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Restriction Fragment Length Polymorphism and Gene Mapping of Two Genes Associated with Milk Composition in Egyptian River Buffalo*

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Abstract: Gene maps and genetic polymorphisms of genes related to the economic quantitative traits as tools for developing more efficient breeding strategies have as targeted animal improvement by the genomic approach. This study aimed to identify the genetic polymorphism and gene mapping of two genes, BTN and ODC, are associated with milk composition in river buffalo. By means of PCR, the amplified fragment of BTN obtained from all tested buffalo DNA was at 501 bp. After digestion of the PCR products with HaeIII, we can distinguish between A and B alleles. The allele A is the most common allele in the Egyptian river buffalo, where the estimated frequencies were 0.89 and 0.11 for alleles A and B, respectively. The PCR products of ODC with a 796 bp fragment were digested with MspI. Fifty four of 70 animals (77.14%) have MspI(+/+) genotype and 13 animals (18.57%) have MspI(+/-) whereas three animals (4.29%) displayed the homologous MspI(-/-) genotype. The assignment of BTN and ODC to river buffalo chromosomes was done by calculating the correlation coefficient (φ) between these two genes and markers representing the river buffalo chromosomes. The results showed that BTN is assigned to the bi-armed buffalo chromosome 2, whereas ODC is assigned to the acrocentric river buffalo chromosome 12.

Key words: River buffalo, BTN, ODC, PCR, RFLP

Introduction

Butyrophilin (BTN) is a trans-membrane protein possessing an exoplasmic N terminus and a single membrane-spanning domain (Jack and Mather, 1990) and comprises 40% by weight of the total protein composition of the bovine milk-fat globulin membrane (Mather *et al*., 1980). This protein is constitutively secreted by surface budding associated with milk lipid production (Franke *et al*., 1981). Consequently, the potential involvement of the BTN gene on milk composition in dairy cattle is of interest (Taylor *et al*., 1996).

Ornithine decarboxylase (ODC) is the rate determining enzyme in the biosynthesis of polyamines, which are essential for protein biosynthesis, DNA replication and cellular differentiation (Pegg, 1986). In some species, ODC has been implicated as a candidate gene affecting growth rate (Bulfield *et al*., 1988; Gray and Tait, 1993). In dairy cattle, animal selection has resulted in an approximately doubling of milk production over the last 40 years (Fallert and Liebrand, 1991). The genetic variants of ODC could be associated with mammary gland activity or interact with trophic hormones during lactation, thus affecting milk performance in dairy cattle (Yao *et al*., 1996).

The closeness between cattle and river buffalo is useful in studying the genetic polymorphisms and constructing the buffalo genome map. The chromosome homology between cattle and river buffalo

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chromosomes indicates gene mapping conservation and also the genetic homology between these two species (El Nahas *et al*., 2001).

The aim of this study was to assign BTN and ODC genes to river buffalo chromosomes using somatic cell hybrids and also to detect the genetic polymorphisms of these two genes in buffalo using PCR-RFLP.

Materials and Methods

Gene Mapping

Somatic Cell Hybridization

Somatic cell hybrids used in this study were produced by fusing river buffalo lymphocytes, in the presence of polyethylene glycol, with cells of hypoxanthine phosphoribosyl transferase deficient ($HPRT^-$) Chinese hamster cell line wg3h (Echard *et al*., 1984). Fused buffalo-hamster cells were isolated from the parental cells, using hypoxanthine-aminopterine-thymidine (HAT) selective media (Hondt *et al*., 1991).

Genomic DNA Extraction

Genomic DNA was extracted from buffalo whole blood, Chinese hamster cell line and buffalo-hamster hybrid cells according to established protocols (Blin and Stafford, 1976; Shih and Weinberg, 1982; Adkison *et al*., 1988). Briefly, the cells were incubated overnight in a shaking water-bath at 37°C in lysis buffer with 20% sodium dodecyl sulfate and proteinase K. Nucleic acids were extracted once with phenol, saturated with TE buffer, followed by extraction with phenol-chloroform-isoamyl alcohol (25:24:1) and this was followed by extraction with chloroform-isoamyl alcohol (24:1). To the final aqueous phase, 0.1 volume of 2.5 M Na acetate and 2.5 volume of cold 95 % ethanol were added. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The DNA was finally dissolved in an appropriate volume of 1X TE buffer. DNA concentrations were determined and diluted to the working concentration of 50 ng μL^{-1}, which is suitable for polymerase chain reaction.

