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Genetics

Microsatellite mapping of the gene causing weaver disease in cattle will allow the study of an associated quantitative trait locus

(ataxia/linkage/QTL)

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ABSTRACT **A genetic disease in cattle, progressive degenerative myeloencephalopathy (weaver disease), is associated with increased milk production. This association could result from population stratification, from a pleiotropic effect of a single gene, or from linkage disequilibrium between the gene causing weaver disease and a quantitative trait locus (QTL) for milk production. To test these hypotheses, we performed an extensive linkage study in a bovine pedigree segregating for the weaver condition and identified a microsatellite locus (*TGLA116*) closely linked to the weaver gene (z_{max}, 8.15; θ, 0.03). *TGLA116* and, by extension, the weaver locus were assigned to bovine synteny group 13. This microsatellite can be used to identify weaver carriers, to select against this genetic defect, and to study the effect of the corresponding chromosomal region on milk production in Brown Swiss and other breeds of cattle.**

The majority of traits selected in livestock production are quantitative traits; i.e., the individual phenotype reflects the action of several genes, confounded by environmental effects. Mapping the underlying genes or quantitative trait loci (QTLs) should allow for marker-assisted selection, which is expected to increase the rate of genetic progress.‖ However, because the contribution of individual QTLs to the overall phenotypic variance may be modest, their mapping may require the study of very large population samples. Several strategies have been proposed to reduce the number of individuals to be analyzed. These strategies include selective genotyping, progeny testing, interval mapping, the simultaneous search for multiple QTLs, and the use of DNA pools (1–3, **).

An alternative strategy consists of exploiting the associations that are sometimes found in selected populations between an unrelated monogenic trait and a quantitative trait under selection. Association between a monogenic and a quantitative trait can result from population stratification, from the pleiotropic effect of a single gene on both the monogenic and the quantitative trait, or from genetic linkage between the gene underlying the monogenic trait and a QTL. In the last two cases, genetic mapping of the monogenic trait leads to the concomitant mapping of a QTL. Examples of such associations in domestic animals include hyperkalemic periodic paralysis and muscularity in Quarter horses (4), malignant hyperthermia and the porcine stress syndrome/pale soft exudative meat syndrome (PSS/PSE) in pigs (5), and weaver disease and increased milk production in cattle (6).

The weaver syndrome in cattle (7–11) is characterized by the appearance, between 5 and 8 months of age, of progressive signs of pelvic limb paresis, ataxia, and proprioceptive placing deficits. Affected animals are mentally alert and reveal normal motor and sensory reflexes. Long-standing cases exhibit atrophy of the flexor and extensor muscles of the hip and stifle. Both males and females have atrophied gonads. Histological examination reveals extensive Wallerian degeneration in the white matter of the spinal cord, with formation of spheroids; lesions are most severe in the thoracic segments of the cord and involve dorsal, lateral, and ventral funiculi. Occasional axonal swelling is observed in brainstem nuclei, and selective degeneration and loss of Purkinje cells occur in the cerebellum. Extraneural lesions have been described but are considered secondary to central nervous system lesions. The weaver syndrome is almost exclusively observed in the Brown Swiss breed of cattle and is characterized by a distinct familial pattern. The mode of segregation is compatible with a simple recessive determinism.

Recently, Hoeschele and Meinert (6) reported a significant association between weaver disease and production traits in Brown Swiss cattle. On average, weaver-carrier animals produce 690 kg more milk and 26.2 kg more fat annually than their homozygous normal contemporaries. The linkage strategy that was used to demonstrate the association eliminates population stratification as the cause of the association. Although one cannot exclude a pleiotropic effect of a single gene, the alternative hypothesis of tight linkage between the gene responsible for weaver disease and a gene with a major effect on milk production is at least equally likely. If linkage disequilibrium were to exist between the defective weaver allele and a favorable allele at the QTL, selection against the weaver phenotype would be counterbalanced by selection for increased milk production and would result in balanced polymorphism at both loci.

