Inheritance of gluten-sensitive enteropathy in Irish Setters

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Objective—To establish a model for inheritance of gluten-sensitive enteropathy (GSE) in Irish Setters.

Animals—44 dogs of a 6-generation family of Irish Setters with GSE and 7 healthy Irish Setters.

Procedure—Phenotype of each dog was determined after oral administration of gluten in the weaning diet, using morphometric evaluation of jejunal biopsies (all generations) and measurement of small intestinal permeability by use of a lactulose-rhamnose permeation test (generations 1, 2, and 3). Overall probability for each of 4 genetic models of inheritance (autosomal recessive, autosomal dominant, sex-linked recessive, and sex-linked dominant) accounting for segregation of partial villus atrophy within the entire family was calculated.

Results—The autosomal recessive model was most tenable and was 56,250 times more likely to account for segregation of partial villus atrophy than the autosomal dominant model, assuming disease prevalence of 0.8%. Both sex-linked models were untenable. These conclusions were robust to the error attached to estimation of disease prevalence. High intestinal permeability without morphometric jejunal abnormalities in 4 of 20 dogs in the 3 youngest generations suggested heterogeneity of lesions associated with GSE.


Presently, 9,381 genetic diseases are recognized in humans, compared with approximately 350 genetic diseases in dogs. Although the number of inherited disorders recognized in dogs is relatively small, genetic analysis of an increasing number of multifactorial diseases in this species, ranging from hip dysplasia to idiopathic epilepsy in Labrador Retrievers, has been performed in recent years. A survey of canine genetic diseases documented 281 disorders, of which 30% had an autosomal recessive mode of inheritance. Predominance of recessively inherited diseases in dogs contrasts with the large number of autosomal dominant disorders in humans, and is a consequence of genetic homogeneity of purebred dog populations and the ease for selecting against dominant traits in dogs. The inbreeding common in purebred dogs promotes expression of recessive or polygenic traits by maximizing homozygosity, and it magnifies the deleterious influence of genetically abnormal dogs that may be overrepresented because of their conformity to breed standards.6

This phenomenon is believed to underlie many of the breed-specific diseases in dogs, such as gluten-sensitive enteropathy (GSE) in Irish Setters. Clinical signs of GSE include inappetence, poor growth, and diarrhea, and pathologic changes include partial villus atrophy in the jejunum and increased intestinal permeability.7,8 It is a disease of concern to veterinarians and has potential as a model for celiac disease (CD) of humans, a condition with which it shares clinicopathologic features.9

Few dogs of other breeds have been identified with gastrointestinal gluten sensitivity, and even those failed to demonstrate pathologic features of chronic GSE.10,11 Gluten-sensitive enteropathy appears to be breed-specific, but it also has features of familial aggregation.12,13 Although both of these observations suggest a genetic cause, little is presently known about familial transmission of this disease. Such knowledge is of importance for the design of effective control strategies for GSE in Irish Setters and for molecular genetic analysis of this disease.

The objective of the study reported here was to establish a model of inheritance of GSE in Irish Setters on the basis of breeding studies within a colony of affected dogs. To determine the disease status of Irish Setters in the study, a screening procedure for GSE in puppies was developed on the basis of results published elsewhere.14

Materials and Methods

Dogs—Six gluten-sensitive Irish Setter puppies were the offspring of a mating between a male dog with GSE owned by a breeder and a gluten-sensitive female dog in the colony, which was from a line of affected Irish Setters (Fig 1) maintained at the Royal Veterinary College, as described.14 The male dog was unrelated to the female, had a history of poor weight gain and inappetence, and had a 90-kd serum glycoprotein that has been associated with gluten sensitivity.15 Phenotype of the female dog was established by controlled gluten administration for 6 weeks, followed by measurement of villus height and permeability.15 For comparisons, data were obtained from comparable examination of biopsy specimens and permeability measurements per-
formed in 7 healthy Irish Setter puppies owned by various breeders.11

