Proc. Assoc. Advmt. Breed. Genet. 21: 53-56

FINE-MAPPING SINGLE NUCLEOTIDE POLYMORPHISMS ON *BOS TAURUS* CHROMOSOME 26 AFFECTING ADIPOSE MYRISTIC ACID

D. Lu[1], S.M. Hickey[2], N.G. Cullen[2] and S. Miller[1] Invermay
[1] Agricultural Centre, Mosgiel 9053, New Zealand
[2]Ruakura Research Centre, Hamilton 3240, New Zealand

SUMMARY

It may be desirable to change the genetic selection program of cattle to make them produce less myristic fatty acid (alternatively C14:0) in milk and adipose fat, providing food products to consumers with enhanced health attributes. As phenotypic measures on an industry-wide basis are not practical, a genomic test related to levels of C14:0 would provide a viable method to enhance animal selection programs. Therefore it is important to identify genome segments and nucleotides that control the trait. The study reported herein was able to locate a region of ~250Kb on chromosome 26 that accounted for 26.47% of the phenotypic variation in C14:0. Two of eight haplotypes of this region were found to reduce C14:0 significantly in subcutaneous fat of beef cattle.

INTRODUCTION

Dietary fatty acids, especially C14:0, have a major influence on lipoprotein concentrations in human plasma (Katan *et al*. 1994; Adamsson *et al*. 2014), which in turn affects cardio-vascular health (Mozaffarian *et al*. 2005). Those fatty acids that increase undesirable cholesterol in humans, are mainly derived from milk fat (Gunstone *et al.* 1994), and beef meat (Youssef *et al.* 2012). Therefore reduction of those fatty acids in milk and beef is desirable.

From a genetics perspective, the proportion of C14:0 to total lipid amount shows evidence of being under genetic control (Tait *et al.* 2008; Bouwman *et al.* 2011). Its heritability in beef adipose fat was reported to be 0.50 (Tait *et al.* 2008). This means it is possible to select cattle for the reduction of C14:0 in meat. Selecting animals via traditional progeny testing is time consuming due to a long generation interval and the complexity of measuring C14:0, would result in slow genetic gain. Current DNA technology can help facilitate the genetic improvement process *via* marker assisted selection or genomic selection. For this to happen, it is important to identify single nucleotide polymorphisms (SNP) that are associated with levels of C14:0.

Morris *et al.* (2007; 2010) identified quantitative trait loci (QTL) on *bos taurus* autosomes (bta) 15, 19, 26, 27 and 29 for C14:0 in adipose fat. However, the QTL regions covered long segments of chromosomes, for example, 18-29cM on bta 26 (Morris *et al.* 2010). Using higher density genetic markers would allow for fine mapping genomic regions in association with the trait.

The study reported here was aimed at refining the QTL regions reported by Morris *et al.* (2007; 2010) for C14:0 in a New Zealand experimental population of Jersey-Limousin backcrosses.

MATERIALS AND METHODS

Animals and Phenotype. Records of fatty acid profiles were available on 406 backcrossed Jersey (J) x Limousin (L), which were sired by three JxL bulls *via* artificial insemination with J and L dams. The animals were raised on pasture and slaughtered at 22-28 months of age. Subcutaneous fat from over the *longissimus dorsi* muscle was used to extract fatty acids and nine (C14:0, C14:1, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1 and C18:2) were measured, and presented as percentage of the total of the nine fatty acids. The mean for C14:0 was 3.41±0.48. More details of the animals and phenotype measurement are described by Morris *et al.* (2010).

Genotypes. Genotypes were available on only 160 heifers and 106 steers born in 1996-1997. These animals formed three half-sib families with 74, 94 and 98 progeny. The genotyping was

performed on DNA extracted from blood, ear tissues, and meat samples. A total number of 54,609 SNP were typed across the bovine genome by Delta Genomics Laboratory (Edmonton, Alberta, Canada), using the Illumina BovineSNP50 Beadchip (Illumina Inc., San Diego, USA). Animals with a call rate less than 90% were removed. Quality control filtered out SNP that had a minor allele frequency less than 1%, or a spurious location, or a GC score less than 0.15. Missing genotypes were imputed using FImpute v2.2 (Sargolzaei *et al.* 2014), which makes use of both family and population information. A subset of 39,988 SNP on 29 autosomes was used for subsequent analyses.

