The Booroola F gene is excluded from close genetic linkage with either the haemoglobin ß or follicle stimulating hormone ß loci

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ABSTRACT

Genetic variation in the beta chain of haemoglobin (HBB) and the beta chain of follicle stimulating hormone (FSHB) was analysed for linkage with the Booroola F gene in large half-sib families or a backcross Romney x Booroola Merino flock where the Booroola F gene was segregating. The half-sib families were highly informative at the HBB locus with 6 of 7 sires heterozygous (AB) for the two alleles. Recombination between the HBB and F gene loci were observed in all six families and the F gene was significantly excluded from within 15 centimorgans (cM) of HBB. A cDNA probe for the FSHß gene detected an insertion/deletion polymorphism close to exon 3 of the gene. Recombination was observed in 4 pedigrees in the half-sib families and the backcross flock and the F gene was excluded from within 5 cM of the FSHB gene.

Keywords Genetic linkage, recombination, Booroola F gene, haemoglobin, FSH.

INTRODUCTION

The Booroola Merino breed carries a major autosomal gene, designated the F gene, that increases ovulation rate (Piper and Bindon, 1982; Davis *et al.*, 1982). Specific physiological differences have been identified in females carrying the F gene (for reviews see Bindon and Piper, 1986; McNatty and Henderson, 1987), but the genetic mutation responsible for the increased ovulation rate is unknown. The only method available to determine the phenotype of females carrying the putative gene is repeat measurements of ovulation rate, while the phenotype in males must be determined by progeny test or pedigree records. Therefore a marker for the gene is essential for the sheep industry to effectively use the Booroola F gene.

In healthy sheep, protein electrophesis of HBB resolves two common autosomal alleles (A and B) and a significant association between the HBB B allele and the F gene in Booroola Merino x Romney ewes has been reported (Dratch *et al.*, 1986). This association was determined by comparing HBB types in groups of animals with and without the F gene and not by the methods of genetic linkage within pedigrees. Genetic linkage can be defined as the occurrence of two loci sufficiently close together on a chromosome such that the loci are inherited together and must be determined by examining the inheritance of a marker and the gene of interest in suitable pedigrees. Linkage between HBB and the F gene must be demonstrated before HBB can be considered as a possible marker for the F gene.

One reason for interest in HBB as a marker locus for the F gene is that HBB is on the same chromosome as the FSHB in both humans (chromosome 11) and cattle (chromosome 15). Circulating concentrations of FSH are higher in FF compared with ++ ewe lambs at 6 weeks of age (Montgomery *et al.*, 1989), and in both intact and ovariectomised adult ewes (McNatty *et al.*, 1987; McNatty *et al.*, 1988). FSHB gene transcripts for both cattle (Maurer and Beck, 1986) and sheep (Mountford *et al.*,1989) and the FSHB gene in cattle (Kim *et al.*, 1988) have recently been cloned and sequenced. The cloned FSHB gene sequences can be used as probes to detect restriction fragment length polymorphisms (RFLPs) around the FSHB genes to directly test whether alterations around the FSHB gene locus in sheep represent the genetic mutation responsible for the increased ovulation rate of sheep carrying the F gene.

The aims of these experiments were to identify genetic variation around the ovine FSHB gene and to test for genetic linkage between HBB, the FSHB gene

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and the Booroola F locus in pedigrees where the F gene is segregating.

MATERIALS AND METHODS

Animals

DNA samples were collected from unrelated individuals in a Romney flock and a Booroola Merino flock to screen for RFLPs around the FSHB gene. The alleles at the HBB and FSH loci were scored in individuals in flocks where the Booroola F gene was segregating. Samples were obtained from a backcross Booroola Merino x Romney flock where ewes carrying the F gene were backcrossed through successive generations to Romney sires purchased from ram breeding flocks. The pedigrees consisted of 2-4 generations. All Romney sires used in the flock were homozygous non-carriers (++) at the F locus. Samples were also obtained from the sires and all available daughters (range 10-43) of 7 progeny tested rams that were heterozygous for the F gene. Dams mated with the progeny-test rams were all homozygous non-carriers (++) at the F locus, but were never individually identified and no samples could be obtained from the ewes. Venous blood was taken in fractions of 10-15 ml using heparin as an anticoagulant and duplicate samples processed for protein analysis or DNA extraction. DNA was extracted from white blood cells following Proteinase K digestion (Boehringer Mannheim, West Germany) using methods involving phenol extraction or high salt precipitation (Montgomery and Sise, 1990).

Protein Electrophoresis

Haemoglobin types were resolved by starch gel electrophesis using Tris borate (electrode : 500mM , gel : 10 mM) buffer pH 8.6. Lysates of washed red blood cells were loaded either fresh or after storage at -80°C and electrophoresis continued for 4 h at 200 volts. Haemoglobin typing was consistent with the method used by Dratch *et al.*, (1986).

