

LYST gene expression in Iraqi water buffaloes (*Bubalus bubalis*) with Chediak-Higashi syndrome

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Abstract

The present study sought to identify Chediak-Higashi syndrome (CHS) in Iraqi water buffaloes and characterize the lysosomal trafficking regulator (LYST) gene associated with this syndrome. A total of 65 buffaloes consisting of 25 black buffaloes, 25 buffaloes with black-white patches, and 15 white buffaloes were included from Central and Southern regions of Iraq. The relative reverse transcription quantitative polymerase chain reaction (RT-qPCR) results demonstrated under-expression of LYST gene in line with CHS clinical and hematological profile. Both Iraqi white buffaloes (*Bubalus bubalis*) and black buffaloes LYST genes were added to the National Center for Biotechnology Information database (accession numbers MN148890.1 and MN614089.1, respectively). This study confirmed association of clinical and hematological profile of CHS in buffaloes with LYST gene expression. In addition, the RT-qPCR approach was found to be the most efficient and cost-effective approach for detection of CHS in buffaloes. This study further differentiated CHS from other genetic diseases with similar clinical profile (Albinism).

Keywords: Chediak-Higashi syndrome, LYST gene, buffalo, *Bubalus bubalis*,

الخلاصة

سعت هذه الدراسة إلى رصد متلازمة "شيدياك-هيجاشي" (CHS) في جاموس الماء العراقي وتوصيف جين "منظم النقل الليزوزومي (LYS)" المرتبط بهذه المتلازمة. شملت الدراسة 65 رأساً من الجاموس (25 أسود اللون، و25 مرقطاً باللونين الأسود والأبيض، و15 أبيض اللون) من المنطقتين الوسطى والجنوبية في العراق. وأظهرت نتائج تقنية تفاعل البوليميراز المتسلسل الكمي بالنسخ العكسي (RT-qPCR) انخفاضاً في مستوى التعبير الجيني لجين LYST، وهو ما يتوافق مع السمات السريرية والدموية للمتلازمة. وقد أدرجت تسلسلات جين LYST الخاصة بكل من الجاموس العراقي الأبيض (*Bubalus bubalis*) والجاموس الأسود في قاعدة بيانات المركز الوطني لمعلومات التكنولوجيا الحيوية (تحت أرقام التسجيل MN148890.1 و MN614089.1 على التوالي). وأكدت الدراسة وجود ارتباط بين السمات السريرية والدموية لمتلازمة "شيدياك-هيجاشي" لدى الجاموس ومستوى التعبير عن جين LYST كما تبين أن تقنية RT-qPCR هي الطريقة الأكثر كفاءة وفعالية من حيث التكلفة للكشف عن هذه المتلازمة في الجاموس. وعلاوة على ذلك، ميزت الدراسة بين متلازمة "شيدياك-هيجاشي" وأمراض وراثية أخرى ذات سمات سريرية مشابهة (مثل المهق).

الكلمات المفتاحية: متلازمة شيدياك-هيجاشي، LYST جين، الجاموس، الجاموس المائي

Introduction

Chediak-Higashi syndrome (CHS) is an inherited genetic disease that has been identified in several animals including cattle and humans. [1,2,3] CHS causes partial or complete oculocutaneous albinism, hypopigmentation, abnormal bleeding and associated with immunodeficiency features such as low immunity linked with a reduction in immune cell function [4, 5, 6]

CHS was first described in Hereford cattle, where it was found to be characterized by abnormal leukocytes, hypopigmentation, photophobia, high the leukocytes and most

granular cells [7, 8]. Furthermore, these granules were found to exhibit the ultrastructural characteristics of lysosomes [9, 10; 11; 12; 13].

Cattle with different genotypes of CHS are known to have an autosomal recessive gene. Recently, the lysosomal trafficking regulator (LYST) gene (also known as CHS1), which encodes the membrane-associated protein that regulates intracellular protein trafficking, has been identified as one such autosomal recessive gene. The incidence of inherited bleeding disorder accompanied by hypopigmentation or a light-colored coat has also been reported in

cattle species [14,15,16, 17].

Furthermore, calves with CHS show a greater tendency toward bleeding, abnormal granules that vary in size within the granular leukocytes (neutrophils, eosinophils, and basophiles), a decreased number of dense granules within the platelets, and partial albinism. The incidence of CHS among pedigree cattle and the autosomal recessive mode of inheritance for the syndrome have previously been investigated where the bovine *LYST* gene was found localized in bovine chromosome 28, which is responsible for CHS in cattle [17]. Additionally, a comparison of the nucleotide sequences of the mutant *LYST* gene between normal and affected cattle (buffaloes) indicated a syndrome-specific nucleotide substitution that caused the amino acid substitution of histidine for arginine [18; 19].

