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Marker Identification for Mastitis and its Association in Thai-Friesian Cattle in Northern Thailand

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Abstract: The objective of this research is to find molecular markers for mastitis which are associated with diseased and non-diseased cows in Northern Thailand (Chiang Mai and Lamphun) by using Amplified Fragment Length Polymorphism (AFLP) and studying the association between discovered markers and mastitis. Three DNA sequences (A3, A7 and A8) among 12 sequences generated by AFLP which differ between cows with and without mastitis showed a similarity with previously described genes: PDZ domain containing 1 (PDZK1) and sodium channel protein type 8 subunit alpha (SCN8A). Four Single-Nucleotide Polymorphisms (SNP) were detected: G>T, T deletion and C>T (SCN8A-10 and SCN8A-54). Association analysis between genotypes of each gene and phenotype (diseased or non-diseased and somatic cell count) showed that SNP SCN8A-54 is associated with mastitis ($p<0.01$) and subclinical mastitis ($p<0.01$). This study demonstrated new molecular markers associated with bovine mastitis of the Thai-Friesian cattle breed in northern Thailand.

Key words: DNA marker, mastitis, AFLP, PDZK1, SCN8A, Thailand

INTRODUCTION

Bovine mammary infection (mastitis) is a complex disease that can cause severe economic problems among affected herds in many countries (Janzen, 1970; Blosser, 1979; Beck *et al*., 1992; Hortet and Seegers, 1998; Rajala-Schultz *et al*., 1999) including reduced milk production (both quantity and quality), increased veterinary treatment costs and in some cases, necessary culling of the infected animals (Wojdak-Maksymiec *et al*., 2006). The results of mammary inflammation mostly occur from the introduction and multiplication of pathogenic microorganisms such as *Staphylococcus aureus* and *Streptococcus agalactiae*. In terms of pathogenesis and symptoms of infection, mastitis is classified into subclinical and clinical types. In Northern Thailand, the prevalence of subclinical mastitis is high (ranging from 33.3-83.3%) in smallholder dairy farms (Boonyayatra and Chaisri, 2004).

Because of the high heritability (h^2) of Somatic Cell Count (SCC) which is closely associated with clinical mastitis (Lund *et al*., 1994), SCC value has been widely used for mastitis resistance selection in dairy cattle. Moreover, mastitis is a disease influenced by many genes and numerous environmental factors. Therefore, identification of genetic markers associated with mastitis resistance or susceptibility would allow producers to decrease costs related to mastitis by improving herd health through animal selection. Many studies have reviewed the Quantitative Trait Loci (QTL) for clinical and subclinical mastitis, Somatic Cell Count (SCC) and Somatic Cell Score (SCS) in many cattle breeds (Heyen *et al*., 1999; Schrooten *et al*., 2000; Klungland *et al*., 2001; Kuhn *et al*., 2003; Ashwell *et al*., 2004). Independent studies have repeatedly confirmed the potential value of fine mapping of QTL. However, additional markers in QTL should be identified to describe the effects or mechanisms of QTL affecting mastitis via specific sequences or by the genes themselves.

The Thai-Friesian crossbreed improved by the Thailand Department of Livestock Development is the main breed of dairy cattle of Thailand. This breed was created by the crossbreeding using Thai native cattle, Zebu cattle with Brahman, Red Sindhi and Holstein-Friesian cattle, resulting in a >75% Holstein-Friesian crossbreed that can adapt to tropical weather while producing more milk than Zebu cattle. Thai-Friesian cattle have been developed for 60 years yet their heredity associated with mastitis resistance or susceptibility has never been studied. Hence, this is the first experiment for

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mastitis marker identification within the Thai-Friesian genome. The objectives of this research were marker investigation for mastitis and studying the Single-Nucleotide Polymorphisms (SNPs) affected by mastitis in Thai-Friesian cattle.

MATERIALS AND METHODS

DNA samples: Blood samples were collected from 70 Thai-Friesian crossbreed cattle of smallholders in Chiang Mai and Lamphun, Northern Thailand with permission and consent obtained from the owners of the small holder dairy farms. These cattle were categorized into two groups: diseased and non-diseased groups using their case histories and SCC. Genomic DNA was isolated by standard SDS/proteinase K method and phenol-chloroform extraction.

