Inheritance of pancreatic acinar atrophy in German Shepherd Dogs

E. Michael Moeller, BS; Jörg M. Steiner, Dr med vet, PhD; Leigh Anne Clark, BS; Keith E. Murphy, PhD; Thomas R. Famula, PhD; David A. Williams, VetMB, PhD; Mary E. Stankovics, DVM; Amy S. Vose, BA

Objective—To assess the heritability of pancreatic acinar atrophy (PAA) in German Shepherd Dogs (GSDs) in the United States.

Animals—135 GSDs belonging to 2 multigenerational pedigrees.

Procedure—Two multigenerational pedigrees of GSDs with family members with PAA were identified. The clinical history of each GSD enrolled in the study was recorded, and serum samples for canine trypsin-like immunoreactivity (cTLI) analysis were collected from 102 dogs. Dogs with a serum cTLI concentration ≤ 2.0 µg/L were considered to have exocrine pancreatic insufficiency (EPI) and were assumed to have PAA.

Results—Pedigree I consisted of 59 dogs and pedigree II of 76 dogs. Serum cTLI concentrations were measured in 48 dogs from pedigree I and 54 dogs from pedigree II. A total of 19 dogs (14.1%) were determined to have EPI, 9 in pedigree I (15.3%) and 10 in pedigree II (13.6%). Of the 19 dogs with EPI, 8 were male and 11 were female.

Conclusions and Clinical Relevance—Evaluation of data by complex segregation analysis is strongly suggestive of an autosomal recessive mode of inheritance for EPI in GSDs in the United States. (*Am J Vet Res* 2002;63:1429–1434)

Pancreatic acinar atrophy (PAA) is a degenerative disease of the exocrine pancreas, mainly seen in **German Shepherd Dogs (GSDs)** and rough-coated Collies, that leads to **exocrine pancreatic insufficiency (EPI)**.[1-5] Affected dogs typically have clinical signs of EPI by 5 years of age, but some dogs may have signs as early as 13 months of age.[2,3,5] Clinical signs include polyphagia, weight loss, voluminous feces, and steatorrhea.[3,4,6-8] Feces are light in color, loose in texture, and can be quite malodorous.[3,4,6-8]

Findings on histologic evaluation of pancreatic biopsy specimens from dogs with PAA include atrophy, scattering, and disorganization of pancreatic acinar cells.[4,5] Electron microscopy of pancreatic tissue reveals degenerative changes of acinar cells as early as 6 weeks of age.[5] Abnormalities include dilation of the rough endoplasmic reticulum and extensive fusion of zymogen granules.[5] As the disease progresses, the tissue loss becomes more extensive and leads to a rapid loss of exocrine pancreatic function.[5] Islets of Langerhans are usually unaffected by the degenerative process.[6]

A number of tests have been developed to aid in the diagnosis of EPI. The fecal soybean stimulation test, fecal proteolytic activity, and N-benzoyl-L-tyrosyl-P-aminobenzoic acid absorption have all been used for the diagnosis of EPI.[5,9] Unfortunately, all of these tests are either cumbersome to perform, unreliable, or both and have been replaced by the measurement of serum **canine trypsin-like immunoreactivity (cTLI)** by use of a radioimmunoassay.[10] The reference range for this assay is 5.0 to 35.0 µg/L, with a value of < 2.5 µg/L being diagnostic of EPI.[10] Serum cTLI concentration has been reported to be 100% sensitive and specific for EPI and, thus, is clinically useful for the diagnosis of EPI.[10] In fact, the high sensitivity and specificity of serum cTLI concentration for a diagnosis of EPI make this disease an ideal candidate for evaluation as a hereditary disease. By use of this assay, the disease status of any family member can be assessed easily. Recently, an assay for measurement of fecal elastase has been introduced.[11] However, this assay is associated with some false-positive results, making it inferior to the measurement of serum cTLI concentration.

In 1977, Weber and Freudiger[1] analyzed a pedigree composed of 19 GSDs with EPI and 33 unaffected GSDs. All 19 affected dogs were found to have a common ancestor born in 1918. Eighteen of the dogs were inbred more than once with a descendant of this dog. On the basis of the degree of inbreeding within this pedigree, Weber and Freudiger[1] hypothesized that chronic EPI was an autosomal recessive trait.

