Complex segregation analysis of deafness in Dalmatians

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Objective—To use pedigree analysis to evaluate the feasibility of a major locus model for deafness in Dalmatians.

Animals—605 purebred Dalmatians from 42 families.

Procedure—Hearing loss was evaluated through the brainstem auditory-evoked response. Dogs were classified into mutually exclusive categories: normal hearing, unilaterally deaf, or bilaterally deaf. Information was collected on sex, coat color, presence or absence of a color patch at birth, and eye color. Statistical analyses were performed by use of regressive logistic models designed for complex segregation analysis. Genetic correlations among eye color, deafness, and color patch were estimated.

Results—Prevalence of hearing loss was 11% for dogs classified as unilaterally deaf and 5% for dogs that were bilaterally deaf. Complex segregation analysis detected statistical evidence of a single allele with an expected frequency of 0.21 that had an effect on the prevalence of deafness. Results of analyses suggested that this locus cannot completely explain the inheritance and incidence of deafness in Dalmatians. Genetic correlation estimates among deafness, eye color, and color patch revealed strong interrelationships among these characteristics.

Conclusions and Clinical Relevance—To reduce the incidence of hearing loss in Dalmatians, unilaterally deaf, blue-eyed dogs should not be considered as potential parents. (*Am J Vet Res* 2000;61:550–553)

Research investigators as well as breeders agree that deafness in Dalmatians is genetic in origin, but there is debate concerning the potential effect of a single locus on the expression of hearing loss. Simple Mendelian single-locus transmission can be eliminated as a meaningful genetic model; however, the possibility that 1 locus may have a major effect on deafness remains.

A variety of single genes that affect hearing loss in humans and mice have been isolated.[1] In humans, the *TECTA* gene on chromosome 11 is a dominantly inherited mutation that results in hearing loss,[2] whereas in mice, recessive mutations in the *MYO15* myosin gene cause profound deafness.[3] The human homolog to mouse *MYO15* is *DFNB3*; recessive mutations in this gene cause analogous hereditary deafness.[4] These genes encode proteins considered to be critical in sensory hair cell function.[2-4]

If it were known that Dalmatians possessed a single gene with large effects (ie, a major locus) on the inheritance of deafness, breeders could construct meaningful selection guidelines to reduce the prevalence of this disorder. Likewise, if statistical analysis revealed that a single locus is not segregating in Dalmatians, alternative breeding programs could be constructed. The objective of the study reported here was to use pedigree analysis to evaluate the feasibility of a major locus model for deafness in Dalmatians.

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Materials and Methods

Dogs—Data from 605 Dalmatians from 42 families were collected at the Animal Dermatology Clinic in Sacramento, Calif. Only 552 (264 females, 288 males) of these dogs had known deafness records; an additional 53 dogs (parents of dogs with deafness records) with unknown deafness phenotype were included to help build appropriate pedigrees. This set of data was distinct from the set of deafness records collected to aid in the estimation of the heritability of deafness.[5]

Brainstem auditory-evoked response (BAER)—Phenotypes for hearing loss were measured by means of the BAER, which allowed discrimination among dogs with normal hearing and those with bilateral and unilateral deafness. The BAER test was conducted with an evoked potential unit and recording system that includes an oscilloscope, a stimulation unit, a computer, a plotter, and a printer.[a] Prior to evaluation, dogs were sedated by administration of acepromazine (0.5 mg, SQ, for puppies; 0.1 mg/kg of body weight, IV, for adults). Each ear was tested separately with insert earphones that produced a click stimulus at 70 dB (normal hearing level) to 1 ear and a "white noise" of 40 dB masking sound to the opposite ear. Three platinum electrodes were placed subcutaneously for the test; 1 electrode was placed at the top of the head midway between the intercanthal line and the external occipital protuberance (vertex-V), 1 electrode was placed between the scapulae, and 1 electrode was placed over the right zygomatic arch just below the pinna, to act as a ground. The tested ear received 500 alternating click stimuli, at a rate of 10 clicks/s. Hearing was evaluated by interpretation of a series of peaks generated by the BAER. The existence of peaks was an indication of normal hearing, whereas lack of peaks indicated failure of the tested ear to function normally.