Irish Setters that were to be used for genetic studies comprised an F1 and a backcross litter. The F1 litter comprised 5 dogs (3 male, 2 female) derived from an outcross mating between a gluten-sensitive male dog in the colony and a healthy female (noncolony) that did not share common ancestors with the male for the preceding 5 generations. Phenotype of the female dog was determined by controlled mating between 1 of the F1 male dogs and a full-sibling female, as described.4 Villus height was measured from crypt mouth to villus tip, as defined.50

**Statistical analyses**—Phenotype was determined on the basis of plasma lactulose-rhamnose ratios (L:R) measured when dogs were 8 and 16 weeks old and villus height (VH) measurements performed when dogs were 16 weeks old, as described.4 Villus height was expressed as a 95% confidence interval for measurements made on 50 to 100 villi examined in each dog. Potential subgroups of dogs were identified prior to designation of phenotype by means of cluster analysis of standardized permeability and VH data, using the Ward minimum variance method.16

Reference thresholds for plasma L:R and VH were assigned by choosing values that would allow segregation of known healthy and gluten-sensitive dogs. Data for each of the F1 and backcross dogs were then reviewed to enable assignment of phenotypes on the basis of the chosen reference thresholds.

**Genetic analysis**—Binomial probability theory formed the basis for statistical comparison of 4 genetic models: autosomal recessive (AR), autosomal dominant (AD), sex-linked recessive (SLR), and sex-linked dominant (SLD). For the purpose of genetic analysis, the gluten-sensitive phenotype was identified on the basis of partial villus atrophy (PVA) alone, which was indicated when any part of the 95% confidence interval for VH measurements was less than the reference threshold. Point prevalence of GSE was estimated by questioning people participating at a large national Irish Setter dog show; of 734 dogs, 6 were identified with clinical histories and permeability measurements compatible with GSE (confirmed in 4 dogs by results of histologic evaluation of jejunal biopsy specimens). Therefore, statistical analyses were performed by using an estimated disease prevalence of 0.8%. However, because owners may have been reluctant to reveal health problems of their dogs, and some dogs may have been fed gluten-free diets, analyses also were performed by using prevalence estimates of 1.6 and 8%, for comparative purposes.

Overall probability (P) of each genetic model that accounted for segregation of PVA within the entire family of dogs was calculated as the product of binomial probabilities for individual generations (Appendix 1). The model most likely to account for segregation of PVA on this basis was then evaluated by \( \chi^2 \) analysis (Appendix 2). Overall P values for each of the models were calculated by use of a computer software program.
Mean inbreeding coefficient of the entire family of dogs was computed by use of a computer software program, using the Quaas-Henderson iterative method.

Results

Phenotypic characteristics of F1 and backcross dogs—All gluten-sensitive dogs had ≥ 1 permeability measurement (plasma L:R) > 0.2, whereas all healthy dogs had plasma L:R < 0.2; therefore, the reference threshold for plasma L:R was assigned a value of 0.2. Similarly, VH measurements for healthy dogs were > 800 µm, whereas values for gluten-sensitive dogs were < 800 µm; therefore, the reference threshold for VH was assigned a value of 800 µm, for comparison with values of dogs of unknown gluten sensitivity status. Partial villus atrophy was considered to be evident when the lower limit of the 95% confidence interval was < 800 µm.

Permeability and VH data for the F1 and backcross dogs were determined. The 95% confidence intervals of VH in the 5 F1 dogs ranged from a lower limit of 821 µm to an upper limit of 1,075 µm, whereas the L:R ratios ranged from 0.01 to 0.35 (Fig 2). The 95% confidence intervals of VH in the backcross dogs ranged from a lower limit of 456 µm to an upper limit of 1,104 µm, whereas the L:R ratios ranged from 0.08 to 0.32. Reference thresholds were applied under the assumption that affected dogs would have PVA and increased intestinal permeability, as described in other reports of GSE. Unequivocal ascertainment of phenotype under this assumption was possible in 2 F1 and 6 backcross dogs; however, 1 of the F1 dogs (dog 18) had congenital megaoesophagus, precluding permeability