Materials and Methods

Animals

A total of 65 Iraqi buffaloes of different ages and both sexes from the Central and Southern regions of the country were included in this study. The animals were divided into three groups on the basis of color: black (n = 25), partially pigmented (mixed) (n = 25), and white (n = 15). The assessments were performed on the included animals directly in the field.

Phenotyping characteristics

The phenotyping characteristics were determined on the basis of descriptions and photographs. More specifically, information was obtained from the owners concerning the buffaloes' morphological description, hair and skin pigmentation and color, skin thickening (as determined using a caliper gauge), eye (iris) color and photophobia, age, sex, pigmented–non-pigmented body percentage, and pedigree (based on information about the sire and dam). A G-spin DNA Extraction Kit (cat. no. 17045, Intron Biotechnology, Korea) and EDTA tubes were used in the analysis of the blood samples collected from the three phenotypes of buffaloes. The genomic DNA was extracted

from the whole blood using an Intron DNA Purification Kit in order to perform the genotyping analysis. The DNA extraction was performed according to the manufacturer's recommendations [13].

Primers and primer design

The second primer (LYST1) utilized in this study, which is specific to Iraqi water buffalo, was designed on the basis of our prior experience and examined many times for the gene mutant before being used as described below.

Gene-specific primer (LYST2)

The gene-specific primer (LYST2) comprised 108 base pairs. The forward and reverse primers can be illustrated as follows. Forward: 5'-GAAAATTACAGCAGAAGTCCTTGG-3'. Reverse: 5'-TGACAAACATAAGTATTAGTAGGAGG-3'.

Diagnosis of the gene-specific primer (LYST1) (designed)

On the basis of our prior experience, the primer LYST1 was designed to consist of 280 pb. The sequencing of this gene can be illustrated as follows (as synthesized by Integrated DNA Technologies, Canada).

Forward: 5'-GACTTGCCAGGTTTTGCAGG-3'.

Reverse: 5'-GGAAATGCTGGGAGGCTTCT-3'.

The product size is 280 base pairs.

RNA extraction

The RNA extraction was performed using a Direct-zol™ RNA MiniPrep Kit (R2051, Zymo Research, USA). The *LYST* gene expression levels were estimated by means of a reverse transcription quantitative polymerase chain reaction (RT-qPCR). To confirm the expression of the target gene, a relative real-time SYBR Green-based RT-qPCR assay was performed. The primer sequences for *LYST 1* were prepared according to the recommendations of William *et al.* (2003), synthesized by Integrated DNA Technologies (Canada), and stored when

lyophilized at -20°C.

Real-time RT-qPCR analysis of the *LYST* gene expression

$$\Delta Ct \text{ (patients)} = Ct \text{ (patients)}_{\text{mean}} - Ct \text{ (reference)}_{\text{mean}}$$

$$\Delta Ct \text{ (Controls)} = Ct \text{ (controls)}_{\text{mean}} - Ct \text{ (reference)}_{\text{mean}}$$

$$\Delta\Delta Ct = \Delta Ct \text{ (patients)} - \Delta Ct \text{ (controls)}$$

The normalized target gene expression level was $2^{(-\Delta\Delta Ct)}$ (Livak, and Schmittgen, 2001, 2008; Al-Khafaji, 2015). The *LYST* gene expression was successfully determined using a new molecular technique, namely RT-qPCR and a specific primer as described previously. The accuracy of the gene amplification product was determined on the basis of the cycle threshold (Ct) value for the triplicate reactions. The data derived from the real-time experiments were interpreted according to the Ct values, which

were calculated from the cycles and proportional to the starting copy number of the target (logarithmic scale) used for the amplification of the mRNA. The point at which the fluorescence signal increased above the baseline represented the Ct, which was inversely related to the amount of starting template. This meant that a high Ct value indicated a low level of gene expression or amplification, whereas a low Ct value indicated a high level of gene expression or amplification [17, 20, 21, 22, 13].

Results

Gene expression of *LYST* gene in Iraqi buffaloes: Relative gene expression

The results from real time PCR (RT-PCR) are shown as curves representing each sample in Figure 1



Figure 1: Buffalo calf with Chediak-Higash syndrome

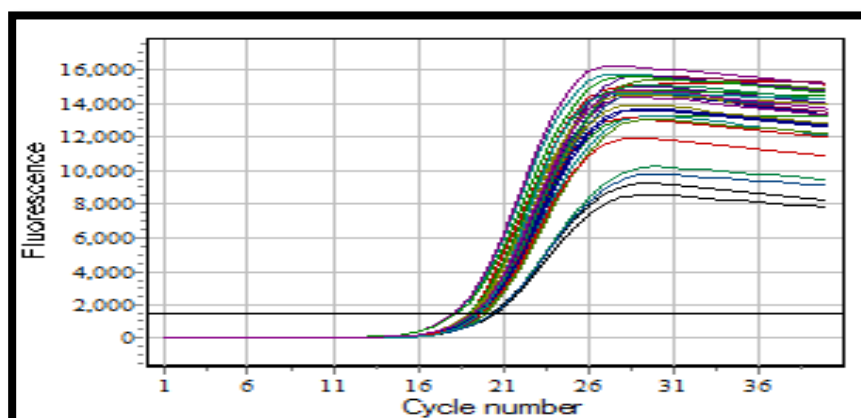


Figure 2. RT-qPCR results for sampled Iraqi buffaloes.

