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New Insights on Immune and Metabolic Candidate Genes Assessing Resistance/Susceptibility to Digital Dermatitis in Italian Buffaloes (*Bubalus bubalis*)

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DUE to the inadequate inheritance of resistance to digital dermatitis (DD), breeding for it is difficult. There may be a connection between genetic and metabolic variables and the development of DD. In this study, 80 pure Italian buffaloes were utilized to examine the relationship between immunological and metabolic gene mutations and digital dermatitis (DD). The incidence and history of digital dermatitis were screened, and the animals were divided into two groups: the affected group (n = 40), and the resistant (healthy) group (n = 40). Ten millilitres of blood were collected from each buffalo for DNA extraction. PCR-DNA sequencing of immune (*TLR4*, *IL-8*, *CATH, IL-17, IL-13, DEFB123, ASB16,* and *SCART1*) and metabolic (*CMPK2, POPDC3, KIF26A, SYT8, ASPG, NRXN2, RND1, GPHN, LPCAT1, FPGT, TNN13K,* and *NDUFS6*) genes revealed coding single nucleotide polymorphisms (SNPs) between DD healthy and affected buffaloes. A substantial variation was observed in the frequency of genes, based on Fisher's exact test of discovered SNPs. The identified SNPs could be employed in marker-assisted selection for digital dermatitis resistance/ susceptibility in buffaloes. These findings propose a hopeful approach for limiting digital dermatitis in buffalo through discriminating breeding of animals established on genetic markers accompanying genetic resistance to infection.

Keywords : Candidate gene, Buffalo, Digital dermatitis.

Introduction

The domestic water buffalo relates to the genus *Bubalus bubalis* [1]. Entirely European buffaloes are claimed to be of the Mediterranean breed, which comprises those from Italy, Egypt, Greece, Bulgaria, Syria, and Turkey [2]. The second-most valuable species in the world for milk production is the buffalo [3]. Although Egyptian-raised dairy cow breeds produce more milk than buffalo do, the price of buffalo milk is almost three times more than that of dairy cattle [4]. The Italian buffalo is perhaps the one that has been studied the most extensively worldwide, and considerable information about its genetic makeup, practical knowledge, pathology screening, hygiene, and superiority of goods is readily available in public databases [5]. It is also well renowned for having the highest milk production in the world [6]. Egypt has extra buffaloes than Italy does, however the Egyptian buffaloes are much less efficient, which results in less milk production. This is largely because Italy has put in a huge amount of work to track, select, breed, and improve the feeding practices for its buffalo populations [7].

Hoof issues are the third most common motive for early culling in dairy cattle worldwide,

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subsequently mastitis and reproductive failures [8]. In contrast to claw horn lesions such as sole ulcers, toe ulcers, white line lesions, sole haemorrhages, and interdigital hyperplasia, which are considered noninfectious traits, infectious traits typically, denote skin injuries such as digital dermatitis (DD), interdigital dermatitis (ID), and heel horn erosion (HHE). Due to its high incidence, discomfort, and negative effects on welfare and the livestock industry's bottom line, digital dermatitis (DD) is currently one of the most prevalent concerns with cattle feet [9]. The presence of large amounts of mud or a typically damp surrounding is a major hazard feature, according to epidemiologic research, and this was later established in an effective trial infection model [10,11]. According to Zinicola et al. [12], digital dermatitis is a polymicrobial condition; nonetheless, the fastidious, anaerobic, and highly mobile spirochetes of the genus Treponema (also known as treponemes) are thought to be the main culprits [13].

Although housing variations may contribute to differences in occurrence within and between breeds, new research suggests that genetic backgrounds may also play a role in susceptibility differences [14-16]. A superficial dermatitis of the distal area of the foot is the disease's defining feature. The lesions first appear as painful, round or oval erosions, but they can quickly advance to filiform warts with matted hair or granular, strawberry-like lesions [17]. Finally, the development of a dry, painless crust that is firmly attached to the healthy skin beneath indicates recovery [18]. The majority of lesions are found along the coronary band or at the skin-horn junction of the heel bulbs on the palmar/plantar aspect of the foot, particularly the hind foot [17]. There may also be involvement of the skin that surrounds the dorsal interdigital cleft of the foot [13, 19]. Clinical DD lesions were divided by Döpfer et al. [13] into five phases (M0-M4), primarily on the basis of a visual examination of the lesions.

