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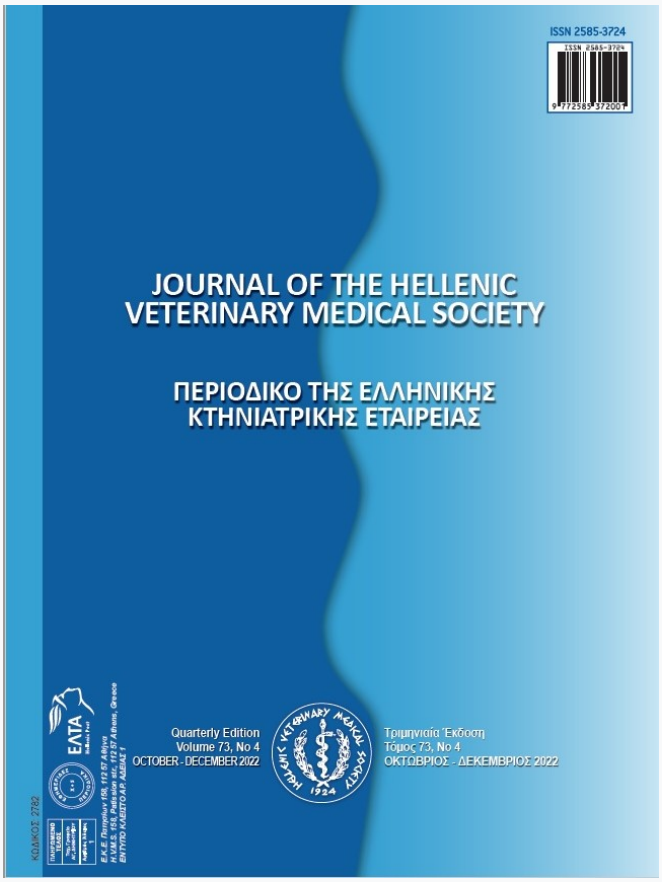

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Research article
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Genetic polymorphisms of *MASP2*, *TG5,* and *DQA1* genes in Holstein and Brown Swiss dairy cows

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ABSTRACT: The objective of this study was to investigate polymorphisms of *MASP2, TG5* and *DQA1* genes using PCR-DNA sequencing inseventy Holstein, and Brown Swiss dairy cows (35 cows each). Blood samples were collected from each animal into tubes containing disodium EDTA as an anticoagulant for DNA extraction. PCR was carried out for amplification of 305-bp of *MASP2,* 545-bp of *TG5* and 373-bp of *DQA1*genes DNA sequencing assessment elaborated nucleotide sequence variations in the forms of single nucleotide polymorphisms (SNPs) between two breeds. The SNPs identified in the investigated genes for the Holstein breed were A46G in the *MASP2* gene and C371T in the *TG5* gene. A characteristic T53C SNP was also reported for the Brown Swiss breed in the *DQA1* gene.Chi-square revealed a significant variation in distribution of all identified SNPs (P <0.001). Consequently, identified SNPs in the investgated genes could be efficeient for characterization of Holsteins, and Brown Swiss breeds.

Keywords: DNA sequencing; gene polymorphism; dairy breeds

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INTRODUCTION

The diversity within and between cattle breeds is closely linked to their origin, history, and evolution (Bradley et al., 1996; MacHugh et al., 1997; Troy et al., 2001). A crucial element of biodiversity conservation is in situ protection of indigenous, native breeds of cattle constituting a reservoir of unique combinations of genes and alleles, which in thefuture may appear highly valuable, e.g. for the creation of new livestock genotypes (Bulla et al., 2013). Although diversity between the world's cattle breeds does not allow the species to be classified as endangered, the loss of local breeds is an irreversible and irreplaceable erosion of genetic resources (Taberlet et al., 2008; FAO, 2011). The genetic structure can be characterized using a variety of molecular markers, depending on the research problem (Groeneveld et al., 2010). In recent years, analysis of single nucleotide polymorphisms (SNP) has begun to dominate this area. New technologies using SNP or whole-genome scanning may revolutionize previous achievements in biodiversity assessment and genetic characterization of breeds, providing therefore a more thorough understanding of the molecular basis of functional diversity (Groeneveld et al., 2010). There is considerable interest in the application of molecular genetic technologies in the form of specific DNA markers that are associated with various QTL to promote more efficient and relatively easy selection and breeding of farm animals with advantages for inheritable traits such as growth rate, body weight, carcass merit, feed intake as well as milk yield and composition (Spelman and Bovenhuis, 1998).

