Contributed paper

GENOME-WIDE ASSOCIATION ANALYSIS FOR TEMPERAMENT IN AUSTRALIAN SHEEP

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SUMMARY

In livestock, temperament traits such as flight speed and agitation are important indicators of management, survival, and welfare. Multiple studies have reported a low to moderate heritability for these traits. Identifying the genomic regions associated with temperament could help to find candidate genes and processes involved in defining these traits and this could be helpful in genomic prediction of phenotype or breeding value. This study aimed to identify genomic regions associated with flight speed and agitation. We used imputed whole-genome sequences from animals with records for flight speed (n = 8,737) and agitation (n = 8,586). The heritability for agitation was 0.18 ± 0.03 and 0.14 ± 0.02 for flight speed respectively. Three and five QTL regions were associated with agitation (on Chr3, Chr4, and Chr20) and flight speed (on Chr 1, Chr13, Chr15, and Chr26), respectively. The identification of these genomic regions provides further knowledge on the genetic mechanism involved in temperament traits providing alternative tools to improve sheep breeding programs. Further analysis is needed to find links between agitation and flight speed with production traits.

INTRODUCTION

Animal temperament has been proposed as a potential indicator of the physical, physiological, and psychological state of the animal in production systems which also defines welfare. Temperament can be assessed through behavioural traits such as agitation and flight speed, with both traits having shown a low to moderate heritability in sheep (Dodd *et al*. 2014). However, a better understanding of the whole genome associated regions and underlying genes involved could help to understand behavioural traits. Previously, a study on single nucleotide polymorphism (SNPs) on only four genes (*SLC6A4*, *TPH2*, *OXTR,* and *HTR2A*) identified SNPs on *TPH2* and *HTR2A* associated with behaviour in sheep (Ding *et al*. 2020), suggesting that a genome-wide association study (GWAS) can provide further information to better understand the involved biological process. This study aimed to identify the genetic regions and candidate genes associated with temperament traits such as flight speed and agitation in sheep.

MATERIALS AND METHODS

Animals and phenotypes. In total, 8,771 genotyped animals were used from the Information Nucleus Flock with records for flight speed (N = 8,737) and agitation (N = 8,586) obtained between 2008 and 2010. Lambs were produced by artificial insemination across eight farms within Australia and there were pure Merino or Merino crosses. A comprehensive description of the breeds is provided in van der Werf *et al*. (2010). A complete description of the recorded traits is provided in Dodd *et al*. (2014). In summary, studied traits were measured at post-weaning age. Lambs were subjected to an isolation test to record agitation by measuring with an agitation meter the number of vibrations caused by movement within the isolation box over a 30 second period. The flight speed corresponded to the speed at which the lamb crosses a specific distance.

Genotypes. Low-density genotypes (50k) were imputed to high-density and finally to sequence level to keep ~31 million SNPs after quality control to remove SNPs with minor allele frequency less than 0.01, deviation from Hardy-Weinberg equilibrium ($P < 10^{-10}$), and missing genotypes >

5%. A detailed description of the imputation is provided in Bolormaa *et al*. (2019).

Statistical analysis. Each trait was normalized using square root and $\log_{10}$ for agitation and flight speed, respectively. Genetic parameters and genetic correlations for agitation and flight speed were estimated in ASReml v4 (Gilmour *et al*. 2015) using the pedigree in an animal model and bivariate model, respectively. The model fitted fixed effects as age, birth type (BT), month, flock (N = 8), year (N = 3), sex, management group (MG), interactions, and an error term (e). The animal id and breed proportion (GG) were fit as random for flight speed (model 1) and agitation (in addition to dam; model 2). Based on the animal model, phenotypes we adjusted for mentioned fixed and random effects.

$$y = \mu + BT + month + age + flock + year + GG + MG*year*flock + e \quad (1)$$

$$y = \mu + BT + month + age + flock + year + sex + MG + dam + GG\ flock*year + e \quad (2)$$

The adjusted phenotypes and imputed sequences were used to perform a GWAS in GEMMA (Zhou *et al*. 2012) software with the model $\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Z\alpha} + \boldsymbol{e}$, where $\boldsymbol{y}$ is a vector of phenotype, $\boldsymbol{X}$ is the incidence matrix for the fixed effects, $\boldsymbol{\beta}$ is the vector of fixed effects (SNPs), $\boldsymbol{Z}$ is the incidence matrixes to relate random additive genetic effects with the phenotypes, $\boldsymbol{\alpha}$ correspond to the vector of direct additive genetic effects effect with $\boldsymbol{\alpha} \sim N(0, \boldsymbol{G}\sigma_\alpha^2)$ where $\boldsymbol{G}$ is a genomic relationship matrix and σ_α^2 is the additive genetic variance; and e is a vector of residual effects. A normal distribution was assumed for the additive genetic effects. QTLs were identified based on a false discovery rate < 0.1, which corresponded to a threshold of $-\log_{10}(9\text{x}10^{-08}) \geq 7$, and a 1 Mb window from the significant SNPs.

