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SEARCHING FOR SNPS THAT AFFECT SHEEP ROBUSTNESS: *CYP17* SNP AFFECTS BEHAVIOURAL RESPONSES TO PSYCHOLOGICAL STRESS

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SUMMARY

The ability of animals to adapt to stress is not only an animal health and welfare concern, but also influences reproduction potential and robustness. An important pathway involved in the stress response is the hypothalamic-pituitary-adrenal axis (HPAA) that results in the release of cortisol from the adrenal gland. In this study the cortisol responses of South African Merinos were measured to assess HPAA responsiveness to stress and relate it to behavioural stress responses to flock-isolation. The experiment was structured according to a 2×2 statistical design, with *CYP17* genotype (*WT1/WT1* vs. *WT1/WT2*) and selection line (H-line vs. L-line) as factors. Selection line criteria was based on divergent selection for (H-line) or against (L-line) maternal multiple rearing ability, where the H-line generally outperformed the L-line in terms of reproduction, animal welfare and resistance to certain pathogens. The *CYP17* genotype is involved in the biosynthesis pathway of cortisol. In the present study the *CYP17* genotype showed a significant influence on behavioural stress responses, where three parameters of the flock-isolation test were affected ($P<0.05$), namely the number of bleats uttered, the urinating frequency and the average distance from a human operator. It is suggested that the *CYP17* genotype affects behavioural responses via its effects on cortisol production, and that the SNPs located within the *CYP17* genotype may have application in marker-assisted selection of sheep.

INTRODUCTION

The ever increasing global population continues to place pressure on improving the 'efficiency' of animal production to meet local and global demands. It is, however, a difficult task to improve animal production in a commercial practice by means of genetic progress if the environment in which the animals are raised does not support the full expression of their genetic potential (Mormède *et al.* 2011). It is thus important to include robustness-related traits in breeding objectives, since 'robustness' is described as the ability to combine a high production potential with resilience to stressors, which allows for the unproblematic expression of a high production potential in a wide variety of environments (Beilharz 1998; Knap and Rauw 2009). In this respect, the hypothalamic-pituitary-adrenal axis (HPAA) plays an important role in adaptation to stress, via the release of cortisol from the adrenal cortex.

The Directorate Animal Sciences of the Western Cape Department of Agriculture at Elsenburg research farm in South Africa embarked on a strategic breeding program in 1986, where selection responses to divergent selection for maternal multiple rearing ability was assessed. The assumption was that selection for this trait would include characteristics for both fitness (increased lamb survival) and 'efficiency' of animal production (number of lambs reared per joining) as suggested by Snowder and Fogarty (2009). Two distinct Merino lines (upward selection: H-line vs. downward selection: L-line) were established that showed a marked divergent response in overall reproduction and animal welfare (Cloete and Scholtz 1998; Cloete *et al.* 2004; 2005a; 2005b; 2009; Scholtz *et al.* 2010; 2011). These lines differed in their behavioural responses to flock-isolation during an arena test (Cloete *et al.* 2005a; 2010), as well as in their cortisol responses to insulin-induced hypoglycemic stress (Van der Walt *et al.* 2009; Hough 2012), where the H-line generally displayed a superior ability to adapt to stressful situations than the L-line.

The present study investigates the contribution of the *CYP17* genotype towards the cortisol response to physiological stress and its implications for behavioural responses to flock-isolation stress (psychological stress). The *CYP17* genotype is considered, since it encodes for an enzyme, namely cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17), that plays a key role in the cortisol biosynthesis pathway (Miller and Auchus 2011). This paper studies the role of selection line and *CYP17* genotype in responses of sheep to induced hypoglycemia and flock isolation.

MATERIALS AND METHODS

Animals and breeding program. All animals belonged to a South African breeding program that commenced in 1986, where Merino sheep have been divergently selected for maternal multiple rearing ability (Cloete *et al.* 2004, 2009). Records of progeny from the 2001-2008 birth years were used, which included their behavioural performances in the arena test (Cloete *et al.* 2005a; 2010) and *CYP17* genotypes (for complete protocol of SNP genotyping, refer to Hough *et al*. 2013; Hough 2012). Records were grouped in a 2 X 2 factorial design, according to selection line (H- vs. L-line) and *CYP17* genotype (homozygous *WT1/WT1* vs. heterozygous *WT1/WT2*), and assigned the following abbreviations: H_E for H-line heterozygous *WT1/WT2*; H_O for H-line homozygous *WT1/WT1*; L_E for L-line heterozygous *WT1/WT2*; and L_O for L-line homozygous *WT1/WT1*. Arena test records were available for 260 H_E-, 74 H_O-, 53 L_E-, and 13 L_O-grouped sheep.

Hypoglycemic stress test. Merino sheep in the above mentioned breeding program (11 H_E-, 6 H_O-, 15 L_E-, and 6 L_O-grouped rams, 2-6 years of age) were subjected to intravenous administration of human insulin at a dose of 0.1 IU/kg body mass, after which blood samples were collected at times: 0, 15, 30, 60 and 120 min post challenge. Blood plasma glucose and free cortisol was determined with radioimmunoassay. Since it is not only the magnitude of the cortisol response that is important, but also the duration of the cortisol output the cortisol responses to hypoglycemic stress was expressed as the area under the curve (AUC) from measurements over 2 hours. Ethics approval was obtained from the Departmental Ethics Committee for Research on Animals (DECRA ref: R08/21) of the Western Cape Department of Agriculture.