Numerous elements, including herd management, nutrition, hormones, metabolism, lactation stage, flooring system, environment, and breed have an impact on the prevalence of DD [20]. The incidence of lameness in dairy cattle is significantly influenced by diet and energy metabolism. Numerous studies have suggested a link between energy supply and the emergence of metabolic foot diseases [21]. According to Somers et al. [22], the prevalence of DD is connected to how much concentrate supplementation for dairy animals is augmented after parturition. Gomez et al. [23] have looked into the possibility that dietary supplements' positive effects on immune responses and skin quality could lessen the prevalence of DD. One method to lower the occurrence of DD on a dairy farm is to optimize management practices [24]. Another tactic would be to enhance claw health via genetic selection [25,26]. For both genetic evaluations and risk factor assessments impacting BDD in a herd, the frequency and course of affected animals can be tracked using the well-known M-scoring system [27,28].

The role of host genetic variables is maintained by estimates of moderate-to-high heritability and a variable immunological reaction for various cow sorts concerning their susceptibility to BDD [23,29]. Numerous millions of animals have had their genotypes examined since dairy buffaloes began to use genomic selection. The majority of genetic variants employed in dairy cattle genomic studies are single nucleotide polymorphisms. Genomic research has significantly improved the genetic architecture of many economically significant characteristics and illnesses, which has also assisted in maximizing genetic gain through selection [30]. Several potential genes linked to inflammatory progressions have been identified as of late [31,32]. Changes to a single nucleotide in an animal's genetic sequence are known as single nucleotide polymorphisms (SNPs). Because this kind of testing is accessible and the rising accuracy of genetic merit prediction, genotyping has grown in popularity [33].

Recent studies employed genome-wide association analysis to identify new genes specifically for dairy cattle's sensitivity to DD [34,35]; however, no preceding studies have inspected SNPs in these genes and their relation to vulnerability to DD. Additionally, earlier studies focused on the relationship between immunological genes and the prevalence of DD in cattle [31,32]; prior to now, no research has examined the polymorphism of immunological and metabolic genes and their association with DD in buffaloes.

As a result, this study's goal was to employ a PCR-DNA sequencing methodology for recognizing candidate immune (*TLR4, IL-8, CATH, IL-17, IL-13, DEFB123, ASB16, and SCART1*) and metabolic (*CMPK2, POPDC3,*

KIF26A, SYT8, ASPG, NRXN2, RND1, GPHN, LPCAT1, FPGT, and NDUFS6) genes with underlying alternates influencing the prevalence of digital dermatitis resistance/ susceptibility in Italian buffalo.

Material and Methods

Ethics statement

Mansoura University's policies were followed, and the Ethical Committee provided its agreement to the methods used in this investigation for collecting samples and caring for animals. The study's protocol was acknowledged by Mansoura University Animal Care and Use Committee (MU-ACUC) (code VM.R.22.09.8).

Animals and sampling

Eighty pure Italian buffaloes outstretched on a private farm in the Delta region of Northern Egypt were utilized in this study. All pet owners provided formal, full approval for the usage of their animals. The selection of the animals was on the basis of farm records, a clinical check of the foot, and a screening of the occurrence and background of digital dermatitis (DD) over several months to distinguish between DD-unaffected animals and those with DD. Therefore, they were consigned into 2 groups: affected group (n =40), and the resistant (healthy) group (n = 40). All lame buffaloes were identified by the herd, and the sorts of lesions were noted. For this investigation, a random selection of 40 buffaloes with ongoing DD lesions in their hind claws over the heel bulb was made. At walk, buffaloes had a clinical assessment.

In a divided dairy barn, buffaloes were grown in accordance with the amount of milk they produced. Three times a day, they were milked, and the sum of milk each one produced was noted. Total mixed ration (TMR) for buffaloes was altered to meet anticipated needs for both reproduction and output. The crude protein content of the TMR was determined to be 12.5%, the net energy meant for breastfeeding to be 2.4 Mcal/kg, the neutral detergent fiber to be 31%, and the acid detergent fiber to be 19%. A ten milliliter sample of blood was taken by puncturing the jugular veins of every buffalo. The samples were placed inside a vacuum tube with an anticoagulant to collect total blood (EDTA or sodium fluoride). At -20°C, blood samples were placed in a freezer until the following DNA isolation.

DNA isolation and Polymerase Chain Reaction (PCR)

Using the Gene JET whole blood genomic DNA extraction kit, genomic DNA was recovered from the full sample of blood and the manufacturer's instructions (Thermo scientific, Lithuania). Nanodrop examined DNA of extreme purity and concentration. Using PCR, the subsequent immune (*TLR4*, *IL-8*, *CATH, IL-17, IL-13, DEFB123, ASB16,* and *SCART1*) and metabolic (*CMPK2, POPDC3, KIF26A, SYT8, ASPG, NRXN2, RND1, GPHN, LPCAT1, FPGT, TNN13K,* and *NDUFS6*) genes' parts of coding sites (CDS) were cloned. The *Bubalus bubalis* sequence that appeared in PubMed was used to create the primer olignoucleotides.

