Mammalian Genome 8, 218–221 (1997).

Mammalian Genome

Towards construction of a canine linkage map: establishment of 16 linkage groups

F. Lingaas,[1] A. Sørensen,[1] R.K. Juneja,[2] S. Johansson,[2] M. Fredholm,[3] A.K. Winterø,[3] J. Sampson,[4] C. Mellersh,[4] A. Curzon,[4] N.G. Holmes,[5] M.M. Binns,[5] H.F. Dickens,[5] E.J. Ryder,[5] J. Gerlach,[6] E. Bäumle,[7] G. Dolf[7]

[1]Norwegian Kennel Klub and Department of Morphology, Genetics and Aquatic Biology, Section of Genetics, P.O. Box. 8146 Dep., N-0033 Oslo, Norway
[2]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7023, 750 07 Uppsala, Sweden
[3]Department of Animal Science and Animal Health, Division of Animal Genetics, The Royal Veterinary and Agricultural University, Bülowsvej, 13, DK-1870, Fredriksberg C, Copenhagen, Denmark
[4]The Department of Biochemistry, University of Leicester, University Road, Leicester, LE1 7RH, UK
[5]Centre for Preventive Medicine, Animal Health Trust, PO Box 5, Newmarket, Suffolk CB8 7DW, UK
[6]Medical Technology and Medicine, Michigan State University, B228 Life Science, East Lansing, Michigan 48824-1317, USA
[7]Institute of Animal Breeding, University of Berne, Bremgartenstrasse 109 a, 3012 Berne, Switzerland

Received: 3 September 1996 / Accepted: 15 November 1996

Over the last few years great efforts have been put into the genetic and physical mapping of several mammalian species. Among mammals, human and mouse maps are the most developed, but significant progress has also been made in economically important agricultural species like cattle, sheep, and pig. Microsatellite loci have formed the basis of these genetic maps because they display significant polymorphism and are evenly distributed throughout the genome. An international collaboration (DogMap) was established in 1993, with the initial aim of generating a low-resolution canine marker map. Details about the aims, participants, rules, etc. of DogMap can be found on the WWW at http://ubeclu.unibe.ch/itz/dogmap.html. One important long-term objective of the work is to establish tools to identify the gene(s) responsible for inherited disease, thereby offering the possibility to develop strategies that could reduce the incidence of such diseases through systematic selective breeding.

The combination of genetic mapping with the physical assignment of the markers to specific chromosomes has been successful for most animal species with a well-developed gene map. The complex karyotype of the dog, consisting of 78 chromosomes, many of which are similar in size and banding pattern, makes physical mapping difficult. At the moment only 21 autosomes have an internationally accepted karyotype (Świtoński et al. 1996), and cytogenetic identification of canine chromosomes will probably rely on both traditional banding as well as indirect identification with specific probes, anchor loci, and chromosome paints.

This study presents a linkage analysis of 94 polymorphic loci and provides the first linkage groups from the International DogMap collaboration.

At the First International DogMap Meeting (1993) a common reference panel for typing polymorphic markers was established; this was the first such panel for canine genetic mapping. The reference panel, including the parents, consists of a total of 129 dogs of pure-bred German Shepherd (35 offspring) and Beagles (71 offspring). Details of the family structure are given in Fig. 1.

In the present study, 94 genetic markers were typed in the reference panel (Table 1). Five of the loci were protein polymorphisms, one was a tandem repeat in the von Willebrand factor gene, and the rest were microsatellites (3 tetranucleotide and 85 dinucleotide repeats). The five protein polymorphisms consisted of four blood plasma proteins; transferrin (TF), alpha 1 - protease inhibitor (PI), alpha 1B-glycoprotein (A1BG), apolipoprotein A4 (APOA4) and erythrocyte enzyme superoxide dismutase 1 (SOD1). The number of alleles detected in the reference family varied between 2 and 11, with a mean value of 4.8 alleles. An overview of the marker loci, number of alleles, as well as the proportion of heterozygotes in the parents, is given in Table 1.

