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Research Article

Molecular Detection of Selected Genetic Polymorphisms in Growth Hormone and Insulin Like Growth Factor 1 Genes in Indigenous Sudanese Baggara Cattle

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Abstract

The study involved 127 bulls of two geographically separate strains of Sudanese Baggara zebu cattle (Nyalawi and Mesairi). The target was to investigate two polymorphisms in growth hormone gene (M57764) and insulin like growth factor1 gene (000162.1). PCR-RFLP was used to genotype DNA samples and DNA sequencing was used to check the accuracy of genotyping results using selected samples. Available sequences were analyzed using BioEdit and MEGA6 softwares. Some population genetic measures in the two strains of Baggara zebu cattle were investigated. PCR-RFLP revealed that the two Baggara cattle strains have high genetic similarity at position 2141C>G of the *GH1* gene (monomorphic showing the ancestral allele C/C). There were no differences between the two strains at position -472C>T of the *IGF-1* gene promoter. The mutant homozygote (TT) was detected in the Mesairi strain only with a frequency of 0.016. The heterozygote (CT) genotype existed in the two strains with low allele frequencies (0. 068 and 0.079 for Nyalawi and Mesairi respectively). Moreover, three mutations were identified in exon5 of the *GH1* sequence including two silent mutations at positions 2230 (C>T) and 2291(A>C) and a third transition mutation at position 2258 (C/T) detected in the sequences of the two strains. In conclusion, the two strains were found to be genetically similar at target positions. The detected mutation at exon 5 of *GH1* (2258 (C/T) should be validated.

Keywords: *Nyalawi, Mesairi, Polymorphisms, IGF-1, GH1*

Yerli Sudan Baggara Sığırında Büyüme Hormonu ve İnsülin Benzeri Büyüme Faktörü 1 Genlerinin Seçili Genetik Polimorfizmlerinin Moleküler Tespiti

Öz

Bu çalışma bölgesel olarak farklı iki (Nyalawi ve Mesairi) Sudan Baggara zebu sığırlarından toplam 127 boğa üzerinde gerçekleştirilmiştir. Çalışmanın amacı bu iki farklı ırk arasında büyüme hormonu geni (M57764) ve insülin benzeri büyüme faktörü 1 genindeki (000162.1) polimorfizmi araştırmaktır. DNA örneklerinde genotiplendirme için PCR-RFLP kullanıldı. Seçili örneklerde genotiplendirmenin doğrulunu kontrol etmek amacıyla DNA sekanslaması kullanıldı. Mevcut sekanslar BioEdit ve MEGA6 yazılımları kullanılarak analiz edildi. Her iki Baggara sığır ırkında bazı popülasyon genetiği ölçümleri araştırıldı. PCR-RFLP sonuçları her iki Baggara sığır ırkında *GH1* geninin 2141C>G pozisyonunda yüksek genetik benzerlik olduğunu ortaya koydu (monomorfik atasal allel C/C görünümü). Her iki ırk arasında *IGF-1* geninin -472C>T pozisyonu bakımından bir fark belirlenmedi. Sadece 0.016 sıklıkta olmak üzere Mesairi ırkında mutat homozigot (TT) belirlendi. Düşük allel sıklıklarında olmak üzere (0. 068 Nyalawi ırkında ve 0.079 Mesairi ırkında) heterozigot (CT) genotip mevcuttu. Ayrıca, *GH1* geninin ekson 5 bölgesinde üç mutasyon tespit edildi. Bunlardan ikisi 2230 (C>T) ve 2291(A>C) pozisyonlarında sessiz mutasyon ve diğeri 2258 (C/T) pozisyonunda geçiş mutasyonuydu. Sonuç olarak, her iki ırkın incelenen hedef poziylonlar bakımından genetik olarak benzer oldukları belirlendi. *GH1* geninin ekson 5 bölgesinde tespit edilen mutasyon (2258 (C/T) ileriki çalışmalarda değerlendirilmelidir.

Anahtar sözcükler: *Nyalawi, Mesairi, Polimorfizm, IGF-1, GH1*

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INTRODUCTION

The Sudan is one of the largest African countries. It is predominantly an agricultural country with a significant development potential, especially in agriculture and livestock production. The local Sudanese cattle breeds belong to the Bos indicus species often referred to as Zebu type. The population of indigenous cattle was estimated at 40 million heads [1]. Kenana and Butana are the main dairy breeds, while western Baggara is the major beef producing cattle breed in Sudan and some neighbouring African countries used for local consumption and export [2]. About 90% of the cattle population is owned by pastoralists mainly concentrated in three major regions, mainly, Western Sudan, the homeland of Baggara cattle. The Baggara cattle and their different strains constitute about 80 percent of the country's total population of cattle.

Nyalawi and Mesairi are two important Baggara cattle strains. The two strains have different characteristic phenotypic morphologies. Both strains are main producers of beef for local consumption and export. However, Nyalawi strain consistently reported as having superior phenotypic beef characteristics [3].