Phenotypic characteristics—Information regarding coat color, eye color, sex, pedigree information, and the presence or absence of a color patch at birth was recorded for all puppies in each litter. Phenotypic characteristics of the parents were also recorded, when known.

Statistical analyses—A regressive logistic model was used to assess the presence of a major gene for deafness. Specifically, a linear model was fitted to the logistic likelihood variable θ such that:

$$\theta_i = \alpha + \delta_P Z_{Pi} + \xi_1 X_{1i} + \xi_2 X_{2i} + \xi_3 X_{3i} + \xi_4 X_{4i}$$

where α represents the equivalent of an intercept (the baseline log odds), Z_{Pi} is a term based on the phenotype of the parents of dog i, X_{1i} through X_{4i} are explanatory variables, and δ_P and ξ_1 through ξ_4 are unknown variables that can range from $-\infty$ to $+\infty$. For our analyses, X_{1i} was 0 for black and white dogs, 1 for liver and white dogs; X_{2i} was 0 for dogs born without a patch and 1 for dogs born with a patch; X_{3i} was 1 for dogs with 2 pig-

mented (nonblue) eyes and 0 otherwise; X_{4i} was 1 for dogs with 1 pigmented eye and 1 blue eye and 0 otherwise. For major gene models, the variable α can be redefined for 3 separate means (eg, α_j for j = 1,2,3), 1 for each putative major locus genotype,[6] whereas the variable Z_{Pi} allows for the polygenic-based covariance between parent and offspring. An assumption of equal parental contribution (ie, the regression of progeny information on parent information is assumed equal for male and female parents) is implied in the model parameterization. Values for Z_{Pi} take the form of indicator variables (0,1; presence, absence) depending on the hearing phenotype of the parents.[7] Alternative models for parental contributions can also be defined[8]; specifically, models that reflect an unequal contribution from each parent. Such models were also considered in our analyses and permitted evaluation of the possibility that the genetic contribution of 1 parent had a greater impact on development of hearing than that of the other parent.

Estimation of the unknown variables, including the expected frequency of the putative major locus under Mendelian transmission and calculation of conditional major locus probabilities given pedigree and phenotypic information was performed with a commercially available software package.[b]

Additional analyses were undertaken to estimate genetic correlation among deafness, eye color, and the existence of a color patch at birth. A threshold model (a multiple-trait extension of models used to evaluate the heritability of deafness[8]) was used to estimate unknown genetic covariances or correlations. Because the analysis was performed on observations of deafness, eye color, and color patch, only coat color and sex were used as fixed effects in the threshold model. Variance and covariance components were estimated with a public domain computer program.[9]

Results

Among the 552 dogs for which hearing status was determined, 464 (84%) had normal hearing, 58 (11%) were deaf in 1 ear, and 30 (5%) were deaf in both ears (**Table 1**). Prevalence of hearing loss was greater in dogs with 1 or 2 blue eyes than in dogs that did not have blue eyes.

Estimates of variables from several regressive logistic models with and without terms to account for major locus inheritance were recorded (**Table 2**). Comparison of likelihood statistics, as well as **Akaike's Information Content (AIC)**[10] values, supported the conclusion that a model that included the effects of a major locus provided the best explanation for inheritance of hearing loss in this population of Dalmatians. The best fit was provided by a major locus of "general" effect[b] (a single locus for which the putative heterozygote effect is free to fall at any value between the 2 homozygote effects). The generalized likelihood ratio test statistic comparing the general major gene model and the no major gene model was l = 31.0 with 3 *df* ($P < 0.001$); accordingly, the substantial decrease in the AIC, coupled with large changes in the likelihood, was evidence that the general major gene model provided the best fit to the data. By the same criterion, a recessive and dominant genetic model had improvement in the AIC when compared with the no major locus model.

