

1 Genetic characterization of Standard Poodles from the United  
2 States and the United Kingdom and how it relates to  
3 geography and sebaceous adenitis disease status  
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15

16 **Definitions**

17 **Mendelian-** The pattern of inheritance of simple genetic traits (traits caused by a mutation in a  
18 single gene) is often referred to as Mendelian, following the classic inheritance studies done on  
19 the common flowering pea by Gregor Mendel.

20 **Complex genetics** – Traits that are caused by the collective effects of numerous genes are  
21 referred to as being complex or **polygenic**. The term Mendelian inheritance is not usually applied  
22 to complex traits, because Mendel's studies dealt with simple or **monogenic** inheritance.

23 **Heritability-** The degree to which a genetic trait is under genetic control. Disorders such as  
24 autoimmunity and cancer may only be 30-50% heritable, with epigenetic and environmental  
25 triggers playing a role in the remaining disease prevalence.

26 **Epigenetic-** Epigenetic changes are alterations in DNA that occur after birth as a result of a  
27 variety of extrinsic and intrinsic processes affecting the genetic code. Epigenetic changes, once  
28 they occur, are often heritable. Epigenetic changes explain why even identical twins grow more  
29 and more dissimilar in appearance, personality, and disease predilection over time.

30 **Locus or loci** – A locus is the specific site on a chromosome where a given gene is found.

31 **Single nucleotide polymorphisms (SNPs)** - A genetic variation in the sequence of DNA that  
32 occurs when a single nucleotide (A, T, C or G nucleotides) is changed is referred to as a SNP  
33 (pronounced snip). Mutations in SNPs, such as an A to T or C to G, occur rarely in evolution. A  
34 mammalian genome has millions of SNPs, but each SNPs has only two possible alleles.

35 **Short tandem repeat (STR)** - A STR is a pattern of two or more nucleotides in the non-coding  
36 regions of the genome that are repeated in a sequential manner, e.g., ...CGCGCGCGCG... (di  
37 STR), ...AATAATAATAAT... (tri STR) or ...CGGGCGGGCGGGCGG... (tetra STR). Such  
38 regions mutate frequently compared to SNPs and are reflected by a change in size (number of  
39 repeat elements). STRs are much more polymorphic than SNPs and can have a large number of

40 alleles. Their polymorphic nature and relatively rapid evolution make them valuable tools to  
 41 determine genetic changes that have occurred over the last hundred and thousands of years rather  
 42 than over hundreds of thousands of years.

43 **Mitochondrial DNA (mtDNA)** – mtDNA is found in the cytoplasm of cells in structures called  
 44 mitochondria. mtDNA is passed from cells of the mother to cells of the fetus through the ovum.  
 45 Sequences from certain regions of mtDNA are used to trace maternal origins.

46 **Y SNPs and Y STRs**- The Y chromosome is the most genetically stable of all chromosomes..  
 47 Therefore, there are a limited number of SNP and STR differences in coding and noncoding  
 48 regions that have occurred during the evolution of various male lineages. These STR and SNP  
 49 differences are powerful tools in tracing more recent as well as ancient paternal lineages.

50 **Genome** – The genome contains all of an individual's hereditary information. The dog genome  
 51 consists of 78 chromosomes; 38 pairs of autosomes and one pair of sex chromosomes (XY or  
 52 XX).

53 **Genome wide association study (GWAS)** - GWAS tests for the presence of genetic variants in  
 54 one population (case or affected) versus another (control or unaffected). GWAS uses genetic  
 55 markers (usually SNPs, but sometimes STRs) that are evenly and closely spaced across each  
 56 chromosome of the genome. If a certain marker is significantly more common in case than  
 57 control individuals, it strongly suggests that the genetic cause for the trait is linked directly or  
 58 indirectly to a gene or genes on or near that position of the genome.

59 **Autosomal DNA**- An autosome is a chromosome other than the sex chromosomes (X and Y).  
 60 Autosomes contain the genomic DNA.

61 **Indigenous dogs** – Dogs still existing today and loosely attached to villages in under-developed  
 62 countries throughout the world. Most indigenous dog populations have been randomly breeding  
 63 for thousands of years and are therefore repositories of the original dog DNA.

64 **Alleles** – Each gene is made up of two identical or nearly identical copies (alleles), one inherited  
 65 from the sire and one from the dam. Alleles often exist in a number of slightly different genetic  
 66 forms (**polymorphisms**). When the exact same form of a gene is inherited from each parent, the  
 67 alleles are said to be **homozygous**, and if different, **heterozygous**.

68 **Genotype**- Genotype refers to the specific allele makeup of the individual with reference to the  
 69 specific trait being considered.

70 **Haplotype**-A haplotype occurs whenever specific alleles on specific genes are always inherited  
 71 as a block, i.e., they are linked to each other. Alleles of the three DLA class II genes frequently  
 72 form three-locus haplotypes. Haplotypes can be involve alleles at a small number of genes or can  
 73 encompass regions of the genome containing many genes.

74 **Dog leukocyte antigen (DLA) complex**- All vertebrate animals possess a large group of genes,  
 75 usually loosely or tightly linked to each other and on a single chromosome, which code for  
 76 proteins important in regulating immune responses and disease processes such as autoimmunity.  
 77 The general term for this region across species is the **major histocompatibility complex**  
 78 (MHC). The DLA is the name given to the MHC of the dog and it is composed of four major  
 79 classes of genes, I, II, III, and IV.

80 **DLA class II genes**- The DLA class II region on canine chromosome 12 is one part of the larger  
 81 DLA. The class II region contains a dozen or more genes that are involved with immune  
 82 recognition. Three genes called **DRB1**, **DQA1** and **DQB1** code for proteins that help form  
 83 cellular receptors important for the recognition of foreign substances by cells of the immune  
 84 system and the production of antibodies.

85 **Zygoty** - Zygoty refers to similarities in alleles at a specific genetic locus or loci (haplotypes).  
86 If the two alleles are identical, the alleles are said to be **homozygous**, and if different,  
87 **heterozygous**.

88 **Linkage disequilibrium (LD)** - LD refers to the randomness of alleles at two or more genetic  
89 loci, either within a region of the same chromosome (e.g., the DLA) or on different  
90 chromosomes. LD occurs when the genetic type (genotype) at one loci are not inherited  
91 independently of each other. The DLA is an example of a region of high LD, because many of  
92 the genes and their alleles are inherited dependently (non-randomly) rather than independently  
93 (randomly) of each other.

94 **Hardy-Weinberg Equilibrium (HWE)** - The HWE principle holds that genetic variation in a  
95 population will remain constant from one generation to the next in the absence of factors that  
96 disrupt random mate selection. Although an ideal, HWE is seldom achieved because of  
97 disruptive pressures (man-made as well as natural) against random mate selection. This is  
98 especially true for breed development, regardless of species.

99

## 100 **I. Summary**

101

102 This study has two objectives; 1) to compare genetic diversity within Standard Poodles from the  
103 United States (US) and the United Kingdom (UK), and 2) to search for possible genetic  
104 associations with sebaceous adenitis in the breed. A total of 233 Standard Poodles (149 from the  
105 US, 84 from the UK) were used in the overall study. Pedigrees were analyzed for relatedness and  
106 28% of US dogs and 38% of UK dogs were found to have the same individual or individuals  
107 appear more than once within three generations. This was the first indication of ongoing  
108 inbreeding. Standard Poodles from the US and UK, regardless of SA status, shared a major  
109 matriline (A for US dogs) or matriline (A and B for UK dogs), and a single patriline (D1D5).  
110 Matriline and patriline were shared with many other modern breeds and with indigenous  
111 (village dogs) in SE Asia. Matriline B and C in US dogs (20% of US population) and F and H  
112 in UK dogs (8% of UK population) appeared largely free of SA. UK dogs from matriline B  
113 were about one half (13% vs 26%) as likely to be SA affected. Therefore, SA appeared to have  
114 entered the breed through matriline A. About one half of the genome (20 chromosomes) was  
115 scanned using single tandem repeat (STR) markers, each detecting 3-9 alleles (genetic variants)  
116 per locus. Based on comparative allele frequencies at each STR locus, US and UK populations  
117 were found to be closely related but genetically distinguishable. Therefore, the two populations  
118 share a common gene pool in the relatively recent past and their ancient paternal origin was  
119 traced to village dogs in present day Taiwan and the Phillipines. Analysis of the STR markers  
120 indicated some degree of either inbreeding or population substructure (i.e., differing bloodlines