In 1980, Westermarck et al[12] investigated the inheritance of PAA in GSDs in Finland. Measurement of fecal proteolytic activity was performed by use of radial enzyme diffusion to determine the disease status of each dog.[12] This study included 59 GSDs from 2 different kindreds that had the same male progenitor. The first kindred had at least 1 affected dog in each of 4 litters. This evidence further supported an autosomal recessive inheritance of PAA in GSDs. However, Westermarck[12] pointed out that on the basis of his data, the mode of inheritance could also be dominant with incomplete penetrance.

Received Oct 26, 2001.
Accepted Apr 29, 2002.
From the Gastrointestinal Laboratory (Moeller, Steiner, Williams), and the Department of Veterinary Pathobiology (Clark, Murphy), College of Veterinary Medicine, Texas A&M University, College Station, TX 77843; The Seeing Eye, PO Box 375, Morristown, NJ 07963 (Stankovics); the Vose German Shepherd Kennel, Paw Paw, IL (Vose); and the Department of Animal Science, University of California Davis, Davis, CA 95616 (Famula).
Supported by a grant from the AKC Canine Health Foundation.
This material was presented as an abstract at the 2000 American College of Veterinary Internal Medicine Veterinary Medical Forum, Seattle, Wash.
The authors thank Dr. Carolyn Sarna for technical assistance.
Address correspondence to Dr. Steiner.

Recent work indicates that PAA might be an autoimmune-mediated disease. More specifically, it is theorized that PAA progresses through the following 2 stages: 1) lymphocytic pancreatitis, when there is active destruction of acinar tissue, and 2) end-stage EPI, during which atypical parenchyma, ductal structures, and adipose tissue replace acinar tissue.[13] Thus, PAA in GSDs may represent an autoimmune disorder that is caused by a gene inherited in an autosomal recessive fashion. Because previous studies have been conducted outside the United States, the purpose of the study presented here was to determine the inheritance of PAA in GSDs in the United States.

Materials and Methods

A questionnaire was sent to veterinarians who had GSD patients with low serum cTLI concentrations as determined by previous analysis of serum samples at the Gastrointestinal Laboratory at Texas A&M University. Veterinarians were asked for permission to contact the owners of the dogs. Owners were then asked to provide information about the breeders they obtained their dogs from. Finally, breeders were contacted for family information and for participation in our study. Several families of GSDs having family members with EPI were identified, and 2 pedigrees were selected because dogs belonging to several generations were available. Many dogs related to these dogs previously determined to have EPI were identified, and as many dogs as possible were tested for EPI. No discrimination was made between dogs that had clinical signs of EPI and those that did not. Dogs previously determined to have EPI were retested when possible.

A single serum sample was collected from each dog, stored in a 10-mL red-top evacuated tube, and sent to the Gastrointestinal Laboratory for measurement of serum cTLI concentration by radioimmunoassay.[a] The serum cTLI concentration was used to determine the disease status for each dog. Dogs with a serum cTLI concentration of ≤ 2.0 µg/L were considered to have EPI, and it was assumed that EPI was caused by PAA. For dogs that were retested (ie, because they had no clinical signs of EPI but did have a low serum cTLI concentration previously), the most recent serum cTLI concentration was reported. Additional blood samples for future extraction of DNA were also collected and sent to the Gastrointestinal Laboratory.

Statistical analysis—Logistic regression models developed for complex segregation analysis were used to assess the possible segregation of a single locus with a large effect on PAA in our pedigrees.[14] For a review of complex segregation analysis, see Lynch and Walsh.[15] This technique, which contrasts possible modes of inheritance, is purely statistical, using pedigree information, disease status, and sex to identify a pattern of transmission. The data are fit to various models of transmission, and a likelihood ratio is calculated for each of these models. The likelihood ratio is a measurement of how well the data fit the model, and a *P* value is calculat-

