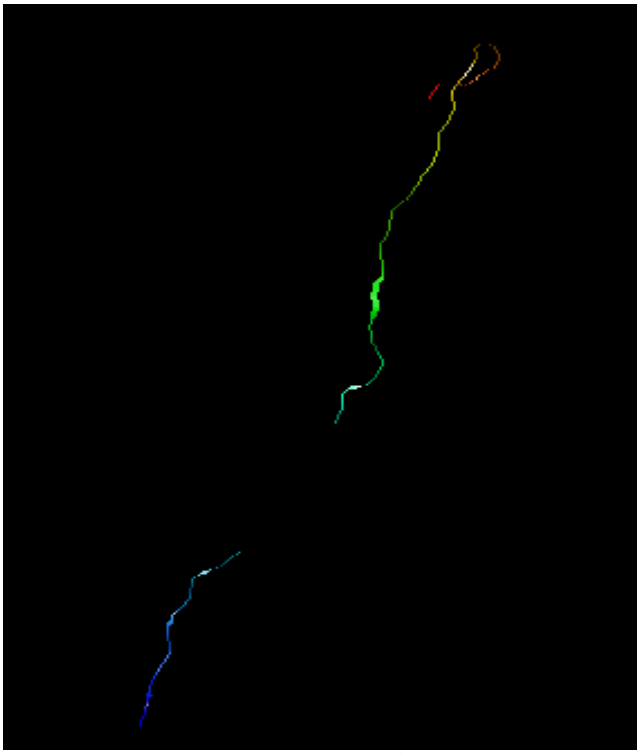

Figure-2a. Tertiary protein structure of AKS18 *CD14* gene

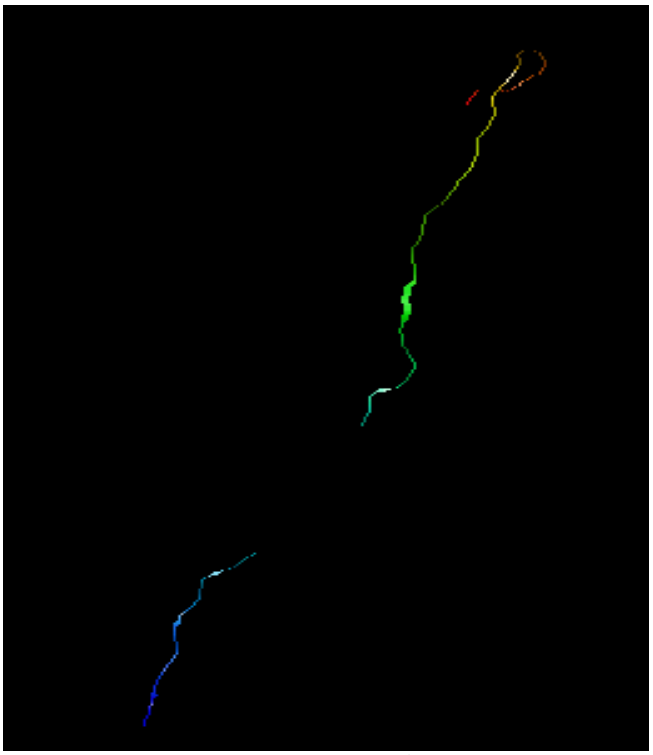

Figure-2b. Tertiary protein structure of CRS20 *CD14* gene

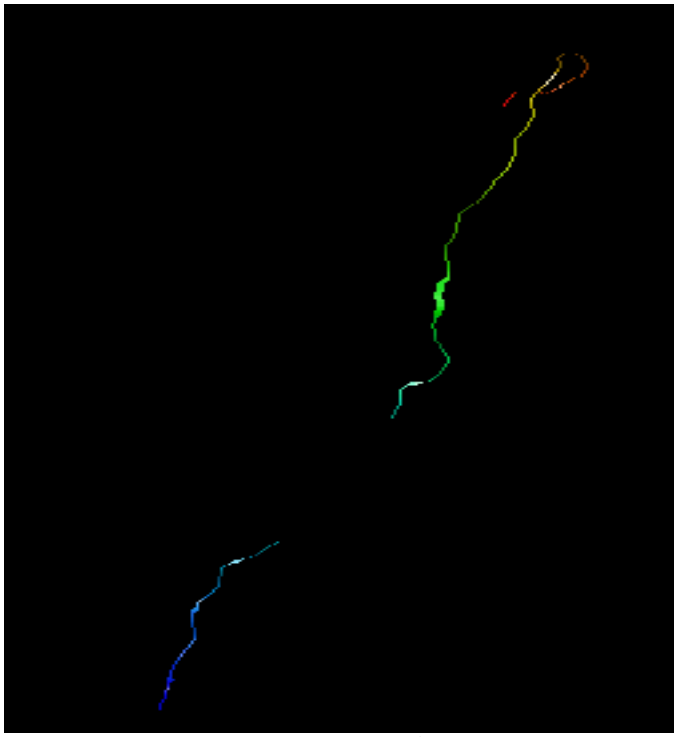