121 based on geography or non-random selection?) within dogs from both the US and UK.  
122 Although there were minor genetic differences between US and UK Standard Poodles in general,  
123 there were no discernible differences between SA affected and unaffected dogs from the same  
124 geographic regions. This observation tends to confirm more detailed analysis of the genomes  
125 using 172,000 single nucleotide polymorphism (SNP) markers. These studies also failed to  
126 identify genetic differences that would segregate SA affected from healthy dogs. Comparisons  
127 were then made in the region on chromosome 12 that contained genes of the major  
128 histocompatibility complex (MHC). This region, known as the dog leukocyte antigen (DLA)  
129 complex in dogs, contains a large number of genes that are involved with the recognition of  
130 foreign substances (antigens), the ability to differentiate self- from non-self-proteins, and genes  
131 that regulate the type and intensity of the immune response. A small region of the DLA (dog  
132 MHC) contains three genes that regulate the recognition of foreign antigens that evoke an  
133 antibody response. These genes are collectively known as the DLA class II genes. Each of the  
134 three genes (*DRB1*, *DQAI* and *DQBI*) contains two possible alleles (genetic variants) – one is  
135 inherited from the mother and one from the father. In most purebred dogs, including the  
136 Standard Poodle, each of the DLA class II genes are composed of from 4 to 13 different alleles.  
137 *DRB1* is the most genetically diverse of the class II genes, while *DQA1* is the least diverse (i.e.,  
138 most conserved in evolution). US Standard Poodles were more diverse in the DLA class II genes  
139 than UK Poodles. Certain alleles at each of the three DLA class II genes are frequently linked to  
140 a specific allele on the other two genes, forming what is known as a DLA class II haplotype.  
141 The DLA class II alleles of the Standard Poodles form 14 different haplotypes (i.e., possible  
142 combinations of alleles). These haplotypes exist in a heterozygous (the haplotype from one  
143 parent is different than the haplotype contributed by the other parent) or homozygous (the  
144 haplotype from sire and dam are the same). Ninety four percent of US and 92% of UK Poodles  
145 were either heterozygous (~40%) or homozygous (~50%) for a single major DLA class II  
146 haplotype (*DRB1\*01501/DQA1\*00601/DQBI\*02301*), but showed some differentiation in the  
147 frequency and geographic distribution of the 13 less common (minor) haplotypes. However, as  
148 with the more genome wide association studies, no difference were observed in the distribution  
149 of major and minor DLA class II haplotypes between SA affected and unaffected dogs from the  
150 same country. This was unexpected, because varying degrees of genetic association is usually  
151 found between certain DLA class II haplotypes and various autoimmune disorders in other pure

152 breeds. Genetic diversity within the DLA region was also tested by a technique called zygosity  
153 mapping. Zygosity mapping provides a visual measure of genetic diversity within the DLA  
154 region, and in this study, the gold standard for genetic diversity in the DLA was an ancestral  
155 outbred population of village dogs from SE Asia. Zygosity maps in the DLA of Standard Poodles  
156 show a significant loss of diversity compared to their SE Asian ancestors, with some individual  
157 Standard Poodles being virtually identical across the entire region.

158 Standard Poodles are quite inbred, but no more so than a number of other pure breeds.  
159 The degree of inbreeding is made more apparent by studies within the DLA region, and  
160 particular in the DLA class II genes. The DLA region, and especially the DLA class II genes, is  
161 normally under what is called high linkage disequilibrium (i.e., genes and their alleles tend to be  
162 inherited as blocks from each parent rather than as independently segregating entities).  
163 Therefore, these regions of the genome are much more susceptible to the effects of inbreeding  
164 than other regions of the genome. The high level of homozygosity in the DLA and DLA class II  
165 regions of Standard Poodles is a strong indication that similar regions of homozygosity exist in  
166 other parts of the Standard Poodle genome. Genes associated with disease traits are frequently  
167 found within such regions of homozygosity.

168 Genetic associations for SA were also not identified in the DLA region as a whole or in  
169 the DLA class II region in particular. This was somewhat unexpected, because associations  
170 between almost all other autoimmune diseases and the DLA class II region have been previously  
171 reported. This can be interpreted in two manners. It is possible that SA is not linked to genes in  
172 the DLA or DLA class II regions of the genome, or that an association exists but is present in  
173 almost all Standard Poodles (i.e., it is fixed in the breed), making it extremely difficult to detect.  
174 This latter possibility was supported by the extremely high prevalence (90%) of a single DLA  
175 class II haplotype in both US and UK Poodles.

176 Although preliminary studies such as this, as well as much denser whole genome scans,  
177 have failed to identify a genetic association for SA, circumstantial evidence supports a genetic  
178 component to the disease. The heritability of autoimmune disorders in humans, and in several  
179 breeds where it has been determined, has ranged from 30-50%. The remaining 50-70% of  
180 disease has been associated with epigenetic changes and environmental triggers. Epigenetic  
181 changes to DNA occur after birth as a result of aging, radiation, toxic substances, and intrinsic  
182 transpositions of genes caused by certain types of inherent processes. Environmental triggers

183 include things such as infections, traumas, toxic exposures, stresses, etc. To further confound  
184 genetic association studies, autoimmune diseases in humans and dogs do not follow a simple  
185 Mendelian mode of inheritance, which means that the portion of disease risk attributable to  
186 genetic factors is the sum total of risks imposed by a number of genes. Genetic association  
187 studies with complex genetic traits require a much greater number of case and control animals, a  
188 much larger number of genetic markers, and careful consideration of the confounding effects of  
189 population substructure. Unfortunately, the ease with which simple Mendelian traits have been  
190 identified in dogs, sometimes with as few as five affected dogs, has led people to believe that  
191 identifying genetic associations (and ultimately the development of genetic tests) for complex  
192 traits such as autoimmunity and cancer would be equally simple.

193         Studies not detailed herein demonstrated that Addison's disease and SA are probably not  
194 part of the same autoimmune syndrome. SA appears to have entered the breed through dogs  
195 from the major maternal haplotype (type A), and is largely free from dogs with minor maternal  
196 haplotypes, especially C. However, Addison's disease occurs at similar prevalence in all  
197 maternal haplotypes, and selection for C would probably not reduce the Addison's disease  
198 prevalence.

199         Although preliminary studies have not identified a genetic association for either SA or  
200 Addison's disease in the Standard Poodle using high density SNP arrays and increased numbers  
201 of case and control animals, it does not mean that finding such an association will be impossible.  
202 Increasing the numbers of case and controls tested by high density SNP arrays may still yield an  
203 association, but the number of case animals may have to be many hundreds and even thousands  
204 to demonstrate a significant association. Two alternative approaches may be more viable. The  
205 first would be to use a large number of STR markers (>800) across the genome rather than the  
206 SNP markers. STR loci are much more polymorphic (variable) and have evolved and changed  
207 much more recently than SNP markers. Therefore, they may better reflect genetic mutations and  
208 associations that have developed over the last several hundred years. A third possibility would  
209 be to use Miniature Poodles for controls, because they are much more likely to be free of the SA  
210 trait. If the trait for SA is fixed in Standard Poodles, healthy Miniature Poodles with no history  
211 of SA, may be useful controls for identifying the genetic basis of SA in Standard Poodles.  
212 However, before doing this, a detailed genetic analysis of Miniature Poodles would have to be  
213 done, and only those dogs with close genetic relationships to Standard Poodles should be

214 included in such a study. Although many people consider Miniature Poodles to be genetically  
215 similar to Standard Poodles, differing only in size, evidence from other researchers suggests that  
216 they may be more genetically distinct than believed. Regardless of which approach or  
217 approaches should be pursued, far more money will be required for research and much better  
218 participation will be required from owners of SA affected dogs in submitting DNA.

219

## 220 **II. Introduction to SA study in Standard Poodles**

221

222 The Standard Poodle is known for its temperament, intelligence, and outstanding coat.  
223 However, as with most pure breeds, it has its own set of health problems. The Poodle Health  
224 Registry database lists over 50 major health disorders of Standard Poodle  
225 (<http://www.poodlehealthregistry.org>), ten of which are of an autoimmune nature. These  
226 autoimmune diseases include sebaceous adenitis (SA), Addison's disease (hypoadrenocorticism),  
227 immune mediated hemolytic anemia (IMHA), chronic active hepatitis, diabetes mellitus (type I),  
228 immune mediated thrombocytopenia (IMTP), masticatory myositis, lupus erythematosus (discoid  
229 and systemic), symmetrical lupoid onychodystrophy, and hypothyroidism. The 2010/2011  
230 health survey by the PCA Foundation placed the prevalence of SA in Standard Poodles at 2.7%,  
231 Addison's disease 2.5%, hypothyroidism/thyroiditis 1.8%, IMHA 1.0%, chronic active hepatitis  
232 0.7%, and IMTP 0.3%. Assuming that the majority of Standard Poodles suffer from only one  
233 autoimmune disease, the overall prevalence of autoimmune disease among US Standard Poodles  
234 would be approximately 9%.