We have initiated linkage studies to map the weaver locus and the hypothesized linked QTL in the bovine genome. In this article, we report tight linkage between a microsatellite marker, *TGLA116*, and the weaver locus in Brown Swiss cattle.

MATERIALS AND METHODS

Pedigree Material. Blood samples were collected from 33 weaver-affected animals reported to the American Brown

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Abbreviations: QTL, quantitative trait locus; lod, logarithm of odds.

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‖Soller, M. & Beckman, J. S., Second World Congress on Genetics Applied to Livestock Production, October 4–8, 1982, Madrid, Vol. 6, pp. 396–404.

**Plotsky, Y., Cahaner, A., Haberfeld, A., Lavi, U. & Hillel, J., Fourth World Congress on Genetics Applied to Livestock Production, July 23-27, 1990, Edinburgh, Vol. 13, pp. 133–136.

Swiss Cattle Breeders Association (Beloit, WI). Diagnosis of the weaver condition was confirmed by visual examination of the animals by experienced observers, and in some cases, by histological examination of the central nervous system after necropsy (10). Whenever possible, samples were also collected from informative sires, dams, and siblings. Parentage relationships were verified by analysis of the marker genotypes. All animals with discordant genotypes were eliminated from the study.

Semen samples were obtained from an additional 137 bulls to represent the general Brown Swiss population.

Markers and Genotyping. All individuals were genotyped for 82 multisite haplotypes (12), 38 variable number of tandem repeat markers (13), and 233 bovine dinucleotide microsatellites (14) that had been developed as part of the construction of a primary bovine DNA marker map. Multisite haplotypes and variable number of tandem repeat genotypes were generated as described (12, 13). Two to four microsatellite systems were amplified simultaneously in 10-μl reaction volumes from 30 ng of each template DNA. Reagent concentrations were 75 mM KCl, 15 mM Tris·HCl (pH 8.4), 2.25 mM $MgCl_2$, 0.02% gelatin, all four dNTPs (each at 0.3 mM), 1 mM of each primer, AmpliTaq (0.05 unit/μl), and [α-^{32}P]dCTP (0.1 μCi/μl; 1 Ci = 37 GBq). PCRs were set up with a Biomek 1000 robotic station (Beckman) and carried out in Techne MW2 devices (Cambridge, U.K.). Samples were denatured at 95°C for 5 min and cycled 30 times under the following conditions: 93°C for 1 min, 60°C for 1 min, and 72°C for 1 min. After addition of 1 vol of formamide dye and denaturation at 95°C for 5 min, each product (2 μl) was electrophoresed on a 7% acrylamide gel containing 32% (vol/vol) formamide, 5.6 M urea, 135 mM Tris, 45 mM boric acid, and 2.5 mM EDTA. The gels were autoradiographed for 2 h to overnight.

The following primer sequences were used for the amplification of the marker that showed linkage to the weaver locus: TGLA116.UP1, 5′-GCACAGTAATAAGAGTGATGGCAGA-3′; TGLA116.DN1, 5′-TGGAGAAGATTTGGCTGTGTACCCA-3′.

Linkage Analysis. Linkage analyses were performed with the LINKAGE package, Version 5.1 (15), on a Sparcstation II (Sun Microsystems, Milpitas, CA).

Synteny Mapping. Hybrid bovine–rodent somatic cells were prepared by fusion as described by Womack and Moll (16). *In vitro* amplification of the *TGLA116* microsatellite was performed in 50-μl reaction volumes using the same buffer and cycling conditions as above. Aliquots (100 ng) of hybrid DNA or of the corresponding rodent and bovine controls were used as templates. PCR products were electrophoresed on 3% NuSieve TM agarose gels with TAE (0.04 M tris acetate/0.001 M EDTA), stained with ethidium bromide, and visualized under ultraviolet irradiation. Segregation patterns were interpreted according to Chevalet and Corpet (17).