Figure-3a. Tertiary protein structure of AKS11 *CD14* gene

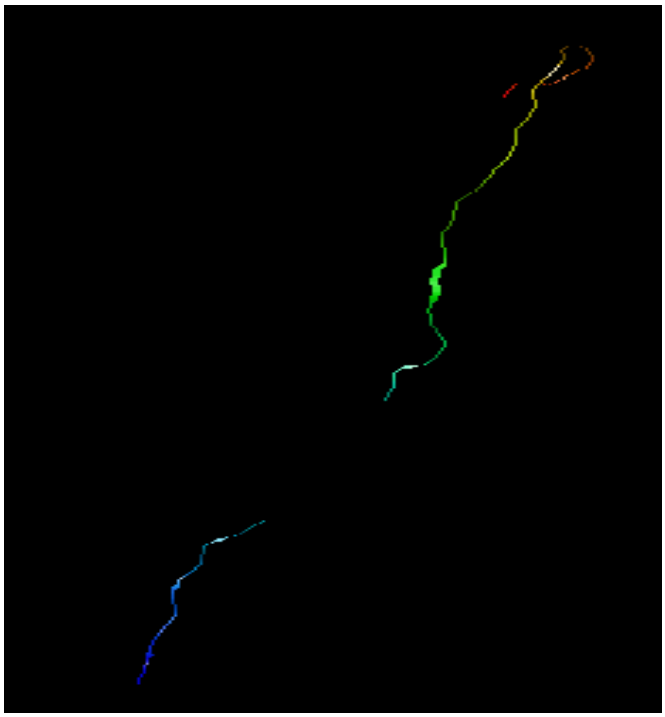

Figure-3b. Tertiary protein structure of CRS12 *CD14* gene

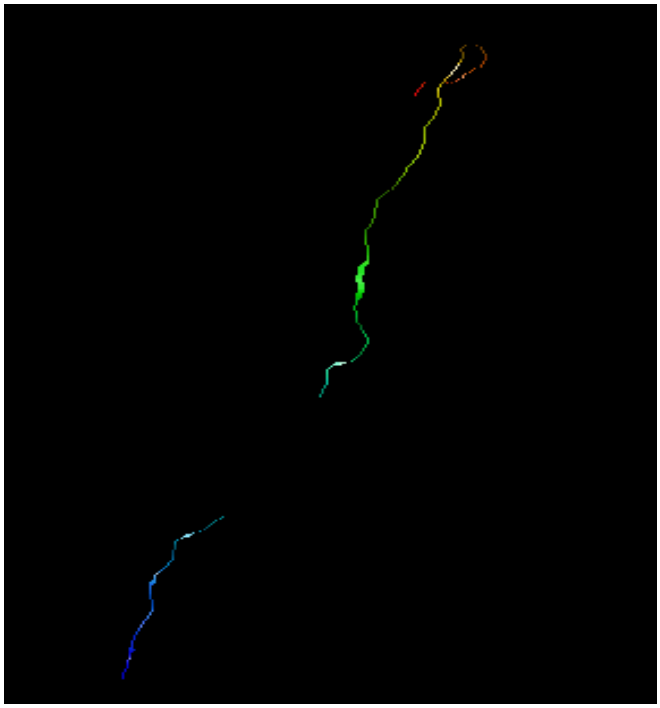

Figure-4a. Tertiary protein structure of CRS14 *CD14* gene

