A NOTE ON LINKAGE BETWEEN THE ANGORA AND *fgf5* GENES IN RABBITS

MULSANT P., ROCHAMBEAU H. DE[†], THÉBAULT R. G.[‡]

[*] INRA, Laboratoire de Génétique Cellulaire. BP 27, 31326 CASTANET CEDEX, France.

[†] Station d'Amélioration Génétique des Animaux. BP 27, 31326 CASTANET CEDEX, France.

[‡] INRA, Domaine pluridisciplinaire du Magneraud. BP 52, 17700, SURGÈRES, France.

ABSTRACT: The angora mutation in mice corresponds to a large deletion in the *fgf5* (fibroblast growth factor 5) exon 1. Two pairs of primers were chosen in the human *fgf5* coding sequence in order to amplify two fragments of the rabbit gene. One biallelic polymorphism was demonstrated in each fragment. The four alleles were cloned and sequenced. A random sample of unrelated angora (n=17) and wild-type control animals (n=15) were analysed for both polymorphisms. The observed haplotype frequencies show a clear separation between the two populations. Informative families were created by backcrossing to measure linkage between the angora and *fgf5* loci. No recombinant was observed between them, in 56 meiosis, proving a tight linkage between both genes.

Key words: rabbit, angora gene, mice, fibroblast growth factor 5 gene.

INTRODUCTION

Angora recessive mutations that cause the production of abnormally long hair have been detected in several species, including mice, rabbits, goats, dogs and cats. Evidence was reported that mice deficient in fibroblast growth factor 5 (*fgf5*), produced by gene targeting are of angora phenotype, and that the natural angora mutation corresponds to a large deletion of the *fgf5* exon 1, and part of upstream sequences (HÉBERT *et al.,* 1994; SUNDBERG *et al.,* 1997). As in mice, the angora mutation in rabbits causes an increase in the length of anagen VI (ROUGEOT and

Correspondence: P. MULSANT
E-mail: mulsant@toulouse.inra.fr

Tʜᴇ́ʙᴀᴜʟᴛ 1984). We therefore investigated linkage in rabbits between the *angora* and *fgf5* loci, using Single Strand Conformation Analysis (SSCA) of PCR-amplified fragments of the *fgf5* gene.

MATERIALS AND METHODS

Rabbit *fgf5* sequences were not available in data banks. Two pairs of primers were thus designed in the human coding sequence (accession number M37825). The primers were chosen to amplify two fragments of about 350 and 290 bp, corresponding to most of human exons 1 and 3, respectively. Primer sequences and PCR conditions for amplification of rabbit DNA are presented in Table 1. Reactions were carried out on 150 ng of genomic DNA in a total volume of 40 µl. Reaction mixtures contained 1 µM of upstream and downstream primers, 200 µM of each dNTP, MgCl2 at the indicated concentration and 1U of Taq DNA polymerase (Appligene) in the manufacturer's buffer. Annealing (at the indicated temperature) and elongation times were 45 sec.

Polymorphism of the two fragments was analysed on a random sample of unrelated angora (n=17), and wild-type control animals from INRA commercial breeds (n=15). Five rex rabbits, 5 rabbits coming from strain 1077 and 5 rabbits

Table 1: Primers and PCR conditions.

exon		primer sequence	PCR conditions		
			cycles	annealing temp.	[Mg] mM
1	F1	CCA GAA TCA GCC CTA CAA GAT GCA C	34	65°	1.5
	R1	GAT GGA AAC CGA TGC CCA CTC TGC			
3	F3	CCT ATG CCT CAG CAA TAC ATA GAA CT	30	58°	2.5
	R3	ATC CAA AGC GAA ACT TGA GTC TG			

coming from strain 2066 made the wild-type sample. PCR fragments were submitted to SSCA in 5% glycerol-containing 10%-acrylamide gels (49:1, overnight migration at room temperature, 15V/cm). For each fragment, the different alleles detected by SSCA were cloned and sequenced by dye-terminator analysis. Allele sequence comparison revealed a EarI polymorphism in exon 1 and a TaqI polymorphism in exon 3. PCR-RFLP assay was performed in 15 µL with 3 units of enzyme at 37°C for 3 hours under the conditions specified by the manufacturer.

To measure linkage between the *angora* and *fgf5* loci, an informative population of 56 young rabbits was created by backcrossing rabbits heterozygous at each locus (2 males and 5 females) with angora individuals. The heterozygous rabbits were derived from a rex by angora cross.