AFLP method: AFLP (Amplified Fragment Length Polymorphism) analysis was done using VspI (Fermentas, USA) and TaqI (Fermentas, USA) followed by adapter ligation, pre-selective and selective amplification with 120 pairs of VspI and TaqI selective primers. The results of VspI and TaqI in this study were unable to distinguish differences in band patterns between mastitis-resistant and susceptible cows. Thus, EcoRI (Fermentas, USA) and TaqI were selected for this study instead. The sequences of EcoRI and TaqI adapters and primers used in this experiment are shown in Table 1. Pooled genomic DNA of each group was digested using 10 units of TaqI at 65°C for 3 h followed by 10 units of EcoRI for 3 h at 37°C in a total volume of 30 μL. Double-stranded adapters were ligated to the restriction fragments for 2 h at 37°C following addition of 10 pmol of EcoRI adapter, 10 pmol of TaqI adapter, 1X of T4 DNA ligase buffer, 0.33 mM of dATP and 5 units of T4 DNA ligase (Fermentas, USA) in a total volume of 30 μL. The ligase solution was subsequently diluted to 300 μL using 1X TE buffer. Adapters and primers were obtained from Bio Basic Inc. (Canada). Amplifications were carried out in two steps as recommended by Vos *et al*. (1995). Pre-amplification was performed using: 5 μL of the restriction/ligation mix; 0.25 mM each of EcoRI and TaqI preamplification primers carrying one selective nucleotide; 0.625 mM of dNTP; 3 mM of $MgCl_2$ and 1 unit of Taq DNA polymerase in 1X PCR buffer (Vivantis, Malaysia) in a final volume of 20 μL. Reactions were incubated for 30 cycles (denaturation at 95°C for 30 sec annealing at 56°C for 60 sec, extension at 72°C for 60 sec with the final extension at 72°C for 10 min) followed by dilution to 400 μL in 1X TE buffer. Selective PCR amplifications of 5 μL of the diluted product were manipulated with the following components: 25 nM of each selective primer (EcoRI and TaqI), 0.625 mM of dNTP, 1.5 mM of $MgCl_2$ and 1 unit of Taq DNA polymerase in 1X PCR buffer. This step was performed in 13 cycles of 95°C for 30 sec, 65°C for 30 sec and 72°C for 60 sec. In each cycle, the annealing temperature was decreased by 0.7°C down to 56°C followed by 23 cycles with an annealing temperature of 56°C with a final extension at 72°C for 10 min. Reactions were size-fractionated by electrophoresis using 6% denaturing polyacrylamide gel (19:1 acrylamide: bisacrylamide) at 1200 Vh in 0.5X TBE buffer. Prior to loading, samples were denatured for 10 min at 95°C. The gel was visualized by 0.2% silver staining.

Table 1: DNA sequences of adapters and primers

Primer	Sequence (5'-3')
EcoRI adapter	CTC GTA GAC TGC GTA CC AAT TGG TAC GCA GTC TAC
TaqI adapter	GAC GAT GAG TCC TGA C CGG TCA GGA CTC AT
EcoRI primer +1	GAC TGC GTA CCA ATT CA
TaqI primer +1	GAT GAG TCC TGA CCG AC
EcoRI primer +3	GAC TGC GTA CCA ATT CAN N
TaqI primer +3	GAT GAG TCC TGA CCG ACN N

Cloning and sequencing of AFLP fragments: Selected bands (differentially present between cattle with and without mastitis) were excised from the gels and soaked in 20 μL of 1X PCR buffer (Vivantis, Malaysia). DNA was eluted by incubation at 95°C for 10 min and used as a PCR template under selective amplification conditions using the same primers as in the selective amplification of AFLP assays. The amplification PCR products were cloned using a T and A Cloning Vector Kit (RBC Bioscience, Taiwan) according to the manufacturer's instructions. After checking the product size of the original DNA fragments using M13 universal primer, white positive colonies were picked up and cultured for 18 h in LB medium and then plasmids were isolated. The sequence of the insert fragment was determined by Applied Biosystems Automated Sequencer (1st Base, Singapore).

