Molecular marker within Major Histocompatibility Complex linked with general growth in sheep

Manzoor R. Mir[1*] and H. Geldermann

Department of Animal Breeding & Biotechnology,
University of Hohenheim, Stuttgart, Germany.

Abstract

The genetic diversity of a microsatellite marker linked to MHC-DRB1 gene in German Merino sheep was studied and association of this marker with growth trait in German sheep was ascertained. This experiment constituted four consecutive lambing among seven Merinoland rams and 249 ewes and a total of 16 MHC-DRB1 microsatellite alleles were detected, ranging from 353 bp to 857 bp. For ewes carrying the allele 394 and 857 bp the birth weight of lambs was about 400 g higher as compared to the residual group of ewes. Additionally, some genotype classes in ewes and rams too have variant effect on the growth traits in sheep. The observed associations could be due to differences in disease resistance, cell recognition or tissue differentiations between carriers of various MHC haplotypes which can in-turn affect the individual growth performance.

Key words: Sheep, DRB1, Microsatellite, Growth, QTL, MHC

Introduction

The Major Histocompatibility Complex (MHC) is a group of linked genes found in all vertebrates, playing a significant role in immune system, development, metabolic and endocrine system (Rupp *et al*., 2007). The MHC genes could be divided in three classes, of which loci of the MHC class I and II genes encode membrane-bound proteins that play a key role in the initiation of the immune response. Genes of class II are the most variable genes of vertebrates and are important to humoral immune system (Stear *et al*., 2005). Certain MHC class II genes especially DRB gene and a microsatellites markers within DRB1 gene is extremely useful in parentage and individual identification, breeding control including tracing of quantitative trait loci (QTL) and identification of gene introgression for livestock improvement (Duarte *et al*., 2005). There are 76 described alleleic sequences for the expressed DRB genes in cattle and 54 sequences for this locus are stored in GeneBank for sheep (Griesinger *et al*., 1999). Even inside a population, the number of alleles is high and reached 10 or more. This high number of alleles is maintained by a selective advantage of heterozygote individuals. Inside of exon 2 of DRB1 locus, a microsatellite is found and genetic variation of the DRB gene along with its associated microsatellite can be used as a marker for MHC variability (Geldermann *et al.,* 2006). Even if the primary function of MHC gene product is their role in the immune response and the defense of the body against pathogens (Rupp *et al*., 2007), effects of this chromosomal region to growth trait have been extensively studied in domestic animals (Gautschi and Gaillard 1990 and Mallard *et al*., 1991). Effect of some background genes

within MHC have been postulated to have an influence on the birth weight, weaning weight and litter size in the pigs. In this experiment, we have used the polymorphic microsatellite (GT)n(GA)m genotyping within intron 2 of the DRB1 gene (Geldermann *et al.,* 2006). for identifying association of MHC with the growth trait in German Merinoland sheep.

Materials and Methods

Experimental design: The experiment was conducted for four mating periods, involving 637 sheep which included 07 rams, 249 ewes and 381 lambs, at the research station of University of Hohenheim, Stuttgart, Germany. Young, virgin female sheep were mated with the same ram for two consecutive periods in order to establish feto-maternal immune response. After two pregnancies, ewes were taken out of the experiment.

Molecular Techniques: 10 ml blood or spleen samples were collected from live and dead animals respectively. DNA was extracted from the respective material using

[1]**Corresponding author**: ***Present address**: Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & A.H., Sher-e Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alustang, Post Bag no: 135, Srinagar, PIN: 190006, J & K State, India