The bovine mannose-binding lectin-associated serine protease (*MASP2*) gene is located on chromosome 16, consisting of 11 exons. It encodes a protein of 686 amino acids. Polymorphisms in the *MASP-2*gene have a strong link to autoimmune disorders (Wu et al., 2015). *MASP2* gene polymorphisms and MASP2 serum levels are associated with several inflammatory disorders and infections. MASP2 is the top biomarker for the diagnosis of HCC(M2) and upper respiratory tract infection in children (Xiong et al., 2015). The levels of MASP2 in sepsis patients differ according to age, higher levels were found in neonates and lower levels were found in older patients compared to healthy controls (Świerzko et al., 2016). Low levels increase the susceptibility to HIV and HCV/HBV co-infection (Boldt et al., 2016; Silva et al., 2018). Moreover, *MASP2* gene has been associated with rheumatoid arthritis (Goeldner et al., 2014), the susceptibility to tuberculosis when the SNPs (rs2273346 and rs6695096) of MASP2 occurs (Chen et al., 2015), and increased risk of ischemic stroke, the SNP (rs147270785_A alleles) of *MASP2* has been shown to protect against ischemic stroke (Tsakanova et al., 2018). It can be also used as a prognostic biomarker for liver cancer (Ding et al., 2014) and colorectal cancer (Ytting et al., 2011).

The thyroglobulin 5′ leader sequence (*TG5*) gene is one of the longest mammalian genes. It is found in the centromeric region of the 14th chromosome, consisting of 37 exons in cattle (Gan et al., 2008; Ardicli et al., 2018). It has two alleles, TG5T and TG5C resulting in three genotypes, TG5TT, TG5CC, and TG5CT.*TG5* gene is considered as one of the vital polymorphic candidate genes encoding hormones such as prolactin and somatotropin, which determine the level of dairy and meat productivity in cows. It is a precursor of the thyroid iodothyronine hormones (thyroid hormone, the triiodothyronine (T3), and thyroxine (T4) hormones), which adjust various physiological and biochemical processes in virtually all body tissues by regulating gene expression. Moreover, thyroid hormones influence lipid metabolism and adipocyte development into adipose tissue. Consequently, the thyroglobulin gene is a functional and positional candidate gene that influences fat accumulation in tissues, including milk (Dolmatova et al., 2020). A single-nucleotide polymorphism in the 5′ untranslated region of the *TG5* gene is used for marker-based selection targeted at raising marbling (Carvalho et al., 2012). Various investigations have revealed that the thyroglobulin gene polymorphism has been associated with fat metabolism, especially intramuscular fat (Anton et al., 2012; Selionova et al., 2019), as well as quality and quantity of milk yield (Anton et al., 2012; Tyul'kin et al., 2013; Zinnatova and Zinnatov, 2014).

The bovine *DQA1* gene is a highly polymorphic gene located on the short arm of chromosome 23. It is one of the Major Histocompatibility Complex type-A genes (MHC-Class II), which belonged to the Immunoglobulin superfamily with the most polymorphism (Vandre et al., 2014).The major histocompatibility complex (*MHC*) genes are called bovine leukocyte antigen (BoLA) genes in cattle. They play a critical role in the induction and regulation of immune responses. Also, they are considered as candidate markers for several diseases and immunological traits in bovine (Takeshima and Aida, 2006). Although the BoLA region appears to be arranged similarly to the MHC

