Quantification of the association between intervertebral disk calcification and disk herniation in Dachshunds

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Objective—To quantify the association between intervertebral disk calcification and disk herniation in Dachshunds.

Design—Longitudinal study.

Animals—61 Dachshunds that had been radiographically screened for calcification of intervertebral disks at 2 years of age in other studies. Thirty-seven of the dogs had survived to the time of the present study and were ≥ 8 years of age; 24 others had not survived.

Procedures—Radiographic examination of 36 surviving dogs was performed, and information on occurrence of disk calcification at 2 years of age were obtained from records of all 61 Dachshunds. Information on occurrence of disk herniation between 2 and 8 years of age was obtained from owners via questionnaire. Associations between numbers of calcified disks and disk herniation were analyzed via maximum likelihood logistic regression.

Results—Disk calcification at 2 years of age was a significant predictor of clinical disk herniation (odds ratio per calcified disk, 1.42; 95% confidence interval, 1.19 to 1.81). Number of calcified disks in the full vertebral column was a better predictor than number of calcified disks between vertebrae T10 and L3. Numbers of calcified disks at ≥ 8 years of age and at 2 years of age were significantly correlated.

Conclusions and Clinical Relevance—Number of calcified disks at 2 years of age was a good predictor of clinical disk herniation in Dachshunds. Because of the high heritability of disk calcification, it is possible that an effective reduction in occurrence of severe disk herniation in Dachshunds could be obtained by selective breeding against high numbers of calcified disks at 2 years of age. (J Am Vet Med Assoc 2008;233:1090–1095)

The strong phenotypic relationship between chondroid metaplasia and hypochondroplastic dwarfism suggests that a major genetic factor in chondroid metaplasia is attributable to a pleiotropic effect of the hypochondroplasia gene. It is unlikely that the conditions are caused by 2 distinct genes because that would imply a specific founder effect in multiple breeds. Furthermore, both conditions are characterized by abnormal chondrocyte differentiation. In Dachshunds, severe disk degeneration with calcification is reportedly highly heritable (heritability estimate, 0.47 to 0.87). This high heritability of disk calcification was estimated in Dachshunds via plain radiography performed during the optimum period (ie, at 24 to 30 months of age, when the number of visibly calcified disks appears to reach a maximum). Heritability of disk calcification within the breed indicates involvement of genes other than the gene encoding hypochondroplasia. Furthermore, disk degeneration with calcification in dogs with severe disease appears to be continuous in nature, which suggests that the degenerative disease is a multifactorial trait. An association between disk calcification and disk herniation appears logical because both conditions are
associated with severe degeneration of the disk. Several studies\textsuperscript{8,13,14} have revealed that the incidence of disk herniation is significantly higher in dogs in which calcified disks are detected during radiographic examination, compared with the incidence in dogs without calcified disks. However, there is high variation in reported incidences of herniation over various periods in dogs with calcified disks (range, 19\% to 45\%) and in dogs without calcified disks (range, 4\% to 6\%). This variation is possibly attributable to differences in study designs. For example, dogs in the aforementioned studies\textsuperscript{8,13,14} were radiographically evaluated at different ages (range, 1 to 11 years). The period during which dogs were monitored for disk herniation also varied considerably between and within these studies. Thus, the association between disk calcification and disk herniation was not quantified in controlled conditions. Furthermore, the incidence of disk calcification within the individual dogs was not measured.

As indicated by high heritability estimates for severe disk degeneration with calcification in Dachshunds,\textsuperscript{8} an effective response (change of population mean) may be obtained via selection against disk calcification (as evaluated at 2 years of age) without changing other features characteristic of the breed. However, the effect of selective breeding on disk herniation depends on the strength of the association between disk calcification and herniation. The purpose of the study reported here was to quantify the association between disk calcification and the occurrence of disk herniation among Dachshunds ≥ 2 and ≤ 8 years of age.

**Materials and Methods**

**Animals**—The study included 61 Dachshunds that were radiographically screened for calcification of intervertebral disks at 2 years of age as part of their participation in other studies.\textsuperscript{10,11} The dogs were initially evaluated at the clinic only to participate in those studies. At the time our study was conducted (2004 through 2005), the 61 dogs were ≥ 8 years of age. Thirty-seven of the 61 Dachshunds were still alive; the other 24 had since died. Of the surviving dogs, 36 were radiographically reexamined for calcification of intervertebral disks (1 owner declined), and historical data regarding occurrence of disk herniation were obtained from owners for all 61 dogs by use of a questionnaire. For the statistical analysis, dogs were considered to have a history of clinical disk herniation when a diagnosis of clinical disk herniation had been made by a veterinary practitioner and the dogs had been euthanized or treated surgically or medically on the basis of this diagnosis.