The expected frequency of the putative deafness gene was 0.21 under the general transmission model and as high as 0.41 if the model was restricted to recessive inheritance (in which only a homozygous susceptible dog, AA, would have unilateral or bilateral deafness). Because the combined prevalence of unilateral and bilateral deafness was 16%, frequency of the susceptibility allele in the recessive model was increased. Assuming that heterozygotes (ie, presumed AB individuals) could have unilateral or bilateral deafness (the assumption underlying the general model of transmission) allowed the model to decrease the frequency of the susceptibility allele while maintaining the population frequency of affected individuals at 16%.

Table 1—Phenotypic classification of 552 Dalmatians (No. of dogs) with or without a color patch that had normal hearing or were unilaterally or bilaterally deaf

Phenotype	Normal hearing	Unilaterally deaf	Bilaterally deaf
No color patch			
Pigment/pigment	354	42	21
Pigment/blue	27	9	4
Blue/blue	9	5	4
Color patch			
Pigment/pigment	70	2	1
Pigment/blue	4	0	0
Blue/blue	0	0	0

Pigment/pigment = Both eyes were pigmented (brown). Pigment/blue = 1 eye was pigmented, 1 eye was blue. Blue/blue = Both eyes were blue.

Table 2—Variable estimates for regressive logistic models fit to a trichotomous deafness phenotype with different baseline log odds for different phenotypic classes of each putative major locus genotype (AA, AB, and BB)

Variable	No major gene model	General major gene model	Recessive major gene model	Dominance major gene model
Frequency of susceptibility Allele A	NA	0.21 (0.04)	0.41 (0.09)	0.14 (0.11)
Baseline, α	–1.46 (0.55)	NA	NA	NA
Baseline, α_{AA}	NA	25.74 (3.95)	1.34 (0.97)	0.57 (0.87)
Baseline, α_{AB}	NA	–0.43 (0.78)	–2.64 (0.97)	0.57 (0.87)
Baseline, α_{BB}	NA	–4.84 (2.29)	–2.64 (0.97)	–2.40 (0.96)
Parent, δ	–0.39 (0.17)	–1.24 (0.53)	–0.96 (0.35)	–0.70 (0.25)
Coat color covariate	0.13 (0.35)	0.49 (0.61)	0.05 (0.58)	0.10 (0.49)
Color patch covariate	–1.71 (0.61)	–2.56 (1.22)	–1.74 (0.81)	–1.59 (0.69)
Pigment/pigment covariate	–1.54 (0.49)	–3.04 (1.20)	–2.75 (0.84)	–2.55 (0.85)
Pigment/blue covariate	–0.58 (0.58)	–1.53 (1.25)	–1.42 (0.84)	–1.33 (0.85)
–2 ln likelihood	568.4	537.4	546.0	547.8
Akaike's Information Content	580.4	555.4	562.0	563.8

Values in parentheses indicate SE.
NA = Not applicable.
See Table 1 for key.

Table 3—Variable estimates for regressive logistic models fit to a trichotomous deafness phenotype with different baseline log odds for different phenotypic classes for each putative major locus genotype (AA, AB, and BB) classified by sex

Variable	No major gene model Equal parent	General major gene model Equal parent	General major gene model Separate parents
Frequency of susceptibility Allele A	NA	0.09 (0.04)	0.14 (0.09)
Baseline, α female	–1.21 (0.56)	NA	NA
Baseline, α male	–1.38 (0.56)	NA	NA
Baseline, α_{AA} female	NA	11.9 (9.6)	2.38 (3.45)
Baseline, α_{AB} female	NA	1.19 (1.1)	0.60 (1.15)
Baseline, α_{BB} female	NA	–2.70 (0.85)	–3.28 (0.92)
Baseline, α_{AA} male	NA	1.72 (3.63)	0.11 (3.15)
Baseline, α_{AB} male	NA	–0.13 (0.88)	–0.22 (0.92)
Baseline, α_{BB} male	NA	–2.05 (0.88)	–2.32 (0.88)
Female parent contribution	NA	NA	0.25 (0.49)
Male parent contribution	NA	NA	–2.12 (0.66)
Equal parent contribution, δ	–0.38 (0.17)	–0.86 (0.32)	NA
Coat color covariate	0.11 (0.35)	0.11 (0.52)	–0.02 (0.57)
Color patch covariate	–1.71 (0.61)	–2.10 (0.87)	–1.81 (0.78)
Pigment/pigment covariate	–1.69 (0.50)	–2.38 (0.63)	–2.73 (0.82)
Pigment/blue covariate	–0.73 (0.58)	–1.38 (0.74)	–1.64 (0.87)
–2 ln likelihood	567.8	536.9	536.0
Akaike's Information Content	581.8	560.9	562.0

