E001

Allelic variation of the D4 dopamine receptor polymorphic region in two dog breeds, Golden Retriever and Shiba

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The dog (*Canis familiaris*) has the longest history among domestic animals, and more than 400 breeds have so far been established around the world. Purebred dogs are significantly different from each other in their behavioral traits, suggesting that some behavioral traits are under genetic control. The D4 dopamine receptor (D4DR) polymorphic region, which is possibly related to the personality trait known as novelty seeking in humans, was examined in 52 dogs from two breeds (Golden Retriever and the Japanese indigenous breed Shiba) by PCR and DNA sequencing of each allele. Golden Retrievers and Shibas are relevant breeds in Japan, and their behavioral traits such as excitability, aggression, and playfulness are quite different. The polymorphic region of the dog D4DR gene was composed of 39- and 12-base pair (bp) units, and six alleles were identified based on the difference of number and/or order of these units. Intra- and inter-breed allelic variations were observed. Two alleles (435 and 447 bp in length) were observed in Golden Retrievers and the frequency of the short 435 bp allele was dominant. On the other hand, the long 447 bp and 549 bp alleles were common among five alleles observed in Shibas. These findings suggest that allele frequency varies significantly between different breeds, and hence analysis of the polymorphism in D4DR might be of use for understanding the behavioral traits of dogs.

E002

Parentage and identity testing by means twelve microsatellites in Dogs

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The Laboratorio Gruppi Sanguigni, which has been carrying on for years a support activity for the genetic improvement of domestic animals in Italy, has set up a protocol for parentage and individual identification in dogs. This protocol, based on STRs (Short Tandem Repeats) analysis through PCR, involves the use of twelve highly polymorphic microsatellites co-amplified in a single multiple PCR. The observed microsatellites were: (AHT140, AHT121; AHT137; 2001, 2137; CPH3; ZUBECA26; DGN3; 2161; DGN14; DGN13; ZUBECA25).

The procedure is almost fully automated: the amplification reaction is accomplished in a thermal cycler and samples electrophoresis and sizing analyses are carried out by using the GeneScan and Genotyper software of the automatic sequencer ABI Prism 377.

To demonstrate the validity of this protocol we analysed 120 non-sib dogs of four different breeds (30 German Shepherds, 30 Setter, 30 Labrador and 30 Yorkshire). Parentage exclusion probability has been calculated for each locus and for the all 12 loci together and is equal to 0.999. If one of the parents is sure, 999 out of 1000 mistakes in the attribution of the other parent can be identified.

Besides, individual identification, as the probability of finding two identical genotypes for 12 loci, has been calculated in each of the four breeds.

E003

A comparative radiation hybrid map of the X-chromosome of the Dog

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The dog serves as an animal model for several human diseases including X-chromosomal diseases. To facilitate comparative genetic analysis, a linkage map and a radiation hybrid map of the dog genome have recently been established. Whereas the canine X-chromosome is the largest chromosome in the dog, only a few markers have been mapped to it. Using a commercially available whole genome radiation hybrid (RH) panel we were able to map 15 microsatellite markers, 25 genes and 19 STSs to the X-chromosome, extending the total number of mapped markers to 59, covering an estimated 830 cR. Nine distinct groups of markers could be established with an average spacing of 18,8 cR_{3000}, 13 markers remained unlinked.Using FISH analysis, 5 markers could be mapped physically to the p- or q-arm of the X-chromosome resulting in the addition of 2 new groups to the comparative map. One group includes FH2985, which is in former maps linked between AR and CHM/ PGK1 in a region known to be on dog Xq13. In our map we have clear linkage of this marker to the p-arm of the X-chromosome. Nineteen other markers mapped to 5 other RH groups consistent with their syntenic map position. Comparison with the human X-chromosome map revealed synteny up to 234 cR for the genes *TIMP-ALAS2-AR-IL2Rγ-XIST.* The extended and revised map presented in this poster will serve as preliminary map for the X-chromosome and makes comparative mapping with other species possible. More markers are needed to create a complete map of the dog X-chromosome.

E004

Comparison of *GALK1* exon sequences in four breeds of dogs affected by juvenile cataracts.

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Juvenile cataract is a heritable disorder in a number of dog breeds. The cause of juvenile cataracts has not been identified in dogs and no genetic tests exist to identify affected or carrier animals. Cataracts occur in many breeds, and vary in age of onset and severity. Therefore, it is likely that more than one gene is responsible for the numerous forms of cataract in purebred dogs.

We have chosen to look at galactokinase (*GALK1*) as a candidate gene for canine juvenile cataracts. The pattern of cataract occurrence in certain breeds parallels that of humans with galactokinase deficiency. That is, puppies develop cataracts as early as 8-9 weeks and these rapidly progress, producing blindness by 1 year of age. Cataracts are the only apparent effect of the mutation. The mode of inheritance is autosomal recessive.

Using PCR primers based on published human *GALK1* sequence, (Stambolian, et. al. 1995) we amplified and sequenced product from genomic dog DNA. BLAST results indicated an average of 89% homology between the dog sequence and the human *GALK1* exon sequence. Exon-specific primers were made based on the canine sequence.

