Reporting of Haplotypes with Recessive Effects on Fertility

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Abstract

Genomic discovery of five haplotypes with recessive effects on fertility requires new automated tracking methods for QTL causing embryo loss in breeding programs. Most of the losses are early in gestation. Approximate locations of the five QTL were refined using crossovers detected within the pedigree. Of the top 100 available proven bulls for net merit, 15 Holsteins, 21 Jerseys, and 14 Brown Swiss are carriers of these haplotypes. Beginning with August genomic evaluations, carrier status is reported for all 127,588 genotyped animals in the North American database but is slightly less accurate for those with 2,900 markers or for imputed dams. Breeders should continue to use mating programs and index selection instead of direct selection against these haplotypes because their additive economic effects are small and are included in evaluated fertility traits.

Key words: genomics, fertility, embryo loss, genetic load

Introduction

Several harmful recessive factors have been examined, confirmed, and reported to breeders of each dairy breed. In 2011, Holstein Association USA added brachyspina (BY; Agerholm and Peperkamp, 2007) to its list of recognized defects after inheritance was confirmed by a lack of homozygous haplotypes in the genotypes of live animals and by phenotypic effects on U.S. fertility data (VanRaden *et al.*, 2011b). The methods used to confirm BY were then used to search across the whole genome for similar haplotypes that had no homozygotes. Of the top 12 haplotypes including BY that had the most expected homozygotes but none observed, 5 new and BY were confirmed as having effects on fertility consistent with lethal recessive inheritance. These haplotypes are termed source haplotypes and often trace mainly to just one source ancestor. A descendant may receive the whole haplotype or just a part if a crossover occurs.

The current report provides additional information on economic effects, use of crossovers for fine mapping, accuracy of detection with 2,900 (3K) markers or imputed dams, and inheritance of haplotypes HH1, HH2, and HH3 in Holsteins, JH1 in Jerseys, and BH1 in Brown Swiss.

Methods

Haplotype segments of about 75 markers spanning 4-7 million DNA base pairs were obtained from version 2 of program findhap.f90 (VanRaden *et al.*, 2011a). Haplotypes that had the largest numbers of homozygotes expected but none observed were examined as potential causes of embryo loss. To estimate the losses, a carrier sire by carrier maternal grandsire interaction effect was added to the sire conception rate (SCR) model of Kuhn and Hutchison (2008). In U.S. conception rate and daughter pregnancy rate evaluations, spontaneous abortions are coded as failures but are coded as successes in most other national evaluations that use nonreturn rates (NR). To estimate time of embryo loss, SCR interactions were compared to various NR (60, 100, and 140) interactions using the same model. Conception rate included all losses during gestation whereas NR included only early losses. Economic effects of the haplotypes are small when embryo loss is early.

Crossover haplotypes contain part of the source haplotype and part of some other haplotype. Crossovers can be detected directly from genotyped animals within the pedigree or indirectly from partially shared segments. Only crossovers detected directly in the pedigree by findhap were used in this research. For each crossover, the last marker known to be from

the first parental haplotype and the first marker known to be from the second parental haplotype are output. A gap may remain between those two markers if the parental haplotypes are identical in that region, some markers are not called, or both parents were heterozygous and could not be phased so that the point of crossover is not located exactly. Crossovers that occur in maternal ancestors are often not detected when dams are not genotyped or imputed.

Fine mapping was accomplished by checking if any genotyped live animals had both the source haplotype and a crossover haplotype. The database included 65 genotypes taken from embryos, and these are excluded from fine mapping because they could be homozygous for the lethal mutation. Also, only the 78,465 50K genotypes were used to ensure accuracy. If a portion of a crossover haplotype from the source becomes homozygous with the source haplotype in a live animal, that portion of the source haplotype is ruled out as containing the lethal mutation. For example, if a live animal received the source haplotype from one parent and the left 20 markers of the source haplotype from the other parent, the left 20 markers are ruled out. After processing all crossover haplotypes, the area not ruled out is the suspect area.