a standard phenol-chloroform extraction protocol. The microsatellite in exon2 of the DRB1 gene was amplified by the polymerase chain reaction (PCR) in a thermocycler (Biometra) using standard ingredients of PCR reaction, including 200 ng of genomic DNA as a template in a reaction volume of 25 µl along with following primer set:
5'GGGGGATCCGCTTCGACAGCGACTGGGGCG3'and 5'CTGACCCAGAKTGAGTGAAAGTATC3' (K = G or T) (Griesinger *et al*., 1999). First cycle of the PCR were 3 min at 94°C, 1 min at 60°C, and 1 min at 72°C followed by 30 cycles with 30 sec at 94°C, 1 min at 60°C and 1 min at 60°C and finally one cycle with 30 sec at 94°C, 1 min at 60°C and 5 min at 72°C. Separation of the PCR product for fragment length analysis was carried on ALF-DNA sequencer. 1 µl of the PCR product and 3 µl of ALF loading buffer was mixed, denatured at 90°C for 2 min and chilled on ice before loading on the ALF gel. Electrophoresis was performed in 0.6 % TBE at 1500 V, 4 mA, 34 W and 50°C for 7 hours. Analysis of the data for fragment lengths was carried with the ALF-Fragment Manager Evaluation software in relation to external standards.

Data description:During the research period, production parameters were recorded for ewes and lambs and fed to a data base. The genotype information of DRB1 microsatellite from all animals was supplemented to the above records in data files. For the ewes the pregnancy status after each mating season (0=non-pregnant, 1=pregnant), the number of lambs born and number of lambs reaching an age of more than 40 days were recorded. Additionally, the birth weight of each lamb at different stages was measured. The resulting data sets including the data per ewe and lamb along with ram's information respectively were analyzed for the association between the DRB1 microsatellite alleles of the ewe, ram and lamb and the growth trait.

Statistical analysis:ANOVA analysis was done with the procedure General Linear Models (GLM) of the statistical package SAS. To correct the variation due to environment, important environmental factors were considered to each trait in ANOVA model. Allele frequencies, heterozygosity and deviation from Hardy Weinberg equilibrium were also calculated (SAS version 6.00, 1994).

Results and Discussion

A total of sixteen alleles, viz: 353, 374, 380, 383, 386, 389, 394, 400, 405, 411, 420, 430, 443, 455, 803 & 857, based on the number of base pairs were detected in the experimental flock at this locus (Table 1). The most frequent alleles were 411, 405, 394 and 383 and accounted for 63.3 % of the allele frequency in the entire flock. Out of 92 genotypes found at DRB1 microsatellite locus in German Merinoland sheep, 394/411 (7.1 %), 411/411 (6.0 %), 383/405 (6.0 %), 383/411 (6.0 %) and 405/411 (9.8 %), 394/405 (6.8 %), 394/411 (5.4 %) were reported most frequent genotypes (Table 1) in parents and offsprings respectively. Using a pooled test no significant deviation from the Hardy-Weinberg equilibrium could be detected. The number of alleles corresponds to higher allele numbers found for the expressed DRB locus in other sheep breeds (Schwaiger *et al*., 1993), as well as in cattle (Giovambattista *et al*., 1996).

Growth traits were measured for lambs and included birth weight, weaning weight, weight upto weaning and daily gain upto weaning. With respect to the association between MHC-DRB1 microsatellite variants and growth traits; genotype of rams and ewes (Table: 2 & Figure: 1 & 2 were considered for the respective effects. Positive effects ($p<0.01$) were observed for ram 1 (389/411), ram 2 (383/383) and ram 6 (405/420), in comparison to ram 5 (405/803). Ram 4 (455/803) was significantly negative against the rams possessing positive effects with the $p<0.05$ except the ram 2 and 3 with $p<0.01$. The MHC-DRB1 microsatellite genotype classes of ewes 353/394 ($p<0.05$), 374/411 and 394/411 ($p<0.01$) were associated with positive effects on birth weight whereas the genotypes 389/411 ($p<0.05$) and 411/411 ($p<0.01$) were associated with negative effects. Specific alleles in the ewes were observed to have their effect on birth weight and weaning weight in the lambs however, daily gain up to weaning was unaffected by MHC-DRB1 alleles of ewes. For the allele 394 in ewes, positive effects were analyzed for birth weight of lambs ($p<0.001$) with a difference of about 400 g when compared to the residual group. A positive effect was also recorded with the allele 857 in comparison to the residual class with $p< 0.05$ and allele 443 had a negative effect on birth weight ($p<0.05$). The allele class of 353 and 389 too had negative effects on growth traits ($p<0.01$). In lambs three alleles viz: 383, 394 and 400 were associated positively with higher birth weight and daily gain up to weaning.

