Proc. Assoc. Advmt. Anim. Breed. Genet. 19:247-250

THE EFFECT OF TELOMERE LENGTH VARIATION ON LIFETIME PRODUCTIVITY TRAITS IN SHEEP

G.S. Nattrass[1], R.G. Banks[2] and W.S. Pitchford[3]

[1]SARDI – Livestock and Farming Systems, Roseworthy, SA 5371
[2]Meat and Livestock Australia, C/- University of New England, Armidale, NSW 2351
[3]School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA 5371

SUMMARY

Telomere DNA length exhibits an age-related decline in humans and it is emerging as a potential biomarker for longevity and fitness. As telomere DNA length in humans is a heritable trait, we assessed whether variation in telomere DNA length in sheep correlated with Australian Sheep Breeding Values (ASBVs) for a range of production traits. The genetic relationship between telomere length and ASBVs was generally low, with the highest associations observed for birth weight (0.14), fatness (-0.14; CFAT) and two wool quality traits; staple strength (-0.1) and coefficient of variation in fibre diameter (0.15).

INTRODUCTION

Telomeres are repetitive segments of DNA which form protective caps on the ends of chromosomes. Telomeres are highly conserved between eukaryotic species and consist of specialised DNA structures composed of many thousands of copies of the same tandem repeat sequence (TTAGGG). Mammalian chromosomes shorten by a small amount after each mitotic cycle. This shortening is associated with a loss of telomere DNA from the terminal ends of chromosomes. The telomeres protect the ends of chromosomes from irreversible DNA damage as cells divide and replicate. An age-related decline in telomere length is evident in humans, especially early in life, and between middle age and old age (Aubert and Lansdorp 2008). The inverse relationship between telomere length and human chronological age has been proposed as an indicator of biological aging, which could be a useful predictor of general health and mortality. This notion is supported by the strong biological connection between shortened telomeres and cellular replicative senescence (Hemann *et al*. 2001) and loss-of-function mutations in telomere maintenance genes that cause inherited premature aging disorders (Armanios *et al*. 2005).

In humans, a link between telomere length and mortality has already been established and evidence suggests that telomere length contributes to the age-related decline in physical function and fitness. Therefore, the objective of this project was to investigate whether a genetic relationship exists between telomere length and Australian Sheep Breeding Values (ASBVs), specifically those associated with traits measuring lifetime productivity. The telomere length of 120 ewes, ranging in age from 1-7 years, was measured with quantitative PCR (qPCR), to ascertain whether telomere length is related to age and could be used as a biomarker for predicting genetic merit for performance traits in sheep.

MATERIALS AND METHODS

Blood sampling and genomic DNA extraction. Blood samples were collected from 120 ewes aged 1-7 years at Oaklea Genetics, Mount Gambier, S.A. The ewes sampled were as divergent as possible in their index values and were sired by 52 different rams which were balanced and dispersed across the 1 – 7 year age range. A blood sample from each animal was collected into K_3EDTA Vacuette tubes (Greiner, Germany) and spotted on FTA Elute Microcards (Whatman, USA). Three genomic DNA extraction methods were employed on the sheep blood samples.

Genomic DNA was extracted from whole blood using the Ultraclean DNA Blood Isolation Kit (MoBio, USA) and the DNeasy Blood and Tissue Kit (Qiagen, Germany). Genomic DNA isolated with the Qiagen kit underwent an additional ethanol precipitation step to remove PCR inhibitors. A hole punch was used to obtain four 3mm diameter sections from dried blood spots on FTA blood cards. Genomic DNA was recovered from the FTA card punches with the Gensolve Whole Blood DNA Recovery kit (Genvault, USA) and then purified using the modified method of McClure et al. 2009.

Real-time PCR measurements on genomic DNA extracted from whole blood and FTA cards. Quantitative PCR (qPCR) was performed on the 3 batches of sheep genomic DNA (n = 120), referred to herewith as Qiagen gDNA, MoBio gDNA and FTA gDNA. Triplicate qPCR measurements were performed on each sample using a telomere-specific assay (Cawthon 2009) and an assay targeting myostatin (GDF8; Table 1). The myostatin assay was used to normalise the telomere data. qPCR was performed with the PowerSYBR reagent (Applied Biosystems, USA) on a 384 well real-time PCR machine (7900; Applied Biosystems, USA). The MoBio gDNA and FTA gDNA were diluted 1:20 in 10mM Tris-HCl (pH 8.0) prior to qPCR, while the Qiagen gDNA was used undiluted. Each 384 well plate contained a standard curve consisting of a 4-fold serial dilution of pooled gDNA (1:4, 1:16, 1:64 and 1:256). The standard curve was used to calculate the PCR efficiency of each real-time PCR assay and provide the data for the relative quantification.

