

## Final Report

**Grant #/Title:** 2272/Localization of the Gene for Sebaceous Adenitis in the Akita Dog by Homozygosity Mapping

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**Start Date:** 9/01

### Narrative Section

#### Progress related to specific objectives of the project

Ten Akita dogs affected with sebaceous adenitis (SA) and at least nine (up to 29) control Akita dogs were genotyped with 163 microsatellite markers comprising the multiplexed canine minimal screening set of genome markers (MSS-1) (Richman, 2001) with Genotyper 2.0 software. For the control dogs, nine were screened individually for all markers and for a majority of the markers, additional controls were screened in four pools of five dogs each.

Homozygosity mapping is based on the inheritance of two identical copies of the disease locus and its surrounding chromosomal region from a common ancestor. A microsatellite marker allele that is present in this conserved chromosomal region will follow the allele of the disease. Therefore, we were most interested in identifying those markers that were homozygous for a single allele in the affected group. Six such markers were found (see Table 1), unfortunately, the controls were also homozygous for this allele and thus these potential markers could not be used to diagnose sebaceous adenitis as these markers are likely monomorphic in the Akita breed.

**Table 1. Microsatellite markers with a single allele in 38 Akita dogs**

MSS-1 Marker	Allele Size (bp)	Location
FH2062	131	CFA 02
C13.900	126	CFA 13
C28.176	155	CFA 28
FH2377	314	CFA 34
C37.172	186	CFA 37
Pez7	195	unlinked

Next we examined the data for markers displaying an allelic imbalance, in which one allele predominated in the affected group, suggesting that the marker was located close to the causative mutation, but far enough away to allow some recombination. Six such markers were identified (see Table 2). To assess for whether there was a definable association between an allele and sebaceous adenitis, Fisher's exact test (Graph Pad) was used to compare the frequency of the allele of interest between the affected and control groups. As all six of these markers were multiallelic in the Akita, the

most associated allele was selected and the other alleles were combined into a single alternative group in order to perform the test. By convention, a p value < 0.0001 is the statistical significance needed to consider a marker linked to a disease gene for a whole genome scan. The resultant p values ranged from 0.150 to 0.015. To be thorough, additional markers available from the second generation screening set (generously supplied by Dr. Keith Murphy) were genotyped in all affected dogs and 10-20 control dogs for the two chromosomes containing markers that received a p value < 0.05. (CFA 10 and CFA 20). Six new markers were examined on each chromosome and none showed an association with sebaceous adenitis.

**Table 2. Microsatellite markers with an allelic imbalance in SA affected dogs compared to**

MSS-1 Marker	# of alleles	Allele size (bp)	Location	p-value
<b>C02.608</b>	<b>3</b>	<b>139, 137, 143</b>	<b>CFA 2</b>	<b>p=0.053</b>
<b>FH2316</b>	<b>7</b>	<b>318, 326, 330, 322, 332, 336, 334</b>	<b>CFA 3</b>	<b>p=0.128</b>
<b>FH2537</b>	<b>7</b>	<b>150, 154, 146, 172, 170, 164, 168</b>	<b>CFA 10</b>	<b>p=0.015</b>
<b>AHT 130</b>	<b>7</b>	<b>116, 122, 106, 104, 120, 118, 110</b>	<b>CFA 18</b>	<b>p=0.150</b>
<b>REN 55 P21</b>	<b>2</b>	<b>231, 233</b>	<b>CFA 20</b>	<b>p=0.015</b>
<b>FH2233</b>	<b>10</b>	<b>272, 280, 284, 358, 396, 400, 404, 408, 412, 416</b>	<b>CFA 21</b>	<b>p=0.062</b>

