VALIDATION OF COMMERCIAL DNA TESTS FOR QUANTITATIVE BEEF TRAITS

R.L. Quaas[1], J. Li[1], R.M. Thallman[2], A.L. Van Eenennaam[3], R.L. Fernando[4] and C. Gill[5]

[1] Department of Animal Science, Cornell University, Ithaca, NY 14850, USA
[2] U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933, USA
[3] Department of Animal Science, University of California, Davis, CA 95616, USA
[4] Department of Animal Science, Iowa State University, Ames, IA 50011, USA
[5] Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

INTRODUCTION

Panels of genetic markers associated with quantitative beef cattle traits have recently become commercially available. One of the first questions producers ask is whether these DNA-based tests perform in accordance with the claims of the companies marketing them. In the USA, the National Beef Cattle Evaluation Consortium (NBCEC, www.NBCEC.org) has undertaken a validation process to independently verify associations between genetic tests and traits as claimed by the commercial genotyping company through the analysis of phenotypes and genotypes derived from reference cattle populations. The genotyping company requests the validation of their claims and is responsible for genotyping DNA samples. The NBCEC performs the analysis to determine whether there is an association between the results of the genetic test and the phenotype for the claimed trait. The validation process is a partnership of the owners of DNA and phenotypes (e.g., breed associations) and commercial testing companies, facilitated by the NBCEC.

MATERIALS AND METHODS

Tests. Two GeneSTAR® (Bovigen LLC, www.bovigen.com) marker panels and one Igenity panel (Merial, www.igenity.com) have gone through the validation process. The GeneSTAR® Quality Grade marker panel is comprised of a single nucleotide polymorphism (SNP) in the 5' leader sequence of the thyroglobulin gene (TG5, denoted "M1"; Barendse *et al*., 2004) and an anonymous SNP (denoted "M2"; unpublished). The GeneSTAR® Tenderness panel is comprised of a calpastatin SNP (denoted CAST-T1; Casas *et al*., 2006) and a μ-calpain SNP (*CAPN1* 316, denoted T2; Page *et al.,* 2002). Igenity *Tender*GENE™ is a marker panel consisting of two μ-calpain SNPs (denoted *CAPN1* 316 & 4751; Page *et al.,* 2002; White *et al*. 2005), and a calpastatin SNP (denoted UoG-CAST; Schenkel *et al.,* 2006). These two tenderness tests therefore share a common μ-calpain SNP (*CAPN1* 316) and each has a calpastatin SNP, but the latter are not the same SNP. All genotyping was done by the respective companies.

Sample populations. The phenotypic data and DNA were collected as part of the Carcass Merit Project (Thallman *et al.,* 2005). These data are "owned" by the various participating breed associations. Each commercial testing company selected the breed groups to be used for the validation and then reached an agreement with the respective breed associations. Bovigen chose to validate their two GeneSTAR® marker panels on both Charolais- and Hereford-sired cattle. The former were out of commercial Angus dams; the latter primarily Hereford or Hereford x Red Angus dams. The Igenity *Tender*GENE™ validation used the same Charolais-sired cattle plus cattle sired by Red Angus (Red Angus and Red Angus cross dams), Brangus (Brangus and Brangus cross dams) and Brahman (Brahman dams) bulls.

Phenotypes. Traits analyzed were 14-d post-mortem Warner-Bratzler shear force (tenderness) and subjectively recorded marbling score ("quality"). The latter was also analyzed as percent qualifying as USDA grades of Choice or Prime.

Statistical Analysis. The basic model was y = CG + Marker Effect + Sire + e where CG denotes a fixed contemporary group (same breed type, feedlot, sex, harvest date) and Sire was a random effect. All cattle were AI-sired (one insemination) so within a CG there was minimal age difference. The Marker Effect for the GeneSTAR® panels was defined two ways. In the first definition, the markers effects were assumed equal and additive so that the Marker Effect was just the regression on the number of favorable alleles summed across markers. The second definition also assumed additivity, but allowed for different magnitude of marker effects, i.e., included a regression on number of favorable alleles for each marker locus. For the Igenity *Tender*GENE™ marker panel, the Marker Effect was similar to the second definition above except that because there were two linked markers (i.e., *CAPN1* 316 & 4751), the regression was on the expected number of copies of each of the four haplotypes (one of which was rare). Haplotype frequencies were estimated and analyses carried out with SAS Proc HAPLOTYPE and Proc Mixed respectively. Results are available at the website http://www.nbcec.org.