Polymerase Chain Reaction (PCR)

The primers used in this study are basically of cattle origin but because of very high degree of nucleotide sequence conservation between cattle and river buffalo, these primers likely to give amplification in buffalo too. These primers were used successfully in PCR amplification for buffalo DNA.

A PCR cocktail consists of 1.0 µM upper and lower primers and 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin (w/v), 0.1 % Triton X-100 and 1.25 units of Taq polymerase. The cocktail was aliquot into tubes with 100 ng DNA of buffalo, hamster or hybrid cells. The reaction mixture was overlaid with sterile mineral oil. The reaction was cycled for 1 min at 94°C, 2 min at an optimized annealing temperature that is determined for each primer (Table 1) and 2 min

Table 1: The DNA sequence and the information of the primers used

Gene	Primer sequence 5`----------3`	PCR conditions	Reference
BTN	(BTN9) TGG AGC TCT ATG GAA ATG GG	1 min 94°C	Taylor *et al*. (1996)
	(BTN11) CTA CCC AAC AGG AAG AAA CAG	2 min 65°C	
		2 min 72°C	
ODC	ACC ACA GGA TAT GCA GAC TGG	1 min 94°C	Yao *et al*. (1996)
	GCA CCC ATG TTC TCA AAG AGC	2 min 61°C	
		2 min 72°C	

at 72°C for 30 cycles. The PCR reaction products were electrophoresed on 3% agarose gel containing ethidium bromide.

Statistical Analysis

The segregation profile (presence or absence) of buffalo-specific PCR product for each gene was studied in the 45 somatic cell hybrids. The assignment of these two genes to buffalo chromosomes was done by calculating the correlation coefficient (φ) between these two genes and markers representing buffalo chromosomes (Chevalet and Corpet, 1986).

$$\text{Correlation coefficient } (\varphi) = \frac{ab - bc}{\sqrt{(a+b)(a+c)(d+b)(d+c)}}$$

Where:

a = Number of hybrids which are positive for both markers
b = Number of hybrids which are positive for first marker and negative for the second marker
c = Number of hybrids which are negative for first marker and positive for the second marker
d = Number of hybrids which are negative for both markers

The two markers are considered syntenic (are located on the same chromosome) when (φ) exceed 0.69 and asyntenic (are located on the different chromosomes) if (φ) is less than 0.69.

Genetic Polymorphism

Animals

Experimental materials for the genetic polymorphisms comprised of blood samples from 70 unrelated buffaloes reared in different farms from different locations in Egypt.

Genomic DNA Extraction

Genomic DNA was extracted from whole blood by phenol-chloroform method with minor modification. Ten milliliter of blood taken on EDTA was mixed with 25 mL cold sucrose-triton and the volume was completed to 50 mL by autoclaved double distilled water. The solution was mixed well and the nuclear pellet was obtained by spin and discarding the supernatant. Nucleic acids were extracted as previously described above in gene mapping.

Polymerase Chain Reaction (PCR)

The same primers which used for gene mapping of these two genes were used to detect genetic polymorphisms of BTN and ODC genes in river buffalo. A PCR cocktail was aliquot into tubes with 100 ng of buffalo DNA. The reaction was cycled for 1 min at 94°C, 2 min at an optimized annealing temperature (Table 1) and 2 min at 72°C for 30 cycles.

RFLP and Agarose Gel Electrophoresis

Twenty microliter of PCR product were digested with 10 units of each restriction enzyme used in this study in a final reaction volume 25 μL. The reaction mixture was incubated at 37°C in water bath for 5 h. After restriction digestion, the restricted fragments were analyzed by electrophoresis on 2.5% agarose/1X TBE gel stained with ethidium bromide. The 100 bp ladder was used as molecular size marker. The bands were visualized under UV light and photographed with yellow filter on black and white film.

Results

Genetic Polymorphism of BTN Gene

By means of PCR, the genomic buffalo DNA was amplified using oligonucleotide primers, BTN9 and BTN11, were designed from the published cDNA sequence of bovine BTN gene. The amplified fragment obtained from all tested buffalo DNA (70 animals) was at 501 bp. The PCR products were digested with *Hae*III endonuclease. After digestion, we can easily distinguish between A and B alleles. Allele A has a characteristic digested fragment at 316 bp, whereas in allele B, this fragment was further digested into 2 fragments at 283 and 33 bp. There are three common digested fragments at 162, 13 and 10 bp for both alleles. The three small fragments 33, 13 and 10 bp not easily demonstrated.