Microsatellites were typed in five laboratories using primers radiolabeled with [γ-33 P] ATP and manual sequencing gels or fluorescently labeled primers and an automated sequencer (Phar-

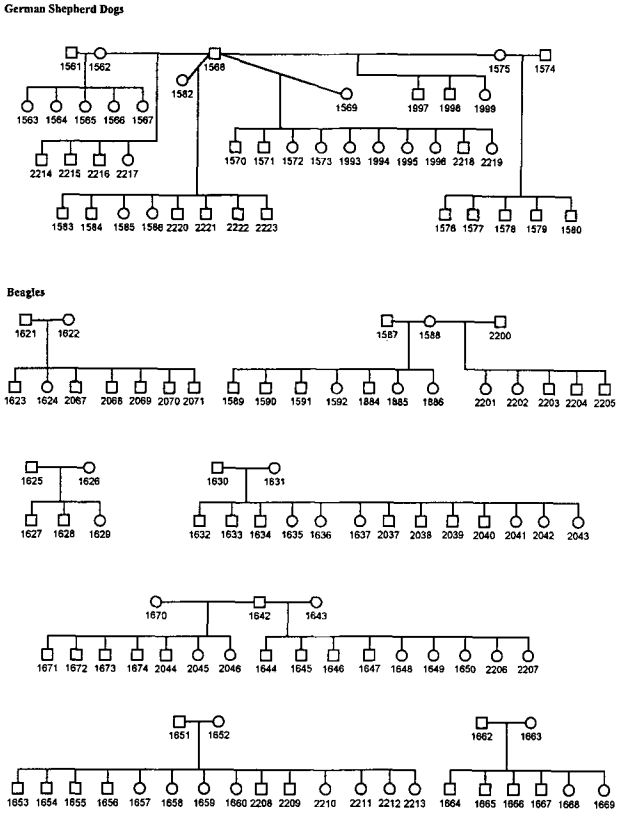

Fig. 1. Reference panel for typing polymorphic markers, consisting of a total of 129 dogs of pure-bred German Shepherd (35 offspring) and Beagles (71 offspring).

Correspondence to: F. Lingaas

Table 1. Overview over markers used for typing in the reference panel of DogMap.

Locus	No. of alleles	Proportion of heterozygotes in parents	References
176	5	0.522	Ostrander et al. 1995
188	2	0.261	Ostrander et al. 1995
191	2	0.348	Ostrander et al. 1995
339	5	0.696	Ostrander et al. 1995
349	4	0.652	Ostrander et al. 1995
359	5	0.739	Ostrander et al. 1995
363	5	0.348	Ostrander et al. 1995
371	4	0.783	Ostrander et al. 1995
383	3	0.783	Ostrander et al. 1995
403	3	0.522	Ostrander et al. 1995
453	7	0.565	Ostrander et al. 1995
473	11	0.870	Ostrander et al. 1995
618	6	0.565	Ostrander et al. 1995
629	5	0.348	Ostrander et al. 1995
630	7	0.522	Ostrander et al. 1995
2001	7	0.826	Francisco et al. 1996
2010	4	0.696	Francisco et al. 1996
2054	7	0.522	Francisco et al. 1996
2088	4	0.435	Francisco et al. 1996
A1BG (alpha1B-glycoprotein)	2	0.261	Juneja et al. 1987
AHT101	6	0.522	Holmes et al. 1993a
AHT103	6	0.696	Holmes et al. 1995
AHT104	2	0.304	Holmes et al. 1995
AHT107	3	0.522	Holmes et al. 1993a
AHT109	3	0.348	Holmes et al. 1993a
AHT110	3	0.217	Holmes et al. 1993a
AHT111	5	0.565	Holmes et al. 1993a
AHT115	2	0.130	Holmes et al. 1993b
AHT118	5	0.391	Holmes et al. 1995
AHT119	4	0.261	Holmes et al. 1995
AHT121	7	0.826	Holmes et al. 1995
AHT123	4	0.348	Holmes et al. 1995
AHT125	6	0.652	Holmes et al. 1994
AHT127	3	0.391	Holmes et al. 1995
AHT129	5	0.609	Unpublished
AHT133	5	0.522	Holmes et al. 1995
AHT135	4	0.565	Unpublished
AHT138	4	0.522	Holmes et al. 1995
AHTf66	6	0.609	Unpublished
AHTk120	6	0.522	Unpublished
AHTk20	4	0.609	Fischer et al. 1996
AHTk207	3	0.478	Unpublished
AHTk211	5	0.696	Unpublished
AHTk292	6	0.304	Unpublished
AHTk32	5	0.435	Unpublished
AHTk39	5	0.522	Unpublished
APOA4 (apolipo-protein A4)	2	0.391	Juneja et al. 1989
CPH 8	5	0.435	Fredholm and Winterø 1995
CPH 1	3	0.435	Fredholm and Winterø 1995
CPH 10	7	0.652	Fredholm and Winterø 1995
CPH 11	11	0.478	Fredholm and Winterø 1995
CPH 12	2	0.304	Fredholm and Winterø 1995
CPH 13	8	0.609	Fredholm and Winterø 1995
CPH 14	4	0.609	Fredholm and Winterø 1995
CPH 15	4	0.435	Fredholm and Winterø 1995
CPH 16	6	0.522	Fredholm and Winterø 1995
CPH 19	6	0.391	Fredholm and Winterø 1995
CPH 2	4	0.565	Fredholm and Winterø 1995
CPH 20	3	0.348	Fredholm and Winterø 1995
CPH 21	7	0.522	Unpublished
CPH 3	6	0.652	Fredholm and Winterø 1995
CPH 4	4	0.304	Fredholm and Winterø 1995
CPH 5	3	0.391	Fredholm and Winterø 1995
CPH 6	7	0.435	Fredholm and Winterø 1995
CPH 7	7	0.522	Fredholm and Winterø 1995
CPH 9	5	0.435	Fredholm and Winterø 1995
CXX.123	6	0.435	Ostrander et al. 1993
CXX.130	6	0.522	Ostrander et al. 1993
CXX.140	5	0.522	Ostrander et al. 1993
CXX.147	3	0.435	Ostrander et al. 1993
CXX.176	5	0.522	Ostrander et al. 1995
CXX.2	5	0.435	Ostrander et al. 1993
CXX.20	7	0.696	Ostrander et al. 1993
CXX.213	6	0.739	Ostrander et al. 1993
CXX.250	6	0.826	Ostrander et al. 1993
CXX.251	3	0.174	Ostrander et al. 1993
CXX.279	6	0.652	Ostrander et al. 1993
CXX.30	7	0.565	Ostrander et al. 1993
CXX.69	4	0.609	Ostrander et al. 1993
LEI 004	3	0.696	Holmes et al. 1993a
LEI 015	5	0.609	Mellersh et al. 1994
LEI 030	4	0.739	Unpublished
LEI001	4	0.652	Holmes et al. 1993a
LEI005	8	0.826	Unpublished
LEI024	4	0.391	Unpublished
LEI025	5	0.391	Unpublished
LEI028	3	0.391	Unpublished
LEI032	4	0.435	Unpublished
MichiganCO4107	5	0.696	Yuzbasiyan-Gurkan et al., 1996
PI (alpha1-protease inhibitor)	2	0.087	Juneja et al. 1987
SOD 1 (superoxide dismutase 1)	2	0.130	Baur and Schorr, 1969.
TF (transferrin)	3	0.348	Juneja et al. 1987
VIAS-D10	4	0.348	Primmer and Matthews 1993
VWF	3	0.522	Shibuya et al., 1994

