SHORT COMMUNICATIONS

Genetic polymorphisms of milk epithelial mucin of yaks*

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Abstract Milk epithelial mucin (MUC1) of yaks was separated by SDS-polyacrylamide gel electrophoresis. A total of nine types of MUC1 were revealed in 427 yaks from five yak breeds including Maiwa yak, Jiulong yak, Tianzhu white yak, Qinghai yak and Tibetan yak. The molecular weights of MUC1 are from 163 kD to 208 kD, most of which are larger than those of bovine. Population genetic analysis shows that the gene frequency and genotype frequency of yak MUC1 differ among breeds, with relatively high gene heterozygosity. The five yak breeds studied fall into two groups according to their milk MUC1 gene frequency, suggesting that milk MUC1 exhibits specificities for local yak groups.

Keywords: yak, milk, genetic polymorphism, MUC1.

The epithelial mucin (MUC1) is a high molecular weight intergral membrane glycoprotein located on the apical plasma membrane of epithelial tissues and organs such as mammary gland, saliva gland, uterus and trachea[1,2]. During milk secretion MUC1 enters into milk along with milk fat globular membrane. The concentration of MUC1 in milk is about 40 mg/L[3]. Analysis of milk MUC1 of mammals has revealed its unique genetic polymorphism. Except for mouse, milk MUC1 from many species including human, bovine, goat, horse and guinea pig exhibits polymorphisms on SDS-gel[4—7], showing one or two codominant alleles from each parent. This polymorphism relates to the variation in the number of a tandem repeated 20 amino acids in MUC1. Huott analyzed milk MUC1 in 119 Holstein cows and detected five alleles, with MUC1 molecular weight of 193 kD[8].

Up to now, little information is available on the function of milk MUC1. Milk MUC1 is highly glycosylated and rich in proline in its structure, existing on the surface of epithelial cells as long filaments. It is proposed that this structure may tend to protect the epithelial cell from invasions of microorganisms and breach by chemicals including enzymes[6]. Hens studied Holstein milk MUC1 and found that individuals which have a larger molecular weight of milk MUC1 contain high concentrations of fat, protein in milk and higher milk yield[9]. The functions of MUC1 in human and animal reproduction have also been proposed in recent years[10]. We have carried out primary studies about MUC1 polymorphisms in yak and goat, and have observed rich polymorphisms in both species[11,12]. Because MUC1 is expressed as various numbers of tandem repeats (VNTR), it is of significance to understand the functions of VNTR in genome. In this experiment, five main yak breeds in China were used to study milk MUC1 polymorphisms in order to provide useful data for studying yak characteristics and biodiversity. This study may also be valuable for seeking new methods to prevent bovine mastitis, disease-resistant breeding, molecular maker of milking performance, and mammary gland biology research.

1 Materials and methods

1.1 Experimental animals

The experimental yaks included Maiwa yak ($n=108$), Jiulong yak ($n=98$), Tianzhu white yak ($n=104$), Qinghai yak ($n=67$) and Tibetan yak ($n=50$). The milk samples were collected from Hongyuan County and Jiulong County in Sichuan Province, Tianzhu County in Gansu Province, Yushu

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Region in Qinghai Province, and Naqu Region in Tibet. All of the yaks were grazed on natural grassland according to local management. Milk samples were obtained by hand milking during July to September. The preservative NaN_3 was added to samples to achieve a concentration of 0.02%, then the samples were frozen and transferred to our laboratory and stored at −20 ℃ until analysis.

1.2 Gel electrophoresis of milk MUC1

Milk samples from the individual yaks were thawed and subjected to denaturing gel electrophoresis according to the method of Patton and Muller[4]. The whole milk was mixed with the sample buffer containing β-mercaptoethanol and heated in boiling water for 5 min and separated with SDS-PAGE. The separating gel was made with acrylamide at the concentration of 6%, and stacking gel contained 3% acrylamide. A volume of 10 μL of whole milk was loaded on the gel. Following electrophoresis, MUC1 bands in the gels were silver stained, and their molecular weights were calculated according to protein standards.

1.3 Statistics

Gene heterozygosity and effective allele number were calculated using software package SPSS (version 11.5).