Documentary evidence of the direct effects of MHC genes on growth is expanding, however, well documented evidence of MHC is on the immune system and disease resistance (Rupp *et al*., 2007). Different QTL's for growth and back fat in Chinese pig have been found in the MHC region as well as outside the MHC region; besides gene for meat quality has also been identified within MHC region (Geldermann *et al.,* 2006). Aroviita *et al*., (2004) has identified certain HLA-DRB1 alleles which were associated with higher intrauterine growth of the fetus, a precursor of the normal post natal growth of the individual. Previous reports pointed out

a significant association between serologically defined MHC class I polymorphism and growth in domestic animals (Beever *et al*., 1990). An earlier finding by Gruszczynska *et al*., (2000) has described the association for birth weight due to certain breed specific haplotypes within the MHC region. Specific DRB1 microsatellite alleles were associated with growth traits in this study. Allele 394 which occurred at a flock allelic frequency of 14.1 %, was associated ($p<0.001$) with the birth weight of lambs. The alleles 443 and 857 were also associated with high birth weights and the allele 400 showed significant positive associations with weaning weight of lambs and daily weight gain. Allelic effects in rams, ewes and lambs were almost equal. Allelic variants at or near MHC confer some selective advantage to the individuals measured as differential fetal survival and fetal growth (Melnick *et al*., 1981). Our results are in agreement with Jung *et al.,* (1989) who found some SLA-RFLP haplotypes associated with different growth traits. Studies on experimental animals suggest that the effects of MHC on growth and development are due to the genes closely linked to class I, class II and class III loci (Kostyu 1994) which increase the mothering ability of the ewes that also contribute to the survivability of the lambs. Moreover, MHC polymorphism has indirect effects on the growth traits e.g. high resistance to nematode infection (Buitkamp *et al*., 1996), as animals with increased susceptibility to diseases have reduced growth capacity. MHC heterozygosity has been has been identified by Penn *et al*., (2002) to confer a selective advantage against different types of infections which ultimately enhances the growth, health and survival of the animal. Furthermore, Mallard *et al*., (1991) has reported that certain haplotypes appears to influence birth weight and weaning weights. Heavier piglets tend to have a survival advantage and usually are phenotypically favored by producers. The DRB1 microsatellite alleles which were associated negatively to the growth traits may also have some detrimental linked genes (t complex) or homozygous state of *Growth and Reproduction complex* (*grc*) gene may be associated with the alleles having negative effects on growth trait in this breed (Melhem *et al*., 1993). DRB1 microsatellite homozygosity in ewes in the present study did not influence the growth traits, though homozygotes had lower values for the most traits and these results are in agreement with Grignola *et al*., (1995) who found that heterozygote bulls also had no better growth rates.

This study conclusively point to different immunological influences due to the MHC which effects the general growth of the sheep. The effects could be due to the influence of MHC proper or some linked genes to the MHC and needs to be investigated thoroughly with large number of microsatellite markers.

Acknowledgements

Authors are thankful to H. Bartenschlager for statistical analysis and valuable suggestions. The first author is grateful to German Academic Exchange Service (DAAD) for the financial support during the doctorate studies.