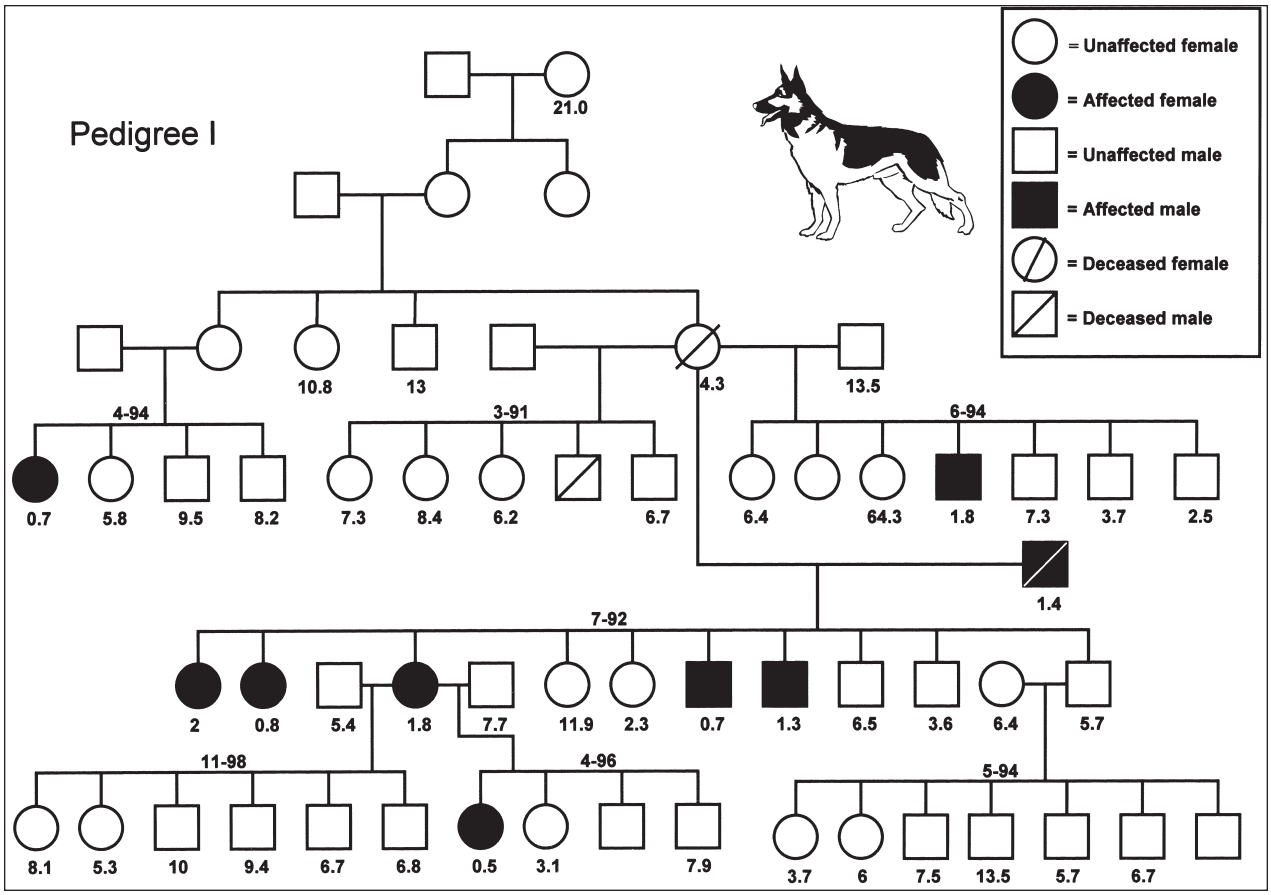

Figure 1—Pedigree I. This family of German Shepherd Dogs consisted of 59 dogs, 48 of which had serum cTLI concentrations measured. Nine of the 48 dogs (4 males and 5 females) had a serum cTLI concentration of ≤ 2.0 µg/L. Two dogs had low serum cTLI concentrations of ≤ 2.0 µg/L but were asymptomatic. The value below each individual is the serum cTLI concentration (µg/L) used to determine the disease status (phenotype) for that dog. Values above a litter indicate the month and year each litter was born.

ed to determine a significant difference between likelihood ratios. The data were fit to the various models by use of a software program.[b] All dogs belonging to either of the pedigrees were used in the complex segregation analysis. Dogs with a serum cTLI concentration ≤ 2.0 µg/L were considered affected, and all other dogs were considered unaffected. A value of $P \leq 0.05$ was considered significant.