Linkage Disequilibrium. Pairwise linkage disequlibrium (LD) was estimated using $r^2 = \frac{D^2}{f(A).f(a).f(B).f(b)}$ (Hill and Roberson 1968), with $D = f(AB) - f(A).f(B)$, where $f(AB)$ is the estimated frequency of haplotype AB using the observed genotype frequency (McVean 2007) and assuming Hardy-Weinberg equilibrium, $f(A), f(B), f(a), f(b)$ being the observed frequencies of alleles *A*, *B*, *a*, *b*, respectively. The metric r^2 was computed using software Snppld v1.0 (Sargolzaei 2010).

Statistical analyses. Genome-wide association analysis was carried out to identify chromosome segments that potentially harbour SNP or haplotypes in strong association with C14:0. The trait was adjusted for fixed effects, including breed of dam (J or L), farm of birth (n=3), birth type (single or twin) within breed of dam, slaughter group (sex and year included), sire family, then fitted in the following model $y_{ij} = \mu + \beta X_{ij} + e_{ij}$, where μ is the overall mean, y_i the adjusted C14:0 for animal *i*, β the regression coefficient for genotype X {0,1,2} at locus *j*, e_{ij} the residual.

Haplotype phase. Haplotypes were reconstructed for only chromosome regions that had high LD between pairs of SNP and contained SNP highly associated with C14:0, using Beagle software v3.3.2 (Browning and Browning 2007). Haplotype effects were estimated from the statistical model mentioned above by replacing genotypes with haplotypes.

RESULTS AND DISCUSSION

Genome-wide association. Significance levels of SNP from the association analysis are presented in Figure 1. The two peaks were observed at *rs41921177* (19:51326750; FDR= 1.03E-6) and *rs110857021* (26:21832456; FDR = 1.03E-6). Within 500kb of *rs41921177* are four other SNP significant at FDR<5% and gene *FASN* (fatty acid synthase), which is reported to be associated with fatty acid composition in beef muscle (Zhang *et al.* 2008; Yokota *et al.* 2012), and milk fat (Roy *et al.* 2006). Two of the four other significant SNPs were within 50kb of gene *FASN*. Within 500kb of *rs110857021* are 14 other SNP significant at FDR<1% and gene *Stearoyl-CoA Desaturase*, which is reported to affect fatty acid composition of adipose tissue in beef cattle (Brooks *et al.* 2011; Yokota *et al.* 2012; Costa *et al.* 2013), and of milk fat (Rincon *et al.* 2012).

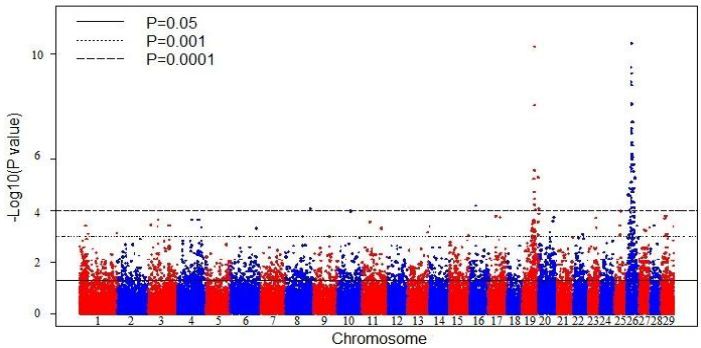

Figure 1. Significance levels of SNP from the association with myristic fatty acid.