SNP identification and genotyping: DNA polymorphisms were detected by comparing the obtained sequence data with the assembled cattle genome (*Bos taurus*) using the BLAST program (Altscul *et al*., 1997) in the NCBI database. Additionally, all sequences were compared using GBrowser via cattle QTL database. Primer pairs for SNP genotyping by PCR-PFLP and Allele-Specific Primer Amplification (ASPA) were designed using Primer Premier 5 software (PREMIER Biosoft International, Palo Alto CA, USA). ASPA reaction using specific primer pairs for each allele was carried out in 20 μL of 1X buffer, 0.625 mM of dNTP, 1 unit of Taq DNA polymerase (RBC Bioscience, Taiwan) and 0.25 nmol of each specific primer with the following profile: 35 cycles at 95°C for 30 sec, 54-61°C for 30-45 sec, 72°C for 30-45 sec (Table 2) and a final extension step at 72°C for 10 min. For PCR-RFLP reaction, the amplified products using the same reaction as in ASPA were digested with 5 units of DdeI restriction

Table 2: DNA fragments in mastitis-resistant and susceptible groups

Fragment No.	Group specificity	Homolog	Length (bp)	E-value	QTLs
A1	ND	*Bos taurus* Chromosome 19 between *Trinucleotide repeat containing 6C* and *Ovarian/Breast septin gamma* genes	127	3e-55	#3409, #3433, #5125, #1391, #1739, #3551, #4383, #5299
A2	ND	*Bos taurus* Chromosome 5 between *Cartilage paired-class homeoprotein 1* and *Endonuclease reverse transcriptase* genes	124	2e-58	#2552, #7067, #3607, #7066, #1559, #1565, #4413
A3	ND	*Bos taurus* Chromosome 3 on *PDZ domain containing 1* gene	85	3e-33	#5663, #2490, #1326, #2549, #2550, #2441, #2470, #2584, #2468, #6053, #6049, #2440, #2469, #2541
A4	ND	*Bos taurus* genomic DNA	76	4e-32	
A5	ND	Not found	86	-	
A6	ND	*Bos taurus* Chromosome 1 on Arginine/Serine-rich coiled-coil 1 gene	188	1e-90	
A7	D	*Bos taurus* Chromosome 5 on Sodium channel *protein type 8 subunit alpha* gene	137	2e-62	#4902, #1365, #1362, #4901, #1301, #1305, #1730, #1376, #7068, #7069, #7125, #7070, #2659, #4495, #4904, #4903, #1363, #2429, #3422, #4973, #7046, #1364
A8	D	*Bos taurus* Chromosome 3 on PDZ domain containing 1 gene	84	1e-32	#5663, #2490, #1326, #2549, #2550, #2441, #2470, #2584, #2468, #6053, #6049, #2440, #2469, #2541
A9	D	*Bos taurus* Chromosome 1 on 5' of *Interleukin 20 receptor beta* gene	85	2e-35	
A10	ND	*Bos taurus* Chromosome 29 on 5' of *odz/odd oz/ten-m homolog 4* gene	207	2e-90	#1316, #1344, #4651, #1664, #1665, #1373, #2593, #2612, #1670, #1671, #7152
A11	ND	*Bos taurus* Chromosome 24 between *SDCCAG 33* variant protein and Zinc binding alcohol dehydrogenase domain containing 2 genes	193	2e-93	
A12	ND	*Bos taurus* Chromosome 6 between *Zinc finger protein 518B* and *Heparan sulfate D-glucosaminyl 3-O-sulfotransferase 1* genes	178	5e-80	

enzyme (Fermentas, USA) for 4 h at 37°C in a total volume of 10 μL. The products were separated by 2% agarose gel electrophoresis and visualized with ethidium bromide staining.

