CONGENITAL HYPOTRICHOSIS IN THE SICILIAN VALLE DEL BELICE SHEEP

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INTRODUCTION

Sheep of the Valle del Belice breed are reared widely throughout Sicily (Italy), with about 60 000 mature ewes. Both male and female Valle del Belice lambs can be born without any hair development. Such affected animals, called hypotrichotic sheep (Figure 1), have no hair development under the wool coat and have a lower density of wool fibres.

Figure 1. Hypotrichotic Valle del Belice sheep

Congenital hypotrichosis is present in several mammalian species and consists of a partial or complete absence of hair coat at birth. The hypotrichotic phenotype, was already described in the Karakul (Nel, 1964) and Australian Poll Dorset (Dolling and Brooker, 1966 ; Mackie and McIntyre, 1992) sheep breeds. These authors hypothesised that an autosomal recessive gene is responsible for this disorder, and assumed that the gene was inherited from distant ancestors of the sheep. So far no genetic study in the ovine species has been carried out to verify this hypothesis. If the disorder is under recessive genetic control, the hypotrichotic phenotype is observed only when the allele is present in the population with reasonable frequency. Hypotrichotic lambs in the Valle del Belice flocks are slaughtered soon after birth because the farmers do not accept them. At the same time all animals with a normal phenotype, including heterozygous carriers, remain in the flocks and these ewes and rams are used as reproducers. The farmers believe that the female offspring of carrier rams have a higher milk production.

Furthermore farmers often use sires from their own flock. Therefore the hypotrichotic phenotype has spread throughout the Valle del Belice population. This paper presents a study on the congenital hypotrichosis disorder in the Valle del Belice population.

MATERIAL AND METHODS

Description of the hypotrichotic phenotype in the Valle del Belice population. In order to test the genetic control of the hypotrichotic disorder planned matings (*test-cross* and *back-cross*) have been undertaken. With the aim of excluding chromosomal structural anomalies, the cytogenic analysis of the karyotype of affected and normal animals was carried out. Blood samples from 17 Valle del Belice ewes with normal (n = 2) and hypotrichotic (n = 15) phenotypes were collected. The normal individuals were most probably, with regard to their pedigree, heterozygous for the hypotrichotic mutation. One ml of blood was inoculated into a bottle containing 9 ml of RPMI culture medium and 1 % of pokeweed mitogen, an agent which causes lymphocytes to become mitotically active in cell culture. The cells were incubated at 37°C for 72 hours. Two hours prior to harvesting, 0.1 ml of Colcemid solution (final concentration : 0.1 mg/ml) was added to the cell culture. The cells were transferred to a centrifuge tube (one tube for each cell culture) and spun at 1 200 rpm for 10 minutes. The supernatant was removed and 10 ml of pre-warmed 0.075 M of KCl solution was added. The solution was incubated at 37°C for 10 minutes and then spun at 1 200 rpm for 5 minutes. The supernatant was removed and 10 ml of fresh fixative (made up of 1 part acetic acid to 3 parts methanol) was added drop by drop. The solution was spun at 1 200 rpm for 10 minutes. The cell pellet was re-suspended in a small volume of fresh fixative and dropped on a clean microscope slide and dried. This was then subjected to QFQ banding, and the stained metaphase became evident ; QFQ banding is a valuable chromosome identification tool. Quinacrine is an acridine dye that binds to DNA by intercalation. The base composition of the isolated chromatin fractions affects the fluorescence of this dye, and the banding pattern of whole chromosomes appears to be greatly influenced by any variation in the protein composition of the chromosomes. The slides were stained by a 30 seconds treatment in 0.005% Quinacrine Mustard. In addition in order to present a cytological and anatomic description skin biopsies and autopsies were analysed. Subsequently, in a different experiment thirty-four skin samples (diameter 5 - 8 mm) from 17 randomly selected Valle del Belice individuals (12 hypotrichotic and 5 normal) were taken, two samples from the stifle region of each animal. The samples were kept in formalin at 10 %. An autopsy on a two-months-old hypotrichotic lamb was undertaken. Portions of a variety of tissues including liver, heart, lung, kidney, thymus, eye, trachea, spleen, pancreas, rumen, bladder and skin sections of four different regions (stifle, cheek, chest, and limbs) were taken and fixed in phosphate buffered 10 % formalin ; and processed routinely for histopathology.

