The TG5 DNA marker test for marbling capacity in Australian feedlot cattle

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Abstract. We tested the TG5 polymorphism in the 5' leader sequence of the thyroglobulin regulatory DNA sequence on 1750 cattle drawn at random from a large feedlot over approximately 9 months in 28 separate days of sampling. The cattle were mainly Angus or Shorthorn grain-fed for 160-240 days. We have confirmed the earlier reported association between the '3' allele and higher marbling scores indicating that a DNA test based on the polymorphism should be robust for predicting marbling potential. This test detects one of the genes that contributes to the genetic potential for marbling.

Introduction

Marbling potential in beef cattle is difficult to predict so the genetic selection for this trait is not simple.

Firstly, the degree of flecking of the fat is scored into categories by eye, so there is always a statistical disconnect between intra-muscular fat percent and marbling score primarily due to comparing a continuous measure to a categoric measure.

Secondly, marbling scores are obtained after slaughter in chiller rooms so there is a long period between the use of a bull and the recording of the performance of its offspring. This is coupled to the long time it takes for high marbling scores to develop, so tests on juvenile animals are unlikely to yield much differentiation between individuals.

Finally, sires do not express high levels of marbling. These are not insuperable obstacles, however they make the selection for marbling an expensive and time consuming activity. The hope is that DNA markers can be used that will aid in the selection for marbling by addressing the genetic material directly.

Several DNA markers, representing 3 cattle genomic regions (and hence at least 3 genes) were described as associated with marbling (Barendse 1997). Like most traits of an organism, marbling score is the result of an interaction between genes and the environment, so many genes are expected to show associations to marbling, with a range of impacts on the trait. There will be several genes with useful effects on marbling potential but no canonical "marbling gene" superior to all others.

We are interested in genes that contribute to the normal variation for marbling and that are not associated with large pleiotropic effects on other traits. One of these DNA markers is the TG5 polymorphism which occurs in the 5' leader sequence of the Thyroglobulin gene. It shows a genotypic association to realised marbling score in long fed cattle in which animals that possess the '3' allele, ie., both '23' and '33' genotypes have higher marbling scores on average than those that have the '22' genotype (Barendse 1997). The '3' allele is defined as being delta T (-537bp; GATT) while the '2' allele is defined as being delta C (-537bp; GATC), the delta locations defined in terms of the beginning of the start of the first exon.

In this study we examine the genotypes at the TG5 polymorphism in cattle obtained from a single feedlot in which the animals were fed to standard commercial endpoints. The objectives of this were: 1) to confirm that the '3' allele is consistently associated with higher marbling score, 2) to determine whether the DNA marker could be used as a feedlot screening tool and 3) to determine whether these effects can still be seen in feeding regimes used in standard commercial practise.

Materials and Methods

Cattle were sampled from the Australia Meat Holdings (AMH) Toowoomba feedlot using the following schema.

To determine whether the TG5 polymorphism would be applicable in real world conditions we tried to maximise the diversity of cattle within breeds. In feedlots it is generally not possible to obtain parentage of cattle while in the cattle industry at large there is a relatively small pool of sires that are used compared to the total number of cattle that are available. To prevent the study from being dominated by a few sires, resulting in the influence of some sires altering the effects that are to be measured, we chose to maximise the number of vendors while sampling. We obtained the breed of the cattle, the vendor, the date of entry, the time spent in the feedlot, the initial, final and hot dressed weights as well as the chiller

assessments for P8 fat, eye muscle area and AUSMEAT marbling score for each animal. Animals were mainly of the Angus and Shorthorn breed. Since slaughter groups are not tidy a small number of steers of other breeds were included.

DNA was extracted from the blood samples using the following method. A ten ml sample of blood (EDTA as anti-coagulant, Becton Dickonson vacutainers) was lysed in 40 mls of chilled buffered water (79 mg Sodium Bicarbonate, 7.6 g Ammonium Chloride per litre) for at least 15 minutes.

White cells were collected by centrifugation and the pellet was completely and vigorously resuspended in 4.5 mls of 1 x Phosphate Buffered Saline. Then 0.25 mls of 0.5 M di-Sodium Ethylene Diamine Tetra-acetic Acid (EDTA) (pH 8.0), 0.25 mls of 10% Sodium Dodecyl Sulphate (SDS) and 50ml of Proteinase K (Boehringer; 10 mg/ml in water) were added, the tubes rolled gently to mix and the samples incubated overnight at 37 degrees Celsius.