Statistical analysis: Genotype and allele frequencies were calculated by simple allele counting according to the Hardy-Weinberg equilibrium (Falconer and Mackay, 1996). The association between genotypes and mastitis traits was evaluated by χ^2-test and the association between genotypes and subclinical mastitis traits was performed by the General Linear Model Procedure (GLM) using SPSS version 14 software (SPSS Inc., Chicago IL, USA) according to the following statistical model:

$$y_{ij} = \mu+G_i+D_j+(GD)_{ij}+e_{ij}$$

Where:

y_{ij} = Observation of the SCC
μ = Population mean
G_i = Effect of ith genotype
D_j = Effect of jth subclinical mastitis traits
$(GD)_{ij}$ = Effect of ijth combination of genotype and subclinical mastitis
e_{ij} = Random residual error

RESULTS AND DISCUSSION

Marker detection: AFLP analysis between mastitis-resistant and susceptible cattle revealed 12 different DNA bands. All bands were isolated, re-amplified, cloned and sequenced to obtain DNA sequences. A genome comparison with the assembled *Bos taurus* genome in the NCBI database showed that 10 DNA sequences were similar to the *B. taurus* genome. Besides, these DNA sequences could be identified their location on the *B. taurus* chromosome (Table 3). The remaining sequences were similar to the *B. taurus* genome but these sequences were unable to identify their location on the *B. taurus* chromosome and another sequence did not match with *B. taurus* and other genomes.

Table 3: Identified SNPs in *PDZK1* and *SCN8A* gene

Gene (Accession no.)	Sequence	SNP position	Genotyping method
PDZK1	CCAA(G/T)GGTA	3 G>T	ASPA
(NW_001494736)	GATT(T/-)CCTG	63 T deletion	PCR-RFLP by DdeI
SCN8A	ACTG(C/T)GTGG	10 C>T	ASPA
(NW_001495013)	AAGA(C/T)CAAT	54 C>T	ASPA

From the results of sequence alignment using the BLAST program, there are four genes related to cancer, immune regulation and inflammation processes: ovarian/breast septin gamma (Ov/Br septin, Septin9), PDZ domain containing 1 (PDZK1), sodium channel protein type 8 subunit alpha (SCN8A) and interleukin-20 receptor beta (IL20Rβ). In this study, only two genes (*PDZK1* and *SCN8A*) were selected for association analysis to develop molecular markers

Table 4: Oligonucleotide primers, amplicon sizes and corresponding annealing temperatures of each SNP

Gene	SNPs	Primer name	Sequence (5'-3')	Length of product (bp)	Annealing temp (°C)
PDZK1	T deletion	PDZK1-T del	CAC ATG CAC GAG GGT ATC TC GGC TGC TGG AAA GGT TGG T	889	56
	G>T	PDZK1-ND	CAT CTC GAC ACA GTT CCC ATA CTG TGG CTT TGG GTG GT	309	57
		PDZK1-D	ATA ACC GTG TCT CAC TTT CG GAG TCA CTG CTG CAC CAT GGA	260	61
SCN8A	10 C>T	SCN8A-10ND	GAA GGG TCT CCT GAT TGG T GGT CTA TTT GAG CGG ATG A	339	57
		SCN8A-10D	TGT GCC TTC CTG CGT CTC AAT AAG CGG GAA GAT CAA TCA	484	54
	54 C>T	SCN8A-54ND	TAT CCA ACA CAG TCT CCA CG GGT CTA TTT GAG CGG ATG A	297	58
		SCN8A-54D	TGT GCC TTC CTG CGT CTC ACA CTT GCC ACT GTG TGG AG	527	55

Underlined letters are the mutation points

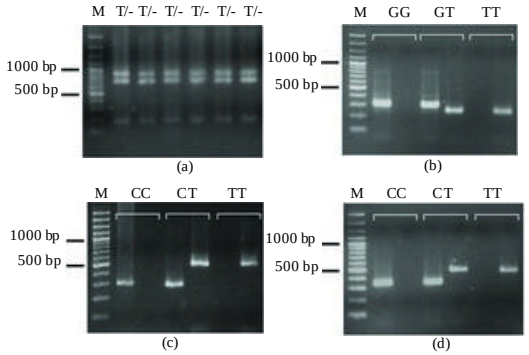

Fig. 1: Polymorphism patterns of 4 SNPs. Polymorphisms of PCR fragments for: a) SNP T deletion; b) G>T; c) 10 C>T and d) 54 C>T. The genotypes are shown at the top of the lanes. Lane M refers to 100 bp plus DNA Ladder (Fermentas, USA)

because of the homology of obtained sequences with the sequences within genes (intron 3 for *PDZK1* and intron 1 for *SCN8A*).