Several genes were putatively controlling growth in animals, in which the somatotropic axis plays a key role. Growth hormone *(GH1)* and insulin-like growth factor 1 *(IGF-1)* genes act and mediate the growth of bones and muscles [4]. The two genes are considered as candidate genes for predicting growth and meat quality traits in animal genetic improvement since they play a key role in growth regulation and development [5]. Growth hormone *(GH),* a single chain polypeptide, was shown to stimulate fatty acid mobilization, amino acid uptake, DNA, RNA and protein synthesis and that it regulates cell division and tissue hypertrophy. It is known that *GH* is the main regulator of postnatal somatic growth, stimulating anabolic processes such as cell division, skeletal growth and protein synthesis and is involved in nutrient partition [6]. The bovine GH gene has several genetic variants including a characterized substitution C>G (leucine/valine) at position 2141 *(rs:4193484)*. The insulin like growth factor 1 *(IGF1)* has many diverse effects; it catalyses skeletal muscle hypertrophy through initiating protein synthesis, and blocking muscle atrophy. The decline in growth hormone secretion and plasma *IGF-1* concentrations is known to occur with age [7]. IGF1 plays an essential role in mammalian reproduction. *IGF* system has a significant relationship with prenatal growth and the growth and differentiation of the mammary gland [8].

The *IGF-1* gene, in comparison to GH1 gene, is extremely conserved among species and few polymorphisms are described including the -472C>T *(rs:109763947)* polymorphism at the 5'region of the gene.

The present study aimed at screening the two cattle strains for the single nucleotide polymorphism 2141C>G in the *GH1* gene and the -472C>T in the *IGF-1* gene. The two mutations were not previously reported in Baggara cattle. The study also used available sequences of the two genes to search for other mutations if they exist and investigate some population genetic measures in the two strains of Baggara zebu cattle (Nyalawi and Mesairi).

MATERIAL and METHODS

Ethics Approval

According to the Animals Use in Research Committee (AURC) of Khartoum University, this study does not require any special approval.

Animals and Sampling

The present investigation was carried out on 127 Baggara bulls that belong to two strains (Nyalawi and Mesairi). All animals were on average 4-5 years old finished in a feedlot near Ganawa commercial abattoir in Omdurman locality, Khartoum State, Sudan. One hundred and twenty seven Baggara blood samples were taken from the jugular vein of each bull using vacutainers containing EDTA as an anticoagulant and held at 4°C. Samples were transferred to the Centre of Excellence, Genetic and Molecular Biology Laboratory at the Department of Zoology Science, Faculty of Science, University of Khartoum where they were kept in a refrigerator pending genotyping and sequencing analysis.

Genomic DNA Extraction

Blood samples (3-5 mL) were collected in EDTA tubes. DNA was extracted following conventional methods [9] procedure with some modifications. Briefly, two ml of lysis buffer were added, followed by 10 µL of proteinase K, 1 mL of guanidine chloride and 300 µL of NH4 acetate. The mixture was incubated over night at 37°C, and then 2 mL of pre-chilled chloroform was added. The upper layer was collected -after centrifugation- to a new tube and DNA was precipitated by absolute ethanol. DNA was washed with 70% Ethanol and the pellet was allowed to dry. The pellet was re-suspended in 200 µL ddH_2O and DNA stock solution was stored at -20°C. The quality of the extracted DNA was measured using Nano-Drop Spectrophotometer (ND 1000) and agarose gel electrophoresis.

Genotyping of GH1 and IGF1 Genes

The growth hormone gene *(GH1)* locus was analyzed targeting a 404 bp fragment covering the sequence containing the 2141C>G missense mutation in exon 5. Similarly, a 249 bp fragment containing the C/T mutation at position -472 in the 5-non coding region of the *IGF-1* gene relative to the start of transcription site was also analyzed. Amplification procedure of the targeted *GH1* [10] was conducted using the forward primer:

F: 5′-TAGGGGAGGGTGGAAAATGGA and the reverse R: 5′ GACACCTACTCAGACAATGCG-3′ and the procedure for the IGF-1 [11] using the primers F: 5′-ATTACAAAGCTGCCTGCC CC-3′ and R: 5′-ACCTTACCCGTATGAAAGGAATATACGT-3′. The resulting PCR fragments were digested using restriction enzymes (Alu1and SnaB1 for the two genes, respectively).

Sequence Analysis and Alignment Procedures

One PCR product of each different genotype of the *GH1* and *IGF-1* genes were sequenced to confirm the detected genotypes using ABI 3730xl DNA Analyzers, with BigDye Terminator v3.1. PCR products were purified prior to sequencing and were sequenced in both directions. Resulted sequences were aligned and compared using BioEdit program in addition to selected available GenBank sequences representing *GH1* (EF592533 and EF592534) and *IGF1* (AF404761, KF202095 and KM111250) genes. The number of base substitutions per site between sequences and standard error estimate (s) analyses were conducted using the Tajima-Nei model [12]. Evolutionary analyses (Molecular Evolutionary Genetic Analysis) were conducted by MEGA6 [13]. The gene and genotype frequencies were calculated using Falconer and Mackay [14] Method.

