

Short Communication

Assignment of Canine MSS1 Microsatellite Markers to Chromosomes by Linkage Data

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Recent advances in mapping the canine genome have led to an increase in the number of linkage studies aimed at dissecting the genetic causes of many hereditary diseases that affect the domestic dog. The first step in developing molecular tools for a whole genome scan was the characterization of a set of microsatellite markers, termed minimal screening set 1 (MSS1), that provided an estimated coverage of 10 cM. A limiting factor in use of the MSS1 is not all of the 172 MSS1 markers have been localized to specific chromosomes. Seventy-five of the markers were positioned on a total of 15 chromosomes with the original publication of the MSS1. The localization based on linkage data of 14 additional MSS1 markers to chromosomes using CRIMAP v. 2.4 to build a linkage map of 113 MSS1 markers that were polymorphic in a kindred of Dalmatians is reported here.

Keywords: Canine; MSS1; Microsatellite markers; Linkage map; CRIMAP

There are more than 450 canine hereditary diseases (Nicholas, 2003), and many of these have genetic causes and clinical presentations virtually identical to various human hereditary diseases. This makes the dog an ideal model for the study of simple and complex human hereditary diseases. To this end, linkage studies to dissect the genetic causes of canine hereditary diseases are increasingly common (Patterson *et al.*, 2002; Todhunter *et al.*, 2002; Werner *et al.*, 2002).

The first step in performing a whole genome scan in the dog was the characterization of a set of microsatellite markers (Richman *et al.*, 2001) that is termed minimal screening set 1 (MSS1).

The resolution of this marker set was estimated at 10 cM, but not all of the 172 markers in the set are localized to specific chromosomes by linkage or radiation hybrid (RH) data. Each MSS1 marker's position was reported based on placements on previous linkage maps (Werner *et al.*, 1999; Mellersh *et al.*, 2000). Seventy-five of the MSS1 markers were positioned on a total of 15 chromosomes, including the X chromosome (Werner *et al.*, 1999; Mellersh *et al.*, 2000). Another 20 MSS1 markers were positioned on chromosomes by Mellersh *et al.* (2000) while Werner *et al.* (1999) positioned those same 20 MSS1 markers in linkage groups. The remaining 77 MSS1 markers were positioned in linkage groups (Werner *et al.*, 1999; Mellersh *et al.*, 2000). Despite the lack of definitive positions for all the markers in the MSS1, statistical analysis showed that 77% of the canine genome was within 10 cM of at least one MSS1 marker (Richman *et al.*, 2001). The MSS1 was multiplexed (Cargill *et al.*, 2002) to provide a more efficient method for whole genome scans.

An updated RH map comprised of 3270 markers providing 1 Mb resolution was recently constructed (Guyon *et al.*, 2003). Examination of the locations of the MSS1 markers with respect to this map revealed 68.6% ($N = 118$) MSS1 markers had been positioned on chromosomes, an increase of 43 markers (Richman *et al.*, 2001). A table of the MSS1 markers listed according to locations on the 1 Mb RH map (Guyon *et al.*, 2003) and linkage data from other studies, can be found at <http://www.cvm.tamu.edu/cgr/dallinkagemap.htm>. Each marker's current

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location on the RH map is given as well as the marker's previously reported location (Richman *et al.*, 2001). A reference is also given for the original publication of each marker.

In addition to the updated RH map (Guyon *et al.*, 2003), a second-generation screening set, termed minimal screening set 2 (MSS2), was characterized. The MSS2 provides 9 Mb resolution achieved with 327 markers, all positioned on the RH map. The benefits of the MSS2 over the MSS1 are obvious (e.g. known marker placement and better coverage of the genome). However, many investigators invested money, time and effort in using the MSS1 prior to the recent publication of the MSS2. Therefore, mapping of MSS1 markers not included in the 1 Mb RH map will benefit those using the MSS1.

The multiplexed MSS1 was used in a whole genome scan of a Dalmatian kindred (Cargill *et al.*, 2004). The specific objectives of this work were to collect genotypes for the MSS1 markers and build a genetic linkage map based on data from the Dalmatian kindred.

Genomic DNA was isolated from 117 dogs (54 males, 63 females) of the aforementioned Dalmatian kindred ($N = 266$). The remaining 149 dogs of the kindred were unavailable for collection of DNA samples. The PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) was used to extract genomic DNA from either whole blood or buccal swabs according to the manufacturer's specifications.

MSS1 microsatellite markers were amplified and resolved in multiplex sets as described

(Cargill *et al.*, 2002). The software program Genoprob version 2.0 (Thallman *et al.*, 2001a,b) was used to compute phase and genotype probabilities based on the full pedigree and the RH map positions. Additionally, Genoprob was used to detect likely genotyping errors. Genotypes with an error probability greater than 0.70 were checked against the original GENESCAN data file and corrections were made when necessary.

A linkage map for each canine autosome was constructed using CRIMAP v. 2.4 (Green *et al.*, 1990). Initially, each chromosome was built individually using the BUILD option based on markers appearing on the RH map (Guyon *et al.*, 2003). MSS1 markers not appearing on the RH map were localized to chromosomes using the TWOPOINT option and a LOD threshold of 3.0 (Lander and Kruglyak, 1995). Unmapped markers showing linkage to a chromosome were incorporated into the map using the BUILD option. The FLIPS option was used to evaluate local permutations of marker order. Finally, the CHROMPIC option was used to identify spurious double recombinants and to facilitate the identification and correction of genotyping errors.