235 Sebaceous adenitis in dogs was first described in detail by Scott (1). The disease has been  
236 reportedly recognized in a number of pure breeds of dogs (2), but is most prevalent in Akitas (2,  
237 3), Standard Poodles (3, 4), English Springer Spaniels (4), and Havanese (5). Detailed  
238 histopathologic and immunohistopathologic descriptions of lesions of sebaceous adenitis have  
239 been reported by Scott (1), Reichler et al (2), Gross and colleagues (6) and Rybnicek et al (7).  
240 Lesions often appear as hair loss in the region of the head (face, ears, neck) (Fig. 1). The  
241 subsequent disease can evolve slowly or quickly, and be relatively localized or generalized to the  
242 body. It can also undergo spontaneous regression at times. A subclinical form also exists,  
243 wherein biopsies show characteristic inflammation centered on sebaceous glands but without

244 outward signs of disease of the coat. Furthermore, there is not always a direct relationship to  
245 histologic lesions and outward clinical signs (2).

246 Dunstan and Hargis (8) were the first to suggest that sebaceous adenitis was a simple  
247 Mendelian trait, but no heritability studies were published. However, the patterns of disease  
248 occurrence among related individuals and highly variable age at onset (very young to aged dogs)  
249 is not entirely compatible with a simple recessive trait. Preliminary GWAS carried out in the  
250 UK on 20 SA affected and 28 healthy Standard Poodles using moderately dense SNP arrays  
251 (22,362 SNPs) failed to show a simple Mendelian association in any region of the genome with  
252 disease (9). A more robust GWAS using SA affected dogs from the US and UK using more  
253 than twice the number of dogs and eight times the number of SNPs also failed to find a definitive  
254 genetic association with disease (unpublished data, 2011). Although whole genome scanning has  
255 so far failed to demonstrate a genetic basis for SA in Standard Poodles, there is little doubt that  
256 genetic factors play a role in the disease. Breeders often associate disease risk with certain  
257 matings and bloodlines and some blame excessive inbreeding (10) as a factor in the increasing  
258 disease prevalence. The most common genetic link with autoimmune disease in both dogs and  
259 humans to date has been with genes in the MHC (HLA in humans and DLA in dogs).  
260 Autoimmune disorders occur disproportionately in pure breeds and often associate with specific  
261 dog leukocyte antigen (DLA) class II haplotypes, especially when they are in the homozygous  
262 state (reviewed in 11,12). Human autoimmune disorders are also frequently associated with  
263 genes in HLA complex, as well as genes controlling T cell regulation, and genes involved with  
264 the production of immunoglobulins (13).

265 Given the difficulty in identifying a genetic association for SA in Standard Poodles using  
266 high density genome wide association studies (GWAS), a decision was made to take a step back  
267 and to more thoroughly analyze the basic genetic makeup of Standard Poodle populations in the  
268 US and UK. Maternal mtDNA and Y SNP/STR haplotypes for the breed were determined, as  
269 well as genetic diversity and population structure based on 24 highly polymorphic STR markers  
270 spread across 20 chromosomes. The DLA region, including the DLA class II genes, was also  
271 interrogated by high density SNP scan, sequencing of class II alleles, and zygosity mapping.  
272 Zygosity maps of the DLA region of SA affected and healthy Standard Poodles were compared  
273 to similar maps derived from village dogs of SE Asia, which are living representatives of the

274 ancestors of modern Standard Poodles. These various genetic parameters were then compared in  
275 SA affected and healthy Standard Poodles from both the US and UK.

### 276 **III. Scientific methods used in study**

#### 277 **A. Standard Poodle case and control samples**

278 One hundred forty nine Standard Poodles from the US and 84 dogs from the UK were enrolled in  
279 the study. Forty nine dogs from the US and 23 dogs from the UK suffered from SA. DNA  
280 containing samples were collected as either 2-5 ml EDTA blood (US dogs) or air dried buccal  
281 swabs using cytological brushes (UK dogs).

282 Pedigrees of all dogs included in the study were screened for relatedness to three generations  
283 Pedigrees were either submitted with the sample or downloaded from the American Kennel Club  
284 (AKC) and Kennel Club UK websites. After removing dogs related to the level of grandparents,  
285 107/149 dogs remained from the US (36 SA affected and 71 unaffected) and 52/84 from the UK  
286 (13 affected and 39 unaffected). Therefore, 28% of dogs from the US and 38% from the UK,  
287 regardless of SA status, had the same animal appear at least twice within three breeding  
288 generations.

289 Analyses were performed on randomly related dogs and on the unrelated subset of these dogs  
290 (sharing no common relative through the level of grandparents). Analyses were performed on  
291 both randomly related dogs and unrelated dogs. However, qualitative results were the same  
292 regardless of degree of relatedness; therefore, only analyses based on the full data set of  
293 randomly related dogs were presented in most tables and figures.

294

#### 295 **B. Indigenous Bali street dog samples**

296

297 DNA was extracted from buccal swabs from 26 randomly selected indigenous dogs from the  
298 streets of Bali (14). Bali street dogs are ancient descendants of dogs migrating from SE Asia  
299 (14) and maintain the broad genetic diversity of their ancestors (14, 15).

300

#### 301 **C. DNA extraction**

302

303 DNA was extracted from whole EDTA blood or cytological brushes using Qiagen Genra  
304 Puregene Blood Kit according to the manufacturer's instructions.

305

#### 306 **D. Determination of paternal and maternal haplotypes**

307

308 Y chromosome haplotypes were determined for 17 SA affected and 48 unaffected male Standard  
309 Poodles from the US and 12 SA affected and 29 unaffected from the UK using a panel of 11 Y-  
310 SNPs (16). The SNPs were assayed using a Sequenom MassARRAY Compact 96 using iPLEX  
311 Gold technology. Primer sequences for Y-SNPs were previously reported (16). Ninety one male  
312 Standard Poodles from the US, including 30 SA affected and 61 unaffected dogs were also tested  
313 with a panel of seven Y-STR markers. Primer sequences and allele sizes for these markers have  
314 been previously reported (17).

315 Mitochondrial DNA (mtDNA) haplotypes were determined for 28 SA affected and 75  
316 unaffected Standard Poodles from the US and 23 SA affected and 58 unaffected dogs from the  
317 UK by sequencing 655 bp of the mitochondrial control region between nt 15452 and 16107 as  
318 previously described (18). Primer sequences, conditions for PCR, cleaning of PCR products, and  
319 sequencing have been previously reported (12).

320

#### 321 **E. Genetic diversity using STR markers**

322

323 Twenty four STRs located on 20 different autosomes were used in the study. Repeat motif,  
324 chromosome assignment, known allele numbers and allele size range for this set of markers have  
325 been previously reported (12).

326

#### 327 **F. DLA Class II genotyping**

328

329 Alleles of the DLA class II genes, DRB1, DQA1, and DQB1, were determined for 47 SA  
330 affected and 90 unaffected Standard Poodles from the US and 23 affected and 61 unaffected  
331 Poodles from the UK by sequence-based typing using published locus-specific intronic primers  
332 (20, 21). PCR reactions, purification of PCR products, and sequencing procedures have been  
333 previously described (12).

### 334 **G. DLA wide SNP typing**

335 DNA from 34 SA affected and 24 unaffected dogs from the US, and 23 SA affected and 16  
336 unaffected dogs from the UK were tested on CanineHD Genotyping BeadChips. Data from 150  
337 SNP markers overlapping the DLA region (base 3802975 to 5672682) of CFA12 were extracted  
338 from the genome wide association study (GWAS). Thirty five of these SNPs were discarded for  
339 being monomorphic, leaving usable data from 115 SNPs across the entire DLA region. Genome  
340 and DLA wide SNP associations were determined by PLINK analysis with MAF >0.05, call rate  
341 >90%, and 50,000 permutations (21).

### 342 **H. Data analysis**

343 Haplotype frequencies (mtDNA and DLA) between US and UK populations and between  
344 affected and unaffected Standard Poodles were compared using Chi-square tests of  
345 independence, with rarer haplotypes pooled to ensure that <20% of expected cell frequencies  
346 were <5 cases (22). Calculation of descriptive statistics, expected ( $H_E$ ) and observed ( $H_O$ )  
347 heterozygosity, and tests of Hardy Weinberg equilibrium were performed using Arlequin v3.1  
348 (23), as were coefficients of inbreeding ( $F_{IS}$ ) within populations and fixation indices ( $F_{ST}$ )  
349 between populations. Tests for gametic (“linkage”) disequilibrium were performed using  
350 Genepop on the Web (v 4.0.10) (24). Sequential Bonferroni adjustments were applied to P-  
351 values to avoid inflation of type I errors due to repeated performance of Hardy-Weinberg and  
352 Gametic Equilibrium tests (25). Because numbers of individuals differed between US and UK  
353 samples, a rarefaction procedure performed in program HP-rare was used to effectively equalize  
354 sample sizes for these estimates based on the lowest numbers of genes sampled from any  
355 population and locus (26). Statistical comparison of averages across loci was based on 95%  
356 confidence intervals calculated from the Z distribution (21). Principle Coordinate Analysis  
357 (PCoA) was performed using GenAlex v6.41 (27).