A pedigree confirmation code was developed to aid breeders in tracking the accuracy of haplotyping. Four generations of ancestors were checked and if a heterozygous ancestor was found in a pathway not blocked by a genotyped noncarrier, the animal's carrier status was coded as confirmed.

The five haplotypes that had no homozygotes and had conception rates consistent with loss of homozygous embryos are listed in Table 1 along with earliest genotyped bull heterozygous for the haplotype (source ancestor). Locations on *Bos taurus* autosomes (BTA) are from the UMD 3.0 map. Along with the source haplotype, several additional crossover haplotypes were detected within the pedigree. The crossover haplotypes were used in fine mapping to narrow the interval containing the causative mutation for four of these from 75 markers down to 8 to 30 markers. For two of the five source haplotypes, actual intervals were wider than 75 markers because an adjoining segment to the left or right also had no homozygotes and similar effects on fertility.

Accuracies of determining carrier status from 3K and 50K genotypes were compared by reducing 50K to 3K genotypes and then imputing from 3K back to 50K. The test included 500 carriers and 500 noncarriers and a total of 1000 animals for each haplotype. The BH1 haplotype was not tested because of too few Brown Swiss 50K genotypes. Repeatabilities of carrier status for imputed dams, 3K, and 50K genotypes were also examined across months by counting numbers of previous animals that changed status as additional animals were added to the database.

Heterozygous animals can be detected in three ways. Method 1 detects haplotypes using both genotype and pedigree databases. Method 2 tests only if a particular haplotype could be part of the animal's genotype but does not include information from the pedigree. Method 3 uses the causative mutation and is preferred but is not available for newly discovered and some previous conditions. Methods 1 and 2 were compared for each of the source haplotypes affecting fertility; crossover haplotypes were excluded from the analysis. Method 3 could not be compared because the causative mutations are not known yet.

Results and Discussion

Many of the top animals in each breed are now known to be heterozygous for the five source haplotypes with confirmed effects on fertility. Table 2 gives overall frequencies of animals that are heterozygous for only the source haplotype or also including the crossover haplotypes. Numbers of bulls in the top 100 are from April 2011 data and include available young bulls for net merit but only daughter-proven bulls for breed indexes. Similar statistics are given for BY.

Table 1. Locations of haplotypes affecting fertility and the earliest heterozygous ancestors.

Haplo-type	BTA, Mbase	Earliest known heterozygote
BY	21, 19–23	Sweet-Haven Tradition
HH1	5, 62–68	Pawnee Farm Arlinda Chief
HH2	1, 93–98	Willowholme Mark Anthony
HH3	8, 92–97	Glendell Arlinda Chief, Gray View Skyliner
JH1	15, 11–16	Observer Chocolate Soldier
BH1	7, 42–47	West Lawn Stretch Improver

Table 2. Overall frequency of heterozygotes and number in the top 100 bulls for net merit (NM$) and breed association indexes (TPI, JPI, PPR).

	Carriers in top 100		Carrier frequency	
Haplo-type	NM$	Breed index	Source only	Including crossovers
BY	4	1	5.83	5.93
HH1	1	3	3.24	4.18
HH2	2	3	3.66	3.75
HH3	12	20	4.84	4.84
JH1	21	24	21.79	23.07
BH1	14	10	14.83	14.83

Table 3 reports how many animals had both the source and a crossover haplotype, the number of markers remaining in the suspect area, number of crossover haplotypes that included the full suspect area, and numbers of crossovers detected within the suspect area. Animals with crossovers in the suspect area might or might not contain the lethal mutation depending on its exact location. Breeding trials could mate such animals to known carriers to narrow further the suspect areas automatically, because the fine mapping programs are run every month. The animal's status could be labeled as inconclusive, but many other crossover haplotypes exist that are not detected within the pedigree and are labeled as noncarriers. Currently only crossover haplotypes that include all of the suspect area are labeled as carriers, and the remainder are labeled as noncarriers. Thus, reported status is conservative, and some animals that have the defect are not reported as heterozygous.