RESULTS

Tight Linkage Between the *TGLA116* Microsatellite Marker and the Weaver Gene. Analysis of the available pedigree information revealed that 31 of the 33 weaver cases referred to us could be traced to at least one of three sires that were acknowledged weaver carriers (identified as F1, F2, and F3). A pedigree was constructed to represent all relationships involving those three lines (Fig. 1). As no other common ancestor was a known weaver carrier, those pathways were ignored. Two totally unrelated cases were ignored, as they could provide no linkage information.

The pedigree represented in Fig. 1 is characterized by 17 consanguinity or mating loops. Because likelihood calculation taking all 17 loops into consideration would require a prohibitively long computation time with available algorithms, the pedigree was divided in two parts. Pedigree A deals with the gene flow from founder sires F1 and F2 to affected individuals, and pedigree B addresses the gene flow from founder sire F3 to affected animals. The 11 individuals located in the intersection trace back to founder F1 or F2 in pedigree A and founder F3 in pedigree B; as a consequence they were duplicated and included in both subpedigrees. To avoid extracting redundant information from these individuals, however, the relationships between the parents located in pedigree B were ignored when calculating likelihoods for pedigree A and vice versa. For example, seven of the affected individuals residing in the intersection are the offspring of founder sire F2 (marker genotype 13), located in pedigree A. When computing likelihoods for pedigree B, these individuals are considered to descend from seven unrelated sires, all with marker genotype 13. The resulting subpedigrees are still characterized by inbreeding and mating loops (two for pedigree A and five for pedigree B); however, these numbers of loops can be dealt with reasonably efficiently by using the available algorithms. To calculate the likelihoods and as requested by the LINKAGE programs (15), the remaining loops were "broken" at the individuals marked by a $ sign in Fig. 1. The choice of the individual at which to break a given loop, however, does not affect the resulting likelihoods.

It should be noted that partitioning pedigrees as done in this study can lead to information loss due to the fact that we ignore loops and to the reduction of full sibs in the intersection to half sibs. Visual examination of the genotypes, however, showed that this does not occur in this particular case.

In an initial screening, two-point logarithm of odds (lod) scores were generated using ILINK, with simultaneous estimation of the maximum likelihood allelic frequencies at the marker locus. The frequency of the weaver allele was set at 5% by following Hoeschele and Meinert (6), and a penetrance of 1.00 and identical recombination rates in both sexes were assumed.

The 78th marker tested, microsatellite *TGLA116*, gave a lod score of 7.72 at 3% recombination (Table 1). This marker has been characterized, in all populations studied so far, by three easily distinguishable codominant alleles (Fig. 2). The maximum-likelihood allelic frequencies estimated from the weaver pedigree were 0.26, 0.17, and 0.57, respectively.

Dividing the pedigree as described will affect the estimates of allelic frequencies. We reevaluated allelic frequencies in the Brown Swiss population from the sample of 137 sires by gene counting. This yielded the following values: 0.20, 0.27, and 0.53. By using these frequencies, we calculated a maximum lod score of 8.15 at 3% recombination (Table 1). Recombination rates yielding lod scores 1 unit below the maximum (0.0015 and 0.125) define the 95% confidence interval for the estimated recombination rate.

Under the assumption of full penetrance, one recombinant can be recognized in our pedigree, although with the information available we cannot determine which of the two individuals marked by a ? sign in Fig. 1 is the true recombinant. It is noteworthy that both individuals are part of the same inbreeding loop and that this obligate recombination event would have gone unnoticed if the corresponding loop had been ignored in the analysis.

Eighteen chromosome homologues carrying the weaver allele can be recognized in this kindred. At the *TGLA116* locus, 33% of these chromosomes carry allele 1, 17% carry allele 2, and 50% carry allele 3. These percentages are in accordance with linkage equilibrium between the two loci.