See Tables 1 and 2 for key.

Table 4—Mean conditional probability of major genotype classification (of putative major gene class, AA, AB, or BB) for dogs with complete phenotype and pedigree information, classified by their deafness phenotype by use of variable estimates from the general major gene model

Putative major gene class	Normal hearing	Unilaterally deaf	Bilaterally deaf deaf
AA	0.00	0.27	0.28
AB	0.27	0.61	0.62
BB	0.73	0.12	0.10

Results of several logistic models suggested that sex did not have an important effect on the prevalence of deafness (**Table 3**). Among female dogs, 16 were bilaterally deaf, 29 were unilaterally deaf, and 219 had normal hearing. Among male dogs, 14 were bilaterally deaf, 29 were unilaterally deaf, and 245 had normal hearing. By use of χ^2 analysis, ignoring all other factors contributing to deafness, the χ^2 value was 0.55 with 2 *df* and a probability of $P = 0.76$. Results of a likelihood ratio test[11] also suggested that sex was not a determining factor for deafness; comparison of the likelihood values of the no major locus models (Table 2 and 3) yielded a test statistic of $\lambda = 0.6$, which has a χ^2 distribution with 1 *df* and a cumulative probability, $Pr(\chi^2 < 0.6) = 0.44$. On the basis of comparison of likelihood and AIC values between male and female parent contributions (Table 3) and the general major gene model (Table 2), male and female parent contributions to deafness were not statistically different.

Mean conditional major locus probabilities for dogs with complete phenotypic and pedigree information were determined (**Table 4**). Values were computed only for the best model, which included a general transmission major gene effect, in which putative heterozygous dogs were included in the affected classes. Among dogs that were bilaterally deaf, mean probability of homozygosity for a putative major gene for deafness was 0.28. Heterozygotes appeared to be the most likely genotypic class for dogs with deafness. Dogs with normal hearing had a high probability of being homozygous for the putative "normal" allele, as expected, although the probability of being heterozygous for the putative deafness allele with a normal hearing phenotype was 0.27. Affected dogs (unilateral and bilateral deafness) also had a small probability (0.10 to 0.12) of being homozygous for the "normal" allele at the putative major locus, suggesting that although a major locus model was the most likely explanation for the data, explanations other than a strict single locus model must also be considered.

Eye color and the presence of a color patch were also associated with hearing loss and had strong genetic interrelationship. In a threshold model, heritability estimates for color patch, eye color, and deafness were 0.38, 0.85, and 0.32, respectively. Color patch was negatively correlated with eye color ($r = –0.48$) and deafness ($r = –0.53$). Of particular concern to Dalmatian breeders was the negative correlation between color patch and deafness; because a color patch is undesirable, breeders who select against this trait increase the likelihood of deafness in future litters.

Discussion

The principal objective of the study reported here was to evaluate the feasibility of a putative major locus model for deafness in Dalmatians; we concluded that a major locus may be an important influence on expression of hearing loss. However, the frequency of the deafness allele was low, suggesting that the 16% prevalence of hearing loss in the population we studied must be the result of more than 1 gene. A single locus may evidently play a role in the expression of hearing loss, but alone, such a locus cannot explain prevalence of the disease entirely; for example, a simplistic model in which dogs with a homozygous major locus are bilaterally deaf and heterozygous dogs are unilaterally deaf was not supported by our analyses. Nevertheless, there was evidence to suggest that 1

locus may be playing an important role in the development of normal hearing.