Table 1. Oligonucleotides used for quantitative PCR measurements on sheep genomic DNA

Gene	Assay name	Primer sequences (5' - 3')
Telomere	TelGC	Forward: ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT Reverse: TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA
GDF8	oMST_In2	Forward: TGGAGTTCGTCTTTCCAACC Reverse: GGAAGGCAGAGTGATGAAGG

Data normalisation and statistical analysis. The relative quantification strategy used in this study to measure telomere length involved the determination in each sample of the amount of telomere DNA (T) and the amount of a single copy reference gene (S). The myostatin gene, which is present in the ovine genome in a single copy, was used as the reference gene to normalise the telomere data between samples within each batch of genomic DNA. For each sample, the telomere repeat copy number and the myostatin gene copy number were measured with qPCR, and in order to adjust all the samples back to the same quantity of genomic DNA, T was normalised to S by determining the (T/S) ratio for each sample. The factor by which the T/S ratio of the samples differs from a reference DNA sample was used to determine relative telomere lengths (Cawthon 2009). The T/S ratio was used to examine the relationship between telomere length and age.

The relationship between telomere length and ASBVs was examined using ASREML (Gilmour et al. 2006). Two mixed models were fit to the telomere data. Model 1 contained fixed effects of the covariate (myostatin), main effect of replicate (6 levels which were a function of sample (whole blood or FTA card), kit (MoBio, Qiagen, FTA), day (30th October or 13th November) and replicate on a given day (2 for MoBio on 30/10 and 2 for Qiagen on 13/11)), the interaction between replicate and the covariate, then random effects of replicate. A variance structure was then placed on the random effect so that 6 separate variances were estimated for each replicate and all covariances were also estimated, resulting in a correlation matrix.

RESULTS AND DISCUSSION

Relationships between measures of telomere length and ASBVs. Telomere length was measured on all 3 preparations of genomic DNA (MoBio, Qiagen and FTA). The level of PCR

inhibitors contained in the different DNA preparations had a profound effect on the telomere data. Additional purification steps were employed to reduce the amount of PCR inhibitors in the Qiagen and FTA DNA preparations. However, this introduced significant technical variation that resulted in poor correlations between the 3 DNA extraction kits (Table 2). Since the MoBio kit produced the highest quality genomic DNA, requiring no additional purification, statistical analyses of telomere length variation were focussed on this data. The correlations between telomere length and ASBVs were generally quite weak. The highest genetic associations were observed for birth weight (0.14), fatness (-0.14; CFAT) and two wool quality traits; staple strength (-0.1) and coefficient of variation in fibre diameter (0.15).

Table 2. Variances* (on diagonal) and correlations (above diagonal) between methods *(Co)variances in units of $\log_n$(telomere copy number)^2

Sample	Kit	Day	Rep	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
Blood	MoBio	30/10	1	0.13	0.97	0.82	0.26	0.30	0.01
Blood	MoBio	30/10	2		0.09	0.82	0.21	0.28	0.05
Blood	MoBio	13/11	3			0.11	0.31	0.37	0.05
Blood	Qiagen	13/11	4				0.11	0.81	0.17
Blood	Qiagen	13/11	5					0.22	0.22
Card	FTA	13/11	6						0.60

Telomere length in sheep did not exhibit an age-related decline. A decline in telomere length with increasing age was not detected. Irrespective of animal age, the variation in telomere length between animals within a particular age group appeared similar. With specific reference to the MoBio data, telomere length within each age group varied about the mean by 30-50% (Figure 1). Given that sheep telomeres are estimated to be around 20kb (Alexander *et al*. 2007), telomere lengths in this study are quite heterogeneous at any given age which is similar with telomere length data from humans.