2 Results

2.1 Polymorphism of yak milk MUC1

Milk MUC 1 was very sensitive to silver staining, and stained much faster than other milk proteins although its concentration is very low in milk. Yak milk MUC1 showed one or two bands of different mobility on the SDS-gel (Fig. 1).

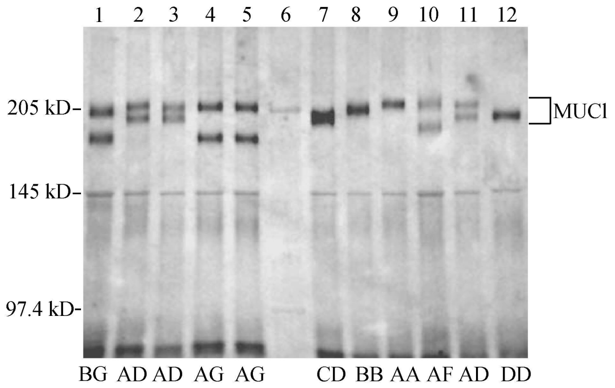

Fig. 1. SDS-PAGE of milk MUC1 from Tibetan yak. Lane 6 is the standard protein marker, others are Tibetan yak milk; letters beneath the figure are phenotypes of MUC1.

Thirty-one phenotypes (genotypes) of milk MUC1 were detected in yaks with nine alleles (Table 1 and 2). Allele frequency of MUC1 in the five yak breeds was B> F> A> C> G> D> E> H> I. In the groups, the dominant genotype of milk MUC1

Table 1. Genotype distribution of yak milk MUC1

Genotype	Maiwa yak (n=108)	Jiulong yak (n=98)	Tianzhu white yak (n=104)	Qinghai yak (n=67)	Tibetan yak (n=50)
AA	3	7	7	3	2
AC			3		
AD	1			2	5
AE			2	1	1
AF	6	11	4		
AG				16	6
BB	14	21	39	17	5
BC	1		2		
BD	5		1		1
BE	6	4	2	1	
BF	19	30	16		
BG				8	3
BH					2
BI				3	
CC	18		10		6
CD	4				

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Genotype	Maiwa yak (*n*=108)	Jiulong yak (*n*=98)	Tianzhu white yak (*n*=104)	Qinghai yak (*n*=67)	Tibetan yak (*n*=50)
CE	2				
CF	6		8		
CG					7
CH					3
DD	1			1	3
DE	1			1	
DF	5	1	1		
DG	2			3	1
EE	2	4		1	
EF			2		
EI				1	
FF	12	20	7		
GG				8	2
GI				1	
HH					3

Numbers in the table are the number of yak samples

showed the following difference: AA and AF in Maiwa yak; AA, AF, FF in Jiulong yak; BB in Tianzhu yak, AG and BB in Qinghai yak. No dominant genotype of milk MUC1 was observed in Tibetan yak.

Table 2. Allele frequency of milk MUC1 in five yak breeds

Allele	Molecular weight (kD)	Maiwa yak	Jiulong yak	Tianzhu white yak	Qinghai yak	Tibetan yak	Total frequency of allele
A	208	0.0602	0.1275	0.1106	0.1865	0.1600	0.6448
B	205	0.2731	0.3878	0.4760	0.3433	0.1600	1.6402
C	200	0.2269		0.1586		0.2200	0.6055
D	196	0.0926	0.0051	0.0096	0.0597	0.1300	0.2970
E	187	0.0602	0.0612	0.0385	0.0448	0.0100	0.2147
F	185	0.2778	0.4184	0.2067			0.9029
G	179	0.0092			0.3284	0.2100	0.5476
H	172					0.1100	0.1100
I	163				0.0373		0.0373

In the five yak breeds, the heterozygous type of MUC1 showed the following difference: 45.4% in Maiwa yak, 54.1% in Jiulong yak, 62.5% in Tianzhu white yak, 44.8% in Qinghai yak, 42.0% in Tibetan yak. Most of these band patterns were correlated with high molecular weight (>200 kD).

