CITRULLINEMIA, MARKER FOR ECONOMICALLY IMPORTANT TRAITS?

R. D. Shanks, Y. C. Huang and J. L. Robinson
Department of Animal Sciences
University of Illinois
Urbana, IL 61801
United States

SUMMARY

Bovine citrullinemia was not useful as a marker for milk, fat or protein production because 1) the frequency of defective allele was low and 2) within family differences were nonsignificant between homozygous normal and heterozygous half-sibs. Several genetic and phenotypic traits were tested to detect differences between normal and heterozygotes for citrullinemia. Collectively a plot of probability of significance by standardized regression coefficient for 53 traits approximated a normal distribution. Unique differences in performance between genotypes were not detected. Age at first classification was 4.5 months younger for heterozygotes than for cows normal at citrullinemia locus.

INTRODUCTION

Homozygous recessive condition of citrullinemia is manifested by increasing ammonia and decreasing arginine concentrations in blood during first 24 hr after birth (Harper et al., 1986; Healy et al., 1990). Affected newborn calves display severe neurological dysfunction and death within 1 wk as a result of a deficiency of argininosuccinate synthetase (E.C.6.3.4.5). This enzymes catalyzes the conversion of citrulline to argininosuccinate as part of the urea cycle. A build-up of ammonia predicates neurological problems which may appear as unsteady gait, aimless wandering, apparent blindness, head pressing, collapse, convulsions and death. Affected calves do not show clinical symptoms at birth because *in utero* the dam eliminates the accumulations of ammonia.

Detection methods based on polymerase chain reaction can distinguish normal cows from heterozygotes for defective citrullinemia allele (Dennis et al., 1989). The defective allele for citrullinemia is detectable because a single-base substitution (C→T) eliminates a restriction site. The restriction enzyme *AvaII* will hydrolyze the normal allele but not the defective one. Detecting other potential differences between homozygotes for normal allele and heterozygotes at citrullinemia locus is the objective of this study.

Citrullinemia was discovered in Australia and incidence of heterozygotes has been estimated as high as 10% with approximately one of every 250 calves born being affected (Healy, 1991). The progenitor of most cases in Australia was Linmack Kriss King, who had traveled from Canada to England and then to Australia. His semen was also distributed internationally and he had a pronounced effect on the Friesian breed in Australia (Healy et al., 1990). His sire, Gray View Crisscross, was also detected as a heterozygote for citrullinemia based on analysis of frozen semen from Crisscross (Healy et al., 1991). Five of 17 offspring of Crisscross also had been identified as heterozygotes (Healy et al., 1991). Incidence of heterozygotes among Holsteins in the United States has been estimated at less than .5% as only one of 367 bulls from the top 400 Type-Production Index (TPI) was detected as a heterozygote, Bull-C (Robinson et al., 1993). Genetic impact of Crisscross and Bull-C on U.S. Holsteins has been small because each had fewer than 2000 offspring registered with Holstein Association of America. Diagnosis of Bull-C as a heterozygote from his semen was confirmed because 26 of 51 daughters of Bull-C were tested and identified as heterozygotes (Robinson et al., 1993).

Defective allele for citrullinemia is a detectable marker in Holsteins, although it was not found in either Al Guernsey or Jersey bulls (Robinson et al., 1993). Incidence in U.S. Holsteins

is low but reported incidence in Australia may suggest possible use as a marker for milk production. Objective was to evaluate potential differences between cows normal and heterozygous for citrullinemia on economically important traits.

MATERIALS AND METHODS

Performance information was requested from Holstein Association on the 51 daughters of Bull-C tested for citrullinemia (Robinson et al., 1993). Cooperation of Tom Lawler and Lynn Pancake in supplying this information was appreciated. This information contained traits considered to be economically important but should not be considered inclusive of all economically important traits. The 51 daughters of Bull-C were from 23 herds in 12 states.

Effects of citrullinemia on 53 traits were explored by a parsimonious, one-way fixed model. Univariate analysis of each trait was conducted but recognition of lack of independence among traits is necessary for reasonable interpretations. Traits investigated included genetic estimates, phenotypic observations and management variables. Milk, fat, protein and type surfaced in many of the traits analyzed. Traits were measured on individual, or maternal grandsire. Sire data was not analyzed because all 51 cows were progeny of the same sire, Bull-C.

Thirty-seven different maternal grandsires (MGS) had descendants among the progeny of Bull-C. Nine MGS had two or more descendants; 6 descendants for one MGS, 3 descendants for another and 2 descendants for 7 MGS. Among MGS with multiple descendants, all but two MGS had both normal and heterozygous descendants. The two exceptions each had two heterozygous descendants. Analysis of MGS traits considered each descendant to have a unique MGS and therefore more significant differences may be detected than justified. Differences between genotypes for citrullinemia were standardized (Neter et al., 1990) and then plotted versus level of significance.