macia ALF, ABI 377). Two-point linkage analysis was carried out with the computer program package FASTLINK 3.0P (Cottingham et al. 1993). As the maximum number of haplotypes is restricted in the analysis with FASTLINK, we first conducted a two-point analysis involving all 94 loci. Only then did we conduct multipoint analyses for ordering purposes exclusively, including only those loci that were linked.

Based on two-point linkage analysis, 43 of the 94 loci could be assigned to 16 different linkage groups (Table 2) which if they correspond to different chromosomes would place linkage groups on about half of the canine chromosomes. Seven linkage groups comprised more than two loci, and of these three could be ordered (Fig. 2). Three of the microsatellites genotyped have also been assigned to chromosomes by FISH. The present standard canine karyotype includes all chromosomes down to 21; at present, it is not possible to unambiguously resolve the smaller chromosomes. Using the physical mapping data (Fischer et al. 1996), we can assign two linkage groups to chromosomes (L13 to *Canis familiaris* 20 (CFA20), and L16 to CFA18); a third linkage group, L8, can be assigned as belonging to a group of the smaller chromosomes.

Assuming that the size of the haploid canine genome is approximately the same as in other mammals (30 M) the 16 linkage groups bracket about 6.5% of the haploid genome (1.9 M). If we further assume that our family material would allow us to detect linkage within 10 cM of a given marker, that is, 10 cM on either side of a linkage group and 10 cM on either side of an unlinked marker, our 16 linkage groups together with the 51 unassigned markers, the total of the 94 investigated loci, potentially cover 15 M, which is about 50% of the haploid genome.