358 A Bayesian model-based method that utilizes genotype frequencies, with no prior  
359 information on population of origin, was used to assess substructure within the data set (28, 29).  
360 The admixture model with correlated allele frequencies was employed.

### 361 **IV. Results**

362

**363 A. Maternal haplotypes**

364

365 Seven distinct mtDNA (maternal) haplotypes were identified among 103 randomly related  
366 Standard Poodles (28 SA affected, 75 unaffected) from the US and 81 Poodles (23 SA affected  
367 and 58 unaffected) from the UK (Table 1). GenBank accession numbers corresponding to the  
368 seven mtDNA haplotypes identified in this study are given in Table 1.

369 Maternal haplotype diversity ( $1 - \text{sum of squared frequencies}$ ) (30) was 0.47 for US dogs and  
370 0.41 for UK dogs; these frequencies were not significantly different between the two countries  
371 ( $F_{ST} = 0.019$ ; Chi square, 2;  $df = 0.17$ ;  $P = 0.92$ ). Therefore, dogs from the US and UK countries  
372 were then pooled for subsequent comparisons. Haplotype frequencies differed significantly  
373 between unaffected and affected dogs ( $F_{ST} = 0.160$ ; Chi square, 2;  $df = 6.3$ ;  $P = 0.04$ ), including  
374 a two-fold difference in haplotype diversity between unaffected (0.51) and SA-affected (0.25)  
375 dogs. This difference was caused by a greater frequency of the most common haplotype in the  
376 SA affected dogs and of minor haplotypes in unaffected dogs (Table 1).

377 Maternal haplotype frequencies of Standard Poodles used on studies of SA in US dogs were  
378 compared to frequencies found for US Standard Poodles with Addison's disease (Table 2). SA  
379 appears to have entered the population from dogs with the major A maternal haplotype, while  
380 dogs with minor maternal haplotypes B were relatively free of SA and dogs with haplotype C  
381 were all healthy. The role of maternal haplotype is not as clear for Addison's disease, i.e., no  
382 major or minor haplotype was significantly more or less frequent between affected and healthy  
383 dogs.

384

**385 B. Paternal haplotypes**

386

387 The 91 SA affected and unaffected dogs from the US and UK shared a single Y SNP haplotype  
388 (AGAAGACCTCC), which is found in village dog populations from across SE Asia, a region to  
389 which most modern breeds trace their ancestry (15). All of these male dogs also shared an  
390 identical Y-STR haplotype. The Y STR markers and their alleles in parentheses were: MS34A  
391 (172), MS34B (176), MS41A (206), MS41B (219), 990.35.4 (127), 650.79.2 (120/134), and

392 650.79.3 (122/124). This haplotype has been designated as D1D5, and is the most common Y-  
393 STR haplotype among breed dogs (17).

394

### 395 **C. Partial genome scan using 24 STR markers on 20 autosomes**

396

397 All 24 autosomal STRs were polymorphic in both US and UK Standard Poodles, yielding 172  
398 alleles. The average (across loci) observed heterozygosity ( $H_o=0.576$ ) was significantly ( $P <$   
399  $0.0001$ ) lower than average expected heterozygosity ( $H_e=0.622$ ), indicating population  
400 substructure within the total sample. Allele frequencies differed significantly between US and  
401 UK poodles ( $F_{ST} = 0.024$ ;  $P < 0.0001$ ), but not between affected vs. unaffected dogs within  
402 either the US ( $F_{ST} = 0.001$ ,  $P = 0.19$ ) or the UK ( $F_{ST} = -0.010$ ,  $P = 0.99$ ).

403 Six of the 24 STR loci in the US and two loci in the UK were significantly out of Hardy-  
404 Weinberg equilibrium, including one locus (INRA21) in both populations (Table 2). After  
405 sequential Bonferroni corrections to adjust for differences in sample size, 13 locus pairs in the  
406 US and nine different locus pairs in the UK (of 276 pairwise combinations in each population)  
407 were out of normal equilibrium. These findings were consistent with inbreeding or population  
408 substructure in both US and UK populations. The coefficient of inbreeding was statistically  
409 significant in these populations albeit low, with  $F_{IS}$  estimated across loci at 0.07 (SE = 0.013) in  
410 the US and 0.05 (SE = 0.023) in the UK. To obtain comparisons of allelic richness between  
411 populations that were not biased by sample sizes, we rarified estimates to 100 genes (i.e., 50  
412 dogs per population), yielding allelic richness estimates averaged across loci of 5.6 (95% CI: 5.1-  
413 6.1) vs. 5.2 (95% CI: 4.7-5.8) alleles per locus in the US and UK, respectively. This difference  
414 was not significant across populations. Heterozygosity was also not significantly different  
415 between SA affected and healthy Standard Poodles within either the US or UK populations  
416 (Table 3).

417

### 418 **D. Population structure of US and UK dogs, SA affected and healthy**

419

420 A principal coordinate analysis (PCoA) plot measuring genetic similarities among randomly  
421 related dogs from US and UK populations based on autosomal STRs showed a degree of  
422 differentiation by country of origin (Fig. 2a). However, SA affected dogs were indistinguishable

423 from unaffected dogs from within their own geographic regions (Fig. 2b, 2c). A blind cluster  
424 analysis (i.e., the program was not given information on geographical or SA status) was  
425 performed in Structure using only unrelated Poodles from the US and UK to investigate patterns  
426 of substructure. An analysis using  $K = 2$  was conducted for comparison and the US and UK dogs  
427 segregated by geographic origin (Fig. 3). Use of four genetic clusters ( $K = 4$ ) was indicated as  
428 the optimum based on the log probability of the data (Fig. 3), but the analysis at  $K = 4$  failed to  
429 differentiate more than the two geographic populations. Therefore, regardless of  $K$ , blind  
430 analyses provided no evidence that SA affected and unaffected dogs segregated as distinct  
431 subpopulations among either US or UK Standard Poodles.

432

### 433 **E. Genetic comparisons of the DLA and DLA class II regions of SA affected and healthy** 434 **dogs from the US and UK**

435

436 Exon 2 sequencing of DLA-DRB1, -DQA1 and -DQB1 loci was conducted on Standard Poodles  
437 from both the US ( $n=137$ ) and UK ( $n=84$ ) (Table 3). Twelve DRB1, seven DQA1 and nine  
438 DQB1 alleles were identified among US and UK Poodles. One DRB1 allele (tentatively  
439 designated drb001v) was unique, but differed from DRB1\*00101 by a single nucleotide. This  
440 suggested that it occurred as a mutation in the more modern period of breed development.

441 The known alleles formed fourteen three-locus haplotypes (Table 4). The proportion of  
442 heterozygous genotypes ( $H_o$ ) did not differ significantly between affected and unaffected dogs in  
443 the US ( $H_o = 0.50$ ; Fisher Exact  $P = 0.16$ ) or UK ( $H_o = 0.44$ ; Fisher Exact  $P = 0.25$ ). Nor was  
444 there a significant deviation from Hardy-Weinberg equilibrium in the US ( $H_e = 0.50$ ; Chi square,  
445 2;  $df = 0.87$ ;  $P = 0.65$ ) or the UK ( $H_e = 0.47$ ; Chi square, 2;  $df = 0.54$ ;  $P = 0.76$ ). There also were  
446 no significant differences between haplotype frequencies of SA affected vs. unaffected dogs in  
447 the US (Chi-square, 5;  $df = 1.64$ ;  $P = 0.90$ ) or in the UK (Chi-square, 5;  $df = 2.52$ ;  $P = 0.77$ ).  
448 However, haplotype frequencies differed slightly ( $F_{ST} = 0.016$ ) but significantly between US and  
449 UK dogs (Chi-square, 5;  $df = 24.7$ ;  $P = 0.0002$ ). This was due mainly to the relative occurrence  
450 and frequencies of minor haplotypes (Tables 4, 5).

451 Although the numbers of individuals with minor haplotypes was too small to render  
452 significance, it is noteworthy that several minor haplotypes were found only in unaffected dogs.  
453 These haplotypes included 00101/00101/00201 and the novel alternative haplotype

454 001v/00101/00201 that was created by the mutation of DRB1\*00101 to form DRB1\*001v  
455 (1.67% in US dogs and 1.64% in UK dogs), and 00201/00901/00101, 01101/00201/01302,  
456 010011/00201/01501 (1.68% in US dogs and 0.82% in UK dogs). If dogs possessing these  
457 haplotypes were indeed free of SA, hundreds and perhaps thousands more SA affected as well as  
458 unaffected dogs would have to be DLA class II haplotyped in order to confirm such an  
459 association. Likewise, no significant relationship between minor maternal and DLA class II  
460 haplotypes was evident. If such a relationship had existed, it would have lent some credibility to  
461 a lack of association with certain minor DLA class II haplotypes and SA.