Because of the way it was collected, our pedigree exhibits ascertainment bias and, as a consequence, distortion of segregation at the disease locus. Any marker characterized by segregation distortion of undefined origin is expected to generate a spurious linkage. We excluded that eventuality by

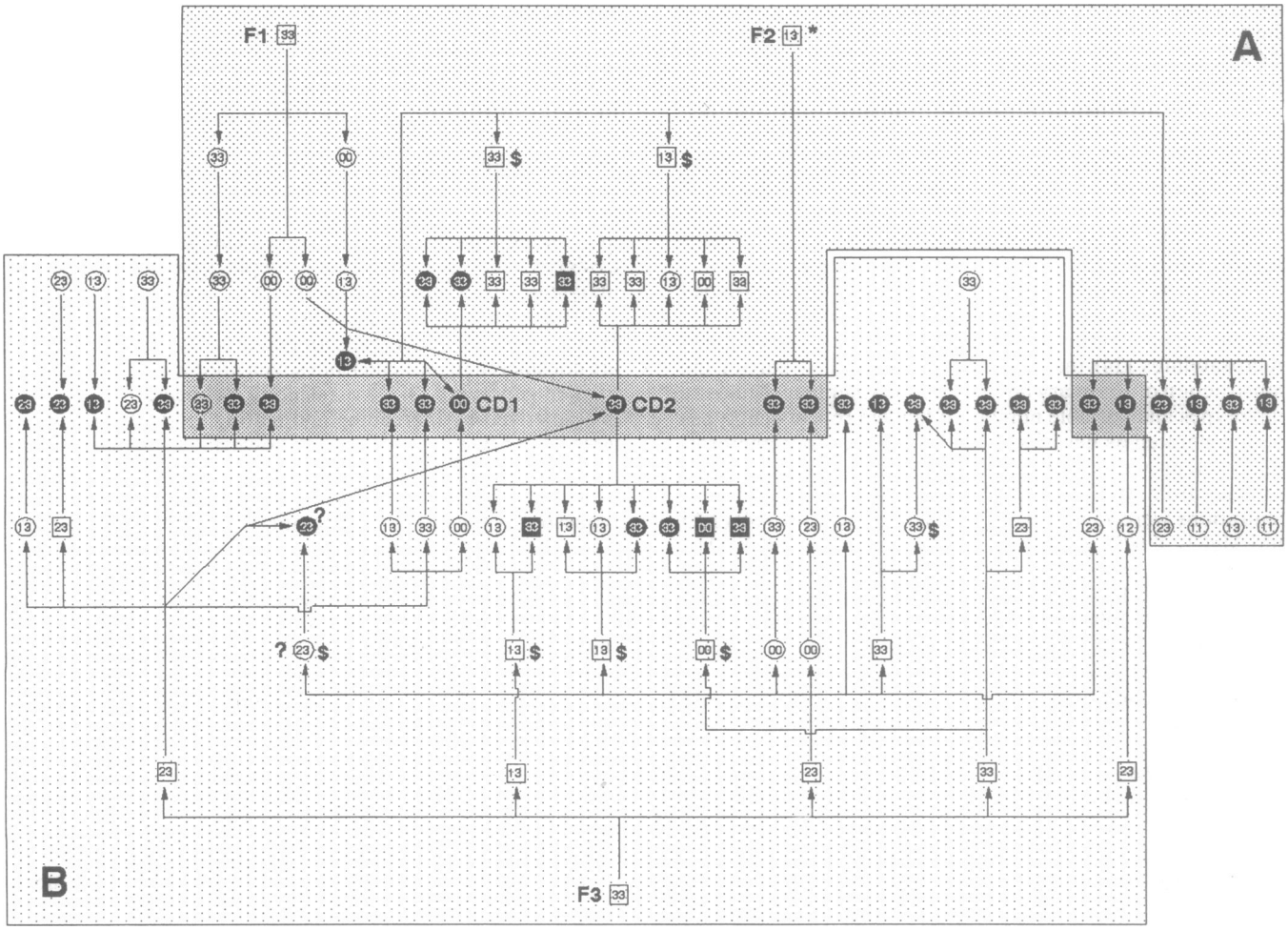

FIG. 1. Brown Swiss cattle pedigree segregating for weaver disease. Affected individuals are represented by solid circles (females) or squares (males). Parent–offspring relationships are symbolized by arrows. Individuals are only represented if genotyped for *TGLA116*, unless they are part of a pathway that leads to one of the three founder carrier sires F1, F2, and F3. The genotype for the *TGLA116* microsatellite marker is reported inside the symbol for each individual. To perform the linkage analysis and to deal with the large number of inbreeding and mating loops, the pedigree was divided into two halves, A and B. Remaining loops were cut at the individuals marked by a $ sign. Under the assumption of full penetrance, this pedigree has an obligate recombinational event involving one of the two individuals marked by a ? sign. Twenty-five of these animals were involved in a carrier detection program, in which suspected-carrier sires were mated to affected dams (CD1 and CD2) by means of induced multiple ovulation and embryo-transfer procedures.

showing Mendelian segregation of *TGLA116* in several unrelated pedigrees (data not shown).