RESULTS

Citrullinemia was not a useful marker for economically important traits associated with production of milk, fat or protein. None of the traits representing biology were significantly different ($P > .05$) between cows normal or heterozygous for citrullinemia. Interpretation of analysis of genetic estimates and phenotypic measurements were consistent. Least squares means by genotype for citrullinemia are listed in Table 1.

Figure 1 contains plot of probability of significance by standardized difference between normal and heterozygous cows. As expected, the plot depicts a normal distribution of probabilities. The 3 traits with significant differences were age at first classification, reliability of type and confidence range for type.

DISCUSSION

Age at first classification, reliability for type and confidence range for type are not independent traits. Why age at first classification was greater for normal cows was unknown. Five of 7 cows with age at classification equal to or greater than 38 months were normal for citrullinemia. Were differences in age at first classification spurious? Perhaps, first classification scores for the 7 older cows were 76, 76, 78, 79, 81, 82, 83, which were very similar to scores of younger cows. Scoring later as a management decision was not a function of the appearance of the cows. Age at first classification influenced reliability for type because the older cows had less information available for evaluation. The product moment correlation between age at first classification and reliability for type was -.83. Five normal cows were classified only once. These cows included 4 of the 5 oldest normal cows at first classification (Age $\geq$ 38 mo). All heterozygotes classified were classified at least twice but only 2 were equal to or older than 38 months. Limiting the data to those cows < 38 mo., reduced the correlation to -.26. Much of the

association between age and reliability was a function of the few cows that were older when first classified. Product moment correlation between age at first classification and confidence range for type was .83 reflecting the expected -1.0 association between reliability type and confidence range type. The range is greater for cows with less reliability.

By chance alone, 3 traits were expected to be significant if 60 independent traits were evaluated. Significance ($P < .05$) was observed for 3 traits but the 3 traits were not independent and reflected a cascade effect.

REFERENCES

DENNIS, J.A., HEALEY, P.J., BEAUDET, A.L., and O'BRIEN, W.E. (1989) Proc. Natl. Acad. Sci. USA 86:7947-7951.

HARPER, P.A.W., HEALY, P.J., DENNIS, J.A., O'BRIEN, J.J., and RAYWARD, D.G. (1986) Aust. Vet. J. 63:378-379.

HEALY, P.J., DENNIS, J.A., CAMILLERI, L.M., ROBINSON, J.L., STELL, A.L., and SHANKS, R.D. (1991) Aust. Vet. J. 68:155.

HEALY, P.J., HARPER, P.A.W., and DENNIS. J.A. (1990) Aust. Vet. J. 67:255-258.

NETTER, J., WASSERMAN, W., KUTNER, M.H. (1990) Applied Linear Statistical Models. 3rd Edition, Richard D. Irwin, Homewood, IL. p.289

ROBINSON, J.L., BURNS, J.L., MAGURA, C.E., and SHANKS, R.D. (1993) J. Dairy Sci. 76:853-858.

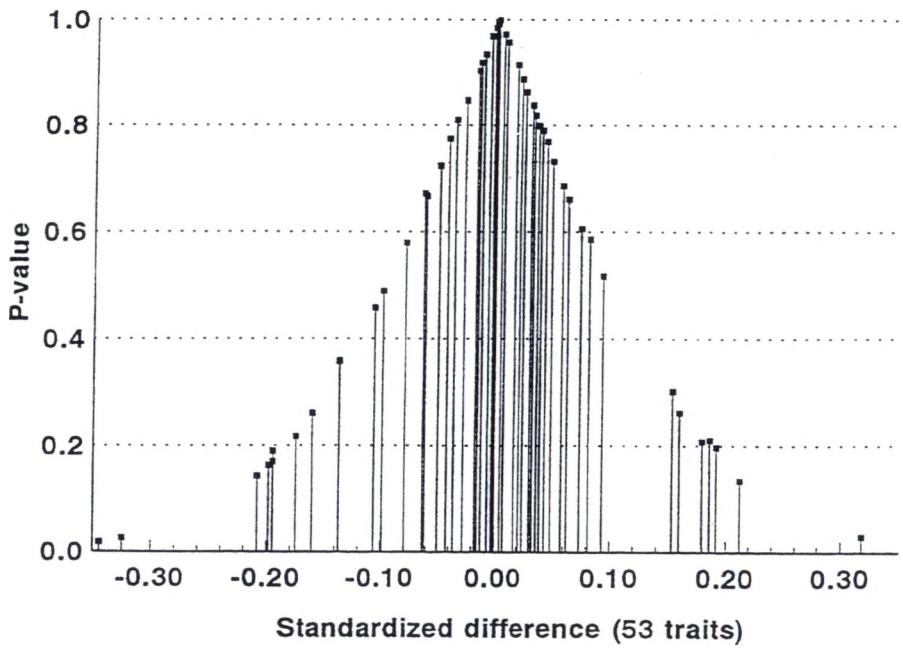

Figure 1. Standardized difference between cows heterozygous or normal for citrullinemia.

