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Original article

A comparison between the β-globin gene clusters of domestic sheep (*Ovis aries*) and Sardinian mouflon (*Ovis gmelini musimon*)

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Summary – The organization of the β-globin gene cluster of wild Sardinian mouflons with different haemoglobin phenotypes has been analyzed by means of Southern blot with probes specific for ε^{IV}- and β^{F}- globin genes. With three endonucleases (*Bam* HI, *Eco* RI, and *Hind* III), Sardinian mouflons with Hb B, Hb BM and Hb M show identical restriction patterns to sheep with Hb A, Hb AB, and Hb B, respectively. Therefore, in Sardinian mouflons as in domestic sheep, the β-globin gene cluster shows two haplotypes characterized by the triplication or duplication of an ancestral four gene set ($\varepsilon-\varepsilon-\psi\beta-\beta$).

domestic sheep / Sardinian mouflon / haemoglobin / β-globin cluster

Résumé – Comparaison du groupe de gènes de la globine-β du mouton domestique (*Ovis aries*) et du mouflon de Sardaigne (*Ovis gmelini musimon*). *L'organisation du groupe de gènes de la globine-β chez des mouflons de Sardaigne montrant divers phénotypes d'hémoglobine a été analysée par buvardage de Southern avec des sondes spécifiques pour les gènes* ε^{IV} *et* β^{F}. *Avec trois endonucléases (*Bam *HI,* Eco *RI et* Hind *III), les mouflons caractérisés par les phénotypes d'hémoglobine B, BM et M montrent des patrons de restriction identiques aux moutons ayant les phénotypes d'hémoglobine A, AB et B respectivement. Par conséquent, le groupe de gènes de la globine-β manifeste dans ces deux espèces deux haplotypes caractérisés par la duplication ou le triplement d'un ensemble de quatre gènes ancestraux :* $\varepsilon - \varepsilon - \psi\beta - \beta$.

mouton / mouflon de Sardaigne / hémoglobine / globine-β

INTRODUCTION

The domestic sheep (*Ovis aries*) β-globin gene cluster shows two common haplotypes: the A haplotype bearing the adult HBB^A allele and the B haplotype bearing the adult HBB^B allele (Garner and Lingrel, 1988, 1989). The A haplotype is similar to the goat (*Capra hircus*) β-globin gene cluster (Townes et al, 1984) and shows the triplication ($5'\varepsilon^{I}-\varepsilon^{II}-\psi\beta^{I}-\beta^{C}-\varepsilon^{III}-\varepsilon^{IV}-\psi\beta^{II}-\beta^{A}-\varepsilon^{V}-\varepsilon^{VI}-\psi\beta^{III}-\beta^{F}3'$) of an ancestral four gene set ($\varepsilon-\varepsilon-\psi\beta-\beta$) characterized by two embryonic genes (ε), one pseudogene ($\psi\beta$), and one gene (β) whose expression varies as a function of ontogenic development and physiological conditions. In fact, β^{C}, β^{A}, and β^{F} genes are expressed during juvenile, adult, and fetal life, respectively (Huisman et al, 1969). The β^{C} and β^{A} switch is reversible under particular physiological or experimental conditions (anaemia, hypoxia or under administration of erythropoietin) (Huisman et al, 1967; Boyer et al, 1968). The B haplotype, lacking the whole juvenile four-gene set, is duplicated ($5'\varepsilon^{I}-\varepsilon^{II}-\psi\beta^{I}-\beta^{B}-\varepsilon^{III}-\varepsilon^{IV}-\psi\beta^{II}-\beta^{F}3'$). Therefore, sheep homozygous for the HBB^B allele do not exhibit the property of $\beta^{B} \rightarrow \beta^{C}$ switching as they do not possess the β^{C} gene. Since domestic sheep β^{A} and β^{B} allelic chains differ by at least seven scattered amino-acid residues and no intermediate haplotype has been found, it has been proposed that this polymorphism is the product of genetic isolation followed by admixture (Boyer et al, 1966). Manwell and Baker (1976) suggest that man has played a role in generating haemoglobin polymorphisms in domesticating sheep by hybridizing individuals that would otherwise be geographically isolated. Southern blot analysis, using β-like globin genes as probes and several endonucleases, strongly supports the polyphyletic origin of the domestic sheep. In fact, by means of this technique, A and B haplotypes can be easily distinguished since they differ in both number and length of restriction fragments and show no intermediates (Di Gregorio et al, 1987; Rando et al, 1989).