The polymerase chain reaction blend was run in a thermal cycler with a finishing capacity of 100 µL. Each reaction volume employed the ensuing components: 1 µL of each primer, 25 µL of PCR master mix (Jena Bioscience, Germany), 5 µL DNA, and 68 µL H2O (d.d. water). The first denaturation temperature was 94 °C for eight minutes to the reaction mixture. The cycling cycled through 30 cycles of denaturation at 94 °C for 1 minute, annealing at ranged from 60 to 64 °C for 45 seconds for 45 seconds, extension at 72 °C for 45 seconds, and a final extension at 72 °C for 8 minutes. Samples were deposited at 4 °C, and demonstrative PCR investigation outcomes were detected with agarose gel electrophoresis. After that, the fragment pattern was observed using a gel documentation system under ultraviolet light.

DNA sequencing and polymorphism detection

Prior to DNA sequencing, primer dimmers, nonspecific PCR product, and other impurities were unconcerned. A PCR purification kit (Jena Bioscience # pp-201s/Germany, Jena, Germany) was used in accordance with the manufacturer's instructions to purify PCR products of the anticipated size (amplicon) [36]. The PCR products of target sequence for investigated genes were measured by means of Nanodrop (Uv-Vis spectrophotometer Q5000/USA) to yield high-quality and guarantee adequate concentrations and purity of the amplified fragments [37]. For identifying SNPs of the examined markers in DD healthy and affected buffaloes, PCR products having the goal sequence were directed for forward and reverse DNA sequencing.

PCR products of the target sequence were sequenced with an ABI 3730XL DNA sequencer

(Applied Biosystems, Waltham, MA, USA) and the enzymatic chain terminator technique [38]. Data from DNA sequencing are evaluated using Chromas 1.45 and BLAST 2.0 programs [39]. SNPs were discovered as variations within the healthy and affected animals for the considered indicators. The MEGA4 tool was used to identify differences in the amino acid sequence of the examined markers between the enrolled buffaloes based on a sequence alignment [40].

Statistical analysis

The statistics program Graphpad was utilized to conduct the statistical investigation (Graphpad prism for Windows version 5.1, Graphpad software, Inc., San Diego, CA, USA). Using Fisher's exact test to analyze the dissemination of the revealed SNPs between the two groups, it was statistically strong-minded whether there were variances in the incidence of immune and metabolic marker SNPs between DD healthy and affected animals.

Results

PCR-DNA sequencing of immune and metabolic genes

PCR-DNA sequencing results of immune and metabolic genes discovered nucleotide sequence dissimilarity in the formula of SNPs linked with digital dermatitis resistance/susceptibility between DD healthy and affected buffaloes (succumbed to GenBank with accession numbers gb|OQ692982|, gb|OQ692983|, gb|OQ692984|, gb|OQ692985|, gb|OQ718328|, gb|OQ718329|, gb|OQ730437|, gb|OQ730438|, gb|OQ730439|, gb|OQ730440|, gb|OQ730441|, gb|OQ730442|, gb|OQ730443|, gb|OQ730444|, gb|OQ730445|, gb|OQ730446|, gb|OQ730447|, gb|OQ730448|, gb|OQ730449|, gb|OQ730450|, gb|OQ730451|, gb|OQ735302|, gb|OQ735303|, gb|OQ784297|, and gb|OQ784298|). All detected SNPs were validated by nucleotide sequence differences between the studied genes in the research animals and reference sequences found in GenBank. Between DD healthy and affected buffaloes, there was a substantial variation in the frequency of genes related to immune and metabolism, according to the chi-square analysis of discovered SNPs (Tables 1 and 2).

The distribution of SNPs and the kind of mutation for DD healthy and affected buffaloes are shown in Table 1. Four recurrent SNPs were found when the *TLR4* gene (326-bp) was sequenced. Synonymous mutations 13L, 25T, and 51P occurred as a result of three 37CT, 75TC, and 153TC SNPs, respectively. One 299GA SNP had a non-synonymous mutation that changed the amino acid G100E. 105AG involved a synonymous mutation, 35E. 152CA and 209TC involved a non-synonymous change, causing the amino acid to be substituted P51H, and S70F respectively; were found in DNA sequences of the *IL-8* gene (466-bp). The *CATH* gene (517-bp) contained three recurrent non-synonymous SNPs: 59GT, 148GT, and 350GA, which caused the amino acids C20F, G50C, and R117H to be substituted, respectively. Two recurrent SNPs were found when the *Il-17* gene's DNA was sequenced (600-bp): 189AG included the synonymous mutation 63S and 217GA contained the non-synonymous mutation D73N. One recurrent non-synonymous SNP, 52GA induced replacement of amino acid A18T, was found in the *IL-13* gene (399-bp). For the *DEFB123* gene (480-bp), two observed recurrent non-synonymous SNPs, 108GT and 356CT, resulted in the substitution of Q36H and P119L, respectively. The 360-bp *ASB16* gene's DNA sequence revealed two identified frequent synonymous SNPs, 126CT and 228GT, which were associated with the amino acids 42S and 76A. Four recurrent SNPs in the *SCART1* gene (505-bp) were discovered using DNA sequencing; two of these, 222AG and 255AG, resulted in synonymous mutations, 74V and 85Q, respectively. While the non-synonymous mutation brought about by the SNPs 382AG and 425GC led to the substitution of the amino acids C142S and R228G, respectively.