Of the 173 multiplexed MSS1 markers, 149 were polymorphic in the Dalmatians, 13 were monomorphic, and 11 could not be resolved for > 50% of the Dalmatians despite multiple optimization attempts. Additionally, 119 of the MSS1 markers had previously been placed on the 1 Mb RH map and 109 of these markers were amplified in the Dalmatians. Of the remaining 54 MSS1 markers not

TABLE I Linkage and positions of 14 MSS1 markers that had not been mapped on the canine radiation hybrid panel, mapped using data from a kindred of Dalmatians

CFA	Marker	cM*	LOD [†]	Mb [‡]	CFA	Marker	cM*	LOD [†]	Mb [‡]
3	FH2137	0.0	3.93	nm	9	FH2566	0.0	6.31	nm
	FH2531	28.6		nm		GALK1	13.5		1.0
	C02864A	42.4	4.96	nm	11	CXX750	90.5	6.32	nm
	FH2316	63.7		67.7		C11873	111.5		80.4
7	C071000	0.0	5.48	nm	15	FH2278	107.0	14.12	71.7
	FH2174	15.4		55.0		C02608	110.3		nm
	FH2201	22.5	3.25	70.6	17	FH2321	9.9	15.91	21.5
	C03895	27.0		nm		PEZ2	17.3		nm
	FH2396	37.0	4.00	nm	18	CPH5	36.1	9.33	36.1
	FH2581	62.4		76.8		WILMSTF	0.0		nm
	8	FH2301	74.0	15.16	nm	25	FH3010	10.9	6.60
FH2138		49.4	nm		C25213		17.9	50.9	
C08618		55.2	9.99	74.4	FH2087A	28.8	4.50	nm	

*Sex averaged positions (in Kosambi centiMorgans, cM) generated using Dalmatian marker data and CRIMAP. †Twopoint LOD score. ‡Radiation hybrid map position (in megabases, Mb) as reported by Guyon *et al.* (2003); nm = not mapped on the RH map.

placed on the 1Mb RH map, 28 markers had previously been placed on chromosomes by linkage data (and all 28 were amplified in the Dalmatians). There are 26 markers that had not been placed previously on a chromosome by linkage or RH mapping (25 were amplified in the Dalmatians).

A linkage map of the polymorphic markers was built with CRIMAP and can be found at <http://www.cvm.tamu.edu/cgr/dallinkagemap.htm>. The order of the markers on each chromosome corresponds to the order as listed in the 1Mb RH map (Guyon *et al.*, 2003). In total, 14 MSS1 markers that were not positioned on the RH map (Guyon *et al.*, 2003) were added to the linkage map. The 14 markers are shown in Table I with linkage results to markers mapped on the RH map (Guyon *et al.*, 2003).

Using the Dalmatian data, of the aforementioned 14 markers, three markers (CXX750, CXX608 and PEZ2) were never mapped to any chromosome by linkage or RH mapping. CXX750 was previously placed in linkage group S4/L3 (Mellersh *et al.*, 1997), but mapped to CFA11. CXX608 was previously placed in linkage group S6/L12 (Ostrander *et al.*, 1995), but mapped to CFA15. PEZ2 was previously an unlinked marker (Neff *et al.*, 1999), but mapped to CFA17.

Of the aforementioned 14 markers, 4 markers (C02864A, FH2087A, C03895 and FH2566) were mapped to different chromosomes than those implicated with previous linkage data. C02864A and FH2087A were previously mapped to CFA02 (Mellersh *et al.*, 1997; Neff *et al.*, 1999), but mapped to CFA03 and CFA25, respectively. C03895 was previously mapped to CFA03 (Neff *et al.*, 1999), but mapped to CFA07. FH2566 was previously mapped to CFA26 (Werner *et al.*, 1999), but mapped to CFA09.

The remaining 7 markers (FH2137, FH2531, C071000, FH2396, FH2301, FH2138 and WILMSTF) of the aforementioned 14 markers were mapped to the same chromosomes suggested by previous linkage data. FH2137 and FH2531 were previously mapped to CFA03 by linkage (Francisco *et al.*, 1996; Werner *et al.*, 1999). C071000, FH2396 and FH2301 were previously mapped to CFA07 by linkage (Mellersh *et al.*, 1997; Neff *et al.*, 1999; Werner *et al.*, 1999). FH2138 was previously mapped to CFA08 by linkage (Francisco *et al.*, 1996). WILMSTF was previously mapped to CFA18 by linkage (Neff *et al.*, 1999).

Placing these 14 markers on the linkage map gives a total of 113 polymorphic MSS1 markers with known locations. With respect to data for the Dalmatian kindred, chromosomes CFA19, CFA31, CFA34 and CFA35 have only one polymorphic marker while chromosomes CFA12, CFA33, CFA36, CFA38 and CFY have no polymorphic markers. Approximately 12 chromosomes (CFA02, CFA04, CFA05, CFA06, CFA07, CFA08, CFA09, CFA13, CFA14, CFA16, CFA19 and CFA23) have significant gaps (> 20cM) in marker coverage based on

the physical size of these chromosomes (Guyon *et al.*, 2003).

The placement of three MSS1 markers that were not placed on chromosomes previously adds to the utility of the MSS1. Mapping of seven MSS1 markers confirms previous linkage data. The placement of four MSS1 markers on chromosomes different than those determined through previous linkage analysis, highlights the need to place all of the MSS1 markers on the RH map. Forty MSS1 markers are still not placed on a chromosome by either the 1Mb RH map (Guyon *et al.*, 2003) or the linkage map based on the marker data from the Dalmatians.

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