RESULTS

Genotyping of Growth Hormone1 Gene (GH1)

The missense mutation polymorphism in the *GH1* which was reported to occur in a single nucleotide causing a C/G substitution resulting in the replacement of leucine with valine was not present in the studied populations. The digestion of the 404 bp PCR product *(Fig. 1)* with the *Alu*1(AG/CT) endonuclease enzyme resulted in one restriction pattern which was assigned as homozygous genotype CC that produced four fragments (185-132-51-36 bp) in all animals under study *(Fig. 2)*. There was a complete absence of both homozygous (TT) genotype (236-132-36 bp) and the heterozygous (CT) genotype (236-185-132-51-36 bp) in all animals under study *(Table 1)*.

Bovine GH1 Gene Sequencing

Sequence Comparison: The results of the present study showed that Baggara cattle (Nyalawi and Mesairi) were homozygous for the 2141C allele (monomorphic for the Leucine variant) in the 2141C>G SNP (leucine to valine CTG/GTG) *(Fig. 3* and *Fig. 7-A)*. Similar results were found

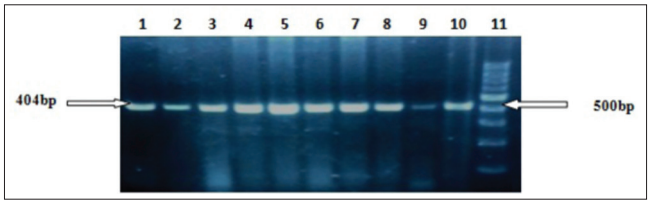

Fig 1. Agarose gel electrophoresis (2%) displaying PCR amplification of exon5 of the Baggara *GH1* Lanes:1-10 PCR products of samples. Lane 11: 100 bp DNA Ladder

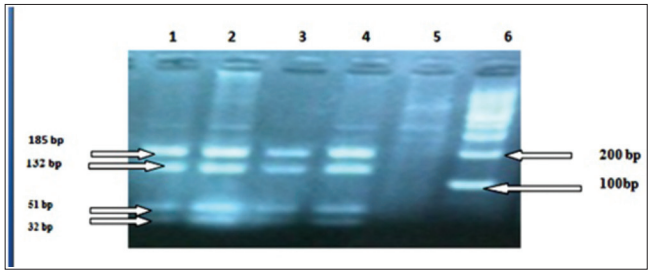

Fig 2. Agarose gel electrophoresis (2%) displaying *Alu*1 restriction digest on an amplified portion of exon 5 of the Baggara *GH1*. Lanes: 1-4 representing CC genotype (185+132+51+36 bp). Lane 5: negative control. Lane 6: 100bp DNA ladder

Table 1. *Genotypic and allelic frequencies, χ2 test values and diversity parameters for the 2141C>G (Leu/Val) substitution of GH1 and the-472 C>T of the IGF-1 gene*

Baggara Strains (Number)	Gene Name	Genotype	*No.	GF	Allele	AF	χ2 /HWE	He
Nyalawi (64)	GH1	CC	64	1	C	1		0
		CG	0	0	G	0		
		GG	0	0				
	IGF-1	CC	51	0.864	C	0.932	0.407 NS	0.1453
		CT	8	0.136	T	0. 068		
		TT	0.0	0.0				
Mesairi (63)	GH1	CC	63	1	C	1		0
		CG	0	0	G	0		
		GG	0	0				
	IGF-1	CC	54	0.857	C	0.921	1.085 NS	0.1455
		CT	8	0.127	T	0.079		
		TT	1	0.016				

** The given numbers are those of DNA samples successfully amplified GF: Genotype frequency, AF: Allelic frequency, χ2 (HWE): Hardy-Weinberg equilibrium χ2 value, Hardy-Weinberg equilibrium (P>.05), He: Gene heterozygosity*

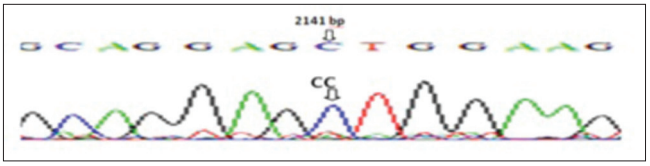

Fig 3. The homozygous (CC) at the 2141 position of the *GH1* detected in both Nyalawi and Mesairi cattle strains

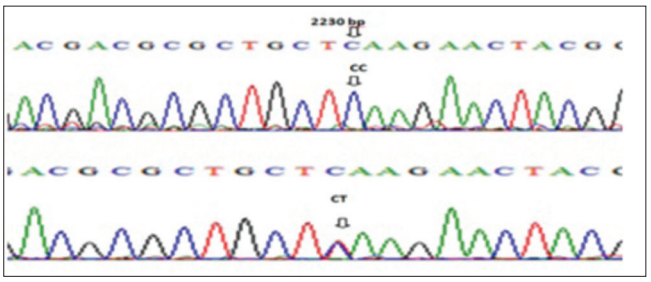

Fig 4. The silent mutation at the position 2230bp (Leucine to leucine, CTC/CTT) of the *GH1* detected in Nyalawi sequence

