Genetic markers in Inverdale (FecX) sheep

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ABSTRACT

The Inverdale gene (FecX) is carried on the sheep X-chromosome. Males carrying the Inverdale gene always pass a copy to their daughters, but not to their sons. Females carrying a single copy will pass the gene on to half of their offspring (both male and female). Progeny testing to determine which rams carry the gene, and which offspring have inherited, is time-consuming. Successful management of the Inverdale gene will require a simple, quick, inexpensive test to distinguish carriers and non-carriers.

A research programme has been initiated to develop a DNA test using DNA markers which will identify carrier animals at birth. Our initial strategy to find suitable markers was to build a map of genes and DNA markers on the sheep X-chromosome as a framework for looking for the Inverdale gene. We have determined the relative positions of twenty genes and DNA markers on the X-chromosome by looking at the pattern of inheritance of the X-chromosome markers in sheep pedigrees established in flocks at Invermay and Tuatapere. We estimate that 80% of the chromosome has been eliminated in our search to the Inverdale gene. Efforts are now directed towards identifying the particular region of DNA in which the Inverdale gene might lie. Suitable markers must be close enough to the Inverdale gene to be always inherited with Inverdale. Once such markers are identified a test can be developed to determine the Inverdale status of animals. Adaptation of the current techniques will enable DNA to be isolated from a few drops of blood, a mouth swab, or the ear tissue from a newborn animal.

Keywords: X-chromosome; genes; linkage; DNA markers; sheep (Ovis aries).

INTRODUCTION

The Inverdale gene (FecX) is located on the X-chromosome in sheep (Davis *et al.*, 1991a). Previous papers have discussed the advantages that this gene confers, some of the potential disadvantages, and the present necessity for progeny testing and laparoscopy to determine carriers of the gene (Davis *et al.*, 1993; 1994). It is clear that we need some way of telling at a young age which animals carry the gene and which do not. To test for this we will need to find either the Inverdale gene itself, or a DNA marker located near the Inverdale gene.

This paper describes the progress that we have made in constructing a map of the sheep X-chromosome, our search for the Inverdale gene and what our expectations are for the future.

Sheep DNA is estimated to contain 100,000 genes which are spread among 27 pairs of chromosomes. There are 26 pairs of autosomes and two sex chromosomes (XY in males and XX in females). The sheep X-chromosome is the fourth largest chromosome and is estimated to contain about 5% of the total DNA content of the cell (Ohno, 1973). The X-chromosome is known to carry a number of genes common in all mammals. Two well-known examples are the genes for red/green cone pigments (RCP and GCP) and blood clotting factor (F9). When defective, these genes cause colour blindness and haemophilia B. When this work began, very little was known about the relative locations of genes on the sheep X-chromosome. We set out to find and order genes on the sheep X-chromosome to provide a framework within which to locate the Inverdale gene. To do this we used an approach called linkage analysis which involves testing X-chromosome genes in individuals from large families to look for common inheritance of adjacent genes.

Genes and Markers

Genes are arranged along the DNA of a chromosome in a defined order that is stably inherited from one generation to another. A gene is a region of DNA that has a specific function (and usually codes for a protein), whereas a marker can be thought of as a region of DNA which may or may not have a specific known function, but which is also stably inherited. A marker may be part of a known gene or lie between genes on the chromosome. Known genes and markers were both used in this study. Known genes were analysed by restriction fragment length polymorphic (RFLP) differences between the two copies of the gene carried in each individual (Montgomery *et al.*, 1992). All our other markers were simple dinucleotide repeats (microsatellites) which exhibited length variations between individuals (Crawford *et al.*, 1991).

From our knowledge of the X-chromosomes in other species it is apparent that the same genes are present on the X-chromosomes of all mammals. This phenomenon was predicted by Ohno (1973) and appears to hold true for all placental mammals. However, the shape (position of the centromere) and the banding patterns differ between human, mouse (Fig. 1), cattle and sheep (Hediger *et al.*, 1991) X-chromosomes, and the orders of genes along the X-chromosome differ widely between species (Fig. 1). Therefore, although we were able to look to the X-chromosomes from other species to find suitable genes and markers, we could not infer an order for these markers along the sheep X-chromo-

some. Gene and microsatellite markers for this study were either obtained from other laboratories around the world that had isolated copies of X-chromosome genes and markers, or made in our own laboratory by cutting sheep X-chromosome DNA into tiny fragments and isolating dinucleotide repeat sequences.

FIGURE 1: Diagram of human and mouse X-chromosomes. Some known genes are indicated by their abbreviated symbols.