Following this, 2.4 mls of a 5 M Sodium Chloride/0.2% 2-Mercaptoethanol solution was added, the solution mixed on a roller for 5 minutes and then 5 mls of Analytical Grade Chloroform was added.

The samples were mixed gently for at least 60 minutes after which the organic phase was separated from the aqueous phase by centrifugation. The aqueous phase (top) was removed with a large-bore glass pipette and the DNA precipitated with an equal volume of chilled iso-propanol. The spooled DNA was fished out on a sealed glass pipette and transfered to 70% Ethanol to de-salt overnight. The spool of DNA was then transfered to 1 x TE (10 mM Tris, 1 mM EDTA, pH 8.0). This is a modified procedure of Barendse *et al.* (1993) and of Mullenbach *et al.* (1989). Genotypes at the TG5 polymorphism were sampled using Single Strand Conformational Polymorphism (SSCP) methods as described previously (Barendse *et al.* 1993; Barendse 1997).

In essence, the DNA fragment was amplified using the primers TG5U2 5'ggg gat gac tac gag tat gac tg3' and TG5D1 5'gtg aaa atc ttg tgg agg ctg ta3' in a polymerase chain reaction (PCR) using Taq I polymerase in the manufacturers buffer (Biotech International) with an annealing temperature of 55 degrees Celsius.

The fragments were radioactively labelled in the reaction using 32P dCTP, separated on 8% acrylamide gels at 3 W overnight and detected using autoradiography. The polymorphism involving the '2' and '3' alleles can also be detected as a restriction fragment length polymorphism (RFLP). Instead of radiolabelling, the DNA is amplified as before without 32P dCTP, and after the reaction is completed, 5 u of MboI (New England Biolabs) was added and the sample incubated at 37 degrees Celsius for 1 hour. Isoschizomers of MboI do not work as efficiently and require purification of the DNA before restriction (data not shown). The fragments are separated on 4% agarose gels (3% wide range agarose and 1% standard agarose; Sigma and Promega) with 0.5 x Tris Borate EDTA pH >8.3 (TEB) gel and electrophoresis buffer with Ethidium Bromide incorporated in the gel and buffer. Fragments were detected under UV transillumination and recorded on Polaroid Instant Film.

The genotypic results were compared to the marbling scores using generalised linear models (GLM) based on the Poisson distribution implemented using S-PLUS (McCullagh and Nelder 1989; S-PLUS handbook; Venables and Ripley 2000). The Poisson distribution was used since marbling score is a categoric trait and as such, contingency tables are generated of numbers of individuals at each marbling score.

Factors in the feedlot were tested to determine whether they had an influence on the marbling score. Those that did were incorporated into the model and the genotypes were treated as fixed effects. The genotypes at TG5 were also compared to measures of feedlot and chiller performance using GLM appropriate to the distribution of the traits.

Results

DNA was obtained for 1750 cattle, 853 were Angus, 773 were Shorthorn, 106 were of 9 breeds and 18 were unknown (Table 1). There were 411 vendors represented with a median of 2 steers per vendor with quartiles of 1 and 5 steers per vendor. Sampling was halted when it became clear that the median number of steers per vendor was likely to increase. All available factors were tested for impacts on marbling. However, after the day of slaughter and the breed of origin were fitted, no other factor was statistically significant (Table 2). The number of days in the feedlot is shown plotted against the realised marbling score for each animal and it is clear that time in the feedlot has little influence on the marbling score (Figure 1). The genotype for TG5 is statistically significantly associated with marbling score (Table 3). The effect of genotype on marbling is shown as the interaction term