2.2 Genetic variation of yak milk MUC1

Gene heterozygosity and effective allele number were calculated based on the frequency of alleles. The five yak breeds showed high gene heterozygosity, large effective allele number and high genetic variations. The gene heterozygosity of milk MUC1 in Jiulong yak and Tianzhu white yak were relatively low, and their genetic variations were lower than other yak breeds studied (Table 3).

Table 3. Genetic variation of yak milk MUC1

Breed	Gene heterozygosity	Effective allele number
Maiwa yak	0.781	4.564
Jiulong yak	0.655	2.894
Tianzhu white yak	0.688	3.208
Qinghai yak	0.733	3.783
Tibetan yak	0.827	5.787

Five yak breeds in China were clustered based on milk MUC1 allele frequency. These yaks fell into two groups: Maiwa yaks clustered with Tianzhu white yaks, then with Jiulong yak; Qinghai yaks clustered with Tibetan yaks. Euclidean genetic distance among the five yak breeds was obtained based on milk MUC1 analysis (Table 4 and Fig. 2).

Table 4. Euclidean genetic distances in five yak breeds of China

Breeds	Maiwa yak	Jiulong yak	Tianzhu white yak	Qinghai yak	Tibetan yak
Maiwa yak	0				
Jiulong yak	0.311	0			
Tianzhu white yak	0.245	0.274	0		
Qinghai yak	0.504	0.541	0.455	0	
Tibetan yak	0.395	0.592	0.473	0.341	0

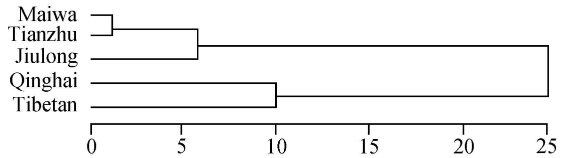

Fig. 2. Cluster analysis of five yak breeds in China.

3 Discussion

Yak milk MUC1 assembled most of other mammals, showing one or two electrophoretic bands differed in mobility on SDS-gel[4−7], and exhibited rich polymorphisms, suggesting that yaks also contain various numbers of tandem repeats in the gene coding for MUC1. The molecular weights of yak MUC1 in this experiment were generally higher than those of Holsteins[8]. It was deduced that there exist more tandem repeats in the gene coding for MUC1. The milk MUC1 size is dependent on the number of 20-amino acid repeat units, and affected by its carbohydrate moiety. Huott et al. proved that bovine MUC1 gene structure was the main factor determining its molecular weight[8]. So, the nine molecular weight types of milk MUC1 in yaks may represent nine loci.

A lower genetic variation of milk MUC1 in Jiulong yak was found in this experiment. It might be related to the local management of Jiulong yak under which the emigration of Jiulong yak was adopted and the immigration of foreign yak breeds was forbidden. The factor mentioned above might lead to a relatively closed yak population. The gene heterozygosity of Tianzhu white yaks was also low, suggesting that this breed was relatively pure. This result is in accordance with the single color characteristic of Tianzhu white yaks. Cluster analysis was carried out in five yak breeds based on their genetic distances calculated from milk MUC1 allele frequency. The five yak breeds fell into two groups: one is Maiwa yak, Tianzhu white yak and Jiulong yak; the other is Qinghai yak and Tibetan yak. In view of ecological and geographic conditions, Jiulong yaks live in the high mountain region, while Maiwa yak and Tianzhu white yaks live in the high plateau region. The reason why they clustered into one group might be the emigration of Jiulong yaks in Maiwa yak breeding. Some researchers classified Chinese yaks into two ecological groups based on ecological environment and appearance of yaks: high mountain type and high plateau type. Jiulong yak and Maiwa yak are two typical types of high mountain type and high plateau type, respectively. However, in this experiment, Jiulong yak and Maiwa yak exhibited similar phenotypes of milk MUC1, which is not in accordance with the above classification of yaks. Because of the rich polymorphism and large effective allele number, as well as being an expressed mini satellite[13], milk MUC1 polymorphism is suitable for analyzing phenotype characteristics of animal breeds. In this experiment, it was found that yaks exhibited high gene heterozygosity and large effective allele number of milk MUC1. Milk MUC1 may become a new and effective genetic marker in marker-assisted selection for quantitative traits and breed classification of yaks. However, milk MUC1 analysis can only be done during lactation; thus, it can be used in performing polymorphism assay of yak milk MUC1 at the gene level. Moreover, in recent years, gene polymorphisms of bovine and goat milk MUC1 have been reported[14, 15].