Table 2 displays the distribution of SNPs and the kind of mutation for DD healthy and affected buffaloes with regard to metabolic genes. One recurrent non-synonymous SNP; 100AC resulted in the substitution of the amino acid S34R, as revealed by DNA sequencing of the *CMPK2* (228-bp). Three recurrent non-synonymous SNPs; 172AT, 184GA, and 292CG cleared during DNA sequencing of the *POPDC3* (477-bp) gene, which resulted in the substitution of the amino acids N58Y, V62I, and Q98E, respectively. DNA sequencing of the *KIF26A* (600-bp) elicited four repeated SNPs; 31GT caused the amino acid A11S to be replaced; 99GA involved a synonymous mutation, 33A; 298TG involved a non-synonymous mutation, resulted in the amino acid S100A being replaced; and 520GC caused the amino acid G174R to be replaced. Two recurrent non-synonymous SNPs were

produced by the *SYT8* gene's (358-bp) nucleotide sequence variants; 58GA and 230TG caused the amino acids P20T and L77R, respectively, to be substituted. One recurrent non-synonymous SNP, 209AG, was elaborated using DNA sequencing of the *ASPG* (474-bp), which led to the substitution of the amino acid Q70R. The *NRNX2* (529-bp) DNA sequence revealed one more recurrent non-synonymous SNP, 164CT, which substituted the amino acid P55L. Two recurrent non-synonymous SNPs were found in the *RND1* gene (591-bp); 50GA and 221AG changed the amino acids R17Q and K74R, respectively. One recurrent non-synonymous SNP was discovered during the sequencing of the *GHPN* gene (393-bp), 124TC modified the amino acid sequence Y42H. Four recurrent non-synonymous SNPs (148AG, 233AC, 284CT, and 386TC) in the *LPCAT1* gene (496-bp) altered the amino acids R50G, E78A, P95L, and L129P, respectively. The nucleotide sequence of the *FGPT* gene (425-bp) revealed one recurrent non-synonymous SNP; H71R resulted from 212AG SNP. For the *TNN13K* gene (538-bp), two synonymous SNPs were found; the results of 153AG and 351TC were 51L and 117T, respectively. The *NDUFS6* gene's (314-bp) sequencing revealed one non-synonymous SNP, 113CT, which altered S38L amino acid sequence.

Discussion

This study employed the PCR-DNA sequencing method to distinguish the immune and metabolic genes in DD healthy and affected buffaloes from a molecular perspective. The findings showed that there were SNPs between the two groups (submitted to GenBank with accession numbers gb|OQ692982|, gb|OQ692983|, gb|OQ692984|, gb|OQ692985|, gb|OQ718328|, gb|OQ718329|, gb|OQ730437|, gb|OQ730438|, gb|OQ730439|, gb|OQ730440|, gb|OQ730441|, gb|OQ730442|, gb|OQ730443|, gb|OQ730444|, gb|OQ730445|, gb|OQ730446|, gb|OQ730447|, gb|OQ730448|, gb|OQ730449|, gb|OQ730450|, gb|OQ730451|, gb|OQ735302|, gb|OQ735303|, gb|OQ784297|, and gb|OQ784298|). Fisher's exact test demonstrated a significant difference in the SNP dissemination among the examined buffaloes of group. When contrasted with the associated GenBank reference sequence, it is noteworthy that the polymorphisms discovered and presented here provide fresh details about the examined genes. Recent research targeted novel genes specifically for dairy cattle's sensitivity to DD using genome wide association analysis [34,35]; however, no research has previously examined these genes' SNPs and their relationship to DD susceptibility.