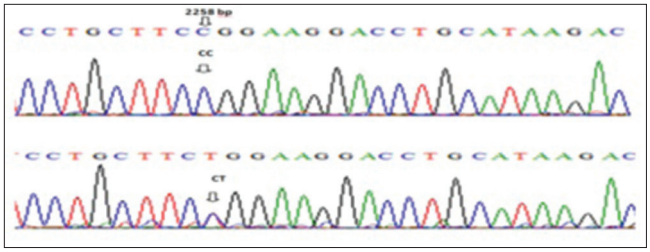

Fig 5. The transition mutation at the position 2258 bp (Arginine to Tryptophan CGG/TGG) GH1detected in both Nyalawi and Mesairi cattle strains

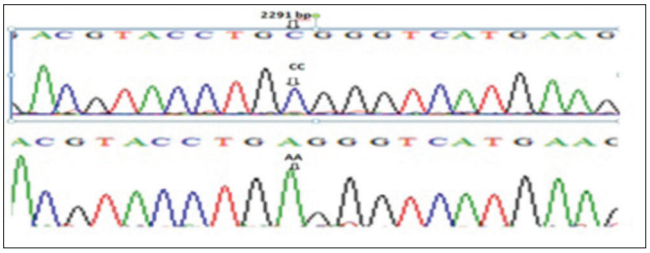

Fig 6. The silent mutation at the position (2291A>C) (Arginine to Arginine AGG/CGG) of the *GH1* gene and was found in Baggara cattle strains, Butana cattle and Kenana cattle

in Butana and Kenana *GH1* gene according to GenBank (EF592533 and EF592534, respectively). Moreover, three non-targeted mutations were identified in exon 5. The first mutation was a C/T transition at position 2230 in which a C nucleotide turned into T nucleotide (C/T) to produce a silent mutation (Leucine to leucine, CTC/CTT) detected only in Nyalawi sequence *(Fig. 4* and *Fig. 7-D)*. A second transition mutation was detected at 2258 bp (C/T) (Arginine to Tryptophan CGG/TGG), which existed in the sequences of the two Baggara strains *(Fig. 5* and *Fig. 7-D),* but it was not found in Butana and Kenana cattle *GH1* sequence according to GenBank records *(Fig. 7D)*. No mutations were detected in exon 5 of the *GH1* gene sequences at A, B, C, F, H and I *(Fig. 7 A,B,C,F,H,I)*.

The third silent mutation was at 2291A>C (Arginine to Arginine AGG/CGG) of the *GH1* gene and was found in Baggara cattle strains, and in the Sudanese Butana and Kenana cattle *(Fig. 6* and *Fig. 7-E)*.

Alignment of sequences also indicated a nucleotide change (C/T) at position 2346 present in Kenana cattle *(Fig. 7-G)* and absent in all study animals and Butana cattle sequences.

The Phylogenetic analysis of Baggara, Kenana (EF592534.1) and Butana (EF592533.1) cattle *GH1* gene sequences *(Fig. 8)* indicated two major clusters. The Baggara strains in one cluster with two sub-branches containing the two strains (Nyalawi and Mesairi) both in the same major cluster. The Baggara strains were not similar to Kenana *GH1* gene (EF592534.1) and Butana (EF592533.1) cattle at that region of *GH1* gene.

Insulin- Like Growth Factor1 Gene

The missense mutation (on chromosome 5) in the bovine Insulin like growth factor 1 *(IGF1)* gene causes the replacement of cytosine (C) by thymine (T) at position -472 in the 5'noncoding region of the *IGF-1* gene. The digestion of the 249 bp *(Fig. 9)* PCR products was carried out with the restriction endonuclease enzyme SnaBI (TAC/GTA). Three restriction patterns were obtained; the uncut pattern was assigned as the homozygote wild type genotype (CC) (249 bp), the heterozygote genotype (CT) produced three fragments (249, 223 and 26 bp), while the homozygous (TT) produced two fragments (223 - 26 bp) *(Fig. 10)*.

The gene and genotype frequency of the IGF1 gene were as shown in *Table 1*. The wild type C variant frequency was the highest among the two cattle strains. The chi- square test values indicated that the two strains were at Hardy-Weinberg equilibrium *(Table 1)*. The heterozygosity (He) was the same (0.145) in the two cattle strains.

IGF1 Gene Sequencing

In addition to the GenBank *IGF1* gene sequence (AC: 000162.1), the obtained *IGF1* sequences of Baggara strains were aligned and compared with other available *IGF1* gene sequences in the GenBank (AF404761, KF202095 and KM111250) using BioEdit program. The transition mutation in the *IGF1* gene (C>T) was found in the homozygous state (TT) in the Mesairi subtype only, whereas the heterozygote genotype (CT) was detected in both strains *(Fig. 11* and *Fig. 12-C)*. This SNP was also found in Bos taurus according to GenBank (AF404761 and KF202095). Another SNP (-468 bp; T-A; Not registered) was observed in both Bos taurus (AF404761) and Bos indicus (KM111250) and absent in the third sequence *(Bos taurus,*