The relative gene expression was tested using RT-qPCR after the extraction of RNA by Zymo research kit, and the results of gene expression for each sample after calculating the folding were shown in the table (1).

Table 1. Relative gene expression for Iraqi buffaloes

Sample No.	Black			White						Mixed					
	Ct ref.	Ct	ΔCt	Ct ref.	Ct	ΔCt	ΔΔCt	Folding	Ct ref.	Ct	ΔCt	ΔΔCt	folding		
1	19.1	31.9	12.8	1	19.4	32.2	12.8	0.3	0.812252	1	19.7	31.3	11.6	-0.9	1.866066
2	19.4	34.3	14.9	2	19.4	33.5	14.1	1.6	0.329877	2	19.3	30.1	10.8	-1.7	3.24901
3	19.3	33.3	14	3	19.4	32.1	12.7	0.2	0.870551	3	19.2	31.4	12.2	-0.3	1.231144
4	19.2	33.9	14.7	4	19	31.9	12.9	0.4	0.757858	4	20.4	32.8	12.4	-0.1	1.071773
5	19.6	31.6	12	5	19.4	33.1	13.7	1.2	0.435275	5	19.7	30.6	10.9	-1.6	3.031433
6	19.3	30.9	11.6	6	19.3	32.9	13.6	1.1	0.466516	6	18.3	30.1	11.8	-0.7	1.624505
7	20.5	30.5	10	7	18.2	30	11.8	-0.7	1.624505	7	19.5	30.2	10.7	-1.8	3.482202
8	19.4	32.4	13	8	18.1	32.9	14.8	2.3	0.203063	8	19.1	30.4	11.3	-1.2	2.297397
9	20	30.4	10.4	9	20.4	30.9	10.5	-2	4	9	19.6	29.5	9.9	-2.6	6.062866
10	20.4	32	11.6	10	19.8	32.1	12.3	-0.2	1.148698	10	19.2	32.1	12.9	0.4	0.757858

Group	ΔCt	$\Delta\Delta Ct$	Folding
White buffaloes	12.92	0.42	0.747425
Mixed (patches buffaloes)	11.45	-1.05	2.07053
Black buffaloes	12.5		

The results of relative gene expression for each sample is presented in Table 1. The findings revealed an increased level of gene expression in animals with mixed colors when compared to black animals. In contrast, gene expression levels were decreased in white compared to black buffaloes.

Discussion

The gene expression amplified by RT-qPCR and isolation of Iraqi buffaloes *LYST* cDNAs, after made the calculation for the real time PCR results data, the relative RT-qPCR results revealed that there was clear correlation between the genotype and phenotype this represented by varying range of clinical signs this due to the missense mutation in the *LYST* gene which lead to altered the proteins.

However, the strong *LYST* gene expression represented by the black animals where there is no change in the clinical signs and no ultration in the coat color that meant whenever the gene expression is strong whenever no clinical signs and the animal were normal and without mutant *LYST* gene, therefore the present study regard it as control group.

The milder *LYST* gene expression represented in the patches animals where the buffaloes carry or has weak gene expression so it has few clinical signs or mild degree of the Chediak-Higashi Syndrome. This was clear and corresponding to the reported clinical sings of the patches buffaloes in the present study, and the folding results reported 2.07053.

The lesser of *LYST* gene expression in the relative RT-qPCR represented in the white water buffaloes where the folding result was less than one (<1) that mean the *LYST* gene have no sense expression to form the black coat or pigmented skin, this weak gene expression explain the clear correlation manifested by the clear clinical signs in the Chediak – Higashi Syndrome buffaloes with the first clinical signs the white coat color or no pigmented while the patches buffaloes may be didn't express well for the color changes because it have a carry mutant

allele.

All the three results above were corresponding to the results reported by [14, 22., 23, 24, 25, 26, 27, 28, 29, 30]

Finally, the *LYST* gene of the Iraqi white buffaloes reported in the National Centre for Biotechnology Information (NCBI) with accession number MN148890.1, moreover, the *LYST* gene of the black Iraqi buffaloes reported in the NCBI with accession number MN614089.1. and this regard as very important step to establish this study.

Conflicts of interest

There are no conflicts of interest.

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