The result showed that the allele A is the most common allele in the Egyptian river buffalo where the genotype AA was displayed in 58 animals (82.86%) and the genotype AB was displayed in 8 animals (11.43%) whereas the homologous BB was displayed in 4 animals (5.71) (Fig. 1). The estimated frequencies were 0.89 and 0.11 for alleles A and B, respectively.

Genetic Polymorphism of ODC Gene

The buffalo genomic DNA was amplified using PCR primers designed to amplify a 796 bp fragment enclosing the sequence from the eighth intron to the eleventh exon in the bovine ODC gene. The PCR products were digested with *Msp*I endonuclease. The polymorphic *Msp*I site is the only site present in the amplified 796 bp, which is restricted into two fragments at 556 and 240 bp when the *Msp*I site is present. After digestion, we can differentiate between three different genotypes of ODC gene. MspI(-/-) gave undigested fragment at 796 bp, MspI(+/+) gave 2 fragments at 556 and 240 bp and the genotype MspI(+/-) is characterized by 3 fragments at 796, 556 and 240 bp (Fig. 2).

The result showed that the frequencies of the MspI(+) and MspI(-) were 0.86 and 0.14, respectively. Fifty four of 70 animals (77.14%) have MspI(+/+) genotype and 13 animals (18.57%) have MspI(+/-) whereas three animals (4.29%) displayed the homologous MspI(-/-) genotype.

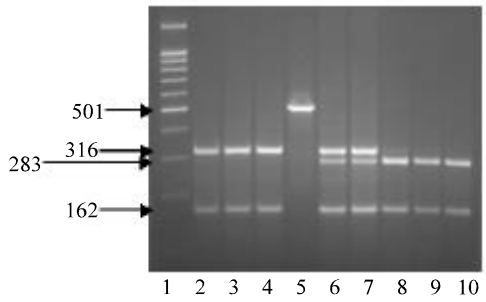

Fig. 1: The electrophoretic pattern obtained after digestion of PCR amplified buffalo BTN products with HaeIII
Lane 1: 100 bp ladder marker, Lanes 2-4: Homozygous AA genotype , Lane 5: 501 bp undigested PCR product, Lanes 6-7: Heterozygous AB genotype, Lanes 8-10: Homozygous BB genotype

Table 2: The correlation coefficient (φ) of segregation of tested genes and markers representing syntenic groups and buffalo chromosomes

Syntenic group	Buffalo chromosome	Markers	BTN (φ)	ODC (φ)
U1	5q	HUJ614	0.38	0.23
U2	10	CGA	0.22	0.04
U3	4q	IGFI	0.53	0.12
U4	20	CSSM18	0.42	0.07
U5	11	NP	0.02	0.39
U6	6	CYM	0.34	0.09
U7	5p	OBCAM	0.50	0.09
U8	24	ELN	0.61	0.16
U9	18	GPI	0.13	0.14
U10	1q	CD18	0.01	0.04
U11	14	PRNP	0.32	0.13
U12	21	CSSM6	0.22	0.33
U13	8	INHBA	0.03	0.30
U14	19	MAP1B	0.29	0.01
U15	7	ADH2	0.02	0.03
U16	12	LGB	0.19	**1.00**
U17	2q	INHA	**0.95**	0.19
U18	3q	CSSM47	0.09	0.10
U19	16	HBB	0.14	0.10
U20	2p	PRL	**1.00**	0.19
U21	3p	MAP2C	0.00	0.00
U22	9	LDLR	0.13	0.15
U23	17	DU23S1	0.13	0.13
U24	15	BSPN	0.27	0.02
U25	1p	ANT1	0.22	0.18
U26	23	G10P1	0.00	0.55
U27	13	F10	0.36	0.13
U28	22	YES1	0.33	0.02
U29	4p	ETH1112	0.49	0.16
X	X	G6PD	0.21	0.19
Y	Y	ZFY	0.27	0.11

Two markers are syntenic when φ>0.69 at an error rate Q = 0.025 and probability for correct decision p = 0.96