462 In order to avoid bias from closely related dogs, DLA class II haplotype zygosity was  
463 calculated using only dogs unrelated to three generations. About one-half of all unrelated US  
464 and UK Standard Poodles were homozygous for various DLA class II haplotypes (Table 4).  
465 These proportions were virtually identical to those of SA affected vs. unaffected dogs from the  
466 same countries. Although there were some difference in the types of low frequency haplotypes  
467 between US and UK dogs, the 01501\*00601\*02301 haplotype, either in a homozygous or  
468 heterozygous state, was found in about 94% of US and UK dogs (Table 5).

469 Zygosity mapping was done within a region on CFA12 from base 3802975 to 5672682,  
470 which includes the entire DLA, for SA affected and unaffected Standard Poodles from the US  
471 (Fig. 4). SA affected and unaffected dogs were each separated into two groups based on  
472 zygosity. The first group was largely heterozygous across the DLA. The second group was  
473 defined by a large region of homozygosity extending from nucleotide positions 4547874 to  
474 5412195, with relative heterozygosity both upstream and downstream of this region. The DLA-  
475 DRB1, -DQA1 and -DQB1 genes are found at approximate positions 5,155,200 to 5,311,100.  
476 There was a tendency, although not quite significant, for a greater proportion of SA affected  
477 dogs to be in the homozygous group. Fourteen of 24 (58%) unaffected dogs were homozygous  
478 for either major or minor alleles across most of the DLA region (Fig. 4), compared to 16/21  
479 (76%) of SA affected dogs. The major 01501\*00601\*02301/01501\*00601\*02301 DLA class II  
480 haplotype, regardless of SA status, was found almost exclusively among the homozygous dogs  
481 (Fig. 4). Similar zygosity mapping was done on affected and unaffected dogs from the UK with  
482 virtually identical results to that found for US Standard Poodles (data not shown). Identical  
483 zygosity mapping was carried out using the same 115 DLA SNPs on 26 randomly selected  
484 indigenous (street) dogs from the Island of Bali, Indonesia (Fig. 4). Bali street dogs showed a

485 much greater level of heterozygosity across the entire DLA region than SA affected or  
486 unaffected Standard Poodles from the US

487

## 488 **V. Discussion**

489

### 490 **A. Breed history and breeding bottlenecks**

491

492 Difficulties in identifying significant associations by genome wide association studies using  
493 modern arrays containing over 172,000 SNPs across the entire genome led us to back-track a  
494 step and in order to obtain a better understanding of genetic diversity and population structure  
495 between US and UK Standard Poodles, whether healthy or SA affected. Before doing such basic  
496 genetic studies, it was important to review the history of the Standard Poodle as a breed to better  
497 evaluate our findings. Although Standard Poodles have existed in more or less their present form  
498 since the 1600's, the breed has evolved mainly over the last century (31-33). Dogs from the  
499 Meadoware, Hill Hurst and Red Brook kennels dominated the breed early in the century, but  
500 their contributions were soon supplanted by dogs from other kennels (32). The most noteworthy  
501 was the Labory Kennels of Switzerland and a dog named Anderl von Hugelberg. Anderl lived in  
502 the 1920's and is perceived as the "Adam" of modern Standard Poodles (32). A further  
503 bottleneck occurred with the Wycliffe line, which traces its origins to the late 1950's. This line  
504 was created around five Standard Poodles from the then dominant Anderl von Hugelberg line,  
505 with only minor contributions from several other lines (33). The Wycliffe line was subsequently  
506 enlarged and further refined by extensive inbreeding and became extremely popular around the  
507 world. The proportion of Wycliffe ancestry among Standard Poodles in the US, UK and  
508 Scandinavia progressively increased to 40-50% by 1980 in dogs and has remained at that level to  
509 the present day (33). Two autoimmune disorders, sebaceous adenitis and Addison's disease,  
510 parallel the Wycliffe line in time of appearance and increasing popularity (33).

511

### 512 **B. Maternal and paternal lineages of modern Standard Poodles (haplotypes)**

513

514 All Standard Poodles share a single paternal (Y chromosome) haplotype based on a panel of  
515 11 Y SNPs. This particular Y haplotype is rooted deep in village dog populations from across SE

516 Asia and is common among western dogs regardless of breed (15). The ancient origin of the Y  
517 SNP markers limited their usefulness in determining more recent male founders. However,  
518 STRs on the Y chromosome have proven to be more useful in resolving modern paternal  
519 lineages (15, 17). Sixty seven Y STR haplotypes have been identified among 50 modern breeds  
520 of dogs, and D1D5 of Standard Poodles is the most common of these haplotypes (15). D1D5 is  
521 also one of the most common haplotypes in the VGL canine forensic database and predominates  
522 in breeds that share characteristics with the Standard Poodle, e.g., Airedale Terrier, Maltese  
523 Terrier, Bichon Frise, Borzoi, German Short-haired Pointer, Komondor, and Norfolk Terrier.  
524 The D1D5 paternal haplotype is also common among village dogs from Taiwan and the  
525 Philippines (15).

526 Sequences within the hypervariable region I of mtDNA have proved useful in determining  
527 maternal origins in a number of dog breeds (34). Seven mtDNA haplotypes were identified in  
528 this study, US and UK Poodles each possessed five mtDNA haplotypes, three of which were  
529 shared and two being unique to each population. Standard Poodles with SA exhibited a higher  
530 frequency (88%) of mtDNA haplotype A than did unaffected Poodles (70%), which had a  
531 correspondingly higher frequency of rare haplotypes B-H (30%) and, consequently, higher  
532 mitochondrial diversity. Although mtDNA polymorphisms have been associated with  
533 susceptibility to autoimmune disease in laboratory mice (35), it is more likely that the association  
534 is more likely a consequence of autosomal selection.

535 The predominance of mtDNA haplotype A in US and UK Standard Poodles supports what is  
536 known about the recent history of the breed and a bottleneck occurring with the advent of the  
537 Wycliffe line in the 1950's. The minor mtDNA haplotypes observed in the present study may be  
538 remnants of lines that were more common prior to the 1950's. It is noteworthy that these minor  
539 maternal lines remain relatively free of SA, suggesting that SA entered the breed with what has  
540 now become the dominant maternal lineage.

541

### 542 **C. Genetic diversity and population structure of US and UK Standard Poodles based on** 543 **autosomal STRs**

544

545 All approaches of data analysis, *F*-statistics, PCoA, and admixture analysis, suggested that  
546 US and UK dogs were closely related but not indistinguishable, as to be expected given their

547 independently selected breeding over the past 25 to 50 generations. The implication of this  
548 degree of population substructure on GWAS using cohorts of Standard Poodles from both the US  
549 and UK is unknown. However, indications of population substructure were also seen with the  
550 autosomal STR markers. The effect of population substructure could influence the minimum  
551 number of case and control dogs from each country required for GWAS, as well as the manner in  
552 which data is analyzed.

553

#### 554 **D. Genetic interrogation of the DLA and DLA class II regions of US and UK Standard** 555 **Poodles**

556

557 Studies of the DLA and DLA class II region were conducted with two objectives in mind. The  
558 first objective was to identify a DLA class II association with SA, especially because  
559 autoimmune diseases in other pure breeds have been almost always associated with specific  
560 haplotypes (reviewed in 11, 12). Sequencing of the three DLA class II genes detected 12 DRB1,  
561 7 DQA1 and 9 DQB1 alleles forming 14 three-locus haplotypes. These were among the 245  
562 DRB1, 39 DQA1, and 79 DQB1 alleles and over 200 three-locus haplotypes previously  
563 identified from purebred and indigenous dogs around the world (Kennedy LJ, personal  
564 communication). The present findings for DLA class II alleles and haplotypes were similar to  
565 those reported by Kennedy (36) on 81 Standard Poodles from the US and UK. She reported 9/12  
566 of the same DRB1 alleles, 5 of the 7 DQA1 alleles, and 4/9 of the same DQB1 alleles. The  
567 frequency of the various haplotypes was also similar, with 01501/00601/02301 being present in  
568 105/162 (65%) chromosomes in her study. Differences between US and UK dogs, when they  
569 occurred, were seen mainly with minor alleles and their relative frequencies. Kennedy reported  
570 DLA class II haplotypes and zygosity of Standard Poodles from the US, Canada and the UK (37,  
571 38). Eleven haplotypes were identified among 31 samples from around the world and 10 from  
572 among 50 samples submitted by the Animal Health Trust, UK. About one-half of these dogs  
573 were homozygous for the DLA class II genes and 90% of these dogs were homozygous for the  
574 same major haplotype, DRB1\*01501/DQA1\*00601/DQB1\*02301. Although DLA and DLA  
575 class II diversity appeared low in Standard Poodles, it was nonetheless greater than in breeds  
576 such as the Italian Greyhound (12) and Pug Dog (11). However, like the Pug Dog, the frequency  
577 distributions of alleles and haplotypes were highly skewed because of differences in the number

578 and frequencies of minor haplotypes. Nonetheless, DLA haplotype diversity in an outbred  
579 population of village dogs in Bali, Indonesia (39) displayed heterozygosity in a single locus,  
580 DQA1 (Heterozygosity = 0.825) that was 70% higher than observed in this study for the entire  
581 tri-locus DLA haplotype of Standard Poodles (0.49) in the present study .