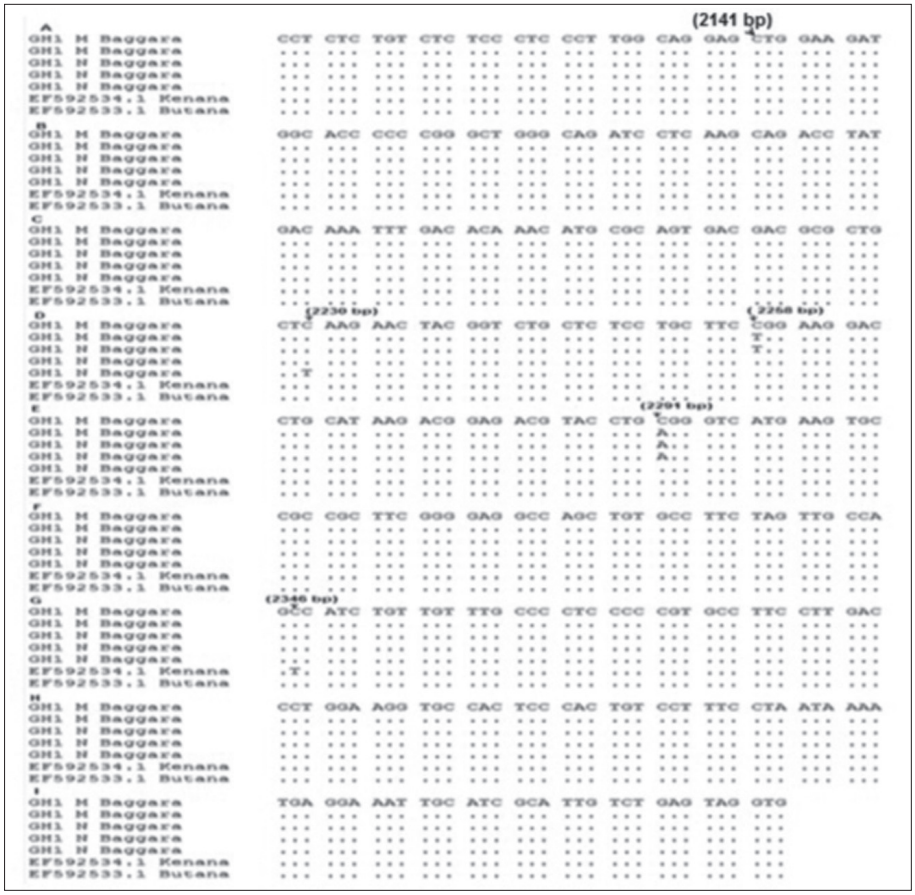

Fig 7. Comparison of the *GH1* (exon5) sequences of Baggara, Kenana and Butana cattle* Accession numbers: EF592533 and EF592534 are the sequences of Butana and Kenana of *GH1* gene in the GenBank. M: Mesairi strain, N: Nyalawi strain. Aligned sequences of complete exon5 and flanking region are presented in 9 patches and given letters for simplicity (A, B, C, D, E, F, G, H & I)

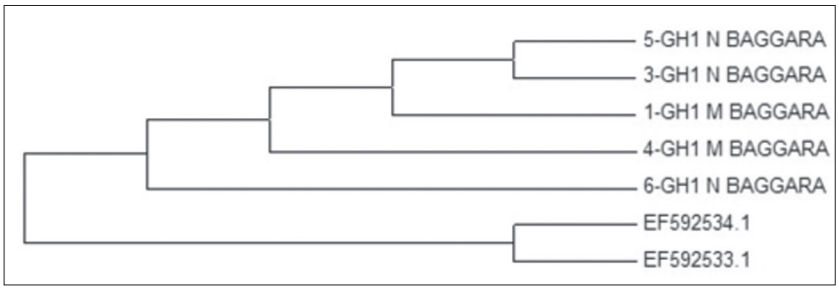

Fig 8. Phylogenetic tree of the *GH1* gene sequences of Baggara, Kenana (EF592534.1) and Butana (EF592533.1) cattle

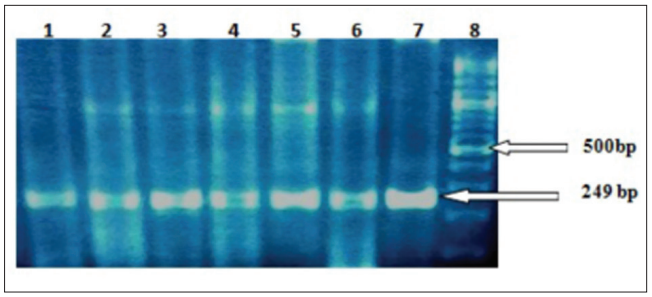

Fig 9. Agarose gel electrophoresis (2%) displaying PCR amplification of Baggara *IGF1* 5'- flanking region. Lanes: 1-7 PCR products of samples. Lane 8: 100 bp DNA Ladder

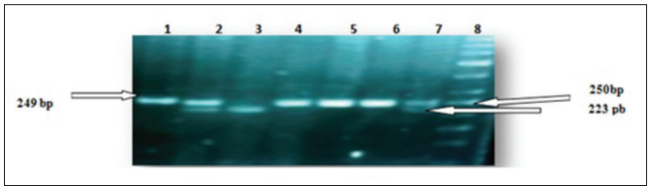

Fig 10. Agarose gel electrophoresis (2%) displaying SnaB1 restriction digest of an amplified portion of Baggara *IGF1* gene in the 5'- flanking region. Lanes: 1, 4, 5 and 6 representing CC genotype (249 bp). Lane 3 representing TT genotype (223+26). Lanes 2 and 7 representing CT genotype (249+223+26). Lane 8: 50 bp DNA ladder

KF202095), while it was presented as homozygote TT in both Baggara cattle strains *(Fig.12 -C)*. No mutations were detected at in the A and B parts of the sequence *(Fig. 12 A,B)*.