Our study is the first to demonstrate this connection using the immunological and metabolic gene sequences of the buffalo (*Bubalus bubalis*) that have been published in PubMed. It's also important to highlight that, to the best of our knowledge, no studies have previously looked at the polymorphism of immune and metabolic genes and their connection to DD in buffaloes. However, earlier studies focused on the connection between immunological genes and the prevalence of DD in cattle. For instance, SNPs in the *IL8* and *TLR4* markers have been proposed as potential markers for the resistance/susceptibility of Holstein cattle to digital dermatitis [31]. Refaai et al. [32] also indicated that cattle digital dermatitis is linked to an overactive innate immune response brought on by the keratinocytes. The authors noticed IL8 expression significantly increased in the M2-M3 stages of the illness, and immunohistochemistry labeling identified keratinocytes as the source, demonstrating a critical role for keratinocyte-derived IL8 in the pathogenesis of DD. In the same vein, functional variants linked to the *CMPK2* and *ASB16* genes have been shown to affect bovine digital dermatitis [41]. Additionally, Watts et al. [42] noted that beta–defensin is an important indicator of active DD. The authors also noted that cathelicidins and IL-10 appeared to be significant in disease progression or in response to therapy. Vermeersch et al. [43] further on noticed in both acute and chronic digital dermatitis lesions in dairy cattle, the IL-17F-driven inflammatory pathway has been continuously activated.

Infectious qualities are typically associated with metabolic processes, whereas noninfectious features are typically associated with immune-related genes [8]. Because a variety of factors, such as immunity and metabolism, affect the occurrence of DD [20]. As a result, it was anticipated that several immunological and metabolic markers would be linked to DD susceptibility. Regarding immune genes, our results cleared that DNA sequencing of *TLR4*, *IL-8*, *CATH, IL-17, IL-13, DEFB123, ASB16,* and *SCART1* genes revealed nucleotide sequence variants between DD healthy and affected buffaloes. Lipopolysaccharide (found in Gram-negative bacteria) and lipoteichoic acid (found in Gram-positive bacteria) are distinguished by the cell surface membrane protein TLR4 [44]. In the active DD (M1 and M2) stages, bovine TLR4

skin transcriptional expression was greater.[42] According to numerous studies [45,46]. IL-8 is reputed to perform a function during the host immunological response, in neutrophil chemotaxis and has been linked to the pathophysiology of both BDD and other spirochaete-associated diseases like Lyme disease [47]. It has been demonstrated that T. phagedenis phylogroup spirochaetes cause IL-8 to be upregulated in bovine macrophages [48]. Cathelicidins and β-defensins are innately antibacterial and immunomodulatory host defense peptides that have undergone evolutionary conservation [49]. Skin epithelial cells and neutrophils both release cathelicidins and β-defensins, which, when activated by invasive infections, can facilitate bacterial death and neutrophil recruitment [49].

IL-17 signaling is known to play a part in barrier tissues like the skin's defense against Gram-negative bacteria [50]. Inflammatory skin pathologies such as psoriasis [51], the observation that disease-associated IL-17 signaling pathways are only considerably enriched in fibroblasts following pathogenic challenge supports spirochaete-associated Lyme arthritis and syphilis [52]. Given that a possible target for human psoriasis therapies is IL-17, all of this leads to a possible character for IL-17 in the pathophysiology of BDD and may provide therapy possibilities [51]. Similar to the actions of the cytokine IL-4, which is closely related to IL-13, IL-13 has an impact on immune cells [53]. Nevertheless, it is thought that IL-13 is the primary mediator of the physiological alterations brought on by allergic inflammation in many tissues [54]. IL-13 possesses anti-inflammatory capabilities, while being largely linked to the onset of airway illness [53]. Real-time PCR was used by Refaai et al. [32] to identify the expression of the IL13 gene in cases of Digital dermatitis in cattle. Comparing the diseased group to the control group, transcriptional levels of IL13 were lower in the sick group.

Within inflammatory signaling pathways, the gene ankyrin repeat and SOCS box containing 16 (ASB16) interact with a variety of effector molecules [55]. Additionally, ASB proteins are crucial for maintaining a balanced immune response because they act as a subset of E3 ubiquitin ligases' substrate recognition unit, which is critical in the control of protein activities [56]. In light of this, ASB16 was chosen as a probable candidate gene for BDD. The protein that is only present in a specific subset of delta gamma T cells that is produced by the Scavenger Receptor Family Member Expressed on T Cells (*SCART1*) gene serves to detect important infections. [57]. Butty et al. [34] reported that *SCART1* gene was associated with hoof fitness attributes in Holstein dairy cattle based on Genome-wide association analysis.