SNPs identification: SNP identification of the *PDZK1* and *SCN8A* genes showed four polymorphisms. Details of all SNPs are shown in Table 3. Two SNPs were identified in intron 3 of the *PDZK1* gene.

We found that DNA fragments A3 (appeared in the mastitis-resistant group) and A8 (found in the mastitis-susceptible group) were complementary to each other. Two SNPs were also detected in the *SCN8A* gene in intron 1. To study polymorphisms and association analysis for those SNPs, PCR-RFLP and ASPA were selected for genotyping.

Polymorphisms of *PDZK1* and *SCN8A* genes: Genotyping was performed by PCR-RFLP for SNP T deletion in the *PDZK1* gene. Digestion of PCR products with DdeI restriction enzyme resulted in fragments of

Table 5: Distribution of genotypic and allelic frequencies in each SNP

		Genotypic frequency			Allelic frequency	
Gene	SNPs	GG	GT	TT	G	T
PDZK1	T deletion	0.00	1.00	0.00	0.50	0.50
	G>T	0.38	0.29	0.33	0.53	0.47
		CC	CT	TT	C	T
SCN8A	10 C>T	0.29	0.40	0.31	0.49	0.51
	54 C>T	0.19	0.20	0.61	0.29	0.71

Table 6: Association analysis between SNPs and mastitis traits

		Chi-square test		
Gene	SNP	Comparison I	Comparison II	General linear model
PDZK1	G>T	0.774	0.026*	0.545
SCN8A	10 C>T	0.493	0.286	0.293
	54 C>T	0.000**	0.000**	0.004**

*p<0.05 and **p<0.01

889 bp for the T allele and 712 and 177 bp for the T deletion allele (-). Genotyping of SNP G>T of the *PDZK1* gene and 10 C>T and 54 C>T of the *SCN8A* gene were carried out by ASPA. Primer pairs for each allele are shown in Table 4. Only a heterozygous genotypic pattern (T/-) was produced as the result of DdeI restriction. Three bands could be distinguished on the gel which were the products of two alleles (T and (-) allele). For ASPA genotyping, three genotypic patterns were produced in SNP G>T of the *PDZK1* gene and SNP 10 C>T and 54 C>T of the *SCN8A* gene which were the products of two alleles (G and T or C and T). The resulting electrophoretic patterns are shown in Fig. 1. Genotypic and allelic frequencies are shown in Table 5.

Association analysis: The results of association analysis among mastitis groups by P^2-test are shown in Table 6. SNP 54 C>T was associated with mastitis ($p<0.01$) in both comparisons I and II. Moreover, SNP G>T of the *PDZK1* gene was associated with mastitis ($p<0.05$) in comparison II. The SNP T deletion of the *PDZK1* gene was not included in these analyses because of its genotypic frequency. When analyzed using GLM procedure for subclinical mastitis, SNP 10 C>T and 54 C>T

Table 7: Least square means and standard error for SCC ($x10^3$) in each SNP

	Genotype					Genotype			
SNPs	GG	GT	TT	p-value	SNPs	CC	CT	TT	p-value
G>T	1247.456±286.430	2169.591±494.304	115.917±371.256	0.277	10 C>T	4168.109±549.934[a]	2268.232±302.474[b]	428.261±311.505[c]	0.000**
	-	-	-	-	54C>T	2850.722±322.719[a]	402.060±334.046[b]	1093.833±295.777[b]	0.000**