Figure 2—Intestinal permeability (top) and villus height measurements (bottom) of healthy control (dogs 1 to 7) and gluten-sensitive (dogs 8 to 13) Irish Setters following oral administration of gluten after weaning. Plasma lactulose-rhamnose concentration ratios were determined 8 (●) and 16 weeks (■) after initiation of oral administration of gluten. Dogs 8 and 13 were not evaluated at 16 weeks. Villus height measurements (95% confidence intervals) were determined from jejunal biopsy specimens obtained 16 weeks after initiation of oral administration of gluten. Dashed lines indicate reference threshold values.

Figure 3—Dendrogram of results of cluster analysis for measurements of intestinal permeability (plasma lactulose-rhamnose concentration ratios determined when dogs were 16 weeks old) and villus height (lower limits of 95% confidence intervals) in healthy control (C1 to C7) dogs and F1, backcross, and parental generation Irish Setters with GSE (dogs 8 to 13, 15 to 17, and 19 to 27). Notice clusters containing affected (A), unaffected (U), and possible intergrade (I) dogs. Scale on bottom indicates semi-partial R² values.
Table 1—Probabilities of 4 genetic models of inheritance of gluten-sensitive enteropathy in 6 generations of Irish Setters. fa = Frequency of dominant allele. fa = Frequency of recessive allele. P(6) to P(1) = Probabilities of models accounting for segregation within individual generations 6 to 1. P(1...6) = Overall probability. AR = Autosomal recessive. AD = Autosomal dominant. SLR = Sex-linked recessive. SLD = Sex-linked dominant. * = Estimated disease prevalence was 0.008, but robustness of calculations was verified with prevalences of 0.016 and 0.08. Allele frequencies for sex-linked models are listed to indicate the basis for subsequent calculations; equal prevalence of disease in males and females (assumed to enable calculation of allele frequencies) suggested that sex-linkage was not evident. NA = Not applicable. M = Male. F = Female.

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Intestinal permeability and VH are 2 established phenotypic expressions of GSE. 7,8 Quantitation of PVA was accomplished by comparison of VH in the dogs of our study with the lower limit of the 95% confidence interval of measurements made in unaffected dogs, which allowed detection of subtle or patchy lesions that may not have been detected by evaluation of mean VH alone. Choice of the reference threshold for intestinal permeability was complicated by overlap between the highest plasma L:R of healthy dogs and the lowest ratios of gluten-sensitive dogs, confounding interpretation of values close to the threshold. Furthermore, reference thresholds were assigned on the basis of measurements from a limited number of known gluten-sensitive and healthy Irish Setters. For these reasons, cluster analysis of permeability and VH data was performed to determine whether discrete groups of dogs could be identified without use of reference values; broad clusters of dogs that were designated as affected and unaffected could be identified. However, cluster analysis and attempts to assign phenotype by reference to threshold values of VH and intestinal permeability suggested a third group of dogs with increased plasma L:R but without decreased VH in the jejunum.
There are several possible explanations for these findings. Because lesions of PVA in GSE often have an uneven distribution, jejunal biopsy may not have obtained affected tissue, although we tried to avoid this error by use of an appropriate study design and by obtaining a large number of biopsy specimens from each dog. Furthermore, host factors such as total number and species of enteric bacterial flora may have influenced intestinal permeability, although clinical signs of bacterial overgrowth were not detected and bacteriologic culture of feces did not detect enteric pathogens. Among environmental factors, housing of the dogs was considered as a possible influence on intestinal permeability, because the backcross dogs were housed in kennels and had opportunity for enteric bacterial loading by coprophagia or could have been influenced by psychoneural factors, such as stress of confinement or boredom; on the other hand, F1 progeny were reared in a home environment. However, F1 and backcross dogs were represented in the group with discordant results for VH and plasma L:R, which argued against a major influence of environmental factors on intestinal permeability of these dogs.

