mapped, is not a hotspot of chromosomal breakpoints seen in canine tumours. The hotspots that have been found in the dog genome so far, include chromosomes 1, 19 and 25 which are preferentially involved in chromosomal fusions[4]. The X-chromosome of the dog, in contrast, is frequently affected by structural aberrations. Therefore, in contrast to humans, the activation of *HMGA1* as a result of chromosomal translocations does not seem to play a considerable role in canine tumours. This may be due to the fact that the corresponding changes are not able to induce benign tumours in the dog or to stimulate their growth. Alternatively, there may be factors favouring the occurrence of the structural changes in humans which are lacking in dogs.

No homology has been found between human chromosome 6, to which the *HMGA1* is mapped, and canine chromosome 23, rather human chromosome 6 shares homology with canine chromosome 22 and 8[5]. In our FISH studies no metaphase shows signals on these dog chromosomes.

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Contribution of a new set of canine microsatellites to the knowledge of the canine genetic map

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Source/description: Canis familiaris DNA was digested with *Sau*3AI and fragments ranging 700–1000 bp in length were ligated into the *Bam*HI site of a pre-digested and de-phosphorylated pUC18 vector (Amersham Pharmacia Biotech, Amersham, UK) and transformed into *Escherichia coli* SURE® cells (Stratagene, La Jolla, CA, USA). A total of 1200 recombinant clones were pooled in groups of 10 and the pools were spotted onto a nylon membrane, screened with digoxigenin-labelled probes $(TG)_{10}$ and $(AAAT)_7$, and the positive pools were screened individually to isolate the final positive clones. Ten positive clones were sequenced in an ABI-310 sequencer (Applied Biosystems, Foster City, CA, USA) from which six

Table 1 Features of the microsatellites, including heterozygosis (H), polymorphic information content (PIC)[2], effective number of alleles (ENA)[3], number of informative meiosis (NIM), linkage group and chromosomal location.

Locus	GenBank Accession no.	Primer sequences (5′ → 3′)	Repeat unit	Size range (bp)	Annealing T (°C)	Alleles (*n*)	H	PIC	ENA	NIM (%)[1]	Assigned linkage group	Chromosome location
UCMCF12	AY059379	GGAGCTCTCTGCGCAGTATG ATCTGTAGTAAAGCTGAC	$(AAAT)_{12}$	135–151	58	5	51.4	48.1	2.1	78 (36)	L28/33	CFA16
UCMCF40	AF210623	TTTACAACCTAAATGTCCTTG CATTCCTTTTCATGGTTAAT	$(GT)_{20}$	103–121	58	9	67.44	63.5	3.1	110 (51)	L19	CFA5
UCMCF54	AF210621	CATGGAGCCTGCTTCTCC GCGCTTTCTCCAAGTAACAG	$(CT)_{13}(GT)_9$	154	50	1	0	0	1	–	–	–
UCMCF71	AF210622	ACTCAAAAGCCATCTTGTCACA AAGAAGCTGCAGGAGCTACG	$(AC)_{14}$	142–156	58	7	69.78	66.9	3.3	69 (32)	L26	CFA1
UCMCF96	AF210620	GGTCGACTCTAGAGGATCTGG GAGCAGCAGCCTGGACTAC	$(TG)_{19}(AG)_{14}$	225–241	63	8	82.74	80.5	5.8	136 (64)	L18/33	CFA10
UCMCF117	AF448484	GAGGATCCTGGAGTCTCGG CTTGCTTGGAACTAGGTACTTGC	$(CT)_6$	186	58	1	0	0	1	–	–	–

true-positive clones were obtained (60%) and are detailed in Table 1. Sequences were checked against public database using the BLAST[1] facility and it was observed that clones *UCMCF71* from nt591 to nt696 and *UCMCF117* in positions 1–49 showed high levels of sequence identity (>80%) with the *C. familiaris* t-RNA family derived short interspersed nucleotide element (SINE) described by Bentolila *et al.*[2] (GenBank AJ239530–49). *UCMCF54* contains not only a SINE at nucleotide positions 9–52 (identity = 98% with AJ239533) but also a *C. familiaris* LINE-1 element ORF-2 mRNA (GenBank AB012223) in positions 194–328 (82% of sequence identity). Primer pairs for PCR amplification of the microsatellite of the positive clones were designed using the program PRIMER 0.5 (GCG Software Package, University of Wisconsin, WI, USA).