**Radiographic examination**—Plain spinal radiography was performed on each dog, and ≥ 5 laterolateral images were obtained to capture the vertebral column from vertebrae C2 through S1. Foam pads were used for optimal positioning to ensure full exposure of all vertebral disks. The images were produced by use of the same radiographic equipment\textsuperscript{8,10,11} as was used in the previous studies,\textsuperscript{8,10,11} in which the dogs were radiographically evaluated at 2 years of age.

Interpretation of radiographs that were obtained from the 36 surviving dogs at ≥ 8 years of age was performed by 2 radiologists individually, then the radiologists conferred to reach a consensus. One of the radiologists had performed the evaluations in the other studies\textsuperscript{10,11} (2 blinded evaluations, followed by 1 evaluation for a definitive diagnosis). For radiologic evaluations of dogs at 2 years of age, radiographic images from the other studies were used.

**Questionnaire**—A questionnaire was mailed to owners of 90 dogs from the other studies,\textsuperscript{10,11} and owners of 29 dogs declined to participate or could not be found. Of the remaining 61 owners, all responded to the questionnaire, but 1 declined to participate in the follow-up radiographic examination. Questions asked included whether the dog had a history of signs of spinal disease or lameness and, if so, whether the signs were provoked by a specific incident and whether the dog was examined and treated by a veterinarian (including diagnosis and outcome). Owners were also asked about the current status and degree of activity of the dog, whether it climbed stairs, and whether it was reluctant to jump. In the event that the dog had died, owners were asked to identify the reason. Questionnaire data regarding the nonsurviving dogs were confirmed (or clarified) via telephone interview; those regarding the nonsurviving dogs were confirmed via owner interviews at the time dogs received follow-up radiographic examinations.

**Statistical methods**—Maximum likelihood logistic regression\textsuperscript{13} was performed by use of a statistical software package\textsuperscript{15} to estimate the association between disk calcification at 2 years of age (predictor variable) and subsequent detection of signs of clinical disk herniation (outcome variable). Three predictor variables were created on the basis of the location of calcified disks within the vertebral column at 2 years of age. Three models were compared with inclusion of one or more of those predictors: number of calcified disks at 2 years of age, number of calcified disks from T10 through L3 at 2 years of age, and number of calcified disks from C2 through T10 and from L3 through S1 at 2 years of age. These models were constructed from the full dataset of 61 dogs. Goodness-of-fit of each model was assessed via the Hosmer-Lemeshow test, and various models were compared via AIC values.\textsuperscript{16}

In addition, the number of calcified disks in dogs at ≥ 8 years of age was evaluated as a predictor for previous cases of clinical disk herniation on the basis of data from the 36 surviving dogs that were radiographically evaluated at 8 years of age. Linearity of the logits of continuous predictor variables (eg, number of calcified disks) was evaluated by categorizing the continuous variable into 5 classes and plotting the log odds of the probability against the median of each class.

The correlation between numbers of calcified disks in the 36 surviving dogs at 2 and ≥ 8 years of age was evaluated via linear regression. A Fisher exact test was used to test for association between number of calcified disks at 2 years of age and risk of euthanasia because of disk herniation.

**Results**

**Animals**—Of the 61 dogs that were used in the study, questionnaire data aided in the identification of 22 (36\%)
with a history of clinical intervertebral disk disease. All dogs had a history of veterinarian-diagnosed disk herniation that led to euthanasia (n = 8, including 3 dogs with prior surgery), surgery (8), or conservative treatment with anti-inflammatory drugs (5). It was assumed that the diagnosis at the different veterinary clinics at which the dogs were evaluated was correct, although criteria applied in achieving the diagnosis were not investigated. Signs of severe back pain were detected in 1 dog, but that dog recovered without veterinary treatment. No additional information regarding lameness was reported.

All 61 dogs (surviving and nonsurviving) underwent radiographic evaluations at 2 years of age. In dogs with $\geq 9$ calcified intervertebral disks at 2 years of age, 7 of 8 had a subsequent history of severe disk herniation and 5 of 8 had been euthanatized because of veterinarian-diagnosed disk herniation before 8 years of age (Table 1). Of the 19 dogs with $> 4$ calcified disks at 2 years of age that died, 12 had a subsequent history of disk herniation and 7 were euthanatized because of disk herniation. In dogs with $\leq 2$ calcified disks, disk herniation was not reported as a cause of death.