We believe there are three likely reasons why an association between a marker and sebaceous adenitis was not found in this study. 1) There is a high mutation rate in the microsatellite DNA near the causative mutation that disrupts linkage disequilibrium between these loci. Data on the frequency of such occurrences within the canine genome is lacking and although this possibility is unlikely, we cannot rule it out. 2) The marker allele linked to the causative mutation is a common allele and is present in the chromosomes of the controls, obscuring linkage disequilibrium. Whether this has occurred in the set of markers used will only be known when a linked marker is discovered and markers from this study near the linked marker can be identified. 3) The locus for SA is not in close proximity to any of the markers examined. Sets of 103 to 213 markers have been used to identify linkage for six canine diseases (Yuzbasiyan-Gurkan, 1997; Acland, 1998 and 1999; Lingaas, 1998; Lin, 1999; Jonasdottir, 2000) however, the number of markers provided by the MSS-1 is associated with gaps in coverage of the canine genome and may not be sufficient to establish linkage for all diseases. This is not the first time this marker set has not allowed for defining linkage in a heritable canine disease. A number of groups have recently failed to detect linkage using this marker set (K. Murphy, pers. comm. 2003; Dukes-McEwan, 2002).

Although we were not able to establish an association with a microsatellite marker and the disease, we continue to believe that linkage disequilibrium and homozygosity mapping can be used very effectively in canine breeds even with markers spaced 10-20cM apart. Recently, Hyun et al (2003) tested this technique by using a low resolution whole genome scan to confirm the location of the gene for copper toxicosis in Bedlington terriers. Interestingly, this group found that evidence for linkage to this disease would have been missed using classical linkage analysis with the same markers. This group reports that linkage disequilibrium extended up to 33cM in this breed around the mutation site. Additionally, we have found a similar result in our own laboratory this year. We identified a microsatellite marker approximately 18cM from a gene containing a mutation causing a severe, recessive scaling disorder in Jack Russell terriers. When allele frequencies for that marker were compared between five affected dogs and 14 unaffected dogs with the Fisher's exact test, a p value <0.0001 was achieved.

An initial concern that Akitas have such extensive genetic homogeneity that identification of linkage would be hindered proved unfounded. Only six of the 163 markers screened were monomorphic (3.7% of the markers analyzed). This is lower than the 10% monomorphism reported by Dukes-McEwan in 48 Newfoundland dogs screened for cardiomyopathy. For completeness and to aid further studies in the Akita breed, Table 3 contains a list of those markers in which we found limited polymorphism (defined here as 2 or 3 alleles).

**Table 3. Microsatellite markers with limited polymorphism in 38 Akita**

MSS-1 Marker	# alleles	Allele size (bp)	Location	MSS-1 Marker	# alleles	Allele size (bp)	Location
AHT 132	3	165, 176	CFA 02	FH2441	3	122, 124, 128	CFA 21
C02.608	3	137, 147, 145	CFA 02	REN 49 F22	3	144, 150, 152	CFA 22
C07.1000	3	105, 110, 114	CFA 07	REN 49 C08	2	174, 178	CFA 22
FH2144	3	347, 354, 359	CFA 08	C22.745	2	270, 278	CFA 22
FH2149	2	156, 202	CFA 08	FH2079	3	276, 274, 278	CFA 24
C12.852	3	99, 101	CFA 12	AHT 140	3	101, 107	CFA 25
C13.391	2	167, 171	CFA 13	REN 01 O23	3	183, 189, 204	CFA 26
FH2060	3	217, 222, 229	CFA 14	C26.733	3	258, 262, 268	CFA 26
AHT 139	2	148, 152	CFA 15	RVCE	2	124, 128	CFA 27
FH2175	2	245, 254, 264	CFA 16	LEI.1F11	3	166, 168, 170	CFA 30
AHT 131	2	106, 110	CFA 16	CPH 2	2	94, 98	CFA 32
AHT 124	2	130, 132	CFA 19	FH2516	2	182, 186	CFA 36
AHT K209	3	92, 94, 98	CFA 20	AHT 106	2	78, 70	unlinked
PRKCD	2	124, 134	CFA 20	TAT	3	157, 163, 165	unlinked
REN 55 P21	2	231, 233	CFA 20	CXX.452	2	168, 173	unlinked
FH2018	3	147, 151	?	FH2550	3	445, 453, 459	unlinked

We believe that this study sets a standard for a cost-effective method to screen the canine genome with a set of microsatellite markers. Pooling of DNA worked well for determinations of alleles within the controls and we believe that for future studies, one could screen pooled samples initially and then perform genotypes on markers of interest individually, reducing the total number of reactions. Importantly, we also were able to use multiplexing of the PCR reactions to save labor and reagent costs.