region in humans, there are significant differences. The BoLA region of chromosome 23 has been divided into two independent sub-regions by a third of the chromosome's length due to a substantial rearrangement in the class II region (Amills et al., 1998; Band et al., 1998). The functionally expressed *DQ* and *DR* genes are found in the class IIasubregion, while genes with unknown functions, such as *LAP2*, *DYA*, *DYB*, *TAP1*, *TAP2*, *DMA*, *DMB*, *LMP7*, *DOB*, and *DOA* are found in the class IIbsubregion. The principal class II restriction elements for CD4 T-helper cells are the DR and DQ molecules, which are produced by the BoLA gene (Aida, 1995; Glass et al., 2000). Each BoLA haplotype produces only a single DR product and one or more DQ products. On the other hand, human MHC class II expresses several DR and DQ products. *DQ* genes are duplicated in about half of the typical class II haplotypes, both sets of *DQ* genes can be expressed. This duplication in association with the *DQA* and *DQB* genes polymorphisms significantly increases variation at the cell surface due to inter-andintra-haplotype pairing of DQ a- and b chains.Duplicated *DQ* genes produce DQ restricted T-cell clones while a single DQ gene pair produce the majority of DR restricted clones in cattle (Glass et al., 2000). It is found that duplicated *DQ* haplotypes complicate the use of restriction elements in cattle. Therefore, cattle can express a variety of class II gene products, any of which could contribute positively or negatively to the immunological response to specific antigens. Cattle have one *DRA* gene, three *DRB* genes (only one of which, DRB3, is thought to be functionally significant), and one or two *DQA* and *DQB* genes, depending on a haplotype (Andersson et al., 1986; Sigurdardóttir et al., 1992). Five *DQA* and five *DQB* loci are located in the DQ region, with exon 2 of the *DQA1*, *DQA2*, *DQB1*, and *DQB2* genes being highly polymorphic(Davies et al., 1997; Russell et al., 1997).

There is little information on the molecular characterization of *MASP2, TG5,*and *DQA1* genes in European breeds of cattle (Ibeagha-Awemu et al., 2007; Schennink et al., 2009). Although research has elaborated associations between these studied genes polymorphism and productive traits in a single breed, there is however a lack of studies regarding genetic polymorphisms of these genes in Holstein and Brown Swiss breeds of cattle. Consequently, the objective of the present study was to characterize thegenetic structure of Holstein, and Brown Swiss breeds of cattle using polymorphism at loci of functional genes encoding *MASP2*, *TG5*, and *DQA1*via DNA sequencing approach.

MATERIALS AND METHODS

Ethics statement

The collection of samples and care of the animals used in this study followed guidelines for experimental animals established by Research Ethics Committee, Faculty of Veterinary Medicine; Mansoura University (code Ph. D/58).

Animals and experimental samples

SeventyHolstein and Brown Swiss dairy cows (35 cows of each breed) were used in this study. Animals belonged to a private farm located at Ismailia desert road, Ismailia Governorate, Egypt. Animals were in the third lactation season and were raised in a commercial dairy herd of approximately 450 animals. Cows were 3 years of age on average and 450 kg of average body weight. Animals were housed in a cubicle (free-stall/feedlot) barn with straw-bedded stalls and a slatted floor that was scraped regularly. They were fed a total mixed ration (TMR), milked twice/day, and artificially inseminated. The annual milk production per cow averaged 8500 kg energy-corrected milk. Blood samples were collected from each animal into tubes containing disodium EDTA as an anticoagulant for DNA extraction.

DNA extractionand Polymerase Chain Reaction (PCR)

Extraction of the genomic DNA from whole blood was done using Gene JET whole blood genomic DNA extraction kit following the manufacturer procedure (Thermo Scientific, Lithuania). The purity and concentration of DNA samples were screened by Nanodrop for further analysis.

PCR was carried out for amplification of fragments of 305-bp of*MASP2,* 545-bp of *TG5,* and373-bp of *DQA1*genes.The primers used in the amplification are shown in Table 1. The polymerase chain reaction mixture was done in a final volume of 100 µL in a thermal cycler. Each reaction volume contained 6 µL DNA, 41 µL H_2O (d.d water), 50 µL PCR master mix (Jena Bioscience, Germany), and 1.5 µL of each primer. The reaction mixture was subjected to an initial denaturation temperature of 94°C for 4 minutes. The cycling proceeded for 30 cycles of 94°C for 1 minute for denaturation, annealing temperatures (as shown in Table 1) for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. Samples were held at 4 °C and representative results of PCR analysis were detected by agarose gel electrophore-

Table 1 Forward and reverse primer sequence, length of PCR product and annealing temperature for *MASP2*, *TG5* and *DQA1* genes