The dog represents the most differentiated domesticated species in terms of the number of distinct breeds and offers many possibilities for basic genetic research not found in other species. Hundreds of years of selective breeding, cross-breeding, line-breeding and inbreeding have created dramatic phenotypic and genetic differences, and there may be fixation, or at least very high allele frequencies, for putative major genes or QTLs. There is extreme variation in many traits including weight, speed, behavior patterns, aggression, and hunting methods; this offers a wealth of opportunities for mapping single major loci as well as quantitative trait loci.

Among dog breeders and dog owners there is great concern about inherited diseases, particularly those with a simple recessive

Table 2. 16 linkage groups defined by typing of the reference panel of the international DogMap collaboration.

Linkage group	locus 1	locus 2	Recombination fraction	lod score
L1	AHT125	AHT118	0.021	10.54
		2010	0.133	9.94
		363	0.071	9.32
		CXX.130	0.150	9.04
	AHT118	2010	0.159	3.24
		363	0.001	6.62
	2010	363	0.155	4.03
L2	LEI 024	LEI 025	0.058	9.17
		CPH 8	0.111	5.94
	LEI 025	CPH 8	0.021	10.83
L3	AHT 109	LEI 030	0.178	3.58
		CXX.69	0.078	5.64
		CPH 3	0.073	6.48
	LEI 030	CXX.69	0.051	9.60
		CPH 3	0.122	8.30
	CXX.69	CPH 3	0.016	14.31
L4	AHT 135	LEI 004	0.054	19.05
L5	TF	453	0.030	13.27
		CXX.123	0.048	7.65
	453	CXX.123	0.018	9.56
		CPH 6	0.100	7.53
L6	PI1	618	0.039	5.38
L7	LEI 005	CXX.279	0.056	23.34
L8	LEI 032	CXX.213	0.218	3.54
		AHTk120	0.185	3.45
	CXX.213	AHTk120	0.083	8.18
L9	LEI 001	CXX.20	0.145	4.82
L10	AHT 133	CXX.359	0.070	8.99
L11	CPH 11	CPH 1	0.001	5.12
L12	CPH 9	CXX.188	0.100	3.89
		CXX.2	0.065	6.93
L13	630	CPH 16	0.122	4.07
		AHTk20	0.085	8.22
L14	AHT127	CPH 13	0.069	10.13
L15	VIAS-D10	CPH 20	0.083	3.63
L16	AHTk292	AHTk32	0.077	3.86

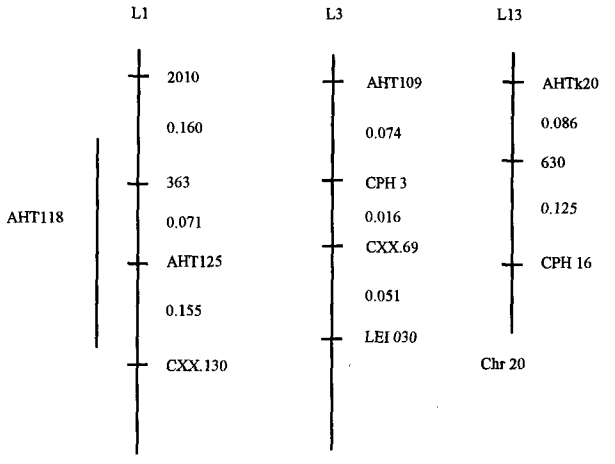

Fig. 2. Ordering of the linkage groups L1, L3, and L13. Linkage group 1 was established by ordering 2010-AHT125-CXX.130 using ILINK (odds 1.67E + 3) and integrating 363 with LINKMAP (odds 7.41E + 3). The locus AHT118 cannot be placed properly with LINKMAP but lies between 2010 (odds 5.59E + 5) and CXX.130 (odds 2.55E + 3). Linkage group 3 was established by placing LEI 030 relative to AHT109-CXX.069 with LINKMAP (odds 1.01E + 3) and integrating CPH 3 with LINKMAP (odds 9.20E + 2). Linkage group 13 was established by placing AHTk20 relative to 630-CPH 16 (odds 2.18E + 4). AHTk20 anchors L13 on Chr 20 (Chr 20). The distances indicated are given in Kosambi morgans and based on two-point linkage analysis.

mode of inheritance, which are the most numerous. The frequency of inherited diseases is alarmingly high in many dog breeds, and these disorders have proved difficult to eradicate by traditional breeding methods. The diagnosis of an inherited disease in a litter usually results in the exclusion of close relatives from further breeding. This may have a dramatic impact on effective population sizes, especially in small inbred populations, and may also reduce breeding progress for other important traits. The identification of genes involved in frequently occurring inherited diseases will allow breeders to avoid crossing two dogs that are carriers for the same recessive disorder. This will have an immediate, dramatic impact on the frequency of dogs suffering from inherited diseases. At the same time, genetic testing permits breeders to do carrier × normal crosses to maintain and improve other beneficial traits in their lines. Mating such carrier males with females homozygous negative for the disease will not produce animals with the disease, and selection against carriers of disease alleles may be performed in the next generation. The existence of canine homologs of human inherited diseases offers attractive possibilities for the development and testing of treatments for human inherited diseases in these canine disease models.