In Phylogentic analysis, *IGF1* gene sequence of the Nyalawi and Mesairi clustered within the same main cluster of Bos indicus while the Bos taurus clustered in a different isolated cluster *(Fig. 13)*.

Selected sequences were submitted at the GenBank and

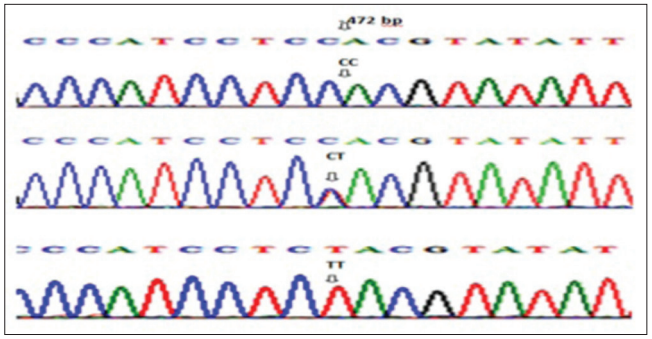

Fig 11. The chromatogram of the sequenced 5'-noncoding region *IGF1* gene showing homozygote (CC and TT) and the heterozygote (CT) genotypes

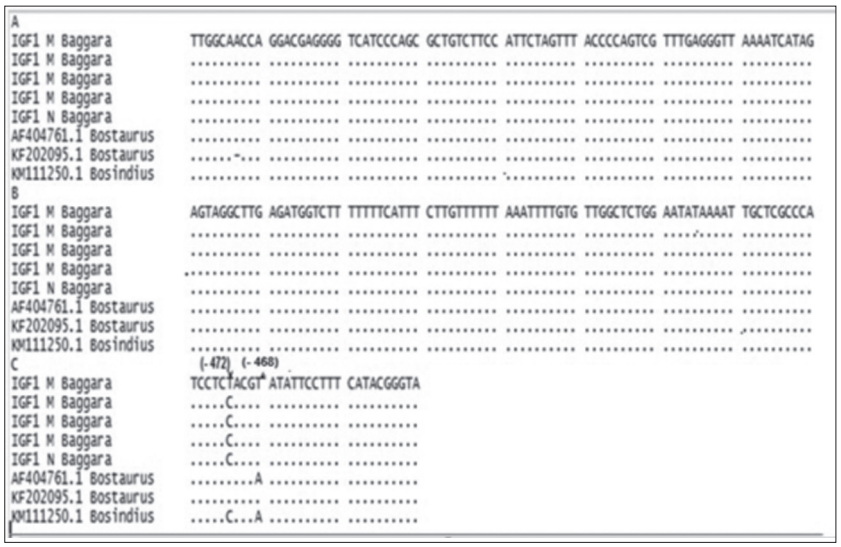

Fig 12. Comparison of the *IGF1* gene sequences of Baggara, Bos taurus (AF404761 and KF202095) and Bos indicus cattle (KM111250). M: Mesairi strain. N: Nyalawi strain. Aligned sequences of partial 5' UTR are presented in 3 patches and given the letters A, B, & C

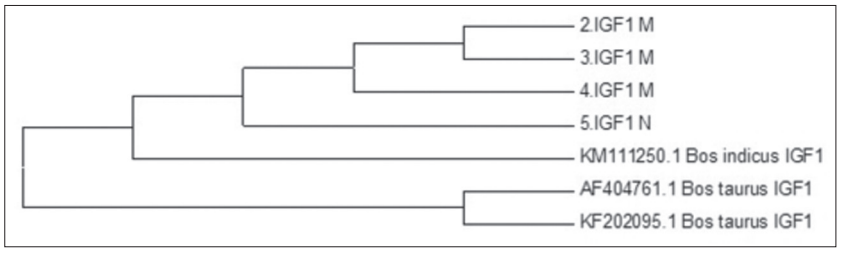

Fig 13. Phylogenetic tree of the *IGF1* gene sequences of Baggara, Bos taurus and Bos indicus cattle. M: Mesairi strain, N: Nyalawi strain

the following accession numbers were given for the *GH1* and *IGF1* respectively:

MG879304, MG879305, MG879306, MG879307, MG879308 and MG879299, MG879300, MG879301, MG879302, MG879303.

