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ASSOCIATION BETWEEN GENETIC MARKERS AND GROWTH TRAITS IN MERINO AND INDONESIAN THIN TAIL (ITT) BACKCROSS SHEEP

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ABSTRACT

The association between markers and quantitative measurements was investigated to detect quantitative trait loci (QTL) for growth traits in a population of four reference families of paternal half-sib backcross Merino and Indonesian Thin Tail (ITT). A total of 137 microsatellite markers were used in genome-wide scan covering 26 autosomal chromosomes. Performance data was analyzed for all backcross sheep included birth weight, 90-day and 180-day adjusted body weights as growth traits. Statistical and genetic analysis was conducted online using QTL Express. The study indicated the existence of 5 putative QTL located on ovine chromosomes (OAR) of 2, 5, 7, 18 and 23 (birth weight on OAR5, 90-day weight on OAR 2, 7 and 18 and 180-day weight on OAR 18 and 23). Indicated QTL were for birth weight on OAR 5, for 90-day weight on OAR 2, 7 and 18, and for 180-day weight on OAR 18 and 23. The QTL on chromosomes 5, 7 and 18 were the most significant ($P \leq 0.01$) based on chromosome-wide permutation testing. The peak F ratio for the QTL region on chromosome 7 was located at 8cM flanked by BM3033 and RNS5/BRN whilst the peak location for QTL on OAR 18 was located at 96cM and flanked by CSSM018 and TMR1/AKT1 markers. Potential gene identification was carried out through NCBI website, suggesting strong positional candidate genes for growth traits on chromosome 18.

Key Words: *Microsatellite Marker, Growth Trait,QTL, Sheep*

INTRODUCTION

Nowadays, identification of biologically complex traits based on molecular analysis is possible with studying on an association of genetic markers and quantitative traits. The ability to identify which chromosomal region flanking loci with large effect or termed as quantitative trait loci (QTL) is first step leading to gene discovery. Molecular analysis involving the phenotypic measurements will direct the study to close to the gene (s) or putative gene (s) of interest traits. Recent study involved molecular and bioinformatics strategies in gene discovery for meat traits (Hagen *et al.*, 2005).

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Application of advanced knowledge in the genetic basis of complex production traits can lead to shortcut in the breeding program. For example an application of advanced knowledge in breeding technologies is now possible to use as marker-assisted selection (MAS). When animal brings the favorable QTL alleles based on markers closely linked to putative production genes or the gene is known, direct selection for favorable alleles could be possible.

Since the sheep mapping has been developed from time to time (Crawford et al., 1995, Maddox *et al.*, 2001, 2002), genetic studies based on genetic markers are now more facilitated to search for putative gene (s) of interest quantitative traits. Some studies have reported findings genes affecting the growth or meat development, such as *Callipyge* gene in Dorset (Cockett et al., 1996), *Carwell* gene (linked to callipyge) in Australian Poll Dorset (Bank 1997), genes close to Callipyge and Carwell in crossing Suffolk and Texel (Walling et al., 2002). This study was therefore designed to investigate whether the Merino and ITT backcross sheep brings the growth gene (s) or another gene (s) that affects on growth traits.

MATERIALS AND METHODS

Backcross sheep population

Four reference families were established to generate backcross sheep lamb population. Two differences genetically in weight types were suggested for this QTL studies. Merino represented a large size sheep while Indonesian thin tail (ITT) represented a small size sheep. Four F1 sires derived from crossing of Merino ewes and ITT Sires. These four F1 sires were crossed back to Merino ewes to established 382 backcross sheep.

Phenotypic records

Body weights were recorded at birth, at ages of 90-day and at age of 180-day. Those live body weights were adjusted as growth traits.

DNA Collection

Individual DNA of all backcross included four F1 sires and their ancestors (Grandsires and granddames) were collected from white blood cells of red blood following the method of Montgomery and Sise (1990).

Genotyping

A genome-wide scan was used for QTL mapping using 137 informative markers covering the 26 autosomal sheep chromosomes. Allele scoring was carried out by at least two researchers (Crawford *et al.* 1995) and also analyzed by software included in the sequencer or DNA analyzer machine for genotyping. Genotyping was undertaken using a semi-automatic Li-COR DNA Analyzer Gene ReadIR 4200.