References

1. Aroviita, P., Partanen, J.; Sistonen, P.; Teramo, K. and Kekomaki, R. (2004): High birth weight is associated with human leukocyte antigen (HLA) DRB1*13 in full-term infants. Eur. J. Immunogenetics. 31: 21 – 26
2. Beever, J.E., George, P.D.; Fernando, R.L.; Stormont, C.J. and Lewin, H.A. (1990): Association between genetic markers and growth and carcass traits in paternal half-sib family of Angus cattle. J. Anim. Sci. 68: 337 – 344
3. Buitkamp, J., Filmether, P.; Stear, M.J. and Epplen, J.T. (1996): Class I and Class II Major Histocompatibility Complex alleles are associated with fecal egg counts following natural, predominantly Ostertagia circumcincta infection. Parasit. Rev., 82: 693 – 696
4. Duarte, L.B.M., Moraes, J.C.F. and Weimer, T.A. (2005): Diversity of microsatellites linked to FSHß gene, their usefulness for individual identification and association with reproductive performance. Cienc. Rural, 35: 145-149.
5. Gautschi, C. and Gaillard, C. (1990): Influence of major histocompatibility complex on reproduction and production traits in swine. Anim. Genet. 21: 161 – 170
6. Geldermann, H.; Mir, M.R.; Kuss A.W.; Bartenschlager H. (2006): OLA-DRB1 microsatellite variants are associated with ovine growth and reproduction traits. Genet. Sel. Evol. 38: 431 - 444
7. Giovambattista, G., Golijow, CD., Dulout, FN., Lojo, MM, (1996): Gene-frequencies of DRB3.2 locus of argentine creole cattle. Animal Genetics 27: 55-56
8. Griesinger, I, Pruser, F.; Siemienski, K. and Geldermann, H. (1999): Extreme fragment lengths differences of the microsatellite in the expressed MHC-DRB gene of Merinoland sheep. Anim. Genet., 30: 77 - 78.
9. Grignola, F., Mao, I.L.; Mejdell, C.; Lie O and Solbu, H. (1995): Association of alleles for the class I bovine lymphocyte antigens with conformation, semen traits and growth rate of young bulls. J. Dairy Sci. 78: 908 – 913.
10. Gruszczynska, J.; Charon, K.M.; Kitlinska, J.; Szydlowski, M. (2000): The influence of OLA-DRB1 (MHC class II) gene polymorphism on lamb body weight and weight gain in Polish Heath sheep. J. Appl. Genet., 41: 1001 – 1112
11. Jung, Y.C.; Rothschild, M.F., Flanagan, M.P., Christian, L.L. and Warner, C.M. (1989): Association of restriction fragment length polymorphisms of swine leukocyte antigenclass I genes with production trtaits of Duroc and Hampshire boars. Anim Genet. 20: 79 - 91
12. Kostyu, D.D., (1994): HLA: fertile territory for developmental genes? Crit. Rev. Immunol. 14: 29 – 59
13. Mallard, B.A., Kennedy, B.W. and. Wilki, B.N (1991): The effects of swine leukocyte antigen haplotype on

the birth and weaning weights in miniature pigs and the role of statistical analysis in this estimation. J. Anim. Sci. 69: 559 – 564.
14. Melhem, M.F., Kunz, H.W. and Gill, T.J. (III), (1993): A major histocompatibility complex linked locus in the rat critically influences resistance to diethylnitrosamine carcinogenesis. PNAS 90: 1967 – 1971.
15. Melnick, M., Jaskoll, T. and Slavkin, H.C. (1981): The association of H-2 haplotype with implantation, survival and growth of murine embryos. Immunogenetics 14: 303 - 308.
16. Penn, D.J., Damjanovich, K. and Potts, W.K. (2002): MHC heterozygosity confers a selective advantage against multiple-strain infections. PNAS 99: 11260 – 11264.
17. Rupp, R; Hernandez, A. and Mallard, B.A. (2007): Association of bovine leukocyte antigen (BoLA) DRB 3.2 with immune response, mastitis and production and type traits in Canadian Holsteins. J. Dairy Sci., 90: 1029 -1038.
18. Schwaiger, F.W., Weyers, E.; Epplen, C.; Brun, J.; Ruff, G.; Crawford A. and Epplen, J.T (1993): The paradox of MHC-DRB exon/intron evolution: a-helix and ß-sheet encoding regions diverge while hypervariable intronic simple repeats coevolve with ß-sheet codons. J.Mol. Evol., 37: 260 – 272
19. Stear, M.J., Innocent G.T. and Buitkamp, J. (2005): The evolution and maintenance of polymorphism in the major histocompatibility complex. Vet. Immunol. Immunopathol., 108: 53 – 57
20. SAS (1994): SAS/STAT User's guide, Edition 6.2 SAS institute Inc. Cary.