Primer	Forward	Reverse	Annealing Temperature (°C)	Length of PCR product(bp)	Reference
MASP2	5′-GTTTGTGGGAG GAATAGTGTC -3′	5-′-AGTTAAGTAGTG GAAGAGTGGC -3′	60	305	[60]
DQA1	5′′-TCAATTTCTTC TTTCACTTTGCT-3′	5′-GGTTTGAAGGGGT AGATTAATAAA-3′	58	373	[59]
TG5	5′′-GGGGATGACTA CGAGTATGACTG-3′	5′-GTGAAAATCTT GTGGAGGCTGTA-3′	55	545	[21]

Table 2. Distribution of SNPs of *MASP2*, *DQA1,* and *TG5* in Holstein and Brown Swiss cattle

		Breeds				
Gene	SNP type	Holstein (N=35)	Brown Swiss (N=35)	Total animals (n=70)	Fisher's exact	P-value
Masp2	A46G	21	----	21/70	20.82	< 0.0001**
DQA1	T53C	----	-----	15/70	43.45	< 0.0001**
TG5	C371T	19	----	12/70	57.85	< 0.0001**

sis.The fragment patterns were then visualized under U.V using a gel documentation system.

DNA sequencing and polymorphism detection

Before DNA sequencing, removing primer dimmers, nonspecific bands, and other impurities was done. As described by Boom et al., (1990), purification of PCR products with the expected size was carried out using a PCR purification kit following the manufacturer procedures (Jena Bioscience # pp-201×s/Germany). Quantification of PCR product was carried out using Nanodrop (Uv-Vis spectrophotometer Q5000/USA) in order to yield high products and to ensure enough concentrations and purity of the PCR products (Boesenberg-Smith et al., 2012). To detect single nucleotide polymorphisms in the investigated genes between the two breeds, PCR products with target band were sent for DNA sequencing in forward and reverse directions using ABI 3730XL DNA sequencer (Applied Biosystem, USA), depending on the enzymatic chain terminator technique developed by Sanger et al., (1977).

Analysis of DNA sequencing data was carried out by chromas 1.45 (http://www.technelysium.com.au) and blast 2.0 software (Altschul et al., 1990). Differences were classified as single-nucleotide polymorphisms (SNPs) between PCR products of investigated genes and reference sequences available in GenBank. Based on DNA sequencing data alignment, amino acid sequence variation between the two breeds was performed using the MEGA4 software package(Tamura et al., 2007).

Statistical analysis

Statistical analysis was performed using Graphpad statistical software program (Graphpad prism for Windows version 5.1, Graphpad software, Inc., San Diego, CA, USA). Difference in the frequencies of SNPs in *MASP2*, *TG5,* and *DQA1*between Holstein and Brown Swiss breeds was statistically evaluated using (Crosstabs).Fisher's exact test was carried out to compare the distribution of the identified SNPs.

Gene and genotypic frequencies

Gene and genotypic frequencies were calculated by allele simple counting (Falconer and Mackay, 1996). Chi-square was carried out to test Hardy-Weinberg equilibrium and show genotype distribution in the Holstein and Brown Swiss cattle populations.

RESULTS

Molecular characterization of *MASP2, TG5* and *DQA1* genes

PCR-DNA sequencing revealed a variation in nucleotide sequence in form of SNPs between Holstein and Brown Swiss breeds (Table 2). Fisher's exact test showed a significant difference in the frequencies of studied genes between the two breeds. DNA sequencing of the *MASP2* gene (305-bp) revealed

Table 3. Type and position of SNPs in *MASP2*, *DQA1* and *TG5* genes and corresponding amino acid number and type

Gene	SNP type	SNP position	Breed/no of animals harboring SNP	Type of mutation	Amino acid number and type
MASP2	A/G	46	Holstein/ 21	Nonsynonymous	16 K to E
DQA1	T/C	53	Brown Swiss/ 15	Nonsynonymous	18 F to S
TG5	C/T	371	Holstein/ 12	Nonsynonymous	124 S to F

Figure 1. Representative DNA sequence alignment of *MASP2* gene (305-bp) between Holstein and Brown Swiss cattle and reference sequence available in GenBank gb|XM_027564920.1|. Asterisks represent similarity. H is Holstein and B is Brown Swiss

