Polymorphism Of The *DQA2* Gene In The Chios Dairy Sheep Population And Its Association With Footrot

A. I. Gelasakis[*], G. Arsenos[*], J. G. Hickford[†], A. Psifidi[*], H. Zhou[†] and G. Banos[*]

Introduction

The *DQA2* gene, located at *ovar* MHC class II region, has been found to modulate the response of sheep to footrot (Escayg et al. 1997). This is a highly polymorphic gene and a 5-point scoring system (1= the most resistant, 5= the most susceptible) has been developed to classify alleles according to resistance to footrot (Hickford 2001), based on research involving meat and wool breeds of sheep. However, the gene has not been studied in dairy breeds. In Greece, the most prolific dairy breed is Chios. A genetic improvement program has been running over the last few years in 66 flocks with emphasis on milk production. In these flocks, footrot is gradually becoming a challenging issue warranting investigation. The objectives of this study were to assess the frequency of polymorphism of the *DQA2* gene in the Chios dairy breed of sheep and to assess its association with footrot prevalence in the population.

Materials and methods

Animals. A total of 400 Chios dairy sheep (385 ewes and 15 rams) raised in 30 flocks were randomly selected. Eighteen (18) of these animals were diagnosed with footrot at the time of sampling, reflecting a footrot prevalence of 4.7%.

Blood collection and DNA isolation. Blood samples were collected from the jugular vein of individual sheep using a vacutainer needle and Vacutainer® Blood Collection K_2 EDTA Tubes. Samples were immediately stored in a portable fringe (0-4ºC) before being transferred to the lab. DNA used in PCR amplification was isolated using NucleoSpin® Blood (Macherey-Nagel Gmbh & Co. KG) following the manufacturer's protocol.

PCR - single strand conformational polymorphism (SSCP). The ovine *DQA2* gene was typed using PCR-single strand conformational polymorphism (SSCP) technique, as described by Hickford et al. (2004). Briefly, the polymorphic exon 2 of the ovine *DQA2* gene was amplified using *DQA2*-specific primers (ACTACCAATCTCATGGTCCCTCT and GGAGTAGAATGGTGGACACTTACC). The *DQA2*-specific primers amplify both *DQA2* and *DQA2-like* sequences. Amplification was carried out in a 20-μl reaction containing 50 ng

[*] Department of Animal Production, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Box 393, 54124 Thessaloniki, Greece.

[†] Department of Agricultural Sciences, Faculty of Agriculture and Life Sciences, Lincoln University, Box 84, 7647, Christchurch, New Zealand.

genomic DNA, 0.25 μM of each primer, 150 μM dNTPs (Eppendorf, Hamburg, Germany), 0.5 U *Taq* DNA polymerase (Qiagen, Hilden, Germany) and 1× the reaction buffer (containing 1.5 mM $MgCl_2$). After initial denaturing at 94°C for 2 min, 35 cycles at 94°C for 30 sec, 62°C for 30 sec and 72°C for 30 sec were utilized, followed by a final 5 min extension step at 72°C. Amplicons were SSCP analyzed using 14% acrylamide: bisacrylamide (37.5:1; Bio-Rad, Hercules, CA) gels. Amplicons representative of the known *DQA2* sequences were also included in each polyacrylamide gel and their banding patterns were used as standards for determining the alleles present in individual sheep.

Statistical analysis. Once genotypes were determined, genotypic and allelic frequencies were calculated with counting. The effect of the *DQA2* gene on footrot was assessed as follows:

$$Y_{abcdef} = FYM_i + L_j + S_k + G_l + e_{ijkl} \quad (1)$$

where: Y = presence or absence of footrot (0/1), *FYM* = fixed effect of i^{th} flock by lambing year by lambing month interaction (55 levels), *L* = fixed effect of j^{th} number of lactation (2 levels), *S* = fixed effect of k^{th} sex (2 levels), *G* = fixed effect of l^{th} DQA_2 genotype (12 levels; genotypes with fewer than 10 observations were combined into 1 class) and e = the random residual. In this analysis, genotype accounted for both additive and dominance effects on footrot. In a separate analysis, the effect of genotype in model (1) was replaced by the substitution additive effect of each allele, namely the values 0, 1 and 2 were used to denote presence of 0, 1 and 2 allele copies, respectively, in a genotype.

Results and discussion

A total of 20 *DQA2* alleles and 78 genotypes were detected in the sampled population. Table 1 shows frequencies of the 11 most prevalent genotypes. Genotypes with fewer than 10 observations were combined into a single class. Genotype KK was the single most frequent one followed by KW. In a study of 3 non-dairy sheep breeds (German Mutton Merino, German Merino and German Blackheaded Mutton), Ennen et al. (2009) found 21 alleles and 96 genotypes with B1D being the most frequent genotype (6.3%). In our population, the B1D frequency was only 1.3%, suggesting a different genetic background of Chios dairy breed compared to meat and wool breeds. Since a random sample was obtained in the present study, the derived frequencies are considered representative of the population.

Table 2 shows frequencies of the *DQA2* alleles. Allele K was the most common followed by W, L and B. No comparison with other dairy breeds may be made due to lack of pertinent literature but these alleles were found to be much more rare in non-dairy breeds such as Romney, Merino and Corriedale (Hickford et al. 2004). In the latter study, H and G were the most frequent alleles (15.8% and 14.5%, respectively).