Results

A total of 135 dogs were evaluated in our study. Serum cTLI concentration was measured in 102 of the 135 (75.6%) dogs . Nineteen of these 135 dogs (14.1% or 18.6% of the 102 dogs tested) had EPI, 8 of which were male and 11 female. The first family of GSDs consisted of 59 dogs, 48 of which had serum cTLI concentrations measured (**Fig 1**). Nine of those 59 (15.3%; 18.8% of the 48 dogs tested) dogs, including 4 males and 5 females, had serum cTLI concentrations ≤ 2.0 µg/L. Two dogs had serum cTLI concentrations of ≤ 2.0 µg/L but were asymptomatic for EPI. The second family of GSDs consisted of 76 dogs, 54 of which had serum cTLI concentrations measured (**Fig 2**). Ten of the 76 (13.2%; 18.5% of the 54 dogs that were tested) dogs , including 4 males and 6 females, had serum cTLI concentrations of ≤ 2.0 µg/L. Thirty-three of the dogs were not tested for various reasons, including death at birth, death before clinical signs warranted testing, and lack of cooperation by the owner for sample collection.

Several litters from parents, of which at least 1 parent was affected, had unaffected individuals. Conversely, there were several litters with affected individuals from unaffected parents. Pedigree I represents data from 7 complete litters, whereas pedigree II represents data from 10 complete litters (**Table 1**). There were 2 dogs that did not have a diagnosis of EPI prior to being tested for the purpose of our study. Because both of these dogs had no clinical signs of EPI, these dogs were classified as having subclinical disease. One dog was retested for confirmation and also had a severely low serum cTLI concentration (≤ 2.0 µg/L) at the time of the second evaluation. The other dog died before a second sample could be collected. The serum cTLI concentration for this dog was 1.4 µg/L, suggesting that this dog was affected.

When using a complex segregation analysis, dogs can either have the phenotype or not have the phenotype in question. Therefore, a cutoff point was established to distinguish affected dogs from unaffected dogs. The cutoff value chosen for our study was a serum cTLI concentration of ≤ 2.0 µg/L. Any dog with a serum cTLI concentration of ≤ 2.0 µg/L was considered affected, whereas all other dogs were considered unaffected.

Complex segregation analysis revealed that the most substantial contrast of likelihood ratios was between a model with no major locus, which included