We initially proposed using DNA from archived, formalin-fixed, paraffin embedded skin biopsy sections for genotyping a portion of the affected dogs. Because of the time and expense incurred by the need for whole genome amplification of these samples in order to have sufficient DNA to test all markers, we found it was more expedient to screen a subset of affected dogs and then planned to test only markers of interest on the DNA obtained from archived samples. In this investigation, no markers gave results that demanded testing these additional samples. From our studies, DNA from archived paraffin embedded tissues could be used to perform genotyping but there was a higher rate of reactions that failed to amplify than with DNA obtained from blood or cheekswabs. Since we initiated this study, newer techniques to amplify the whole genome (such as GenomiPhi, Amersham) have become available and these methods may be more robust than the standard degenerate oligonucleotide primed PCR (DOP) used in this investigation.

In summary, after completing the study as proposed, none of the 163 markers tested showed an association with SA in Akitas. However, the techniques used were successful and by performing this study, a sizable proportion of the Akita genome has been examined and can be excluded, narrowing the search for the causative mutation in future investigations.

### **Presentations**

Credille KM, Dunstan RW. What is known about sebaceous adenitis now? Poodle Club of America National Specialty Show, Upper Marlboro, Maryland, June 2002.

### **Publications**

Greer KA, Cargill EJ, Clark LA, Cox ML, Tsai KL, Dunstan RW, Venta PJ, Credille KM, Murphy KE. Digging up the canine genome: Developments in canine genomics. (in press, *Cytogenetics and Genome Research*, February 2004).

### **References**

- Acland GM, et al. A novel retinal degeneration locus identified by linkage and comparative mapping of canine early retinal degeneration. *Genomics* 59:134-142, 1999.
- Acland GM, et al. Linkage analysis and comparative mapping of canine progressive rod-cone degeneration (prcd) establishes potential locus homology with retinitis pigmentosa (RP17) in humans. *PNAS* 95:3048-3053, 1998.
- Dukes-McEwan J, Jackson IJ. The promises and the problems of linkage analysis by using the current canine genome map. *Mamm Genome* 13:667-672, 2002.
- Hyun C, et al. Prospects for whole genome linkage disequilibrium mapping in domestic dogs. *Mamm Genome* 14:640-649, 2003.
- Jonasdottir TJ, et al. Genetic mapping of a naturally occurring hereditary renal cancer syndrome in dogs. *PNAS* 97(8):4132-4137, 2000.
- Lin L, et al. The sleep disorder canine narcolepsy is caused by a mutation in the Hypocretin (Orexin) receptor 2 gene. *Cell* 98:365-376, 1999.
- Lingaas F, et al. Genetic markers linked to neuronal ceroid lipofuscinosis in English Setter dogs. *Animal Genetics* 29:371-376, 1998.
- Richman M, et al. Characterization of a minimal screening set of 172 microsatellite markers for genome-wide screens of the canine genome. *J Biochem Biophys Methods* 47:137-149, 2001.
- Yuzbasiyan-Gurkan V, et al. Linkage of a microsatellite marker to the canine copper toxicosis locus in Bedlington terriers. *Am J Vet res* 58(1):23-27, 1997.