MUC1 exists in many tissues of mammals. The high sialic acid content of MUC1 confers a strong negative charge to epithelial cell surfaces, which may aid in keeping the ducts and alveoli open and preventing the cell adhesion[3]. It was also proposed that the size of milk MUC1 may be related to their capability as a barrier to prevent mastitis[3]. Thus, a likely potential application of milk MUC1 concerns preventing bovine mastitis. The molecular weight of yak milk MUC1 is relatively high. It is an interesting hypothesis that the higher molecular weight of MUC1 might provide yaks with a more powerful ability against mastitis. In fact, clinical mastitis is seldom observed in yaks. The polymorphisms of milk MUC1 of the main yak breeds in China are of importance in understanding the characteristics of local yak breeds or population and improving the yak breeds.

References

1 Patton S., Gendler S. J. and Spicer A. P. The epithelial MUC1 of milk, mammary gland and other tissues. Biochim. Biopys. Acta 1995, 1241: 407—424.

2 Pemberton L., Taglor-Papadimitriou J. and Gendler S. J. Antibodies to the cytoplasmic domain of the MUC1 mucin show conservation throughout mammals. Biochem. Biophy. Res. Commun., 1992, 185: 167—175.

3 Patton S. Some practical implications of the milk mucins. J. Dairy Sci., 1999, 82: 1115—1117.

4 Patton S. and Muller D. Genetic polymorphism of the epithelial mucin, PAS-Ⅰ, in milk samples from the major dairy breeds. J. Dairy Sci., 1992, 75: 863—867.

5 Patton S. Detection of large fragment of the human mucin MUC1 in feces of breast-fed infants. J. Pediar. Gastroenterol Nutr., 1994, 18: 225—230.

6 Johnson V. G., Greenwalt D. E., Held H. W. et al. Identification and characterization of the principal proteins of the fat-globule membrane from guinea-pig milk. Eur. J. Biochem., 1985, 151: 237—244.

7 Patton S. and Patton R. S. Genetic polymorphism of PAS-Ⅰ, the mucin-like glycoprotein of bovine milk fat globule membrane. J. Dairy Sci., 1990, 73: 3567—3574.

8 Houtt M. L., Josephson R. V., Hens J. R. et al. Polymorphic forms of the epithelial mucin, PAS-Ⅰ (MUC1), in milk of Holstein cows (*Bos taurus*). Comp. Biochem. Physiol., 1995, 111B: 559—565.

9 Hans J. R., Rogers G. W., Huott M. L. et al. Association of the epithelial mucin, PAS-Ⅰ with yield, health, and reproductive traits in Holstein dairy cows. J. Dairy Sci., 1995, 78: 2473—2480.

10 Goulart L. R., Vieira G. S., Martelli L. et al. Is MUC1 polymorphism associated with female infertility? Reprod. Biomed. Online, 2004, 8(4): 477—482.

11 Zheng Y. C., Peng X. W., Zhong G. H. et al. Genetic polymorphisms of epithelial mucin, MUC1, in yak milk. Acta Veterinaria et Zootechnica Sinica, 2002, 3: 235—238.

12 Peng X. W., Zhong G. H., Zheng Y. C. et al. Genetic polymorphisms of epithelial mucin, MUC1, in goat milk. Zoological Res., 2002, 23(1): 15—18.

13 Lancaster C. A., Peat N., Duhig T. et al. Structure and expression of the human polymorphic epithelial mucin gene: an expressed VNTR unit. Biochem. Biophys. Res. Commun., 1990, 173: 1019—1029.

14 Rasero R., Sacchi P., Rosati S. et al. Molecular analysis of the length polymorphic MUC1 gene in cattle. J. Anim. Breed. Genet., 2002, 119: 342—349.

15 Sacchi P., Caroli A., Cauvin E. et al. Analysis of the MUC1 gene and its polymorphism in *Capra hircus*. J. Dairy Sci., 2004, 87 (9): 3017—3021.