DISCUSSION

The implementation of genomics creates many opportunities for beef cattle production through increased genetic progress and the inclusion of new traits of economic importance in the selection programs. The identification of potential SNPs in selected breeds or environments will make the exploitation of novel genomic selection methodologies in farm animals possible. Better genetic characterization of local breeds can help increase selection intensity and decrease generation interval. Local breeds in Sudan are generally poorly characterized and there is the possibility of detecting new mutations. In the current study, two important Sudanese Baggara cattle (Nyalawi and Mesairi) were targeted for genotyping in two important candidate genes in beef production. Sequencing was performed for the purpose of confirmation and to detect new mutations if any. The results of genotyping of the SNP 2141C>G (Leu/Val substitution) showed that all Nyalawi and Mesairi bulls in our study were homozygous for 2141C allele (Leu variant). A previous report [3] mentioned that the genotyping results (using PCR-RFLP) for Nyalawi and Mesairi (Baggara cattle) in exon 5 showed similarity between the strains although they were phenotypically different. Musa et al.[15] reported that the 2141C variant (Leu) appeared to be monomorphic in all studied animals of Kenana and Butana cattle. The mutation was also not found in Bali cattle [16].

Another group [17] found that the substitution at the same position of the *GH1* occurred with allele frequencies of 0.85 and 0.15 for C and G alleles, respectively, in Podolian cattle in Southern Italy. The excess of homozygotes (100% CC) in the current study, which resulted in the disequilibrium, may reflect a series of events such as inbreeding, selection, genetic drift or population subdivision. However, some mutations were detected in the sequenced region the *GH1* sequence of Baggara strains including the 2291A>C SNP. According to GenBank, the mutation was previously reported in Butana, Kenana, Pakistani Dhanni, Red Sindhi, Sahiwal and Kamori cattle breeds *GH1* sequence (accession numbers EF592533, EF592534, DQ307369, DQ307370, EF451794 and EF451795, respectively).

Two other mutations were also detected in Sudanese Baggara *GH1* sequence. The first mutation is 2230 C>T SNP which appeared in the Nyalawi subtype only. A similar result was mentioned by another group in Indonesia [18] who reported the same C>T SNP as a new mutation in

exon five of Aceh cattle (Banda Aceh and Indrapuri). The second mutation (2258 bp C>T) appeared in the two strains of Baggara cattle. The 2258 C>T SNP was previously associated with average daily weight gain (ADG) and carcass weight (CWT) [19]. The high allele frequency of the favorable allele (C) in both European and Asian Bos taurus breeds indicated that selection for genetic improvement of ADG and CWT is associated with the SNP or a locus at Linkage disequilibrium with the SNP [19]. There is a need to further investigate this important mutation in all strains of Baggara cattle. However, according to GenBank records those two SNPs were not reported in Butana and Kenana cattle.

The results of PCR-RFLP also indicated that Baggara cattle strains showed the existence of the targeted SNP of the *IGF1* gene. Genotype frequencies were: 86% (CC) and 14% (CT) in Nyalawi subtype compared to 86% (CC), 13% (CT) and 2% (TT) in Mesairi. Using Chi-square ($\chi 2$) test, both Nyalawi and Mesairi strains were shown to be at Hardy-Weinberg equilibrium ($\chi 2$=0.407and 1.085, respectively) in this locus indicating low exchange of alleles between populations, large population size, and no selective pressure for or against any genotype.

The TT genotype was found only in the Mesairi subtype at a low allele frequency. The reported frequency of the T allele (0.079) was very low in Mesairi subtype in comparison to other reports in which allele frequencies of T and C alleles were 0.64 and 0.36, respectively, in Angus cattle [11], 0.48 and 0.52 in Polish Holstein-Friesian cattle [20], 0.55 and 0.45 in a population of Holstein cattle [21], 0.56 and 0.44 in two commercial lines of dairy cattle [22] and 0.54 and 0.46 in Polish Holstein-Friesian heifers [23].

Our results disagree with those previously published [3] in Sudan using the two Baggara strains in which only the homozygous CC genotype was found. They considered that the reason for the differences in growth rate between Bos taurus and Baggara cattle could be due to nutritional factors. One study [24] proposed that allele B (C allele) is characteristic to indicine populations as it was fixed in a Nellore population.

A potential expression changing SNP was detected at -468 bp (T-A). This SNP was presented in homozygous TT in all sequenced samples and was only observed in both *Bos taurus* (AF404761) and Bos indicus (KM111250). Preliminary analysis indicated changes at putative transcription factor binding sites (PROMO software). The ancestral allele could be any of the detected alleles. Such SNPs might have significant importance in population and evolutionary studies.

The newly detected mutations were not previously reported in Baggara cattle and their frequencies should be validated. The transition mutation in the *GH1* sequence at 2258 bp (C/T) position which existed in the sequences of the two Baggara strains and its association with growth traits should also be investigated. More SNPs need to be screened among Sudanese Baggara cattle to reveal the genetic profile of this important cattle ecotype in Sudan and other African countries using high throughput techniques.

REFERENCES

1. Elniema A, Abdelhadi OMA, ElEmam MB, Salih A: Husbandry management system and its effect on improvement of Sudanese indigenous livestock types in the peri-urban region of Khartoum North Province (KNP). *Global Vet,* 6 (1): 51-55, 2011.