Both tables 1 and 2 attest to the polymorphic nature of the *DQA2* gene in the Chios breed.

The overall effect of genotype on prevalence of footrot was statistically significant ($P<0.05$). Genotype LK was associated with decreased and KK with increased prevalence of footrot ($P<0.05$; Table 1), suggesting that it is possible the effect of L to be better manifested in an interaction with K.

Table 1: *DQA2* genotypes, frequencies and effect on footrot in the Chios dairy sheep

		Linear Model		
Common nomenclature	**Genotypic frequency**	**Effect**	**S.E.**	**P- value**
BL	2.6	-0.036	0.021	0.085
LK	7.0	-0.030	0.012	0.012
KK	12.0	0.004	0.009	0.025
BO	3.4	0.012	0.010	0.251
BW	4.2	0.013	0.011	0.208
WW	3.6	0.021	0.020	0.290
OK	5.5	0.029	0.027	0.295
DK	3.4	0.038	0.052	0.466
BK	6.0	0.071	0.297	0.812
EK	2.6	0.082	0.073	0.263
KW	8.3	0.112	0.262	0.668
Remaining 67 genotypes	41.4	0.149	0.089	0.095

The effect of individual alleles, assessed with an allele substitution model, was found not significant (P>0.05) in all cases except for E (Table 2). A single copy of E was associated with 7.3% higher susceptibility to footrot; a similar result was reported by Ennen et al. (2009) for meat and wool producing breeds. Allele E has been also classified as relatively susceptible to footrot by Hickford (2001) with a score of 4 (Table 3). In fact, alleles E and L were the only alleles among the susceptible ones (scores 4 and 5) detected in the Chios population. An additional observation is that the frequency of alleles B1 and G that are classified as most resistant by Hickford (2001) is substantial in the Chios breed (Table 3); however, the resistance of these alleles was not confirmed in the present study.

The resistance of allele L was also not found in our study. This notion is supported by results of Ennen et al. (2009). Also, in the resistance scale proposed by Hickford (2001; Table3), allele L was rated as susceptible. Unrated alleles were found in relatively high frequency in the Chios breed (Table 3) warranting further research.

Admittedly, the diversity of *DQA2* alleles within the breed combined with the low number of footrot cases make rating of alleles according to susceptibility to footrot rather difficult; it should be noted here that the prevalence found in our sample (4.7%) is very close to the population average (5.0%) suggesting that these results are representative of the population.

Conclusion

A high diversity at the ovine *DQA2* gene locus was revealed in the Chios dairy breed, suggesting that genetic selection may be successful in rendering the breed resistance to footrot. The next step is to investigate the susceptibility to footrot of unrated genes, which were found in high percentage in Chios dairy sheep.

Table 2: *DQA2* alleles, frequencies and effect on footrot in the Chios dairy sheep

Common nomen-clature	Labeling accredited by the ISAG	Nomenclature valid in combination with *DQA2*– like sequence	Allelic frequency (%)	Linear model		
				Effect	S.E.	P-value
B1	0601		11.3	0.068	0.025	0.782
B2	0602		1.7	-0.004	0.072	0.951
C1	08011		1.4	-0.053	0.064	0.406
D	0103		5.5	-0.009	0.036	0.794
E	1101		4.4	0.073	0.036	0.045
G	0101	1401	1.3	-0.000	0.071	1.000
J′	0701	1301	1.7	-0.005	0.056	0.926
J1	0401	1501	8.2	-0.001	0.030	0.968
J2′	0702	1601	1.2	-0.068	0.069	0.322
J2′′	0702	1501	3.0	0.003	0.039	0.941
K	0301		31.7	-0.005	0.016	0.750
L	0501		11.4	-0.035	0.024	0.153
W	0603	1101	14.4	0.017	0.021	0.407
C2, J′′ Q, H, J2, J, U			2.8	Non-significant		

Table 3: Frequencies of the alleles in the Chios breed and classification by level of resistance to footrot as proposed by Hickford (2001)

Score*	*DQA2* allele	Frequencies in the Chios breed (%)
1	B1, G	12.6
2	C1, F1, J, J′, J2′, J′′, Q, S	5.4
3	B2, C2, D, K, H, J2	40.4
4	E, J1′, L	15.8
5	I, F2	0
Unrated	T, U, B3, W, J1, J2′′	25.8

*1 = Most resistant to footrot, 5 = Most susceptible to footrot

Acknowledgements

The Chios Sheep Breeders' Cooperative "Macedonia" for collaboration and the Greek State Scholarships Foundation for financial support to the first author.

References

Ennen, S., Hamann, H., Distl, O., *et al.* (2009). *Small Rumin. Res.*, 86:22-25.

Escayg, A.P., Hickford, J.G. and Bullock, D.W. (1997). *Res. Vet. Sci.*, 63:283-287.

Hickford, J.G. (2001). In: *Proc 31st Meeting of the Sheep and Beef Cattle Society of New Zealand*, pp. 75-79.

Hickford, J.G., Zhou, H. and Fang, Q. (2004). *J. Anim. Sci.,* 85:577-582.