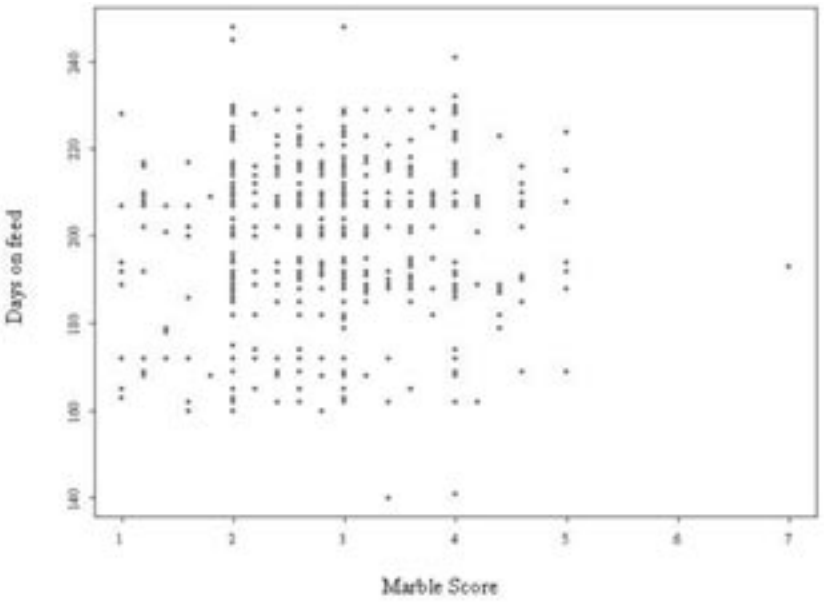

Figure 1. Marble score in cattle in relation to days on feed.

Table 1. Characteristics of the cattle sample

Steers	1750
Breeds	Angus, Shorthorn, Mixed
Angus	853 steers
Shorthorn	773 steers
Mixed	106 steers
Unknown	18 steers
Mixed	Murray Grey
	Crossbred Angus or Shorthorn
	Charolais cross
	Limousin cross
	Red Angus
	Shaver Cross
	Composite
	Simmental cross
	Santa Gertrudis cross
Vendors	411
	Median: 2 steers per vendor
	Bottom quartile: 1 steer per vendor
	Top quartile: 5 steers per vendor
Days on which cattle were sampled	28
	Median: 60 steers
	Mode: 49 steers (5 days)
	Mean: 61.9 steers
	Minimum: 30 steers
	Maximum: 85 steers
	9 days with more than one breed

marbling:genotype and is added last in the analysis. The distribution of genotypes vs raw marbling scores shows that '33' genotype shows on average higher marbling scores than either the '23' or '33' genotype (Figure 2). There is a clear increase in gene frequency from one marbling extreme to another (Table 4). The TG5 genotypes show a small effect on feedlot liveweight gain where gain is measured as the difference between the final and initial weight normalised by the number of days spent in the feedlot (Table 5). The least squared means show that the '33' genotypes showed higher rates of gain than the '22' or '23' genotypes (Table 6). These TG5 genotypes were not found associated with any other of the measured traits.

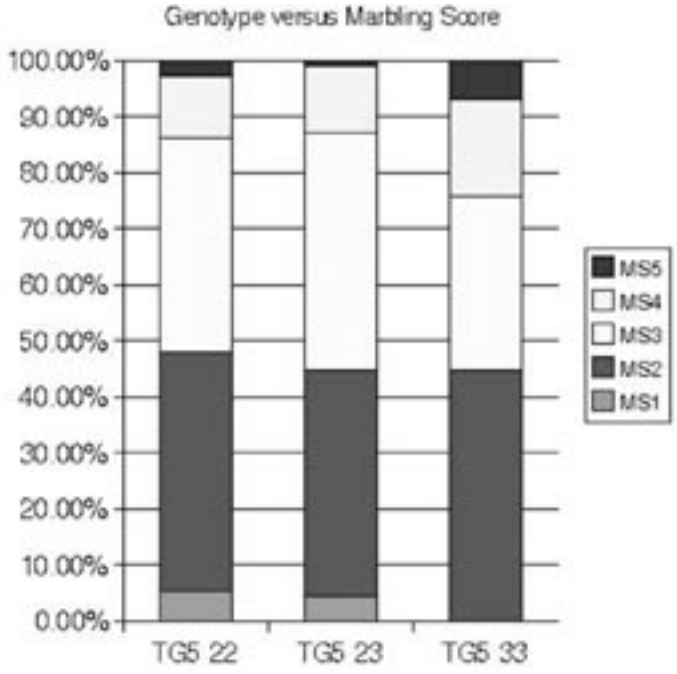

Figure 2. Effect of TG5 genotype on marble scores.

Discussion

The TG5 genotypes are consistently associated with marbling score. Not only are genotypes associated with marbling score as previously reported (Barendse 1997) but in this study we find the third example where the

Table 2. The effect of various feedlot measures on marbling score. Marbling score here is scored to 0.2 of a marbling score. (Response: marfine) (Terms added sequentially (first to last).