2. MARF (Ministry of Animal Resources, Range and Fisheries): Department of Statistics and Information, Khartoum - Sudan, 2008. http://www.idosi.org/gv/gv6(1)11/7.pdf

3. Alsiddig MA, Babiker SA, Galal MY, Mohammed AM: Phenotypic characterization of Sudan Zebu cattle (Baggara Type). *Res J Anim Vet Sci,* 5, 48-52, 2010.

4. Sellier P: Genetically caused retarded growth in animals. *Domest Anim Endocrinol,* 19, 105-119, 2000. DOI: 10.1016/S0739-7240(00)00071-0

5. Breier BH: Regulation of protein and energy metabolism by the somatotropic axis. *Domest Anim Endocrinol,* 17, 209-218, 1999. DOI: 10.1016/S0739-7240(99)00038-7

6. Goodman HM: Growth hormone and metabolism. **In,** Schreibman MP, Scanes CG, Pang TP (Eds): The Endocrinology of Growth, Development, and Metabolism in Vertebrates. 93-115, Academic Press, Diego, CA, USA,1993.

7. Corpas E, Harman SM, Blackman MR: Human growth hormone and human aging. *Endocr Rev,* 14, 20-39, 1993. DOI: 10.1210/edrv-14-1-20

8. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A: Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igfr). *Cell,* 75, 59-72, 1993. DOI: 10.1016/S0092-8674(05)80084-4

9. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res,* 16, 1215, 1988.

10. Yao J, Hayes F, Aggrey SE, Kuhnlein U: Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holstein. *Genetics,* 144 (4): 1809-1816, 1996.

11. Ge W, Davis ME, Hines HC, Irvin KM, Simmen RCM: Associations of a genetic marker with blood serum insulin-like growth factor-1 concentration and growth traits in Angus cattle. *J Anim Sci,* 79, 1757-1762, 2001.

12. Tajima F, Nei M: Estimation of evolutionary distance between nucleotide sequences. *Mol Biol Evol,* 1, 269-285, 1984. DOI: 10.1093/oxfordjournals.molbev.a040317

13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S: MEGA6 molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol,* 30 (12): 2725-2729, 2013. DOI: 10.1093/molbev/mst197

14. Falconer DS, Mackay TFC: Introduction to Quantitative Genetics. Longman, 4th ed., Essex, U.K, 1996.

15. Musa LMA, Reissmann M, Ishag IM, Rahamtalla SA, Brockmann G, Peters JK: Characterization of the growth hormone gene (GH1) in Sudanese Kenana and Butana Cattle Breeds. *J Anim Prod Adv,* 3 (2): 28-34, 2013.

16. Jakaria R, Noor R: Analysis on Alu-i growth hormone (GHAlu-I) gene in Bali cattle. *J Indonesian Trop Anim Agric,* 36 (2): 77-82, 2011. DOI: 10.14710/jitaa.36.2.77-82

17. Dario C, Carnicella D, Caroli A, Bufano G: Analysis of growth hormone GH1-AluI polymorphism in Podolica cattle breed. *XXXIX International Symposium "Meat Science and Research"* 10 June, 303-308, Roma, Italy, 2004.

18. Sari EM, Noor RR, Sumantri C, Yunus M, Han JL, Muladno:

Identification of single nucleotide polymorphism on growth hormone gene in Aceh cattle. *Media Peternakan,* 21-25, 2013. DOI: 10.5398/medpet.2013.36.1.21

19. Lee JH, Lee YM, Lee JY, Oh DY, Jeong DJ, Kim JJ: Identification of single nucleotide polymorphisms (SNPs) of the bovine growth hormone (bGH) gene associated with growth and carcass traits in Hanwoo. *Asian-Australas J Anim Sci,* 26 (10): 1359-1364, 2013. DOI: 10.5713/ajas. 2013.13248

20. Siadkowska E, Zwierzchowski L, Oprządek J, Strzałkowska N, Bagnicka E, Krzyżewski J: Effect of polymorphism in IGF-1 gene on production traits in Polish Holstein-Friesian cattle. *Anim Sci Pap Rep,* 3, 225-237, 2006.

21. Hines HC, GE W, Zhao Q, Davis ME: Association of genetic markers in growth hormone and insulin-like growth factor I loci with lactation traits in Holsteins. *Anim Genet,* 29 (1): 69, 1998.

22. Li C, Basarab J, Snelling WM, Benkel B: Assessment of positional candidate genes myf5 and IGF1 for growth on bovine chromosome 5 in commercial lines of *Bos taurus*. *J Anim Sci,* 82, 1-7, 2004. DOI: 10.2527/2004.8211

23. Szewczuk M, Bajurna M, Zych S, Kruszyński W: Association of insulin-like growth factor I gene polymorphisms (IGF1/TasI and IGF1/SnaBI) with the growth and subsequent milk yield of Polish Holstein-Friesian heifers. Czech. *J Anim Sci,* 58, 404-411, 2013.

24. Curi RA, de Oliveira HN, Silveira AC, Lopes CR: Association between IGF-I,IGF-IR and GHRH gene polymorphisms and growth and carcass traits in beef cattle. *Livest Sci,* 94, 159-167, 2005. DOI: 10.1016/j.livprodsci.2004.10.009