The lod scores reported here were obtained under the assumption of full penetrance. An estimate of the actual penetrance was obtained from 25 animals from our pedigree that were involved in a carrier-detection program. Five suspected-carrier sires were mated to affected dams by means of induced multiple ovulation and embryo-transfer procedures (Fig. 1). One of the five sires generated five normal offspring and was, therefore, considered homozygous normal (probability of type I error, 3%). All four of the other sires were diagnosed as weaver carriers, as they each produced at least one affected offspring. Altogether, of the 13

Table 1. Lod scores for *TGLA116* vs. weaver

θ	0.00	0.01	0.02	0.03	0.04	0.05	0.10
a	$-\infty$	7.52	7.69	7.72	7.69	7.64	7.11
b	$-\infty$	7.96	8.13	8.15	8.12	8.06	7.49
c	3.94	3.90	3.84	3.76	3.68	3.58	3.05

For a, weaver, 5% allelic frequency, full penetrance; *TGLA116*, marker allele frequencies of 0.26, 0.17, and 0.57, estimated from the weaver pedigree. For b, weaver, 5% allelic frequency, full penetrance; *TGLA116*, marker allele frequencies of 0.20, 0.27, and 0.53, estimated from 137 Brown Swiss sires. For c, weaver, 10% allelic frequency, 50% penetrance; *TGLA116*, marker allele frequencies of 0.20, 0.27, and 0.53, estimated from 137 Brown Swiss sires.

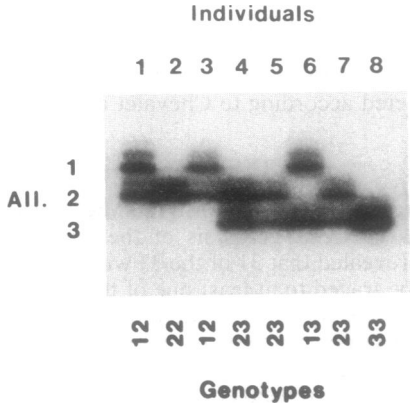

FIG. 2. Patterns were obtained by PCR amplification of the *TGLA116* microsatellite, electrophoresis in denaturing acrylamide gels, and autoradiography. Lanes contain DNA from each of eight unrelated Brown Swiss cows. *TGLA116* is characterized in all breeds tested so far by the three alleles (All.) shown here; the corresponding genotypes are indicated.