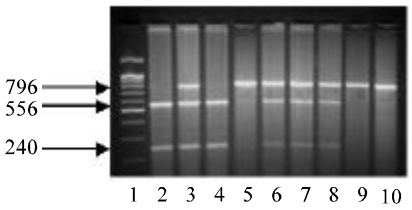

Fig. 2: The electrophoretic pattern obtained after digestion of PCR amplified buffalo ODC with MspI Lane 1: 100 bp ladder marker, Lanes 2 and 4: Homozygous +/+ genotype, Lanes 3 and 6-8: Heterozygous +/- genotype, Lane 5: 796 bp undigested PCR product, Lanes 9-10: Homozygous -/- genotype

Gene Mapping of BTN and ODC Genes

The assignment of BTN and ODC to river buffalo chromosomes was done by calculating the correlation coefficient (φ) between these two genes, BTN and ODC and markers representing the syntenic groups and river buffalo chromosomes (Table 2).

BTN is segregated dependently with the INHA, the marker of syntenic group U17 and buffalo chromosome 2q (Hondt *et al*., 2000) with a φ value 0.95. Also, BTN is segregated with PRL, the

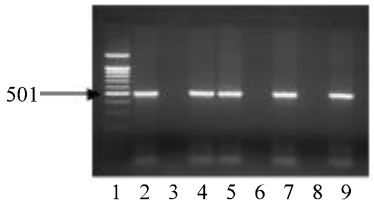

Fig. 3: Ethidium bromide-stained gel of amplified PCR products representing BTN primer
Lane 1: 100 bp ladder marker, Lane 2: Buffalo control, Lane 3: Hamster cell line control, Lanes 4, 5, 7 and 9: Positive hybrids for BTN primer, Lanes 6 and 8: Negative hybrids for BTN primer

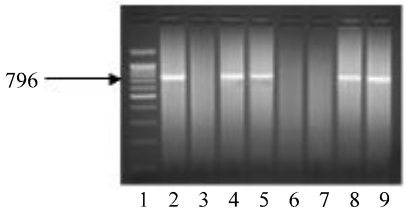

Fig. 4: Ethidium bromide-stained gel of amplified PCR products representing ODC primer
Lane 1: 100 bp ladder marker, Lane 2: Buffalo control, Lane 3: Hamster cell line control, Lanes 4, 5, 8 and 9: Positive hybrids for ODC primer, Lanes 6 and 7: Negative hybrids for ODC primer

marker of syntenic group U20 and buffalo chromosomes 2p (Othman and El Nahas, 1999) with a φ value 1.00. So, BTN is assigned to the bi-armed river buffalo chromosome 2. Figure 3 shows a representative ethidium bromide-stained gel of the positive and negative hybrids for BTN marker.

From Table 2, ODC is segregated dependently (φ = 1.00) with LGB, the marker of syntenic group U16 and buffalo chromosome 12 (Othman and El Nahas, 1999). ODC was segregated independently from the other markers where the highest φ value did not exceed 0.55, which was reported between ODC and G10P1, the marker of U26 and buffalo chromosome 23. Due to this syntenic relationship between ODC and LGB, ODC is assigned to the acrocentric river buffalo chromosome 12. Figure 4 shows a representative ethidium bromide-stained gel of the positive and negative hybrids for ODC marker.

Discussion

The river buffalo is an economically important livestock species in many Asian and Mediterranean countries. Its genetic improvement, especially in reproductive performance and quantity of meat and milk production, ranks high among agricultural research needs of these countries. Gene maps and genetic polymorphism of genes related to the economic quantitative traits as tools for developing more efficient breeding strategies have as targeted animal improvement by the genomic approach.

The objectives of this study were to identify the genetic polymorphism and gene mapping of two genes, BTN and ODC, are associated with milk composition in river buffalo. The aim was done using the polymerase chain reaction and hybrid panel resulted from the fusion between river buffalo lymphocytes and cells of the HPRT-deficient Chinese hamster cell line.

Butyrophilin is a glycoprotein of the immunoglobulin super-family that is secreted in association with the milk-fat-globulin membrane from mammary epithelial cells (Ogg *et al*., 1996). Butyrophilin is structurally related to a number of genes belonging to the immunoglobulin super-family (IgSF) including B30.2, human ret finger protein (RFP), rat myelin oligodendrocyte glycoprotein (MOG) and chicken B blood system (B-G) protein (Vernet *et al*., 1993).