## Financial Section

Year 1 (Sept. 1, 2001 through Aug. 31, 2002)	
Allocated:	\$31,779
Expenditures:	
Salary, wages and benefits	\$14,743
Supplies	\$4,573
Microsatellite markers	\$2,500
Genotyping lab services	<u>\$1,202</u>
Total expenditures	\$23,018
Year 2 (Sept. 1, 2002 through Aug. 31, 2003)	
Allocated:	\$32,389
Expenditures:	
Salary, wages and benefits	\$29,612
Supplies	\$4,330
Microsatellite markers	\$2,500
Genotyping lab services	<u>\$3,320</u>
Total expenditures	\$39,762
Year 3- no cost extension period (Sept. 1, 2003 through Mar. 31, 2004)	
Allocated:	0
Expenditures:	
Salary, wages and benefits	0
Supplies	\$186
Microsatellite markers	0
Genotyping lab services	<u>\$1,072</u>
Total expenditures	\$1,258
Total Allocated:	\$64,168
Total Expenditures:	\$64,038

## **Lay Summary**

In the fall of 2001, the Comparative Dermatology Laboratory at Texas A&M University was funded by the Canine Health Foundation and the Akita Club of America to scan the Akita genome using the most validated set of DNA markers available at the time to determine if a DNA-based test for the skin disease sebaceous adenitis (SA) in the Akita breed could be established. SA is a devastating disease in Akitas because it is relatively common, clearly heritable and has no curative therapy. Because this disease seldom shows itself until sexual maturity and because a small group of affected dogs may never develop obvious clinical signs, it can be very difficult for breeders to determine which dogs to breed. For these reasons, all agree that SA is a disease very much in need of a genetic test.

Because of the problems in distinguishing SA-affected from normal dogs, studies utilizing conventional linkage analysis based on pedigrees cannot be used reliably. Instead, our approach was to associate a DNA marker with SA by defining the markers found in a large number of carefully selected normal and unequivocally affected dogs. Akitas appear to be ideal for this type of investigation because of their history of genetic bottlenecks and growth within relatively isolated populations.

163 DNA markers were tested in our groups of SA affected and unaffected dogs. None of these markers could be associated with SA. Our most likely explanation for this finding is that the gene containing the mutation that causes SA was not in close proximity to any of the markers examined. It is becoming clear that the current set of DNA markers does create gaps in covering the canine genome and therefore, a number of recent studies performed with similar sets of markers have had results like ours. In short, luck was not with us.

Although the capriciousness of biology is disappointing, this study is not without value to canine genomic research. First, through this project we have examined a large portion of the Akita genome and have eliminated these areas from further consideration for the locus of this disease, thus narrowing the search for a marker for SA. Second, all of the techniques we proposed and performed in this study worked well and can be used in future canine studies. Third, one concern for this project was that Akitas would not demonstrate sufficient genetic variation to detect an association with a disease. Based on our data, this does not appear to be the case. Lastly, we continue to believe that the approach we have selected will be effective for finding markers for hereditary diseases in purebred dogs and in fact, while our study was on-going, a paper supporting this technique was published. In summary, this study shows that with the development and examination of more DNA markers covering the canine genome, a DNA-based test for SA will be a reality for the Akita.

The members of the Comparative Dermatology Laboratory, along with our co-investigators in the Canine Genetics Laboratory and in the Laboratory of Molecular Medicine and Canine Genetics at Michigan State University, wish to express their deep appreciation for the support of the Akita Club of America and the AKC Canine Health Foundation and to the Akita owners and veterinarians who have contributed their time and samples for this important study.



February 6, 2004

AKC Canine Health Foundation  
Attn: Ms. Erika Werne  
P.O. Box 37941  
Raleigh, NC 27627-7941  
Phone: 919-334-4010

Dear Erika,

Enclosed please find my final report for Grant No. 2272: "Localization of the Gene for Sebaceous Adenitis in the Akita Dog by Homozygosity Mapping". I have also included a summary of the results in lay language that can be forwarded to the Akita Club of America for use in their publications, if desired.

Sincerely,

Kelly M. Credille, DVM, PhD  
Asst. Professor