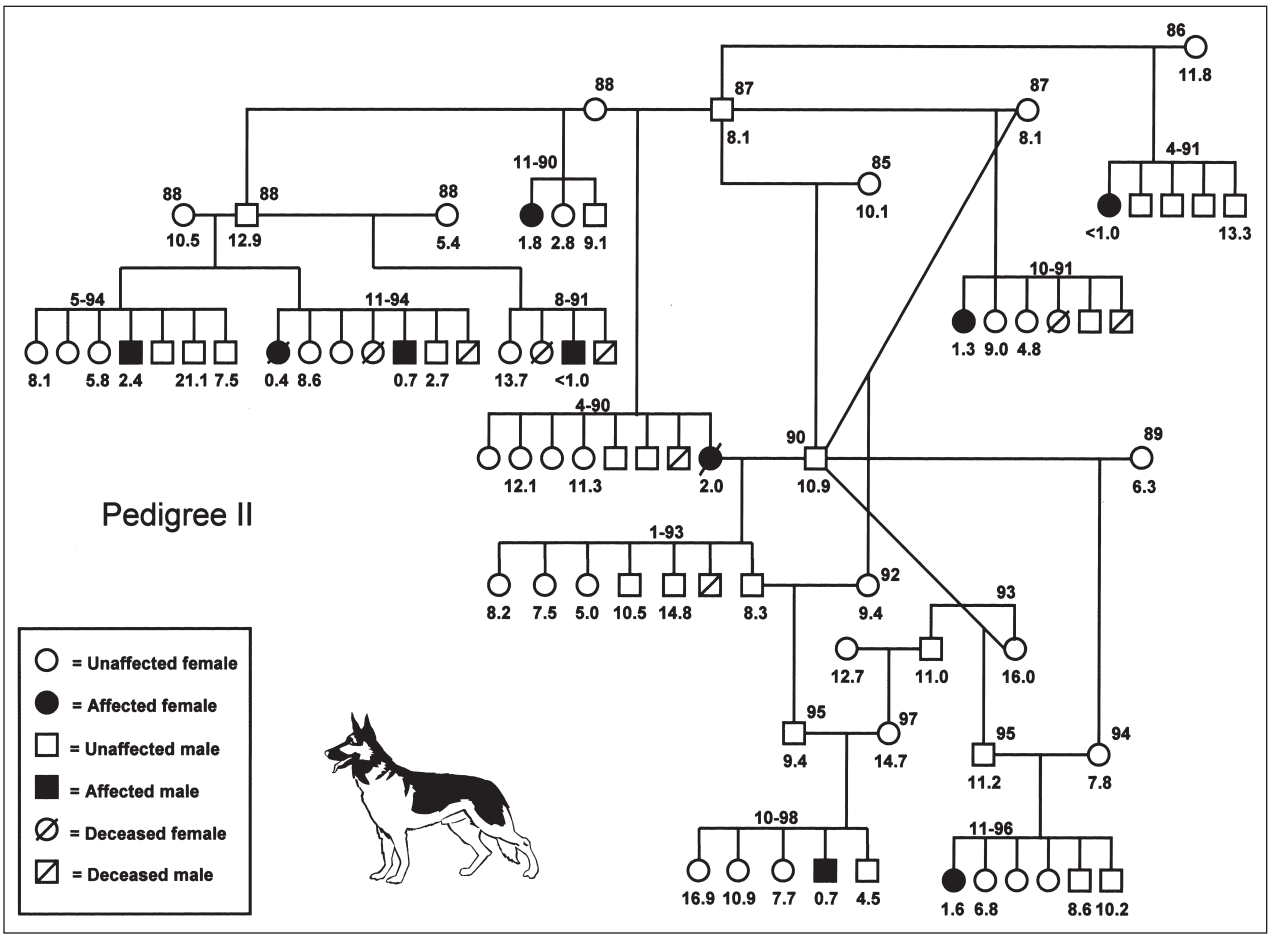

Figure 2—Pedigree II. This family of German Shepherd Dogs consisted of 76 dogs, 54 of which had serum cTLI concentrations measured. Ten of the 54 dogs (4 males and 6 females) had a serum cTLI concentration of ≤ 2.0 µg/L. *See* Figure 1 for remainder of key.

Table 1—Number of affected dogs with exocrine pancreatic insufficiency (EPI) in litters of German Shepherd Dogs from 2 pedigrees

Dogs	No. of affected dogs: Parents	No. of affected dogs: Litter	Total No. of dogs in litter
Pedigree I			
Litter 1	0	0	5
Litter 2	0	0	7
Litter 3	0	1	4
Litter 4	0	1	7
Litter 5	1	0	6
Litter 6	1	1	4
Litter 7	1	5	10
Pedigree II			
Litter 1	0	1	3
Litter 2	0	1	4
Litter 3	0	1	5
Litter 4	0	1	5
Litter 5	0	1	6
Litter 6	0	1	6
Litter 7	0	1	7
Litter 8	0	1	8
Litter 9	0	2	7
Litter 10	1	0	7

Table 2—Parameter estimates (± SE) from the logistic regression model in complex segregation analysis of pancreatic acinar atrophy (PAA) in German Shepherd Dogs