582 The second objective of studying the DLA was to use a small region of the genome as a  
583 window into what may be happening in other regions of the genome. Although the DLA region  
584 is normally under high linkage disequilibrium, the degree of homozygosity within both the DLA  
585 and the DLA class II regions was much higher than would be expected. This was most noticeable  
586 in comparative zygosity mapping between Standard Poodles and their ancestral SE Asian village  
587 dog relatives. Zygosity maps of indigenous dogs showed a much greater degree of  
588 heterozygosity, much smaller blocks of homozygosity, and a greater use of minor alleles. The  
589 loss of genetic diversity in the DLA and DLA class II was mirrored by indications of inbreeding  
590 and the occurrence of population substructure between SA affected and healthy dogs, as  
591 determined allele frequencies at the 24 autosomal STR loci.

592 There was no significant association between susceptibility to sebaceous adenitis in Standard  
593 Poodles in the present study and any DLA class II haplotype. However, it is possible that a major  
594 disease association existed with 01501/00601/02301, in which case it could have gone  
595 undetected due to a near fixation of this haplotype within the breed. This would have rendered  
596 the numbers of case and control dogs woefully insufficient. Using the SA case and control  
597 population from the US in a genetic power calculation  
598 (<http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html>), and assuming that the high risk allele  
599 (haplotype) frequency was 0.701, the SA prevalence in the breed 2%, the relative risk in the  
600 heterozygous state 0.88 and in the homozygous state 1.14, the linkage disequilibrium ( $D'$ ) 0.7,  
601 the marker allele frequency 0.701, the number of cases 35, df 1, and the control to case ratio 2.0,  
602 2,629 cases would have been required to yield a probability of 0.05 at 80% power.

603

#### 604 **E. Complex genetics and SA in Standard Poodles**

605

606 Finally, the present study did not address complex genetics as it relates to autoimmune diseases  
607 such as SA. Autosomal genetic associations in human autoimmune diseases are largely  
608 polygenic, a pattern that has also been seen in SLE related disease in Nova Scotia Duck Tolling

609 Retrievers (41, Addison's disease in Portuguese Water Spaniels (42), and a multiple autoimmune  
610 disease syndrome of Italian Greyhounds (12). Therefore, it is not surprising that the genetics of  
611 autoimmune diseases in humans has been highly elusive, as elegantly stated by Johannesson and  
612 colleagues (43) – “from disease to genes: the monogenic success and the polygenic failure.”  
613 The discovery of simple Mendelian traits with surprisingly small numbers of case and control  
614 dogs has been remarkably easy in dogs, but studies of complex traits such as autoimmunity or  
615 epilepsy will likely be as challenging as they have been in humans (12, 41, 42, 44).

616

#### 617 **F. Where can you find Standard Poodles free of SA?**

618

619 Trafficking of Standard Poodles between the US, Canada, UK and Scandinavia has obviously  
620 been quite extensive throughout the century and therefore dogs in countries where the breed is  
621 popular are likely to be quite related. Therefore, in absence of a specific genetic test, it may be  
622 important for Standard Poodle breeders to search for remnants of bloodlines, possibly based on  
623 minor mtDNA types (or minor DLA class II types if they can be shown to be unaffected), which  
624 remain free of autoimmune disorders such as SA and Addison's disease. Such lines may exist in  
625 a few older kennels, or more likely, in parts of the world less influenced by the bottlenecks of the  
626 1920's and 1950's. In the absence of definitive genetic markers for SA susceptibility, a  
627 recommendation has been made to break this bottleneck by crossing Standard Poodles with  
628 Miniature and Toy Poodles (37), which appear to have a much lower prevalence of SA and  
629 Addison's disease. However, detailed knowledge of the genetics of Miniature Poodles would be  
630 important in the implementation of such a breeding scheme and without tests to identify  
631 individuals SA carriers, crossing to Miniature Poodles and then backcrossing to re-establish the  
632 desired Standard Poodle phenotype may recreate the original problem. Furthermore, such a  
633 breeding scheme, besides requiring careful genetic monitoring, could easily take a decade more  
634 and thousands of offspring to prove an effect.

635

#### 636 **VI. Acknowledgements**

637

638 Funding for this study was provided by the Poodle Club of America Foundation. We are also  
639 grateful for partial matching funds from the Center for Companion Animal Health, UC Davis.

640 We wish to also thank the staff of the Veterinary Genetics Laboratory (VGL), UC Davis for  
641 running STR parentage panels and SRY haplotyping and Angel Del Valle for assisting in  
642 mtDNA and DLA class II sequencing. Beth Wictum, head of the Veterinary Forensics Unit of  
643 the VGL kindly allowed us access to a large mtDNA sequence database of pure breed dogs. We  
644 are also grateful for assistance rendered by the Standard Poodle Club UK.

645

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758 **Tables**  
759

760 Table 1. The incidence and frequency of maternal or mitochondrial (mtDNA) haplotypes in  
761 Standard Poodles  
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mtDNA Type (GenBank#)	% in VGL forensic data set	US		UK	
		SA (%)	Control (%)	SA (%)	Control (%)
<b>A</b> (AB622536)	0.7	26 (92.9)	56 (77.8)	19 (82.6)	37 (63.8)
<b>B</b> (AB622568)	1.1	0	7 (9.7)	3 (13.0)	15 (25.9)
<b>C</b> (AB622564)	1.6	0	8 (11.1)	0	0
<b>D</b> (AB622557)	1.8	1 (3.6)	1 (1.4)	0	0
<b>F</b> (AF531740)	0	1 (3.6)	0	0	2 (3.5)
<b>G</b> (AY706505)	0	0	0	1 (4.4)	2 (3.5)
<b>H</b> (AB622517)	5.4	0	0	0	2 (3.5)

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765 Table 2. Maternal haplotypes of Standard Poodles from the US used in independent SA and  
766 Addison's disease studies. SA appears to have entered the population from dogs with the major  
767 A maternal haplotype. Dogs with minor maternal haplotypes B are relatively free of SA, while  
768 dogs with haplotype C are all healthy. The role of maternal haplotype is not as clear for  
769 Addison's disease; no haplotype is significantly more or less frequent between affected and  
770 healthy dogs.  
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Maternal haplotype (GenBank#)	US Standard Poodles – SA study		US Standard Poodles – Addison's study	
	Sebaceous adenitis (%)	Healthy (%)	Addison's (%)	Healthy (%)
<b>A</b> (AB622536)	41 (91.11)	60 (77.92)	36 (76.6)	76 (78.35)
<b>B</b> (AB622568)	2 (4.44)	8 (10.39)	8 (17.02)	11 (11.34)
<b>C</b> (AB622564)	0	8 (10.39)	2 (4.26)	8 (8.24)
<b>D</b> (AB622557)	1 (2.22)	1 (1.3)	0	2 (2.06)
<b>F</b> (AF531740)	1 (2.22)	0	0	0
<b>G</b> (AY706505)	0	0	0	0
<b>H</b> (AB622517)	0	0	1 (2.13)	0
<b>TOTAL</b>	45	77	47	97

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Table 3. Microsatellite locus-specific observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, heterozygote deficit ( $F_{IS}$ ), and rarified (to 100 genes) estimates of Allelic richness (RAR) for Standard Poodles from the US and UK.