Linkage disequilibrium was estimated for pairs of all SNP on bta 19 and 26. Figure 2 displays the LD heatmap for chromosome segments of approximately 500kb on each side of *rs41921177* and *rs110857021*. Single nucleotide polymorphism *rs41921177* on bta 19 did not appear to have strong LD with its surrounding SNPs, while SNP *rs110857021* on bta 26 was highly correlated ($r^2 \geq 0.6$) with five other SNPs immediately adjacent to it. This finding supports the number of SNPs found significantly associated with C14:0 in these two chromosomal regions. Regions around *rs41921177* might need denser SNPs to capture higher LD, enabling higher confidence in identifying nucleotides that are causative for differences in C14:0. The region of six consecutive SNPs (~250kb) on bta 26 was further investigated, using phased haplotypes.

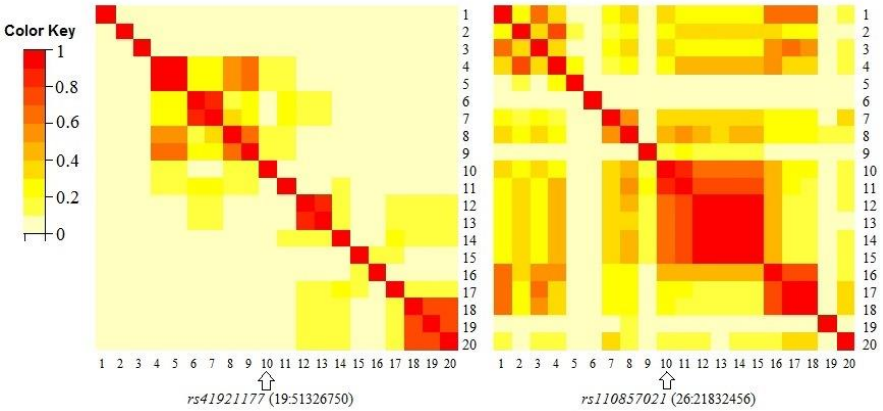

Figure 2. LD heatmap for segments of chromosomes 19 and 26.

Haplotype analysis. Eight haplotypes were found out of the six SNP region mentioned above. Their frequency is presented in Table 1. Four haplotypes with very low frequency (<1%) were grouped together and given code hap1. Five haplotypes (hap1-5) were used in a linear regression to estimate haplotype effect (Table 2).

Table 1. **Distribution of haplotypes on bta 26**

Haplotype (Code)	Sire1	Sire2	Sire3	Freq (%)
CACGGA (hap1)	1	0	4	0.94
CATAAC (hap2)	41	25	67	25.00
CCCAAC (hap1)	0	0	1	0.19
CCCGGA (hap3)	4	6	6	3.00
TACGGA (hap1)	0	0	1	0.19
TCCAGA (hap1)	0	0	1	0.19
TCCGGA (hap4)	96	151	105	66.17
TCTAAC (hap5)	6	6	11	4.32

Table 2. **Haplotype effect**

Hap	Effect	P value
hap4	0	N/A
hap1	-0.07 ± 0.27	> 0.05
hap2	-0.68 ± 0.09	< 0.001
hap3	-0.63 ± 0.22	< 0.005
hap5	-0.03 ± 0.19	> 0.05

In the analysis, effects of hap1, hap2, hap3, hap5 were contrasted against the effect of hap4. Hap4, as the most frequent haplotype among the studied animals, and a common haplotype among the three sires, appeared to be associated with the highest percentage of C14:0 in adipose fatty acids. Hap2 was the second most frequent haplotype, and appeared to be associated with a reduction in C14:0. Hap3 differed from hap4 only at locus *rs110857021*, where *T* in hap4 was replaced by *C* that subsequently caused a significant reduction in C14:0 (P<0.005). The ~250kb segment of bta 26 accounted for 26.47% of the phenotypic variation in C14:0.

CONCLUSION

Chromosome regions were refined, which were previously reported to be associated with C14:0. A very high LD segment of ~250kb on bta 26 was located accounting for 26.47% of the variation in C14:0 in the research animals. Two haplotypes (hap2 and hap3), which had a combined frequency of 28% and caused a reduction of myristic fatty acid, should be tested for association with other economically important traits, and tested in other cattle breeds or populations.