Table 2. Concordancy analysis for *TGLA116*

Synt	Chr	Conc	Corr	Synt	Chr	Conc	Corr
1		75	0.47	16		70	0.30
2		53	0.07	17	8	84	0.66
3	5	65	0.20	18		75	0.45
4	21	75	0.43	19	15	55	−0.02
5	10	45	−0.04	20	23	70	0.30
6		70	0.34	21	19	50	0.10
7		45	−0.10	22		50	−0.18
8		55	0.17	23		65	0.38
9	18	69	−0.18	24	14	60	0.24
10		60	0.24	25		33	−0.25
11		65	0.21	26		35	−0.48
12		60	0.12	27		60	0.12
13		92	0.84	28		55	0.17
14		37	−0.29	29		65	0.31
15	6	65	0.31	X	X	40	−0.24

Synt, synteny group; chr, chromosome; conc, percent concordancy; corr, correlation coefficient.

offspring generated from these four sires, eight were diagnosed as weavers. These data support the assumption of full penetrance. Nevertheless, we tested the effect of reduced penetrance by generating lod scores by assuming a penetrance as low as 50% and a concomitant increase of the weaver allele frequency to 10%. Under these assumptions, we still obtained a highly significant lod score of 3.94 with no obligate recombinant (Table 1).

We conclude that the microsatellite marker *TGLA116* is tightly linked to the bovine weaver locus. None of our other anonymous markers has yet shown linkage to either *TGLA116* or to weaver disease.

Tentative Assignment of *TGLA116* and the Weaver Gene to Bovine Synteny Group 13. *TGLA116* was mapped to bovine synteny group U13 in a panel of somatic cell hybrids (18) (Table 2). *TGLA116* showed a 92% concordance for U13, when the pattern of the *met* protooncogene was used as reference. However, the segregation pattern of *TGLA116* was 100% concordant with the pattern of three of the five genes previously mapped to U13 in this panel, inhibin-βA and T-cell receptor γ and β chain genes; using 25 hybrids, it showed only two discrepancies with *met* and three with *PGY3* (data not shown).

DISCUSSION

We have identified close linkage (3% recombination) between a microsatellite marker, *TGLA116*, and the gene causing weaver disease in cattle. Cosegregation between this marker and the disease confirms the hypothesis of a simple recessive determinism, with full penetrance, for bovine progressive degenerative myeloencephalopathy.

We found no evidence of linkage disequilibrium between the weaver and marker locus. This indicates that the mutation is probably fairly old despite the recent identification and description of the disorder (7). An alternative explanation would be the occurrence of frequent neomutations at the weaver locus, although this seems difficult to reconcile with the breed-specific distribution of the disorder.

Available algorithms to compute pedigree likelihoods (19) do not deal very efficiently with the complex pedigree structures characterizing domestic animal species, because of the large number of inbreeding and mating loops. To some extent these limitations can be alleviated by partitioning the pedigree with duplication of the appropriate individuals, as shown in this work. However, this strategy is cumbersome and can potentially lead to loss of information. This is illustrated in this study by the fact that the only obligate recombination between the marker and the disease locus would have been overlooked if the inbreeding loop involving the recombinant individual had been ignored. There is a clear need to develop more efficient algorithms to calculate likelihoods related to complex pedigrees. Gibbs sampling (20) or random-walk methods (21) may prove more appropriate for such pedigrees.

The *TGLA116* marker will allow breeders to select efficiently against weaver disorder without having to rely on lengthy and expensive progeny testing procedures. Because of the breeding structure of dairy populations, the marker will be used primarily for determining the risk status of offspring from valuable sires that are known carriers of the weaver condition. The present study has identified eight carrier sires that were also heterozygous for *TGLA116* and has defined the linkage phase of the marker with the weaver locus in these bulls. Marker genotypes will be informative for segregation of the paternal allele in 85–95% of offspring (depending on the genotype of the sire), if the genotype of the dam is available, and in 60–76% of offspring, if it is not. For these offspring the weaver status can be predicted with a high degree of accuracy that is limited, however, by the 3% recombination rate between marker and disease locus and by our degree of confidence in the estimated frequency of weaver disease in the Brown Swiss population generally. For more complex pedigrees, risk calculations can be performed using available software such as the LINKAGE programs (15). Despite the limitations inherent in genetic diagnosis based on a linked marker, *TGLA116* should allow a rapid reduction of the frequency of the disease gene to a level where it will no longer represent a significant problem for the breed.