	Df	Deviance Resid.	Df	Resid. Dev	Pr(Chi)
NULL			614	425.0780	
Day	9	29.1056	605	395.9724	0.0006220
Breed	2	7.3655	603	388.6068	0.0251533
Vendor	184	141.0148	419	247.5920	0.9919853
Time	1	0.3126	418	247.2793	0.5739238
Weight	1	1.1130	417	246.1663	0.2914249
P8 fat	1	0.026	416	246.1367	0.8715631
Gain.rate	1	0.0600	415	246.0767	0.8064873
Hdw	1	0.0394	414	246.0373	0.8426280
Day:breed	1	2.0730	413	243.9643	0.1499289
Day:time	9	8.7514	404	235.2129	0.4605270
Day:weight	9	4.2565	395	230.9564	0.8937367
Day:P8fat	9	9.7231	386	221.2334	0.3733663
Day:gain.rate	9	6.7926	377	214.4407	0.6586987
Day:hdw	9	3.8950	368	210.5457	0.9181832
Breed:time	1	0.3066	367	210.2390	0.5797466
Breed:weight	2	0.4836	365	209.7554	0.7851945
Breed:P8 fat	2	3.4952	363	206.2602	0.1741922
Breed:gain.rate	2	0.1182	361	206.1420	0.9426323
Breed:hdw	2	1.1940	359	204.9461	0.5499225
Time:weight	1	0.0450	358	204.9011	0.8320947
Time:P8 fat	1	0.9136	357	203.9875	0.3391579
Time:gain.rate	1	0.1420	356	203.8455	0.7064238
Time:hdw	1	0.1211	355	203.7245	0.7278718
Weight:P8 fat	1	0.9436	354	202.7808	0.3313485
Weight:gain.rate	1	0.0477	353	202.7332	0.8271823
Weight:hdw	1	0.4773	352	202.2559	0.4896680
P8 fat:gain.rate	1	0.0048	351	202.2512	0.9449918
P8 fat:hdw	1	2.6293	350	199.6219	0.1049109
Gain.rate:hdw	1	0.1832	349	199.4386	0.6685960

Table 3. The association between TG5 genotype and marbling score (Analysis of Deviance Table, Poisson model, Response: score). (Terms added sequentially (first to last).)

	Df	Deviance Resid.	Df	Resid. Dev	Pr(Chi)
NULL			539	3366.707	
Breed	2	86.758	537	3279.949	0.0000000
Day	27	260.975	510	3018.974	0.0000000
Genotype	2	1141.545	508	1877.430	0.0000000
Marbling	4	1288.672	504	588.757	0.0000000
Breed:day	6	25.581	498	563.176	0.0002666
Breed:genotype	4	2.759	494	560.417	0.5988828
Breed:marbling	8	32.354	486	528.063	0.0000805
Day:genotype	54	50.369	432	477.693	0.6152116
Day:marbling	108	228.707	324	248.987	0.0000000
Genotype:marbling*	8	16.078	316	232.908	0.0412742

*TG5 genotype significantly (P<0.05) affects marble score

Table 4. Gene frequency change in TG5 in extreme marbling score. M1 and M4+ are marbling score 1 and marbling score 4 and above respectively. f(3) is the frequency of the '3' allele. G(adj) is the value of G with the Williams correction.

Genotype	M1	M4+
g22	71	143
g23	27	72
g33	0	12
f(3)	0.14	0.20

G(adj) = 9.64, 2 df, P < 0.009

Table 5. Generalised linear model of liveweight gain as a function of several feedlot characters, fixed factors and DNA markers. (Analysis of Deviance Table, Gaussian model; Response: (gain<2.5). (Terms added sequentially (first to last).)
*TG5 genotype significantly (P<0.001) affects liveweight gain

	Df	Deviance Resid.	Df	Resid. Dev	F Value	Pr(F)
NULL			1176	0.9991504		
Breed	2	0.01266390	1174	0.9864865	7.390225	0.0005326
Day	27	0.02184458	1147	0.9646419	0.969834	0.5091034
Hdw	1	0.00011094	1146	0.9645310	0.132988	0.7154244
P8 fat	1	0.00142609	1145	0.9631049	1.709481	0.1913287
Ema	1	0.00023533	1144	0.9628695	0.282093	0.5954411
Mark1	15	0.01750987	1129	0.9453597	1.399294	0.1396183
Mark2	9	0.00710742	1120	0.9382523	0.946645	0.4832997
Mark3	26	0.00853282	1094	0.9297194	0.393402	0.9974246
TG5*	5	0.02124945	1089	0.9084700	5.094424	0.0001267

Table 6. Analysis of Variance of liveweight gain (LWG) with breed and genotype as factors. The breeds are Angus, Mixed and Shorthorn. The genotypes* for TG5 are t12, t13, t22, t23, t24, and t33. Not all genotype means are estimable.

