RFLP MARKERS AND THEIR RELATIONSHIP WITH A QTL IN SHEEP

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ABSTRACT

Restriction fragment length polymorphisms (RFLP's) were observed in EcoRI-, HindIII- and TaqI- digested Thai Longtail sheep genomic DNA probed with ovine FSH cDNA. RFLP was also detected in EcoRI-digested DNA of the same sheep breed with ovine thymus genomic DNA. No polymorphism was found in follistatin and ovine growth hormone (OGH) loci of the same sheep breed.
When an analysis of variance between genotypes of the simulated backcross data on body weight at 9 months was done, effect of FSH genotypes showed significantly different effects on body weight but no such differing effect of genotypes of the thymus locus on body weight was observed.

INTRODUCTION

In recent years RFLP linkage maps have been reported in sheep (Di Gregorio et.al.,1991; Foley and Beh, 1992). Although RFLP has been suitably replaced for genetic variation studies by techniques involving minisatellite and then microsatellite markers (RAPD, STMS etc) recently, RFLP markers are still being assessed for their relationship with quantitative trait loci (QTL). The present paper shows RFLP markers in some loci in nuclear DNA of sheep and a simple method to estimate the relationship of the markers with some growth traits.

MATERIAL AND METHODS

Blood samples from 32 Thai Longtail sheep were obtained from University of Malaya's nucleus herd of sheep. Total genomic DNA was extracted from sheep white blood cells according to the method of Hallerman et al (1988). A 5-10 µg sample of DNA was digested separately with the following endonucleases: EcoRI, HindIII, BamHI and TaqI (Cao, 1992). DNA restriction fragments and lambda DNA-*Hind*III digest size markers were electrophoresed horizontally on 0.8% agarose gels in 0.5 x TBE buffer. Electrophoresed gels were placed on a UV transilluminator and photographed with Polaroid type 667 films with 2-3 seconds exposure. Southern blotting to transfer DNA fragments from agarose gels to nylon membranes was performed under neutral condition using the vacuum blotting method (Olszewska and Jones, 1988). Radiolabelled probes[32P] for hybridization (Feinberg and Vogelstein , 1983) were prepared by Multiprime DNA labelling system and Megaprine DNA labelling system (Cao,1992).
Hybridization of labelled probes to immobilized target DNA was carried out by using Hybaid Mini Hybridization oven. After washing of the blots the membranes were blotted on filter briefly. Each membrane was then wrapped up in cling film, exposed to an X-ray

film in an X-ray cassete and then autoradiographed at -70°C for 7 days after which the autoradiographs were analysed for the hybridization patterns of -digested DNA at various loci.

Details of c-DNA/genomic DNA probes used are given in Table 1.

Table 1: c-DNA/genomic DNA probes used in the study

Clone number	33	19	p RP1	FS 2.0
Clone name	Ovine FSH CDNA	Ovine growth hormone cDNA	Ovine thymus genomic DNA	Ovine follistatin cDNA
Plasmid vector*	pKC 3	pGEM 3	pUC 18	pUC 18
Insert size	570 bp	830bp	1.3 Kb	801bp
Source	U. Queensland	U. Queensland	U. Otago	U.Otago

*Antibiotic resistance of all plasmids is to penicillin

Detail procedures concerning the preparation of the probes for the purpose of this study from the supplied probes either in the form E.*coli* stabs or plasmid DNA have been outlined by Cao (1992). Hybridization patterns at the FSH, Thymus, Follistatin and OGH loci were observed using all four endonucleases mentioned above.

Data on body weight of sheep at 9 months for Thai Longtail sheep was collected from the data files of the University farm. Limited data on similar body weight of Cameroon sheep was also available from the record book. Based on the limited parental data of two breeds a simulation study to generate growth data of 100 animals each for F1 and Backcross 1 (F1 x Thai Longtail) was performed using Statistical Analysis of Microsoft Excel Version, 2002.

To investigate the effect of genotypes on the QTL (in this case the locus controlling the body weight at 9 months) a simple single factor model was used involving the backcross data:

$Yi = \mu + Gi + e$,

Where Yi= body weight at 9 months

μ= Mean

Gi= Effect of Genotype within each locus

e= residual error for each observation

All data were analysed with GLM procedure of MINITAB.