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Genetic and Statistical analyses

Markers and genetic marker distances were referred from Maddox *et al* (2001). Online QTL Express software through website http://qtl.cap.ed.ac.uk was accessed for QTL Analysis with elucidation of Seaton *et al.* (2002). Two tests of Permutate Experiment Wide and Permutate chromosome wide were performed at levels of 5% dan 1%. A chromosome wide threshold for statistical significant was calculated for each chromosome based on a permutation test of 1000 iterations. Putative gene (s) was investigated through GenBank while the gene description was analyzed based on OMIM (Online Mendelian Inheritance of Man).

RESULTS AND DISCUSSION

The QTL analysis of phenotype data, molecular data and sheep map data for all observed body weight traits (birth weight, 90-day and 180-day) indicated that there were the existence of 5 putative QTL located on ovine chromosome (OAR) 5 for birth weight, on OAR 2, 7 and 18 for weight 90-day and on OAR 18 and 23 for weight 180-day. The QTL on OAR 5, 7 and 18 were the most significant ($P \leq 0.01$) based on chromosome-wide permutation testing (Table 1).

Those identified significance QTL ($P \leq 0.01$) on OAR 5, 7 and 18 were analyzed for gene investigation through GenBank-NCBI. The QTL locations, flanking markers, homology of OAR to human chromosome and segment of putative gene locations for all analyzed weight traits were presented in Table 2.

Putative gene on OAR 5 was located between flanking markers MCM527 and BMS1247 with *CAST* gene was identified for birth weight in sheep. Symbol of *CAST* gene is described as *calpastatin* that associated with growth trait. On the OMIM reference, *calpastatin* is described as nature inhibitor to *calpain* activity that affects on muscular dystrophy (OMIM 114090, 2005). Putative gene on OAR 7 (homolog to human chromosome 14) was located between markers RNS/BRN and BMS1620 with *SSTR1* gene was identified for weight 90-day. *STTR1* gene is notation of *somatostatin receptor 1.* Somatostatin spreads throughout central neural system and peripheral tissues such as abdominal, gut and pancreas (OMIM 182451, 2005).

Table 1. Significance threshold of birth weight, body weight at 90-day and 180-day

Traits	Chrom	F-Value	Chromosome-Wide		Experiment-Wide	
			5%	1%	5%	1%
BW	5	4.42	3.2373	4.3577**	5.4262	6.4222
BW90	2	4.43	4.0092*	5.3145	5.3728	6.5258
	7	4.33	3.2942	4.0787**	5.3728	6.5258
	18	4.13	3.2773*	4.7927	5.3728	6.5258
BW180	18	4.3	3.1801	4.0585**	5.1151	6.3466
	23	3.21	2.9808*	3.6954	5.1151	6.3466

Chromosome wide: significant at the individual chromosome level
Experiment wide: Significant at level of the whole 26 chromosomes
* Significant at 5% ($p<0.05$), ** Significant at1% ($p<0.01$)

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Tabel 2. QTL location and flanking markers for birth weight (BW), weights on 90-day (BW90) and 180-day (BW180)

Traits	QTL Location (cM)	Distance (cM)	Flanking Markers	Homolog to Human Chrom	Segment (Mb)
BW:					
5**	112	40,4	MCM527-BMS1247	5	82.36 –106.889
BW90:					
2*	84	6,4	MCM505-BMS1341	9	34.712–74.436
7**	8	49,9	BM3033-RNS5/BRN	14	0 – 22.077
18*	96	21,3	CSSM018-TMR1/AKT1	14	98.509-104.318
BW180:	96	21,3	CSSM018-TMR1/AKT1	14	98.509-105.138
18**	48	23,0		18	0.89 -46.065
23*			CSSM31-MCM136		

Identification of putative gene on OAR 18 (homolog to human chromosome 14) was found between flanking markers CSSM018 and TMR1/AKT1 but could not find gene (s) related to growth traits. However, as reported by Cockett et al. (1994) that the CSSM018 marker is strongly linked to the location of *callipyge* gene. The *callipyge* (*CLPG*) gene locates at the end of OAR 18. The *CLPG* gene was first detected due to the significance effect of muscular hypertrophy (Haley 2001). *CLPG* gene was identified as genome mutation in sheep that corresponds to muscular hypertrophy of fiber muscular fast-twitch at hind leg (Koohmaraie et al. 1995; Carpenter et al. 1996). Cockett et al. (1996) and Feking et al. (1998) have defined as polar overdominance at the ovine *callipyge* locus. In addition, they described polar overdominance as genetic characterization of a locus showing a unique inheritance model that only heterozygous individual offspring inherits mutation from their sire phenotype expression.

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