In our opinion, the discordance between VH values and plasma L:R indicated an intermediate grade of GSE. The clinical spectrum of CD in humans, which has prompted such terms as active, silent, latent, and potential, has been established. A pathologic spectrum is also recognized, ranging from infiltrative lesions characterized only by increased density of intraepithelial lymphocytes to destructive and hypoplastic lesions characterized by subtotal villus atrophy. The finding of diarrhea in patients with infiltrative lesions and lack of clinical signs in some patients with destructive lesions emphasizes the lack of a simple correlation between clinical and pathologic features of CD. Similar complexity may also characterize GSE in dogs, in which intermediate-grade lesions may be detected by measurement of permeability but not by morphometric features of jejunal biopsy specimens. Authors of a report of plasma L:R in Irish Setters proposed the possibility of subclinical GSE in dogs with increased intestinal permeability and a 90-kd serum glycoprotein marker of GSE. Heterogeneity of results in our study agrees with this concept and suggests that gluten in the diet of genetically predisposed Irish Setters may give rise to a spectrum of clinical signs, the severity of which may depend in part on secondary host or environmental factors, such as metabolic demands of active growth or pregnancy, or composition of the population of enteric flora.

Genetic analysis of GSE was based on segregation of dogs with signs of gluten sensitivity after controlled oral administration in accordance with an established protocol. Phenotype was assigned on the basis of VH alone, because dogs with plasma L:R that were inconsistent with VH values may have represented an intermediate phenotype, the importance of which remains unclear. Further understanding of the anatomic pathways by which permeation markers cross the intestine may eventually allow definitive interpretation of results of permeability studies and enable refinement of genetic models.

In our study, outcross mating of an affected dog with an unaffected dog was performed, followed by a mating between 1 of those F1 progeny and a full-sibling female of the male parent. An alternative strategy may have been to mate an affected Irish Setter with a healthy dog of an unrelated breed, followed by backcross mating between 1 of those F1 progeny and the affected parent. This approach would have avoided the possibility that the healthy Irish Setter used in the outcross mating carried susceptibility alleles; however, modifier genes within the Irish Setter genome may be necessary for expression of GSE, and crossbred dogs may not express gluten sensitivity even though they may carry major susceptibility alleles. For this reason, studies were restricted to Irish Setters.

Mean inbreeding coefficient within the Irish Setter colony was 0.18, suggesting close genetic relationship between family members. Use of inbred dogs may have facilitated genetic analysis by minimizing phenotypic variation attributable to genetic background, allowing unhindered expression of major susceptibility loci. Results of studies in a colony of Keeshonds with inherited conotruncal defects illustrated this principle. In that colony, the autosomal susceptibility locus was discovered only in dogs that had been inbred over 10 generations, in contrast to results of studies of outbred dogs, which erroneously suggested polygenic inheritance. Inbreeding may facilitate genetic studies but has the undesirable consequence of disclosing recessive susceptibility alleles. For example, inbreeding in Dutch Bouvier des Flandres has been correlated with osteochondrosis, food allergy, autoimmune disease, neoplasia, and hypoplastic trachea. Cluster analysis has also confirmed lack of genetic heterogeneity in populations of Dutch Labrador Retrievers, Irish Wolfhounds, and Bedlington Terriers and has revealed familial aggregation of breed-specific diseases, which suggests the genetic influence of certain founder dogs.

Transmission of GSE in the family of Irish Setters in our study was compatible with the AR model. Autosomal dominance was much less likely to account for segregation of affected and unaffected dogs, and simple sex-linked models were excluded. Although sex-linked recessive and dominant inheritance patterns were considered in our analysis for the sake of completeness, the phenomenon of Lyonization precluded rigid distinction between these 2 models. However, even assuming incomplete penetrance, the probability that either mode of inheritance was appropriate for GSE was negligible. At least 1 AR susceptibility locus has been implicated in transmission of CD, and a single major AR locus may be responsible for GSE, as is true for a large proportion of genetic traits in dogs. Recessive transmission may be responsible for the increased prevalence of certain disorders in specific breeds of dogs, reflecting the undesirable consequence of homozygosity in progeny of dogs that share close genetic relationship.