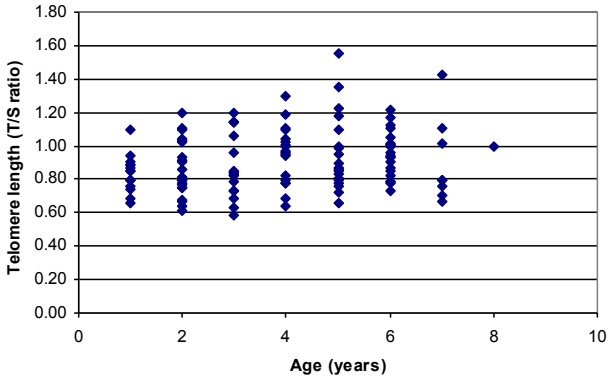

Figure 1. Relative telomere length plotted against age. The T/S ratio of a reference sample, an 8 year old ewe, was set as 1.00 and all other samples were expressed relative to this value.

CONCLUSIONS

This study was undertaken to investigate the feasibility of developing a diagnostic test that assessed telomere length in sheep at a relatively young age for the purpose of accurately predicting

lifetime productivity. In this preliminary study, little evidence was found to support the hypothesis for a genetic relationship between telomere length and lifetime productivity traits, making it unlikely that a diagnostic test measuring telomere length will aid in the prediction of lifetime productivity traits in sheep.

An age-related decline in telomere length was not detected in this study using an even distribution of sheep aged between 1 and 7 years. In contrast, telomere length is known to shorten in ovine fibroblasts when they are cultured *in vitro* and telomere length attrition has been calculated at 1kb per year in the skin of Dorset cross sheep aged 1 month to 36 months of age (Alexander *et al*. 2007). A closer inspection of the telomere length data reported for sheep skin, indicates that the greatest decline in telomere length occurred between measurements made at 1, 6 and 12 months of age, whereas the telomere lengths between 1-3 years of age remained unchanged. Therefore, telomere length in the skin of sheep appears to decline in the first year of life and then remain constant for the next couple of years, or potentially longer as observed in blood leucocytes in this study. This conclusion is supported by findings of a longitudinal study conducted on baboons, where telomere length in blood leukocytes of 4 animals declined 2-3kb in the first year of life with negligible attrition observed over the next 3 years (Baerlocher *et al*. 2007). Even though 2 baboons had an average telomere length of ~25kb at birth and the other 2 were only ~15kb, the telomeres of all 4 animals declined by a similar amount in the first year of life.

A longitudinal study examining telomere length attrition in sheep tissues with high rates of cell turnover could be warranted. A relationship between telomere length and lifetime productivity traits may still be established if tissue types that are closely linked to phenotypic variation are examined at the right stage of postnatal growth. For example, the wool follicle is constantly turning over cells, so dramatic changes in telomere length early in life could have life-long consequences on the production of certain types of wool, especially if animals with relatively short telomeres at birth lose a significant portion of their telomeres in the first 12 months of life.

ACKNOWLEDGMENTS

We wish to thank Don Pegler from Oaklea Genetics, S.A. for his willingness to participate in this project, and for his efforts collecting the blood samples from his flock. We gratefully acknowledge Meat and Livestock Australia for providing the funding to undertake this research.

REFERENCES

Alexander B., Coppola G., Perrault S.D., Peura T.T., Betts D.H. and King W.A. (2007) *Mol. Reprod. Dev.* **74**: 1525.

Armanios M., Chen J.L., Chang Y.P., Brodsky R.A., Hawkins A., Griffin C.A., Eshleman J.R., Cohen A.R., Chakravarti A., Hamosh A. and Greider C.W. (2005) *Proc. Natl. Acad. Sci. U .S. A* **102**: 15960.

Aubert G. and Lansdorp P.M. (2008) *Physiol. Rev.* **88**: 557.

Baerlocher G.M., Rice K., Vulto I. and Lansdorp P.M. (2007) *Aging Cell* **6**: 121.

Cawthon R.M. (2009) *Nucleic Acids Res.* **37**: e21.

Gilmour A.R., Butler D., Cullis B.R. and Thompson R. (**2006**) ASREML user guide.

Hemann M.T., Strong M.A., Hao L.Y. and Greider C.W. (2001) *Cell* **107**: 67.

McClure M.C., McKay S.D., Schnabel R.D. and Taylor J.F. (2009) *BMC Res. Notes* **2**: 107.