RFLP Analysis

From 3-10 µg of DNA were digested with appropriate restriction enzymes using 5 units per µg of DNA under the manufacturer's recommended conditions. The restricted DNA was run in a 0.8% agarose gel in TAE buffer (pH 7.8, Tris-acetate 5 mM, 1 mM EDTA) at a constant voltage overnight. The gels were photographed under UV light, and transferred to Hybond N+ (alkaline transfer, Amersham, England) according to the manufacturer's recommended procedures.

A bovine FSHB cDNA probe (Maurer and Beck, 1986) was verified by restriction analysis and isolated insert was labelled with a^{32}P-dCTP by nick translation (Rigby *et al.*, 1977). The filters were pre-hybridised for at least 30 min in 6xSSC (1xSSC = 15 mM sodium citrate, 150 mM sodium chloride), 5xDenhardt's (100xDenhardt's=2% Ficol, 2% polyvinylpyrrolidone, 2% BSA), 0.5% SDS, 20 µg/ml denatured sonicated salmon sperm DNA at 63°C and hybridised overnight at 63°C after addition of the labelled probe. The filters were rinsed in 2xSSC, 0.1% SDS, washed twice in 2xSSC, 0.1% SDS at 63°C (15 min and 30 min) and washed once in 0.5xSSC,1.0 % SDS at 63°C for 10 min. The filters were autoradiographed using Kodak XAR-5 films at -80°C with intensifying screens. Filters were reprobed after stripping twice in 0.1% SSC, 0.1% SDS heated to 100°C and poured over the filters at 15 min intervals.

Data analysis

Phenotypes at the F locus were assigned from at least 2 records of ovulation rate (Davis *et al.* 1982). In the backcross flock, the number of records available ranged from 2 records for ewes born in 1987 up to 10 records for ewes born in 1978. Daughters of progeny test rams had 2 - 4 records of ovulation rate. Two-point lod scores (z) at different recombination fractions (theta) were computed for pedigrees in the backcross flock using the computer program MLINK of the LINKAGE package (Lathrop and Lalouel, 1984) assuming equal male and female recombination rates. In the progeny-test pedigrees, all daughters of sires that were heterozygous at the test loci were scored and the data were analysed only for daughters that were homozygous at the test locus. Recombinants were scored directly in the offspring of these rams and the lod scores for different recombination fractions calculated directly for pedigrees where the phase is unknown (Ott, 1985).

RESULTS

The two common alleles at the HBB locus (A and B) were present in the flocks. The mean allele frequencies differed for the backcross flock and in daughters of the half-sib families (Table 1).

TABLE 1 Allele frequencies for protein variation at the Haemoglobin ß(HBB) locus (A and B alleles) and for an insertion/deletion RFLP at the follicle stimulating hormone (FSHB) locus (A1 and A2 alleles) in a backcross Romney x Booroola Merino flock and half-sib daughters of sires heterozygous for the alleles.

Locus	Flock	N	Allele 1	Allele 2
HBB	Backcross	150	0.61	0.39
	Half-sib daughters	164	0.44	0.56
FSHB	Backcross	85	0.31	0.69
	Half-sib daughters	32	0.47	0.53

The half-sib families were highly informative at the HBB locus with 6 of 7 sires heterozygous (AB) for the two alleles. The allele inherited from the sire could be identified in a total of 80 daughters (homozygous AA or BB at the HBB locus) from among the six families. Recombination between the HBB and F gene loci were observed in all six families and the combined lod score calculated for the six families was -3.64 at a recombination fraction of 0.15 (Table 2).

TABLE 2 Analysis of linkage between the F gene and the haemoglobin (HBB) and follicle stimulating (FSHB) loci: Lod scores (z) calculated for different recombination fractions combined for 6 half-sib families for HBB and two half-sib families and 11 pedigrees in a backcross flock for FSHB.

Recombination fraction	HBB	FSHB
0.00	-∞	-∞
0.05	-14.01	-3.22
0.10	-7.10	-1.33
0.15	-3.64	-0.47
0.2	-1.63	-0.04
0.3	-0.03	0.19
0.4	0.29	0.06

The cDNA probe for bovine FSHB detected multiple bands in samples of DNA from Romney and Booroola Merino sheep cut with a number of different restriction enzymes. The band intensity was strong and variable bands were detected with several restriction enzymes tested. A series of experiments demonstrated that variable bands between individuals with different enzymes were caused by an insertion/deletion polymorphism close to exon 3 of the FSHB gene. Segregation of the insertion/deletion polymorphism was demonstrated in a small sheep pedigree (Fig.1) and a larger sample of pedigrees in the backcross flock. The two alleles at the insertion/deletion site (designated A1 and A2) were common in the sheep populations studied, although not at equal frequencies (Table 1).