Locus	US				UK			
	$H_o$	$H_e$	$F_{IS}$	RAR	$H_o$	$H_e$	$F_{IS}$	RAR
AHT121	0.73	0.78	0.06	9.4	0.65	0.76	0.16	9.1
AHT137	0.76	0.78	0.03	6.9	0.65	0.73	0.11	7.0
AHTH130	0.69	0.76	0.09	6.2	0.67	0.81	0.17	6.0
AHTh171-A	0.73	0.71	-0.03	7.9	0.66	0.61	-0.08	5.0
AHTh260	0.46	0.57	0.19*	6.6	0.45	0.52	0.14	6.6
AHTk211	0.39	0.42	0.08	3.7	0.40	0.38	-0.05	3.4
AHTk253	0.70	0.72	0.03	5.0	0.66	0.78	0.15	5.0
C22.279	0.59	0.62	0.04	5.8	0.66	0.68	0.03	5.0
FH2001	0.67	0.72	0.07*	6.3	0.58	0.57	-0.03	4.9
FH2054	0.47	0.56	0.16	6.0	0.52	0.51	-0.03	4.9
FH2328	0.66	0.77	0.15	5.3	0.53	0.79	0.33*	5.7
FH2848	0.19	0.22	0.17*	4.8	0.38	0.40	0.05	6.4
INRA21	0.53	0.62	0.15*	5.8	0.61	0.66	0.07*	4.9
INU005	0.48	0.51	0.05*	3.7	0.53	0.59	0.10	3.8
INU030	0.70	0.69	0.00	5.3	0.64	0.73	0.12	5.0
INU055	0.70	0.69	-0.02	4.8	0.61	0.68	0.10	6.6
LEI004	0.37	0.38	0.03	4.0	0.30	0.32	0.04	4.2
REN105L03	0.49	0.56	0.13	4.6	0.61	0.59	-0.03	4.5
REN162C04	0.47	0.48	0.01	5.8	0.58	0.65	0.11	6.7
REN169D01	0.70	0.72	0.03	6.5	0.61	0.66	0.07	5.9
REN169O18	0.44	0.49	0.10	5.1	0.43	0.42	-0.03	4.5
REN247M23	0.66	0.66	0.01	4.3	0.57	0.53	-0.07	3.6
REN54P11	0.63	0.71	0.12*	4.9	0.79	0.74	-0.07	4.0
REN64E19	0.63	0.65	0.03	5.7	0.80	0.67	-0.19	3.0

780 \*Significant deviation from Hardy-Weinberg equilibrium (HWE) after sequential Bonferroni  
781 Correction. Bonerroni correction adjusts for differences in population sizes. HWE is achieved  
782 when all individuals in the population are randomly breeding. Significant deviations in HWE at a  
783 certain loci may be the result of non-random breeding or population substructure (two or more  
784 subpopulations breeding randomly but somewhat independently of the others).

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788 Table 4. DLA class II haplotype frequency in all randomly related SA affected and control  
 789 Standard Poodles. n= the total number of haplotypes with each dog contributing two haplotypes.  
 790 The percentage of a certain haplotype among individuals in each population is shown in ( ).  
 791

Haplotype			US		UK	
DRB1	DQA1	DQB1	SA n=94 (%)	Control n=180 (%)	SA n=46 (%)	Control n=122 (%)
01501	00601	02301	68 (72.34)	124 (68.89)	36 (78.26)	83 (68.03)
01502	00601	02301	9 (9.57)	17 (9.44)	1 (2.17)	2 (1.64)
01501	00901	00101	6 (6.38)	12 (6.67)	5 (10.87)	22 (18.03)
02001	00401	01303	6 (6.38)	12 (6.67)	2 (4.35)	5 (4.1)
01503	00601	02301	1 (1.06)	5 (2.78)	1 (2.17)	7 (5.34)
00901	00101	008011	1 (1.06)	3 (1.67)	1 (2.17)	0
01501	00601	04901	1 (1.06)	1 (0.56)	0	0
02301	00301	00501	1 (1.06)	0	0	0
00601	005011	00701	1 (1.06)	0	0	0
001v	00101	00201	0	2 (1.11)	0	0
00101	00101	00201	0	1 (0.56)	0	2 (1.64)
00201	00901	00101	0	1 (0.56)	0	0
01101	00201	01302	0	1 (0.56)	0	1 (0.82)
010011	00201	01501	0	1 (0.56)	0	0

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795 Table 5. Zygosity of DLA class II haplotypes in unrelated Standard Poodles from the US and  
796 UK. Homozygous haplotypes are in bold lettering –haplotype from both parent is identical.

797 Heterozygous haplotypes are in regular lettering – haplotype from each parent is different.

Haplotype	US		UK	
	SA n=35 (%)	Control n=69 (%)	SA n=13 (%)	Control n=39 (%)
<b>01501*00601*02301/01501*00601*02301</b>	17 (48.57)	29 (42.03)	7 (53.85)	19(48.72)
01501*00601*02301/01502*00601*02301	5 (14.29)	12 (17.39)	1 (7.69)	0
01501*00601*02301/02001*00401*01303	5 (14.29)	8 (11.59)	1 (7.69)	2 (5.13)
01501*00601*02301/01501*00901*00101	4 (11.43)	6 (8.70)	3 (23.08)	11 (28.21)
01501*00601*02301/01503*00601*02301	1 (2.86)	3 (4.35)	0	2 (5.13)
01501*00601*02301/02301*00301*00501	1 (2.86)	0	0	0
01501*00601*02301/010011*00201*01501	0	1 (1.45)	0	0
01501*00601*02301/01501*00601*04901	0	1 (1.45)	0	0
01501*00601*02301/01101*00201*01302	0	1 (1.45)	0	1 (2.56)
01501*00601*02301/00101*00101*00201	0	1 (1.45)	0	1 (2.56)
01501*00601*02301/00201*00901*00101	0	1 (1.45)	0	0
01501*00601*02301/00901*00101*008011	0	2 (2.90)	1 (7.69)	0
<b>001v*00101*00201/001v*00101*00201</b>	0	1 (1.45)	0	0
<b>02001*00401*01303/02001*00401*01303</b>	0	1 (1.45)	0	0
<b>01502*00601*02301/01502*00601*02301</b>	0	1 (1.45)	0	0
01502*00601*02301/00601*005011*00701	1 (2.86)	0	0	0
01502*00601*02301/00901*00101*008011	1 (2.86)	0	0	0
<b>01503*00601*02301/01503*00601*02301</b>	0	1 (1.45)	0	0
01502*00601*02301/01503*00601*02301	0	0	0	1 (2.56)
<b>01501*00901*00101/01501*00901*00101</b>	0	0	0	1 (2.56)
01501*00901*00101/02001*00401*01303	0	0	0	1 (2.56)

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## Figures

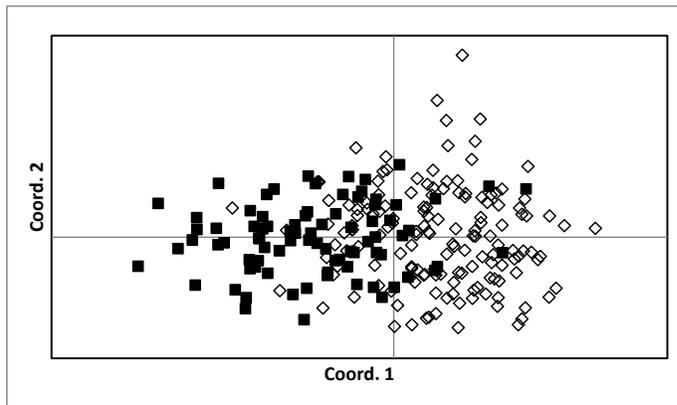


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Fig. 1. Standard Poodle suffering from sebaceous adenitis. The disease often starts on the head, neck and ears and can progress to involve all or large parts of the body.

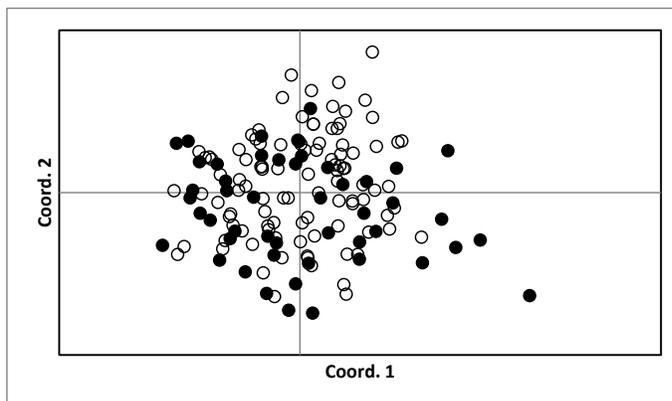
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a. All US vs. all UK Standard Poodles



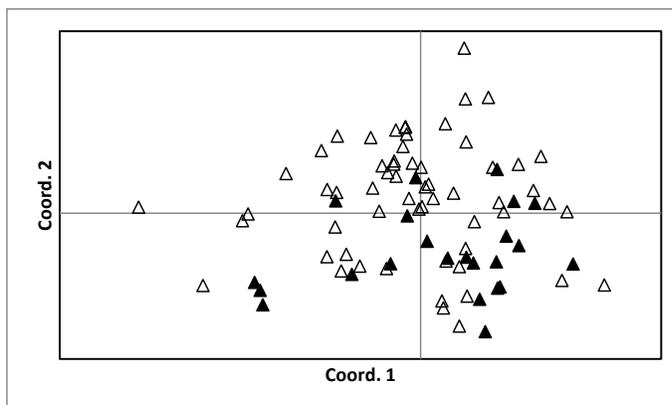
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b. SA affected vs. unaffected US Standard Poodles