**p<0.01

were associated with subclinical mastitis (p<0.01) and polymorphisms of SNP 10 C>T and 54 C>T were significantly different (p<0.01). Animals with genotype CC had the highest SCC in both SNPs as shown in Table 7. In this study, we discovered 12 different DNA fragments in the Thai-Friesian cattle genome using AFLP analysis with EcoRI and TaqI restriction sites. Prior to using EcoRI and TaqI, we had used VspI and TaqI for this approach but these restriction sites revealed indistinguishable DNA patterns with fewer DNA fragments. This should be a result of primers which cannot bind to the DNA template. Vos *et al.* (1995) revealed that both sides of DNA fragments digested by MseI would construct a stem loop from their inverted repeat sequences (T^TAA). VspI had a restriction site (AAT^TAAT) similar to MseI. Therefore, the DNA fragment cut with VspI could occur in the same manner as MseI. When the restriction enzymes were changed to EcoRI and TaqI, clearer DNA patterns were found which were more easily distinguished than when using VspI and TaqI. Ajmone-Marsan *et al.* (1997) reported that a combination of EcoRI and TaqI appears to be the most suitable for producing DNA fragments in an animal genome since the restriction site of TaqI (T^CGA) has a CpG dinucleotide within its sequence which tends to mutate from methylation of cytosine and thymine in vertebrate DNA.

Based on 12 different DNA fragments, only three DNA fragments (A3, A7 and A8) showed similarity to known genes (*PDZK1* and *SCN8A*) within the gene region (introns). SNP identification was performed within these DNA fragments. Although, these fragments were not exhibit homology to the exons of genes which have a direct effect on gene function, many documents have reviewed the importance of mutations within introns via mRNA processing. Mutations mostly occurred at the junctions between introns and exons which affect splicing are usually deleterious. They may cause an exon to be left out of the product, cause an intron to be included or cause splicing to occur at an aberrant site (Lewin, 2008). Moreover, mutations occurring in introns affected DNA methylation in the CpG island and also were found at the promoters of some tissue-regulated genes. Van Laere *et al.* (2003) studied the SNPs of the *IGF-2* gene affecting muscle development in pigs and found that a SNP (G>A) in intron 3 at position 3072 on the CpG island, a hypomethylated paternal allele (Q^{pat}) would increase the level of gene expression and also cause more muscle development. Because this mutation inhibits binding of the repressor protein with the DNA strand, the *IGF-2* gene in pigs increased its gene expression. In addition to the above reasons, both PDZK1 and SCN8A have similar positions as the previously described QTL (PDZK1 for SCC and SCN8A for SCS and clinical mastitis) linked to mastitis in bovines.

PDZK1 encodes the PDZ domain containing 1 protein whose functions include signal transduction, cell polarity, cell differentiation and ion transport. This protein binds with MAP17 protein which involved with cell proliferation (Kocher *et al.*, 1998). PDZK1 is expressed in many types of cancer. Ghosh *et al.* (2000) reported that PDZK1 was overexpressed in early breast cancer influenced by estrogen hormone. This condition was similar to mastitis which occurred only in female cattle. Hence, PDZK1 could be linked to a pathophysiological effect of inflammation processes from an increase of cell differentiation (Schwerin *et al.*, 2003) associated with mastitis.

SCN8A encodes the voltage-gated sodium channel (Nav1.6) expressed in central nervous system and is involved in signal transduction (Smith *et al.*, 1998). Sodium Channel/Voltage-Gated Sodium Channel (VGSC) is expressed in other cells including immune cells such microglia or macrophages (Craner *et al.*, 2005). Although, the effect of VGSC on the immune system is unclear. Roselli *et al.* (2006) found that Na^+ flux flow through VGSC protein was important for lymphocyte activation and proliferation; this arises from a decrease of Na^+ level affecting Na^+/Ca^{2+} exchange and causing decreased Ca^{2+} level during lymphocyte activation. Furthermore prior T-cell activation, Na^+ flux was found. Now a days, VGSC blockers are synthesized for medical use to adjust Immunoglobin (Ig) levels in both mice and humans (Roselli *et al.*, 2006). Thus, SCN8A may be also a candidate gene for the immune response influencing mastitis.