The percentage of genetic variance captured by the top significant SNPs was calculated as $2p_iq_i\alpha_i^2/\sigma^2 * 100$, where σ^2 is the additive genetic variance, p and q are the allele frequency for the SNP, and α_i^2 s the additive effect of the SNP. An additional threshold of $-\log_{10}(1\text{x}10^{-05}) \geq 5$ was used to identify the candidate genes around significant SNPs in a window of 1 Mb. The candidate genes were used in a pathway and gene ontology (GO) analysis performed with ClueGo v2.5.6 (Bindea *et al*. 2009) plugin. The function of candidate genes was further investigated in the literature.

RESULTS AND DISCUSSION

Moderate to low heritabilities were observed (Table 1) for agitation (0.18 ± 0.03) and flight speed (0.14 ± 0.02). Similar heritabilities were previously reported in an overlapping population of sheep for agitation (h^2 = ~0.20; Lennon *et al*. 2009; Dodd *et al*. 2014), and flight speed (h^2 = 0.11; Dodd *et al*. 2014) and cattle (for flight speed h^2 = 0.21; Valente *et al*. 2016). A positive genetic correlation (r_g) of 0.41 was detected between the studied traits, but this was higher than previously reported by Dodd *et al*. 2014 (r_g = 0.20).

Table 1. Heritability and genetic variance for temperament traits in sheep

Trait	h^2 ± SE	V_g	V_p
Agitation	0.18 ± 0.03	0.14 ± 0.02	0.77 ± 0.02
Flight speed	0.14 ± 0.02	0.11 ± 0.02	0.81 ± 0.03

h^2: heritability; V_g: genetic variance; V_p: phenotypic variance; SE: standard error

From the GWAS results, there were three QTLs regions (Figure 1A) identified for agitation that account for 9 % of the total genetic variation on the chromosomes Chr3, Chr4, and Chr20 (Table 2). Within these regions, 15 unannotated and 22 annotated genes were identified from which the top 15 genes are *MED27*, *RAPGEF1*, *UCK1*, *POMT1*, *PRRC2B*, *PPAPDC3*, *FAM78A*, *NUP214*, *AIF1L*, *CDK14*, *FZD1*, *MTERF1*, *AKAP9*, *EDN1*, and *HIVEP1*. For flight speed, five QTLs (Figure 1B) were detected on Chr1 (with two QTLs), Chr13, Chr15, and Chr26 accounting for 16 % of the genetic variance (Table 2). These regions contained 35 annotated genes and 26 unannotated genes,

being *CD47*, *IFT57*, *HHLA2*, *MYH15*, *KIAA1524*, *DZIP3*, *GUCA1C*, *MORC1*, *ELP4*, *PAX6*, *FCRLA*, *FCRLB*, *DUSP12*, *ATF6*, and *CENPA* the top 15 genes in a window of within 1MB from the most significant SNPs.

Most of the candidate genes were previously reported mainly in human studies to schizophrenia (*FCRLA*, *UHMK1*, *RGS4*, *RGS5*, and *DCDC5*; Campbell *et al*. 2008; Stefanis *et al*. 2008), depression (*DUSP12*, *OLFML2B*, *ZFP64*, and *DCDC1*; Wray *et al*. 2012), Alzheimer's disease (*ATF6*, *HSD17B7*, and *DZIP3*; Montibeller *et al*. 2018, Xu *et al*. 2018) and other mental conditions (i.e. stress, bipolar, and anxiety disorders). For agitation candidate genes, fewer studies previously reported their function but similarly, some genes were associated with schizophrenia (*RAPGEF1*, *FZD1*, and *AKAP9*, Igolkina *et al*. 2018, Lui *et al*. 2020) or Alzheimer (*NEDD9*; Li *et al*. 2008).

Table 2. Significant SNPs associated with agitation and flight speed

Traits	Chr	Mb	AF	beta	p-value	% Vg
Flight speed	1	171	0.38	-0.09	5.92E-09	3.56
	1	11	0.02	-0.31	8.12E-09	3.14
	13	79	0.14	-0.11	6.03E-08	2.71
	15	60	0.15	-0.12	1.17E-08	3.40
	26	37	0.03	-0.23	5.34E-08	2.80
Agitation	3	5	0.02	0.25	6.77E-08	2.06
	4	8	0.20	-0.10	1.45E-08	2.32
	4	8	0.14	-0.12	1.13E-08	2.32
	20	43	0.34	0.084	8.64E-08	2.23

Chr: chromosome; Mb: megabase pair; AF; allele frequency; $\%V_g$: percentage of genetic variance.