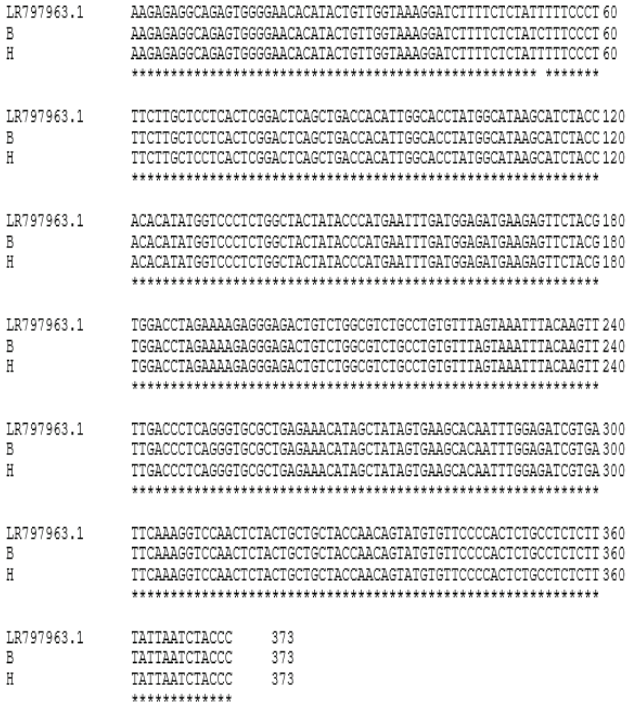

Figure 2. Representative DNA sequence alignment of *DQA1* gene (373-bp) between Holstein and Brown Swiss cattle and reference sequence available in GenBank gb|LR797963.1|. Asterisks represent similarity. H is Holstein and B is Brown Swiss

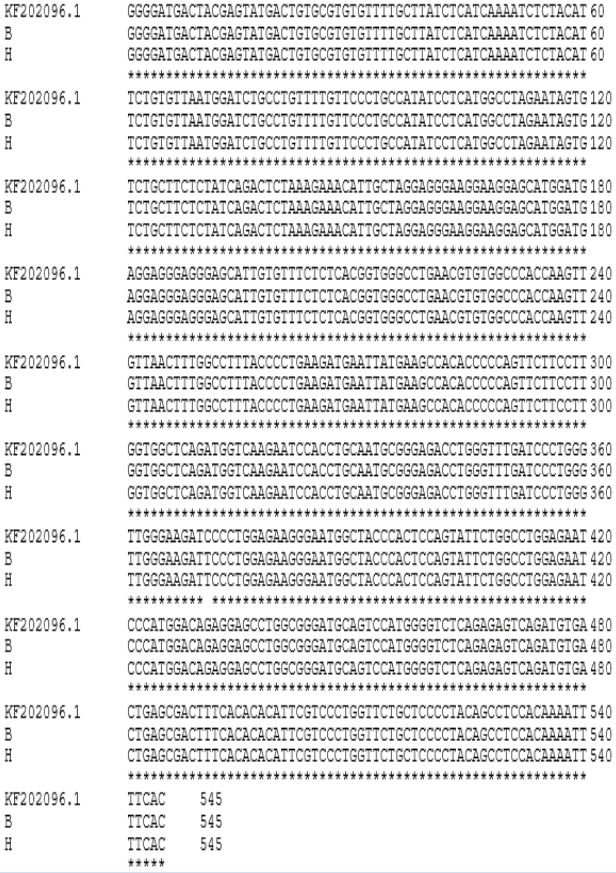

Figure 3. Representative DNA sequence alignment of *TG5* gene (545-bp) between Holstein and Brown Swiss cattle and reference sequence available in GenBank gb|KF202096.1|. Asterisks represent similarity. H is Holstein and B is Brown Swiss

one non-synonymous SNP (A46G) specific for many Holstein cows. In the same line, DNA sequencing of *DQA1* (373-bp) revealed also one non-synonymous SNP (T46C) specific for manyBrown Swiss cows. In the*TGF5* gene, the identified SNP (C371T) was non-synonymous and specific for many Holstein cows. Variations in the nucleotide sequence of *MASP2* gene (305-bp), *DQA1* (373-bp), and *TG5* (545-bp) genes between the two breeds, as well as between these breeds and reference sequences available in GenBankwere classified as single-nucleotide polymorphisms (SNPs) (Figure. 1, 2, and 3). Amino acids sequence variation of the coding regions of *MASP2, TG5,*and *DQA1*genes between two breeds is shown in Table 3. The three identified SNPs in the investigated genes were non-synonymous.