Arena stress test. In this flock-isolation stress test, sheep entered a 10.6 m X 4.0 m arena (marked out in 18 squares) one-by-one, as described by Cloete *et al.* (2005a). The arena was surrounded by wooden panels to prevent escape, but still allowing visual contact with six contemporary group sheep on the opposite side of the arena, behind a split-pole fence, where a human operator was situated on a chair. The operator remained motionless, while the behaviour of the sheep was assessed for 3 minutes according to the following parameters: number of bleats, number of defecation events, number of urinating events, average distance from human operator (meters); and movement based upon the number of boundaries between squares that were crossed (crosses).

Statistical analysis. GraphPad Prism (version 4) software (GraphPad Software, San Diego, California) was used for all statistical analysis. The interaction effects of *CYP17* genotype and selection line was tested with a two-way analysis of variance and a Bonferroni's post-test for each recorded item in the arena test (average distance between the sheep and the human operator, movement in arena depicted by number of squares crossed, number of bleats, number of urinating events and number of defecating events). The selection line × *CYP17* genotype interaction was investigated with a two-way ANOVA of the AUC for the cortisol responses (normalized with glucose concentrations as measurement of the degree of hypoglycemic stress) of each subgroup.

RESULTS AND DISCUSSION

Hypoglycemic stress test. Comparison of the cortisol responses of the *CYP17* genotype × selection line groups with a two-way ANOVA indicated that the interaction between the selection line and the *CYP17* genotype was significant (P=0.0226). Differences in cortisol responses between the *CYP17* genotypes were only found within the L-line (Bonferroni post-test: P<0.05; 2751.5±57.5 AUC for the L_O-group vs. 1765.0±179.0 AUC for the L_E-group). In contrast, cortisol output was independent from *CYP17* genotype in the H-line (Bonferroni post-test: P>0.05; 2528.5±225.5 AUC for the H_O-group vs. 2610.5±37.5 AUC for the H_E-group). These results indicated that the effect of the *CYP17* genotype is dependent on the genetic background of the animal, since it has been shown that the H-line sheep have a superior HPAA function that allows them to adapt to stressful situations more effectively than L-line sheep (Hough 2012).

Arena stress test. The arena test performance of sheep in the H_E (n = 260), H_O (n = 74), L_E (n = 53) and L_O (n = 13) groups were compared. As seen by the sample size, the L-line sheep were poorly represented compared to the H-line, due to the effects of downward selection on the birth rate and survival of L-line animals. These statistics need to be improved in future studies. The behavioural response to stress was tested before one year of age (prior to exposure to various handling procedures) in lambs born from 2001-2008 of which the *CYP17* genotypes were known.

The effect of the selection line and *CYP17* genotype, as well as their interaction, was assessed with a two-way ANOVA, followed by a Bonferroni's post-test, for each arena test item (Table 1). It was found that the *CYP17* genotype (P<0.05), but not the selection line (P>0.05) or its interaction with the *CYP17* genotype (P>0.05), had a significant effect on three out of five of the arena test parameters, namely the number of bleats (P=0.0038, heterozygous *WT1/WT2*: 18.58±0.69 bleats vs. homozygous *WT1/WT1*: 13.76±1.15 bleats); number of urinating events (P=0.0083; heterozygous *WT1/WT2*: 1.45±0.15 events vs. homozygous *WT1/WT1*: 3.45±0.71 events); and the average distance of the sheep from the human operator (P=0.0192; heterozygous *WT1/WT2*: 4.50±0.17 meters vs. homozygous *WT1/WT1*: 4.26±0.28 meters). The animals of the L_E-group on average kept a longer distance (Bonferroni post-test: P<0.05; L_E: 4.21±0.28 meters) from the human operator (signal of stress) compared to the L_O-group (3.05±0.55 meters) that coincided with a higher cortisol response to hypoglycemia (superior stress response). The H_E-group uttered more bleats (17.50±0.76 bleats), but urinated less frequently (1.79±0.23 events) during the arena test than the H_O group (P<0.05; H_O: 12.82±1.23 bleats, 4.01±0.78 urinating events). Although the psychological stress responses of these two H-line groups seemed to be different, their responses to physiological stress (insulin-induced hypoglycemia) were the same. The line differences reported by Cloete *et al*. (2005a) were not evident in the present study. However, selection line tended to interact with *CYP17* genotype for the average distance from the human operator (P=0.06) and the number of defecating events (P=0.10). Future research on more animals is needed to elucidate the separate and combined effects of line and *CYP17* genotype.