This marker should be the starting point toward the isolation of additional markers in this chromosomal region; a panel of markers should increase the power of marker-assisted selection against the weaver defect. Detailed physical mapping of the corresponding region may ultimately lead to positional cloning of the culprit gene.

TGLA116 and, by extension, the weaver locus were tentatively assigned to bovine synteny group 13. This synteny group has as not yet been assigned conclusively to a specific bovine chromosome. Ten other genes have previously been assigned to the same synteny: the *met* protooncogene (*MET*), interleukin 6 (*IL6*), the cluster of homeobox 1 genes (*HOX1*), esterase D (*ESD*), collagen type I-α2 (*COL1A2*), inhibin-βA (*INHBA*), laminin B1 (*LAMB1*), P glycoprotein 3 (*PGY3*), and the T-cell receptor β (*TCRB*) and γ (*TCRG*) chains (21–24, ††).With the exception of *ESD*, which in the human lies on 13q, all other genes in this group are on chromosome 7 in humans: *HOX1*, *IL6*, *TCRG*, and *INHBA* are on the short arm and *COL1A2*, *LAMB1*, *MET*, *PGY3*, and *TCRB* are on the long arm. To our knowledge, none of the spinocerebellar ataxias located to date maps to either chromosome 7 or 13 in the human. Moreover, none of these chromosomes carries an obvious candidate gene for such disorder.

In the mouse, the corresponding genes are scattered across several chromosomes: *IL6* and *PGY3* map to chromosome 5; *MET*, *COL1A2*, *TCRB*, and *HOX1* map to chromosome 6; *LAMB1* maps to chromosome 12; and *INHBA* and *TCRG* map to chromosome 13. Several mutations causing signs of spinocerebellar deficiency have been reported in the mouse. One of them maps to murine chromosome 5 (reeler, *rl*); two map to chromosome 6 [deaf waddler (*dfw*), lurcher (*Lc*)]; and one maps to chromosome 13 [Purkinje cell degeneration (*pcd*)] (24). Finer mapping will be required, however, to determine whether any of these mutant genes could be the

††Neibergs, H. L., Gallagher, D. S., Dietz, A. B. & Womack, J. E., Seventh North American Colloquium on Domestic Animal Cytogenetics and Gene Mapping, July 11, 1991, Philadelphia, Vol. 7, p. 107.

murine homologue of the bovine gene causing the weaver defect.

The marker now identified for weaver disease should allow study of the associated QTL for milk production. As previously mentioned, the observed association could reflect the pleiotropic effect of a single gene. Contrary to hyperkalemic periodic paralysis and muscularity in Quarter horses (4) or malignant hyperthermia and the porcine stress syndrome/pale soft exudative meat syndrome (PSS/PSE) in pigs (5), it seems difficult from a physiological point of view to envisage a single gene affecting phenotypes as diverse as milk production and posture control. Hence, the hypothesis of two closely linked genes seems more likely. The association between milk production and weaver disease reported by Hoeschele and Meinert (6) was not only very strong but was apparently consistent across all families included in the analysis. This observation, in conjunction with the apparent ancientness of the weaver mutation, indicates that the two genes must be very tightly linked. Nevertheless, it may be possible to identify haplotypes that confer a favorable effect on milk production yet carry the wild-type allele at the weaver locus. The frequency of favorable haplotypes could then be increased in the population by marker-assisted selection. Moreover, we will be able to study the contribution of this chromosomal region to variance in milk production in other breeds. Preliminary data point toward segregating QTL for milk production in the same chromosomal region in other dairy cattle breeds.

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