The population of seventyHolstein and Brown Swiss (35 each) was genetically described. Where, the incidence and frequency of genotypes and alleles for each gene were calculated (Tables 4, 5 and 6). The $\chi 2$ -test showed that the genotype distributions in the cattle populationswere notin Hardy-Weinberg equilibrium ($p<0.05$).

DISCUSSION

Despite the increasing number of studies assessing the genetic variability of various cattle breeds around the world, very few of them have focused on characterizing thegenetic diversity of native Central European cattle breeds and analyzed their genetic structure (Gutiérrez et al., 2003; Petrakova et al., 2012). Studies that determined phylogenetic affiliation of various breeds of cattle based on functional gene polymorphism are also uncommon. There was a bias in research towards the assessment of genetic variability of cattle breeds belonging to northern Europe, or towards the determination of phylogenetic affiliation of European and African cattle breeds based on haplotype variation (Gautier et al., (2007). Several studies indicated that functional gene polymorphism is closely linked to specific production traits in cattle, and analysis of variability at gene loci makes it possible to assess the predisposition of animals to a specific type of production (Kamiński and Zabolewicz, 2000; Dobicki et al., 2002; Liefers et al., 2002; Buchanan et al., 2003; Brym et al., 2005; Curi et al., 2006; Komisarek and Antkowiak, 2007; Ghasemi et al., 2009;

Table 4. Frequency of genotypes and alleles for the *MASP2* locus in Holstein and Brown Swiss breeds

Gene	Item	Breed	Number/frequency of genotypes			Allele frequency	
			AA	AG	GG	A	G
MASP2	Observed	Holstein	10/0.29	4/0.11	21/0.60	0.34	0.66
	Expected	N= 35	4.046	15.708	15.246		
		Brown Swiss	35/1.0	-	-	1	0
		N= 35	-	-	-		

Holstein: Chi Square calculated (χ^2) = high significance differences 19.66 Chi Square tabulated (χ^2) at DF 1 and $p< 0.05 = 3.84$.
Brown Swiss: A allele is the only allele at its locus and AA is the only genotype in the population

Table 5. Frequency of genotypes and alleles for the *DQA1* locus in Holstein and Brown Swiss breeds

Gene	Item	Breed	Number/frequency of genotypes			Allele frequency	
			TT	TC	CC	T	C
DQA1	Observed	Holstein	35/1.0	-	-	1	0
	Expected	N= 35	-	-	-		
		Brown Swiss	17/0.49	6/0.17	12/0.34	0.57	0.43
		N= 35	11.3715	17.157	6.4715		

Holstein: T allele is the only allele at its locus and TT is the only genotype in the population. Brown Swiss: Chi Square calculated (χ^2) = high significance differences 14.76 Chi Square tabulated (χ^2) at DF 1 and $p< 0.05 = 3.84$.

Table 6. Frequency of genotypes and alleles for the *TG5* locus in Holstein and Brown Swiss breeds

Gene	Item	Breed	Number/frequency of genotypes			Allele frequency	
			CC	CT	TT	C	T
TG5	Observed	Holstein	13/0.37	7/0.20	15/0.43	0.47	0.53
	Expected	N= 35	7.7315	17.437	9.8315		
		Brown Swiss	35/1.0	-	-	1	0
		N= 35	-	-	-		

Holstein: Chi Square calculated (χ^2) = high significance differences 12.55 Chi Square tabulated (χ^2) at DF 1 and $p< 0.05 = 3.84$.
Brown Swiss: C allele is the only allele at its locus and CC is the only genotype in the population

Karimi et al., 2009; Signorelli et al., 2009; Rahbar et al., 2010).