RESULTS AND DISCUSSION

Hybridization patterns at various loci are shown in Table 2. Polymorphisms were only observed at the follicle stimulating hormone (FSH) locus in DNA digested with EcoRI, and HindIII enzymes. With Eco RI enzyme, polymorphism was shown with 5.0-4.4 fragments. They are genotypically designated as AA (5.7/5.7), Aa (5,7/5.2) and aa (5.2/5.2). The other bands were invariant bands and therefore not considered for designation of genotypes. Also with Hind III enzyme, three genotypes were found (BB-5.0/5.0, Bb-5.0/4.4 and bb-4.4/4.4). Thymus locus showed polymorphism only in the

EcoRI digested DNA. Genotypes in this locus are CC (5.0/5.0), Cc (5.0/3.0), cc (3.0/3.0). No polymorphism was found either in follistatin and ovine growth hormone loci. Polymorphic loci showed varying fragment size while separating. Monomorphic loci showed only one band whose single fragment size or two bands with similar position for all individuals.

Table 2: Hybridization data with various enzymes

Locus	Morph Status	Enzyme	Fragment size (Kb)	Genotype
FSHa	P	Eco RI	5.7, 5.2	AA, Aa, aa
FSHb	P	Hind III	7.5, 5.0, 4.4, 2.1	BB, Bb, bb
	M	Taq 1	5.5	
		Bam HI	-	
Thymus	P	Eco RI	5.0, 3.0	CC, Cc, cc
	M	Hind III	8.5	
	M	TaqI	1.4	
	M	Bam HI	2.3	
Folli-	M	Eco RI	7.6	
statin	M	Hind III	6.0	
	M	Bam HI	14.7, 6.5	
	M	Taq I	4.0	
OGH	M	Eco RI	7.6, 3.7	
	M	Taq I	4.4, 3.8	
		Hind III	-	
		Bam HI	-	

_ No band observed

Assuming allelic polymorphism at FSH loci for two restriction enzymes and at thymus locus for one enzyme, variance analysis was done separately for two loci using the model given in the material and methods section (Table3). In view of the small sample size and the simulation of data, rather than actual data, nothing conclusive could be suggested from this analysis However results indicate genotypes of FSH locus have some varying effect on backcross data on body weight at 9 months but thymus genotypes have similar effect on body weight. In absence of any family study genotype identification is difficult. Hence the designated genotypes are tentative.

Table 3: Analysis of variance of body weight at 9 months (* indicates P<0.05)

Source of variation	df (Mean Squares)		
	FSHa	FSHb	Thymus
Between genotypes	1 (224) *	1(241)*	1(22)
Error	30(67)	30(54)	30(27)

A separate analysis done with three loci combined together did not show any significantly different effect of the combination of genotypes on the body weight. This was because of the small sample size of the individuals typed for RFLP. The data was not subjected to analysis with known computer programmes eg. Mapmaker/QTL available free on the net as this experiment was not done with micro- or mini-satellite markers and the QTL data was basically simulated.

CONCLUSION

RFLPs were observed in two out of four loci studied in 32 Thai Longtail sheep. Because of the small numbers involved, it is suggested that future study of this nature requires more animals in obtaining meaningful results. Perhaps RAPD or STMS based data on polymorphism would be more suitable for investigating the effect of alleles or genotypes on traits controlled by polygenes (QTL). Currently there are many software programmes available for such analysis, some of which are freely available on the net.

REFERENCES

Cao, H. (1992). A study of restriction fragment length polymorphism (RFLP) in sheep. M.Sc. Thesis, University of Malaya, pp.100.

Di Gregorio P., Rando, A., Pieragostini, E and Masina, P. (1991). DNA polymorphism at the casein loci in sheep. Animal Genetics 22: 21-30.

Feinberg, A.P. and Vogelstein, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Analytical Biochemistry 132: 6-13.

Foley, R.C. and Beh, K,J. (1992). Analysis of aminoglobulin light chain loci in sheep. Animal Genetics 23: 31-42.

Hallerman, E.M., Nave A., Soller M. and Beckmnn, J.S. (1988). Screening of Israeli Holstein-Friesian cattle for restriction fragment polymorphisms using homologous and heterologous deoxyribonucleic acid probes. J. Dairy. Science 71: 3378-3389.

Olszewska, E. and Jones, K. (1988). Vacuum blotting enhances nucleic acid transfer. Trends in Genetics 4: 92-94.