Parameter	No Major Locus: Estimate§	No Major Locus: SE	General major locus arbitrary transmission: Estimate§	General major locus arbitrary transmission: SE	General major locus Mendelian transmission: Estimate§	General major locus Mendelian transmission: SE	Recessive major locus Mendelian transmission: Estimate§	Recessive major locus Mendelian transmission: SE
p(a)*	NA	NA	0.017	0.003	0.175	0.074	0.180	0.074
Pooled Base	–2.659	0.404	NA	NA	NA	NA	NA	NA
aa	NA	NA	2.938	2.028	1.033	0.711	1.274	2.416
Aa	NA	NA	33.688	20.319	–68.112	15.647	–11.132	4.643
AA	NA	NA	–11.998	10.921	–9.130	2.421	–11.132	4.643
τ_{aa}	NA	NA	0.499	0.891	1.00	Fixed	1.00	Fixed
τ_{Aa}	NA	NA	0.298	0.524	0.50	Fixed	0.50	Fixed
τ_{AA}	NA	NA	0.001	0.000	0.00	Fixed	0.00	Fixed
Parent †	–0.124	0.249	–4.794	0.28	–3.976	0.832	–4.330	0.822
ln(L)‡	–75.022	—	70.381	—	–71.024	—	–71.915	—

A positive estimate value indicates an increased risk for PAA, whereas a negative estimate value indicates a reduced risk for PAA. The aa genotype, having a positive estimate, is likely to be affected. AA genotypes and heterozygotes, Aa, are least likely to develop PAA.

*Frequency of the putative major allele a. †Natural log of the likelihood. ‡Regression effect for parents. §Estimate for each parameter calculated for the specific regression model.

A = Dominant allele for PAA. a = Recessive allele for PAA. NA = Not applicable. τ = Major locus transmission probabilities for transmission of putative major allele a.

the possibility for polygenic inheritance, and a model with a single major locus exhibiting general Mendelian transmission. The model assuming general Mendelian transmission resulted in a significantly higher likelihood (P = 0.046) than the model assuming no major locus (**Table 2**).

Discussion

From a clinical standpoint, PAA in GSDs is a hereditary disease that can be readily diagnosed because identification of disease status can easily be accomplished by measuring a single serum cTLI concentration. The cTLI assay has been shown to be 100% sensitive and 100% specific for diagnosing EPI in dogs.[10]

The almost even distribution of PAA between males and females in the 2 pedigrees of our study indicates that PAA is not a sex-linked disease. If PAA were inherited as an X-linked disease, we would expect many more males to be affected than females. Also, Y-linked inheritance would produce only affected males, which is not the case for PAA.

Parents lacking clinical signs of EPI produced dogs with EPI. This provides further evidence that the putative trait for PAA is recessive and supports the findings in Finnish GSDs by Westermarck.[3,12] However, the rate of affected dogs is slightly lower than would be expected for a simple autosomal recessive inheritance (ie, 18/102 dogs belonging to complete litters; 17.8% compared with the 25.0% expected for simple autosomal recessive inheritance). This may be explained by a higher rate of stillbirths in affected dogs or by the fact that at the time of analysis, some dogs had not yet reached 4 to 5 years of age. These dogs may develop clinical signs and a low serum cTLI concentration at a later time in life. Finally, we used a cutoff value of 2.0 µg/L for serum cTLI concentration to ensure that all dogs with positive results truly were affected. In contrast, the cutoff value for serum cTLI concentration for EPI currently reported by our laboratory is ≤ 2.5 µg/L. This may have led to a small increase in false-negative results, decreasing the apparent prevalence of the disease.

Small intestinal disease is common in GSDs and may be associated with a slight decrease in serum cTLI concentrations. However, these low serum cTLI concentrations are > 2.5 µg/L. This clinical impression may account for dogs that had serum cTLI concentrations less than the lower limit of the reference range (5.0 µg/L) but still greater than the cutoff value for EPI (2.0 µg/L). For the purpose of our study, dogs with serum cTLI concentrations that fell into this range were considered not affected.

At the time of our study, 17 dogs were not yet 4 years of age when they were tested for EPI. However, 7 of these dogs were siblings of dogs that had already been determined to have EPI. None of these dogs had any signs of EPI. Additionally, none of the remaining 10 dogs, which were not yet 4 years of age and did not have siblings that had previously been determined to have EPI, had any clinical signs of EPI. Although some of these dogs may develop EPI in the future, we consider this possibility unlikely. These dogs were considered unaffected for the purpose of our study.