Two important regulators of T cell-mediated immune response, B7-1 and B7-2 also display significant sequence similarity with BTN, MOG and B-G (Linsley *et al*., 1994). Both B7-1 and B7-2 are members of the IgSF possessing both variable (V)-like and constant (C)-like domains in their extracellular regions. Butyrophilin, MOG and B-G demonstrate significant homology to the V-like domains of B7-1 and B7-2 while BTN also demonstrate significant homology with the C-like domains of these proteins (Linsley *et al*., 1994). Consequently, the chromosomal location of BTN within the MHC of man and cattle and its sequence homology to members of the IgSF may suggest a possible immunologic function (Taylor *et al*., 1996).

Present results showed that BTN is assigned to the short arm of bi-armed river buffalo chromosome 2 where BTN is segregated dependently with PRL, the Marker of BBU2p (Othman and El Nahas, 1999). The same arm of this river buffalo chromosome carries, in addition to PRL and BTN, the histocompatibility complex, class II DR beta 3 (BoLADR3) gene (Hondt *et al*., 2000) which is related to the immune system. The closely chromosomal locations between BTN, PRL and MHC in river buffalo, as in human and cattle (Rhodes *et al*., 2001), may suggest the possible function of BTN in milk composition and immune system in river buffalo.

The analysis of genetic polymorphisms of BTN enables us to identify the genotype which is related to the good milk composition in the river buffalo. Among 70 tested animals characterized by high milk quality, the allele A displayed in a frequency of 0.89 whereas the frequency of allele B was 0.11, indicating the close relation between allele A of BTN gene and high milk quality in river buffalo.

Rhodes *et al*. (2001) assigned BTN gene to the bovine chromosome 23 which is homologous to buffalo chromosome 2p to which we assigned BTN in this work. The estimated frequencies of Alleles A and B for BTN gene in bovine were 0.875 and 0.125, respectively. These frequencies in bovine are nearly resemble to those in Egyptian river buffalo which were declared in this study.

The gene mapping of ODC assigned this gene to buffalo chromosome 12 where is segregated dependently with LGB, the marker of syntenic group U16 and buffalo chromosome 12 (Othman and El Nahas, 1999). β-lactoglobulin (LGB) is the major why protein in the milk of ruminants and several non-ruminant species (Perez and Calvo, 1994). The close mapping between ODC and LGB could be associated with milk-protein biosynthesis.

Present results declared that the presence of polymorphic MspI site (+/+) is associated with high quality and quantity characters of milk in river buffalo where the frequencies of the MspI(+) and MspI(-) were 0.86 and 0.14, respectively, in the tested animals characterized by high milk quantity and quality. In bovine, the allele frequencies of the MspI(+) ODC in two groups of Holstein bulls were 0.771 and 0.923 whereas the frequencies of MspI(-) ODC in these two groups were 0.229 and 0.077, respectively (Yao *et al*., (1996). Also, these frequencies are slightly different than those in the river buffalo animals characterized by high yield of milk as shown in this study.

The genetic variants of ODC could be associated with mammary gland activity or interact with trophic hormones during lactation, thus affecting milk performance in dairy animals like cattle and river buffalo (Yao *et al*., 1996).

As a conclusion, the results of this work enabled us to assign these two important genes on river buffalo chromosomes and also to identify the different alleles of these genes are associated with the best characters of milk in Egyptian river buffalo. This study was considered the first work focused on the gene mapping and genetic polymorphisms of genes associated with milk composition in Egyptian river buffalo which is considered an economically important livestock species in Egypt and these findings will help us to improve the milk production of this species.

References

Adkison, L.R., D.W. Leung and J.E. Womack, 1988. Somatic cell mapping and restriction fragment length analysis of bovine α and β interferon gene families. Cytogenet. Cell Genet., 47: 62-65.

Blin, N. and D.W. Stafford, 1976. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acid Res., 3: 2303-2308.

Bulfield, G., J.H. Issacson and R.J. Middleton, 1988. Biochemical correlates of selection for weight-for-age in chickens: Twenty fold higher muscle ornithine decarboxylase levels in modern broilers. Theo. Applied Genet., 75: 432-437.