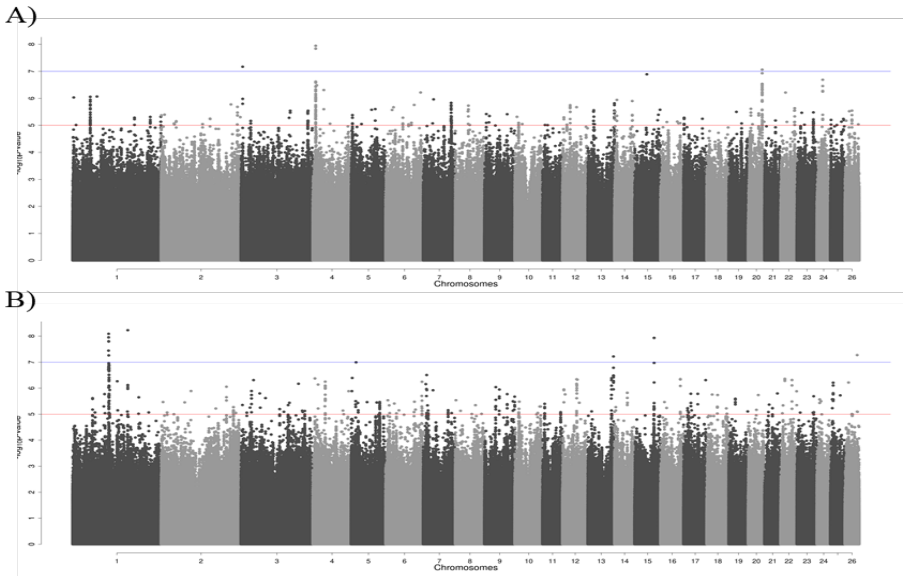

Figure 1. Manhattan plot of GWAS for (a) agitation and (b) flight speed indicating with lines the FDR < 0.1 threshold (blue) and the suggestive threshold (red)

The candidate genes located within 1 Mb of significant SNPs ($-\log_{10}(1x10^{-05})\geq5$) were identified for agitation (582 genes) and flight speed (907 genes) where 53 genes overlapped for both traits. The three most represented gene ontology terms for flight speed were found to be intracellular, regulation of cellular process, and cytoplasmic part; while for agitation the signal transduction, regulation of signaling, and regulation of response to stimulus were the most enriched.

CONCLUSIONS

Genomic regions associated with flight speed and agitation were identified in this study. The significant SNPs in these regions are close to genes previously associated in multiple studies in humans with schizophrenia disorders, depression, and Alzheimer's disease. Further knowledge on the genetic mechanism of behaviour and other important complex diseases can be provided from GWAS on non-model organisms such as sheep. From a production perspective in sheep, the genetic relationships between temperament and carcass traits are required together with economic values to assess the relevance of including these behavioural traits in selection programs, but a consistent recording of these phenotypes is needed to ensure the advantages in genomic selection.

ACKNOWLEDGEMENTS

The authors acknowledge MLA for providing access to the datasets used in the present study.

REFERENCES

Bindea G., Mlecnik B., Hackl H., Charoentong P., Tosolini M., Kirilovsky A., and Galon J. (2009) *Bioinformatics* **25**: 1091.

Bolormaa S., Chamberlain A.J., Khansefid M., Stothard P., Swan A.A., Mason B., and MacLeod I.M. (2019) *Genet. Sel. Evol.* **51**: 1.

Campbell D.B., Lange L.A., Skelly T., Lieberman J., Levitt P., and Sullivan P.F. (2008) *Schizophr. Res.***101**: 67.

Ding L., Maloney S., Wang M., Rodger J., Chen L., and Blache D. (2020) *Brain and Behav*, e12714.

Dodd C.L., Edwards J.H., Hazel S.J., and Pitchford W.S. (2014). *Livest. Sci.* **160**: 12.

Gilmour A.R., Gogel B.J., Cullis B.R., Welham S., and Thompson R. (2015) *Hemel Hempstead: VSN international ltd*.

Igolkina A.A., Armoskus C., Newman J.R., Evgrafov O.V., McIntyre L.M., Nuzhdin S.V., and Samsonova, M.G. (2018) *Front. Mol. Neurosci.* **11**: 192.

Lennon, K. L., Hebart, M. L., Brien, F. D., & Hynd, P. I. (2009) *Proc. Assoc. Advmt. Anim. Breed. Genet.* **18**: 96.

Li Y., Grupe A., Rowland C., Holmans P., Segurado R., Abraham R., and Williams J. (2008) *Hum. Mol. Genet.* **17**: 759.

Liu X., Low S.K., Atkins J.R., Wu J.Q., Reay W.R., Cairns H.M., and Cairns M.J. (2020) *Aust. N. Z. J. Psychiatry* **54**: 902.

Montibeller L., and De Belleroche J. (2018) *Cell Stress Chaperones*: **23**: 897.

Stefanis N.C., Trikalinos T.A., Avramopoulos D., Smyrnis N., Evdokimidis I., Ntzani E.E., and Stefanis C.N. (2008) *Behav. Brain Funct.* **4**: 1.

Wray N.R., Pergadia M.L., Blackwood D.H. R., Penninx B.W.J. H., Gordon S.D., Nyholt D.R., and Sullivan P.F. (2012) *Mol Psychiatry* **17**: 36.

Xu M., Zhang D.F., Luo R., Wu Y., Zhou H., Kong L.L., and Yao Y.G. (2018) *Alzheimer's & Dementia*: **14**: 215.

Zhou X., and Stephens M. (2012) *Nat. Genet.* **44**: 821.