Mapping Animal Genomes : Molecular Tools for Animal Production

Jonathan E. Beever
Department of Animal Sciences,
University of Illinois at Urbana-Champaign

The identification of individual genes that control economically important traits has been an attractive proposition for many years. It is this impetus that has lead to the tremendous effort to characterize animal genomes during the past 5 to 10 years. It is believed that upon characterization, we will develop a set of molecular tools that can be used to predict or enhance livestock performance. The following summary describes the current status of these tools and a practical example of their use in identifying the genetic mutation causing hereditary chondrodysplasia in sheep.

Initial efforts to characterize animal genomes have included the production of various rudimentary genetic linkage maps composed of highly polymorphic genetic markers. Maps such as these have been produced in cattle(Bishop et al., 1994, Barendse, et al., 1994, Georges et al., 1995, Ma et al., 1996), swine (Rorher, et al., 1994 ; Archilbald et al., 1995), sheep(Crawford et al., 1995) and poultry(Bumstead, 1992). Over the past year secondary maps of higher marker density have also been reported for cattle(Barendse et al., 1997 ; Kappes et al., 1997). Currently, there are more than 1,700 genetic markers mapped in cattle and swine, more than 600 in sheep and greater than 1,000 mapped in chickens. In addition to the saturation of genetic maps with anonymous DNA markers, research has also focused on the use of the highly developed human and mouse genome maps for mapping orthologous regions in the less defined agricultural species, i.e., comparative gene mapping. Heterologous chromosome painting, called Zoo-FISH, has broadly defined the boundaries of genome evolution among several species(Solinas-Toldo et al., 1995 ; Chowdhary et al., 1996) and the number of individual structural genes mapped has reached greater than 200 in most agricultural species.

Thus, with the tremendous progress made over the past 5 years we are now equipped with the tools to perform genome-wide searches for genes influencing economically important traits. Indeed, several studies aimed at the identification of genes affection production or performance traits have already been conducted in both cattle and swine resource populations (Georges, et al., 1995 ; Andersson et al., 1994). Although conceptually, animal genome maps were developed to identify genes influencing traits of polygenic inheritance, more immediate success has been in the mapping or identification of loci for single gene traits in several species including fecundity and muscular hypertrophy in sheep(Montgomery et al., 1993 ; Cockett et al., 1994) and double muscling in cattle(Grobet et al., 1997).

Most recently we have identified the gene causing ovine hereditary chondrodysplasia using the above described mapping tools. Ovine hereditary chondrodysplasia, commonly called Spider Lamb Syndrome(SLS), is a semi-lethal congenital disorder that was first identified in young lambs during the mid-1970's. The syndrome has since surfaced in several sheep breeds within the last two decades including North American Suffolks and Hampshires, and U.S. Southdowns, Shropshires and Oxfords. Additionally, there are reported cases of SLS in New Zealand and Australia following the importation of several U.S. Suffolk sheep into Australia in the early 1990's.

Several structural abnormalities are associated with the syndrome including abnormally long, "spider-like"legs, humped and twisted spines, deformed ribs and sternebra, facial deformities, lack of body fat and underdevelopment of muscle. Radiological islands of ossification(Vanek et al., 1989). Histologic examinations of vertebrae and long bones indicate and increase in width of the zone of proliferation, as well as hypertrophy and unevenness of the growth cartilage.

It is believed this disorder arose as a mutation in a Suffolk genetic line that was used heavily during the late 1960's because of desirable production qualities. Breeding studies have established that the gene responsible for the disease has an autosomal recessive mode of inheritance(Thomas and Cobb, 1986). While dramatic culling of all suspected carriers would reduce the frequency of the disease, it is a tedious and expensive process. Progeny testing of potential breeding rams is another method of reducing gene frequency but, it is also costly. Because of the drawbacks of traditional animal breeding methods, a molecular

genetic marker for SLS will be and important tool for elimination the SLS defect from sheep populations. Thus, the objective of the project was to identify a genetic marker that segregates with the SLS gene and subsequently identify the causative mutation.

In 1991, a resource flock segregation for SLS was established at Utah State University.

During 1992－94, blood and/or tissue samples were collected from 39 spider and 86 normal lambs. These lambs were offspring of a heterozygous ram(referred to as "Greyhound") that had been mated to 52 ewes, 14 of which were his daughters. From 1995－97, sample were collected from 39 spider and 135 normal lambs that were offspring of a second heterozygous ram(referred to as "Honeyville"). This ram was mated to 40 ewes, of which 4were his own daughters and 15 were daughters of Greyhound. Forty of these ewes, including 11 "Greyhound" daughters and 1 Honeyville daughter, were obligate carriers of the SLS mutation in that they produced at least one Spider lamb during the time they were in the USU flock.