Table 1. Least squares means for genotypes (Heterozygotes or Normal) for citrullinemia

N	Trait	Heterozygotes	Normals	P	R^2
51	Age at First Calving	24.8±.5	25.8±.5	.17	.038
51	Times Milked Daily	2.54±.10	2.36±.10	.21	.032
51	First Days in Milk	295±4	303±4	.16	.039
51	First Milk Yield, kg	9099±323	9102±330	1.0	.000
51	First % Fat	3.45±.06	3.42±.06	.80	.001
51	First Fat Yield, kg	314±11	310±11	.80	.001
51	First % Protein	3.14±.03	3.20±.03	.14	.043
51	First Protein Yield, kg	285±10	290±10	.72	.003
47	Age at Best Record	39.5±1.9	39.5±1.8	.99	.000
47	Times Milked Daily, Best	2.55±.11	2.36±.10	.21	.035
47	Days in Milk, Best	335±8	338±8	.77	.002
47	Best Milk Yield, kg	12679±465	11844±436	.20	.037
47	Best % Fat	3.48±.07	3.45±.07	.77	.002
47	Best Fat Yield, kg	442±19	409±18	.21	.035
47	Best % Protein	3.11±.04	3.16±.04	.36	.019
47	Best Protein, kg	395±15	374±14	.30	.024
47	Final Score	79.9±.9	79.7±.9	.89	.000
47	Cow TPI	656±34	630±35	.59	.007
47	MGS_TPI	422±42	427±42	.93	.000
45	MGS_Final Score	89.2±1.0	89.6±1.0	.81	.001
51	MGS_PTA Protein, kg	6.1±1.5	6.5±1.6	.85	.001
51	MGS_PTA Fat, kg	7.3±1.7	8.7±1.7	.58	.006
51	MGS_PTA Type	.39±.15	.31±.15	.73	.002
51	MGS_Udder Composite	.31±.19	.25±.19	.82	.001
51	Reliability Milk	53.2±.4	52.5±.4	.26	.026
51	Management Dev. Milk, kg	1337±147	1325±150	.96	.000
51	PTA Milk, kg	654±39	618±40	.52	.009
51	Confidence Range Milk, kg	173.8±.8	175.1±.8	.26	.026
51	Management Dev. Fat, kg	32±6	32±6	.98	.000
51	PTA Fat, kg	15.3±1.6	15.2±1.6	.97	.000
51	Confidence Range Fat, kg	6.98±.04	7.06±.05	.22	.031
51	PTA % Fat	-.088±.012	-.076±.012	.46	.011
51	PTA $ Fat	164±10	157±11	.61	.005
51	Reliability Protein	53.1±.5	52.1±5	.13	.045
51	Management Dev. Prot., kg	35±4	35±4	.90	.000
51	PTA Protein, kg	17.5±1.1	16.9±1.2	.69	.003
51	Confidence Range Prot., kg	5.88±.04	5.91±.04	.49	.009
51	PTA % Protein	-.030±.007	-.025±.007	.67	.004
51	PTA $ Cheese Yield	131±10	128±11	.86	.001
51	PTA $ Protein	153±10	147±10	.66	.004
47	Reliability type	59.8±.8	57.2±.8	.03	.101
47	Type Deviation	.42±.16	.38±.16	.84	.001
47	Age Adjusted Score	80.5±.8	80.5±.8	1.0	.000
47	Parent Average Type	.32±.08	.37±.08	.67	.004
47	PTA Type	.39±.11	.35±.11	.79	.002
47	Confidence Range Type	44.2±.4	45.7±.41	.02	.119
47	Udder Composite	.00±.12	.02±.17	.92	.000
47	First Final Score	76.3±.8	77.9±.8	.19	.038
47	First Age Adjusted Score	79.3±.8	80.4±8	.36	.019
47	Age at First Classification	29.6±1.4	34.1±1.4	.03	.105
42	First Permanent Score	79.9±.9	79.9±1.1	.99	.000
42	First Perm. Age Adj. Score	80.4±.9	80.4±1.0	.97	.000
42	Age at First Perm. Score	53.3±1.7	53.1±1.9	.91	.000