RESULTS AND DISCUSSION

Polymorphism of the two fragments of the rabbit *fgf5* locus

Amplification products of the expected size were obtained on rabbit genomic DNA using human primers specific for *fgf5* exon 1 or 3. An uninterrupted coding sequence was found in each case, with 87% and 91% identity with the relevant human fragment (primers excluded). One biallelic polymorphism was demonstrated in each fragment. The polymorphism in the putative exon 1 (called exon 1 thereafter) did correspond to the insertion at position 217 in the slow allele of three nucleotides, creating a supplementary in phase TCT (serine) codon in a serine stretch (Table 2). The mutation of the slow allele creates an additional EarI site. A single variation was also observed in the 288 bp putative exon 3 fragment, with a T (slow allele) or C (fast allele) at position 58. This transition replaces the leucine present in the slow allele (as in the human and mouse sequences) by a serine. It corresponds to a TaqI polymorphism, with 0 or 1 digestion site in the slow or fast allele, respectively. It was checked that EarI or TaqI digestion of the amplification products effectively yielded the expected fragments (data not shown).

Table 2: Characterisation of the rabbit *fgf5* polymorphisms.

Exon	Allele	Sequence	Amino Acid change	PCR-RFLP Enzyme	Number of sites[1]
1	Fast	ctct--gcc		Ear1	1 (79, 267)
	Slow	ctctTCTgcc	+ Ser	Ear1	2 (9, 143, 127)
3	Fast	ctCga	Ser	Taq1	1 (57, 231)
	Slow	ctTga	Leu	Taq1	0 (288)

[1] Size of the restriction fragments (bp) after enzymatic digestion.

As all tested animals were homozygous for either the exon 1 (angoras) or the exon 3 product (wild-type animals), haplotyping could be performed. The observed haplotype frequencies are presented in Table 3, showing clear separation between the two populations. Among the four possible haplotypes, only three were observed in this sample, the fast/fast haplotype (1) being absent. Both fast and slow homozygotes for the exon 1 fragment were observed among wild-type animals, thus excluding the mutation in this fragment from being the cause of the angora phenotype. The fast allele of exon 3 was not present in the tested wild-type rabbits. A single fast/slow heterozygous animal was detected among the analysed angora rabbits, making it unlikely that this mutation is the cause of the angora phenotype. The same genotype was confirmed in its dam and one sib.

Linkage between the *angora* and *fgf5* loci

Informative families were created by backcrossing to measure linkage between the *angora* and *fgf5* loci. The rare angora animal heterozygous for the exon 3 fragment was not used to breed the backcross rabbits. A total of 56 progeny individuals were obtained in 10 families and genotyped at the *angora* and *fgf5* loci; both fragments of the *fgf5* gene were analysed. No recombinant was observed between the *angora* and *fgf5* loci in 56 (respectively 48) meiosis informative for *fgf5* exon 3 (respectively exon 1). These data show tight linkage between the rabbit *angora* and *fgf5* loci,

Table 3: Haplotype frequencies at the *fgf5* locus amongst angora (n=17) and wild-type (n=15) rabbits.

Haplotype	Allele (SSCA)		Haplotype frequency[1]	
	Exon 1	Exon 3	*angora*	wild-type
1	Fast	Fast	0	0
2	Fast	Slow	0	0.47 (14)
3	Slow	Fast	0.97 (33)	0
4	Slow	Slow	0.03 (1)	0.53 (16)

[1] Number of haplotypes is indicated in parentheses

with a LODmax of 15.65 for a recombination fraction of 0 (The H0 hypothesis was independence between the 2 loci).

Our results strongly suggest that as in mice a defect in the *fgf5* gene may be the cause of the rabbit angora phenotype, although we found no evidence of complete linkage disequilibrium between *angora* and a *fgf5* allele. Between the two polymorphisms detected in the *fgf5* sequence, a preferential allelic association with angora was observed for the fast allele of the exon 3 fragment. This situation can be due either to strong linkage disequilibrium or to different mutations causing the angora phenotype, the most frequent being the T to C transition detected in exon 3. The amplified PCR products covered 520 bp (280 + 240, primers excluded), i.e. about 65% of the 795 bp coding sequence, as estimated from human or murine data. The angora mutation may therefore lie in the remaining third of the coding sequence between exons 1 and 3, or in promoter or intron sequences.

In view of the linkage disequilibrium observed between *angora* and the fast allele of the *fgf5* exon 3, one practical application of our results will be the simple genotyping by either SSCA or TaqI PCR-RFLP of animals heterozygous for the recessive angora mutation. This may be of particular interest in introgression programmes.

Finally our results demonstrate the interest of the accumulated murine and human data for the genetical analysis of less well-known species, such as rabbits. The same approach could be applied to the analysis of the angora mutation in other species. In particular the designed pairs of primers allow efficient amplification on goat genomic DNA (MULSANT, unpublished). Fibroblast growth factor-5 is known to be expressed in the embryo (HAUB and GOLDFARB, 1991; HÉBERT *et al.,* 1990; HÉBERT *et al.,* 1991). The analysis of defects at this locus in angora animals of different species should thus be of help in elucidating the role of fibroblast growth factor 5 in embryonic development.

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