The dermal-epidermal interface between the live and horn tissues of the hoof is not nourished, which gradually degrades and result in a lack of support within the hoof, metabolic disorders typically cause poor hoof quality and more lesions to arise. White line illnesses, hemorrhages, and ulcers may then emerge as a result [58]. The nucleotide sequence variations of the metabolic genes *CMPK2, POPDC3, KIF26A, SYT8, ASPG, NRXN2, RND1, GPHN, LPCAT1, FPGT, TNN13K,* and *NDUFS6* were found between the DD healthy affected groups, according to our findings. Genome-wide association investigation was accomplished to examine the connection between Holstein dairy cow foot health parameters and copy number variations [34]. Twenty potential genes, including *CMPK2, POPDC3, KIF26A, SYT8, ASPG, NRXN2, RND1, GPHN, LPCAT1, FPGT, TNN13K,* and *NDUFS6*, were shown to be linked to these features, according to the results. Nearly all of the genes associated with non-infectious hoof lesions have been shown to be associated with recognized metabolic disorders.

Cytidine/uridine monophosphate kinase 2 (*CMPK2*) determined to be a candidate gene for a potential impact on BDD because it has been demonstrated to be essential for several species' immunomodulatory signaling pathways [59]. This is especially true in connection with bacterial infections [60]. In addition to its function in nucleotide synthesis, mitochondrial CMPK2 also participates in the generation of amino acids and DNA repair machinery, supporting the survival of triggered proinflammatory macrophages [61]. The Popeye Domain Containing 3 (*POPDC3*) gene, which was linked to characteristics of hoof health, encodes a membrane protein linked to muscle dystrophy and serum creatine kinase levels [62]. The gene that may be connected to the other infectious characteristic, DD, is Kinesin Family Member 26A (*KIF26A*). The integrity of the microtubules that the cells employ to generate vacuoles, known as kinesin proteins, is impacted [63]. The infection risk may increase if the less solidified vacuoles are less successful at isolating pathogens in the cell [64].

TABLE 1. Dissemination of SNPs, category of mutation in immune genes for healthy and digital dermatitis buffaloes

Gene	SNPs	Healthy *n*= 40	Digital dermatitis n= 40	Total *n*= 80	Type of mutation	Amino acid number and type	Fisher's exact	P-value
TLR4	C37T	-	24	24/80	Synonymous	13 L	20.82	<0.0001
	T75C	-	31	31/80	Synonymous	25 T	26.89	<0.0001
	T153C	23	-	23/80	Synonymous	51 P	19.95	<0.0001
	G299A	-	19	19/80	Non-synonymous	100 G to E	16.48	<0.0001
	A105G	-	27	27/80	Synonymous	35 E	23.42	<0.0001
IL-8	C152A	30	-	30/80	Non-synonymous	51 P to H	26.03	<0.0001
	T209C	17	-	17/80	Non-synonymous	70 S to F	14.75	<0.0001
	G59T	27	-	27/80	Non-synonymous	20 C to F	23.42	<0.0001
CATH	G148T	15	-	15/80	Non-synonymous	50 G to C	13.01	<0.0001
	G350A	33	-	33/80	Non-synonymous	117 R to H	103.98	<0.0001
IL-17	A189G	-	25	25/80	Synonymous	63 S	78.77	<0.0001
	G217A	28	-	28/80	Non-synonymous	73 D to N	88.22	<0.0001
IL-13	G52A	-	30	30/80	Non-synonymous	18 A to T	94.53	<0.0001
DEFB123	G108T	27	-	27/80	Non-synonymous	36 Q to H	85.07	<0.0001
	C356T	23	-	23/80	Non-synonymous	119 P to L	72.47	<0.0001
ASB16	C126T	-	33	33/80	Synonymous	42 S	103.98	<0.0001
	G228T	18	-	18/80	Synonymous	76 A	56.71	<0.0001
	A222G	22	-	22/80	Synonymous	74 V	69.32	<0.0001
SCART1	A255G	37	-	37/80	Synonymous	85 Q	116.58	<0.0001
	A382G	-	28	28/80	Non-synonymous	142 C to S	88.22	<0.0001
	G425C	14	-	14/80	Non-synonymous	228 R to G	44.11	<0.0001

- TLR4= Toll-like receptor 4; IL-8= Iterleukin-8; CATH= Cathelicidin; IL-17= Iterleukin-17; IL-13= Iterleukin-13; DEFB123= Defensin Beta 123; ASB16= Ankyrin repeat and SOCS box containing 16; and SCART1= Scavenger Receptor Family Member Expressed on T Cells 1.
- A= Alanine; C= Cisteine; D= Aspartic acid; E= Glutamic acid; F= Phenylalanine; G= Glycine; H= Histidine; L= Leucine; N= Asparagine; P= Proline; Q= Glutamine; R= Argnine; S= Serine; T= Threonine; and V= Valine.