Some authors (Bunch et al, 1976; Bunch and Nadler, 1980; Bunch and Nguyen, 1982; Ryder, 1984; Di Gregorio et al, 1987) consider the mouflon as one of the ancestors of the present day domestic sheep, whereas others (Poplin, 1979; Vigne, 1983) claim that it originated by feralization of the first domesticated sheep in the Corsico-Sardinian islands (Neolithic).

In wild mouflons (*Ovis gmelini musimon*) captured in Sardinia, two alleles at the adult β-globin locus have been observed: HBB^B and HBB^M, with frequencies of 0.94 and 0.06 respectively. Both adult β-globin variants in this species are electrophoretically different from those observed in sheep (Naitana et al, 1990). No homozygous individuals for the HBB^M allele had been found (Naitana et al, 1990). In this paper, with the availability of a mouflon homozygous for the HBB^M allele, we compared the organization of the β-globin clusters of wild Sardinian mouflons and domestic sheep with different Hb phenotypes by means of Southern blot analysis.

MATERIALS AND METHODS

Haemoglobin phenotypes of sheep and wild Sardinian mouflons were determined by means of isoelectric focusing in the pH range 6.7–7.7 and by gel electrophoresis of dissociated globin chains (Naitana et al, 1990; Masala et al, 1991). Southern blot

analysis was accomplished on DNA samples obtained from six mouflons selected according to Hb phenotype (3 Hb B, 2 Hb BM, and 1 Hb M) and, as a comparison, from six sheep (2 Hb A, 2 Hb AB, and 2 Hb B). DNA samples were digested with *Hind* III, *Bam* HI, and *Eco* RI and probed with plasmid pG16Ec3Bm2 (containing the 5′ of the goat ε^{IV}-globin gene) and plasmid pGγ5′ (containing the 5′ of the goat β^{F}-globin gene). According to the hybridization conditions reported by Rando et al (1989), these plasmids (a kind gift from JB Lingrel) strongly cross-hybridize with the paralogous genes.

RESULTS AND DISCUSSION

Southern blot analysis of mouflon and domestic sheep genomic DNAs digested with *Hind* III, *Bam* HI, and *Eco* RI and hybridized with ε^{IV} and β^{F} probes demonstrates that mouflons with Hb B, Hb BM, and Hb M show the same electrophoretic patterns as domestic sheep with Hb A, Hb AB, and Hb B, respectively. As an example, figure 1 shows digestion of mouflon and domestic sheep genomic DNA with *Bam* HI and hybridization with the ε^{IV} gene. It can be seen that restriction patterns of mouflons with Hb B and domestic sheep with Hb A (homozygotes for the triplicated haplotype) are characterized by fragments of 9.0, 5.5, and 6.6 kb containing the ε pair genes of the juvenile, adult, and fetal sets, respectively (Rando et al, 1989). On the other hand, restriction patterns of mouflons with Hb M and domestic sheep with Hb B (homozygotes for the duplicated haplotype) are characterized by fragments of 5.7 and 6.6 kb that previous reports show contain the ε pair genes of the adult and fetal sets, respectively (Garner and Lingrel, 1988; Rando et al, 1989). Figure 2 summarizes results obtained with the three endonucleases and the two probes. Thus the Sardinian mouflon shows two haplotypes at the β-globin gene