Perhaps most interesting is how deafness is associated with eye color. As indicated by the genetic correlation of 0.72, these 2 traits obviously overlap genetically. Exploring the basis of the statistical relationship between hearing loss and eye color may shed light on the underlying biological characteristics of each trait. Given the variable estimates from the regressive logistic model (Table 2), the probability of being in a major genotypic class (eg, defining putative major locus genotypes as AA, AB, or BB) given other phenotypic characteristics (eg, coat color, presence of color patch, or eye color) may be calculated. The calculation of such conditional probabilities was a component of the software used in our study.[b] For example, a bilaterally deaf dog with no color patch and blue eyes would have a 0.17 probability of being in the AA class, a 0.28 probability of being AB, and a 0.55 probability of being BB. A dog with pigmented eyes that is bilaterally deaf and does not have a color patch would have probabilities of 0.41, 0.42, and 0.17 for genotypes AA, AB, and BB, respectively. In other words, a blue-eyed bilaterally deaf dog would have a 0.45 probability of carrying at least 1 copy of a deafness allele, whereas a bilaterally deaf dog with pigmented eyes would have a 0.83 probability of carrying at least 1 copy of a deafness allele.

The conclusion from such a comparison of probabilities is that deafness in Dalmatians is a polygenic trait in which 1 locus may provide an important contribution to hearing loss but alone cannot completely explain the expression of this disorder. The role any single locus may play is dependent on the surrounding genome. Dogs with pigmented eyes and normal hearing have a 0.20 probability of carrying at least 1 copy of the putative deafness allele; in other words, dogs may carry this allele and have normal hearing, or, conversely, be deaf without any statistical trace of the allele. Such a conclusion should not be considered surprising or unusual. Results of previous analyses have suggested polygenic inheritance as the most likely basis for deafness, as supported by results of our statistical analyses. The putative deafness allele is one that increases risk, not unlike the alleles *BRCA1* and *BRCA2* that may increase the risk of breast cancer in humans but are not the only factor in the expression of disease.[12]

Results of complex segregation analysis suggested that the search for a deafness allele may have the greatest success in deaf dogs with pigmented eyes. Although the frequency of this allele in the Dalmatian population as a whole may not be large enough for easy detection, its expected frequency in deaf dogs with pigmented eyes is quite high. Unilaterally deaf dogs are likely to carry at least 1 copy of this putative deafness allele. Interestingly, dogs with normal hearing are virtually eliminated (across all color patch and eye color classifications) from consideration as being homozygous for such a disease allele, although, as results of our analysis suggest, dogs with normal hearing may be capable of passing the allele to their offspring.

Results of previous analyses, although sometimes conflicting, have suggested that females are more likely to be deaf than males[8,13,14]; anecdotal evidence from breeders supports this suggestion. However, results of our analysis of 552 medical records did not reveal a significant difference in prevalence of deafness between sexes. Whether this was the result of sampling, differences between the population we studied and those of other studies, or underlying biological characteristics is unknown. This disparity may suggest that the expression of hearing loss is complicated, involving the interaction of many loci in addition to the major locus indicated by results of our analyses.

The genetic correlations among color patch, eye color, and deafness provide a means of decreasing the prevalence of deafness in Dalmatians by selective breeding. Although a genetic test for a single allele may improve breeding decisions, decisions based on the genetic correlations estimated by our analyses may also have a high degree of success. Specifically, selecting only dogs with normal hearing, pigmented eyes, and a color patch (although a color patch is an otherwise undesirable characteristic) could have a substantial effect on reducing the prevalence of deafness.

[a]Cadwell 5200A Evoked Potential System, Cadwell Laboratories, Kennewick, Wash.

[b]Statistical Analysis for Genetic Epidemiology, Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio (US Public Health Service Resource Grant 1 P41 RR03655).

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