TG5	t12	t13	t22	t23	t24	t33
Mean LWG (kg)	1.4965	non-est	1.2711	1.2612	non-est	1.3140
se	0.1256		0.0105	0.0142		0.0426

*TG5 genotype '33' had significantly higher LWG than '23' or '22' genotypes

'3' allele is associated with higher marbling scores. In studies of genetic factors associated with continuous traits there is a distinct possibility that associations may be different in different studies. This can be due to the DNA marker being insufficiently close to the causal mutation, so in one sample the higher trait values are associated with one allele while in a second sample the higher trait values are associated with the alternative allele.

Secondly, the association may be variably detected because the effect due to genotypic substitution is small relative to the overall phenotypic variance in the trait. When the sample sizes are modest there is a chance that this genetic variance will not be statistically significant. By using a relatively large sample to account for the large error variance in dealing with a categoric trait that has a subjective component in its assessment, we have tried to ensure that failure to detect the association will be minimised.

The consistency of the '3' allele associated with higher marbling score in this as well as the two previous samples implies that the TG5 polymorphism is not only a robust test but that the polymorphism is either the causal mutation or is close to the causal mutation (e.g. Edwards 1980). However, in the previous study, which used cattle fed for unusually long periods (well in excess of 300 days), both the '23' and '33' genotypes were associated with higher marbling scores. Indeed, the '33' genotype is usually so rare that the tables are dominated by individuals with the '23' genotype. In this study, the plot of raw marbling score versus genotype does not show a difference in raw marbling score between the 22' and '23' genotypes. This could be due to the animals being fed for a much shorter period (Figure 1), although it should also be borne in mind that plots based on raw marbling scores lumped across breeds and slaughter days may either disguise or exaggerate differences.

Figure 2 is used primarily to show the polarity of the association, that the '3' allele is associated with higher marbling scores. This is confirmed in Table 4. Increasing the frequency of the '3' allele in cattle will have an impact on the proportions of cattle reaching higher marbling scores even when the statistics are viewed in the aggregate, lumped across breeds, days, vendors and and where the ancestry of the cattle is not well known. This would imply that it should be a useful breeding and feedlot screening tool. Increased gain in the feedlot associated with the '3' allele of the TG5 polymorphism is consistent with much earlier descriptions in which goiterogens were shown to increase gain or improve 'fattening' in the feedlot (Burroughs *et al.* 1958; Raun *et al.* 1960).

No association with hot dressed weight or final weight was found and it is not possible to determine whether this is an overall increase in fat since an equivalent of a hot dressed weight at entry to the feedlot is not available.

No association with P8 rump fat thickness was found indicating that selecting cattle on this DNA marker is not expected to change the subcutaneous fat thickness. The cattle sampling methods are appropriate to identifying the gene affecting marbling.

The consistent associations to TG5 implicate the Thyroglobulin gene which codes for thyroglobulin, the molecular store for the thyroid hormones T3 and T4 (tri-iodothyronine and thyroxine respectively). These hormones affect adipocyte growth and differentiation both in vivo and in vitro (Folley and Malpress 1948; Salter 1950; Beato 1989; Ailhaud *et al.* 1992; Darimont *et al.* 1993) and T3 and T4 levels have recently been associated with intra-muscular fat in Wagyu cattle (Mears *et al.* 2001). However, the mechanism by which mutations in the TG gene influence either marbling score directly or whether mutations affect the availability of T3 and T4 and thus marbling score is not known. None of the genes adjacent to TG are known to be implicated in adipocyte differentiation or growth, but negative evidence is not a strong form of confirmation.

Further research in the control and regulation of TG may provide information on the mechanism of action while studies of the adjacent genes could rule them out of contention as genes affecting marbling score.

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W. Barendse extracted DNA, performed the analyses and designed the experiment.

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