Elston et al[16] outline criteria that must be satisfied before accepting a major gene model. The first model to fit is one with no major locus, which includes a term for polygenic inheritance. Alone, this model is unin-

formative, but it will serve as a baseline for future comparisons. The next model is one that includes a parameter for a major locus, an effect expected to pass from parent to offspring on the basis of Mendel's laws. The contrast of these 2 models is insufficient to establish a putative major gene or to have a reduced incidence of false positives. Additional models fitted to the data include a major locus effect but estimate the transmission from parent to offspring.

In our study, the most substantial difference of likelihood ratios was seen between a model with no major locus, which included the possibility for polygenic inheritance and a model with a single major locus exhibiting general Mendelian transmission. The latter model resulted in a higher likelihood, indicating a better fit, and was shown to be significantly (P = 0.046) different from the first model. A recessive Mendelian model was then compared with the original model of PAA having no major gene. The recessive Mendelian model resulted in the maximal likelihood ratio and a significant (P = 0.044) difference. These results support the theory that a major gene is responsible for PAA in these populations.

Other models that were fitted to the data include a dominant Mendelian model and a model with a term for sex differences. These models resulted in likelihood ratio statistics that were not significant (data not shown).

Likelihood ratios for the various models are reported (Table 2), including the "general" major locus model ("general" meaning the locus does behave in a strict dominant or recessive manner). Mendelian transmission of the putative alleles provides a significantly better fit than a "no major locus model." For this comparison, the log of the likelihood ratio is calculated as follows: -2(-75.022 – [-71.024]) = 7.996, with 3 degrees of freedom (P = 0.046). However, a "general major locus model" where the transmission probabilities are estimated from the pattern of inheritance displayed within the data does not provide a significantly better fit than the "general model with fixed Mendelian transmission probabilities" (ie, -2[-71.024 – (-70.381)] = 1.286, with 3 degrees of freedom, P = 0.732). This contrast between the 2 models is suggested by Elston et al[16] to reduce the probability of falsely declaring the presence of a major locus. Alleles of a genuine major locus would have to be transmitted from parent to offspring with probabilities that reflect Mendelian transmission. A test for equal transmission probabilities (not presented) also supports the 3 criteria of a major locus model as described by Elston et al.[16] The recessive major locus model was not significantly different from the general major locus model (ie, -2[-71.915 – (-71.024)] = 1.782, with 1 degree of freedom, P = 0.182), though the recessive model is more parsimonious. Accordingly, we conclude that a major locus with an impact on PAA in GSDs in the United States can be established with the present data. This major locus apparently acts in a recessive, or close to completely recessive, fashion.

Statistical analysis supports the theory that a major gene is responsible for PAA in the pedigrees evaluated in our study. The single major locus model exhibiting general Mendelian transmission had a higher likelihood than the model assuming no major locus. This indicates that the single major locus model has a better fit to the data observed in these pedigrees. In addition, the recessive Mendelian model had the maximal likelihood ratio and significant difference from the model assuming no major gene. Because no other Mendelian models had significant likelihood ratio statistics, these data suggest that the mode of inheritance of PAA in GSDs in the United States is autosomal recessive.

One problem in our study is that for some litters, blood samples could not be collected from all the dogs. For instance, several dogs in pedigree II (Fig 2) were stillborn or died before the disease could have developed. There is no way to exclude the possibility that some of these dogs would have developed PAA later in life. Additionally, other dogs died before follow-up samples could be collected. These losses may affect the observed incidence of PAA and may explain the lower than expected frequency observed in these 2 pedigrees. The only definitive way to determine whether a dog is affected with PAA would be to only include pedigrees that exclusively contain family members that live a full lifespan and in which a determination of disease status is being made shortly before a natural death. Unfortunately, such a study would not be feasible.

We conclude that there is evidence to suggest that PAA is inherited as an autosomal recessive trait in GSDs in the United States. Currently, linkage analysis is being performed by use of a set of 172 microsatellite markers (ie, minimal screening set 1) that spans the entire canine genome.[17] Because no candidate gene is available for PAA, the minimal screening set 1 is screened for a microsatellite marker that cosegregates with the disease.

[a]Diagnostic Products Corp, Los Angeles, Calif.

[b]S.A.G.E., Statistical Analysis for Genetic Epidemiology, Vol 3, Chapter REGD. Release 3.1. Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio: Unpublished data, 1997.

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