Only 36 surviving dogs were radiographically evaluated at $\geq 8$ years of age. In these dogs, the total number of calcified intervertebral disks identified was 83, and of these, only 11 (13%) disks had not been visibly calcified at 2 years of age. At 2 years of age, 139 calcified disks had been identified in the same 36 dogs. The number of calcified disks was higher at 8 years of age than at 2 years of age by 1 calcified disk in 5 dogs and by 2 calcified disks in 1 dog.

**Statistical analysis**—Among the 61 dogs, risk of euthanasia following clinical disk herniation was significantly higher in dogs with $> 4$ calcified disks than in dogs with $\leq 4$ calcified disks (37% vs 4.8%, respectively; $P = 0.003$). When the number of calcified disks was treated as a continuous predictor variable in logistic regression analyses, results indicated a strong association between the probability of clinical disk herniation and the number of calcified disks at 2 years of age (Table 2 and Figure 1). According to that model, the odds of clinical disk herniation increased by a factor of 1.42 (95% CI, 1.19 to 1.81) for each additional calcified disk. When only the number of calcified disks from T10 through T3 was included in the analysis, the odds of clinical disk herniation increased by a factor of 2.49 (95% CI, 1.53 to 4.53) for each additional calcified disk.

Results of Hosmer-Lemeshow tests indicated that the 3 models based on number of calcified disks at 2 years of age fit the data well. Values of AICs indicated that separating numbers of calcified disks into 2 predic-

<table>
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<th>Table 1—Summary of history of presumptive clinical intervertebral disk herniation* (IDH) and cause of death in 61 Dachshunds, by number of calcified intervertebral disks identified during radiographic evaluation of dogs at 2 years of age.</th>
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<tbody>
<tr>
<td><strong>No. of calcified disks</strong></td>
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<td>0</td>
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<tr>
<td>1 or 2</td>
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<td>3 or 4</td>
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<td>5 or 6</td>
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<td>13 or 14</td>
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<td>15 or 16</td>
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</table>

*IDH was diagnosed at various veterinary clinics, and information regarding the diagnostic criteria used was not obtained. †Death occurred before radiographic examination at $\geq 8$ years of age.

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<th>Table 2—Results from logistic regression analyses for predicting the occurrence of clinical disk herniation in Dachshunds (n = 61) according to the number of calcified disks detected at 2 time points (2 and 8 years of age) and within certain regions of the vertebral column.</th>
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<tr>
<td><strong>Predictor</strong></td>
</tr>
<tr>
<td>Calc2year</td>
</tr>
<tr>
<td>CalcT10-L3</td>
</tr>
<tr>
<td>CalcOther and CalcT10-L3</td>
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<tr>
<td>Calc8year‡</td>
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*One unit of change in the OR is the equivalent of 1 calcified disk. †Profile likelihood CI. ‡Analysis was based on data from 36 dogs.

Calc2year = Number of calcified disks at 2 years of age. CalcT10-L3 = Number of calcified disks between T10 through L3 at 2 years of age. CalcOther = Number of calcified disks cranial to T10 and caudal to L3 at 2 years of age. Calc8year = Number of calcified disks at 8 years of age.
Number of calcified intervertebral disks in dogs at ≥ 8 years of age was not significantly (P = 0.67) associated with clinical disk herniation among the 36 dogs evaluated (Table 2). A goodness-of-fit test revealed a difference between predicted and observed values that neared significance (P = 0.053), which was interpreted as indicating low predictive power of the model.

Linear regression analysis of data from the 36 surviving dogs revealed that the numbers of calcified intervertebral disks at 2 and 8 years of age were strongly correlated (P < 0.001; Figure 2). The relationship was as follows:

\[ \text{number of calcified disks at 8 years of age} = 0.104 + 0.57 \times \text{(number of calcified disks at 2 years of age)} + \text{error}. \]

**Discussion**

In chondrodystrophic breeds of dogs such as Dachshunds, predisposition to disk herniation is related to the severity of disk degeneration, which is attributable to impairment of the mechanical function and increased stress on the outer annulus fibrosus.\(^5\) Intervertebral disk calcification coexists with severe disk degeneration, although severe degeneration may develop without visible disk calcification.\(^6\) If one assumes that the liability of disk degeneration within a population of Dachshunds is normally distributed and that there is a liability threshold for disk calcification, selection against disk calcification may be expected to decrease the incidence of disk herniation in dogs. In theory, the well-being of the Dachshund population may be improved even with a mean liability below the threshold for disk calcification. The results of our study indicated a strong association between number of calcified disks at 2 years of age and occurrence of clinical disk herniation at a later age. These findings supported the hypothesis that number of calcified disks at 2 years of age is an indicator of severity (liability) of disk degeneration and thus a measure of the risk of disk herniation.