TABLE 2. Dissemination of SNPs, sort of mutation in metabolic genes in healthy and digital dermatitis buffaloes

Gene	SNPs	Healthy *n*= 40	Digital dermatitis *n*= 40	Total *n*= 80	Type of mutation	Amino acid number and type	Chi value	P-value
CMPK2	A100C	-	29	29/80	Non-synonymous	34 S to R	91.37	<0.0001
	A172T	-	16	16/80	Non-synonymous	58 N to Y	50.41	<0.0001
POPDC3	G184A	-	34	34/80	Non-synonymous	62 V to I	107.13	<0.0001
	C292G	25	-	25/80	Non-synonymous	98 Q to E	78.77	<0.0001
	G31T	36	-	36/80	Non-synonymous	11 A to S	113.43	<0.0001
KIF26A	G99A	-	26	26/80	Synonymous	33 A	81.92	<0.0001
	T298G	19	-	19/80	Non-synonymous	100 S to A	59.87	<0.0001
	G520C	31	-	31/80	Non-synonymous	174 G to R	97.68	<0.0001
SYT8	C58A	-	16	16/80	Non-synonymous	20 P to T	50.41	<0.0001
	T230G	-	36	36/80	Non-synonymous	77 L to R	113.43	<0.0001
ASPG	A209G	29	-	29/80	Non-synonymous	70 Q to R	91.37	<0.0001
NRXN2	C164T	15	-	15/80	Non-synonymous	55 P to L	47.26	<0.0001
RND1	G50A	18	-	18/80	Non-synonymous	17 R to Q	56.72	<0.0001
	A221G	-	36	36/80	Non-synonymous	74 K to R	113.43	<0.0001
GPHN	T124C	-	29	29/80	Non-synonymous	42 Y to H	91.37	<0.0001
	A148G	19	-	19/80	Non-synonymous	50 R to G	59.87	<0.0001
LPCAT1	A233C	30	-	30/80	Non-synonymous	78 E to A	94.53	<0.0001
	C284T	27	-	27/80	Non-synonymous	95 P to L	85.07	<0.0001
	T386C	35	-	35/80	Non-synonymous	129 L to P	110.28	<0.0001
FPGT	A212G	-	28	28/80	Non-synonymous	71 H to R	88.22	<0.0001
TNN13K	A153G	33	-	33/80	Synonymous	51 L	103.98	<0.0001
	T351C	-	25	25/80	Synonymous	117 T	78.77	<0.0001
NDUFS6	C113T	-	32	32/80	Non-synonymous	38 S to L	100.83	<0.0001

- CMPK2= Cytidine/Uridine Monophosphate Kinase 2; POPDC3= Popeye Domain Containing 3; KIF26A= Kinesin Family Member 26A; SYT8= Synaptotagmin 8; ASPG= Asparaginase; NRXN2= Neurexin 2; RND1= Rho Family GTPase 1; GPHN= Gephyrin; LPCAT1= Lysophosphatidylcholine acyltransferase 1; FPGT= Fucose-1-Phosphate Guanylyltransferase; TNN13K= troponin-I interacting kinase; and NDUSF56= NADH:ubiquinone oxidoreductase subunit S6.
- A= Alanine; E= Glutamic acid; G= Glycine; H= Histidine; I= Isoleucine; K= Lysine; L= Leucine; N= Asparagine; P= Proline; Q= Glutamine; R= Argnine; S= Serine; T= Threonine; V= Valine; and Y= Tyrosine.

The Synaptotagmin (*SYT8*) gene is a crucial exocytosis and insulin secretion regulator [65]. This gene's deletion may be linked to a tendency toward a poor energy balance and slowing metabolism, both of which are thought to have the consequence of increasing vulnerability to hoof disorders [8]. L-asparaginases, like ASPG, are essential for the metabolism of amino acids because they catalyze the conversion of asparagine to aspartic acid and ammonia [66]. Butty et al. [34] cited that *ASPG* gene was associated with DD susceptibility in Holstein dairy cattle. Leukocyte adhesion deficit type 3 is impacted by the neurexin 2 (NRXN2), which has been linked to features related to hoof health [67]. In the event that a pathogen is present in the body, this gene may improve the leukocytes' capacity to act.