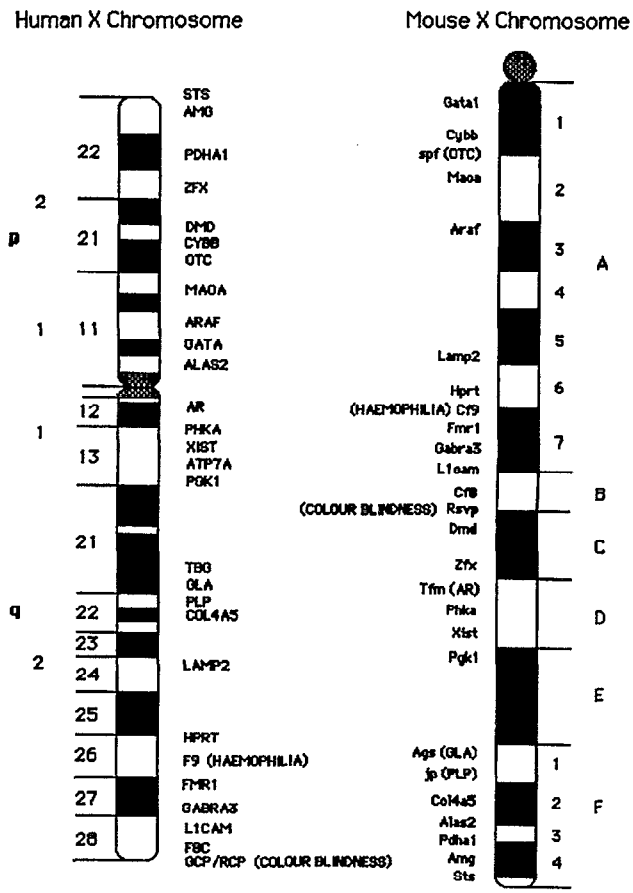
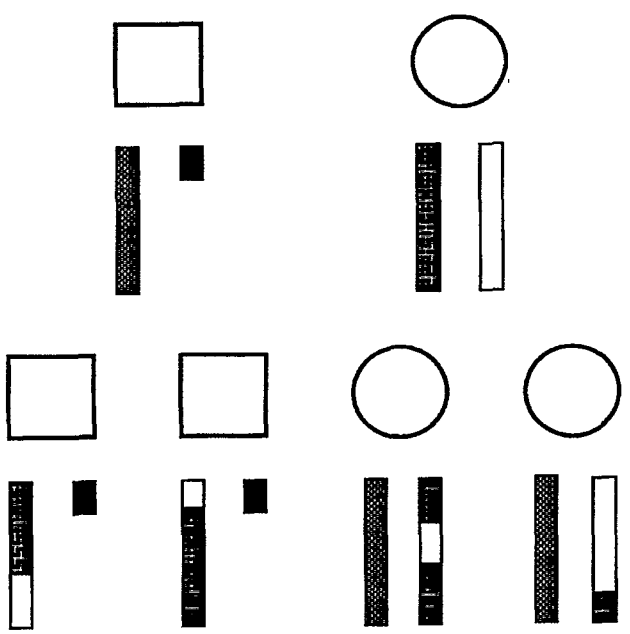


FIGURE 2: Inheritance of the X-chromosome. Long bars illustrate X-chromosomes, the short black bar illustrates the Y-chromosome, circles are females and squares are males.



Linkage

The aim of linkage analysis is to determine the relative positions of adjacent genes or markers on a chromosome. During the production of sperm and eggs for a new offspring, genes along a pair of chromosomes in the parent are shuffled during recombination so that the offspring receives a mixture of genes from each parent. Figure 2 illustrates the inheritance of X and Y chromosomes from two parents to four offspring. Females receive two copies of each X-chromosome gene and males receive one copy. Due to recombination in the female, the X-chromosome inherited from the mother contains a mixture of both of her X-chromosomes, which pair with each other during meiosis and exchange genetic material. The X-chromosome from the male is virtually passed on intact because the very small region of homology between the X and Y-chromosomes only allows pairing at the very tip of the X-chromosome. The closer together two genes are on a chromosome the more likely they are to be inherited together, whereas two genes that lie far apart on a chromosome are much more likely to be passed on separately. This phenomenon allows us to determine the orders of genes along a chromosome based on how often they are inherited together in a large number of animals. We measured the frequency at which individual copies of genes and markers were passed on together in all animals in our pedigrees.