Table 4 shows results of the 3K test. All haplotypes had a slight decrease in accuracy as compared with the 50K test. However, over 95% of test results were the same from the two chips. False negatives occurred more frequently than false positives. Accuracy with actual 3K may be lower because of lower call rate and fewer genotyped ancestors than for 50K. Homozygous animals were found for an average across the five defects of 0.004% of the 46,100 3K animals and 0.02% of the 3,023 imputed dams. Those were assumed to be haplotyping mistakes and were labeled as heterozygotes because no homozygous 50K genotypes were found. In the future, homozygous embryos could be detected, reported, and not implanted as is done in human fertility clinics; however the benefits in dairy cattle may not justify the cost.

Carrier status from 50K genotypes was very stable from month to month as new genotypes were added. On average across the five haplotypes, one animal changed from carrier to noncarrier and one changed from noncarrier to carrier as 3 months of genotypes were added from April to July 2011. However, changes were much larger when segments were repartitioned at new locations because of revised marker edits introduced in August 2011. In some cases, important bulls and their descendants switched carrier status because a crossover haplotype was included in one analysis but not another.

Detection of heterozygotes was very similar using methods 1 or 2. Results in Table 5 show an average of 2.5% false positives and 0.05% false negatives when using method 2 to predict method 1 results, with an overall accuracy of 99.69%. Similarly, Georges *et al.* (2010) reported 2% false positives and 0% false negatives using method 2 to predict method 1 BY status for 1,999 animals. Thus, independent laboratories could provide accurate testing for source haplotypes using method 2 but will miss some crossover haplotypes. Accuracy would be lower if raw rather than edited genotypes (Wiggans *et al.*, 2010) were used. Using 3K genotypes, the method 2 test works poorly (64 to 95% accuracy) if imputation is not done because the haplotypes contain only 3 to 8 3K markers.

Estimation of haplotype effects across the whole genome and using all of those in selection is standard procedure (Calus *et al.* 2008). Thus, patenting the use of one haplotype would make the genomic selection systems already in practice illegal (VanRaden, 2009). Discovery and patenting of the actual QTL should not stop others from tracing the inheritance of haplotypes that affect fertility in their populations, because actual QTL locations are not required for effective selection. Similarly, many countries practice genomic selection for all QTL that affect fat percentage even though one QTL was patented.

Table 3. Numbers of crossovers found and SNP remaining after fine mapping.

	Crossover haplotypes			
Haplo-type	Combined with source	Within suspect area	Contain suspect area	SNP in suspect area
BY	10	66	31	28
HH1	8	51	26	39
HH2	9	38	15	25
HH3	3	54	0	70
JH1	10	22	19	17

Table 4. Number of false positive and negatives using 3K genotypes of 500 carriers and 500 noncarriers.

Haplotype	False positives	False negatives	Error rate (%)
BY	0	11	1.1
HH1	2	32	3.4
HH2	0	9	0.9
HH3	1	14	1.5
JH1	2	32	3.4

Table 5. Comparison of one at a time (method 2) to full haplotyping (method 1) carrier status.

Haplo-	Carriers		Noncarriers		Accuracy
type	N	Errors	N	Errors	(%)
BY	4,425	36	66,242	16	99.88
HH1	2,531	120	68,136	40	99.96
HH2	2,688	30	67,979	1	99.93
HH3	3,437	75	67,230	27	99.97
JH1	1,356	61	4,405	4	98.71
BH1	311	6	1,726	1	99.71

Interactions for NR and SCR in Table 6 reveal that for haplotypes BH1, JH1, HH2, HH3, and for BY, the embryo loss is mainly before 60 days, whereas for HH1 and (CVM) the embryo losses occur throughout gestation. Results for CVM are consistent with Berglund *et al.* (2004), but indicate slightly earlier losses for BY than estimated by Georges *et al.* (2010). Stillbirth effects are small and not significant, but estimates tend to be larger for haplotypes with later effects during gestation.