The actin cytoskeleton is essential for cellular morphology maintenance, tissue integrity, and fibroblast migration during wound healing and tissue repair [68]. Gram-negative bacteria, such as treponemes, are known to use it as a target in their pathogenesis [48]. Human gingival fibroblasts are detached and their expression of filamentous actin is decreased by Treponema denticola. Stress fiber rearrangement is also increased [69]. In fibroblasts treated with T. pedis, the transcription of filamentous actins including Rho Family GTPase 1 (RND1) dramatically decreased [70]. Most intriguingly, *RND1* transcription was elevated in cattle fibroblasts by both T. medium phylogroup and T. pedis. Because of its suppression of the production of actin pressure fibers and damage of integrin-based focal adhesions, the overexpression of the *RND1* gene is linked to cell rounding [71]. Newbrook issued a challenge for Bovine Foot Skin Fibroblasts with Digital Dermatitis Treponemes. The system's utility in simulating the pathophysiology of BDD was highlighted by immunohistochemistry, which revealed elevated RND1 protein expression in BDD lesions.

The folate biosynthesis KEGG pathway was connected to the Gephyrin (*GPHN*) gene. Changes in the metabolism of folate result in an increase in blood metabolites that may have an impact on hoof quality [58]. Fucose-1-phosphate guanylyl transferase (FPGT), the L-fucose pathway includes a key sugar found in complex carbohydrates that are involved in cell-to-cell communication, inflammation, and immune responses. [72]. The protein kinase serine/threonine (TNNI3K) is similarly related to the causes of inflammation [73]. Based on this function, we predict that a marker gene for DD resistance may be found. According to Durán Aguilar et al. [74] the genetic locus on BTA20 has been linked to SCS in Holstein previously, suggesting that its gene content Lysophosphatidylcholine acyltransferase and NADH:ubiquinone oxidoreductase subunit S6 (*LPCAT1* and *NDUFS6*) may have an impact on the battle to mastitis and metabolic disorders, two factors that contribute to financial losses in the dairy industry.

Conclusion

The occurrence of digital dermatitis may be influenced by genetic and metabolic variables. Between DD healthy and affected buffaloes, genetic polymorphisms associated with digital dermatitis resistance/susceptibility were found by PCR-DNA sequencing of immunological and metabolic genes. The discovered SNPs in the examined genes could be used as a substitute marker, making it possible to choose traits for buffaloes susceptibility or resistance to digital dermatitis using genetic markers. Future therapeutic strategies for digital dermatitis may be made possible by the gene targets discovered here.

Conflict of interest

The authors affirm that they have no battle of interest.

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Author contributions

Ahmed Ateya conceived, designed the experiment, performed PCR and wrote the manuscript. Mona Al-Sharif performed DNA sequencing and contributed to writing the manuscript.

Data availability

On judicious request, the corresponding author will provide the information supporting the study's conclusions.

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رؤى جديدة حول جينات المرشح المناعي والتمثيل الغذائي التي تقيم المقاومة / القابلية للإصابة بالتهاب الزوائد الجلدية في الجاموس الإيطالي

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بسبب التوراث غير الكافي لمقاومة التهاب الزوائد الجلدية ، يصعب الحكم عليها من سجلات التربية. قد تكون هناك علاقة بين المتغيرات الجينية والأيضية وتطور التهاب الزوائد الجلدية. في هذه الدراسة ، تم استخدام 80 جاموسًا إيطاليًا نقيًا لفحص العلاقة بين الطفرات الجينية المناعية والتمثيل الغذائي والتهاب الزوائد الجلدية. تم فحص حدوث وتاريخ التهاب الزوائد الجلدية ، وتم تقسيم الحيوانات إلى مجموعتين: المجموعة المصابة (ن = 40) والمجموعة المقاومة (ن = 40). تم جمع عشرة مليلتر من الدم من كل جاموس لاستخراج الحمض النووي. كشف تفاعل انزيم البلمرة المتسلسل وطريقة معرفة التتابع النيكليوتيدى لجينات المناعة (، IL-8 ، TLR4 ASB16 ، DEFB123 ، IL-13 ، IL-17 ، CATH ، و SCART1) والتمثيل الغذائي (، CMPK2 LPCAT1 FPGT ، GPHN ، RND1 ، NRXN2 ، ASPG ، SYT8 ، KIF26A ، POPDC3 و TNN13K و NDUFS6) عن تعدد أشكال النوكليوتيدات المفردة بين الجاموس المقاوم والمصاب بالتهاب الزوائد الجلدية. لوحظ تباين كبير في تكرار الجينات ، بناءً على اختبار فيشر الدقيق لتعدد اشكال النيكليوتيدة المنفردة المكتشفة. يمكن استخدام تعدد اشكال النيكلوتيدة المحددة في الاختيار بمساعدة الواسمات الوراثية لمقاومة / قابلية التهاب الزوائد الجلدية في الجاموس. تقترح هذه النتائج نهجًا للحد من التهاب الزوائد الجلدية في الجاموس من خلال سجلات التربية المميزة للحيوانات المعتمدة على المعلمات الوراثية المصاحبة للمقاومة الجينية للعدوى.