Phenotypes for SLS were determined in the lambs based on three measurements including physical appearance, radiographs of the anconeal process, and histological examination of chondrocytes in the anconeal process and the sternebrae. A lamb that showed angular limb deformities, kyphosis, scoliosis, roman nose and/or sternal displacement was considered positive for physical signs of SLS. A lamb was judged to have positive radiographic signs for SLS if islands of ossification were present around the anconeal process. A lamb exhibiting chondrodysplasia on histological examination was considered histologically positive for SLS. Lambs with two or more positive measurements were recorded as spiders, those with no positive measurements were recorded as normal, and those lambs with only one positive measurement were considered ambiguous(25/301 or 8.3%) and therefore, not included in the data.

A genome scan using microsatellite markers was then initiated. Makers were identified based on information from the existing genome maps of cattle and sheep and were selected to systematically search the genome, having markers spaced about 20 centimorgans(cM) apart. In total, 551 markers were obtained from researchers or commercially synthesized using published primer sequences. Of these markers, 503(91.3%) successfully amplified ovine DNA using protocols developed in the USU laboratory. Greyhound and Honeyville were informative(i.e., heterozygous) for 181(32.8%) and 234(42.5%) of the markers, respectively. After genotyping the pedigrees for 117 markers, significant linkage was detected between a marker and the SLS gene, with a combined lod score of 4.71 at 26% recombination. Three additional markers that had been previously mapped to this region were then used to refine the genetic map position of SLS. A recombination frequency of 0.032 with lod score of 16.22 was obtained for the closest microsatellite marker.

A comparative mapping approach was initiated to identify candidate genes that are located in this genetic region. Comparative mapping data for both sheep and cattle indicate that the distal end of ovine chromosome 6 corresponds to the distal end of human chromosome 4, specifically 4p 16.3(Load et al., 1996). Fortuitously, amongst the possible candidate genes was one causing skeletal genetic defects in humans and mice, thus, making it an obvious choice for investigation. Oligonucleotide primers were designed from the available human and mouse sequences and amplified fragments screened by single－strand conformational polymorphism(SSCP) analysis. Successful amplification was obtained from a fragment containing 5 of the gene's known exons. Within this fragment a SSCP was identified that segregated with SLS in the USU pedigrees. Subsequent cloning and sequencing of the fragment revealed a single point mutation that caused a non－conservative amino acid substitution within a functional domain of the protein.

Over the past 6 months, 1017 samples from more than 45 flocks have been collected to validate the role of the mutation in SLS. Five－hundred forty－nine of these animals were of known SLS genotype－194 homozygous normal, 174 known carriers(parents of spider lambs) and 181 spider lambs. Of the normal and known carrier sheep, the analysis of the mutation accurately classified all 369 properly(i.e, homozygous normal or heterozygous). However, of the 181 spider lambs, 6 were heterozygous for the mutation. These 6 could be the result of human error such as misclassification of phenotype or switching of the DNA samples. Alternatively, these lambs could be providing us, not yet understood, insight to how the

defective gene functions.

In summary, the identification of the gene responsible for SLS is an example of what we can expect as the era of molecular animal breeding comes of age. As our knowledge of animal genomes and the genes within them grows the identification of genes that influence other economically important traits will also increase.

Reference

Andersson, L., Haley, C.S., Ellegren, H. et al.(1994) Science 263, 1771-1774.

Archibald, A.L., Haley, C.S., Brown, J.F., et al.(1995) Mann Genome 6, 157-175

Barendse, W., Armitage, S.M., Kossarek, L.M., et al.(1994) Nature Genet. 6, 227-235

Barendse, W., Vaiman, D., Kemp, S.J. et al.(1997) Mamm Genome 8, 21-28

Bishop, M.D., Kappes, S.M., Keele, J.W., et al.(1994) Genetics 136, 619-639.

Bumstead, N. and Palyga, J.(1992) Genomics 13, 690-697.

Chowdgary B.P., Fronicke L., Gustavsson I. & Scherthan H. (1996) Mamm Genome 7, 297-302.

Cockett, N.E., Jackson, S.P., Shay, T. L., et al.(1994) PNAS 91, 3019-3023.

Crawford, A.M., Dodds, K.G., Ede, A.J., et al.(1995) Genetics 140, 703-724.

Gerges, M., Nielson, D., Mackinnon, M., et al.(1995) Genetics 139, 907-920.

Grobet, L., Martin, L.J.R., Poncelet, D., et al.(1997) Nature Genetics 17, 71-74.

Lord, E., Lumsden, J.E., Da Y., et al.(1996) Mamm Genome 7, 373-376.

Ma R.Z., Beever J.E., Da Y., et al.(1996) J Heredity 87, 261-71.

Montgomery, G.W., Crawford, A.M., Penty, J.M., et al.(1993) Nature Genetic 4, 410-414

Rorher, G.A., Alezander, L.J., Keele, J.W., et al.(1994) Genetics 136, 231-245.

Solinas-Toldo S., Lengauer C. & Fries R.(1995) Genomics 27, 489-96.

Thomas, D.L., Cobb, A.R.(1986) Sheep Magazine 7, 44-46.

Vanek, J.A., Walters, P.A., Alstad, A.D.(1989) JAVMA 194, 244-248.