Regulations that ban importation of all carriers of all QTL that affect fertility hurt genetic progress and are simply trade barriers. If genotyping someday reveals that all bulls possess some bad genes, some countries may force their breeders to stop using genotyped AI bulls and instead require that they use nongenotyped natural-service bulls about which nothing is known. Politicians and veterinarians should let breeders choose sires.

Conclusions

Automatic methods were developed to discover, fine map, and report inheritance of five new recessive factors. This approach could lead to further haplotypes and families being added as numbers of genotypes and phenotypes in the database continue to grow. Recessive factors causing embryo loss should be viewed as QTL affecting fertility and considered along with all other genetic factors (good and bad) that animals possess. Recessive factors exist in all populations and can be managed effectively once discovered by using mating programs or crossbreeding. Frequencies can be reduced optimally by index selection including fertility and all other traits that affect profit.

Acknowledgements

The authors thank Tom Lawlor, Cari Wolfe, Michael Cowan, and members of the National Association of Animal Breeders' Inherited Biochemical Defects Committee for helpful discussions.

Table 6. Estimated interactions for NR, SCR, and stillbirth for previous and new recessives.

Defect	NR60	NR100	NR140	SCR	Stillbirth
CVM	–0.9	–1.5	–1.9	–2.9	1.4
BY	–2.2	–2.3	–2.3	–2.5	–0.4
HH1	–1.1	–1.6	–2.0	–3.1	0.7
HH2	–1.7	–3.0	–2.9	–3.0	1.8
HH3	–3.1	–3.4	–3.4	–3.2	1.0
JH1	–3.7	–3.7	–3.6	–3.7	-0.4
BH1	–2.5	–3.7	–2.9	–3.4	N/A

References

Agerholm, J.S. & Peperkamp, K. 2007. Familial occurrence of Danish and Dutch cases of the bovine brachyspina syndrome. *BMC Vet. Res. 3*, 8.

Berglund, B., Persson, A. & Stålhammar, H. 2004. Effects of complex vertebral malformation on fertility in Swedish Holstein cattle. *Acta Vet. Scand. 45*, 161–165.

Calus, M.P.L., Meuwissen, T.H.E., de Roos, A.P.W. & Veerkamp, R.F. 2008. Accuracy of genomic selection using different methods to define haplotypes. *Genetics 178*, 553–561.

Georges, M., Coppieters, W., Charlier, C., Agerholm, J.S. & Fredholm, M. 2010. A genetic test for Brachyspina and fertility in cattle. Patent application WO2010012690. http://www.wipo.int/patentscope/search/en/WO2010012690

Kuhn, M.T. & Hutchison, J.L. 2008. Prediction of dairy bull fertility from field data: Use of multiple services and identification and utilization of factors affecting bull fertility. *J. Dairy Sci. 91*, 2481–2492.

VanRaden, P.M. 2009. Why we don't patent [editorial]. *J. Anim. Breeding Genet. 126*, 91.

VanRaden, P.M., O'Connell, J.R., Wiggans, G.R. & Weigel, K.A. 2011a. Genomic evaluations with many more genotypes. *Genet. Sel. Evol. 43*, 10.

VanRaden, P.M., Null, D.J., Olson, K.M. & Hutchison, J.L. 2011b. Harmful recessive effects on fertility detected by absence of homozygous haplotypes. *J. Dairy Sci. 94*, submitted.

Wiggans, G.R., VanRaden, P.M., Bacheller, L.R., Tooker, M.E., Hutchison, J.L., Cooper, T.A. & Sonstegard, T.S. 2010. Selection and management of DNA markers for use in genomic evaluation. *J. Dairy Sci. 93*, 2287–2292.