Marker conversion was performed for all four SNPs determined by AFLP analysis. Simple PCR-based methods are the most suitable marker for large-scale genotyping and can be easily applied in Marker-Assisted Selection (MAS) (Sharma *et al.*, 2006). The use of PCR-based markers that are converted from RFLP or AFLP markers is technically simpler, less time-consuming and cheaper

(Collard *et al*., 2005). Therefore, PCR-RFLP was chosen for SNP T deletion genotyping since, their sequence shows the restriction site of the DdeI restriction enzyme whereas the other three SNPs were genotyped by Allele-specific Primer Amplification (ASPA). Genotyping of SNP T deletion shows only a heterozygous pattern (T/-). The only one genotype would come from cross genetics of the Thai-Friesian crossbreed which developed by crossbreeding of Indian cattle (Thai native, Zebu, Brahman crossbred and Red Sindhi crossbred) and Holstein-Friesian cattle. Mendel's first law, the law of segregation, states that when an individual produces gametes, each gamete receives only one allele which can be described by the process of meiosis. In meiosis, each paternal and maternal chromosome is separated and the alleles are segregated into two different gametes. This indicates that allele T and T deletion (-) may be due to the different genetics of the parents, resulting in the only genotype pattern (heterozygous: T/-) in this population.

Association between mastitis (both clinical and subclinical mastitis) and all described SNPs (except SNP T deletion because of its genetic frequency) was analyzed by Chi-square test. A Chi-square test was selected for this association analysis because we were unable to collect the SCC of cattle in the clinical group. The results show that SNP 54 C>T is significantly associated with mastitis in both comparisons ($p<0.01$) and located on QTL#2654 and QTL#4973 which affect SCS and clinical mastitis, respectively. Moreover, association analysis between subclinical mastitis and SNPs was performed using GLM. A significant association was found for SNP 10 C>T and 54 C>T ($p<0.01$) and the least square means of polymorphisms in both SNPs were significantly different ($p>0.01$). Genotype CC had the highest SCC values (4168.109±549.934 and 2850.722±322.719 for SNP 10 C>T and 54 C>T, respectively). Additionally, the study found that genotypes with allele T (CT and TT) were less prevalent than genotypes GG (CC) and GT (CT) but this finding conflicted with the presence of allele T in cattle in the mastitis group using AFLP approach. Previously, the study by Suriyasathaporn *et al*. (2000) indicated that the extremely low value of SCC in cattle with non-infected udders was related to increasing risk of clinical mastitis. Hence, the findings could be confirmed by increasing the sample size to obtain the trend of SCC in each genotype since SCC is affected by many conditions such age, stage of lactation, type of pathogen and period of infection (Harmon, 1994). The other DNA fragments (A1 and A9) which are located near the genes related to cancer and inflammation (Ov/Br Septin and IL20Rβ, respectively) should be investigated. In addition, gene expression of PDZK1 and SCN8A should be verified to study the effect of both genes at the transcription level. Besides, it has been suggested that alleles associated with resistance to one disease could be associated with susceptibility to another disease (Rupp and Boichard, 2003) and positive association between mastitis and high milk yield is well-known (Smaragdov, 2006). Therefore, both disease-resistant and productive traits should be included for MAS to produce dairy cattle that meet both producer and consumer requirements. Finally, to improve the Thai-Friesian breed to become the ideal dairy cattle, further studies of molecular marker identification for economic traits should be performed.

CONCLUSION

Twelve different DNA fragments were identified in Thai-Friesian dairy cattle for mastitis using AFLP approach with EcoRI and TaqI. Two genes concerned with human breast cancer and inflammation were detected which exhibit homology to three DNA fragments (A3, A7 and A8). Four point mutations were detected in two genes (two mutations for PDZK1, G>T and T deletion and two mutations for SCN8A, 10 C>T and 54 C>T). A significant association of SNP 54 C>T was found for mastitis and subclinical mastitis. This identified SNP can be used as a marker for selection of animals and could potentially be used for cattle breeding. However, it will be necessary to confirm the findings by testing a large number of animals before definite conclusions can be made.

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