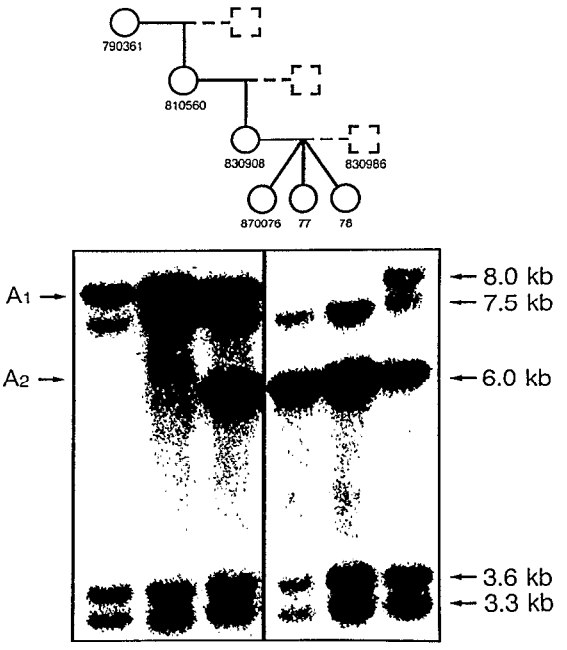

FIG 1 The inheritance of an insertion/deletion RFLP close to the FSHB locus identified by the variable bands (A1 = 8.6 kb, A2 = 6.0 kb) in sheep DNA samples digested with the restriction enzyme MspI.

Two of the sires of the progeny-test families were heterozygous for the insertion/deletion at the FSHB locus. The FSHB allele inherited from the sire could be determined in 10 and 5 daughters from the two sires respectively. Recombination between the FSHB

and F gene loci were observed in 4/10 and 1/5 daughters of the two families. Data from 11 pedigrees within the backcross flock were analysed for linkage between the F gene and FSHB loci. Recombination between the two loci was observed in two pedigrees (z= -∞ at theta = 0.0). The remaining pedigrees yielded little information on recombination since many of the pedigrees were fragmented. The combined lod score (z) calculated from the half-sib and backcross pedigrees was -3.22 at a recombination fraction (theta) of 0.05 (Table 2).

DISCUSSION

These results show that the Booroola F gene is not closely linked genetically to either HBB or the FSHB locus. Genetic recombination was observed in six informative families of half-sib daughters of rams that were heterozygous at both the F gene and HBB loci. The exclusion limit, or recombination fraction at which z = -2, determines the genetic distance around any locus from which the F gene can be excluded (Ott, 1985). One cM is the distance along the chromosome which gives a recombination fraction of one percent. Recombination rates vary for different sites along the DNA, but 1cM is roughly equivalent to a physical distance of 1 million base pairs. The combined estimate for all families indicates that the F locus is not within 15 cM of the HBB locus. Therefore the significant association previously reported between HBB B allele and the F gene (Dratch *et al.*, 1986) is not the result of close genetic linkage between the two loci. The association probably reflects the higher frequency of the B allele in the Merino population where the F gene originated compared with the Romney population (Dratch *et al.*, 1986).

The FSHB gene is located on the same chromosome as HBB in both humans (chromosome 11) and cattle (chromosome 15). Therefore, the FSHB gene was tested for linkage as a marker likely to originate from the same chromosomal location in sheep and because FSH concentrations are higher in F gene carriers. Concentrations of FSH are consistently higher in females carrying the F gene, at 6 weeks of age (Montgomery *et al.*, 1989), and in intact and ovariectomised adult ewes (McNatty *et al.*, 1987; McNatty *et al.*, 1988). A bovine FSHB cDNA probe (Maurer and Beck, 1986) detected variable bands in digests of sheep genomic DNA with several restriction enzymes resulting from an insertion/deletion polymorphism, approximately 2 kb in size, located downstream to exon 3 of the FSHB gene (Montgomery *et al.*, 1990). The half-sib families were less informative at the FSHB locus since only 2 of the 7 sires were heterozygous for the insertion/deletion RFLP. Combined with the pedigrees from the backcross flock, genetic recombination was observed in 4 pedigrees and the F gene was excluded from within 5 cM of the FSHB gene.

A recombination distance of 5 cM far exceeds the distance for direct interactions of controlling regions influencing gene transcription and therefore, the mutation responsible for the increased ovulation rate in sheep carrying the F gene is not the result of changes to the structure of the FSHB gene. Increased transcription of the FSHB gene resulting in higher circulating concentrations of FSH may be the cause of increased ovulation rate in F gene carriers. However, the increased transcription would have to result from the interaction of another gene product with the FSHB gene.

ACKNOWLEDGEMENT

We thank Dr R.A. Maurer, (University of Iowa, Iowa City, USA) for providing the bovine FSHß cDNA clone.

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