Three models with 3 different predictors were tested; each predictor included a different range of vertebral disks in determination of the number of calcified disks at 2 years of age. The thoracolumbar region of the vertebral column is of particular interest because disk herniation in this region has a higher risk of being associated with clinical signs of disease. The OR was higher for numbers of calcified disks in that region than numbers for other regions partly because fewer disks exist in that region, which yields a higher relative importance of any calcified disks, and also because there is a higher probability of clinical signs associated with disk herniation in the thoracolumbar region. However, the models that included the number of calcified disks throughout the entire vertebral column were superior to the model that contained only the number of calcified disks from T10 through L3, as indicated by a lower AIC value. Separating numbers of calcified disks into 2 predictors on the basis of location within the vertebral column did not increase the predictive value of the model. Because a more parsimonious model is preferred, the model that included data from the entire vertebral column as the only variable was considered superior.

In the 36 dogs that were radiographed at ≥ 8 years of age, the number of calcified disks had declined from 139 to 83 at 2 years of age. Only 6 dogs had a slightly (1-2) increased number of calcified disks. These findings supported the hypothesis that development of additional calcified disks after 2 years of
age is a rare condition, as suggested by findings in another study. The correlation between the numbers of calcified disks at 2 and 8 years of age was high. On average, 48% of the calcifications were no longer detectable at ≥8 years of age. However, the number of disks that were no longer evidently calcified within each dog ranged widely (95%, CI 24% to 62%). As revealed in a study of Dachshunds, the number of disks that may resolve over time in some dogs, probably in relation to disk herniation, while in other dogs the number of calcified disks may be unchanged. This may explain why the number of calcified disks in dogs ≥8 years of age in the present study was not correlated with the occurrence of clinical disk herniation. Many of the dogs (10/22 [45%]) in which clinical disk herniation was diagnosed died; an additional 16 dogs died for other reasons. Thus, the sample size of dogs ≥8 years of age was considerably reduced, which limited the likelihood of finding a significant association between the number of calcified disks and the probability of clinical disk herniation. However, the model of that association did not fit the data well, indicating that there was no association between the number of calcified disks and the probability of clinical disk herniation. In the study reported here, 1 of 12 dogs without disk calcifications had disk herniation, and the incidence of disk herniation in the subsample without calcified disks was estimated at 11% in a lifetime (as calculated from the intercept in the model). Investigators in other studies of the association between disk calcification and clinical disk herniation, disk calcification was measured with either scale. The number of calcified disks appears to be a better measure of disk calcification for several reasons. First, none of the disks in Dachshunds can be classified as normal. The step from no visible calcification to 1 visibly calcified disk is merely a small step on a continuous scale of disk degeneration. Second, a dog with many calcified disks will have severe disk degeneration in many of its intervertebral disks. The use of the number of calcified disks as a measure of intervertebral disk degeneration is supported by the high heritability of disk calcification detected on the basis of the continuous scale (ie, number of calcified disks vs a dichotomous scale).

The results of our study also indicated a quantitative association between number of calcified disks and occurrence of clinical disk herniation. The degree of disk degeneration and thus the predisposition to disk herniation may also vary within dogs that do not appear to have calcified disks. On the basis of the hypothesis that disk calcification group is a threshold characteristic, the predisposition to disk herniation in the subpopulation with no calcified disks would depend on the mean liability of disk degeneration in the population and thus occurrence (the mean and distribution) of disk calcification in the population. In the study reported here, 1 of 12 dogs without disk calcifications had disk herniation, and the incidence of disk herniation in the subsample without calcified disks was estimated at 11% in a lifetime (as calculated from the intercept in the model). Investigators in other studies detected a lower incidence (4% to 6% of dogs within the defined study periods) among dogs with no calcification evident on radiographs. This was most likely an underestimation because the observation period was variable (as short as 1 year) and in many situations did not encompass the interval of 4 to 7 years of age, when most disk herniations occur. Also, the true prevalence of disk calcification and incidence of disk herniation may be higher than suggested by the results of these studies because few of the dogs used were radiographed at the optimum age for disk calcification (2 to 3 years of age). In addition, in many situations, the age period with highest risk of disk herniation (4 to 7 years of age) was not included in the evaluation period. The occurrence of clinical disk herniation would also depend on the number of calcified disks, and this information was not evaluated in the other 3 studies. Thus, the results of the study reported here cannot be compared with findings of other studies.