The AR model of GSE has genetic and pathogenic implications. A linkage approach to the genetic dissection of GSE is likely to be productive, because results of the study reported here suggest that a single major locus contributes to disease transmission. Ultimate identification of the major disease gene in GSE holds promise for the molecular diagnosis of affected and carrier Irish Setters and may also provide further insight into the molecular pathogenesis of dietary sensitivity in general.
Appendix 1
Statistical comparison of genetic models

Genetic models were compared by calculating the probability of observed segregation of affected and unaffected offspring among all 6 generations of dogs. Binomial probability of observing a certain number of affected offspring in a litter was given by the equation:

\[ P(\text{affected}) = \binom{\text{n}}{\text{r}} p^\text{r} (1-p)^{\text{n-r}} \]

where \( n \) is the number of combinations of 'r' affected out of 'n' total litter members = \( n!/(r!{n-r!}) \), and \( P_{\text{affected}} \) is the probability of a member of a litter being affected in its generation.

Overall probability, \( P(1...6) \), was the product of binomial probabilities of all generations.

Rationale underlying statistical comparison of the genetic models is illustrated for autosomal recessive (AR) inheritance, in which ‘a’ denotes the allele transmitting disease. Assuming Hardy-Weinberg equilibrium (\( p \)), the probability (\( p \)) that both alleles are ‘a’ (ie, the estimate of disease prevalence), is equal to \( p_a = p \cdot p \), and, therefore, \( p = 1 - p_a \). It follows that \( p(AA) = p_a p_a \), and that \( p(Aa) = 2p_a(1-p_a) \).

The estimate of \( P_{\text{affected}} \) in a particular generation was straightforward when the genotype of each parent was known, but weighted calculations were used when alternative possible genotypes could be inferred with given probabilities. For generations 3 to 6, genotypes of parents were aa; therefore, \( P_{\text{affected}} = 1 \) and genotype of all offspring were aa. Thus, binomial probability for generations 3 to 6 was \( P(3...6) = 1 \). For generation 2, genotype of dog 14 could have been AA or Aa, yielding a \( p \) statistic of 0.840. For generation 1, \( p = 0.480 \).

The \( \chi^2 \) test was performed on extended pedigrees. For generations 3 to 6, genotypes of parents were aa; therefore, \( P_{\text{affected}} = 1 \) and genotype of all offspring were aa. Thus, binomial probability for generation 2 was calculated as: \( P(2) = (P[AA]/[1-p(\text{aa})])(1-p(\text{aa}))/[1-p(\text{aa})] \). For generation 1, \( P(1...6) = (P[1])(P[2])(P[3])(P[4])(P[5])(P[6]) \).

Similar arguments were applied for the other genetic models, allowing comparison of their relative likelihoods.

Appendix 2
Goodness-of-fit test for autosomal recessive inheritance

Observed and expected numbers of affected and unaffected offspring in each generation were compared by means of the \( \chi^2 \) test. For generations 6 to 3, parental genotypes were clearly AA; therefore, observed and expected numbers of affected and unaffected offspring were equal, yielding a \( \chi^2 \) statistic = 0 and a significance probability = 1. For generation 2, the genotype of dog 14 could have been AA or Aa, yielding a \( \chi^2 \) statistic of 0.025, respectively. Using the probabilities of the genotypes AA or Aa as weights (reported in Appendix 1 as \( p(AA)/[1-p(\text{aa})] \) and \( p(\text{Aa])/[1-p(\text{aa})] \), weighted mean of the 2 significance probabilities was calculated for generation 2 was calculated as: \( P(2) = (P[\text{AA}]/[1-p(\text{aa})])(1-p(\text{aa}))/[1-p(\text{aa})] \). Overall probability, \( P(1...6) \), of observing the distribution of affected and unaffected offspring when the AR model was:

\[ P(1...6) = (P[1])(P[2])(P[3])(P[4])(P[5])(P[6]). \]

References