MATERIALS AND METHODS

Flocks

Animals were obtained from two major flocks. The Inverdale pedigree contains 197 animals of known Inverdale carrier status from the AgResearch flock (Davis *et al.* 1992) and the Tuatapere pedigree contains 285 animals farmed by Arnold Gray (Gray and Davis, 1995). The Inverdale gene is segregating through the Tuatapere pedigree from three sires used in the first generation. The combined pedigree has a three-generation structure with 10 rams in the first generation mated to flock Romney ewes, to produce 145 ewes in the second generation. These were mated to 14 rams, and samples were collected from 207 female and 104 male offspring in the third generation. Inverdale carrier status of animals in the Inverdale pedigree was confirmed by progeny testing and presence of streak ovaries following laparoscopic examination (Davis *et al.* 1992).

Procedure of analysis

Blood samples (15 - 50 ml) were obtained from all available animals in the two flocks and DNA was extracted as previously described (Montgomery and Sise, 1990). Correct assignment of parentage is vital for linkage analysis and this was confirmed for each animal by DNA testing using several diagnostic markers. Anomalous animals were not included in the final mapping pedigree. X-chromosome genes and markers from other species were tested for homology to sheep DNA. X-linkage of homologous genes/markers was

confirmed by Mendelian inheritance in small family pedigrees to ensure they were on the X-chromosome in sheep. The relative positions of genes and markers along the sheep X-chromosome was then assigned using the linkage programmes such as CRIMAP (Lander and Green, 1987) modified for use with animal pedigrees (Dodds *et al.*,1993).

RESULTS AND DISCUSSION

Table 1 summarises the results obtained from 127 genes or markers tested. The numbers of markers from various species reflect their current availability. Initially, very few genes were available from species more closely related to sheep. Most genes were obtained from laboratories working in the human medical field. As increasing numbers of microsatellite markers became available from cattle (Barendse *et al.*, 1994; Bishop *et al.*, 1994), and our own markers from sheep DNA (Hanrahan *et al.*, 1994), we concentrated on these more informative markers. Many genes from other species contained sequences which recognised repetitive DNA sequences in sheep DNA. This masked the specific gene in sheep, and these genes were unable to be used. Other genes recognised DNA sequences on chromosomes apart from the X-chromosome and these genes were also unable to be used because they could not be verified as being specifically on the X-chromosome. Only those genes which identified single copy genes in sheep that could be verified as X-linked and inherited in a Mendelian fashion were used in our map. Seven known genes have been mapped to the sheep X-chromosome.

TABLE 1: Summary of numbers of genes and markers analysed for X-linkage in sheep.

GENES

102 markers to 46 known genes on the X chromosome
84 human, 7 mouse, 8 rat, 2 cattle, 1 rabbit

9 were able to identify similar genes in sheep
7 are included in our map

MARKERS

25 markers to regions of DNA not assigned to known genes
2 human, 14 cattle, 6 sheep, 1 pig, 2 hamster

18 were able to identify similar regions of the X chromosome in sheep
13 are included in our map

Microsatellite markers have proved to be more informative. The more distantly related the species, the less likely the marker was to detect a corresponding sequence in sheep, but most cattle markers were able to identify similar DNA regions in sheep. This reflects the extensive homology which has already been demonstrated between sheep and cattle DNA. Thirteen microsatellite markers have been included in this study.

At present our map contains 20 genes or markers, and appears to span most of the sheep X-chromosome. At least two of the markers lie in the pseudoautosomal region of the chromosome. This region is thought to be located near the centromeric tip of the sheep X-chromosome (Dai *et al.* 1994). The position of the Inverdale gene has not been pinpointed exactly and it is not flanked by any of our closely linked markers. However, the markers span most of the chromosome and we estimate that 80% of the chromosome has been eliminated from the search. We are now focussing our efforts on the remaining 20% in order to find the gene. To pinpoint the gene and provide a reliable test we need to find flanking markers on either side of the gene which are close enough to be inherited with the Inverdale gene almost all of the time. At present, DNA is extracted from 20 to 50ml of blood and we need DNA from the animal being tested as well as both parents (and often grandparents) to be able to predict Inverdale status. Our current markers are still not close enough to the gene to be completely accurate so predictions are still verified by progeny testing and laparoscopy. We are developing new markers and aiming for a closely linked marker which will provide an accurate test that is simple, quick, can be automated and requires minimum fuss and sample handling. DNA is present in all cells, so a small sample of ear tissue at tagging, a few wool follicles, a mouth swab or a few drops of blood should be all that is required. To assist with these analyses, and eventually offer a commercial diagnostic facility, AgResearch is currently establishing a commercial DNA service called GenomNZ.

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