RESULTS AND DISCUSSION

The hypothesis of a simple Mendelian segregation was not in disagreement with the pedigree of the hypotrichotic sheep (Finocchiaro, 2001). *Test-cross* matings between hypotrichotic animals always gave hypotrichotic offspring ; furthermore F1 animals backcrossed with hypotrichotic animals gave 50 % hypotrichotic offspring and 50 % normal offspring (heterozygous for the hypotrichotic trait). These results were in accordance with the hypothesis of Mendelian inheritance.

The karyotype of the 17 sheep studied, affected and unaffected, was normal, made of 54 chromosomes and with no chromosomal malformation. Many of the known hypotrichotic disorders in mammalian species like mice and rats generate pleiotropic effects, including athymia (Pantelouris *et al.*, 1973 ; Festing *et al.*, 1978 ; Nehls *et al.*, 1996), immunodeficiency and autoimmune disease (Shultz, 1988), defective cellular immunity (Morrissey *et al.*, 1980), and progressive nephrosis (Meier *et al.*, 1969). The Valle del Belice affected individuals are in good health, with no evidence of immune system dysfunction or unusual susceptibility to skin tumours. The absence of hair however increases temperature susceptibility in hypotrichotic sheep : hypotrichotic animals have serious problems with thermoregulation. This means that affected animals must be kept indoors during summer and winter. It has been noted that hypotrichotic females in different species, including mice and hamsters, do not nurse their young (Crew and Mirskaia, 1931 ; Pinter and McLean, 1970 ; Nixon, 1972) but the Valle del Belice ewes seem to take care of their lambs. Skin sections of hypotrichotic animals are easily recognised, regardless of the analysed region (stifle, limbs, head, and chest). Although the epidermis of the affected individuals appears normal the inner epidermal area shows a continuous stratum of follicle dysplasia. A microscopic examination of the skin reveals very few hair follicles, and those present are dilated and contain either keratinous plug or no hair at all. Sometimes there are primordial inferior follicles, typical of the hair anagen phase. Similar descriptions were given by Mackie and McIntyre (1992) for the Australian Poll Dorset sheep breed, by Bracho *et al.* (1984) for the Hereford cattle breed and recently by Ahmad *et al.* (1998) for humans. The development of the sebaceous and sweat glands in the affected animals appeared normal. The biopsies of the stifle region revealed very few follicles in the catagen and anagen phases. Probably these follicles belong to the wool coat present in the area. Skin sections of a supposed heterozygous hypotrichotic animal showed normal hair development. Clinically, supposed heterozygous animals cannot be distinguished from the normal animals. This result is in accordance with what was already described for other mammalian species, including mouse (Cachon-Gonzalez *et al.*, 1994) and human species (Chicon *et al.*, 1998). A necropsy of a two-months-old hypotrichotic Valle del Belice lamb was carried out in order to present a complete description of the disorder. All the organs analysed presented an animal in good health, with the exception of the cutaneous changes already mentioned. The results of the necropsy are in accordance with what has been reported by Mackie and McIntyre (1992) in the Australian Poll Dorset sheep breed and by Bracho *et al.* (1984) for the Hereford cattle breed.

CONCLUSION

The intention of this paper was to investigate the hypotrichotic phenotype in the Valle del Belice sheep. Hypotrichotic sheep do not appear to suffer in any way, but they do have problems with thermoregulation. According to the results presented here it seems the hypotrichotic disorder in Valle del Belice sheep is controlled genetically. A number of loci are known to cause a hypotrichotic disorder in mammalian species. The most likely candidate gene for the hypotrichotic phenotype in sheep is the so-called *hairless* (hr) gene. The *hairless* gene has been located on mouse chromosome 14 (Cachon-Gonzalez *et al.*, 1994) and on human chromosome 8 (Ahmad *et al.*, 1998 ; Nöthen *et al.*, 1998). The human *hairless* gene is highly homologous to the mouse and the rat *hairless* genes suggesting high conservation among mammals (Ahmad *et al.*, 1998 ; Cichon *et al.*, 1998). Therefore a *"*Candidate gene approach*"* was carried out. A comparative analysis was made of the sheep DNA with human, rat and

mouse genomes. So far the *hairless* (hr) gene was successfully amplified on the ovine genome (Finocchiaro *et al*., 2001). At present the whole sequence of exons 2, 3 and 4 representing about 44 % of the coding sequence, was carefully analysed as a function of the occurrence of the hypotrichotic phenotype (Finocchiaro, 2001). Point mutations were observed in exon 3. These observed mutations in exon 3 might not be the functional mutation, but could be closely linked to the mutation(s) responsible for the lack of hair in hypotrichotic sheep. Further sequencing of the gene, which is currently in progress, could improve the PCR based test for the molecular typing of the mutated hypotrichotic allele, in order to correctly identify the heterozygous animals.

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