PCR conditions: Polymerase chain reaction was performed in a total volume of 10 µl of the following mixture: 10 ng of canine genomic DNA, 5 pmol of each primer, 200 µM of each dNTP, 2 mM $MgCl_2$, 1X PCR Buffer [75 mM Tris–HCl (pH 9.0), 50 mM KCl, 20 mM $(NH4)_2SO_4$, 0.001% BSA] and 0.3 U of thermostable DNA polymerase (Biotools, Madrid, Spain, 28080, E). The cycling programme in a PTC-200™ Programmable Thermal Controller (MJ Research Inc., Watertown, MA, USA) included initial denaturation during 5 min at 95 °C, 30 cycles of 50 s at 95 °C, 50 s at 50–63 °C, 50 s at 72 °C and final extension at 72 °C for 25 min. PCR products were analysed by capillary electrophoresis in an ABI-310 sequencer (Applied Biosystems).

Polymorphism: Polymorphism was revealed by genotyping 10 unrelated individuals of each of the following breeds: Beagle, English Pointer, English Setter, Epagneul Breton and German Shepherd. The observed alleles were named according to their size in base pairs as estimated with the GENESCAN® 2.1 software (Applied Biosystems). Markers *UCMCF54* and *UCMCF117* did not show any polymorphism, but in the rest of markers, heterozygosity (H) and polymorphic information content[3] (PIC) was found to range between 51.5–82.7% and 48.1–80.5%, respectively (Table 1). The effective number of alleles[4] observed ranged between 1 and 5.8.

Mendelian inheritance: Codominant segregation was observed in the Reference Families of the Dogmap Panel[5] including 212 meiosis, where no mutation events or null alleles were observed and the informative meiosis ranged between 69 (32%) and 136 (64%).

Chromosomal location: Linkage analysis was carried out with the software package CRI-MAP 2.4[6]. Markers were assigned to previously described linkage groups[7] and anchored into chromosomes by using the 'twopoint' option, and arranged with the 'build' option. Linkage groups and chromosomal locations of the new loci are shown in Table 1 and depicted in Fig. 1.

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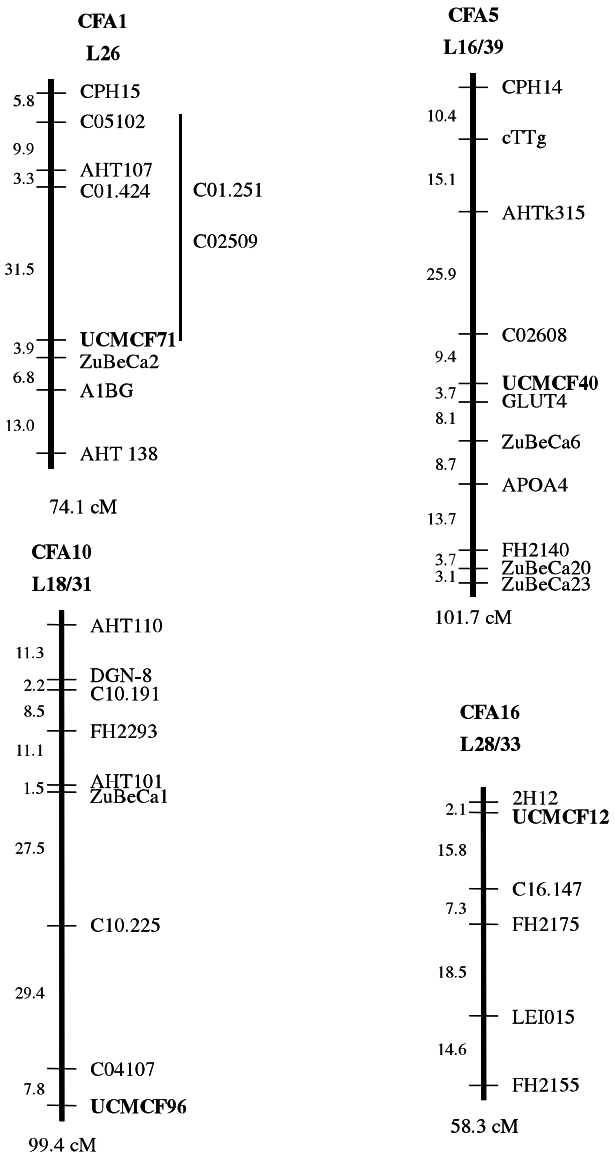

Figure 1 Microsatellite chromosomal location. Distances are given in Kosambi cM. The linkage groups are displayed as sex-averaged maps.

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