These primers were used to amplify *GALK1* exon sequence in four breeds of dogs in which juvenile cataracts occur. Exon sequences of affected dogs, carriers and normal animals were compared and analyzed for potential mutation sites.

E005

Identification of a X-autosome translocation in an intersex dog by chromosome painting

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A 3-year-old female registered Yorkshire terrier had female external genitalia with an enlarged clitoris, uterus and bilateral ovotestis. Cytogenetic studies revealed a 78,XY chromosome complement and a mosaic condition for the X chromosome. In a subpopulation of cells the banding of the distal part of Xp was not in accordance with the one of the partial standardized canine karyotype. A whole X chromosome painting probe derived from this intersex dog was used for fluorescence *in situ* hybridization (FISH) on chromosome metaphase spreads of a normal male dog. The painting was seen on Xq and proximal on Xp and moreover on a small autosome indicating an X-autosome translocation. In order to identify the autosome the same canine probe was hybridized to human metaphase chromosome spreads. This revealed painting on the long arm of the human X chromosome and on human 20p. Using data from comparative chromosome studies the autosome of the sex-reversed dog involved in the translocation is CFA 24.

E006

Microsatellite polymorphism at nine loci in the dog, silver fox and blue fox

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Microsatellite polymorphism was analyzed at nine, previously described, canine loci: CPH1, CPH3, CPH6, CPH11, 2004, 2010, 2140, 2168 and 2319. Altogether, 151 dogs, 91 silver faxes and 53 blue faxes were included in this study. For all loci PCR amplification was successful and in general the length of the product was comparable with date published for the dog. The only exception concerned locus 2140 at which significantly shorter PCR products for silver fox (100 and 108 bp) and blue fox (92-124 bp) were observed, when compared with that of the dog (139-166 bp). Sequencing of this microsatellite revealed in the dog the following repetitive motif $(GAAA)_{10}(A)_2(GAA)_2GAAA$ which showed a quite high similarity to the originally published one - $(GAAA)_n$,. On the contrary, in the silver fox (genotype 108/108) no such motif was found. Interestingly, the two identified alleles: 100bp (p=0.04) and 108bp (p=0.96) segregated regularly in silver fox families. At other loci a variable number of alleles in these species was noticed, i.e. at locus 2004-15 alleles in dogs, 6 alleles in silver foxes and 4 alleles in blue foxes. Heterozygosity (Het) at these loci varied from 0.32 (locus 2140 in blue fox) to 0.93 (locus 2319 in dog). Probability exclusion (PE) for the studied loci was estimated: 0.9996 for dog, 0.989 for silver fox and 0.993 for blue fox.

E007

Mapping gene(s) for inherited skin disease and underlying muscular atrophy: sebaceous adenitis in Standard Poodles and dermatomyositis in Shetland Sheepdogs

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Mapping the causal genetic defect(s) in sebaceous adenitis (SA) in Standard Poodles and dermatomyositis (DM) in Shelties are both problematic since the suspected autosomal recessive inheritance patterns have not been confirmed. Nor have successful treatments for affected dogs been developed for either disease. Age of onset seems more consistent in SA (around two years) with eventual loss of sebaceous glands that lubricate the skin and fur, accompanied by scaling and fur loss in affected areas. DM may strike at nearly any age. Severely affected puppies with atrophied head muscles have been observed. More commonly, the hallmark of DM is barely discernable scabs on ear tips and on extremity friction points, fur loss around the eyes and tail tip occurring in early adulthood or rarely as late as 6-8 years of age) and may progress to muscle degeneration in the same areas. While rare, muscle atrophy prior to skin involvement has been observed in Shelties but is common in related breeds. Environmental "triggers" are suspected in DM, and microbial involvement is under study by other groups. While SA has been observed in about 30 breeds, DM is documented in humans (juvenile) and only a few other breeds. Extensive linebreeding practiced in all of the affected kindreds examined so far, makes it quite possible that multiple gene loci are involved in producing both diseases and also that several defects in one gene might be responsible for the variation in symptoms within the breed or even particular lines. Testing multiple disease models for linkage to a map of informative microsatellite markers is being explored as a strategy with analyses of both nuclear and extended kindreds.

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E008

Microsatellite analysis in South African wild dogs

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The African wild dog (*Lycaon pictus*) is the second most endangered carnivore in Africa. The de Wildt Cheetah and Wildlife trust is the largest and most successful captive breeding centre for wild dogs. In the case of the wild dog the main goal of de Wildt is to breed animals for release in to the wild in order to contribute to other populations. To maintain a genetically diverse population inbreeding must be kept minimal. Analysis of microsatellite loci has been shown to be a valuable tool for such purposes. The aim of the study was to select a set of polymorphic microsatellites to be used routinely in South African wild dogs. Ten microsatellites were selected and typed in 50 dogs of the breeding stock at de Wildt showing up to 24 different alleles. In addition we evaluated a commercially available kit for paternity testing developed for the domestic dog and found that 9 out of 10 systems were also polymorphic (3-10 alleles) in the South African wild dog.