It is known that cortisol can affect behaviour via its effects on the brain (Pryce *et al.* 1988; Da Costa *et al.* 2004; Dwyer *et al.* 2004). Results from the present study indicate that a higher cortisol response from the adrenal cortex is related to less stressed behaviours during flock-isolation, namely smaller average distances maintained from humans and fewer vocalizations of protest. The higher urinating frequency associated with the higher cortisol response might be ascribed to alterations in steroid hormone synthesis, which directs steroid biosynthesis towards cortisol production and away from aldosterone production. Subsequently there is an increase in hormonal signals (via the renin-angiotensin regulating mechanism) to increase urination. The remaining parameters in the arena test might be related to other complex traits, but would seem to not be related to the *CYP17* genotype.

Table 1. Summary of the behavioural responses of sheep during the arena test. Values depict means±SEM and *P*-values from two-way ANOVA with *CYP17* genotype (CG) and selection line (SL) as factors. Traits considered were: average distance from human operator (ADIS), number of crosses (NCROSS), number of bleats uttered (NBL), number of urinating events (NUR) and number of defecating events (NDEF).

	H-line		L-line		*P*-values		
Trait	***WT1/WT1***	***WT1/WT2***	***WT1/WT1***	***WT1/WT2***	**SL**	**CG**	**SL × CG**
ADIS	3.65±0.16	3.79±0.99	3.05±0.55	4.21±0.28	0.7301	0.0192*	0.0646
NCROSS	18.70±1.33	19.40±0.75	15.46±2.54	17.32±1.30	0.1709	0.5096	0.7650
NBL	2.82±1.23	17.50±0.76	11.69±3.11	18.42±1.43	0.9574	0.0038**	0.6017
NUR	4.01±0.78	1.79±0.23	4.54±2.45	2.49±0.68	0.4466	0.0083**	0.9116
NDEF	1.16±0.14	1.06±0.07	0.77±0.26	1.26±0.13	0.6093	0.2852	0.1018

CONCLUSIONS

The present results suggest that the *CYP17* genotype affects cortisol production and behavioural responses to psychological stress, where the presence of *WT1* seems to be more beneficial for adaptation to stress compared to the presence of *WT2*. The effect of the *CYP17* genotype, however, also depends on the genetic background of the animal to cope with stressors. More research is needed understand the interaction between selection line and *CYP17* genotype. The two SNPs within the ovine *CYP17* gene may have application via marker-assisted selection to improve the ability of sheep to cope with stress and to adapt to their environment more effectively.

REFERENCES

Beilharz R.G. (1998) *J. Anim. Breed. Genet.* **115**: 433.

Cloete J.J.E., Cloete S.W.P. and Hoffman L.C. (2005a) *Small Rumin.Res.* **60**: 227.

Cloete, J.J.E., Cloete, S.W.P. and Hoffman, L.C. (2010) Proc. 9th World Cong. Gen. Appl. Livest. Prod., 1-6 Aug 2010, Leipzig. http://www.kongressband.de/wcgalp2010/assets/pdf/0703.pdf.

Cloete S.W.P. and Scholtz A.J. (1998) *Aus. J. Exp. Agric.* **38**: 801.

Cloete S.W.P., Gilmour A.R., Olivier J.J. and van Wyk J.B. (2004) *Aus. J. Exp. Agric.* **44**: 745.

Cloete S.W.P., Scholtz A.J., Cloete J.J.E. and van Wyk J.B. (2005b) *Aus. J. Exp. Agric.* **45**: 1131.

Cloete S.W.P., Misztal I. and Olivier J.J. (2009) *J. Anim. Sci.* **87**: 2196.

Da Costa A.P., Leigh A.E., Man M. and Kendrick K.M. (2004) *Proc. Royal Soc. Lon. B* **271**: 2077.

Dwyer C.M., Gilbert C.L. and Lawrence A.B. (2004) *Horm. Behav.* **46**: 529.

Hough D. (2012) PhD Thesis, Stellenbosch University.

Hough D., Cloete S.W.P., Storbeck K., Swart A.C. and Swart P. (2013) *J. Anim. Sci.* **91**, 1193.

Knap P.W. and Rauw W.M. (2009) In 'Resource allocation theory applied to farm animal production', pp. 210-229, editor W.M. Rauw, CABI Publishing, Wallingford, UK.

Miller W.L. and Auchus R.J. (2011) *Endocr. Res.* **32**(1): 81.

Mormède P., Foury A., Terenina E. and Knap P.W. (2011) *Animal* **5**(5): 651.

Pryce C.R., Abbott A.H., Hodges J.K. and Martin R.D. (1988) *Phys. Behav.* **44**: 717.

Scholtz A.J., Cloete S.W.P., Van Wyk J.B., Kruger A.C.M. and Van der Linde T.C.deK. (2010) *Anim. Prod. Sci.* **50**: 203.

Scholtz, A.J., Cloete, S.W.P., Cloete, J.J.E., Kruger, A.C.M., van Wyk, J.B. and Van der Linde, T.C.de K. (2011) *Proc. Assoc. Advmt. Anim. Breed. Genet.* 19: 175.

Snowder, G.D. and Fogarty, N.M. (2009) *Anim. Prod. Sci.* **49:** 9.

Van der Walt D., Cloete S.W.P., Storbeck K. and Swart P. (2009) *Proc. Assoc. Advmt. Anim. Genet.* **18**: 100.