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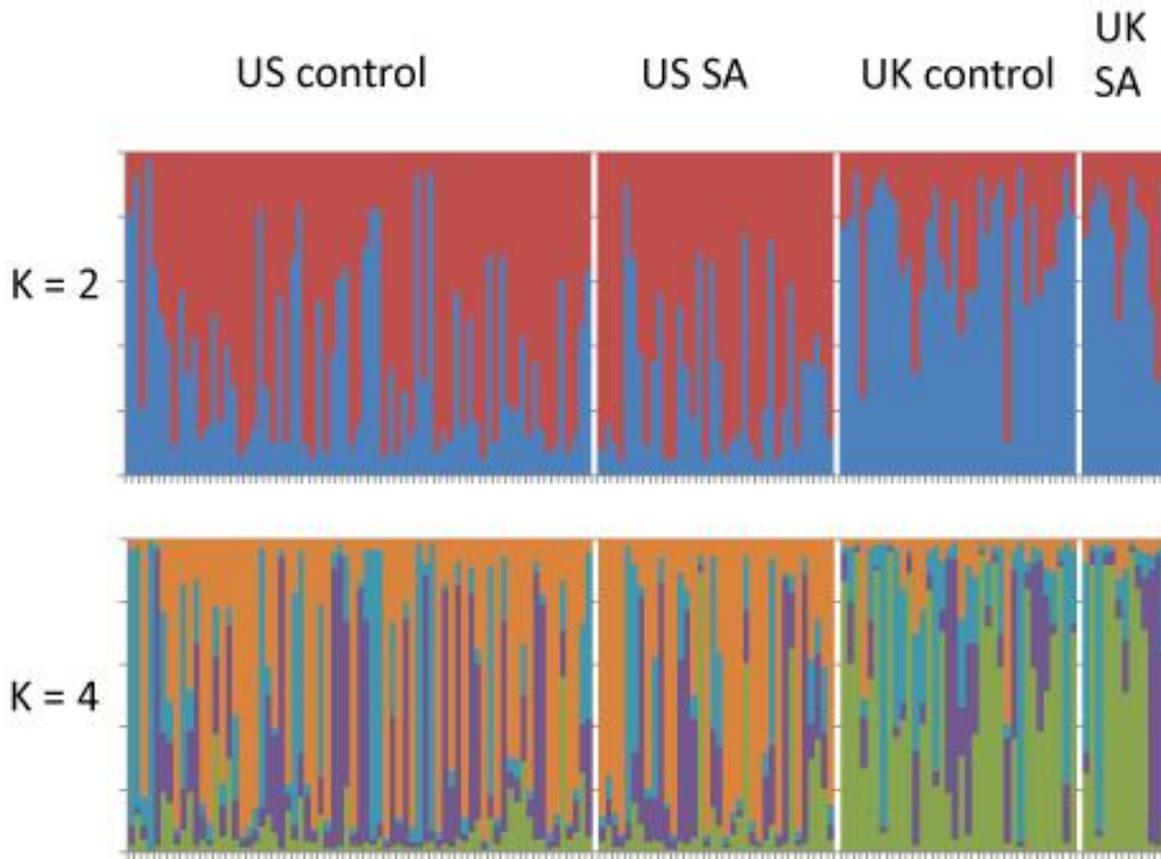
c. SA affected vs. unaffected UK Standard Poodles



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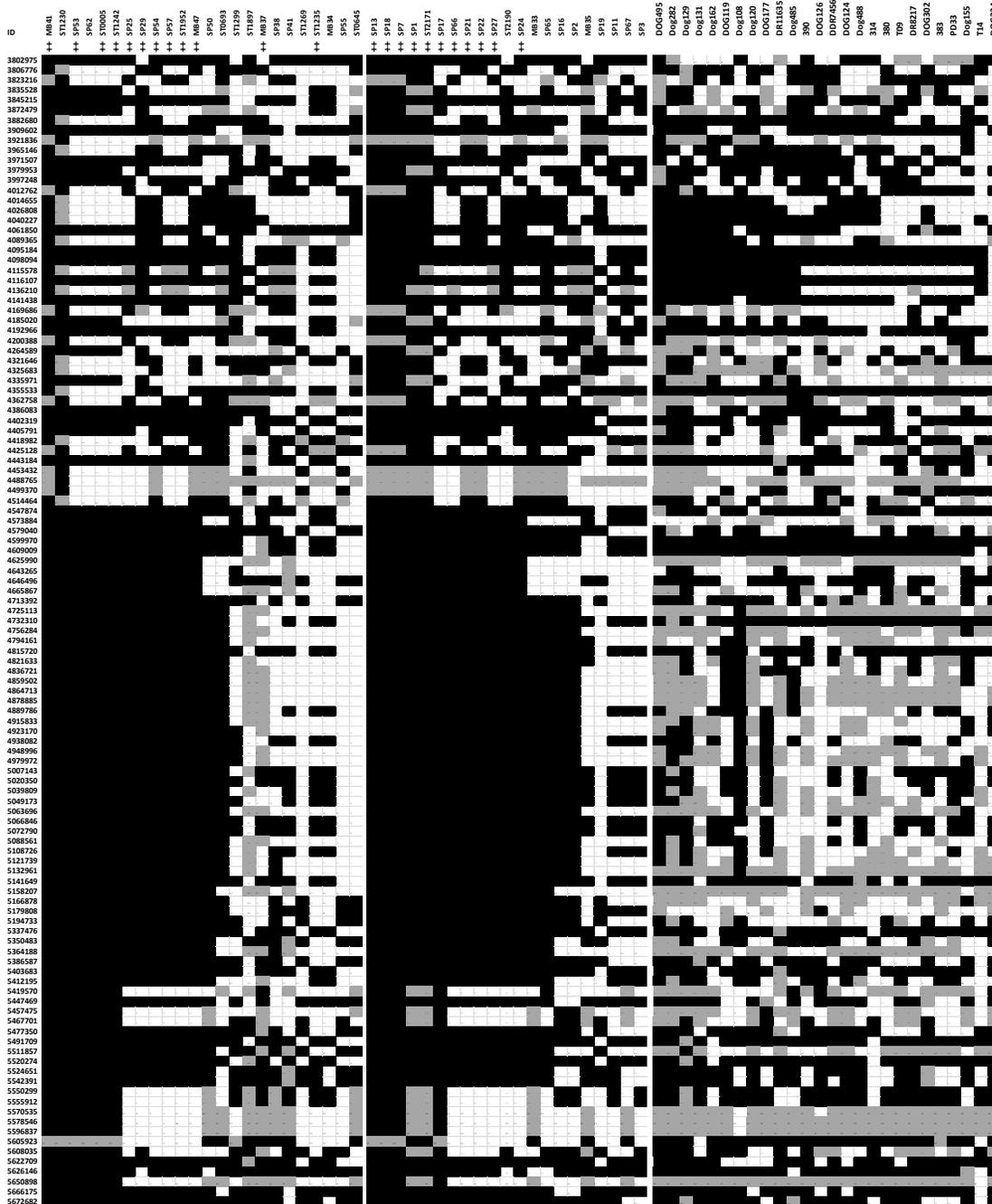
819 Figure 2. PCoA plot based on STR alleles of randomly related Standard Poodles. a) US (open  
820 open diamonds) vs. UK (closed squares); b) unaffected (open circles) vs. SA affected dogs (closed  
821 circles) from the US; c) unaffected (open triangles) vs. SA affected dogs (closed triangles) from  
822 the UK. All of the dogs from the US and UK cluster as two overlapping, yet distinct,  
823 populations. SA affected dogs do not segregate from their healthy relatives in either the UK or  
824 US.

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831 Figure 3. Structure analysis using STRs from unrelated dogs, SA affected and unaffected, from  
832 the US and UK. The actual population to which each dog belonged was not listed and the  
833 program was “asked” to place each animal into distinct populations based on country of origin  
834 and disease status. At K=2, two subpopulations are apparent (red and blue). Blue dominates in  
835 the UK dogs while Red dominates in the US population. Attempts to segregate SA affected and  
836 healthy dogs from the US and UK (four populations predicted) at K=4 fails to isolate affected  
837 from healthy dogs.

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840 Figure 4. Zygosity mapping of across the DLA region of SA affected (left panel) and unaffected  
 841 (middle panel) unrelated Standard Poodles from the US. The right panel shows the zygosity map  
 842 for 26 randomly selected indigenous (village) dogs from Bali, Indonesia. Designations of SNPs  
 843 (far left vertical column) that encompass the DLA class II region are colored grey. The major  
 844 SNP alleles are colored black, the minor homozygous alleles are colored grey, and all  
 845 heterozygous alleles are colored white. Individuals possessing the major DLA class II haplotypes  
 846 01501\*00601\*02301/01501\*00601\*02301 are identified as ++ (second horizontal column at top  
 847 of figure).

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