RESULTS AND DISCUSSION

GeneSTAR® Quality Grade is a marker panel comprised of two markers (denoted TG5 and M2). The frequency of one of the M2 alleles was too low in the Hereford-sired sample population to detect marker effects, so the analysis only included the 387 Charolais-sired × Angus cattle (Table 1). The GeneSTAR® Quality Grade test results did not have a significant association with marbling score in the Charolais x Angus reference population. An increase in quality grade (percentage grading choice or prime) that approached significance (p = 0.06) was associated with substituting favorable alleles of TG5 and M2 in the Charolais x Angus animals.

Table 1. GeneSTAR® (Marbling Score and % Choice & Prime) Quality Grade analysis.

Trait	Effect	Frequency	Estimate	SE	F	p
Marbling Score	GeneSTAR® Quality Grade*		5.7	4.2	1.80	0.18
	TG5**	.22	9.7	5.9	2.65	0.10
	M2**	.21	0.1	7.0	0.00	0.99
% Choice and Prime	GeneSTAR® Quality Grade*		6.2	3.2	3.7	0.06
	TG5**	.22	8.6	4.5	3.6	0.06
	M2***	.21	2.9	5.2	0.3	0.58

* Combined 2-marker panel = total number of favorable TG5 and M2 alleles; value of an average favorable allele.
** Effects of TG5 and M2 favorable alleles.

The association seemed to be primarily associated with the favorable allele of the TG5 marker. In this sample population, each TG5 "star" was associated with an 8.6% increase in the number of cattle grading choice or prime, and each M2 "star" was associated with a 2.9% increase in the number of cattle grading choice or prime. The average effect of a GeneSTAR® Quality Grade star was associated with a 6.2% increase in the number of cattle grading choice or better.

GeneSTAR® Tenderness is a marker panel comprised of two markers (denoted CAST-T1 and *CAPN1* 316-T2). An increase in "tenderness" is associated with substituting a "T" allele at calpastatin (CAST-T1) and a "C" allele at μ-calpain (*CAPN1* 316-T2). The GeneSTAR® Tenderness analysis included 672 animals (387 Charolais × Angus, and 285 Hereford). There was a strong association of both markers with Warner-Bratzler shear force (Table 2).

Table 2. GeneSTAR® Tenderness (Warner-Bratzler shear force, kg) analysis.

Effect	Frequency	Estimate (kg)	SE	F	p
GeneSTAR® Tenderness *		-0.18	0.04	21.1	5.3E-06
CAST-T1**	0.78	-0.14	0.06	5.6	1.8E-02
CAPN1 316-T2**	0.23	-0.21	0.05	15.7	8.1E-05

* Combined 2-marker panel = total number of favorable CAST-T1 and *CAPN1* 316-T2 alleles; value of an average favorable allele
**Effect of CAST-T1 and *CAPN1* 316-T2 favorable alleles.

In this sample population each calpastatin "T" was associated with a decrease of 0.14 kg of Warner-Bratzler Shear Force, and each μ-calpain (316) "C" was associated with a decrease of 0.18-0.21 kg of Warner-Bratzler Shear force. The average effect of a GeneSTAR® Tenderness star was a decrease of .18 kg of Warner-Bratzler shear force. Breeders should not expect gains this large because no herd will consist 100% of the 'least tender' genotype.