Table 1: Allele/ Genotype frequencies of DRB1 microsatellite locus in German Merinoland sheep.

Allele frequencies (bp)	Parents (n)	%	Offspring (n)	%	Total (n)	%
353	15	3.01	2	2.32	7	2.6
374	16	3.27	13	2	3	22
380	21	4.2	14	27	35	34
383	69	13.7	57	108	126	122
386	28	5.6	14	27	42	41
389	26	5.2	20	38	46	45
394	72	14.3	74	140	146	141
400	18	3.6	5	09	23	22
405	65	12.9	85	161	150	145
411	102	20.2	130	246	232	225
420	2	0.4	11	21	13	13
430	3	0.6	7	13	10	10
443	7	1.4	4	0.8	11	11
455	26	5.2	36	68	62	60
803	13	2.6	46	87	59	57
857	21	4.2	6	11	27	26
Genotype frequencies						
383/383	10	4.0	6	2.3	16	3.1
383/394	3	1.2	8	3.0	11	2.1
383/405	15	6.0	10	3.8	25	4.8
383/411	15	6.0	8	3.0	23	4.5
389/411	8	3.2	5	1.9	13	2.5
394/405	11	4.4	18	6.8	29	5.6
394/411	18	7.1	18	6.8	36	7.0
405/411	2	0.8	26	9.8	28	5.4
405/803	3	1.2	8	3.0	11	2.1
411/411	15	6.0	16	6.1	31	6.0
411/455	3	1.2	12	4.5	15	2.9
411/803	2	0.8	12	4.5	14	2.7

Table-2. Association between DRB1 microsatellite genotypes of ewes and growth traits of lambs

Source of varience	n	Birth weight of lamb[3] (kg)	(LS-Means ±SE)	n weight[3] (kg)	Weaning Means ±SE)	(LS-up to Weaning [3] (kg)	Weight gain	(LS-Means ±SE)	Daily gain up to weaning [3] (g)	(LS-Means ±SE)
Ewes genotype[1]										
353/394	11	5.148 [ac]	0.251	9	22.976	1.417	18.008	1.338	191.733	15.123
374/411	14	5.303[ad]	0.225	13	24.885	1.205	19.680	1.138	216.352	12.860
380/411	12	4.792	0.241	10	24.033	1.300	19.004	1.228	204.196	13.882
383/383	10	4.736	0.262							
383/405	22	4.732[bc]	0.179	15	24.506	1.201	19.702	1.134	208.708	12.823
383/411	25	4.881	0.168	17	25.868	1.082	20.970	1.022	229.509	11.549
383/455	9	4.836	0.277							
386/405	18	4.961[cde]	0.201	11	24.356	1.266	19.238	1.195	211.868	13.513
389/411	8	4.246[b]	0.289							
394/405	16	4.864	0.212	13	25.796	1.218	20.929	1.150	222.599	13.002
394/411	30	5.263[ad]	0.159	24	24.639	0.976	19.661	0.921	213.763	10.417
405/455	8	4.992	0.293	7	23.284	1.509	18.389	1.425	195.155	16.112
405/857	8	4.903	0.293							
411/411	33	4.594[be]	0.147	28	24.337	0.876	19.725	0.827	215.671	9.348
Rest	151	4.991[ac]	0.075	140	24.476	0.672	19.629	0.635	211.979	7.175

Sign. (p)[2) r2)] *(0.041) N.S. 0.475, 0.489, 0.478, 0.676

[1)] = 7 observation per genotype;

[2)] Probability at *:p=0.05; n.s.: not significant

[3)] LS-means within columns with characters differ at p< 0.05

* * * * * * * *

* * * * * * * *