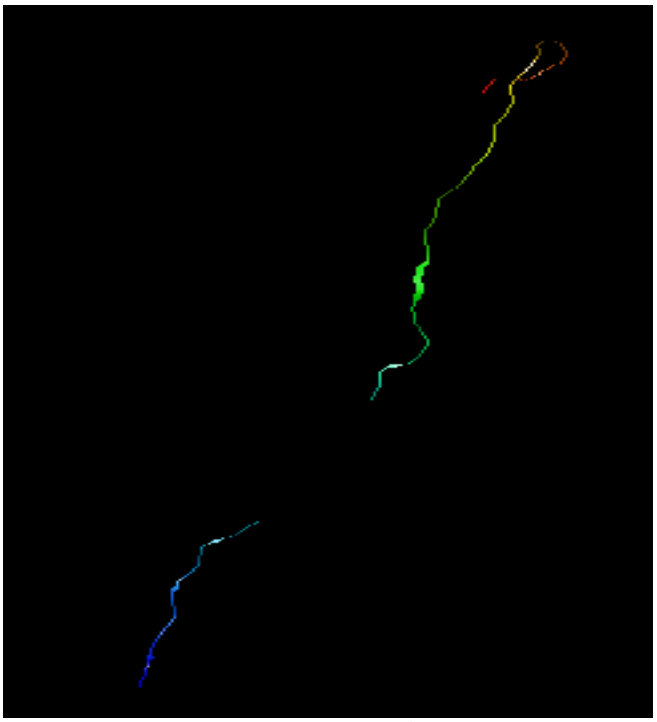

Figure-4b. Tertiary protein structure of CRS18 *CD14* gene

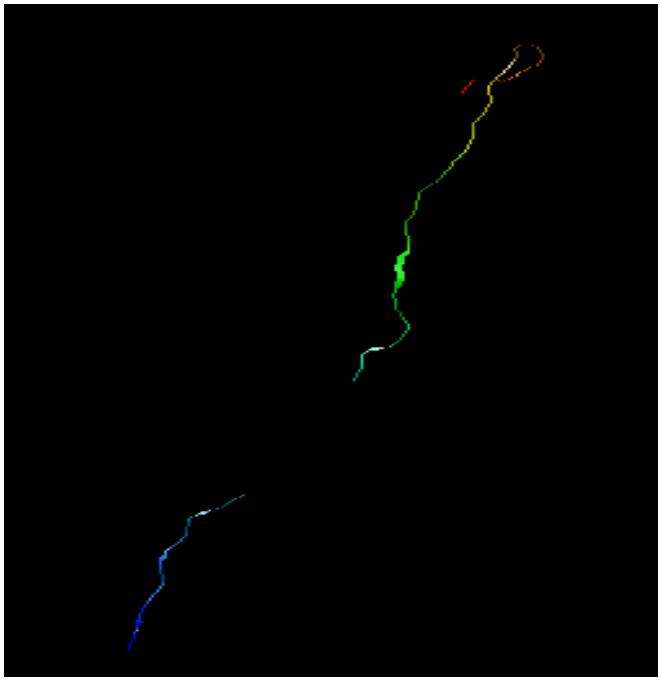

Figure-5a. Tertiary protein structure of CRS19 *CD14* gene

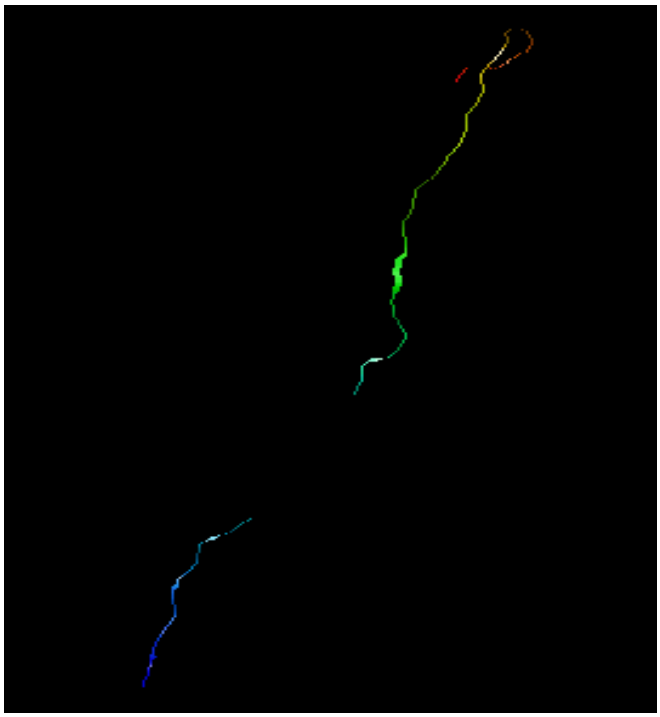