Igenity *Tender*GENE™ is comprised of three markers (UoG-CAST, *CAPN1* 4751, and *CAPN1* 316). An increase in "tenderness" is associated with substituting a "C" allele at calpastatin (UoG-CAST) and a "C" allele at both μ-calpain loci (*CAPN1* 4751 and *CAPN1* 316). In the Igenity *Tender*GENE™ analysis, the calpastatin marker (UofG-CAST) and the μ-calpain haplotypes based on *CAPN1* 4751 and *CAPN1* 316 were each highly significant, and the combination of all three even more so. For UoG-CAST, *CAPN1* haplotype, and the joint UoG-CAST + *CAPN1*, respective p values were 1.8E-04, 4.7E-06 and 1.9E-08. Table 3 shows the decrease in "toughness" (Warner-Bratzler Shear Force, kg) for each of the possible alleles or haplotypes contrasted to the least tender genotype (i.e. UoG-CAST "GG", *CAPN1* 4751 "TT", *CAPN1* 316 "GG") calculated from a combined analysis of 1209 cattle: 181 Brangus, 400 Charolais x Angus cross, 310 Red Angus and 318 Brahman. In this sample population, each calpastatin "C" was associated with a decrease of 0.19 kg of Warner-Bratzler shear force, and substituting the *CAPN1* 4751 "C" - 316 "C" haplotype for the *CAPN1* 4751 "T" - 316 "G" was associated with a decrease of 0.33 kg of Warner-Bratzler shear force.

Table 3. Igenity *Tender*GENE™ (Warner-Bratzler shear force, kg) analysis*.

Marker	Allele/ Haplotype	Sample Frequency	Estimated Effect (kg)	SE
UoG-CAST	C	0.72	-0.19	0.05
	G	0.28	0.00	0.00
CAPN1	C-C	0.16	-0.33	0.07
4751	C-G	0.22	-0.18	0.06
&	T-C*	0.01	0.22	0.23
316	T-G	0.61	0	0.00

* The low number of animals with the T-C haplotype in this study made it difficult to get an accurate estimate of its effect.

Among genotypes with sufficient information there was a 1.04 kg difference in Warner-Bratzler shear force between the best genotype (homozygous C at all three markers) and the least tender (GG-TT-GG at UoG-CAST-CAPN4751-CAPN316, respectively) genotype.

CONCLUSIONS

Associations between three panels of genetic markers and quantitative beef cattle traits as claimed by the companies marketing them were independently verified by the NBCEC. The

GeneSTAR® Quality Grade test results did not have a significant association with marbling score, however the association between favorable alleles of the two markers (TG5 and M2) and increased quality grade (percentage of animals grading choice or prime) approached significance. The association was primarily associated with the TG5 marker. Analyses of the GeneSTAR® Tenderness and Igenity *Tender*GENE™ marker panels showed similar results: strong association of each marker in the panel with sizable Warner-Bratzler shear force effects. These two tenderness tests share a common μ-calpain SNP (*CAPN1* 316), and each has a calpastatin SNP but the latter are not the same SNP.

REFERENCES

Barendse, W., Bunch, R., Thomas, M., Armitage, S.M., Baud, S. and Donaldson, N. (2004). *Aust. J. Exp. Agric. 44*: 669-674.

Casas, E., White, S.N., Wheeler, T. L., Shackelford, S.D., Koohmaraie, M., Riley, D.G., Chase, C.C. Jr., Johnson, D.D. and Smith, T.P.L. (2006). *J. Anim. Sci.* **84** : 520-525.

Page, B.T., Casas, E., Quaas, R.L., Thallman, R.M., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M., White, S.N., Bennett, G.L. Keele, J.W, Dikeman, M.E. and Smith, T.P.L. (2004) *J. Anim. Sci.* **82 :** 3474-3481.

Schenkel, F.S., Miller, S.P., Jiang, Z., Mandell, I.B., Ye, X., Li, H. and Wilton, J.W. (2006). *J. Anim. Sci.* **84 :** 291-299.

Thallman, R.M., Moser, D.W., Dressler, E.W., Totir, R.L., Fernando, R.L., Kachman, S.D., Rumph, J.M., Dikeman, M.E. and Pollak, J.E. (2005) *Proc. Genetic Prediction Workshop Conference*, December 6, 2003 : 5-15.

White, S.N., Casas, E., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M., Riley, D.G., Chase, C.C. Jr., Johnson, D.D., Keele, J.W. and Smith, T.P.L. (2005) *J. Anim. Sci*. **83** : 2001-2008.