In this context, PCR-DNA sequencing was carried out for molecular characterization of fragments of 305-bp of *MASP2,* 545-bp of *TG5,* and373-bp of *DQA1* genes in Holstein and Brown Swiss breeds of cattle exposed to environmental conditions of Egypt. The SNPs identified in the investigated genes for the Holstein breed were A46G in the *MASP2* gene and C371T in the *TG5* gene. A characteristic T53C SNP was also reported for theBrown Swiss breed in the *DQA1* gene.The occurrence of some exclusive SNPs in each breed is probablyrelated to the founder effect associated with origin, history, evolution, and to differences in genetic constituents in each breed (Bradley et al., 1996; MacHugh et al., 1997; Troy et al., 2001). There is little information available on the molecular characterization of productive genes in European breeds of cattle (Ibeagha-Awemu et al., 2007; Schennink et al., 2009). Moreover, this is the first study that reports genetic polymorphisms of *MASP2, TG5,* and *DQA1* genes in Holstein and Brown Swiss breeds. The $\chi 2$ -test showed that the genotype distributions in the cattle populations were not in Hardy-Weinberg equilibrium ($p<0.05$).

Several studies have reported the association of *MASP2, TG5,* and *DQA1*genes polymorphism with production and components in cattle (Al-Waith et al., 2018; Zhang et al., 2019; Dolmatova et al., 2020). However, unlike our study, all of these studies investigated the association of gene polymorphism in only one breed of cattle. Also, all previous studies reported gene polymorphisms using other genetic markers (RFLP and SSCP). The current study explored polymorphisms via SNP genetic marker which may revolutionize previous achievements in conservation decisions, biodiversity assessment, and genetic characterization of breeds, providing, therefore, a more understanding of the molecular basis of functional diversity (e.g. Groeneveld et al., (2010). SNPs analy-

sis could explain the history of European cattle more accurately than other markers (Gautier et al., 2007; Svensson et al., 2007; McKay et al., 2008; Socol et al., 2015). Particular importance is also attributed to SNPs in the search for linkages between a marker with a specific location in the genome and an unknown gene locus. The search for such associations is important because they allow a phenotypic effect to be assessed by identifying its genetic basis (Svensson et al., 2007; McKay et al., 2008). Additionally, DNA sequence alignment revealed novel single nucleotide polymorphisms in *MASP2, TG5,* and*DQA1*genes when matched with the Gen Bank reference sequence (Figure 1, 2, and 3). Interestingly, our results indicated that the polymorphisms identified are reported here for the first time.

Assessment of genetic variation based on functional gene polymorphism can be used both to characterize thegenetic structure of different breeds of cattle and to supplement analysis of genetic diversity based on polymorphisms of genetic markers (Kasprzak-Filipek et al., 2019). In the current study, PCR was carried out to amplify 305-bp of *MASP2,* 545-bp of *TG5,* and373-bp of *DQA1* genes. However, one SNP in each of *MASP2* and *TG5* genes was characteristic for theHolstein breed. Additionally, one SNP in the *DQA1* gene was also characteristic for theBrown Swiss breed. The similarity in the remaining part of the amplified fragments and the common SNPs between the two breeds may be attributed to conducting PCR-DNA sequencing on a conserved part i.e. exon of investigated genes that enables accurate molecular characterization of genes and deciphers physiological differences in milk production and disease resistance between breeds (Singh et al., 2014). Other causes may be the close geographic proximity and gene flow between breeds in the past. It should be noted that all analyzed populations represented local breeds which, in the past, were the most popular breeds in East-Central Europe, before intensification and globalization of agriculture that led to their marginalization. Current genetic resource conservation programs are contributing to an increase in the numbers and to the preservation of valuable gene reservoirs(Kasprzak-Filipek et al., 2019).

It has been suggested that genes related to milk yield and composition traits exist in all autosomal chromosomes in cows. The most important genes affecting the amount and percentage of milk fat are found on *Bos taurus* autosomal chromosomes (BTA) 5, 6, 9, 14, 20, and 26 (Khatkar et al., 2004). It was reported that *MASP2, TG5,* and *DQA1* genes polymorphisms were associated with milk production, mastitis resistance susceptibility, and heat tolerance traits in dairy cows suggesting the phenotypic variation in the latter traits between Holstein and Brown Swiss breeds could be attributed to these genetic loci.

The limitation of this study should be acknowleged; a higher number of animals are needed. Other breeds of cattle should and much information about pedigree and relatedness of animals also should be considered.

CONCLUSION

PCR-DNA sequencing of*MASP2, TG5,* and *DQA-1*genes revealed a nucleotide sequence variation in the form of SNPs between Holstein and Brown Swiss breeds. These findings suggest that variability in thesegenes could be used for efficient characterization and genotyping of European cattle breeds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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