Figure-5b. Tertiary protein structure of CRS15 *CD14* gene

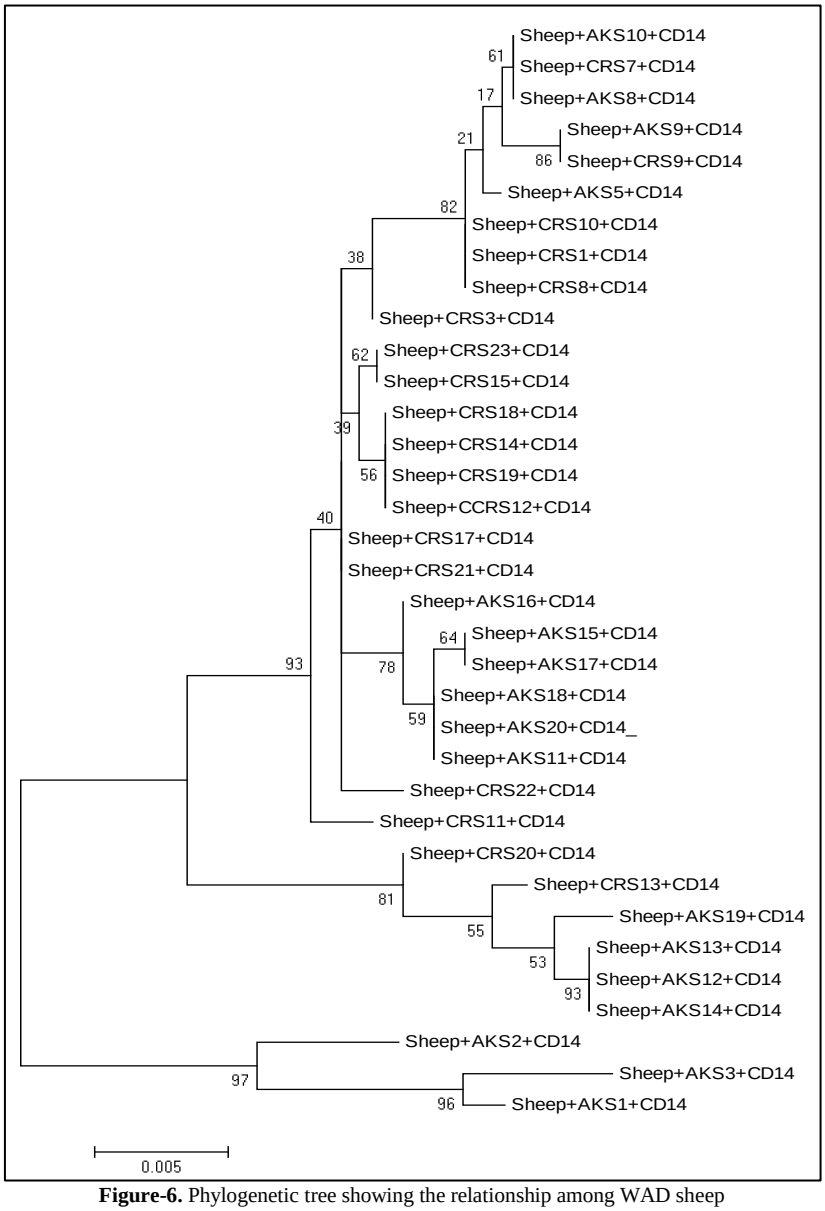

Figure-6. Phylogenetic tree showing the relationship among WAD sheep

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