ACKNOWLEDGEMENTS

The NZ-half of the trial run in conjunction with the University of Adelaide was funded by New Zealand Foundation for Research, Science and Technology, and by J. S. Davies Bequest to the University of Adelaide. Dr C. A. Morris (retired) was the project leader in NZ.

Beef & Lamb NZ Genetics funded the genotyping work and the analysis presented in this study.

REFERENCES

Adamsson, V., Cederholm, T., Vessby, B. and Riserus, U. (2014) *Food & Nutrition Res.* **58**:24114.

Bouwman, A.C., Bovenhuis, H., Visker, M.H.P.W. and Van Arendonk, J.A.M. (2011) *BMC Genetics* **12**:43.

Brooks, M.A., Choi, C.W., Lunt, D.K., Kawachi, H. and Smith, S.B. (2011) *J. Anim. Sci.* **89**(8):2556-2570.

Browning, S.R. and Browning, B.L. (2007) *Am. J. Hum. Genet.* **81**:1084-1097.

Costa, A.S., Silva, M.P., Alfaia, C.P., Pires, V.M., Fontes, C.M., Bessa, R.J. and Prates, J.A. (2013) *Lipids* **48**(4):369-381.

Gunstone, F.D., Harwood, J.L. and Padley, F.B. (1994) 'The Lipid handbook' Chapman & Hall, London.

Hill,W.G.,and Roberson,A. (1968) *Theor.Appl.Genet.* **38**:226–231.

Katan, M.B., Zock, P.L. and Mensink, R.P. (1994) *Am. J. Clin. Nutr.* **60**(suppl):1017-1022.

McVean, G. (2007) 'Handbookofstatistical genetics ' 3rd ed. John Wiley and Sons, New York.

Morris, C.A., Bottema, C.D., Cullen, N.G., Hickey, S.M., Esmailizadeh, A.K., Siebert, B.D. and Pitchford, W.S. (2010) *Anim. Genet.* **41**:589-596.

Morris, C.A., Cullen, N.G., Glass, B.C., Hyndman, D.L., Manley, T.R., Hickey, S.M., McEwan, J.C., Pitchford, W.S., Botteman, C.D.K. and Lee, M.A.H. (2007) *Mammalian Genome* **18**:64-74.

Mozaffarian, D., Ascherio, A., Hu, F.B., Stampfer, M.J., Willett, W.C., Siscovick, D.S. and Rimm, E.B. (2005) *Circulation* **111**(2): 157-164.

Rincon, G., Islas-Trejo, A., Castillo, A.R., Bauman, D.E., German, B.J. and Medrano, J.F. (2012) *J. Dairy Res.* **79**(1):66-75.

Roy, R., Ordovas, L., Zaragoza, P., Romero, A., Moreno, C., Altarriba, J. and Rodellar, C. (2006) *Anim. Genet.* **37**(3):215-218.

Sargolzaei, M. (2010) Snppld v1.0, University of Guelph, Ontario, Canada.

Sargolzaei, M., Chesnais, J.P. and Schenkel, F.S. (2014) *BMC Genomics* **15**:478.

Tait, R.G., Zhang, S., Knight, T., Strohbehn, D. and Beitz, D.C. (2008) *Anim. Industry Report* AS 654, ASL R2285.

Yokota, S., Sugita, H., Ardiyanti, A., Shoji, N., Nakajima, H., Hosono, M., Otomo, Y., Suda, Y., Katoh, K. and Suzuki, K. (2012) *Anim. Genet.* **43**(6):790-792.

Youssef, M.K.E, Mostafa, B.M.D., Seliem, M.A.A. and Hashem, A.M.A. (2012) *Food and Public Health* **2**(2):34-39.

Zhang, S., Knight, T.J., Reecy, J.M. and Beitz, D.C. (2008) *Anim. Genet.* **39**(1):62-70.