Chevalet, C. and F. Corpet, 1986. Statistical decision rules concerning synteny or independence between markers. Cytogenet. Cell Genet., 43: 132-139.

Echard, G., J. Gellin and M. Gillois, 1984. Localisation des genes MPI, PKM2, NP sur le chromosome 3 du porc (Sus scrofa L.) et analyse cytogenetique dune lignee de hamster chinois issue de la DON (wg3h). Genet. Sel. Evol., 16: 261-270.

El Nahas, S.M., H.A. de Hondt and J.E. Womack, 2001. Current status of the river buffalo (*Bubalus bubalis* L.) gen map. J. Heredity, 92: 221-225.

Fallert, R.F. and C.B. Liebrand, 1991. Economic implications of bovine somatotropin for the United States dairy industry. J. Dairy Sci., 74: 12-19.

Franke, W.W., H.W. Heid and C. Grund, 1981. Antibodies to the major insoluble milk fat globule membrane-associated protein: specific location in apical regions of lactating epithelial cells. J. Cell Biol., 89: 485-494.

Gray, A. and A. Tait, 1993. Identification of ornithine decarboxylase as a trait gene for growth in replicated mouse lines divergently selected for lean body mass. Genetical Res., 62: 31-37.

Hondt, H.A.de, Bosma, A.A., Bieman, M. den, Haan, N.A. de and Zutphen, L.F.M. van , 1991. Gene mapping in the river buffalo (*Bubalus bubalis* L.). Genet. Sel. Evol., 23: 104s-108s.

Hondt, H.A., O.E. de, M.F. Othman, A.A. Abdel Samad, H.A. Hassan, S.M. Oraby and El Nahas, 2000. Mapping of eight molecular markers to river buffalo chromosomes and the confirmation of the nature of chromosomes 2 and 3. J. Egypt German Soc. Zool., 31C: 39-48.

Jack, J.W. and I.H. Mather, 1990. Cloning amd analysis of cDNA encoding bovine butyrophilin, an apical glycoprotein expressed in mammary tissue and secreted in association with the milk-fat globule membrane during lactation. J. Biol. Chem., 265: 14481-14486.

Linsley, P.S., R. Peach, P. Gladstone and J. Bajorath, 1994. Extending the B7 (CD80) gene family. Protein Sci., 3: 1341-1343.

Mather, I.H., C.B. Tamplin and M.G. Irving, 1980. Separation of the proteins of bovine milk-fat-globule membrane by electrofocusing with retention of enzymatic and immunological activity. Eur. J. Biochem., 110: 327-336.

Ogg, S.L., M.V. Komaragiri and I.H. Mather, 1996. Structural organization and mammary-specific expression of the butyrophilin gene. Mamm. Genome, 7: 900-905.

Othman, O.E. and S.M. El Nahas, 1999. Synteny assignment of four genes and two microsatellite markers in river buffalo (*Bubalus bubalis* L.). J. Anim. Breed. Genet., 116: 161-168.

Pegg, A.E., 1986. Recent advances in the biochemistry of polyamines in eukaryotes. Biochem. J., 234: 249-262.

Perez, M.D. and M. Calvo, 1995. Interaction of β-lactoglobulin with retinol and fatty acids and its role as a possible biological function for this protein: A review. J. Dairy Sci., 78: 978.

Rhodes, D.A., M. Stammers, G. Malcherek, S. Beck and J. Trowsdale, 2001. The cluster of BTN genes in the extended major histocompatibility complex. Genomics, 71: 351-362.

Shih, C. and R.A. Weinberg, 1982. Isolation of a transforming sequence from a human bladder carcinoma cell line. Cell, 29: 161-169.

Taylor, C., M. Everest and C. Smith, 1996. Restriction fragment length polymorphism in amplification products of the bovine butyrophilin gene: Assignment of bovine butyrophilin to bovine chromosome 23. Anim. Genet., 27: 183-185.

Vernet, C., J. Boretto and M.G. Mattei, 1993. Evolutionary study of multigenic families mapping close to the human MHC class I region. J. Mol. Evol., 37: 600-612.

Yao, J., D. Zadworny, U. Kuhnlein, S.E. Aggrey and J.F. Hayes, 1996. A MspI polymorphism in the bovine ornithine decarboxylase gene and its possible association with selection for milk production in Holstein bulls. Anim.Genet., 27: 283-284.