It is important to differentiate between disk herniation and clinical disk herniation (ie, disk herniation leading to a clinical diagnosis). The high frequency of disk calcification in the thoracic vertebral column, compared with other areas, suggested that this region is the most severely degenerated and thus more disposed to disk herniation. However, clinical disk herniation from T1 through T9 is rare because of the location of the ligamentum interscapulare. The ratio of the width of the spinal cord to that of the spinal canal is high in the thoracic region and low in the lumbar and cervical region. Areas in with a low ratio, disk herniation is more likely to be subclinical. In addition, the dorsal zone of annulus fibrosus is narrow in the thoracolumbar region, and the nucleus pulposus is located dorsally. In combination, these characteristics may explain why most (65% to 75%) clinical cases of disk herniation occur between vertebrae T11 through L2, although high frequencies of disk herniation between vertebrae T12 through L2 and vertebrae L2 through L5 have been detected during pathologic examination of affected dogs. Subclinical disk herniation is likely painful and may cause weak neurologic symptoms (ie, decreased proprioception) but remain undiagnosed because dog owners may not acknowledge the signs. One limitation to our study is that the questions in the questionnaire required subjective responses from owners. In particular, change of behavior attributable to pain may not have been noticed by owners. In severe cases of intervertebral disk herniation that lead to euthanasia, the accuracy of historical information obtained is likely high because owners are likely to remember the cause of death and usually have no incentive to hide that information. Consequently, the incidence of disk herniation in the group of dogs which were alive when our study was performed was likely underestimated, because all but 1 case were severe (ie, clinical signs prompted owners to seek veterinary examination of affected dogs and, for most [19/22] dogs, resulted in surgery or euthanasia).

In theory, some of the Dachshunds classified as having clinical disk herniation may have had other diseases because we did not investigate the criteria that had led to the diagnoses. However, 16 of the 22 dogs presumed to have clinical disk herniation had undergone surgical treatment for disk herniation, had been euthanatized on the basis of the diagnosis, or both. Furthermore, the incidence of clinical disk herniation in Dachshunds is very high (approx 18%). Therefore, the potential for bias from false-positive diagnosis of disk herniation was likely low in our study and should not have affected statistical conclusions. The measurement bias from false-negative diagnoses is likely much more important. Thus, our study estimated
the association between number of calcified disks and acknowledged cases of clinical disk herniation.

Low test variability is crucial when evaluating heritability and associations between identified disk calcification and occurrence of disk herniation. Test error (inter- and intraobserver variation) was not included in our models. However, intertest variability was minimized by use of the same radiographic film and folio, the same radiographic technique, and the same radiologic interpreters throughout the study. Sedation is generally recommended for optimal positioning of the spine but was not used in all dogs because some owners objected (the radiographs were only for research purposes). To overcome this challenge, all images were evaluated during the radiographic session by the same radiologist until images with optimal positioning and exposure were obtained of the entire vertebral column. Importantly, all dogs underwent radiographic evaluation during the optimum period for detection of disk calcification (2 years of age), decreasing the test variability.

The true association between disk calcification and disk herniation may be even stronger than indicated by this study because the identification of episodes of disk herniation is subject to uncertainty when based on owner recall. To be more certain of the association detected in our study, postmortem pathologic examination should be performed on old dogs with a history of disk herniation (possibly supported by information recalled by owners) that underwent radiographic evaluation at 2 years of age. Currently, a radiographic screening program is ongoing in Denmark, increasing future opportunities to obtain postmortem pathologic information on dogs that have been radiographically screened at 2 years of age. The Danish Dachshund Club recommends breeding dogs with ≤ 2 calcified disks but not dogs with > 4 calcified disks. Dogs with 3 or 4 calcified disks should only be used in strategic breeding (ie, only 1 or 2 litters and screening of the progeny before selection for further breeding). These breeding recommendations are supported by the findings in the present study.

Presumptive clinical disk herniation was a frequent cause of euthanasia in dogs with ≥ 4 calcified disks but had not occurred in dogs with < 4 calcified disks. These findings stress the importance of selective breeding against disk calcification. The findings may also be used in small animal practice to identify dogs that are predisposed to disk herniation so that preventive measures can be initiated. Because disk calcification is highly heritable and is strongly associated with disk herniation, a breeding program that screens dogs and selects against disk calcification can be expected to effectively reduce the occurrence of disk herniation in Dachshunds. The utility of such a screening program would depend on low test variability. Evaluation of the development of disk calcification should not be performed before dogs are 24 months of age because the number of visibly calcified disks increases rapidly from 12 to 24 months of age. Further, evaluation should be performed before dogs are 4 years of age because disk calcifications are resorbed in relation to disk herniation and most calcified disks may resolve with increasing age.

Furthermore, the radiographic technique should be standardized and include sedation of all dogs for optimal positioning, and the inter- and intraobserver variation should be minimized.

References


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