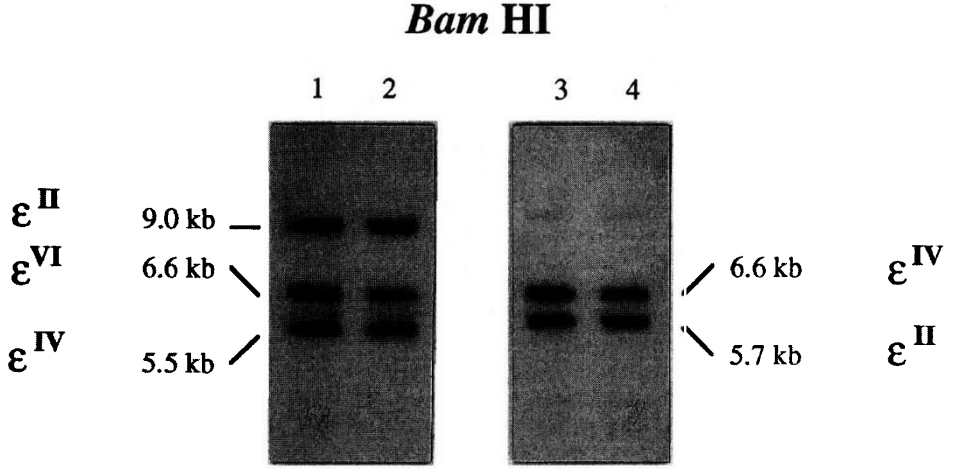

Fig 1. Autoradiograms of mouflon and sheep DNAs digested with *Bam* HI and probed with the goat ε^{IV} gene. Gene nomenclature is according to Garner and Lingrel (1988, 1989). 1: Mouflon Hb B; 2: sheep Hb A; 3: Mouflon Hb M; 4: sheep Hb B.

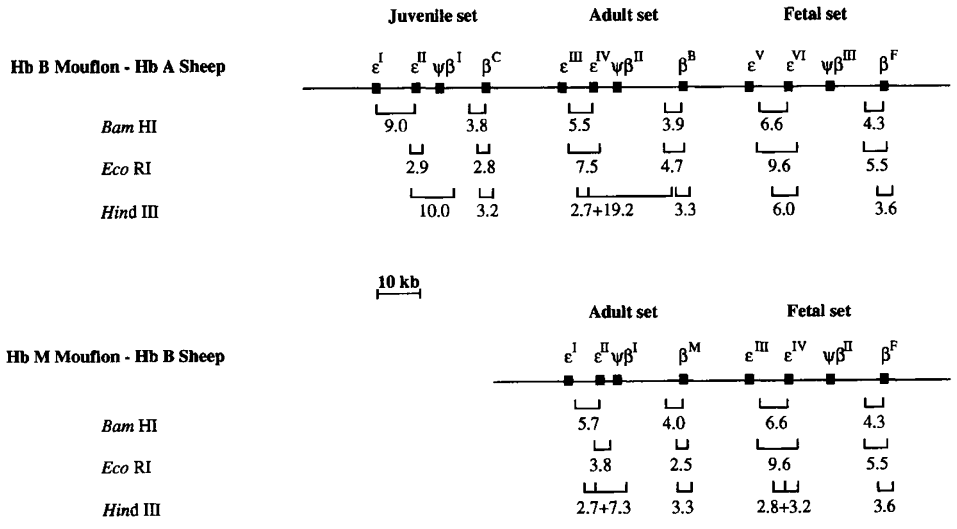

Fig 2. Restriction fragments obtained by hybridizing the goat ε^{IV} and β^{F} probes with DNA samples extracted from mouflons homozygous for Hb B and Hb M and digested with three endonucleases. Fragments of identical length are observed with DNA samples extracted from sheep homozygous for Hb A and Hb B, respectively. Lengths of fragments are given in kilobases. Locations of restriction sites are according to Townes et al (1984), Di Gregorio et al (1987), Garner and Lingrel (1988, 1989), and Rando et al (1989).

cluster, a triplicated one bearing the adult HBB^{B} allele corresponding to the sheep. A haplotype, and a duplicated one bearing the adult HBB^{M} allele corresponding to the sheep B haplotype.

According to the results presented in this paper, the two haplotypes differ not only by the presence/absence of the juvenile set but also by many other mutations evidenced by different restriction endonucleases (see fig 2). The absence of intermediate haplotypes in both sheep (Di Gregorio et al, 1987; Rando et al, 1989) and mouflon confirms the hypothesis that the two haplotypes evolved separately (Boyer et al, 1966) and, at the same time, demonstrates that they were present in ancestor common to both species. According to data presented by Naitana et al (1990), the frequency of the HBB^{B} allele (triplicated switching cluster) is much higher in Sardinian mouflons (0.94) than in domestic sheep and, in particular, in the Sarda breed sheep which is almost monomorphic for the HBB^{B} allele (duplicated non-switching cluster) (Manca et al, 1993) and lives in the same environment. Therefore, it remains to be established whether the marked differences in the frequencies of 'switching' and 'non-switching' chromosomes between Sardinian mouflon and sheep are the result of genetic drift, natural selection or domestication. Masala et al (1991) put forward the possibility of an advantage in synthesizing Hb C in a wild-type niche in the case of mouflon. Evans and Turner (1965) evidenced a certain reproductive advantage of the duplicated cluster in sheep. If we consider that the domestic sheep has been the object of selective pressure for milk, wool, and meat production, it could be that man, through domestication and artificial

selection, is responsible for the high frequency of the duplicated cluster in domestic sheep.

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