Acknowledgments. Work at the Animal Health Trust and University of Leicester was supported by the Guide Dogs for the Blind Association. Work at the Norwegian College of Veterinary Medicine, the Royal Veterinary and Agricultural University, Denmark, and the Swedish University of Agricultural Sciences was supported by the Nordic Ministry of Agriculture.

References

Baur EW, Schorr RT (1969) Genetic polymorphism of tetrazolium oxidase in dogs. Science 166, 1524–1525

Cottingham RW Jr, Idury RM, Schaffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53, 252–263

Fischer PE, Holmes NG, Dickens HF, Thomas R, Binns MM, Nacheva EP (1996) The application of FISH techniques for physical mapping in the dog (*Canis familiaris*). Mamm Genome 7, 37–41

Francisco LV, Langston AA, Mellersh CS, Neal CL, Ostrander EA (1996) A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. Mamm Genome 7, 359–362

Fredholm M, Winterø AK (1995) Variation of short tandem repeats within and between species belonging to the Canidae family. Mamm Genome 6, 11–18

Holmes NG, Mellersh CS, Humphreys SJ, Binns MM, Holliman A, Curtis R, Sampson J (1993a) Isolation and characterization of microsatellites from the canine genome. Anim Genet 24, 289–292

Holmes NG, Humphreys SJ, Binns MM, Curtis R, Holliman A, Scott AM (1993b) Characterization of canine microsatellites. In *DNA Fingerprinting: State of the Science,* S.D.J. Pena, R. Chakraborty, J.T. Epplen, A.J. Jeffreys, eds. (Basel, Switzerland: Birkhauser Verlag) pp 415–420

Holmes NG, Strange NJ, Binns MM, Mellersh CS, Sampson J (1994) Three polymorphic canine microsatellites. Anim Genet 25, 200

Holmes NG, Dickens HF, Parker HL, Binns MM, Mellersh CS, Sampson J (1995) Eighteen canine microsatellites. Anim Genet 26, 132–133

Juneja RK, Arnold ICJ, Gahne B, Bouw J (1987) Parentage testing of dogs using variants of blood proteins: description of five new plasma protein polymorphisms. Anim Genet 18, 297–310

Juneja RK, Gahne B, Lukka M, Ehnholm C (1989) A previously reported polymorphic plasma protein of dogs and horses, identified as apolipoprotein A-IV. Anim Genet 20, 59–63

Mellersh C, Holmes N, Binns M, Sampson J (1994) Dinucleotide repeat polymorphisms at four canine loci (LEI 003, LEI 007, LEI 008 and LEI 015). Anim Genet 25, 125

Ostrander EA, Sprague Jr GF, Rine J (1993) Identification and characterization of dinucleotide repeat $(CA)_n$ markers for genetic mapping in dog. Genomics 16, 207–213

Ostrander EA, Mapa FA, Yee M, Rine J (1995) One hundred and one new simple sequence repeat-based markers for the canine genome. Mamm Genome 6, 192–195

Primmer CR, Matthews ME (1993) Canine tetranucleotide repeat polymorphism at the VIAS-D10 locus. Anim Genet, 24, 332

Shibuya H, Collins BK, Huang TH-M, Johnson GS (1994) A polymorphic (AGGAAT)n tandem repeat in an intron of the canine von Willebrand factor gene. Anim Genet 25, 122

Świtoński M, Reimann N, Bosma AA, Long S, Bartnitzke S, Pieńkowska A, Moreno-Milan MM, Fischer P (1996) Report on the progress of standardization of the G-banded canine (*Canis familiaris*) karyotype. Chromosome Res 4, 306–309

Yuzbasiyan-Gurkan V, Blanton SH, Cao Y, Ferguson P, Li J, Venta PJ, Brewer GJ (1996) Linkage of a microsatellite marker to the canine copper toxicosis locus in the Bedlington Terrier. Am J Vet Res in press